

# Immature stages and new host plant record for *Leucochimona hyphea* (Cramer, 1776) (Lepidoptera: Riodinidae: Riodininae) in the southern Peruvian Amazon

Rodrigo Ccahuana<sup>1,2</sup>, Thalia Corahua-Espinoza<sup>1,5</sup>, Shinichi Nakahara<sup>3,4</sup>, Rafael Tejeira<sup>1,2</sup>, Maryzender Rodríguez-Melgarejo<sup>4,6</sup> and Geoffrey Gallice<sup>1,3</sup>

1. Alliance for a Sustainable Amazon, Hanover, MD 21076, USA; 2. Universidad Nacional de San Antonio Abad del Cusco, Av. de La Cultura 773, Cusco 08000, Perú; 3. McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, FL 32611, USA; 4. Departamento de Entomología, Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Apartado 14-0434 Lima-14, Perú; 5. Universidad Nacional Amazonica de Madre de Dios, Av. Jorge Chávez 1160, Puerto Maldonado, Madre de Dios 17000, Perú; 6. Laboratorio de Entomología, Instituto Argentino de Investigaciones de las Zonas Áridas (IADIZA, CCT CONICET, Mendoza), Casilla de Correo 507, 5500 Mendoza, Argentina. \*Correspondence: info@sustainableamazon.org

Date of issue online: 17 December 2021

Electronic copies (ISSN 2575-9256) in PDF format at: <http://journals.fcla.edu/troplep>; <https://zenodo.org>; archived by the Institutional Repository at the University of Florida (IR@UF), <http://ufdc.ufl.edu/ufir>; DOI: 10.5281/zenodo.5777000

© The author(s). This is an open access article distributed under the Creative Commons license CC BY-NC 4.0 (<https://creativecommons.org/licenses/by-nc/4.0/>).

**Abstract:** We report for the first time the complete immature stages of the Neotropical riodinid butterfly *Leucochimona hyphea* (Cramer, 1776), based on individuals reared in Madre de Dios, Peru. We illustrate all five larval instars, in addition to the pupa and the egg, and provide the duration of each stage. We also provide evidence for the presence of ant organs on A8 of the larvae and pupa of *L. hyphea*, and report its natural host plant at the study site, *Spermacoce latifolia* Aubl. (Rubiaceae: Rubiaceae), which represents a new host record for this species.

**Resumen:** Reportamos por primera vez todos los estadios inmaduros de la mariposa riodínida neotropical *Leucochimona hyphea* (Cramer, 1776), basados en individuos criados en la estación de campo Finca Las Piedras en Madre de Dios, Perú. Se ilustran los cinco estadios larvarios, además de la pupa y el huevo, y se proporciona la duración de cada etapa. También reportamos la presencia de órganos asociados a hormigas en el A8 de las larvas y pupa de *L. hyphea*, así como su planta hospedera nativa en Finca Las Piedras, *Spermacoce latifolia* Aubl. (Rubiaceae: Rubiaceae), el cual constituye un nuevo registro de planta hospedera para esta especie.

**Key Words:** ant organs, host plant, Finca Las Piedras, life history, Madre de Dios

## INTRODUCTION

Over 90% of the species diversity in the butterfly (Papilionoidea) family Riodinidae is found in the Neotropics, with approximately 1,500 species in 142 genera concentrated mostly in the subfamily Riodininae (Callaghan & Lamas 2004; Trujano-Ortega *et al.*, 2018; Zhang *et al.*, 2019; Seraphim, 2019). Based on genomic data, the systematic placement of Riodinidae within Papilionoidea appears to be well-supported, as a sister group of the family Lycaenidae (Espeland *et al.*, 2018). Molecular markers have also contributed to resolving and revising the higher-level classification of Riodinidae (Espeland *et al.*, 2015; Seraphim *et al.*, 2018), in addition to affirming its monophyly and providing insights into the origin of the group. Nevertheless, in-depth study of species-level taxonomy, as well as assessment of generic classification, is still lacking for many riodinid taxa, with relatively few studies on the subject published in recent years (e.g., Hall, 1999, 2005, 2018; Trujano-Ortega *et al.*, 2018; Zhang *et al.*, 2019). While genetic data offers great promise in advancing these aforementioned aspects of riodinid systematics, such data do not shed light on the life

histories of riodinids, which is an area where our understanding lags far behind, as highlighted in studies such as Nielsen & Kaminski (2018). Notwithstanding this situation, filling in the major gaps in our knowledge of riodinid early stage biology is still important and warrants study because of the potential for finding characters to support species-level classification, as well as for documenting their native host plants as discussed below. Furthermore, larvae of certain Riodinidae (e.g., species in the genus *Nymphidium* Fabricius, 1807), along with its sister family Lycaenidae, are known for their associations with ants (myrmecophily) (Campbell & Pierce, 2003), which has important further implications for the evolution of butterflies in these groups (Pierce *et al.*, 2002).

*Leucochimona* Stichel, 1909 is a relatively small genus with nine species in the riodinid subtribe Mesosemiina (Callaghan & Lamas, 2004). Nielsen & Kaminski (2018) documented immature stages of three *Leucochimona* taxa, and summarized host plant information for the group based mainly on existing literature (Beccaloni *et al.*, 2008; Brown, 1992; DeVries, 1997; DeVries *et al.*, 1994; Harvey, 1987; Janzen & Hallwachs, 2018). Based on a personal communication by A. V. L. Freitas,

Nielsen & Kaminski (2018) reported *Richardia brasiliensis* (Rubiaceae) as a host plant of *Leucochimona hyphea* (Cramer, 1776), although its immature stages were not described in that work. *Papilio hyphea* was described by Pieter Cramer based on an unspecified number of specimens from “Indes Occidentales [=West Indies]” (Cramer, 1776: 145). Despite its questionable type locality and the syntype(s) not being found to our knowledge (e.g., Gernaat *et al.*, 2012), the illustration of *Papilio hyphea* associated with the original description (pl. XCII, fig. C) is consistent with species of *Leucochimona* with an ocellus on the forewing and four hindwing bands. Seitz (1932: 209-210) described *Diophthalma* [sic] *hyphea* f. *prosoeca* based on an unspecified number of specimens from “Sparwin-Creek” [French Guiana]. Seitz (1932) mentioned three bands on the hindwing in “f. *prosoeca*”, but four in “*hyphea*”, supported by the illustration associated with the original description of the former (p. 210: Taf. 2). Nevertheless, this figure indicates both “*hyphea*” and “f. *prosoeca*” as originating from the same locality (i.e. “Sparwin-Creek”), and thus excludes the possibility of “f. *prosoeca*” representing a geographic variation. Indeed, Gallard (2017) suggested that “f. *prosoeca*” might be conspecific with *L. matisca* (Hewitson, 1860), but investigating that possibility is beyond the scope of the present study. The same year, Lathy (1932: 65) described *Diophthalma* [sic] *hyphea pallida* Lathy, 1932 based on two females from Chaquimayo, Peru, and stated that this “Peruvian race” was differentiated from the nominate race by its reduced gray coloration. The syntype of *D. hyphea pallida* figured in Warren *et al.* (2017) clearly shows four hindwing bands, supporting conspecificity with the nominate race of unknown origin. Thus, whether “f. *prosoeca*” (i.e., considered as subspecific today, under Article 45.6.4.1 of the ICZN (1999)) should be regarded as conspecific with the nominate race is questionable. Despite these taxonomic complexities reflected in Callaghan & Lamas (2004), the specific epithet “*hyphea*” seems to be the only available name for an Amazonian *Leucochimona* species with an ocellus on the forewing and four distinctive hindwing bands. We thus apply this species-group name at the species level in the present study and await for a future taxonomic study to decide the appropriate subspecific name for the population discussed herein.

We here document the life history of *Leucochimona hyphea* and report its natural host plant based on a population found in the southern Peruvian Amazon, following on from previous related studies (i.e., See *et al.*, 2018; Baine *et al.*, 2019; Nakahara *et al.*, 2020; Teixeira *et al.*, 2021; Ccahuana *et al.*, 2021). These articles are all based on butterfly rearing conducted at the Finca Las Piedras (FLP) field station (Madre de Dios, Peru) in order to contribute to the long-term “Lepidoptera Diversity and Biology Project” conducted at FLP and throughout Madre de Dios by the authors.

## STUDY SITE AND METHODS

As mentioned above, the present study was carried out at Finca Las Piedras (FLP), a 54 ha field research station located approximately 48 km north of Puerto Maldonado in the department of Madre de Dios, Peru (-12.22789, -69.11119; ca. 240 m). The study site is covered mostly by ‘terra firme’ or upland

forest, although regenerating secondary forests, abandoned agricultural fields, as well as *Mauritia* L. f. (Arecaceae) palm swamps are also present in the station’s vicinity. Terra firme forest at the site is dominated by emergent tree species such as Brazil nut (*Bertholletia excelsa* Bonpl. (Lecythidaceae)), ironwood (*Dipteryx micrantha* Harms (Fabaceae)), and other species, particularly in the family Fabaceae; the understory is dominated by various palm species (Arecaceae), a variety of bamboo and other grasses (Poaceae), and Piperaceae. More information regarding FLP is summarized in Baine *et al.* (2019) and See *et al.* (2018), and also available at <https://www.sustainableamazon.org/finca-las-piedras>. Field work was carried out between January and February 2021 by TCE based on prior information and field work conducted by RC in February to July 2020. During the period when rearing was conducted, the air temperature ranged daily from 22.8°C to 27.1°C and daily precipitation was 12.1 mm (average across 55 days of data).

All sampled eggs and larvae were taken into the field laboratory at FLP to monitor their growth and were photographed daily with voucher codes assigned (2021-FLP-IMM-XXX). The immatures were kept in 200 ml, 500 ml and 1 L plastic containers covered with a nylon mesh cloth held in place by an elastic band. The larvae received fresh leaves from their host plant as needed, which were kept fresh using floral water tubes, and the container was cleaned daily. Observations of the head capsules were made using a Novel NSZ-608T microscope and stereoscope. The head capsule graphics were made with Adobe Illustrator version 2019. The measurements of the egg, larval and pupal stages were made by means of pixel counting of high resolution photos using Adobe Photoshop CC, version 2014.2.2. We follow Stehr (1987) for terminology related to immature stages and Cottrell (1984) for terminology related to ant organs. The examined individuals were vouchered and morphological examinations were carried out in the Arachnology and Zoology laboratory of the Universidad Nacional de San Antonio Abad del Cusco, Peru (UNSAAC); the vouchered material is deposited in the collection of the Alliance for a Sustainable Amazon (ASA), Puerto Maldonado, Peru.

## RESULTS

Recorded dates and voucher codes associated with individuals studied in the present article are provided in Table 1. Information regarding the condition of the host plants at the time of sampling for each immatures are provided in Table 2.

### Host plant and behavior

The host plant was identified as *Spermacoce latifolia* Aubl. (Rubiaceae: Rubioideae) (Figs. 1A-G). It is an annual herb with white flowers, brown seeds and a quadrangular stem (Ferrer *et al.*, 2012), and it is distributed from southern Mexico through Central America south to Paraguay (Wiersema *et al.*, 2017). Egg and larvae collections were made from different individuals of the same plant species. The vouchered host plant listed in Table 2 was found in clusters in some areas at FLP, but also as solitary individuals in a clearing by the trail leading to a mature forest that had been selectively logged. The adult female was

**Table 1.** Voucher information and dates recorded for *Leucochimona hyphea* individuals studied in 2021, as well as the single specimen from 2020 used for Fig. 4C.

voucher	date of coll.	egg hatch	L1 to L2	L2 to L3	L3 to L4	L4 to L5	pupation	adult	died
2021-FLP-IMM-0040	27 Jan 21	02 Feb 21	09 Feb 21	16 Feb 21	N/A	N/A	24 Feb 21	07 Mar 21	N/A
2021-FLP-IMM-0094	06 Feb 21	10 Feb 21	16 Feb 21	18 Feb 21	23 Feb 21	28 Feb 21	06 Mar 21	17 Mar 21	N/A
2021-FLP-IMM-0095	06 Feb 21	N/A	10 Feb 2021	N/A	N/A	N/A	N/A	N/A	13 Feb 2021
2021-FLP-IMM-0118	12 Feb 21	16 Feb 21	21 Feb 21	25 Feb 21	01 Mar 21	05 Mar 21	12 Mar 21	22 Mar 21	N/A
2021-FLP-IMM-0122	12 Feb 21	13 Feb 21	N/A	N/A	N/A	N/A	N/A	N/A	17 Feb 2021
2021-FLP-IMM-0124	12 Feb 21	N/A	16 Feb 21	23 Feb 21	dead	N/A	N/A	N/A	N/A
2021-FLP-IMM-0125	12 Feb 21	N/A	N/A	N/A	N/A	N/A	N/A	N/A	14 Feb 2021
2021-FLP-IMM-0126	12 Feb 21	N/A	15 Feb 21	21 Feb 21	25 Feb 21	01 Feb 21	9 Mar 21	20 Mar 21	N/A
2020-FLP-IMM-148A	1 Apr 20	8 Apr 20	22 Apr 20	N/A	N/A	N/A	29 Apr 20	11 May 21	N/A

**Table 2.** Details of sampled individuals of immatures with information on the host plant conditions. All individuals were found on *Spermacoce latifolia* (Rubiaceae, host plant ID 490), on the abaxial surface of mature leaves. Abbreviations: distal (D); middle (M); midrib (B); middle (E); none (NO); light (L); mid (MI).

VOUCHER	DATE COLLECTED (immature)	DISTANCE FROM GROUND (m)	LEAF PROXIMITY TO BASE	LEAF BLADE PROXIMITY	LEAF DAMAGE	STAGE FOUND
2021-FLP-IMM-0040	27 Jan 2021	0.3	M	E	L	egg
2021-FLP-IMM-0094	6 Feb 2021	0.15	M	E	NO	egg
2021-FLP-IMM-0095	6 Feb 2021	0.15	M	B	NO	egg
2021-FLP-IMM-0118	12 Feb 2021	0.3	M	E	NO	egg
2021-FLP-IMM-0122	12 Feb 2021	0.1	D	E	NO	egg
2021-FLP-IMM-0124	12 Feb 2021	0.15	M	B	MI	larva
2021-FLP-IMM-0125	12 Feb 2021	0.2	D	E	L	larva
2021-FLP-IMM-0126	12 Feb 2021	0.2	D	B	L	larva

found laying eggs on April 1, 2020 around 10:00 am by RC (the recorded temperature of the day was 24.5°C, with a minimum of 23°C and a maximum of 29°C). This individual was found hopping from plant to plant until it landed on the stem where it remained for about 5 seconds before laying two eggs on the ventral surface of the leaf. Based on field observations by TCE and RC conducted between 2020-2021, *L. hyphea* was uncommon at the study site, where it was found in association with the host plant during the rainy season (November-April); TCE observed that the host plant was not present at the site during the dry season (June-October). The larval stages were not observed closely in the field, thus we are unable to provide detailed information regarding association with ants. Existing literature (e.g., DeVries 1997; Vélez-Arango *et al.*, 2010) reports members of Mesosemiina to be non-myrmecophilous, but TCE observed ants inside the flowers of some individuals of *S. latifolia*.

### Description of the immature stages

**Overall duration (collection of egg to adult emergence):** 36-39 days (n=4).

**Egg.** (Figs. 2A, B). Creamy yellow, hemispherical with flattened lower surface. Exochorion translucent and patterned with hexagonal cells with small spines at each intersection, micropylar area centrally located at top. Duration: 4-5 days (n=3). Diameter: 0.50 mm (n=3).

**First instar.** (Figs. 2C, D; 3A). Head yellowish (i.e., light tan) with semi-translucent capsule, fused cervical triangle; short primary setae present on epicranium and sides of labrum, front and labrum reddish brown; six stemmata present, all roughly similar in size. Width of head capsule: 0.27 mm (n=1). Body yellowish, appearing lighter after hatching and appearing more greenish after feeding due to gut contents visible via semi-translucent. Gut contents dark green. Setae on dorsal surface dark, and those on lateral side white on all segments, with more setae discernable on prothoracic and anal plate. Anterior tentacle organs (ATOs) absent; tentacle nectary organs (TNOs; i.e., ant-organs) visible on A8. Average body length: 2.6 mm (n=2). Duration: 5-7 days (n=3).

**Second instar.** (Figs. 2E, F). Head capsule with morphology similar to previous instar, except with setae longer and increasing in number. Width of head

capsule: 0.47 mm (n=1). Head yellowish (i.e., light tan) with semi-translucent capsule. Body dark green, similar to previous instar, with apparently increasing number of setae overall, compared to first instar. Anterior tentacle organs (ATOs) absent. TNOs visible on A8. Average body length: 3.36 mm (n=2). Duration: 5 days (n=5).

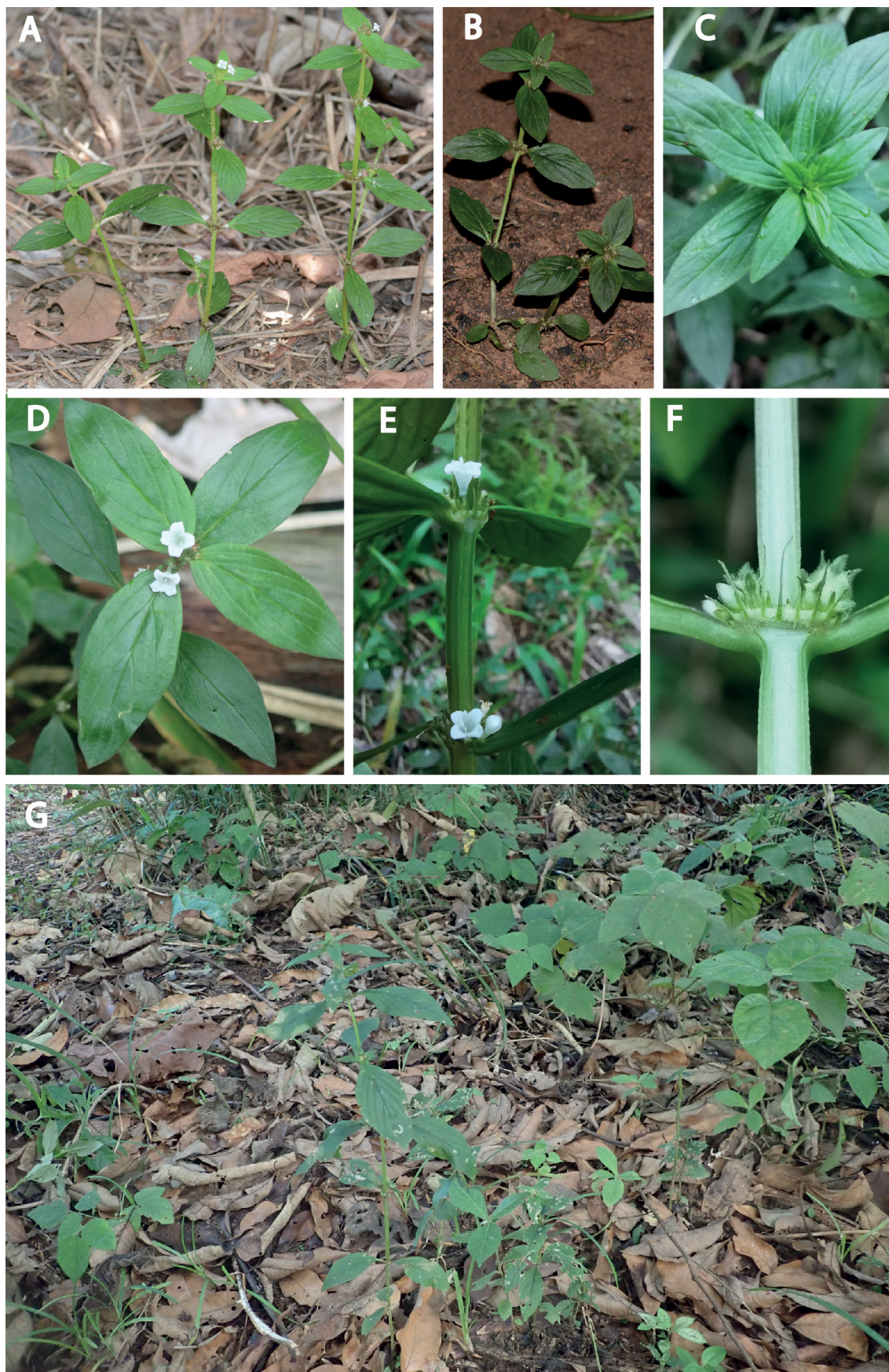
**Third instar.** (Figs. 2G, H). Head capsule morphologically similar to first instar, except with setae longer and increasing in number. Head capsule width: 0.63 mm (n=1). Head yellowish (i.e., light tan) with semi-translucent capsule. Body green, similar to previous stages, white lateral tracheal system visible, with irregular white markings on posterior side of each segment. White spiracles, presence of whitish horizontal line located in middle part of dorsal area. Lateral white setae and dorsal dark setae more numerous and longer compared to previous stages. Anterior tentacle organs (ATOs) absent. TNOs visible on A8. Average body length: 5.4 mm (n=2). Duration: 4 days (n=3).

**Fourth instar.** (Figs. 2I, J, 3B, 4A, B). Head capsule morphologically similar to first instar, except with setae longer and increasing in number. Head capsule width: 0.79 mm (n=1). Head yellowish (i.e., light tan) with semi-translucent capsule. Body green, darker than previous instar. White markings dorsally at posterior end of each abdominal segment bordered with purplish markings dorsally and ventrally. White spiracles, slightly visible yellowish dorsal medial line, lateral white setae and dorsal black setae, longer than previous stage. Anterior tentacle organs (ATOs) absent. TNOs visible on A8. Average body length: 7.9 mm (n=2). Duration: 4 days (n=3).

**Fifth instar.** (Figs. 2K, L, 4A, B). Head capsule morphologically similar to first instar, except with setae longer and increasing in number. Head capsule width: 1.53 mm (n=1). Head green with semi-translucent capsule. Body lighter green than previous instar, white spiracles, dorsal medial line white becoming darker as feeding progressed. White markings at posterior end of each abdominal segments bordered with purplish markings dorsally and ventrally. White speck-like setae present dorsally. Anterior tentacle organ (ATO) absent. TNOs visible on A8. (Fig. 4A, B). Average body length: 11.6 mm (n=2). Duration: 7-8 days (n=3).

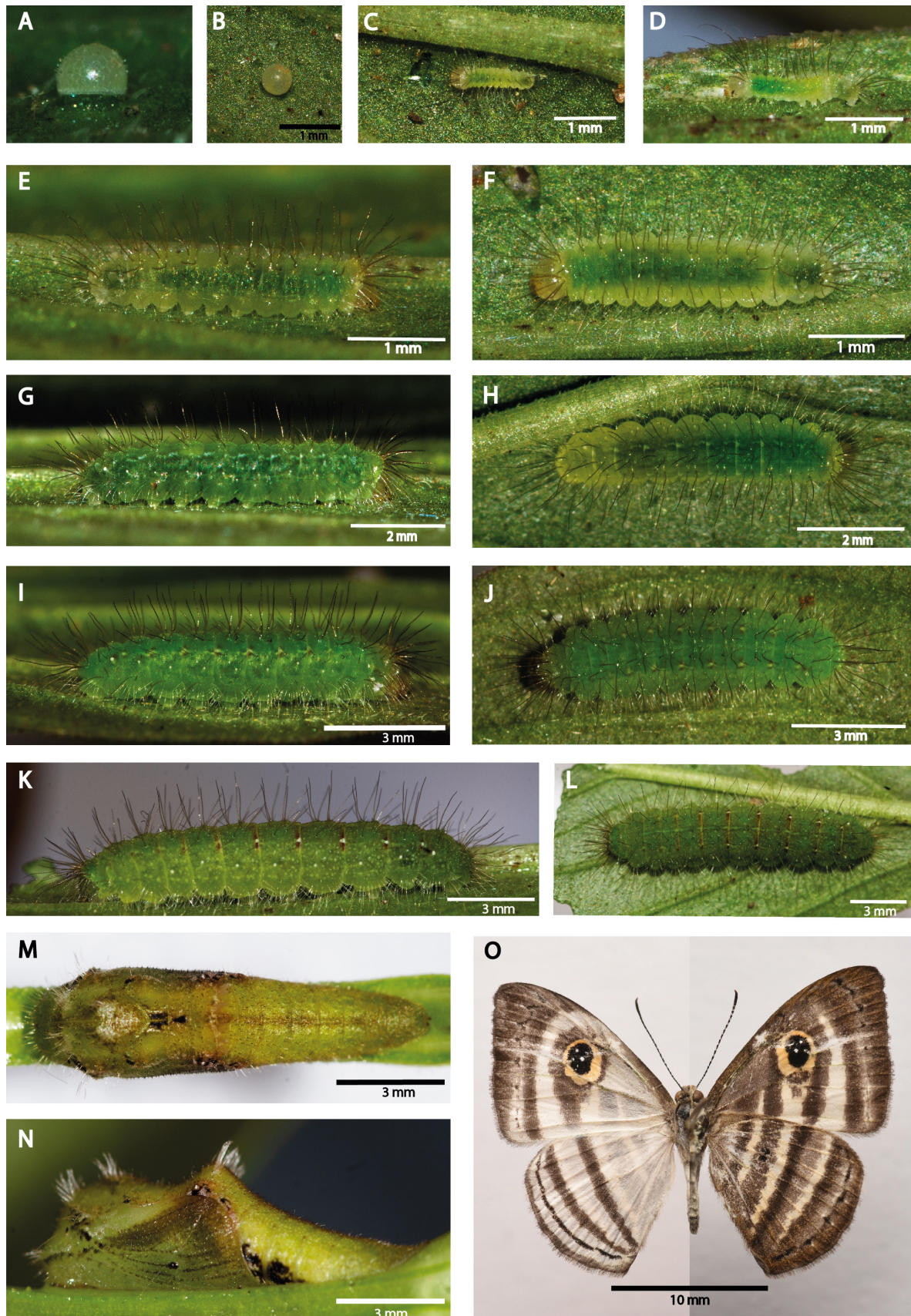
**Pupa.** (Figs. 2M, N). Body green, erect crest present on A2 with translucent setae. Wing pad changed from green to black as adult emergence approached. Pair of tubercles with erect translucent setae on metathorax and prothorax. Irregular black spots in lateral area near edge of wing case from A1 to A3. One black discal spot and numerous markings on proximal portion of wing case. Trace of TNOs visible on A8 based on pupal case (Fig. 4C). Body Length: 11 mm (n=3). Widest point at A2: 4 mm approx. (n=3) Duration: 11 days (n=4).



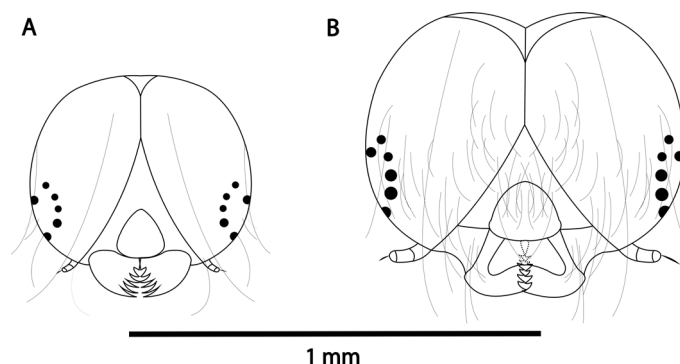


**Figure 1.** Images of the habitat and host plant (*Spermacoce latifolia*) of *Leucochimona hyphea*: A, B) Close-up view of *Spermacoce latifolia*; C) Detailed view of the leaves and the apex; D) Top view of the flowers; E) Lateral view of the flowers; F) Lateral view of the internodes and stipules; G) General view of the plant habitat where the immatures were located.





**Figure 2.** Immature stages of *Leucochimona hyphea*: A) Egg in lateral view; B) Egg in dorsal view C) First instar in dorsal view; D) First instar in dorso-lateral view; E) Second instar in dorso-lateral view; F) Second instar in dorsal view; G) Third instar in dorso-lateral view; H) Third instar in dorsal view; I) Fourth instar in dorso-lateral view; J) Fourth instar in dorsal view; K) Fifth instar in dorso-lateral view; L) Fifth instar in dorsal view; M) Pupa in dorsal view; N) Pupa in lateral view; O) Dorsal (left) and ventral (right) view of the adult of *L. hyphea*. Photographs based on 2021-FLP-IMM-0118.



**Figure 3.** Illustrations of the head capsule: A) First instar (in frontal view), labrum not discernible thus not illustrated; B) Fourth instar (in frontal view). Illustrations based on IMM-FLP-2021-0094.

## DISCUSSION

Beccaloni *et al.* (2008), Janzen & Hallwachs (2018) and Nielsen & Kaminski (2018) provided evidence that the host plants for *Leucochimona* are primarily within the plant family Rubiaceae. There are two exceptions, *Leucochimona icare* (Hübner, 1819) and *L. molina* (Godman & Salvin, 1885), which feed on a species in the family Commelinaceae (*Commelina* sp.) and species in the family Onagraceae, respectively (Janzen & Hallwachs, 2018; Nielsen & Kaminski, 2018). Nielsen & Kaminski (2018), based on work carried out in central Colombia, reported host plants for *Leucochimona lagora* (Herrich-Schäffer, 1853), *Leucochimona icare icare* and *Leucochimona icare matatha* (Hewitson, 1873), all species of Rubiaceae, in addition to *Richardia brasiliensis* as a host plant for *Leucochimona hyphea hyphea*. Here, we report *Spermacoce latifolia* as a new host plant for *L. hyphea*, which is consistent with other records since it is also a member of Rubiaceae. Although members of the diverse subfamily Riodiniinae are known to feed on approximately 70 plant families (Robinson *et al.*, 2010), the host plant records for species in the tribe Mesosemiina are restricted to eight plant families (Beccaloni *et al.*, 2008; Nielsen & Kaminski, 2018). Given that over 1,000 species are known in the Riodiniinae, the host plant breadth of the Mesosemiina, with over 200 species, is perhaps rather narrow given the overall diet breadth of the subfamily.

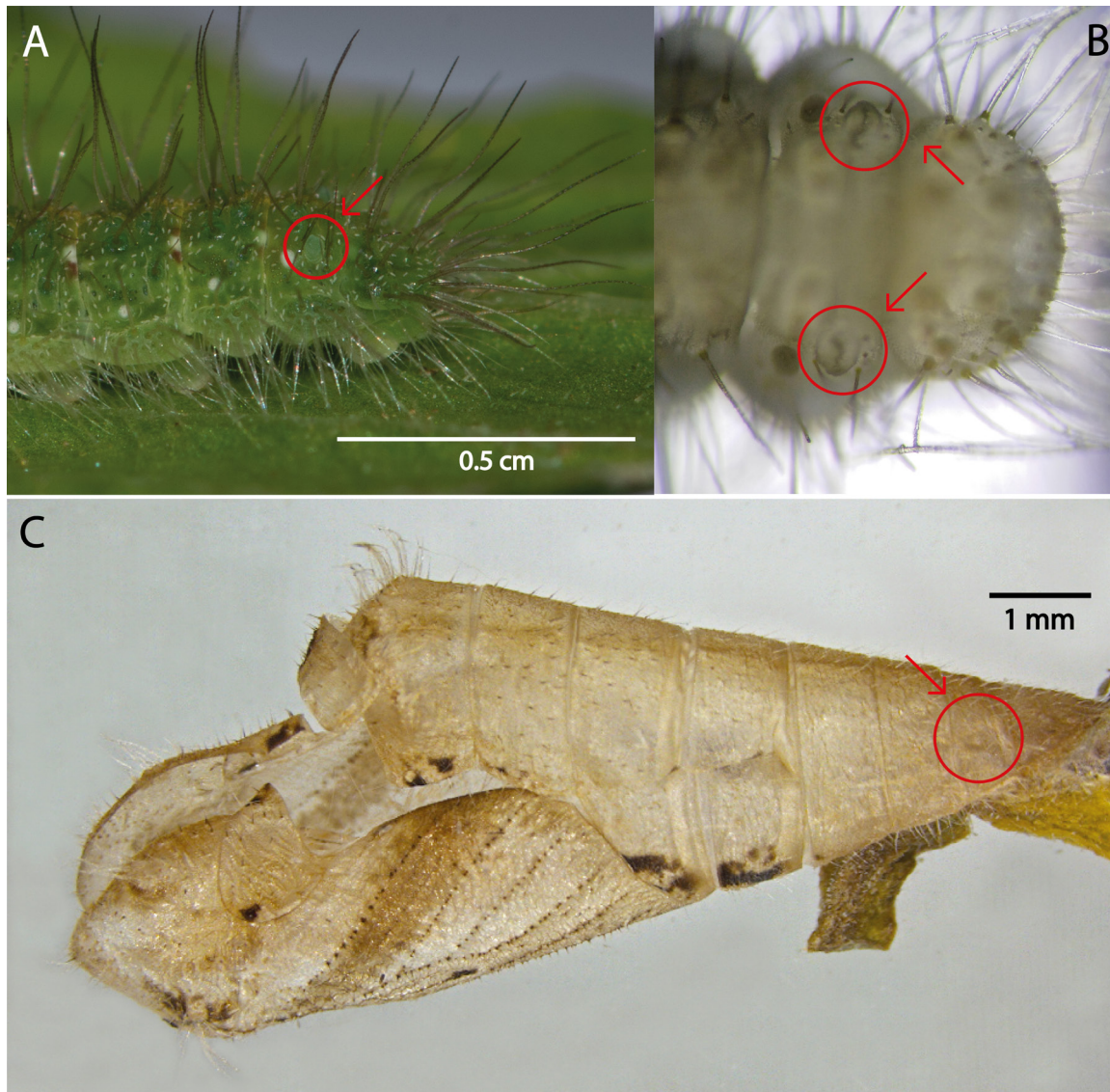
Based on available life history information for *Leucochimona* (Janzen & Hallwachs, 2018; Nielsen & Kaminski, 2018), some species-level morphological differences can be observed between *L. hyphea* and other *Leucochimona* taxa. The general structure of the egg of *L. hyphea* is overall the same as that of other species in *Leucochimona*, namely *L. icare icare*, *L. lagora* and *L. icare matatha*, in showing the same pattern of hexagonal cells all over the hemispherical egg. These species also share small spines at the intersections of the hexagonal cells and the micropylar area on top, although the overall appearance of the eggs of these taxa differ in their hue. The first, second and third larval instars of *L. hyphea* are all generally similar in appearance to these aforementioned three *Leucochimona* species in possessing black dorsal setae and white lateral setae, although some body coloration and marking differences are observed. For example, *L. icare matatha* in its

second instar presents lines of subdorsal white spots, whereas these are absent or not visible in the second instar of *L. hyphea*. These subdorsal white markings are visible in the third instar of all four *Leucochimona* species, and *L. icare matatha* is the only taxon to pass through four larval instars (as opposed to five) among the four taxa, so this fact may explain the difference. Similarly, the head capsule of the third instar of *L. icare matatha* is light greenish, whereas it is more light brownish or yellowish in the third instar of the three other taxa, including *L. hyphea*. The fourth and fifth larval instars of *L. hyphea* differ from the fourth and fifth instars (or penultimate and ultimate instars) of other *Leucochimona* species with known equivalent immature stages (i.e., in *L. lagora*, *L. iphias*, *L. icare icare*, *L. molina*, *L. icare matatha*) by possessing white markings at the posterior end of each abdominal segments bordered with purplish markings dorsally and ventrally. In other *Leucochimona* species, these markings are either absent (or not visible) or present but lack the purple adjacent area. The overall morphology and color of the pupa of *L. hyphea* is similar to that of other *Leucochimona* species, which all exhibit greenish background color with some darker markings. Nevertheless, the prominent crest on the thorax and A2 distinguishes the pupa of *L. hyphea* from other *Leucochimona* species with known pupal stages. In addition, the coloration of A9 and A10 of *L. hyphea* is similar to its more basal abdominal segments, whereas A9 and A10 of the pupal stages of other *Leucochimona* species are different in color compared to the rather greenish A1-A8 (in *L. lagora* and *L. icare icare* the two segments are reddish; in *L. molina* and *L. icare matatha* they are reddish brown).

Like many other butterfly groups in the tropics, information on immature stages for Riodinidae taxa is scarce. Robust phylogenetic hypotheses are increasingly being generated based solely on genetic data (references cited in the introduction), but species-level morphological differences remain useful in further supporting such hypotheses. The study of morphological differences within the immature stages of closely related butterfly taxa is an area that lags far behind that of adult morphological characters. In addition, the accumulation of host plant records can contribute to our understanding of the diversification of these species-rich Neotropical butterfly groups, since, for example, studies have shown an association between diet breadth and diversification in other diverse Neotropical butterfly groups (Mullen *et al.*, 2011). Arriving at a stable species-level riodinid classification, coupled with reliable natural host plant records for the group, would be valuable towards achieving this goal.

Another unexplored area related to the present study is myrmecophily in Riodinidae. Despite the presence of ant organs (TNOs) on A8 in *L. hyphea*, and reports of their presence in other closely related Mesosemiina taxa (e.g., Nielsen & Kaminski, 2018, 2021), the function of these organs is poorly understood in this group of non-myrmecophilous riodinids, although some intriguing discussions in the aforementioned studies hypothesized these organs to play a defensive role. Documentation of tending ants in the field and their potential to interact symbiotically, as well as assessing the function of the ant-organs in *L. hyphea* and closely related non-myrmecophilous riodinid taxa, is a necessary step towards





**Figure 4.** Ant-organs of *L. hyphea*: A) Lateral view of the ant-organ, tentacle nectary organs (TNOs) on A8 based on 2021-FLP-IMM-0094; B) Dorsal view based on 2021-FLP-IMM-0124; C) Trace of TNOs on A8 based on pupal case obtained in 2020 by RC (2020-FLP-IMM-148A).

better understanding of how myrmecophily evolved in certain Riodinidae taxa. As noted by Campbell & Pierce (2003), the locations of these ant organs in thoracic/abdominal segments vary across taxa in Riodinidae and Lycaenidae, thus assessing homology is also an important task towards elucidating how many times myrmecophily has evolved in these lineages. The present study, therefore, fills a gap in our knowledge by documenting the immature stages of *L. hyphea* and reporting the presence of ant-organs, despite no documented association with ants, and provides natural host plant records for *L. hyphea*, to contribute towards a better understanding of riodinid diversity.

#### ACKNOWLEDGMENTS

We are extremely grateful to Joseph See, Joselin Cenvantes, Nathan Duerr and Eric Perlett for laboratory support; Oliver Keller and Bernard Hermier for their help during the preparation of the manuscript; Elnatan Bezerra De Souza for

identifying the host plant and providing relevant information; Lucas Kaminski for providing relevant information; Gregory Nielsen and an anonymous reviewer for helpful comments; the authors wish to thank the National Forest and Wildlife Service (SERFOR) of Peru for granting permission to conduct the field research (permit number: 187-2017-SERFOR / DGGSPFFS). SN thanks the Department of Entomology and Nematology at the University of Florida and the Alliance for the Sustainable Amazon for their support. MRM thanks Diana Silva and Gerardo Lamas for their continuous support, and Consejo Nacional de Investigaciones Científicas y Técnicas - CONICET for a doctoral fellowship.

#### LITERATURE CITED

- Baine, Q., Polo-Espinoza, G., Nakahara, S., Gallice, G. 2019. Immature stages and new host record of *Taygetis rufomarginata* Staudinger, 1888 (Lepidoptera: Nymphalidae: Satyrinae). *Tropical Lepidoptera Research* 29(2): 79-86.

- Beccaloni, G. W., Vilorio, A. L., Hall, S. K., Robinson, G. S. 2008. *Catalogue of the host plants of the Neotropical butterflies / Catálogo de las plantas huéspedes de las mariposas Neotropicales*. In: m3m-Monografías Tercer Milenio, Volume 8. Zaragoza (Spain): Sociedad Entomológica Aragonesa (SEA)/Red Iberoamericana de Biogeografía y Entomología Sistemática (RIBES)/Ciencia y Tecnología para el Desarrollo (CYTED)/Natural History Museum, London, U. K. (NHM)/Instituto Venezolano de Investigaciones Científicas, Venezuela (IVIC). 1-536 pp.
- Brown, K. S. 1992. *Borboletas da Serra do Japi: diversidade, habitats, 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 florestal no sudeste do Brasil*. Campinas, Editora da Unicamp/Fapesp.
- Callaghan, C. J., Lamas, G. 2004. *Riodinidae*, pp. 141-170. In: Lamas, G. (Ed.), *Checklist: Part 4A. Hesperioidea - Papilionoidea*. In: Heppner, J. B. (Ed.), *Atlas of Neotropical Lepidoptera. Volume 5A*. Gainesville, Association for Tropical Lepidoptera; Scientific Publishers.
- Campbell, D. L., Pierce, N. E. 2003. *Phylogenetic relationships of the Riodinidae: Implications for the evolution of ant association*, pp. 395-408, 4 figs., 2 tabs. In: Boggs, C. L., Watt, W. B., Ehrlich, P. R. (Eds.), *Butterflies. Ecology and Evolution taking flight*. Chicago, The University of Chicago Press.
- Ccahuana, R., Tejeira, R., Hurtado, T., Nakahara, S., Rodríguez-Melgarejo, M., Gott, R. J., See, J., Gallice, G. 2021. Immature stages of *Ebusus ebusus* (Cramer, 1780) in the Peruvian Amazon (Lepidoptera: Hesperidae: Hesperinae). *Tropical Lepidoptera Research* 31(2): 90-95.
- Cottrell, C. B. 1984. Aphytophagy in butterflies: its relationship to myrmecophily. *Zoological Journal of the Linnean Society* 80: 1-57.
- Cramer, P. 1776. *De uitlandsche Kapellen voorkomende in de drie Waereld-Deelen Asia, Africa en America. Papillons exotiques des trois parties du monde l'Asie, l'Afrique et l'Amérique*. Amsterdam, S. J. Baalde; Utrecht, Barthelemy Wild and J. Van Schoonhoven & Comp. (8): 133-155.
- DeVries, P. J. 1997. *The Butterflies of Costa Rica and their Natural History. Volume II. Riodinidae*. Princeton, Princeton University Press. xxvii + 288 pp.
- DeVries, P. J., Chacon, I. A., Murray, D. 1994. Toward a better understanding of host use biodiversity in riodinid butterflies (Lepidoptera). *Journal of Research on the Lepidoptera* 31(1): 103-126.
- Espeland, M., Hall, J. P., DeVries, P. J., Lees, D. C., Cornwall, M., Hsu, Y. F., ... & Pierce, N. E. 2015. Ancient Neotropical origin and recent recolonisation: Phylogeny, biogeography and diversification of the Riodinidae (Lepidoptera: Papilionoidea). *Molecular Phylogenetics and Evolution* 93: 296-306.
- Espeland, M., Breinholt, J., Willmott, K. R., Warren, A. D., Vila, R., Toussaint, E. F., Maunsell, S. C., Aduse-Poku, K., Talavera, G., Eastwood, R., Jarzyna, M. A., Guralnick, R., Lohman, D. J., Pierce, N. E., Kawahara, A. Y. 2018. A comprehensive and dated phylogenomic analysis of butterflies. *Current Biology* 28(5): 770-778.
- Ferrer Gallego, P. P., Laguna Lumbreras, E., Roselló Gimeno, R. 2012. *Spermacoce latifolia* Aubl. (Rubiaceae), una especie alóctona nueva en la flora europea». *Orsis: organismos i sistemes* 26: 193-199.
- Gallard, J-Y. 2017. *Les Riodinidae de Guyane*. Published by the author. 191 pp.
- Gernaat, H. B., Beckles, B. G., van Andel, T. R., van Andel, T. R. 2012. *Butterflies of Suriname. A Natural History*. Amsterdam, KIT Publishers. 680 pp.
- Hall, J. P. W. 1999. A revision of the genus *Theope*. Its systematics and biology (Lepidoptera: Riodinidae: Nymphidiini). Gainesville, Scientific Publishers. vii + 127 pp.
- Hall, J. P. W. 2005. *A Phylogenetic Revision of the Napaeina (Lepidoptera: Riodinidae: Mesosemiini)*. Washington, DC, Entomological Society of Washington. [vi] + 235 pp.
- Hall, J. P. W. 2018. *A Monograph of the Nymphidiina (Lepidoptera: Riodinidae: Nymphidiini). Phylogeny, Taxonomy, Biology and Biogeography*. Washington, DC, The Entomological Society of Washington. [10] + 990 pp.
- Harvey, D. J. 1987. *The Higher Classification of the Riodinidae (Lepidoptera)*. PhD dissertation. Austin, University of Texas at Austin. 215 pp.
- ICZN (International Commission on Zoological Nomenclature) 1999. *International Code of Zoological Nomenclature, 4th Edition*. London, International Trust for Zoological Nomenclature.
- Janzen, D. H., Hallwachs, W. 2018. *Dynamic Database for an Inventory of the Macrocarpillar Fauna, and its Food Plants and Parasitoids, of the Area de Conservacion Guanacaste (ACG), Northwestern Costa Rica*. Philadelphia, University of Pennsylvania. <http://janzen.sas.upenn.edu>. Accessed November 2020.
- Lathy, P. I. 1932. New species and races of Erycinidae (Lepidoptera). *Annals and Magazine of natural History* 9(49): 65-74.
- Mullen, S. P., Savage, W. K., Wahlberg, N., Willmott, K. R. 2011. Rapid diversification and not clade age explains high diversity in neotropical *Adelpha* butterflies. *Proceedings of the Royal Society B: Biological Sciences* 278(1713): 1777-1785.
- Nakahara, S., Hoffman, F. L. A., Hoffman, F. L., Gallice, G. 2020. Immature stages of *Magneptychia harpyia* (C. Felder & R. Felder, 1867) (Lepidoptera: Nymphalidae: Satyrinae). *Tropical Lepidoptera Research* 30(1): 33-38.
- Nielsen, G., Kaminski, L. A. 2018. Immature stages of the Rubiaceae-feeding metalmark butterflies (Lepidoptera: Riodinidae), and a new function for the tentacle nectary organs. *Zootaxa* 4524(1): 1-32.
- Nielsen, G. J., Kaminski, L. A. 2021. Subtle life cycle differences between two sympatric *Mesosemia* (Lepidoptera: Riodinidae) butterflies. *Studies on Neotropical Fauna and Environment* 1-10.
- Pierce, N. E., Braby, M. F., Heath, A., Lohman, D. J., Mathew, J., Rand, D. B., Travassos, M. A. 2002. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annual Review of Entomology* 47(1): 733-771.
- Robinson, G. S., Ackery, P. R., Kitching, I. J., Beccaloni, G. W., Hernández, L. M. 2010. *HOSTS - A Database of the World's Lepidopteran Hostplants*. <http://www.nhm.ac.uk/hosts>. Accessed March 2021.
- Seitz, A. 1932. Goyaz-Reise. *Entomologische Rundschau* 49(19): pl. 2, (20): 205-210.
- See, J., Nakahara, S., Gallice, G. 2018. Immature stages of *Splendeptychia quadrina* (Butler, 1869) (Lepidoptera: Nymphalidae: Satyrinae). *Tropical Lepidoptera Research* 28(2): 49-53.
- Seraphim, N. 2019. *Riodinidae Species Checklist: a Preliminary Species Checklist for the Riodinidae*. [https://www2.ib.unicamp.br/labor/site/?page\\_id=805](https://www2.ib.unicamp.br/labor/site/?page_id=805). Accessed October 2021.
- Seraphim, N., Kaminski, L. A., DeVries, P. J., Penz, C., Callaghan, C., Wahlberg, N., Silva Brandão, K. L., Freitas, A. V. L. 2018. Molecular phylogeny and higher systematics of the metalmark butterflies (Lepidoptera: Riodinidae). *Systematic Entomology* 43(2): 407-425.
- Stehr, F. W. 1987. *Immature Insects. Volume 1*. Dubuque, Kendall/Hunt Publishing. 754 pp.
- Tejeira, R., Ccahuana, R., Hurtado, T., Nakahara, S., See, J., Rodríguez-Melgarejo, M., Corahua-Espinoza, T., Gallice, G. 2021. Immature stages of *Chloreptychia marica* (Weymer, 1911) (Lepidoptera: Nymphalidae: Satyrinae: Satyrini). *Tropical Lepidoptera Research* 31(2): 96-100.
- Trujano-Ortega, M., García-Vázquez, U. O., Callaghan, C. J., Ávalos-Hernández, O., Luis-Martínez, M. A., Llorente-Bousquets, J. E. 2018. Two new genera of metalmark butterflies of North and Central America (Lepidoptera, Riodinidae). *ZooKeys* 729: 61-85.
- Vélez-Arango, A. M., Vélez, P. D., Wolff, M. 2010. The life cycle of *Mesosemia mevania* (Hewitson, 1857) (Riodinidae) in a lower montane humid forest in Colombia. *Journal of the Lepidopterists' Society* 64 (1): 23-28.
- Warren, A. D., Davis, K. J., Stangeland, E. M., Pelham, J. P., Grishin, N. V. 2017. *Illustrated List of American Butterflies*. <http://www.butterfliesofamerica.com/>. Accessed March 2021.
- Wiersema, J. H., Delprete, P. G., Kirkbride, J. H., Franck, A. R. 2017. A new weed in Florida, *Spermacoce latifolia*, and the distinction between *S. alata* and *S. latifolia* (Spermacoceae, Rubiaceae). *Castanea* 82(2): 114-131.
- Zhang, J., Shen, J., Cong, Q., Grishin, N. V. 2019. Genomic analysis of the tribe Emesidini (Lepidoptera: Riodinidae). *Zootaxa* 4668(4): 475-488.