Description of the biology and immature stages of *Pagyris ulla* (Hewitson) (Lepidoptera: Nymphalidae: Ithomiini) in the Colombian Andes

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The biology and immature stages of *Pagyris ulla* (Hewitson, [1857]) (Lepidoptera: Nymphalidae: Ithomiini) are described for the first time. The species’ host plant is established to be *Brugmansia candida* Pers. (Solanaceae). The life cycle from eggs to adult under laboratory conditions and ambient temperature took approximately 47 days, and the larvae passed through five instars. The larvae are gregarious, feed at night, and rest during the day in nests made by joining leaves near the apex of the plant stem.

**Keywords:** life cycle; Nymphalidae; Solanaceae; South America

Introduction

Although the tribe Ithomiini (Nymphalidae) is one of the most comprehensively studied within Neotropical Lepidoptera (Willmott and Freitas 2006), basic aspects of the biology and life cycle of many species are still not known; in particular, few papers describe immature instars (Young 1973a, b, 1977, 1978; Freitas 1993; Freitas and Brown 2002, 2005; Willmott and Lamas 2006, 2008; Greeney et al. 2009). The genus *Pagyris* Boisduval 1870, reviewed by Lamas (1986), comprises the species *Pagyris ulla* (Hewitson [1857]), with six subspecies, *Pagyris cymothoe* (Hewitson [1855]), with two subspecies, *Pagyris priscilla* Lamas, 1986 and *Pagyris renelichyi* Neild. 2008; Lamas (1986) initially considered *P. priscilla* as a subspecies of *P. ulla*, but later raised it to species status (Lamas 2004), whereas *P. renelichyi* is considered a close and allopatric relative from the Venezuelan Guiana highlands (Neild 2008). The genus is found in the Andes from Venezuela to Bolivia, and in the Venezuelan Guiana highlands. Of all four described species and their respective subspecies, only the host plants and some morphological characters of immature stages of *P. cymothoe* have been reported (Lamas 1986; Drummond and Brown 1987; Willmott and Freitas 2006). This species’ larvae have been reared on the leaves of *Solanum chlamydogynum* Bitter, *Brugmansia suaveolens* Humb. & Bonpl. ex Willd. (Lamas 1986), *Acnistus arborescens* (L.) Schltdl., *Cuatresia riparia* (Kunth) Hunz. and *Dunalia solanacea* Kunth (Solanaceae) (Drummond and Brown 1987). Descriptions of the biology and the immature stages are important to advance our knowledge of the morphological diversity and ecology of the group. In recent phylogenetic studies,
new characters resulting from descriptions of the biology and immature stages of key species have helped provide a greater understanding of the systematics of Ithomiini (Brown and Henriques 1991; Brown and Freitas 1994; Willmott and Freitas 2006). The description of the immature stages of *P. ulla* may also help better determine the relationships between the genera *Pagyris* and *Placidina* d’Almeida, 1928. According to Brower et al. (2006) *Pagyris* may be paraphyletic with respect to *Placidina*. Information on the life history of *P. ulla* might also help to reconsider the inclusion of *P. cymothoe* within *Pagyris*, through comparison of the immature stages of both species, given that the adult morphological characters used to assign *P. cymothoe* to the genus *Miraleria* Haensch, 1903 are considered weak (Lamas 1986). The objective of this study is to describe the biology of *P. ulla*, its immature stages and the development time for each stage.

**Materials and methods**

Egg masses of *P. ulla* were collected at the Parque Ecológico Piedras Blancas, located in the municipality of Guarne, in the east of the department of Antioquia, Colombia (6°17′40.68″ N, 75°30′4.32″W, WGS84), between 2007 and 2009. The study area lies at an altitude of 2350 m, with an average annual temperature of 15°C (IDEAM 2003), and is classified within the life zone of tropical lower montane moist forest (Holdridge 1987). Eggs were taken to the laboratory and kept in plastic containers at ambient temperature to monitor the different stages until adult emergence. The containers were cleaned daily and fresh leaves of the host plant were added while noting the dates of changes in instars to calculate their duration. Only those egg masses that were observed being laid in the field were used to calculate the development time of the eggs. Larvae from each instar and pupae were fixed in Kahle’s solution; adults were mounted with entomological pins and deposited in the Museo Entomológico e Insectario Piedras Blancas (MEPB). Characters used by Willmott and Freitas (2006) in a cladistic analysis of the tribe are used to describe the immature stadia. Photographs were also taken of each stage.

**Results**

A total of 800 larvae, from seven egg masses, were monitored. Larvae went through five instars and took 23.1 ± 2.15 days (*n* = 417, larvae of different egg masses) to pass from the first through the fifth instar. The whole cycle took 47.5 ± 1.53 days, from oviposition to the emergence of new adults (*n* = 305, larvae of different egg masses).

**Descriptions of immature stages**

**Eggs** (Figure 1A)

The eggs are white, ellipsoid, with a pointed apex and longitudinal ridges without elevated carinae. The four egg masses where females were observed ovipositing were used to record development time. These egg rafts contained a total of 611 eggs, which took 9.7 ± 1.27 days to hatch.
Figure 1. Stages of *Pagyris ulla*. (A) Egg, (B) first instar, (C) second instar, (D) third instar, (E) fourth instar, (F) fifth instar, (G, H) pupa, (I) male: 1 – dorsal and 2 – ventral, (J) female: 1 – dorsal and 2 – ventral.
First instar (Figure 1B)
The head capsule is shiny black, with a few large, scattered white setae. The body is uniformly pale lime-green in colour, without protuberances and covered with large setae along its whole length. The thoracic legs are dark, and the anal plate is sclerotized in an irregular manner. Development time: 4.5 ± 0.49 days (n = 592).

Second instar (Figure 1C)
The head capsule is shiny black, with large scattered setae. Flattened swellings are present along the entire body and even under the spiracles, being slightly larger from A3 through A7 and very small on the thorax. The body is uniformly olive-green in colour, with open, white, U-shaped marks, less conspicuous under the abdominal spiracles. Development time: 3.7 ± 0.83 days (n = 592).

Third instar (Figure 1D)
Similar to the previous stage. Development time: 3.6 ± 0.76 days (n = 582).

Fourth instar (Figure 1E)
Similar to the previous stage. Two discontinuous bands with yellow markings appear above and below the spiracles from T2 through A8. The upper band begins in each segment with a round mark, followed by two smaller round marks at the end of the segment. This pattern is less evident on T2, T3 and A8. A U-shaped mark is present on the lower band of each segment, less evident after A2. The spiracles are black. The lower third of the anal process is sclerotized. Development time: 3.7 ± 0.53 days (n = 557).

Fifth instar (Figure 1F)
The head capsule is uniformly shiny black with large, scattered setae. The body is olive-green, and dark on the ventral side. The cuticle is densely covered with short setae, although some larger and more scattered setae are also present. The lateral swellings on each segment become more evident except on A8 and appear semicircular from a dorsal perspective. The same applies to the markings on the body, with all segments similarly marked, becoming more intense orange in colour. The thoracic legs are shiny black, and the abdominal and anal prolegs have an external black sclerotized process. The anal plate is dark and completely sclerotized. Larvae become yellow-green in colour when entering the prepupal stage. Development time: 7.7 ± 1.07 days (n = 417).

Pupa (Figure 1G, H)
The pupa is globular in shape, with a pupal angle of 180° (the position that the thorax adopts with respect to the abdomen). The pupa is copper coloured with a yellow sheen; the abdomen has a pronounced curve on the posterior half, after the cremaster; A1 and A2 have the same width and a pair of dark dorsal bands is present on the
abdomen, but only continuous on A1 and A2. A series of points are found at the beginning of the other segments. There is a button-shaped protuberance at the base of the wings; ocular capsules are rounded and slightly bulging; wing pads have dark marks, and the discal cell and all veins are darkly delineated. The cremaster is black with a smooth base, without any protuberance. The exuvial holdfast tubercles are strongly sclerotized, covered by a black patch, extending up to the stalk of the cremaster; pupal skins take on a brown coloration after adult emergence. Development time: 14.6 ± 0.82 days (n = 417).

**Biology**

Adults were not common at the study site, and have always been observed flying more than 2 m above the ground along forest edges, near their host plants. On four occasions, a female was observed laying eggs on its host plant, *Brugmansia candida* (Solanaceae) (Vélez et al. 2009), known locally as “Borrachero Blanco”. After flying around the host plant and selecting a leaf at more than 3 m above the ground, the female began the oviposition on the underside of the leaf, near the central vein. A female took approximately 1 hour and 10 minutes to lay 199 eggs. Eggs are grouped and masses are made up of 133–205 eggs (n = 7). After simultaneous hatching, larvae feed on the chorion, and then begin to eat the leaf from the centre, making holes. Larvae remain aggregated until the final instar. From the second instar on, a large amount of silk production is evident. The silk is used to join the leaves closest to the apical part of the plant stem in constructing temporary nests where larvae rest during the day, feeding only at night. Larvae from a single egg mass construct two or three nests next to each other in which they congregate in large numbers. This behaviour was observed both in the field and in the laboratory. Furthermore, larvae can be found during the day within the flower buds, densely grouped, or even resting among the leaf litter close to the base of the host plants. Prepupae attach themselves in pairs or singly, close to the ground; pupae were also found among the leaf litter, under the bark of dead trees, and even in moss, but never on the host plant. Adults always emerge during the morning and begin to fly before midday.

**Discussion**

Nest construction in Ithomiini has been reported so far only for species of the subtribe Dircechina (Willmott and Freitas 2006). The folding or rolling up of host plant leaves is typical in species of *Dirceenna* Doubleday [1847] and *Hyalenna* Forbes 1942. According to Willmott and Freitas (2006) this character is a synapomorphy for the clade Dirceenna + *Hyalenna*. Tying up of nearby leaves with silk has only been reported in *Episcada clausina* (Hewitson, 1876) (Willmott and Freitas 2006). *Pagyris ulla* is therefore the second species with a similar behaviour to *E. clausina* and this observation constitutes the first report of shelter construction within Ithomiini for a species that does not belong to Dircechina.

According to the characters encoded by Willmott and Freitas (2006) for the immature stages of *Pagyris*, characters that were considered as ambiguous synapomorphies, there are differences between *P. cymothoe* and *P. ulla*. In the first instar larvae, the colour of the cephalic capsule of *P. ulla* is dark whereas that of *P. cymothoe* is pale, and the colour of the cremaster stalk in the pupa is black in *P.
ulla whereas that of *P. cymothoe* is red. These differences suggest the ambiguity of these synapomorphies. However, the results of this work do not provide additional data for inclusion of *P. cymothoe* within *Pagyris*.

*Placidina euryanassa* (C. Felder and R. Felder 1860) (Lamas 2004) of the monotypic genus *Placidina* is the sister group of the genus *Pagyris* according to morphological data (Willmott and Freitas 2006). However, *Pagyris* is paraphyletic with respect to *Placidina* based on molecular data (Brower et al. 2006). According to Willmott and Freitas (2006) the following characters support the clade *Pagyris* + *Placidina*: lateral swellings in the abdominal segment eight and the dark ventral colour in last instar larvae, as well as the brown coloration in pupal skins after eclosion, and ocular capsules rounded in the pupae. In addition to *P. cymothoe*, the species *P. ulla* also shares these synapomorphies with *Placidina euryanassa*. A pupal angle of 180° has only been reported in species of the genera *Methona* and *Placidina* (Willmott and Freitas 2006), which are phylogenetically distant within the tribe [see fig. 5. in Brower et al. 2006 and fig. 2. in Willmott and Freitas 2006]. Now this feature is confirmed to exist in *Pagyris* as well. Additionally, *Placidina euryanassa* has a similar biology to that observed in members of *Pagyris*. In Brazil, *Placidina euryanassa* also lays eggs in masses of more than 100, the fifth instar larvae rest near the ground (Freitas 1993), and feed on leaves of plants of the genus *Brugmansia* (*B. suaveolens*) (Freitas 1993). New phylogenetic analyses should make use of these traits to confirm the inclusion of *P. cymothoe* within *Pagyris*.

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References

Brower AVZ, Freitas AVL, Lee M-M, Silva KL, Whinnett A, Willmott KR. 2006. Phylogenetic relationships among the Ithomiini (Lepidoptera: Nymphalidae) inferred from one mitochondrial and two nuclear gene regions. Syst Entomol. 31:288–301.

Brown KS, Freitas AVL. 1994. Juvenile stages of Ithomiinae: overview and systematics (Lepidoptera: Nymphalidae). Trop Lepid. 5:9–20.

Brown KS, Henriques SA. 1991. Solanaceae III: taxonomy, chemistry, evolution. London: Linnean Society of London; 492p. Chemistry, co-evolution, and colonisation of Solanaceae leaves by ithomine butterflies; p. 51–68.

Drummond BA III, Brown KS Jr. 1987. Ithomiinae (Lepid: Nymphalidae): summary of know larval foodplants. Ann Miss Bot Garden. 74:341–358.

Felder C, Felder R. 1860. Lepidopterologische Fragmenten. Part 4. Wien ents. Monats. 4:97–112.

Freitas AVL. 1993. Biology and population dynamics of *Placidula euryanassa*, a relict ithomiine butterfly (Nymphalidae: Ithomiinae). J Lepid Soc. 47:87–10.

Freitas AVL, Brown KS Jr. 2002. Immature stages of *Sais rosalia* (Nymphalidae, Ithomiinae). J Lepid Soc. 56:104–106.

Freitas AVL, Brown KS Jr. 2005. Immature stages of *Napeogenes sulphurina* Bates, 1862 (Nymphalidae, Ithomiinae) from northeastern Brazil. J Lepid Soc. 59:35–37.
Greeney HF, Hill RI, Simbaña WR, Gentry GL. 2009. The immature stages and natural history of *Veladyris pardalis* (Salvin, 1869) in eastern Ecuador (Lepidoptera: Nymphalidae: Ithomiinae). J Insect Sci. 9:1–6. doi: 10.1673/031.009.3501

Hewitson WC. 1876. Illustrations of new species of exotic butterflies selected chiefly from the collections of W. Wilson Saunders and William C. Hewitson. Vol 2. London: John Van Voorst; 300.

Holdridge LR. 1987. Ecología basada en zonas de vida. Quinta impresión. San José Costa Rica: IICA; 216p.

IDEAM. 2003. Informe de precipitación anual 1986–1992 Estación El Vivero. Medellín: Instituto de Hidrología, Meteorología, y Estudios Ambientales IDEAM.

Lamas G. 1986. Revisión del género *Pagyris* Boisduval (Lepidoptera, Nymphalidae: Ithomiinae). An Inst Biol Univ Nac Autón Méx Ser Zool. 56:259–276.

Lamas G. 2004. Atlas of neotropical lepidoptera. Volume 5A. Gainesville (FL): Association for Tropical Lepidoptera; Scientific Publishers; Checklist: Part 4A. Hesperioidea – Papilionoidea. Nymphalidae. Ithomiinae; p. 172–191.

Neild AFE. 2008. The butterflies of Venezuela. Part 2: Nymphalidae II (Acraeinae, Libytheinae, Nymphalinae, Ithomiinae, Morphinae). A comprehensive guide to the identification of adult Nymphalidae, Papilionidae, and Pieridae. London: Meridian Publications; 275.

Vélez A, Duque P, Wolff M. 2009. Mariposas del parque ecológico Piedras Blancas. Guía de campo. Medellín: Fondo editorial Comfenalco Antioquia. Subfamilia Ithomiinae; 99–109.

Willmott KR, Freitas AVL. 2006. Higher level phylogeny of the Ithomiinae (Lepidoptera: Nymphalidae): classification, patterns of larval hostplant colonization and diversification. Cladistics. 22:297–368.

Willmott KR, Lamas G. 2006. A phylogenetic reassessment of *Hyalenna* Forbes and *Dircenna* Doubleday, with a revision of *Hyalenna* (Lepidoptera: Nymphalidae: Ithomiinae). Syst Entomol. 31:419–468.

Willmott KR, Lamas G. 2008. A revision of the genus *Megoleria* (Lepidoptera, Nymphalidae, Ithomiinae). Trop Lepid Res. 18:46–59.

Young AM. 1973a. On the life cycle and natural history of *Hymenitis nero* (Lepidoptera: Ithomiinae) in Costa Rica. Psyche. 79:284–294.

Young AM. 1973b. The life cycle of *Dircenna relata* (Ithomiidae) in Costa Rica. J Lepid Soc. 27:258–267.

Young AM. 1977. Notes on the biology of *Hypothyris euclea* in Costa Rica. Pan-Pac Entomol. 53:104–113.

Young AM. 1978. The biology of the butterfly *Aeria curimeda agna* (Nymphalidae: Ithomiinae: Oleriini) in Costa Rica. J Kans Entomol Soc. 51:1–10.