Immature stages and new host record of *Taygetis rufomarginata* Staudinger, 1888 (Lepidoptera: Nymphalidae: Satyrinae)

Quinlyn Baine[1,2,*], Gabriela Polo Espinoza[2], Shinichi Nakahara[3,4], and Geoffrey Gallice [2,3]

1. Washington State Department of Agriculture, Olympia, WA 98504, USA
2. Alliance for a Sustainable Amazon, Hanover, MD 21076, USA
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
*Correspondence: info@sustainableamazon.org

Date of issue online: 28 October 2019
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.3515367

Abstract: In the present study, the immature stages of the Neotropical euptychiine butterfly *Taygetis rufomarginata* Staudinger, 1888 are described and illustrated in detail for the first time, from specimens collected in Madre de Dios department, Peru. The morphology of all four instars, the egg and pupa, are described and the duration of each stage is recorded. General immature morphology is similar to that of *Taygetis virgilia* (Cramer, 1776), and *T. acuta* Weymer, 1910. The life history of studied specimens is compared to that of *T. rufomarginata* in Brazil and Costa Rica. In addition, we report a species of grass, *Lasiacis ligulata* Hitchcock & Chase (Poaceae), as a new host plant for this species.

Resumen: En el presente estudio, se describen e ilustran a detalle y por primera vez los estadios inmaduros de la mariposa neotropical euptychiina *Taygetis rufomarginata* Staudinger, 1888 en base a especímenes colectados en el departamento de Madre de Dios, Perú. La morfología de los cuatro estadios larvales, el huevo, y la pupa son descritos, y la duración de cada uno es registrada. La morfología general del inmaduro es similar a *Taygetis virgilia* (Cramer, 1776) y *T. acuta* Weymer, 1910. Adicionalmente, se compara la historia de vida de los especímenes estudiados con las de *T. rufomarginata* de Brasil y Costa Rica. Finalmente, se reporta a la gramínea *Lasciacis ligulata* Hitchcock & Chase como una nueva planta hospedera para esta especie.

Key Words: Euptychiina, host plant, larva, Lepidoptera, Madre de Dios, Peru

INTRODUCTION

Butterflies of the subfamily Satyrinae are among the most species-rich and widespread in the world (Ackery *et al.*, 1999). The satyrine subtribe Euptychiina is particularly diverse and contains a large number of species of mostly brown, cryptic, forest floor butterflies, the majority of which are Neotropical (Murray, 2001; Peña *et al.*, 2010). Among the euptychiines, the so-called "*Taygetis* clade" currently includes 9 described genera (*sensu* Peña *et al.*, 2010; Matos-Maraví *et al.*, 2013; Freitas *et al.*, 2016; Nakahara *et al.*, 2018), in addition to at least one genus still remaining to be described to maintain *Taygetis* Hübner, [1819] as monophyletic (Matos-Maraví *et al.*, 2013; Nakahara *et al.*, 2018). *Taygetis* is one of the most species-rich euptychiine genera, with some 30 recognized species and unknown cryptic diversity awaiting to be documented (Lamas, 2004; Matos-Maraví *et al.*, 2013; pers. obs.). Like most euptychiine genera it is purely Neotropical (Matos-Maraví *et al.*, 2013). Despite recent improvements in our understanding of euptychiine systematics (e.g., Nakahara *et al.*, 2018, 2019; Espeland *et al.*, 2019), the genus *Taygetis* has featured in few publications since the several taxonomic changes made in Matos-Maraví *et al.* (2013).

While the taxonomy remains to be resolved in a number of species complexes within *Taygetis* (Nakahara *et al.*, in prep.), even less is known about the larval biology of this genus (Murray, 2001; Kaminski & Freitas, 2008; Freitas, 2017) as few reports on the early stage biology exist; larval stages for only 6 species have been described or illustrated to date (Freitas, 2017). Additionally, intra-specific larval variation has been observed but not well-documented (Murray, 2001; Janzen & Hallwachs, 2018). Immature biology is not only important for our understanding of butterfly systematics and morphological diversity, but also our understanding of trophic interactions in Neotropical forests (Kaminski & Freitas, 2008).

This paper is a continuation of a series of publications by the authors to document early stage biology of euptychiine butterflies following See *et al.* (2018), based on field work conducted at Finca Las Piedras (FLP) in Madre de Dios, Peru. We here describe the biology and morphology of the egg, larva and pupa of *Taygetis rufomarginata* Staudinger, 1888, based on a population found at FLP in 2018. Despite the prior documentation of the penultimate and ultimate larval stages, in addition to the pupal stage (Freitas, 2017; Janzen & Hallwachs,

2018), the complete life cycle of this species has not yet been described and we here present this information for the first time.

MATERIALS AND METHODS

Study Site and Collecting

Samples were collected at Finca Las Piedras (Fig. 1), a 54 ha biological research station located *ca*. 48 km north of Puerto Maldonado in Madre de Dios department, southeastern Peru (-12.22789, -69.11119; *ca*. 240 m). The site is situated approximately 2 km from the Interoceanic Highway that connects the Peruvian highlands with Brazil's Amazonian road network and is at the edge of the agricultural frontier that expanded upon completion of the highway several years previously. The property is covered mostly by selectively logged, upland or '*terra firme*' rainforest but also provides access to regenerating secondary forest, *Mauritia* palm swamps, and active and abandoned agricultural land. To the north, east, and south of the property lie expansive tracts of *terra firme* rainforest that stretch, unbroken, east into the Manuripi National Amazonian Wildlife Reserve in neighboring Bolivia.

Specimens described were collected opportunistically as part of a long-term Lepidoptera diversity and biology study conducted by the Alliance for a Sustainable Amazon, which manages FLP. The goals of the project are to document patterns in the distribution and abundance of Lepidoptera species in southeastern Peru as well as their biology, including host-plant associations. Collection was focused to a stand of mature forest and a section of younger, secondary forest adjacent to a regenerating agroforestry plot. Immatures were collected and brought to the field lab for rearing; associated plants were georeferenced, photographed and flagged, and a number of morphological measurements recorded. The host plant was re-visited throughout the field season, both to search for new immatures and to gather leaves to feed the collected individuals.

Rearing

Immatures were kept in a 1 L plastic container with a mesh cover. The larvae received leaves from their host plant at the rate the plant in the container was eaten or as it wilted. Leaves were kept hydrated using floral water tubes (Aquapic, Floral Supply) that were re-filled daily. Immatures were monitored daily and photographed to document changes using a Nikon D7100 digital camera. After emergence, reared adults were photographed and stored in a container with silica gel to preserve the specimens. One fourth-instar larva was stored in 70% ethanol for morphological analysis. A JEOL JSM-5510LV scanning electron microscope (SEM) was used to record images of fourth instar larval morphology.

Molecular work

Molecular analysis was done at the Washington State Department of Agriculture Plant Pathology and Molecular Diagnostics Lab in Olympia, Washington, USA. Total DNA from two legs of one reared individual (2018-FLP-IMM-004) was extracted using the Chelex method. Legs were added to solution of 5% Chelex® 100 and 0.4 mg/ml Proteinase K and incubated at 56°C for 1 hour, then heated to 99°C for 20 minutes. The cytochrome oxidase subunit 1 (COI) gene was amplified using primer set LCO1490_t1 (5'-TGTAAAACGACG GCCAGTGGTCAACAAATCATAAAGATATTGG-3') and HCO2198_t1 (5'-CAGGAAACAGCTATGACTAAACTTC AGGGTGACCAAAAAATCA-3') (Folmer *et al.*, 1994). Rapid cycle PCR using the method outlined by Wittwer &

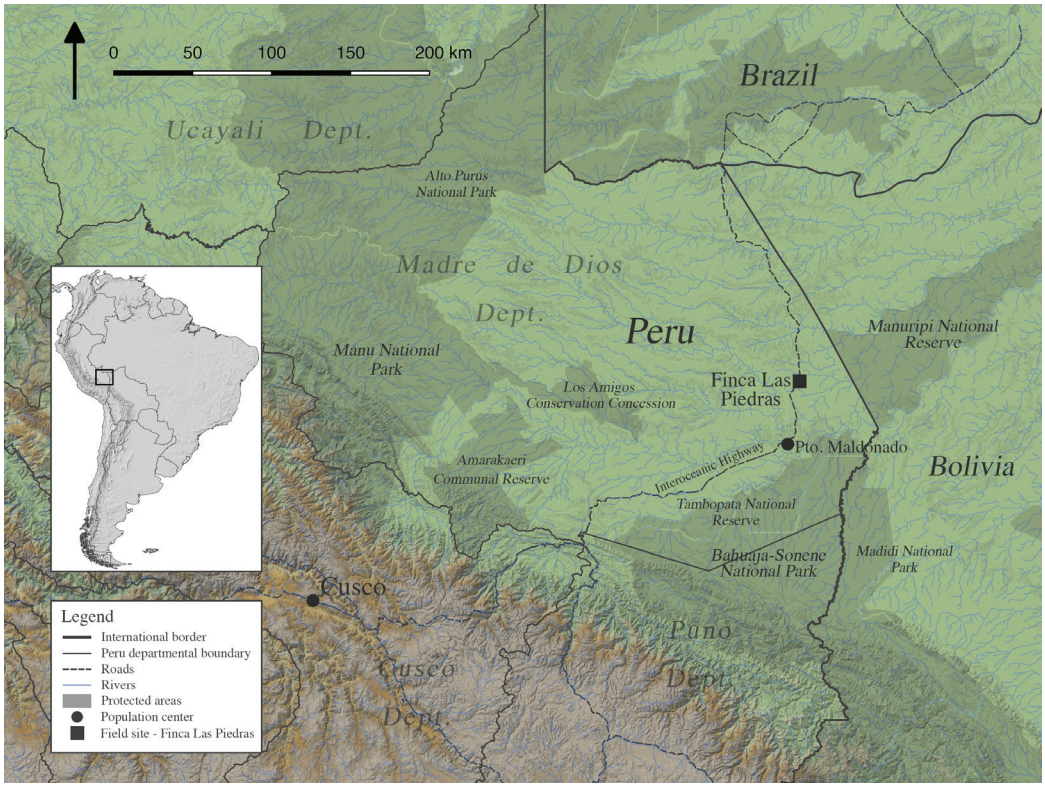

Fig. 1. Map of the field site, Finca Las Piedras, in southeastern Peru.

Garling (1991) was performed using a BioRad iCycler® with 2 µl of supernatant (genomic DNA-containing crude extract) as template and the following reaction conditions: 95°C for 1 minute, followed by 30 cycles of 96°C for 2 seconds, 50°C for 5 seconds, and 72°C for 20 seconds, followed by a final extension at 72°C for 2 minutes. The PCR product was visualized using gel electrophoresis and the remaining product was cleaned using ExoSAP-IT° Express (ThermoFisher Scientific) and sequenced by Genewiz in South Plainfield, New Jersey, USA, using Sanger sequencing.

To clarify the identity of the reared, sequenced sample, the above sequence was incorporated into a dataset including >120 relevant *Taygetis* barcode sequences compiled from unpublished data, in addition to available sequences from GenBank and BOLD (see Table 1). The dataset (657 bp) was aligned using MUSCLE (v 3.8.425) in Geneious (v 11.1.5) (Biomatters Ltd.) and placed in its correct reading frame. The neighbor-joining (NJ) tree was produced by applying all three distance models available in Geneious v 11.1.5 when conducting NJ analysis (Tamura-Nei, Hasegawa-Kishino-Yano, and Jukes-Cantor), each with 1,000 replications of bootstrapping performed as re-sampling method. The consensus tree regarding each of these three different models all grouped 2018-FLP-IMM-004 with 68 other individuals (Table 1) identified as *T. rufomarginata* spanning its range (Mexico-Brazil). Thus, we only provide the NJ tree based on model JC to minimize variants (Fig. 2).

Table 1. GenBank accession numbers for *Taygetis rufomarginata* COI sequences that grouped with 2018-FLM-IMM-004.

Voucher code	Genus	Species	GenBank Acc. No.	Country
DNA97-004	*Taygetis*	*rufomarginata*	AY508579	Cayo, Belize
99-SRNP-15409	*Taygetis*	*rufomarginata*	GU334348	Guanacaste, Costa Rica
NW108-3	*Taygetis*	*rufomarginata*	DQ338812	São Paulo, Brazil
08-SRNP-24499	*Taygetis*	*rufomarginata*	GU649767	Guanacaste, Costa Rica
UN-0416	*Taygetis*	*rufomarginata*	JQ392706	N/A
MAL-04238	*Taygetis*	*rufomarginata*	HM388491	Quintana Roo, Mexico
UN-0417	*Taygetis*	*rufomarginata*	JQ392707	N/A
08-SRNP-21505	*Taygetis*	*rufomarginata*	GU666830	Guanacaste, Costa Rica
GSM-409	*Taygetis*	*rufomarginata*	JQ392696	Peru
PM02-03	*Taygetis*	*rufomarginata*	JQ392700	Antioquia, Colombia
07-SRNP-58852	*Taygetis*	*rufomarginata*	JQ537101	Guanacaste, Costa Rica
MAL-04235	*Taygetis*	*rufomarginata*	GU658830	Yucatán, Mexico
MAL-04237	*Taygetis*	*rufomarginata*	GU658825	Campeche, Mexico
MAL-04236	*Taygetis*	*rufomarginata*	GU658831	Quintana Roo, Mexico
MAL-04234	*Taygetis*	*rufomarginata*	GU658829	Quintana Roo, Mexico
MAL-04233	*Taygetis*	*rufomarginata*	GU658828	Quintana Roo, Mexico
MAL-04232	*Taygetis*	*rufomarginata*	GU658835	Campeche, Mexico
MAL-04230_	*Taygetis*	*rufomarginata*	GU658834	Campeche, Mexico
07-SRNP-58869	*Taygetis*	*rufomarginata*	JQ537099	Guanacaste, Costa Rica
09-SRNP-72771	*Taygetis*	*rufomarginata*	HM885987	Guanacaste, Costa Rica
05-SRNP-4621	*Taygetis*	*rufomarginata*	JQ542415	Guanacaste, Costa Rica
03-SRNP-21804	*Taygetis*	*rufomarginata*	GU334344	Guanacaste, Costa Rica
09-SRNP-65514	*Taygetis*	*rufomarginata*	GU653916	Guanacaste, Costa Rica
08-SRNP-24500	*Taygetis*	*rufomarginata*	GU649769	Guanacaste, Costa Rica
08-SRNP-24498	*Taygetis*	*rufomarginata*	GU649768	Guanacaste, Costa Rica
07-SRNP-3178	*Taygetis*	*rufomarginata*	JQ536331	Guanacaste, Costa Rica
07-SRNP-2221	*Taygetis*	*rufomarginata*	JQ536329	Guanacaste, Costa Rica
07-SRNP-1966	*Taygetis*	*rufomarginata*	JQ535738	Guanacaste, Costa Rica
06-SRNP-101436	*Taygetis*	*rufomarginata*	JQ578221	Guanacaste, Costa Rica
05-SRNP-59421	*Taygetis*	*rufomarginata*	JQ542418	Guanacaste, Costa Rica
04-SRNP-23612	*Taygetis*	*rufomarginata*	GU157587	Guanacaste, Costa Rica
03-SRNP-28019	*Taygetis*	*rufomarginata*	GU334343	Guanacaste, Costa Rica
03-SRNP-17576	*Taygetis*	*rufomarginata*	GU334345	Guanacaste, Costa Rica
03-SRNP-17575	*Taygetis*	*rufomarginata*	GU334350	Guanacaste, Costa Rica
08-SRNP-4816	*Taygetis*	*rufomarginata*	GU666835	Guanacaste, Costa Rica
08-SRNP-32290	*Taygetis*	*rufomarginata*	GU647245	Guanacaste, Costa Rica
08-SRNP-31719	*Taygetis*	*rufomarginata*	GU647235	Guanacaste, Costa Rica
09-SRNP-23765	*Taygetis*	*rufomarginata*	HQ574590	Guanacaste, Costa Rica
08-SRNP-24501	*Taygetis*	*rufomarginata*	GU649762	Guanacaste, Costa Rica
08-SRNP-23366	*Taygetis*	*rufomarginata*	GU647234	Guanacaste, Costa Rica
08-SRNP-22488	*Taygetis*	*rufomarginata*	GU647244	Guanacaste, Costa Rica
08-SRNP-21773	*Taygetis*	*rufomarginata*	GU666829	Guanacaste, Costa Rica
07-SRNP-58671	*Taygetis*	*rufomarginata*	JQ536332	Guanacaste, Costa Rica
07-SRNP-57809	*Taygetis*	*rufomarginata*	JQ536334	Guanacaste, Costa Rica
07-SRNP-2022	*Taygetis*	*rufomarginata*	JQ535741	Guanacaste, Costa Rica
05-SRNP-4789	*Taygetis*	*rufomarginata*	JQ542412	Guanacaste, Costa Rica
05-SRNP-4786	*Taygetis*	*rufomarginata*	JQ542417	Guanacaste, Costa Rica
05-SRNP-4619	*Taygetis*	*rufomarginata*	JQ542414	Guanacaste, Costa Rica
10-SRNP-72675	*Taygetis*	*rufomarginata*	JQ529759	Guanacaste, Costa Rica
07-SRNP-3179	*Taygetis*	*rufomarginata*	JQ536330	Guanacaste, Costa Rica
07-SRNP-2416	*Taygetis*	*rufomarginata*	JQ535668	Guanacaste, Costa Rica
07-SRNP-2222	*Taygetis*	*rufomarginata*	JQ536333	Guanacaste, Costa Rica
UN-0415	*Taygetis*	*rufomarginata*	JQ392705	Guanacaste, Costa Rica
05-SRNP-4622	*Taygetis*	*rufomarginata*	JQ542416	Guanacaste, Costa Rica
07-SRNP-2220	*Taygetis*	*rufomarginata*	JQ536328	Guanacaste, Costa Rica
06-SRNP-103283	*Taygetis*	*rufomarginata*	JN807272	Guanacaste, Costa Rica
05-SRNP-4713	*Taygetis*	*rufomarginata*	JQ542413	Guanacaste, Costa Rica
03-SRNP-21366	*Taygetis*	*rufomarginata*	GU334349	Guanacaste, Costa Rica
02-SRNP-28529	*Taygetis*	*rufomarginata*	GU334347	Guanacaste, Costa Rica
02-SRNP-28528	*Taygetis*	*rufomarginata*	GU334351	Guanacaste, Costa Rica
PM04-11	*Taygetis*	*rufomarginata*	JQ392704	Para, Brazil
2018_FLP_IMM_0004	*Taygetis*	*rufomarginata*	MN158703	Madre de Dios, Peru
PM04-05	*Taygetis*	*rufomarginata*	JQ392702	Para, Brazil
NW129-26	*Taygetis*	*rufomarginata*	JQ392697	São Paulo, Brazil
99-SRNP-15408	*Taygetis*	*rufomarginata*	GU334346	Guanacaste, Costa Rica
08-SRNP-22752	*Taygetis*	*rufomarginata*	GU647260	Guanacaste, Costa Rica
07-SRNP-58851	*Taygetis*	*rufomarginata*	JQ537100	Guanacaste, Costa Rica
07-SRNP-58269	*Taygetis*	*rufomarginata*	JQ537102	Guanacaste, Costa Rica

Fig. 2. Neighbor-Joining tree illustrating similarity of the sequence of the reared individual 2018-FLP-IMM-004 to other *Taygetis rufomarginata* sequences (Jukes-Cantor employed as distance model; bootstrapping values as branch support). "Clade A" represents 7 sequences related to *T. nympha*, and "Clade B" represents 49 sequences related to *T. virgilia*. See Table 1 for GenBank accession numbers for ingroup sequences.

Morphology

All measurements of general aspects of the egg, larval and pupal stages were determined by pixel counts of high resolution photos using ImageJ 1.51j8. Head capsule width was determined by measuring the distance between the most external stemmata (as in Freitas, 2007), using a Nikon SMZ - 1B ESD stereomicroscope, in addition to the same system used for photographing. Head capsule drawing was done using a camera lucida attached to Leica MZ 16 stereomicroscope. Scoli length was determined by measuring the longer scolus of the two, and inter-scoli length was determined by measuring between the bases of the scoli (as in See *et al.*, 2018). Maximum total length for both larvae and pupae were determined by measuring the distance from the front of the head to posterior margin of the 10th abdominal segment in dorsal view (as in Freitas, 2007), this data is presented as an average value. Caudal filament length was calculated by measuring the longer of the two cerci. Terminology for the immature stages description follows Stehr (1987), Torre-Bueno (1989) and Zombori & Steinmann (1999).

RESULTS

Host Plant

The host plant *Lasiacis ligulata* Hitchcock & Chase (Poaceae: Panicoideae) (Fig. 3) (G. Davidse pers. comm., Feb 11 2019), is a grass that grows in disturbed areas and along

Fig. 3. Host plant of *Taygetis rufomarginata* from Finca Las Piedras: **A.** plant in habitat; **B.** detail of mature fruit; **C.** example of leaf fungus pattern.

the forest edge at the field site. The plant produces several pithy stems from a single point in the soil and branches from the nodes. The leaves are compound with a variable number of leaflets. The plant at the time of egg collection measured about 2 m in height and had a foliar diameter of 1.5 m. The flower is a raceme inflorescence with spherical calyxes surrounding reduced white petals. The fruits are similar in size to the mature flower and are dry, brown and spherical (Fig. 3B). Plants were observed flowering in late June and fruiting in July.

Examined Individuals

The first immature collected on April 4th, 2018 was reared from egg to adult stage, identified as a female, and vouchered (voucher code 2018-FLP-IMM-004) (Fig. 4). Many more euptychiine eggs were collected off the same species of host plant with the intention of rearing several more Taygetis specimens. Just one additional *Taygetis rufomarginata* was reared from egg to fourth instar (2018-FLP-IMM-246) (Fig. 5), and was vouchered as a larva and used for SEM images (Fig. 6). Findings from the remainder of these specimens will be treated in subsequent publications (Baine *et al.*, in prep.).

Immature stages description

Egg (Fig. 4A, B). Diameter 1.76–1.80 mm (n=2), spherical, light yellow chorion with several sunken dots laterally and smooth on top. Deposited singly on a mature and a young leaf (n=2), on underside of leaflet along midrib, between center and base of leaflet (n=2) at a height of 1.3 m from ground (n=1). Duration 2–5 days (n=2) from collection.

First instar (Fig. 4C; Fig. 5A). Head capsule width 1.10–1.24 mm (n=2); scoli length 0.25–0.27 mm (n=2); inter-scoli 0.18–0.22 mm (n=2). Head brown with 6 enlarged chalazae, each with one brown setae; also bearing a pair of short scoli on vertex, each with two long narrow brown setae. Body light green, dark green after feeding, smooth, with several longitudinal white stripes arranged as follows: a pair of thin stripes in middorsal area, a thick stripe in subdorsal area very close to another thin stripe in supraspiracular area, a thin stripe farther separated in same area, and a thick stripe in subspiracular area. Bifid caudal filaments very short. Setae elongated and brown. Maximum length 9.65–10.63 mm (n=2); caudal filament length 0.49–0.51 mm (n=2). Duration 6–8 days (n=2).

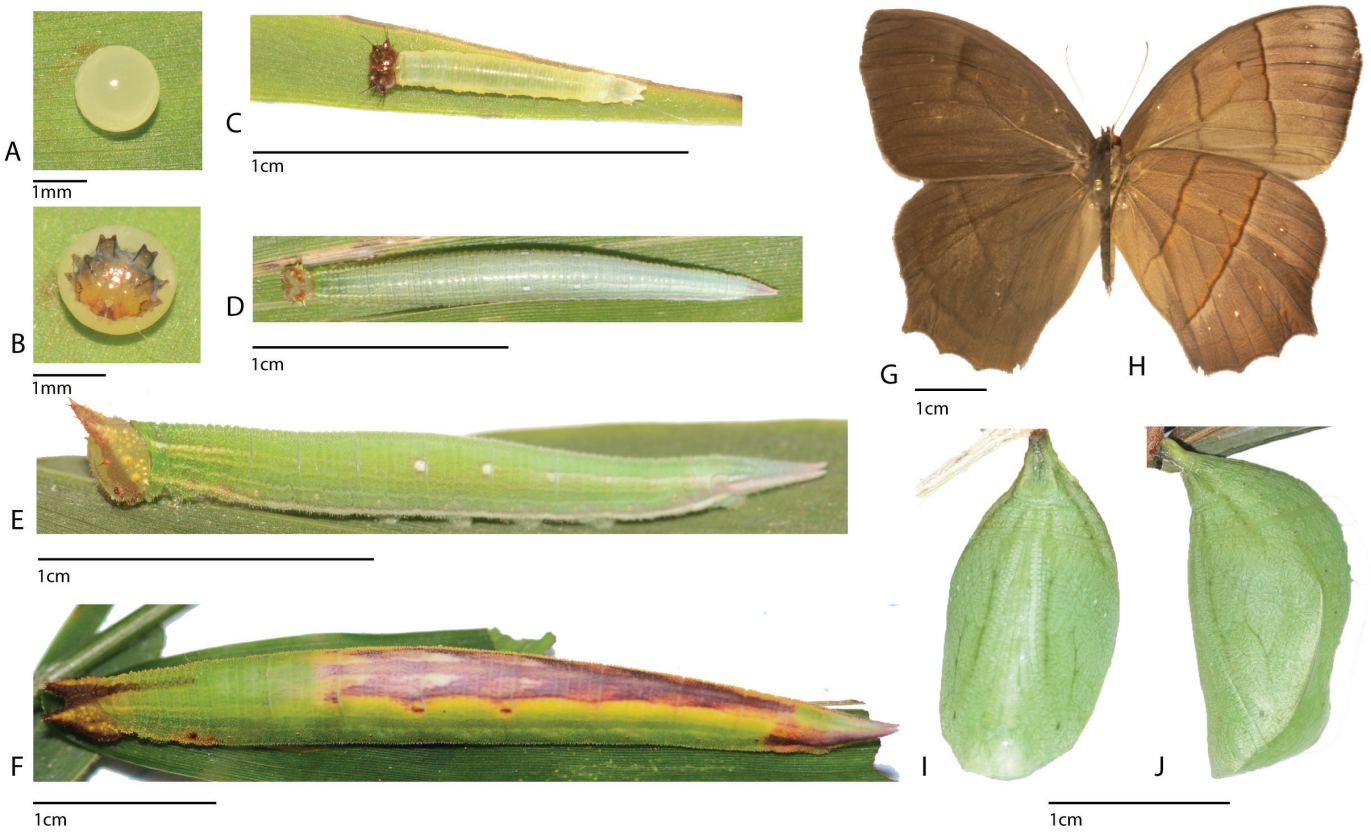

Fig. 4. Life stages of *Taygetis rufomarginata* (2018-FLP-IMM-004): **A**. dorsal view of egg; **B**. dorsal view of egg with visible head capsule prior to hatching; **C**. dorsal view of first instar; **D**. dorsal view of second instar; **E**: lateral view of third instar; **F**: dorso-lateral view of fourth instar; **G**: dorsal view of adult; **H:** ventral view of adult; **I.** dorsal view of pupa; **J.** lateral view of pupa.

Second instar (Fig. 4D; Fig. 5B). Head capsule width 1.67–1.92 mm (n=2); scoli length 0.56–0.57 mm (n=2); inter-scoli 0.30–0.44 mm (n=2). Head has two large scoli and is yellow with a reddish brown band extending from outer side of each stemma around head and converging on vertex; two long reddish brown bands starting on stemmata converging on epicranial suture. Body green in anterior half, then changes to turquoise and ends in light reddish caudal filaments; stripes light green and situated similarly to first instar but subspiracular area has two longitudinal stripes close together, one white and other reddish. Body also has two pairs of white spots dorsal to subdorsal stripes in alignment with first and second pair of prolegs, respectively; bifid caudal filaments short. Maximum length 16.50–17.84 mm (n=2); caudal filament length 1.31 mm (n=1). Duration 7 days (n=2).

Third instar (Fig. 4E; Fig. 5C)**.** Head capsule width 2.45–2.68 mm (n=2); scoli length 0.99–1.09 mm (n=2); inter-scoli 0.56–0.66 mm (n=2). Head very similar to second instar except for reddish-brown bands on anterior part of head that start millimeters from stemmata and end millimeters before reaching epicranial suture. Two additional bands start in middle of ecdysial line and converge on epicranial suture. Posterior of head with two reddish brown bands starting at inter scoli extending parallel until reaching prothorax. Body green, in all aspects similar to second instar but larger; bifid caudal filaments also similar, but longer relative to body length. Maximum length 27.0–31.50 mm (n=2); caudal filament length 2.98–3.02 mm (n=2). Duration 7-8 days (n=2).

Fourth (last) instar (Fig. 4F; Fig. 5D; Fig. 6). Head capsule width 3.67–3.74 mm (n=2); scoli length 1.56–1.71 mm (n=2); inter-scoli 0.63–0.79 mm (n=2). Head with all aspects similar to third instar except for a unique band starting at inter scoli on posterior side of head and converging at prothorax, becoming a single line extending to middorsal area of body in alignment with third pair of legs. Body green; abdominal segments with a dorsal, elongate, brown and white splotch bordered with yellow, similar to discoloration caused by fungus on leaf of host plant; bifid caudal filaments long and brownish. Maximum length 59.75 mm (n=1); caudal filament length 4.22 mm (n=1). Duration 16 days (n=1).

Pupa (Fig. 4I, J). Short, wide, smooth and green. Short, slightly quadrangular and rounded ocular caps, little white pointed spots along post-discal area of forewings, two parallel rows of little white pointed spots along dorsal abdomen without projections; cremaster green. Pupa attached to sheath of a new leaf of host plant. Total length 19.41 mm (n=1). Duration 15 days (n=1).

DISCUSSION

The larval morphology and phenology of *T. rufomargiata* is consistent with available descriptions of closely-related *Taygetis* species in the "*T. virgilia* group", namely *Taygetis virgilia* Cramer, 1776 and *T. acuta* Weymer, 1910, based on comparison of penultimate and last instar (Freitas, 2017). Larvae lack scoli along the body, have short horns on the head capsule, and have long caudal filaments (Freitas, 2017), and the pupa is stout and leaf-like. The last instar larvae of species in this group share a characteristic oblong longitudinal marking along the dorsum. Though this marking in *T. rufomarginata* is brightly colored and visible, it is also remarkably similar to the common discoloration caused by fungus on a *Lasacis* leaf (Fig. 3C). There is some speculation that, instead of aposematism, the defensive strategy of this presumably palatable larva is crypsis by way of camouflage (Freitas, 2017).

Taygetis rufomarginata reared in Costa Rica (Janzen & Hallwachs, 2018), and an individual reared in Brazil (NW-129-63; Freitas, 2017), show similar coloration, patterning and size to those reared in Peru. Average duration from first instar to date of eclosion of individuals reared in Costa Rica (n=21) is about 30 days; the average duration of recent prepupa to eclosion

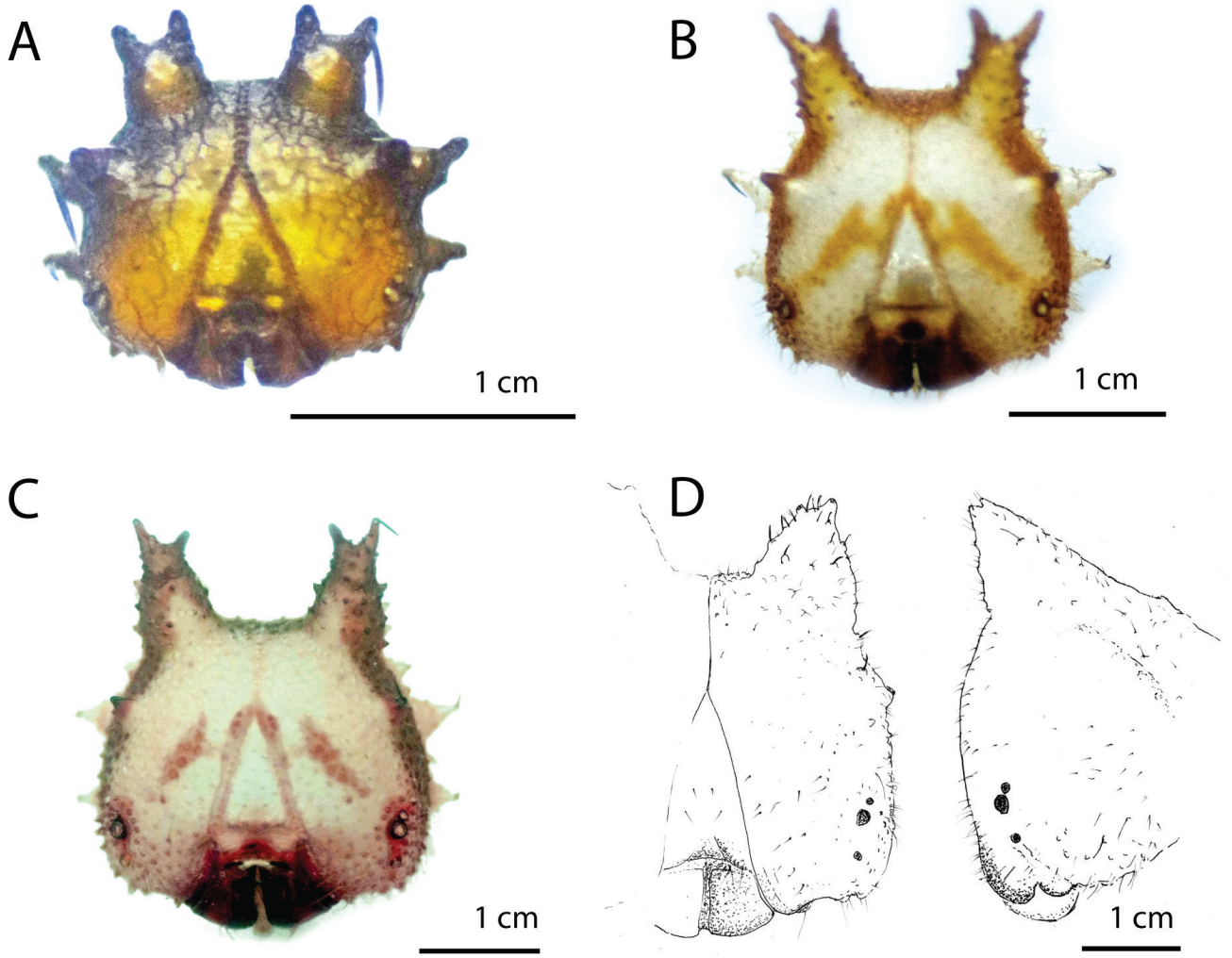

Fig. 5. Head capsule detail of *Taygetis rufomarginata* (2018-FLP-IMM-246): **A.** first instar; **B.** second instar; **C.** third instar; **D.** illustration of fourth instar.

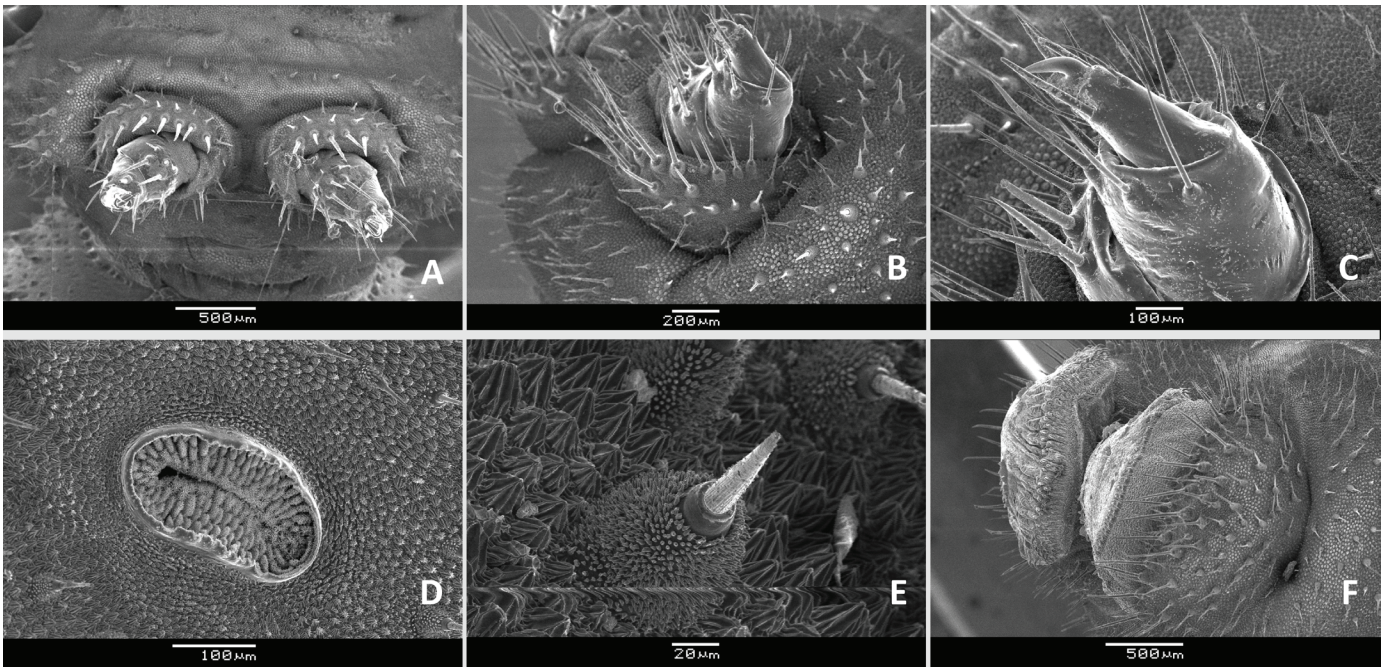

Fig. 6. Scanning Electron Microscopy images of fourth instar larva (2018-FLP-IMM-246): **A.** foreleg in ventral view; **B**. close-up view of foreleg; **C.** magnified view of midleg; **D.** abdominal spiracle; **E.** setae on T1; **F.** last proleg. Scale bar indicated below each image.

is 15.45 days (D. Janzen pers. comm., March 30, 2019). The pupal stage of individuals reared as part of this study are within the range of these values: 2018-FLP-IMM-004 was 16 days (May 15th – May 31th) from prepupa to eclosion. However, the duration of the larval stages was significantly higher: 2018-FLP-IMM-004 was 52 days (April 10th – May 31th) from hatching of the egg to eclosion, and 2018-FLP-IMM-246 was 30 days (July 24th – August 23th) from hatching of the egg to fourth instar. This difference is possibly explained by lab conditions, which in this study were not temperature controlled, but could also point to lower availability of nutrients necessary for larval development in the host plant used in Peru.

Individuals reared in Costa Rica fed on a suite of Poaceae plants, including several *Lasiacis* species, but not *L. ligulata*, which does not occur in Central America (Davidse, 2003; Janzen & Hallwachs, 2018). Future collections of *T. rufomarginata* immatures can help to determine if there is a latitudinal pattern of host plant preference, or simply a suite of acceptable host species throughout its range.

Increased knowledge of the biology of forest understory butterflies contributes to understanding the Peruvian lowland rainforest ecosystem and its fragility in the face of large-scale habitat loss in the region. We hope this identification of a new lepidopteran host plant, as well as this description of the immature stages of a euptychiine butterfly, will support ongoing research of Neotropical invertebrate communities.

ACKNOWLEDGMENTS

We thank Allison Stoiser and other Alliance for a Sustainable Amazon staff, interns and volunteers who supported the collecting and rearing elements of field research at Finca Las Piedras. We also acknowledge Gerrit Davidse for help with host plant identification, Dan Janzen and Winnie Hallwachs for sharing data, Juan Manuel Andía Navarro for help with immature photos, and Ernesto Rázuri for help with Spanish translation. The authors would like to thank Peru's Servicio Nacional Forestal y de Fauna Silvestre (SERFOR) for granting permission to conduct the field research (permit no. 187-2017-SERFOR/DGGSPFFS) and the US Fish and Wildlife Service for permission to import specimens for analysis (declaration confirmation no. 2018SE2284245). SN acknowledges University of Florida's Entomology and Nematology Department for support. QB acknowledges Telissa Wilson and the rest of the Washington State Department of Agriculture Plant Pathology and Molecular Diagnostics Lab for support. The authors thank the reviewers of this work, André V .L. Freitas and Fernando M. S. Dias, for their helpful suggestions.

LITERATURE CITED

Ackery, P. R., de Jong, R., Vane-Wright, R. I. 1999. *The Butterflies: Hedyloidea, Hesperoidea and Papilionoidea*, pp. 263–300. *In*: Kristensen, N. P. (Ed.), *Lepidoptera: Moths and Butterflies. 1. Evolution, Systematics and Biogeography*. Handbook of Zoology, vol. IV. Berlin, Walter de Gruyter. Part 35.

Davidse, G. 2003. Lasiacis, pp. 278–283. *In*: Zuloaga, F. O., Morrone, O., Davidse, G., Filgueiras, T. S., Peterson, P. M., Soreng, R. J., Judziewicz, E. J. *Catalogue of New World Grasses (Poaceae): III. Subfamilies Panicoideae, Aristidoideae, Arundinoideae, and Danthonioideae*. Contributions of the United States National Herbarium. 46.

Espeland, M., Breinholt, J., Barbosa, E. P., Casagrande, M., Huertas, B., Lamas, G., Marín, M. A., Mielke, O. H. H., Miller, J. Y., Nakahara, S., Tan, D., Warren, A. D., Zacca, T., Kawahara, A. Y., Freitas, A. V. L, Willmott, K. R. 2019. Four hundred shades of brown: higher level phylogeny of the problematic Euptychiina (Lepidoptera, Nymphalidae, Satyrinae) based on hybrid enrichment data. *Molecular Phylogenetics and Evolution* 131: 116–124. https://doi.org/10.1016/j.ympev.2018.10.039

Folmer, O., Black, M., Hoeh W., Lutz, R., Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial Cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3(5): 294-299.

Freitas, A. V. L. 2007. A new species of *Moneuptychia* Forster (Lepidoptera: Satyrinae, Euptychiina) from the highlands of southeastern Brazil. *Neotropical Entomology* 36: 919–925.

Freitas, A. V. L. 2017. Immature stages of the Neotropical satyrine *Taygetis acuta* (Nymphalidae: Euptychiina). *Tropical Lepidoptera Research* 27: 1–5.

Janzen, D. H., Hallwachs, W. 2018. *Dynamic Database for an Inventory of the Macrocaterpillar Fauna, and its Food Plants and Parasitoids, of Área de Conservación Guanacaste (ACG), Northwestern Costa Rica*. http:// janzen. sas.upenn.edu. [accessed May 2019]

Kaminski, L. A., Freitas, A. V. L. 2008. Immature stages of the butterfly *Magneuptychia libye* (Linnaeus) (Lepidoptera: Nymphalidae: Satyrinae). *Neotropical Entomology* 37: 169–172.

Lamas, G. 2004. *Nymphalidae. Satyrinae. Tribe Satyrini. Subtribe Euptychiina*, pp. 217-223. *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.

Marín, M. A., Peña, C., Freitas, A. V. L., Wahlberg, N., Uribe S. I. 2011. From the phylogeny of the Satyrinae butterflies to the systematics of Euptychiina. *Neotropical Entomology* 40(1): 1–13.

Matos-Maraví, P., Peña, C., Willmott, K. R., Freitas, A. V. L., Wahlberg, N. 2013. Systematics and evolutionary history of butterflies in the "*Taygetis* clade" (Nymphalidae: Satyrinae: Euptychiina): Towards a better understanding of Neotropical biogeography. *Molecular Phylogenetics and Evolution* 66: 54–68.

Murray, D. 2001. Immature stages and biology of *Taygetis* Hübner (Lepidoptera: Nymphalidae). *Proceedings of the Entomological Society of Washington* 103: 932–945.

Nakahara, S., Willmott, K. R., Mielke, O. H. H., Schwartz, J., Zacca, T., Espeland, M., Lamas, G. 2018. Seven new taxa from the butterfly subtribe Euptychiina (Lepidoptera: Nymphalidae: Satyrinae) with revisional notes on *Harjesia* Forster, 1964 and *Pseudeuptychia* Forster, 1964. *Insecta Mundi* 0639: 1–38.

Nakahara, S., Lamas, G., Tyler, S., Marín, M. A., Huertas, B., Willmott, K. R., Mielke, O. H. H., Espeland, M. 2019. A revision of the new genus *Amiga* Nakahara, Willmott & Espeland, gen. n., described for *Papilio arnaca* Fabricius, 1776 (Lepidoptera, Nymphalidae, Satyrinae). *ZooKeys* 821: 85–152.

Peña, C., Nylin, S., Freitas, A. V. L., Wahlberg, N. 2010. Biogeographic history of the butterfly subtribe Euptychiina (Lepidoptera, Nymphalidae, Satyrinae). *Zoologica Scripta* 39(3): 243–258.

See, J., Nakahara, S., Gallice, G. 2018. Immature stages of *Splendeuptychia quadrina* (Butler, 1869) (Lepidoptera: Nymphalidae: Satyrinae). *Tropical Lepidoptera Research* 28(2): 49–53.

Stehr, F. W. 1987. *Immature Insects*. Dubuque, Kendall/Hunt Publishing. 754 pp.

Torre-Bueno, J. R. 1989. *The Torre-Bueno Glossary of Entomology*. New York Entomological Society in cooperation with the American Museum of Natural History. 840 pp.

Wittwer, C. T., Garling, D. J. 1991. Rapid cycle DNA amplification. *BioTechniques* 10: 76-83.

Young, A. M. 1984. Natural history notes for *Taygetis andromeda* (Cramer) (Satyridae) in eastern Costa Rica. *Journal of the Lepidopterists' Society* 38(2): 102–113.

Zombori, L., Steinmann, H. 1999. *Dictionary of Insect Morphology*, Vol. 4. Walter de Gruyter. 409 pp.