Immature stages of *Splendeuptychia quadrina* (Butler, 1869) (Lepidoptera: Nymphalidae: Satyrinae)

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Abstract: The immature stages of the Neotropical nymphalid butterfly *Splendeuptychia quadrina* (Butler, 1869) are documented herein based on a population found in Madre de Dios, Peru. Larval morphology is illustrated for the first time for the genus. The host plant is a species of bamboo, identified as *Rhipidocladum racemiflorum* (Steud.) McClure (Poaceae: Bambusoideae).

Resumen: Se documentan los estados inmaduros de la mariposa Neotropical de la familia Nymphalidae *Splendeuptychia quadrina* (Butler, 1869) basándose en una población encontrada en Madre De Dios Peru. La morfología larval es ilustrada por primera vez para el género. La planta hospedera es una especie de bambú, identificada como *Rhipidocladum racemiflorum* (Steud.) McClure (Poaceae: Bambusoideae).

Key words: Euptychiina, life history, Madre de Dios

Palabras claves: Euptychiina, estados inmaduros, Madre de Dios

INTRODUCTION

Like many other species in the nymphalid subtribe Euptychiina, species in the genus *Splendeuptychia* Forster, 1964 are found throughout Central and South America, in particular in association with bamboo groves (Beccaloni *et al.* 2008; pers. obs.). Although *Splendeuptychia* appears to be polyphyletic (e.g., Peña *et al.* 2010), *Splendeuptychia* is currently perceived as the most speciose genus among euptychiine butterflies containing ca. 50 species, including many undescribed species (Lamas, 2004; Huertas *et al.* 2009; Huertas, 2011; Brévignon & Benmesbah, 2012). Despite the fact that our understanding of the species richness of euptychiine butterflies has steadily improved during recent years (e.g., Cong & Grishin, 2014; Barbosa *et al*. 2015; Nakahara *et al*. 2018), our knowledge of their early stage biology lags far behind, with relatively few publications on this subject (e.g., Singer *et al.* 1983; Freitas *et al*. 2016a; 2016b; Freitas, 2017). Regarding *Splendeuptychia*, Kendall (1978) and Brown (1992) provided information on host plant records for *S. kendalli* Miller, 1976 and *S. hygina* (Butler, 1877). In addition, Beccaloni *et al*. (2008) listed hostplant records for *S. ashna* (Hewitson, 1869), *S. doxes* (Godart, [1824]) *S. hygina*, *S. itonis* (Hewitson, 1862) and *S. pagyris* (Godart, [1824]) based on personal communications from André Freitas. All *Splendeuptychia* species mentioned above feed on bamboo (Poaceae: Bambusoideae), and *Bambusa aculeata* (Ruprecht) has been identified as larval food plant for *S. kendalli* (Kendall, 1978). Apart from these reports, no further information on the early stage biology of *Splendeuptychia* is available to date.

To contribute to knowledge of *Splendeuptychia* life history, we therefore provide notes here on the early stage biology of *Splendeuptychia quadrina* (Butler, 1869), including illustrations of *Splendeuptychia* larval morphology for the first time.

Fig. 1. Study site, Finca Las Piedras, Madre de Dios, Peru.

MATERIALS AND METHODS

Study area and field work

Adults of *Splendeuptychia quadrina* were observed at Finca Las Piedras (Fig. 1), a biological research station located approximately 2 km from the Interoceanic Highway in Madre de Dios, Peru (Latitude: -12.22789; Longitude: -69.11119), situated in the southwestern Amazon basin. The field site is a 54 ha property that includes mostly mature 'terra firme' or upland rain forest, but also regenerating secondary forest, abandoned agricultural fields, and *Mauritia* palm swamps. Field work was carried out between August and December 2017 after the discovery at the field site of adult *S. quadrina* closely associated with a patch of bamboo within the terra firme forest. Further adults were observed in the field, and eggs and immatures were collected and reared in plastic containers with nylon mesh lids in the laboratory. The host plant was kept fresh in floral water tubes (Aquapic, Floral Supply). Larvae were checked and fed daily and photos were taken (Nikon D750 DSLR camera with AF-S VR Micro-Nikkor 105mm f/2.8G IF-ED lens) of all life stages.

Morphological study

Head capsule measurements were taken by pixel counts of high resolution photos (Adobe Photoshop CC, 2014.2.2 release) obtained using a Cannon EOS 6D and stacked using Helicon Focus 6.7.1 and Helicon Remote (ver.3.8.7 W). Head capsule width was obtained by measuring between the bases of the most widely set stemmata (as in Freitas, 2007). Scoli length was obtained by measuring the longer scoli of the two. Inter-scoli length was obtained by measuring between the bases of scoli. A JEOL JSM-5510LV Scanning Electron Microscope (SEM) was used to take images of fifth instar larval morphology. We follow Peterson (1962) for the terminology used in the immature stages description below.

RESULTS

Examined individuals

Five eggs were collected on 5th Sep 2017, two of which did not hatch. The remaining three that hatched died between the first and second instar; one fourth instar collected on 5th Sep 2017 emerged 20th Sep 2017 as adult and was vouchered (2017 FLP 0053); one first instar collected 5th Sep 2017 emerged 1st Nov 2017 as adult (2017 FLP 0051); one second instar collected on 5th Sep 2017 was preserved in ethanol on reaching fifth instar (UF-FLMNH-MGCL-1036245). Additional immatures were collected in Nov 2017 to obtain better images of various stages, especially the third and fourth instar.

Host plant and adult behavior

The host plant is a species of bamboo, *Rhipidocladum racemiflorum* (Steud.) McClure (Fig. 2), and adults were rarely observed more than 10 m from the patch of this bamboo. The plant is patchily distributed and we know of only one stand at the study site, although more plants have been observed at least several hundred meters away, off the property. The adults were observed roosting at night on the host plant, clinging to the tips of the leaves less than 1m off the ground. On 5th Sep 2017, a pair was found *in copula* at approximately 10:00 am and had separated by 11:00 am (Fig. 5). Oviposition has not yet been observed.

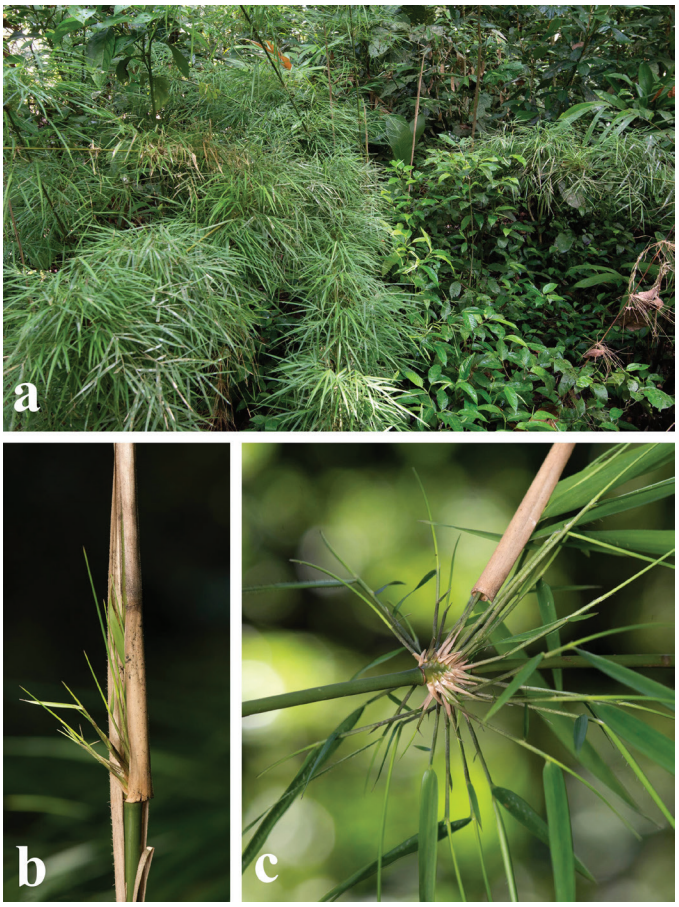

Fig. 2. Host plant of *Splendeuptychia quadrina*, *Rhipidocladum racemiflorum* (Steud.) McClure (Poaceae: Bambusoideae): **a)** bamboo habitat at the edge of a light gap; **b)** growing shoot; **c)** detail of leaves and node.

Description of immature stages

All eggs and immature stages were found between 1–2.5 m off the ground, with eggs laid on the undersides of leaves.

Egg (Fig. 3a). Spherical and cream in color, widest point 0.86 mm in diameter. Three eggs all hatched within 3 days of collection (two failed to hatch).

First instar (Fig. 3b). Head capsule black, with a pair of very short scoli (horns) on vertex. Body pale green, green longitudinal stripes visible in supraspiracular areas; bifid caudal filament. First instar lasted 6 days (n=1). Head capsule width 0.379 mm (n=1); scoli length 0.10101 mm (n=1); scoli length to head capsule height ratio 0.22 (n=1); inter-scoli width 0.194 mm (n=1); caudal filament 0.017 mm (n=1).

Second instar (Fig. 3c). Head capsule dark brown, scoli longer than first instar and easily visible to naked eye. Body somewhat darker green, with thin, light colored dorsal stripe and alternating dark and light stripes in supraspiracular areas; bifid caudal filament. Second instar lasted 7 days (n=1). Head capsule width 0.889 mm (n=1); scoli length 0.450 mm (n=1); scoli length to head capsule height ratio 0.607 (n=1); inter-scoli width 0.387 mm (n=1); caudal filament length 0.491 mm (n=1).

Third instar (Fig. 3d). Head capsule light brown, with scoli and band across dorsal aspect of head darker, as well as mouthparts and area around the stemmata. Body color gray green with lighter longitudinal stripe along dorsal

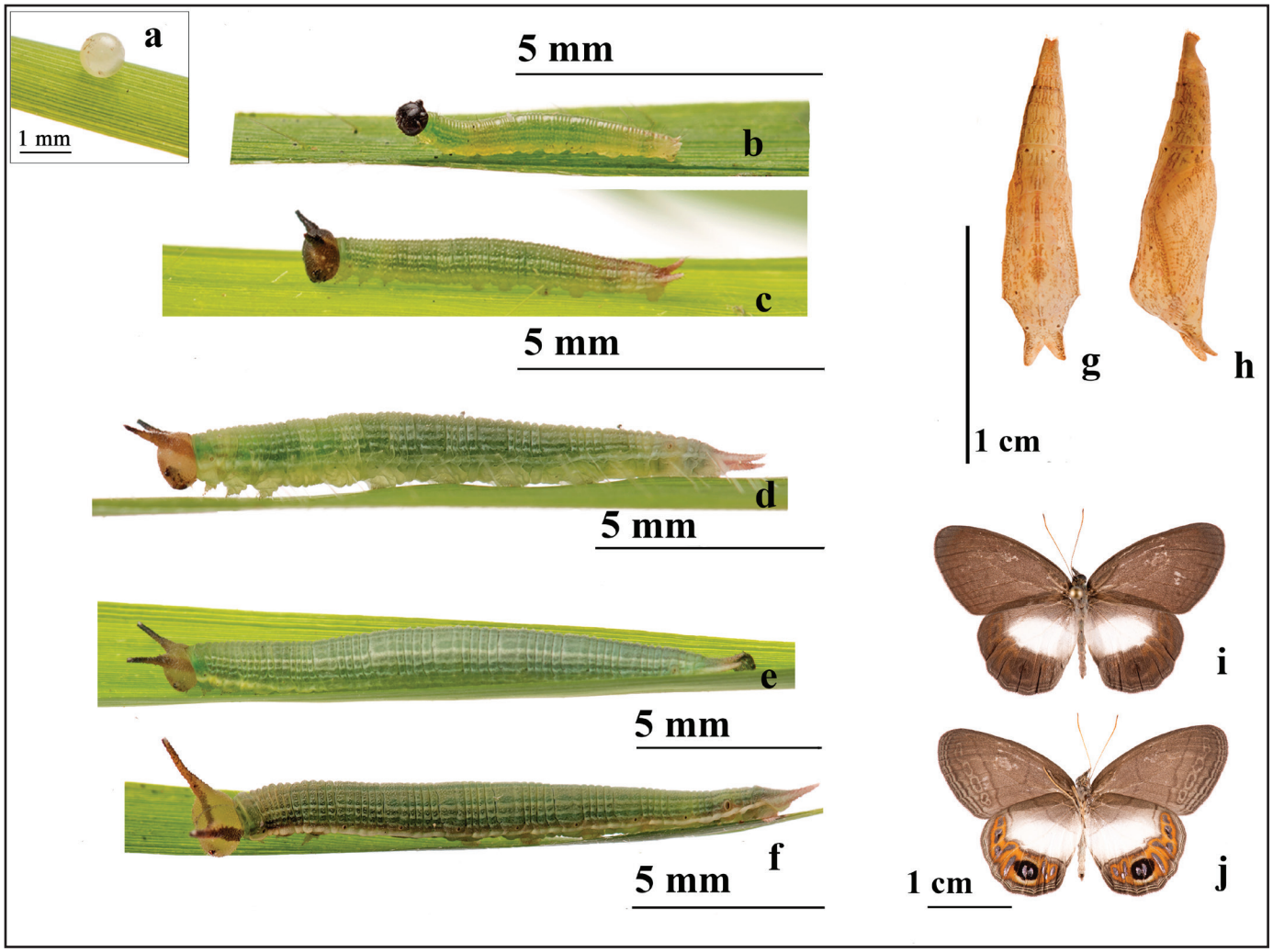

Fig. 3. Immature stages and adult of *Splendeuptychia quadrina*: **a)** egg; **b)** first instar lateral; **c)** second instar lateral; **d)** third instar lateral; **e)** fourth instar lateral; **f)** fifth instar lateral; **g)** pupa dorsal and **h)** lateral; **i)** adult male (FLP 2017 0053), dorsal and **j)** ventral.

and in subdorsal and supraspiracular areas; caudal filament slightly longer than in second instar. Third instar lasted for 8 days (n=1). Head capsule width 1.01 mm (n=1); scoli length= 0.736 mm (n=1); scoli length to head capsule height ratio 0.800 (n=1); inter-scoli width 0.457 mm (n=1); caudal filament length 0.920 mm n=(1).

Fourth instar (Fig. 3e). Head capsule light brown, with dark markings as in third instar; dark stripe running on head posteriorly from each scolus. Body gray green with pale longitudinal stripes as in fourth instar, but pale stripe is present subspiracularly; caudal filament similar in appearance to that of third instar. Fourth instar lasted for 9 days (n=1). Head capsule width 1.34 mm (n=1); scoli length 1.11 mm (n=1); scoli length to head capsule height ratio 1.06 (n=1); inter-scoli width 0.507 (n=1); caudal filament length 1.490 mm (n=1).

Fifth instar (Figs 3f, 4). Identical to fourth instar except for longer scoli; body color yellowish-green, caudal filaments more robust, covered with posteriorly pointing simple setae, as in Fig. 4i. Simple setae appear to cover scoli and entire head, facing outward along the scoli. Fifth instar lasted for 13 days (n=1). Prepupa 14 mm (n=1) in length. Head capsule width 2.77 mm (n=1); scoli length 2.05 (n=1); scoli length to head capsule height ratio 1.22; inter-scoli width 0.9662 mm (n=1); caudal filament length 1.290 mm (n=1).

Pupa (Figs 3g, h). Final instars pupated on side of containers (n=1) or on upperside of leaf (n=1). Pupa long, smooth, slender, light brown, with pointed ocular caps and a pair of spots margined in paler color dorsally posterior to ocular caps, as well as on segment A1. Darker brown chevrons run along dorsal line and supraspiracular region. Pupal stage lasted a mean of 9.5 days (n=2, range= 9, 10).

Adult (Figs 3i, 5). Spread adult was one that emerged from a reared immature (Fig. 3i). Figure 5 shows a mating pair observed at the same bamboo patch where immatures were collected.

DISCUSSION

Based on published larval illustrations for other euptychiine species (e.g, Singer *et al*. 1983; DeVries, 1987; Kaminski & Freitas, 2008; Cong & Grishin, 2014; Freitas *et al*. 2016a; 2016b; Freitas, 2017; Janzen & Hallwachs, 2018), the immature stages of *S. quadrina* are overall morphologically similar to many other euptychiines, although with a number of conspicuous differences. For example, Freitas *et al*. (2016) and Freitas (2017) described the larva of euptychiine species as lacking body scoli and having short head scoli and caudal filaments, and the pupae as short and smooth. Indeed, many illustrations in those aforementioned publications do agree with this description. In contrast, the immature stages of *S. quadrina* are different from other known euptychiine species in possessing rather long, diverging head scoli and pupae with an elongate bifid head. For example, the head scoli appear to be absent in the larva of *Hermeuptychia sosybius* (Fabricius, 1793) and *H. hermybius* Grishin, 2014 (Cong & Grishin, 2014);

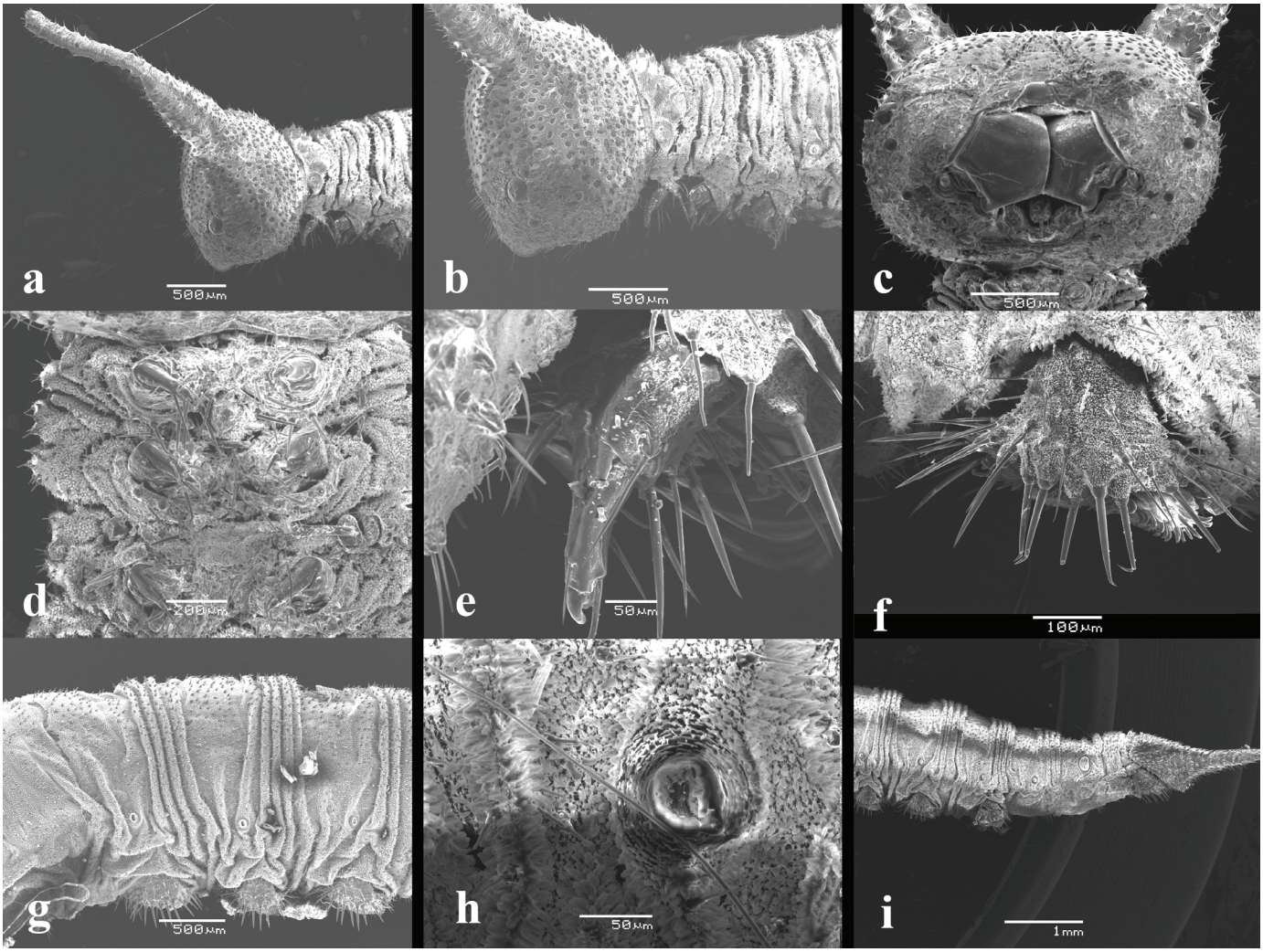

Fig. 4. Scanning Electron Micrographs of fifth instar larva of *Splendeuptychia quadrina* (UF FLMNH MGCL 1036245): **a)** head in lateral view; **b)** detail of head and thorax in lateral view; **c)** head in ventral view; **d)** thorax in ventral view; **e)** prothoracic leg; **f)** proleg, A5; **g)** A3 - A5 in lateral view; **h)** spiracle and detail of the cuticle, A4; **i)** posterior end of abdomen in lateral view.

present but shorter compared to the vertical axis of the head in *Chloreuptychia arnaca* (Fabricius, 1776) (DeVries, 1987); present and rather similar in length to *S. quadrina* in the larva of *Magneuptychia gomezi* (Singer, DeVries & P. Ehrlich, 1983) ("*Cissia*" *gomezi* in Singer et al. 1983: 108, fig. 4N) and *Amphidecta reynoldsi* (Sharpe, 1860), although the latter species do not enter fifth instar and the ultimate stage appears to be fourth instar (Freitas, 2004). In addition, the larva and pupa illustrated in DeVries (1987: 261) as "*Cissia alcinoe*" (now included in the genus *Magneuptychia* Forster, 1964) also resembles *S. quadrina* based on this head character, although this species is likely misidentified and, based on the adult figured in plate 41, it most likely represents a taxon closely related to *Magneuptychia modesta* (Butler, 1867).

Given the polyphyly of *Splendeuptychia* (e.g., Peña *et al*. 2010), immature stage information could help revise the generic classification of species currently in the genus. We thus hope that this short article will be part of a series of forthcoming studies on the life history of *Splendeuptychia*.

Fig. 5. Mating pair of *Splendeuptychia quadrina* from Madre de Dios, Peru.

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