

Early stage biology of two euptychiine butterfly species in the Peruvian Amazon (Lepidoptera: Nymphalidae: Satyrinae: Satyrini)

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Abstract: The immature stages of two euptychiine butterflies (Nymphalidae: Satyrinae), *Paryphthimoides terrestris* (Butler, 1867) and *Magneuptychia iris* (C. Felder & R. Felder, 1867), are described herein and their natural host plants are documented. Notes on the immature stages of these two species were included previously in an unpublished dissertation, although our work is the first to provide satisfactory illustrations of the immature stages of these two taxa. Based on the population found and studied in Madre de Dios, Peru, both species utilize plant species in the family Poaceae. We provide illustrations of immatures, head capsules, and host plants for both species.

Key Words: Euptychiina, Finca Las Piedras, host plant, life history, Madre de Dios.

Resumen: Los estadios inmaduros de dos mariposas euptychiinas (Nymphalidae: Satyrinae), *Paryphthimoides terrestris* (Butler, 1867) y *Magneuptychia iris* (C. Felder & R. Felder, 1867) se documentan y describen, así como también sus plantas hospederas naturales. Los estadios inmaduros de estas dos especies fueron documentados previamente en una disertación no publicada; no obstante, nuestro estudio es el primero en proporcionar ilustraciones de los estadios inmaduros de estos dos taxones. Basado en la población encontrada y estudiada en Madre de Dios, Perú, ambas especies utilizan especies de plantas de la familia Poaceae. Proporcionamos ilustraciones de inmaduros, cápsulas cefálicas y plantas hospederas para estas dos especies.

Palabras clave: Planta hospedera, Finca Las Piedras, historia de vida, Madre de Dios, Euptychiina.

INTRODUCTION

The nymphalid subtribe Euptychiina is a diverse radiation found predominantly in the Neotropical lowlands, with more than 440 described species (excluding synonyms), with true species-richness estimated to exceed 500 species (Hurtado *et al.*, 2021; unpublished data). Members of the subtribe are common elements of butterfly fauna throughout Central and South America, as several inventories focused on different habitat types have shown (e.g., Beltrami *et al.*, 2014; Lamas *et al.*, 1991; Yoshimoto *et al.*, 2018). In particular, the southwestern Amazon basin (i.e., Madre de Dios department in Peru and adjacent state of Acre in Brazil) harbors a particularly high diversity of euptychiines, rivaled only by the slopes of the tropical Andes in terms of its community diversity (unpublished data). Many euptychiine species utilize plants in the family Poaceae, commonly known as grasses, in addition to several species known to feed on plant families such as Arecaceae, Marantaceae, Heliconiaceae, and Selaginellaceae (Beccaloni *et al.*, 2008; Janzen & Hallwachs, 2018). Despite many species

being a common component of butterfly communities in the Neotropical region, our knowledge of euptychiine immature stages and their natural host plant remains highly fragmentary, with approximately 80% of the members lacking any host plant records (Corahua-Espinoza *et al.*, 2022). It is worth investigating this topic because Lepidoptera life history information serves as the basis for understanding species diversity due to their close links with plant distribution and diversity (Brown, 1991; New, 2014), and for developing appropriate conservation strategies that account for species interactions.

The southwestern Amazon rainforest, including Madre de Dios department, is one of the most biodiverse regions on earth, evidenced by a survey of butterflies (Lamas *et al.*, 1991), as well as faunistic studies focused on other vertebrate and invertebrate groups (e.g., Barthelm *et al.*, 2003; von May *et al.*, 2008). It is worth noting that while this region is the heart of diversity for euptychiine butterflies, it is also home to many euptychiine taxa that are not known elsewhere in the Neotropics (e.g., *Caeruleuptychia scripta* Nakahara, Zacca & Huertas, 2017; Nakahara *et al.*, 2018). The southwestern Amazon basin,

therefore, offers an ideal setting to explore the early stage biology of euptychiine species and to generate valuable natural host plant records for future studies. Climate change and other anthropogenic threats, such as road proliferation and associated resource extraction and deforestation (Gallice *et al.*, 2019; Finer & Novoa, 2016; Finer & Mamani, 2021), make documenting the biodiversity of southwestern Amazonia ever more urgent.

The Alliance for a Sustainable Amazon (ASA) (<https://www.sustainableamazon.org/>) is a nonprofit organization working to conserve biodiversity in the Madre de Dios region by conducting research to generate baseline information for science-based conservation. One of ASA's long-term research projects is focused on Lepidoptera diversity and biology, and the study reported here was conducted under the auspices of this project. Fieldwork is primarily conducted at ASA's research facility, Finca Las Piedras (FLP), located approximately 48 km north of Puerto Maldonado in Madre de Dios department, Peru (-12.22789, -69.11119; ca. 240 m). This study is part of an ongoing series of publications that document complete immature stages of butterflies and their host plants, with seven of them focused on euptychiine species (See *et al.*, 2018; Baine *et al.*, 2019; Nakahara *et al.*, 2020, 2022; Tejeira *et al.*, 2021; Ccahuana *et al.*, 2021a, b; Hurtado *et al.*, 2021; Corahua-Espinoza *et al.*, 2022). Following the precedent set by these studies we here report life history information for two additional euptychiine species with their natural host plant records. The generic classification of one species discussed herein, *Magneptychia iris* (C. Felder & R. Felder, 1867), changed recently, and a new generic arrangement has been proposed for this taxon in Andrade *et al.* (2019). As stated in previous relevant studies (e.g., Nakahara *et al.*, 2022), however, this taxonomic change was not supported by a phylogenetic hypothesis of any kind and ongoing molecular work suggests the proposal to be invalid (unpublished data). We thus follow the most widely accepted Neotropical butterfly classification (Lamas, 2004) and use this specific epithet in combination with *Magneptychia* Forster, 1964. Although we do not provide complete information regarding the life history of *Paryphthimoides terrestris* (Butler, 1867), we nevertheless consider this a valuable contribution to our understanding of euptychiine butterfly biology for the reasons outlined above.

MATERIALS AND METHODS

As mentioned above, the study was conducted at FLP field station, a 54-ha property of mature “*terra firme*” rainforest, regenerating secondary forest, active and abandoned agricultural plots, and *Mauritia* L. f. (Arecaceae) palm swamps. The “*terra firme*” or upland forest at FLP is characterized by emergent, tall *Bertholletia excelsa* Humb. & Bonpl. (Lecythidaceae) (i.e. Brazil nut trees) and other species in the family Lecythidaceae such as *Eschweilera coriacea* (DC.) S.A. Mori, *Couratari guianensis* Aubl., and *Couratari macrosperma* A. C. Sm.; *Aspidosperma parvifolium* A.DC. (Apocynaceae); *Hymenaea oblongifolia* Huber, *Tachigali* sp., and *Dipteryx micrantha* Harms (Fabaceae); and *Iriartea deltoidea* Ruiz & Pav., *Euterpe precatoria* Mart. (Arecaceae); the understory consists of a variety of herbaceous bamboo species and other grasses such

as *Pharus virescens* Döll. as reported herein. Samples were collected and processed using procedures published by See *et al.* (2018), Baine *et al.* (2019), Nakahara *et al.* (2020), and Ccahuana *et al.* (2021a, b). Fieldwork was conducted from February to July 2021, in which larvae were collected from mature forest. The larvae were reared in the laboratory at FLP in 500 mL and 1 L plastic containers with mesh covers to monitor growth and development. The containers were cleaned and searched for head capsules daily, photographs were taken frequently (at least twice per instar), and the larvae were provided with fresh leaves from their respective host plants kept fresh using floral water tubes. The samples were vouchered (2021-FLP-IMM-0102; 0242; 0314; 0382; 0386), and head capsules were observed using a Novel NSZ-608T microscope at the Arachnology and Zoology laboratory of the Universidad Nacional de San Antonio Abad del Cusco, Peru (UNSAAC). The head capsule graphics were made with Adobe Illustrator version 2019.23.0. Measurements of the larvae and pupae were determined based on a ruler photographed with immatures and rounded to the nearest tenth of a millimeter. Body length for larvae was measured from the frons to the posterior tip of the caudal filament; pupae length was measured from the base of the cremaster to the anterior tip of the head. Larval terminology follows Stehr (1987). After examination, the preserved adult specimens were deposited in the ASA's Lepidoptera collection in Puerto Maldonado, Peru.

We obtained the first half of mitochondrial gene *cytochrome oxidase I* (COI), the so-called “barcoding region” (*sensu* Hebert *et al.*, 2003) for 2021-FLP-IMM-0314 in order to aid the identification. DNA was extracted from thoracic tissue cut out by scalpel and homogenized using magnetic beads. Subsequent extraction procedures largely followed the protocols of Qiagen's DNeasy Blood & Tissue Kit with 50 µl as an elution volume. The COI barcoding region was amplified using primer pairs LCO (forward, GGTCAACAAATCATAAAGATATTGG) and HCO (reverse, TAAACTTCAGGGTGACCAAAAAATCA), with PCR reaction conditions as in Nakahara *et al.* (2020). This PCR product was purified by the addition of Exonuclease I (New England BioLabs) and rAPid Alkaline Phosphatase (Roche Diagnostics) and incubated with the following thermocycler conditions: 15 min at 37C, 15 min at 80C, hold at 10C. Subsequently, the purified product was submitted for direct Sanger sequencing at Eurofins Genomics in Louisville, Kentucky, USA. The raw sequence was trimmed and manually edited using 4Peaks software and the NCBI Open Reading Frame Finder (ORFfinder). To verify identity, the sequence was aligned with 96 other euptychiines in Geneious Prime (v 2021.2.2, Biomatters Ltd.) using the MUSCLE plug-in (Muscle 3.8.425) set at 10 iterations. The sequences used in alignment were first selected for closely-related euptychiine taxa within the “*Pareuptychia* clade” (*sensu* Murray & Prowell (2005)) plus an outgroup (*Amiga* Nakahara, Willmott & Espeland, 2019 spp.) (unpublished data) and then selected for high coverage of our sequenced region. A neighbor-joining tree was constructed using the Jukes-Cantor genetic distance model through Geneious Tree Builder. The GenBank accession number for 2021-FLP-IMM-0314 is OM524555.

RESULTS

Larvae of the two euptychiine species were collected on leaves of plant species in the family Poaceae, between February and July 2021. Both species were found on different host plant species. Dates for key life history events for each species are summarized in Table 1. Table 2 provides the host plant conditions observed and recorded.

Description of the immature stages

Paryphthimoides terrestris (Butler, 1867)

(Voucher: 2021-FLP-IMM-0102)

Murray (2001) reported five larval stages for *P. terrestris*, although considering the variable infra-specific larval instar numbers observed in euptychiine species (e.g., *Splendeptychia furina* (Hewitson, 1862); Corahua-Espinoza *et al.*, 2022), we decided not to draw conclusions regarding the number of larval stages of this species based on our incomplete life history information.

Penultimate instar (Figs. 1 A, B; 4 A). Head capsule width: 1.4 mm (n=1). Head capsule dark beige with numerous secondary setae and two short scoli terminating in rather rounded tips (scolus length 0.52 mm (n=1)); three semi-transparent chalazae visible on lateral side (M1-3), with primary seta on each of these three chalazae; dark band-like marking visible on frontal part of head capsule, as well as inner side of scoli and

vertex; six setae visible on one side of labrum; six stemmata visible, with first and sixth somewhat semi-transparent and thus insignificant, third stemma largest and closer to second stemma than fourth stemma. Body chartreuse in color with dark brown discoloration dorsally along upper abdominal segments; dark green band extending along middorsal area, well-defined along thorax and absent or insignificant in upper abdominal segments, more defined and brownish along A7-A10; dark brown spiracles, prominent on T1 and A8; ventral prolegs present on A3 to A6, caudal prolegs present on A10; bifid caudal filaments similar in length to A8 in dorsal view. Body length: 12.2 mm (n=1). Duration: Unknown; larva molted four days after collection.

Ultimate instar (Figs. 1 C, D; 4 B). Head capsule width: 1.8 mm (n=1). Head capsule morphologically similar to previous instar (scolus length 0.62 mm (n=1)). Body morphologically similar to previous instar, except coloration; body light beige with gray dorsal discoloration; middorsal band dark brown and more defined along thorax than in previous instar; indistinct, light brown subdorsal stripes extending from base of scoli to T3; jagged subserosal band more defined from A3 to A6. Body length: 19.7 mm (n=1). Duration: 13 days.

Pupa (Figs. 1 E, F). Body and wings cream in color and mottled with dark brown; body short and round with squared ocular caps; spiracles appearing light brownish; small two-toned cream and brown protuberances present in two rows along dorsal area of abdomen; dark brown cremaster, details not discernable based on images. Body length: 8.8 mm (n=1). Duration: 11 days.

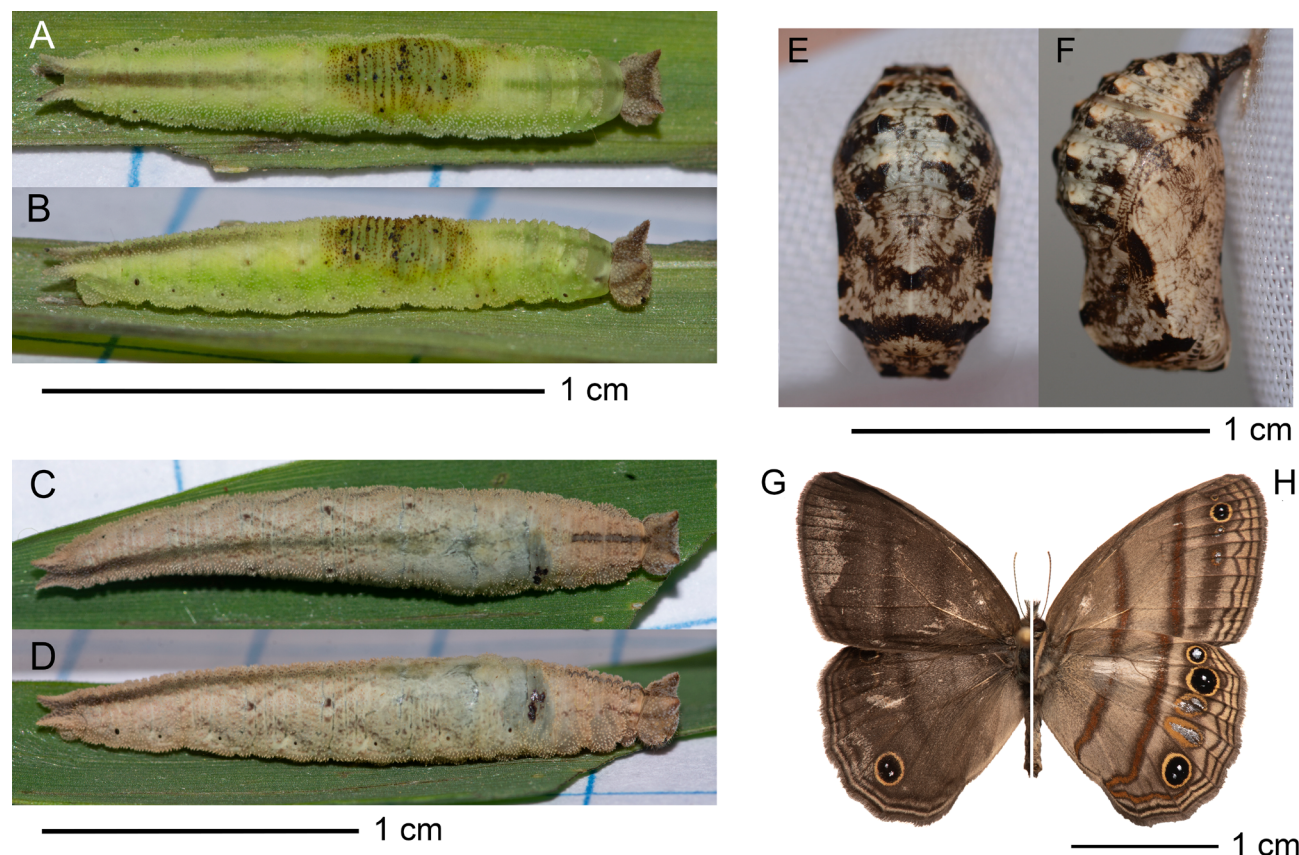


Figure 1. *Paryphthimoides terrestris* life stages: A, B) penultimate instar in dorsal view, lateral view; C, D) ultimate instar in dorsal view, lateral view; E, F) pupa in dorsal view, lateral view; G, H) adult in dorsal view, ventral view.

Table 1. Dates of important life history events recorded for the two euptychiines: *Paryphthimoides terrestris* and *Magneptychia iris*.

Voucher	Taxon	sex	Date of collection	Egg hatch	L1 to L2	L2 to L3	L3 to L4 (Penultimate to ultimate)	Pupation	Adult	Died
2021-FLP-IMM-0102	<i>Paryphthimoides terrestris</i>	male	09/02/2021	no data	no data	no data	13/02/2021	25/02/2021	07/03/2021	N/A
2021-FLP-IMM-0242	<i>Magneptychia iris</i>	female	17/03/2021	no data	no data	no data	25/03/2021	04/04/2021	13/04/2021	N/A
2021-FLP-IMM-0382	<i>Magneptychia iris</i>	male	15/05/2021	no data	16/05/2021	26/05/2021	04/06/2021	16/06/2021	01/07/2021	N/A
2021-FLP-IMM-0314	<i>Magneptychia iris</i>	no data	08/04/2021	12/04/2021	19/04/2021	26/04/2021	06/05/2021	N/A	N/A	10/05/2021
2021-FLP-IMM-0386	<i>Magneptychia iris</i>	male	15/05/2021	no data	no data	no data	no data	27/05/2021	07/06/2021	N/A

Table 2. Details of host plant conditions, location and sampled immature stages. Abbreviations: abaxial (AB); adaxial (AD); distal (D); middle (M); proxima (P); midrib (B); middle (E); margin (IN).

IMM ID	HOST PLANT ID	PLANT GENUS	PLANT SPECIES	DISTANCE FROM GROUND (meters)	LEAF SURFACE (AB/AD)	LF PROXIMITY TO BASE (D/M/P)	LF BLADE PROXIMITY (B/E/IN)	FOUND AS:	LATITUDE	LONGITUDE	FLOWER
2021-FLP-IMM-0102	482	<i>Lasiacis</i>	<i>ligulata</i>	0.3	AB	D	B	larva	-12.22643	-69.11414	no
2021-FLP-IMM-0242	545	<i>Pharus</i>	<i>virescens</i>	0.2	AB	M	E	larva	-12.22792	-69.11108	no
2021-FLP-IMM-0314	558	<i>Pharus</i>	<i>virescens</i>	0.5	AB	P	N	egg	-12.22510	-69.11045	yes
2021-FLP-IMM-0382	558	<i>Pharus</i>	<i>virescens</i>	0.8	AB	P	B	larva	-12.22510	-69.11045	yes
2021-FLP-IMM-0386	558	<i>Pharus</i>	<i>virescens</i>	0.3	AB	M	E	larva	-12.22510	-69.11045	yes

Host plant. *Lasiacis ligulata* Hitchc. & Chase (Fig. 3 A, B, C). Habit: herb, belongs to plant family Poaceae: subfamily Panicoideae: tribe Paniceae. Culms: branched; woody. Culm nodes: glabrous. Leaves: glabrous on adaxial surface and pubescent on abaxial surface; blade base asymmetrical; linear to ovate or lanceolate; blade margins scabrous; blade apex acuminate. Inflorescence: raceme with spherical calyxes surrounding reduced white petals. Fruits: similar size to mature flower and dry, brown, and spherical (<https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:135703-2>). Phenology: flowering early between April and June, and fruiting between July and August (Baine *et al.*, 2019), both in dry season. Distribution: from Caribbean to northern and western South America, between 0-2000 m (Tovar-Serpa, 2009; Bernal *et al.*, 2020).

The plant on which the larva was found was located at the edge of the forest, where this plant species is usually found in the highest abundance at the field site (Baine *et al.*, 2019). At the time of larva collection (2 February 2021), the plant was approximately 1.3 m tall; the larva was found at a distance of 0.3 m from the ground; situated on the abaxial surface of the leaf, near the apex of the blade, in the midrib of the leaf (see Table 2).

***Magneptychia iris* (C. Felder & R. Felder, 1867)**

(Vouchers: 2021-FLP-IMM-0242, 2021-FLP-IMM-0314, 2021-FLP-IMM-0382, 2021-FLP-IMM-0386)

Murray’s (2001) descriptions of egg, five larval stages and pupa for “*Magneptychia ayaya*” (Butler, 1867) is largely in agreement with life stages of *M. iris* documented and illustrated herein. Currently, *Euptychia ayaya* is regarded as a junior subjective synonym of *Neonympha iris* (e.g., Lamas, 2004). Unlike the present study, *Magneptychia iris* went through five larval stages based on individuals from eastern Ecuador (Murray, 2001). However, despite this discrepancy, we believe the in-depth morphological study of immatures conducted in Murray (2001) will complement our documentation of *M. iris* based on a population from southeastern Peru.

Although 2021-FLP-IMM-0314 did not reach the adult stage (see Table 1), the larva closely resembled other known larvae of *Magneptychia iris* studied herein at all stages and

was found to utilize the same host plant species as these other three individuals. In addition, our neighbor-joining analysis of the COI barcode sequence from this specimen resulted in identity matches of 99.844%, 97.738%, and 97.738% respectively with three sequenced individuals identified as *M. iris* based on comparison with the syntype of *Neonympha iris* (images available at: <https://www.butterfliesofamerica.com/>), and thus we consider this individual to be conspecific.

Egg (Fig. 2 A, B). Spherical, semi-transparent, pearl-like, and whitish in color, with polygonal markings covering the entire surface; head capsule visible via translucence 1 day prior to hatching. Diameter: 1.1 mm (n=1). Duration: Unknown, hatched 4 days (n=1) after collection.

First instar (Figs. 2 C, D, 4 C). Head capsule width: 0.74 mm (n=1). Head capsule black; head capsule smooth, with two rectangular scoli (scolus length 0.19 mm (n=1)), each with two primary setae dilated at tip; six chalazae visible (three on each side) in frontal view, each accompanied primary seta dilated at tip; five setae visible on one side of labrum; six stemmata visible, third stemma largest and closer to second stemma than fourth stemma. Body integument creamy-white and appearing semi-translucent thus presumably gut content visible, influencing light green body color; pair of white stripes in subdorsal area, more visible late during first instar; chaetotaxy not fully discernible based on pictures but primary setae bulbous at apex (“weakly bulbed” *sensu* Murray, 2001: Fig. 3.13C), XD1 and XD2 visible on T1; D1, D2, SD1 and L1 apparently present from T1 to A10, D1 and D2 rather short except for terminal abdominal segments, D1 apparently considerably longer than D2 on A8 and A9; ventral prolegs present on A3 to A6, caudal prolegs present on A10; caudal filament short (shorter than A8 in dorsal view). Body length: 5.9 mm (n=1). Duration: 7 days (n=1).

Second instar (Figs. 2 E, F). Head capsule width: 1.1 mm (n=1). Head capsule dark brown; head scoli developed and tapered (compared to first instar; scolus length = 0.49 mm (n=1)), resembling those of the immediately following instar; no visible chalazae; five setae visible on one side of labrum; six stemmata, with first and sixth somewhat semi-transparent and thus insignificant. Body dark-green; pair of white stripes in subdorsal area, indistinct stripe visible laterally, just below white subdorsal stripe; spiracles whitish, prominent on T1

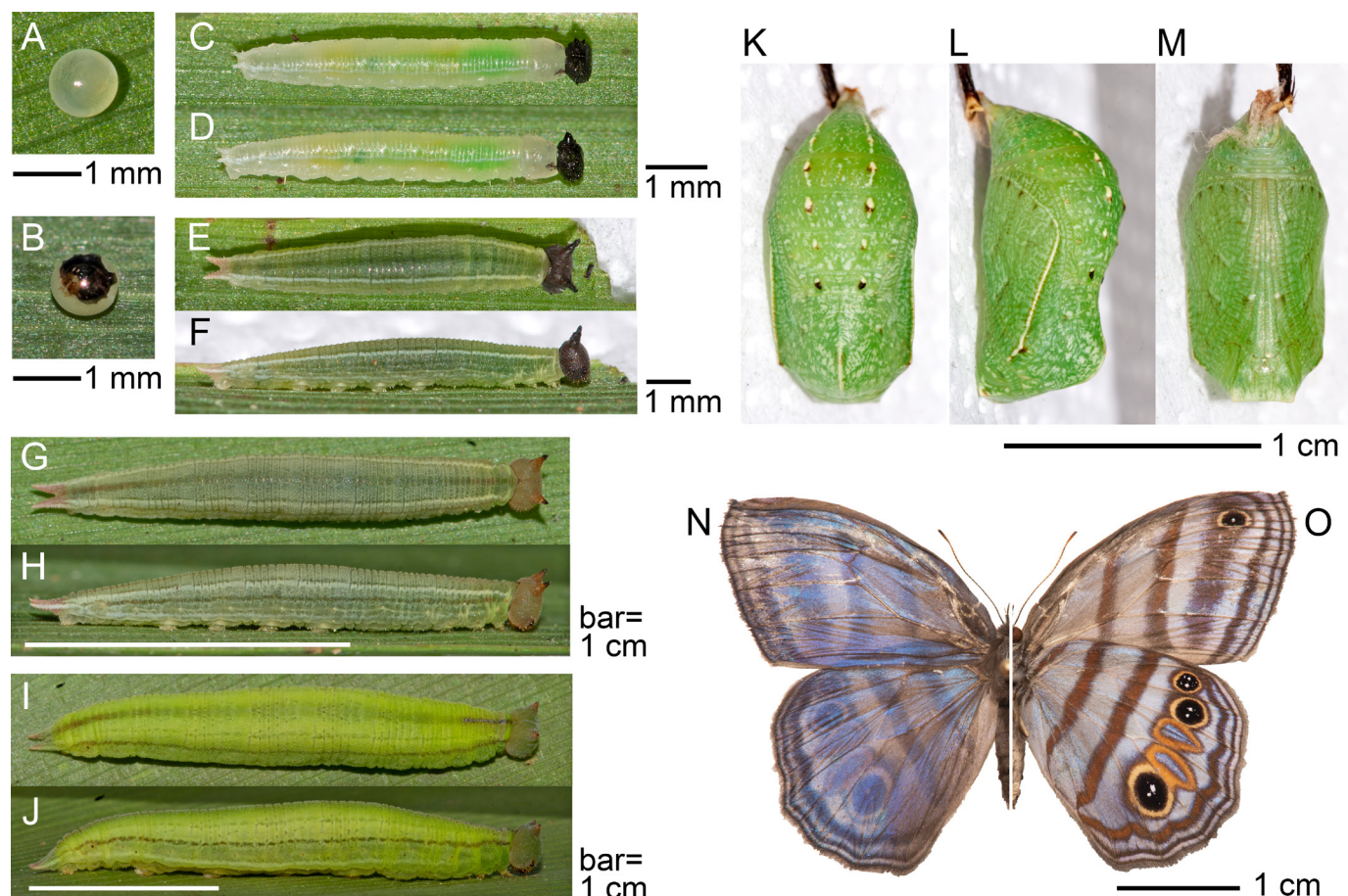


Figure 2. *Magneptychia iris* life stages: A, B) egg in dorsal view, head capsule visible before hatching C, D) first instar in dorsal view, lateral view; E, F) second instar in dorsal view, lateral view; G, H) third instar in dorsal view, ventral view; I, J) fourth (ultimate) instar in dorsal view, lateral view; K, L, M) pupa in dorsal view, lateral view, ventral view; N, O) adult in dorsal view, ventral view. Figures A and B are based on 2021-FLP-IMM-0314; figures C-O are based on 2021-FLP-IMM-0386.

and A8; ventral prolegs present on A3 to A6, caudal prolegs present on A10; caudal filament pink-ish and appearing as equal in length to A8 in dorsal view. Body length: 9.0 mm (n=1). Duration: 7-11 days (n=2).

Third instar (Figs. 2 G, H, 4 D). Head capsule width: 1.8 mm (n=1). Head capsule light olive in color with mild iridescence; scoli short (scolus length 0.74 mm (n=1)) terminating in rather pointy ends, tipped in orange; five setae visible on one side of labrum; six stemmata, with first and sixth somewhat semi-transparent and thus insignificant. Body yellow-green with mild wart projections; dark green longitudinal band along mid-dorsal area of abdomen, fading to maroon along the thorax and near caudal filaments; white and dark green subdorsal longitudinal lines extending from the head to caudal filaments; two pairs of dark green spots along white subdorsal line between abdominal segments A3 and A4 and A4 and A5; maroon spiracles; bifid caudal filaments tipped with orange. Body length: 13.0-15.1 mm (n=2). Duration: 9-10 days (n=2).

Fourth (ultimate) instar (Figs. 2 I, J; 4 E). Head capsule width: 2.2 mm (n=1). Morphology of head capsule similar to previous instar except for darker coloration and scoli appearing stubby compared to head capsule width and terminating in pointy anterior ends (scolus length 0.62 mm (n=1)). Body morphologically similar to previous instar; body brighter in

color; middorsal band more well-defined along prothoracic plate to metathorax and white outlined with maroon in color; bottom subdorsal line and pair of spots along white subdorsal line appear more maroon than previous instar; spiracles brownish, prominent on T1 and A8. Body length: 26.8-28.7 mm (n=2). Duration: 11-12 days (n=2).

Pupa (Figs. 2 K, L, M). Body bright green with scattered white speckles; body overall short, wide and rounded with squared ocular caps; spiracles present as yellow spots; protuberances present in two rows along dorsal area of abdomen as two-toned brown and white dots; cremaster appearing paler, somewhat elongate and gradually narrowing towards distal end. Some hours prior to emergence, the wing shell color changed dramatically from green to black. Body length: 11-11.9 mm (n=2). Duration: 9-15 days (n=3).

Host plant. *Pharus virescens* Döll. (Figs. 3 D, E, F). Habit: herb, belongs to the plant family Poaceae: subfamily Pharoideae: tribe Phareae. Culms: decumbent; 50-100 cm long; rooting from lower nodes. Leaves: elliptic; blade venation oblique from midrib; with well-defined transverse veins; apex acuminate. Inflorescence: in the form of an open panicle; pubescent; spikelets with male and female flowers. Fruits: Caryopsis with adherent pericarp (<https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:415723-1>). Phenology: early



Figure 3. Host plants of two satyrine butterfly species in the Peruvian Amazon. *Lasiacis ligulata*: A) details of inflorescence, B) host plant *in situ* view, C) close-up view of the leaves; *Pharus virescens*: D) details of inflorescence; E) host plant *in situ*; F) close-up view of the leaves and node. Scale bar = 1 cm for figures A & D.

flowering from April; fruiting between May and July (in the dry season), the plants dry up as an annual species at the end of June and in July (in FLP). Distribution: Mesoamerica, northern South America, western South America, and Brazil, between 230-500 m altitude (Bernal *et al.*, 2020).

The plant on which the larva was found was located in an area with a closed canopy and low light incidence. This plant species is typically found along trails and in light gaps within the forest at FLP, but usually not at the forest edge, which likely explains the fact that adults are often found flying in the forest understory away from the edge. At the time of larva collection (March 17, 2021; based on 2021-FLP-IMM-0242), the plant was approximately 0.5 m tall. The larva was found at a distance of 0.2 m from the ground, situated on the abaxial surface of the leaf, in the middle of the blade, between the midrib and the margin of the leaf (see Table 2).

DISCUSSION

Singer & Ehrlich (1991) reported the following host plant records for *Paryphthimoides terrestris* (as *Cissia terrestris*) based on plant material accepted by the females in captivity in oviposition trials in Trinidad: *Cyperus* L. and *Scleria* Bergius (both Cyperaceae); *Lasiacis sloanei* (Griseb.), *Ichnanthus pallens* (Sw.), *Panicum pilosum* Sw., *Panicum polygonatum* Schrad (Panicoideae: Paniceae), *Paspalum conjugatum* Bergius, *Paspalum decumbens* Stapf (Panicoideae: Paspaleae), *Setaria paniculifera* (Steud.) (Panicoideae: Paniceae) and *Tripsacum* L. (Panicoideae: Andropogoneae) (summarized in Beccaloni *et al.* (2008)). However, these records do not reflect the host plant of this species in the natural environment. Murray's (2001) dissertation reported "many grass species" as host plants for *P. terrestris* found in the field in eastern Ecuador (as *Cissia terrestris* or "*Marissa*" *terrestris*), which includes grass genera such as *Eleusine* Gaertn. (Chloridoideae: Cynodonteae),

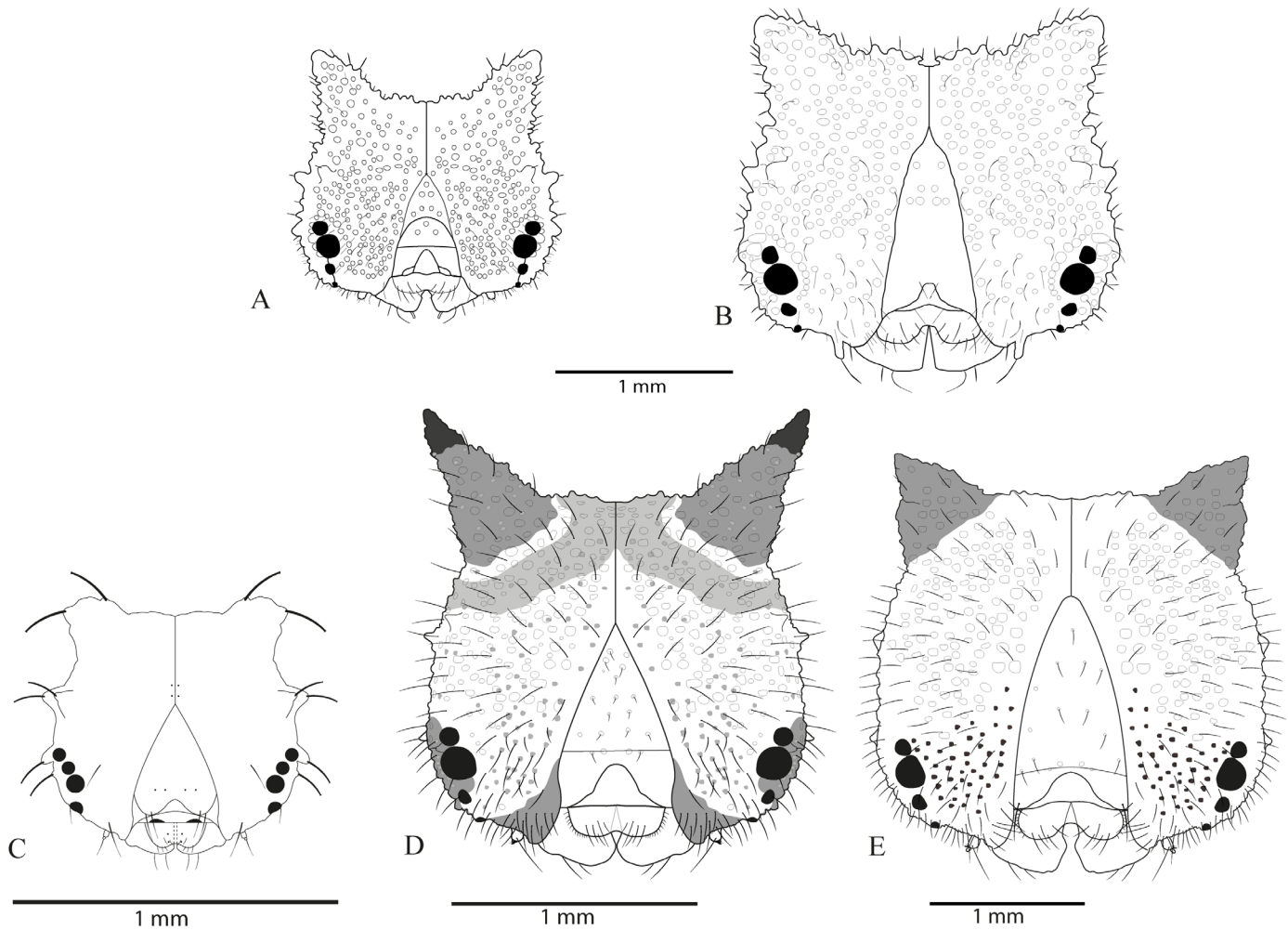


Figure 4. Illustrations of the head capsules: A, B) penultimate and ultimate instar in frontal view of *Paryphthimoides terrestris* (based on 2021-FLP-IMM-0102); C, D, E) first, third and fourth (ultimate) instar in frontal view of *Magneuptychia iris* (based on 2021-FLP-IMM-0382).

Ichnanthus Beauvois (Panicoideae: Paspaleae), *Lasiacis* Hitchc., *Andropogon* L. (Panicoideae: Andropogoneae), *Paspalum* L. (Panicoideae: Paspaleae), *Pharus* P. br., *Orthoclada* P. Beauvois (Panicoideae: Zeugiteae), and *Guadua* Kunth (Bambuseae: Guaduinae). However, the study does not explicitly state which of the genera were utilized by *P. terrestris* and adult images of reared materials were not available to confirm their identification. Notwithstanding this situation, it is reasonable to assume that *P. terrestris* was correctly identified in Murray (2001) considering that the identification of this taxon appears to be correct in the same author's subsequent work, Murray & Prowell (2005). Therefore, our record of *Lasiacis ligulata* as a natural host plant for *P. terrestris* at FLP is the first species-level record of a natural host plant for this taxon. This record is consistent with many other host plant records for euptychiine species, most of which are in the plant family Poaceae. *Lasiacis ligulata* grows in abundance along forest edges at FLP, and this grass species is recorded as a natural host plant for some euptychiine taxa (e.g., *Taygetis rufomarginata* Staudinger, 1888; Baine *et al.*, 2019); it is also accepted in captivity by some species known to feed on other Poaceae species at the study site (e.g., *Paryphthimoides brixius* (Godart, [1824]; Corahua-Espinoza *et al.*, 2022). This observation suggests *P. terrestris*

may well be found on other host plants at FLP. However, based on our field observations, a given euptychiine species appears to be repeatedly found on the same species in the family Poaceae at the study site, such as *Magneuptychia iris* documented herein (four individuals all found on *P. virescens*), and further sustained fieldwork is required to provide insights into the diet breadth of *P. terrestris*. We were unable to find any host plant records for *Magneuptychia iris* in existing studies, including Murray (2001) where this species is documented under the specific epithet *ayaya*, thus, to our knowledge, *Pharus virescens* is the first reported host plant record for this taxon. *Pharus virescens* is not reported as a host plant for any euptychiine taxa in Beccaloni *et al.* (2008) nor Janzen & Hallwachs (2018); however, several additional euptychiine species have been documented on this grass species at FLP (Corahua-Espinoza *et al.*, in prep.). *Pharus virescens* is not present throughout the year at the study site, as it dies back during the dry season; therefore, this observation suggests that this taxon may utilize different host plants which await confirmation based on future fieldwork. Therefore, there remains much to explore in terms of natural host plant use of these two euptychiine species, and the generation of further natural host plant records for euptychiines in the southwestern Amazonian euptychiine diversity hotspot

would be extremely valuable towards exploring this group's diet breadth and in understanding their diversity in the region.

The two euptychiine species in this study are scattered across two major clades recovered within Euptychiina, and our study shows some morphological affinities by comparing taxa with known immature stages within these clades. For example, *Magneuptychia iris* is recovered as a member of the so-called "Pareuptychia clade" (*sensu* Murray & Prowell (2005)) in Espeland *et al.* (2019), and the ultimate instar and pupa overall resemble those stages of *Optimandes eugenia transversa* (Weymer, 1911) and species in *Pareuptychia* Forster, 1964 (Murray, 2001; Willmott *et al.*, 2019), mainly by sharing these following characters: 1) greenish body with a dorsal stripe; 2) head capsule with a pair of short and conical orangeish scoli on vertex; 3) black stemmata (based on live pictures); 4) pupa greenish, short and smooth, with few scattered spots on abdomen. It must be noted that several morphological characters are also discussed in Murray (2001) to support a similar relationship found in that work (((*Pareuptychia occirhoe* (Fabricius, 1776) + *Pareuptychia hesionides* (Forster, 1964))) + *Pareuptychia metaleuca* (Boisduval, 1870))) + *Cepheuptychia cephus* (Fabricius, 1775)) + *Magneuptychia ayaya*). For example, the pore Pb being located postero-basal to P2 (i.e., distal primary seta on scoli) in the first instar is considered as synapomorphic for *Pareuptychia* - *M. "ayaya"* clade in Murray (2001). Albeit there is a limited number of satyrine taxa coded for cladistic analysis in Murray (2001), a matrix consisting of more than 150 characters based on immature stages is remarkable and the work undoubtedly serves as a baseline, as well as a vital foundation, for exploring satyrine immature morphology. *Paryphthimoides terrestris* is a taxon recovered in the so-called "*Splendeuptychia* clade", which is perhaps the most species-rich major clade within the subtribe, only rivaled by the "*Taygetis* clade" (*sensu* Murray & Prowell (2005)) in terms of its species diversity, with both of these two clades containing over 80 described species each. Nevertheless, the available life history information for taxa in the "*Splendeuptychia* clade" remains sparse, reflecting our poor knowledge of euptychiine early stage biology as a whole. For example, despite *Paryphthimoides* Forster, 1964 being recovered as a monophyletic entity accommodating eight species (Zacca *et al.*, 2020), the present study is one of the few sources of information for its natural host plant and immature stages. Murray's (2001) findings on *P. terrestris* and Corahua-Espinoza's (2022) report on *Paryphthimoides brixius* (Godart, [1824]) are the two other existing works that contribute towards knowledge of the early stage biology of *Paryphthimoides*. Nevertheless, Murray's (2001) mature larva image for *P. terrestris* (Fig. 2.12B) is insufficient to discern any characters for consistency and we herein discuss features based solely on a single individual described above. Based on some available studies, as well as unpublished data, on early stage biology regarding taxa in the "*Splendeuptychia* clade" (e.g., Singer *et al.*, 1983; DeVries, 1987; Murray, 2001; Corahua-Espinoza *et al.*, unpublished data), there appear to be two recurring characters observed in this clade: 1) first instar having a semi-translucent body with orangeish stripes; 2) somewhat mottled pupa with bright protuberances along the dorsal surface of the abdomen. Collectively, sustained effort aimed at documenting

immature stages of butterflies in the Neotropics, particularly in diverse regions such as the Peruvian Amazon, is extremely valuable towards filling in gaps where our knowledge lags far behind. Some relevant studies on euptychiine immature stages have shown several putative synapomorphic characters based on caterpillar morphology to refine taxonomic hypotheses (e.g., Corahua-Espinoza *et al.*, 2022; Nakahara *et al.*, 2022), and we continue to contribute towards a better understanding of Neotropical butterfly taxonomy, ecology, and evolution by revealing the life history of butterflies in one of the most diverse regions on the planet.

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