# Immature stages of *Magneuptychia harpyia* (C. Felder & R. Felder, 1867) (Lepidoptera: Nymphalidae: Satyrinae)

**Shinichi Nakahara[1,2], Fjella Livia Adele Hoffman[3], Fabia Luna Hoffman[4] and Geoffrey Gallice [1,5*]**

1. McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA
2. Departamento de Entomología, Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Apartado 14-0434 Lima-14, Perú
3. Schiller-school, Fidelio-F.-Finke str. 15 01326 Dresden, Germany
4. Gymnasium Dresden-Bühlau, Quoherener str. 12 01324 Dresden, Germany
5. Alliance for a Sustainable Amazon, Hanover, MD 21076, USA *Correspondence: info@sustainableamazon.org

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**Abstract:** We here report for the first time the complete life history of *Magneuptychia harpyia* (C. Felder & R. felder, 1867) (Satyrinae: Euptychiina), based on an individual from Madre de Dios, Peru. An egg obtained from a single female was reared on *Lasiacis ligulata* Hitchcock & Chase (Poaceae: Panicoideae) and the larva passed through five instars. Images of the egg, all larval instars, and the pupa are provided herein, in addition to illustrations of the head capsule and information on the duration of each stage. The host plant in nature is unknown.

**Resumen:** En este artículo se reporta por primera vez el ciclo de vida completo de *Magneuptychia harpyia* (C. Felder & R. Felder, 1867) (Satyrinae: Euptychiina), basado en un individuo de Madre de Dios, Perú. Este individuo se obtuvo a partir del huevo, de una hembra criada en *Lasiacis ligulata* Hitchcock & Chase (Poaceae: Panicoideae) y cuya larva pasó por los cinco estadios del ciclo larval. Se proveen fotografías del huevo, todos los estadios larvales, la pupa, así como ilustraciones de la cápsula cefálica. Adicionalmente, se detalla la duración de cada estadio larval. La planta hospedera en la naturaleza es aún desconocida.

**Key Words:** Euptychiina, *Lasiacis ligulata*, Madre de Dios, Peru

## INTRODUCTION

Like many other genera in the nymphalid subtribe Euptychiina, the genus *Magneuptychia* Forster, 1964, with approximately 30 species as conceived by Lamas (2004), has proven to be highly polyphyletic in several phylogenetic studies (e.g., Peña *et al.* 2010; Espeland *et al.* 2019). Recently, Costa *et al.* (2016) and Andrade *et al.* (2019) proposed a taxonomic arrangement for some taxa placed in *Magneuptychia*, but, without a comprehensive phylogenetic assessment with both morphological and molecular data, the validity of these taxonomic rearrangements can not be assessed. Nevertheless, ongoing research by SN and collaborators on the systematics of Euptychiina, in particular including extensive molecular data, has provided a reasonable understanding of the relationships of species currently placed in *Magneuptychia*, and work in progress will substantially revise the generic classification.

At a time when phylogenetic hypotheses are often generated based solely on molecular data, morphological data perhaps play a role more in downstream analyses such as character mapping, rather than in phylogenetic estimation itself. However, historically, morphological characters were used extensively in constructing matrices for phylogenetic study (e.g., Minet, 1991). Regarding butterflies, adult morphological characters are commonly used for coding/scoring characters, but morphological and ecological data derived from the immature stages have also been incorporated in some studies (e.g., Kitching, 1984), providing important synapomorphies in some cases (e.g., Willmott & Freitas, 2006). Recent years have seen a number of contributions on euptychiine life history (e.g., Freitas *et al.* 2016a,b; Freitas, 2017; See *et al.*, 2018; Baine *et al.*, 2019), but our understanding of euptychiine immature stages is still very limited and the complete life cycle and/or detailed descriptions are available for less than 10% of the entire subtribe. We also lack knowledge about which characters are informative and contain phylogenetic signal. With the exception of the type species of *Magneuptychia*, *Papilio libye* Linnaeus, 1767, for which the complete life history has been documented (Kaminski & Freitas, 2008), there exists very little information on the early stage biology of *Magneuptychia* species (Singer *et al.*, 1983; DeVries, 1987; Beccaloni *et al.*, 2008).

Following See *et al.* (2018) and Baine *et al.* (2019), we here document the complete life history of another euptychiine species currently placed in *Magneuptychia* based on a study carried out mainly at Finca Las Piedras (hereafter FLP) in southeastern Peru. *Magneuptychia harpyia* (C. Felder & R. Felder, 1867) is a species distantly related to the type species of the genus, *Papilio libye* Linnaeus, 1767, and thus a new genus will be described based on molecular data, with support from morphology, to harbor the former species and a few other closely related taxa as briefly discussed below (Nakahara, in prep.). Due to the scope of ongoing projects on butterfly life histories being conducted at the FLP, we here document the early stage biology of *M. harpyia* prior to its transferral to the new genus and also further discuss the validity of the subspecific epithet associated with this species, namely *M. harpyia batesi* (Butler, 1867).

## MATERIALS AND METHODS

**Study Sites:** The single individual used for this study was collected at FLP, located about 47 km north of Puerto Maldonado, Madre de Dios department, Peru (-12.22789, -69.11119). See *et al.* (2018) and Baine *et al.* (2019) provide additional information about FLP (see also https://www.sustainableamazon.org/). The individual was reared at FLP from 7 November 2019 to 16 December 2019, and then at the nearby location of Ecocentro from 16 December 2019 to 1 January 2020. Ecocentro is situated about 3 km north of Puerto Maldonado (-12.55714, -69.20655), and serves as a base for promoting agricultural projects based on agroforestry systems in this area. Both temperature and precipitation data were recorded at FLP daily at around 08:00, and this information is provided in Table 1 for the period when the rearing was conducted at FLP.

**Rearing and morphological study:** Rearing was carried out at room temperature and leaves were kept hydrated using floral water tubes and/or 1.5 ml tubes. A female of *Magneuptychia*

**Table 1.** Temperature (in Celsius) and precipitation (mm) data measured daily at FLP for the period when the rearing was conducted at this site. Maximum and minimum temperature represents those of the previous day.

| date | max.temp. (C) | min.temp. (C) | current.temp. (C) | time (am) | precip. (mm) |
|---|---|---|---|---|---|
| 1-Nov-19 | 32.0 | 22.0 | 22.0 | 8:05 | 38.00 |
| 2-Nov-19 | 24.0 | 21.0 | 24.0 | 8:03 | 0.00 |
| 3-Nov-19 | 30.0 | 22.0 | 25.0 | 8:10 | 0.00 |
| 4-Nov-19 | 30.0 | 23.0 | 25.5 | 8:06 | 4.00 |
| 5-Nov-19 | 28.0 | 23.5 | 25.5 | 8:08 | 19.00 |
| 6-Nov-19 | 29.5 | 23.0 | 25.5 | 8:04 | 0.00 |
| 7-Nov-19 | 30.0 | 24.0 | 25.5 | 8:15 | 0.00 |
| 8-Nov-19 | 26.5 | 23.5 | 24.5 | 8:00 | 3.50 |
| 9-Nov-19 | 30.0 | 23.0 | 26.0 | 7:54 | 0.00 |
| 10-Nov-19 | 30.5 | 22.5 | 25.5 | 8:02 | 0.00 |
| 11-Nov-19 | 30.0 | 22.5 | 22.5 | 7:56 | 13.50 |
| 12-Nov-19 | 25.5 | 22.0 | 24.0 | 8:00 | 1.00 |
| 13-Nov-19 | 28.5 | 22.0 | 26.0 | 8:08 | 4.50 |
| 14-Nov-19 | 29.0 | 23.0 | 26.0 | 8:10 | 23.00 |
| 15-Nov-19 | 28.0 | 21.0 | 21.0 | 8:08 | 125.00 |
| 16-Nov-19 | 23.5 | 21.0 | 23.5 | 7:58 | 10.00 |
| 17-Nov-19 | 27.0 | 23.0 | 25.0 | 8:00 | 0.00 |
| 18-Nov-19 | 28.5 | 23.5 | 25.0 | 7:54 | 0.00 |
| 19-Nov-19 | 29.5 | 23.0 | 25.5 | 8:03 | 0.00 |
| 20-Nov-19 | 30.5 | 21.5 | 24.5 | 8:04 | 4.50 |
| 21-Nov-19 | 28.5 | 24.0 | 25.0 | 8:02 | 8.50 |
| 22-Nov-19 | 28.5 | 24.0 | 26.0 | 8:01 | 0.00 |
| 23-Nov-19 | 29.5 | 23.0 | 24.5 | 7:55 | 44.00 |
| 24-Nov-19 | 25.0 | 21.0 | 23.0 | 8:03 | 48.00 |
| 25-Nov-19 | 27.5 | 22.0 | 25.0 | 8:09 | 0.00 |
| 26-Nov-19 | 29.0 | 23.0 | 26.0 | 8:07 | 0.00 |
| 27-Nov-19 | 30.5 | 25 | 27 | 8:09 | 0 |
| 28-Nov-19 | 29 | 24 | 24.5 | 8:15 | 43.5 |
| 29-Nov-19 | 25 | 21.5 | 23 | 8:09 | 22.5 |
| 30-Nov-19 | 25 | 22.5 | 25 | 8:10 | 0.5 |
| **Nov Avg.** | **28.3** | **22.7** | **24.7** | - | **413 (total)** |
| 1-Dec-19 | 28 | 23 | 25.5 | 8:20 | 5.5 |
| 2-Dec-19 | 27.5 | 23 | 23.5 | 8:09 | 68 |
| 3-Dec-19 | 26 | 22 | 24 | 8:00 | 5 |
| 4-Dec-19 | 27 | 23 | 24.5 | 8:05 | 1.5 |
| 5-Dec-19 | 27.5 | 22.5 | 25 | 8:03 | 8.5 |
| 6-Dec-19 | 28 | 23.5 | 25.5 | 8:13 | 0 |
| 7-Dec-19 | 27.5 | 22.5 | 24 | 8:09 | 4 |
| 8-Dec-19 | 28 | 23.5 | 25.5 | 8:00 | 11 |
| 9-Dec-19 | 29 | 24 | 25 | 8:03 | 4 |
| 10-Dec-19 | 28 | 23.5 | 25 | 7:54 | 0.5 |
| 11-Dec-19 | 27 | 23 | 24 | 7:59 | 9 |
| 12-Dec-19 | 26.5 | 24 | 25 | 8:03 | 0.5 |
| 13-Dec-19 | 29 | 23 | 23.5 | 8:08 | 3 |
| 14-Dec-19 | 31 | 23 | 26 | 7:45 | 5.5 |
| 15-Dec-19 | 27.5 | 24 | 25 | 8:07 | 7 |
| **Dec Avg.** | **27.8** | **23.2** | **24.7** | - | **133 (total)** |

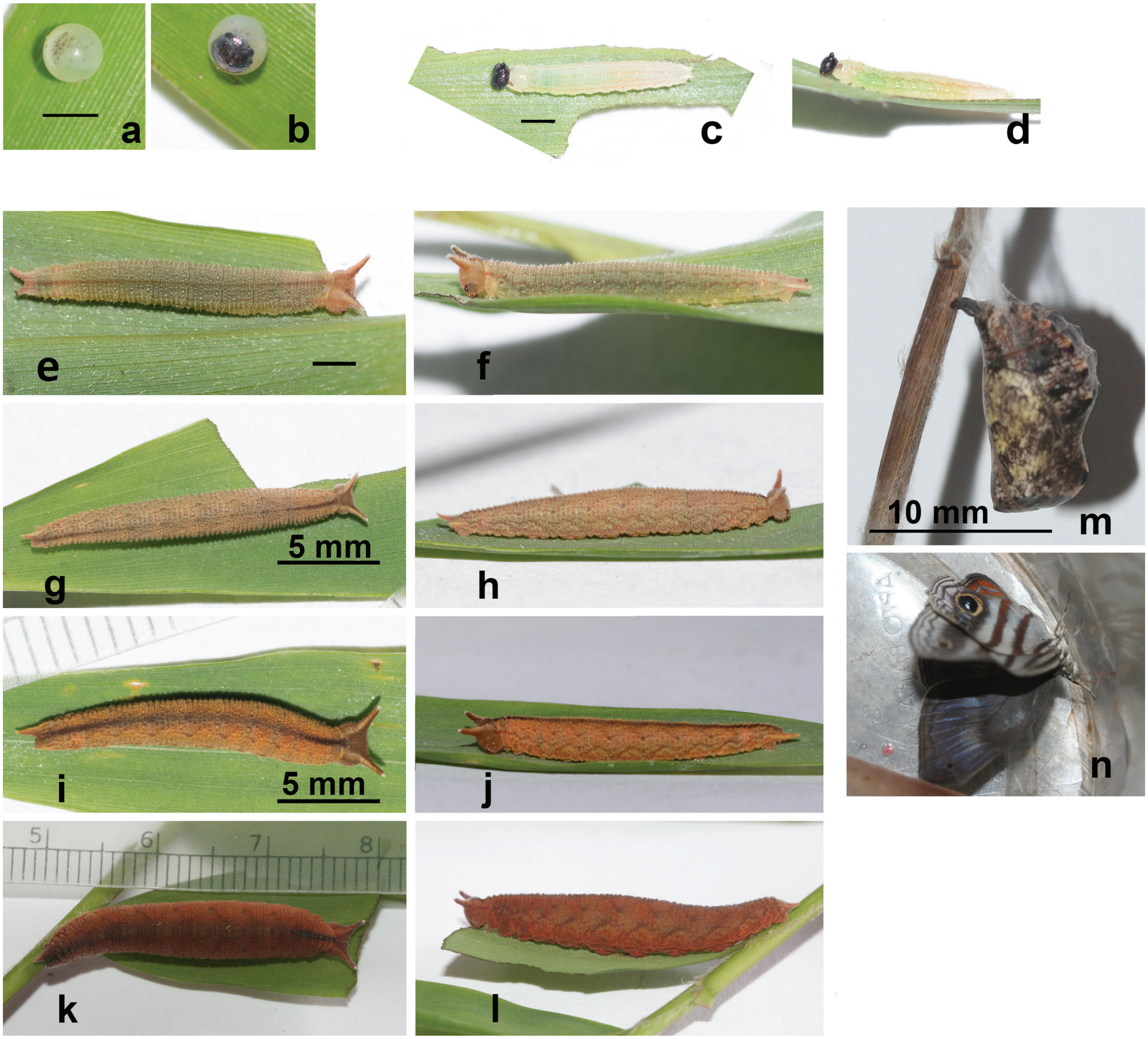


**Fig. 1.** *Magneuptychia harpyia* immature stages: **a, b)** egg in dorsal view, same view with visible head capsule one day prior to hatching; **c, d)** first instar larva in dorsal view, lateral view; **e, f)** second instar larva in dorsal view, lateral view; **g, h)** third instar larva in dorsal view, lateral view; **i, j)** fourth instar larva in dorsal view, lateral view; **k, l)** fifth instar larva in dorsal view, lateral view; **m)** pupa in lateral view; **n,)** adult. Scale bars indicate 1 mm unless indicated otherwise.

*harpyia* was collected on 7 November 2019 inside a *terra firme* forest within the FLP property, and subsequently kept in a 1 L plastic container with a mesh cover. This female received a mature leaf of *Lasiacis ligulata* Hitchcock & Chase (Poaceae: Panicoideae), in addition to few other unidentified grass leaves found at FLP, for oviposition. A single egg was laid on a leaf of *L. ligulata* on 10 November 2019. Subsequently, the egg and the leaf were moved to a 500 ml plastic cup with a closed lid. The caterpillar was checked daily and photographed using a Nikon D7100 digital camera and Canon DS126071. We collected head capsules, which were subsequently examined under stereomicroscope (Leica MZ 16) in order to measure relevant structures (e.g., head capsule width, scoli length). A camera lucida attached to this stereomicroscope was used to prepare head capsule drawings. The terminology for immature stages used herein largely follows Stehr (1987).

## RESULTS

**Description** (n=1 throughout).

**Egg** (Figs 1a, b). Duration 4 days, larva (head capsule) visible via transparency 1 day prior to eclosion; diameter 1.1 mm (all measures approximate), spherical, appearing smooth, whitish with numerous vertical and horizontal ridges defining rather indistinct concave hexagonal facets. The egg hatched on 14 November 2019.

**First instar** (Figs 1c, d; 2a, b). Duration 5 days. Head capsule width 1.1 mm; scoli length 0.2 mm; inter-scoli 0.4 mm. Head blackish, two roundish scoli with hair-like seta visible in anterior view (P1 visible on image of this head capsule taken prior to drawing, thus indicated as such in dotted line in Fig. 2a), height of scoli similar to that of mandible; six chalazae present, each with single seta, six stemmata present, third is largest among these six; body light green, darker after feeding; single brown longitudinal stripe in middorsal area, pair of brown stripes in subdorsal area, these lines more visible late during first instar; caudal filament short. First to second instar molt on 19 November 2019.

**Second instar** (Figs 1e, f). Duration 6 days. Head capsule width 1.5 mm; scoli length 0.9 mm; inter-scoli length 0.8 mm. Head light brownish, scoli developed, height similar to distance between two largest stemmata, inner region darker; chalazae absent, 6 stemmata present, position similar to first instar; body dark green; single brown well-defined longitudinal stripe in middorsal area, a pair of brown stripes in subdorsal area, stripe distally placed from former stripe more prominent; caudal filament developed, similar to head scoli in length. Second to third instar molt on 25 November 2019.

**Third instar** (Figs 1g, h). Duration 6 days. Head capsule width 2.0 mm; scoli length 1.4 mm; inter-scoli distance 1.0 mm. Head morphologically similar to second instar, appearing darker, except for area around epicranial notch; body light brown; single brown well-defined longitudinal stripe in middorsal area, becoming narrow and rather indistinct along several segments in middle; stripes in subdorsal area jagged and rather indistinct; caudal filament developed, slightly shorter than head scoli in length. Third to fourth instar molt on 1 December 2019.

**Fourth instar** (Figs 1i, j; 2c, d). Duration 7 days. Head capsule width 2.9 mm; scoli length 2.0 mm; inter-scoli distance 1.3 mm. Head similar to third instar morphologically, except for appearing darker; body orangish; single brown longitudinal stripe in middorsal area, anterior and posterior few segments more defined; stripes in subdorsal area jagged and poorly defined; caudal filament developed, slightly shorter than head scoli in length. Fourth to fifth instar molt on 8 December 2019.

**Fifth (ultimate) instar** (Figs 1k, l). Duration 12 days. Head capsule width not recorded; scoli length not recorded; inter-scoli distance not recorded. Head morphology similar to fourth instar, in addition to coloration; body orangish; single brown longitudinal stripe in middorsal area, anterior and posterior few segments more defined; stripes in subdorsal area jagged and poorly defined; caudal filament short in comparison with fourth instar. Pupation (final instar turning into a prepupa) on 19 December 2019.

**Pupa** (Fig. 1m). Duration 12 days**.** Total length 1.1 cm. Overall appearing short and rounded, brownish mottled with white in part, rather sparsely dorsally, more densely on wing case; ocular caps not prominent; two rows of reddish small protuberances along dorsal surface of abdomen; cremaster black. Adult eclosion on 1 January 2020.

**Adult** (Fig. 1n). Discussion of the adult morphology, identity and taxonomy of this species will be included in the forthcoming study mentioned above (Nakahara *et al.*, in prep.).

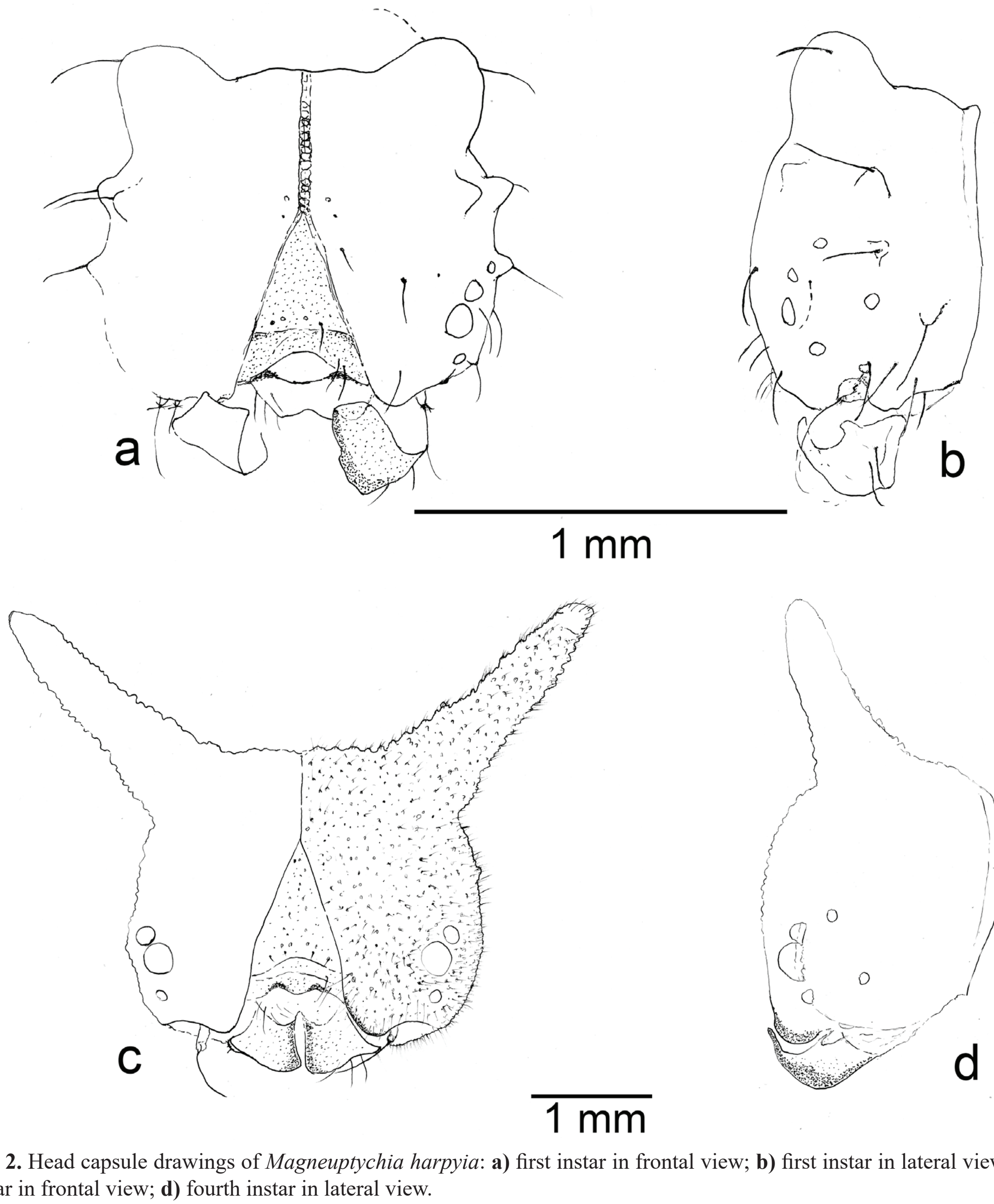


**Fig. 2.** Head capsule drawings of *Magneuptychia harpyia*: **a)** first instar in frontal view; **b)** first instar in lateral view; **c)** fourth instar in frontal view; **d)** fourth instar in lateral view.

**Host plant.** The female laid an egg and the larva easily accepted mature leaves of *Lasiacis ligulata*, but the host plant in nature is unknown. As described in Baine *et al.* (2019), this grass species is common at FLP, especially along forest edges. We did not observe flowering and presence of mature fruit while we were conducting field work in November 2019 through December 2019.

## DISCUSSION

Overall, the larva of *Magneuptychia harpyia* possesses a number of characters which are reported as characteristic and/or visible in available descriptions of euptychiine early stage biology (e.g., Singer *et al.*, 1983; Freitas, 2003, 2004, 2007, 2017; Freitas & Peña, 2006; Freitas *et al.*, 2016a,b, 2018; Kaminski & Freitas, 2008; Cosmo *et al.*, 2014; See *et al.*, 2018; Baine *et al.*, 2019): enlarged 3rd stemma (this is a characteristic of species in the subfamily Satyrinae (Stehr, 1987)); presence of a pair of caudal filaments; smooth body (lack of body scoli); presence of head scoli. Based on the aforementioned publications on euptychiine immature stages, it seems likely that the head capsule of the first instar and remaining instars exhibit some notable morphological differences between them: rounded or reduced head scoli of the first instar develop into horn-like scoli in second instar in many euptychiine species, as shown in this present study, except for species currently placed in *Taygetis* Hübner, [1819] and a single species in *Yphthimoides* Forster, 1964, where the development of head scoli do not change dramatically from the first instar to second instar in some species (e.g., Murray, 2001; Freitas *et al.*, 2012). Furthermore, primary setae of first instar head capsule are spatulate in species in the so-called "*Taygetis* clade" (Murray, 2001; Nakahara *et al*., in review), whereas appearing hair-like in many other known euptychiine species, including *M. harpyia* as described above. It is also worth noting that this transition in the form of head scoli in many euptychiine species seem to be accompanied by a reduction in long primary setae (mainly on the scoli and chalazae), in addition to a reduction in developed chalazae. The head scoli appear to be absent in at least three species of *Hermeuptychia* Forster, 1964 throughout all instars (Cong & Grishin, 2014; Cosmo *et al.*, 2014). On the other hand, passing through five instars is a trait restricted to the minority of Euptychiina, given that about two-thirds of euptychiine species pass through only four instars (references above). Infra-specific variation in terms of instar numbers is known in some nymphalid species (e.g., *Cercyonis pegalis* Fabricius, 1775, (Sourakov, 1995); *Adelpha basiloides* (Bates, 1865), (Willmott, 2003)), in addition to difference between males and females of *Automeris io* (Fabricius, 1775) (Sourakov, pers. comm.). This infra-specific variation is also reported for few euptychiine species such as *Moneuptychia itapeva* Freitas, 2007 (varying from five to six instars) and *Taydebis peculiars* (Butler, 1874) (varying from four to five instars) (Freitas, 2003, 2007). Nevertheless, considering the limited available information for the full life cycle of euptychiines and any other tropical butterfly groups, we are unable to draw a conclusion as to the phylogenetic informativeness of the number of instars and stability within each species.

Based on an unpublished molecular data (Espeland *et al.*, in prep), *Magneuptychia harpyia* is a member of the so-called "*Splendeuptychia* clade", distantly related to the type species of *Magneuptychia*, *Papilio libye* Linnaeus, 1767. Research is thus underway to describe a new genus for *Magneuptychia harpyia* and few other closely related taxa (Nakahara *et al.*, in prep.). The immature stages of *M. harpyia* and *M. libye* differ in several ways, such as the length of the scoli (long in *M. harpyia*, whereas short in *M. libye*) and the body coloration (ultimate instar orangish in *M. harpyia*, whereas more brownish in *M. libye*), but head capsule chaetotaxy was not provided by Kaminski & Freitas (2008), thus making it difficult to compare those characters. It is worth noting that considering their rather "simple" overall appearance, the immature morphology of some satyrine groups may not be informative at either generic-level or species-level. Hill & Tipan (2008) indicated differences in the first instar head capsule chaetotaxy between two species in *Methona* Doubleday, 1847 (Nymphalidae: Ithomiinae), suggesting that chaetotaxy might also potentially provide useful information at species-level classification or higher. However, scrutinizing immature stage morphology and detailed studies, of, for example, head capsule chaetotaxy, is still valuable both for its potential to provide important comparative information, as well as its intrinsic contribution to our knowledge of the early stage biology of Neotropical butterflies.

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