# Description of the immature stages of the high Andean pierid butterfly *Catasticta incerta incerta* (Dognin, 1888) (Lepidoptera: Pieridae)

**Pablo Sebastián Padrón[1, 2] and Ariana Vélez[1]**

1. Laboratorio de Entomología, Universidad del Azuay. Avenida 24 de Mayo 7-77 y Hernán Malo. Cuenca, Ecuador.
2. McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, FL USA. Corresponding author: E-mail: sebastianpadronm@yahoo.com

**Date of issue online**: 24 August 2020
**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.3990651


**Abstract:** As part of a series of papers focusing on the description of immature stages of the genus *Catasticta* Butler, 1870, we here describe for the first time the immature stages of the high Andean butterfly *Catasticta incerta incerta* (Dognin, 1888), from Azuay province in southern Ecuador. The host plant was identified as an aerial hemi-parasitic mistletoe *Antidaphne andina* Kuijt (Santalaceae), which is restricted to high elevations in the Andes. Immature stages were reared *in situ* by enclosing them and the vegetation on which they were feeding in a mesh enclosure. A comparison with the immature stages of *Catasticta philothea* (C. Felder & R. Felder, 1865) from Colombia is presented. Finally, the larval chaetotaxy is illustrated and described.

**Key words:** Andes; Ecuador; host plant; mistletoe; Santalaceae.

**Resumen:** Como parte de una serie de artículos científicos enfocados en la descripción de estados inmaduros del género *Catasticta* Butler, 1870; aquí describimos por primera vez los estados inmaduros de *Catasticta incerta incerta* (Dognin, 1888), una mariposa altoandina de la provincia del Azuay en el sur del Ecuador. La planta hospedera es identificada como un hemi-parásito aéreo *Antidaphne andina* Kuijt (Santalaceae) la cual es restringida a los altos andes. Los estados inmaduros fueron criados in situ usando una funda de crianza que encerraba a los estados inmaduros y a la planta hospedera. Se presenta una comparación entre los estados inmaduros de *C. incerta* con los de *Catasticta philothea* (C. Felder & R. Felder, 1865) de Colombia. Finalmente, se ilustra y describe la quetotaxia de la larva.

**Palabras clave:** Andes; Ecuador; muerdago; planta hospedera; Santalaceae.

## INTRODUCTION

*Catasticta* is one of the most diverse butterfly genera in the Neotropics, with almost 100 described species (Lamas, 2004; Braby & Nishida, 2010; Padrón, 2014), but despite the fact that it has been intensively sampled in the field and several taxa are still being described (Bollino & Padrón 2016; Nakahara *et al.,* 2018), currently we still know very little about its biology, especially the immature stages (Schultze, 1935; Braby & Nishida, 2010; Montero & Ortiz 2013; Callaghan, 2019; Padrón, *et al.,* 2020). Such information is essential to help understand factors controlling species distributions and ecology, and especially the evolution of the group, since it has been suggested that shifts in host plant preference, particularly to aerial mistletoes, could have triggered a burst of diversification (Braby & Trueman 2006; Ferrer *et al.,* 2013; Padrón, 2014). However, without more comprehensive information about host plant interactions it is difficult or impossible to test certain hypotheses about the evolution of the genus. The description, analysis and integration of morphological characters from immature stages could also help to resolve some of the current taxonomic problems of the genus, where intra-specific and inter-specific relationships are unclear (Braby *et al.,* 2006; Padrón, 2014; Wahlberg *et al.,* 2014).

*Catasticta incerta* (Dognin, 1888) is medium-sized, high altitude butterfly restricted to the Ecuadorian Andes between 2500 m to 3500 m (Fig. 1). The species was originally described in the genus *Archonias* by Dognin (1888), based on seven specimens from Zamora and Loja in southern Ecuador. Currently, there are two recognized subspecies, *C. i. incerta* and *C. i. concerta* Eitschberger & Racheli, 1998, with the former restricted to southern Ecuador, from southern Zamora-Chinchipe and Loja to southern Morona-Santiago, and the latter restricted to central and northern Ecuador, from Pastaza to Sucumbíos (Fig. 1). Despite an apparent biogeographical disjunction (that could be a sampling artifact), the taxonomy of this species is not completely resolved. *Catasticta i. concerta* was described by Eitschberger and Racheli (1998), based only on ventral hindwing pattern and coloration, which show high variability among populations. Examination of additional material, and molecular data, are needed to clarify the taxonomy.

Little is known about the biology of this species; males are commonly found flying over vegetation on sunny days in search of hilltops, where they congregate and compete for the perching spots that they use to wait and look out for females for mating. Females, on the other hand, are extremely rare, with Bollino *et al.,* (2002) recently illustrating the female for the first time, based on a specimen from Loja. Despite our efforts to

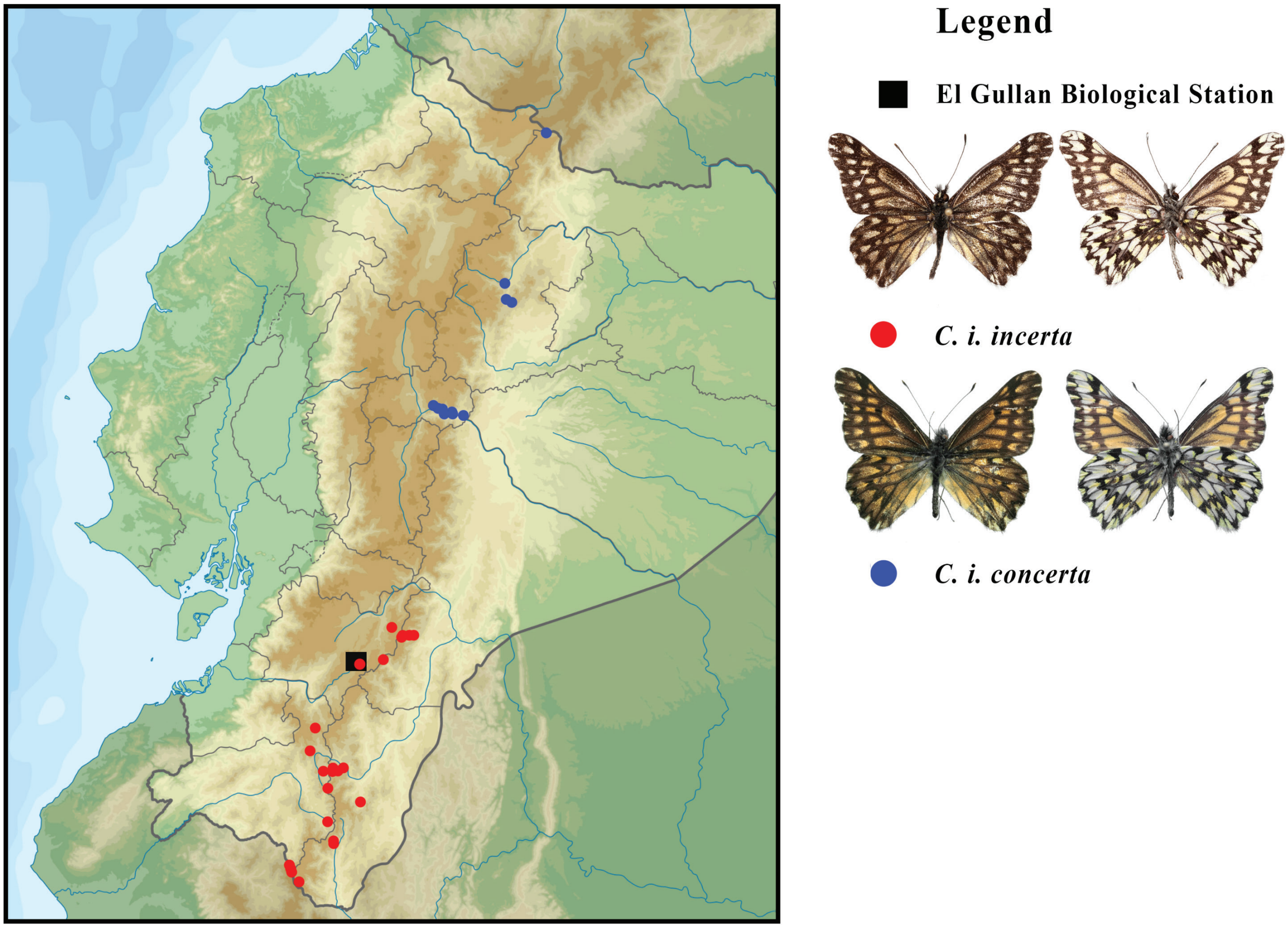



**Figure 1.** Record localities for *C. i. incerta* and *C. i. concerta* in Ecuador and the study location where larvae where found and reared in Azuay province.

locate females ovipositing in the field, we were unsuccessful, but we were able to locate several clusters of eggs in the field and rear them to adult. The purpose of this paper is to provide the first morphological descriptions of the immature stages of *C. i. incerta*, and to provide information about the host plant associations of this species.

## MATERIAL AND METHODS

**Study area:** The study was conducted at El Gullán Biological Station (-3.338116°S, -79.171707°W) in Azuay province, located at 3000 m above sea level in the southern Andes of Ecuador. El Gullán Biological Station contains 136 ha and is located within humid montane scrub (Padrón, 2016). The types of vegetation within the station property include native scrub, grasslands, pastures and plantations of introduced pines. The temperature during the day fluctuates between 7°C to 18°C, and annual precipitation ranges from 250 mm to a maximum of 750 mm. The area has high cloud cover throughout the year with an average wind speed of 20 km/h (Padrón, 2016) (Fig. 1).

**Rearing methods:** Several clusters of eggs were found previously between 2016 and 2018, but despite efforts to rear them under lab conditions, these were unsuccessful, mainly because of the difficulty in obtaining a continuous supply of fresh leaves to feed the larvae. The host plant, a hemi-parasite, is highly dependent on its host plant, and when leaves are removed, they quickly desiccate, in less than a day, making *ex situ* breeding of immature stage very difficult. After several unsuccessful attempts, we decided to rear the immature stages in the field. For this, we used a 'Sock Enclosure' obtained from the American entomological supply company Bioquip, which is a fine mesh bag 10" in diameter by 20" in length, used to cover the host plant and the immatures stages *in situ*.

In April 2019, an egg cohort was found on the underside of host plant leaves at 1.5 m above the ground. The leaves that contained the egg cluster and additional leaves to be consumed by the larvae were enclosed using a 'Sock Enclosure', and every 15 days the immatures were checked and different stages were collected and used for the description. Finally, pupae were transported to a cage were the adults could hatch.

**Morphology:** For a description of the larval chaetotaxy and general morphology of immature stages we followed the terminology of Stehr (1987). Photos were taken using a Canon 5D Mark III DSLR camera with Canon MP-E 65mm f/2.8 1-5x Macro Photo Lens. Eggs were photographed using a Nikon 10x CFI Plan and a Nikon Fluor 20X/0.50 Dic M microscope objectives, and multiple images were combined through focusing stacking technique using Zerene Stacker Software. For morphological measurements, a stereomicroscope Nikon SMZ745T with MSHOT software was used. Illustrations were

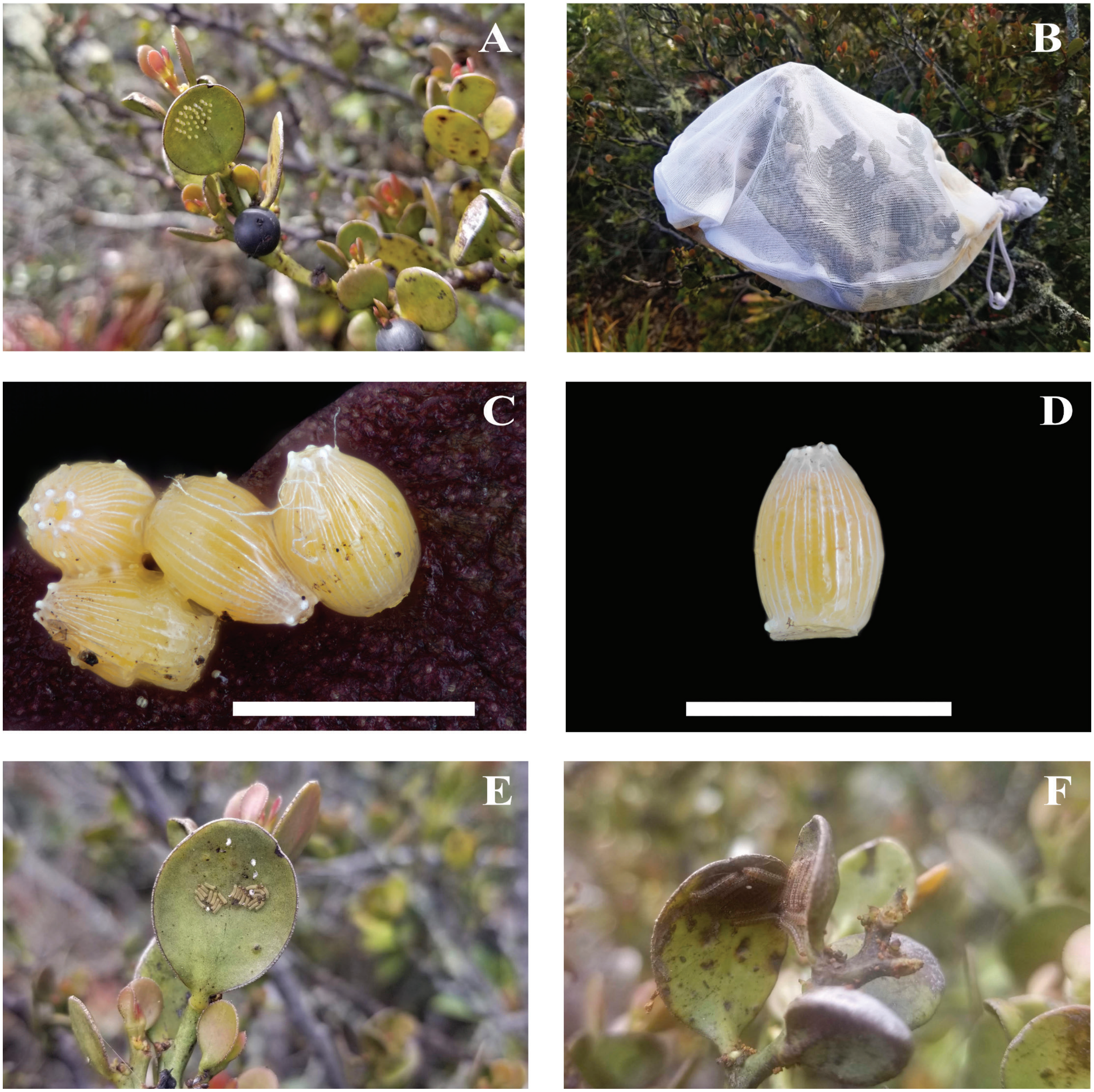



**Figure 2. A.** Cluster of eggs on leaf; **B.** Immature stages and host plant covered with the 'Sock Enclosure' in the field; **C.** Cluster of eggs; **D.** Detail of an egg; **E.** Group of newly emerged larvae; **F.** Second instars actively feeding on host plant. Scale bar: C and D 1 mm.

made using the preserved specimens and photos with the help of a Bamboo Wacom Pen and Inkscape software, and plates were prepared using Adobe Photoshop CS6. Voucher material of the immature stages have been deposited in the Museo de Zoología de la Universidad del Azuay, Cuenca, Ecuador.

## RESULTS

**Eggs:** Egg clusters were found on the undersides of food plant leaves. The number of eggs per cluster varied from 23 to 46, with a mean of 35 (n=4); eggs 0.73 mm high, 0.48 mm wide, color yellow-cream, barrel-shaped, with base flattened and apex narrower; chorion with numerous pale longitudinal ribs (some incomplete), apical rim with six to seven prominent pale protuberances or nodules. (Fig. 2A, C and D).

**First-instar larva:** Head capsule length from mouth to top of head: 0.61mm, width: 0.52 mm; length of body from head to A10: 2.78 mm. Head brown, with colorless primary setae, body orange after hatching, changing to a browner color after consuming food. After eclosion, larvae feed first on the exochorion, after which they actively feed on the ventral epidermis of the mistletoe leaf. During the first and second instar, larvae were gregarious. Gregariousness in other instars was difficult to corroborate, since the confinement of larvae to a small area as a result of the rearing methods might have interfered with their normal behavior (Figs. 2E and 3A).

**Second-instar larva:** Head capsule length from mouth to top of head: 1.14 mm, width: 0.75 mm; length of body estimated from head to A10: 5.7 mm. Head color maroon with colorless primary setae arising from brown-orange chalazae. Body maroon with dark maroon middorsal line and long and dense primary colorless setae next to many black secondary setae. Prothorax with maroon dorsal plate with cream-yellow color spots on the sides (Figs. 2F and 3B).

**Third-instar larva:** Head capsule length from mouth to top of head: 1.30 mm and width: 1.17 mm; length of body from head to A10: 7.55 mm. Head color deep dark maroon with colorless primary setae arising from light maroon chalazae. Body maroon with big cream-yellow spots and dark maroon middorsal line; primary colorless setae arising from cream-yellow chalazae, several additional black secondary setae arising from white chalazae. Prothorax with deep dark maroon dorsal plate, on each side of the dorsal plate are cream-yellow color spots with two cream-yellow chalazae from which arise two notorious long colorless setae (Fig. 3C and D).

**Fourth-instar larva:** Head capsule length from mouth to top of head: 2.33 mm and width: 1.94 mm; length of body from head to A10: 14.64 mm. Head color black with colorless primary setae arising from light maroon chalazae. Body dark maroon and dark maroon middorsal line, more notable yellow chalazae from which arise primary colorless setae, several additional black secondary setae arising from white chalazae. Cream color spots have almost disappeared, nevertheless, segments A2 to A8 have two small light cream spots on side with two notable yellow chalazae from which arise long setae. Prothorax with deep dark maroon dorsal plate, on each side of the dorsal plate are cream-yellow colored spots with two cream-yellow chalazae from which arise two notable long colorless setae (Fig. 3E and F).

**Fifth-instar larva:** Head capsule length from mouth to top of head: 3.17 mm and width: 2.95 mm; length of body from head to A10: 21.58 mm. Similar to fourth instar, except color of body and middorsal line is a lighter maroon and yellow and white chalazae are larger and more prominent (Fig. 3G and H; Fig. 4A, B and C).

**Pupa:** 22.45 mm long (excluding anterior projection), 6.74 mm wide, anterior projection length: 3.35 mm. Pupae pale green with black and orange spots, and extensive black patches that cover almost all dorsal part. Head with a prominent orange anterior projection with black at top, oriented upwards and bifurcated at apex and two smaller black projections posteriorly. Both prothorax and mesothorax have a maroon longitudinal dorsal ridge, which is more prominent on latter.

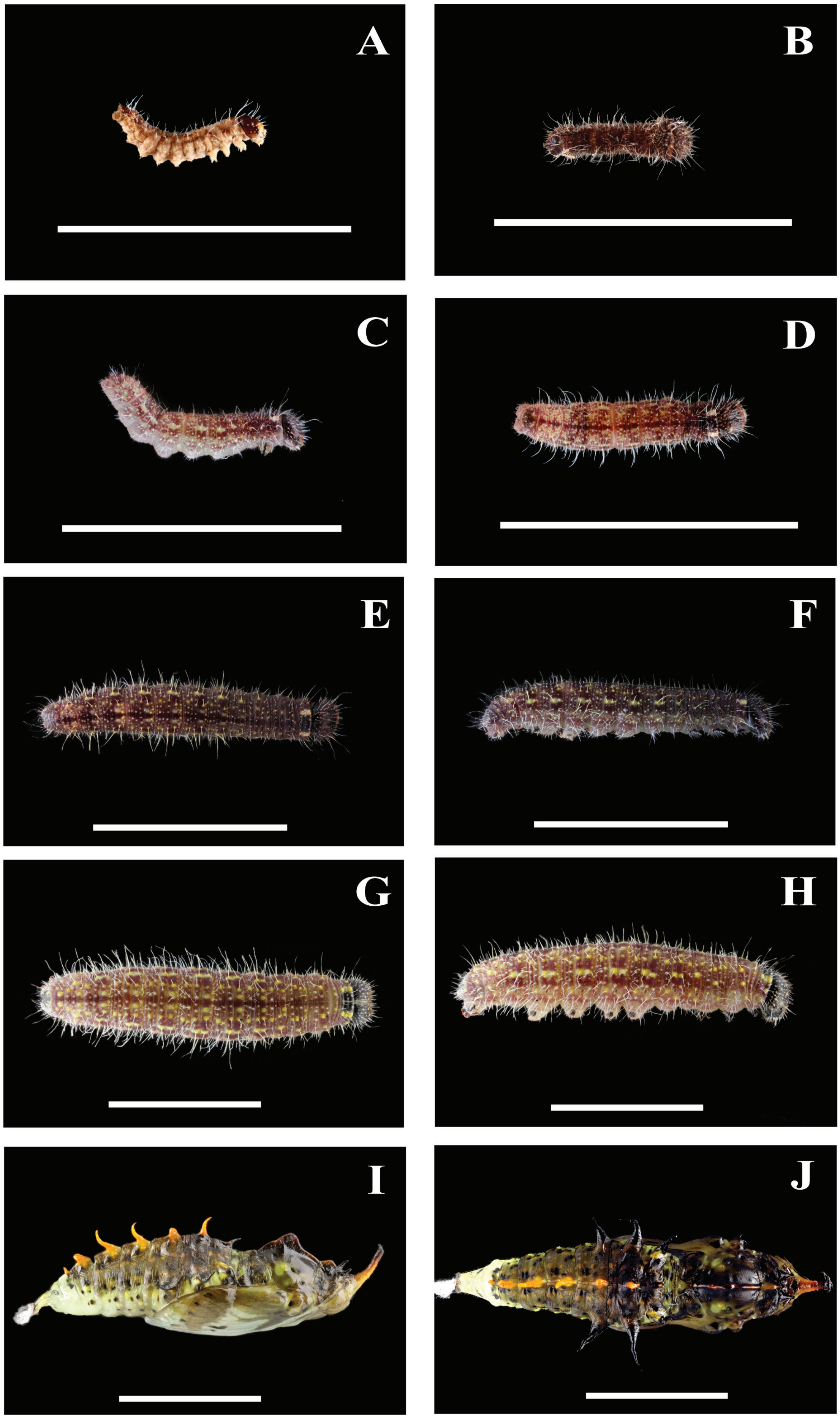
Abdominal segments A2 to A4 have long spine-like black dorsolateral projections; A2 to A8 have on each segment an orange middorsal projection (projections on A3, A5 and A6 larger than on remaining segments), all projections are spine-like with exception of A4 and A7 which are rounded. When agitated, pupa moves abdominal segments (Figs. 3I and J; Fig. 4D).

**Food plant and oviposition:** The larval host plant of *C. i. incerta* was identified as the hemi-parasitic mistletoe *Antidaphne andina* Kuijt*,* family Santalaceae. *Antidaphne* is a Neotropical genus which contains 14 species (Kuijt, 1988), and which is characterized by having small inflorescences, epicortical roots, lanceolate or elliptical leaves and one-seed berry fruit (Kuijt, 1988). Most of the species are limited to South America (Kuijt & Hansen, 2014). *Antidaphne andina* was originally described from a specimen collected in Ecuador in Azuay province, 18 km NW of Cuenca on the trail from Sayausid to Lagunas de Surucuchu, at an elevation of 2880 m, on Compositae (Kuijt, 1988). The type locality is located 54 km from the area where *C. i. incerta* immatures were reared. *Antidaphne andina* is restricted to the Andes from Colombia to Bolivia, and in southern Ecuador it is commonly found at high elevations (Kuijt, 1988), where it parasitizes a wide range of hosts, including *Weinmannia* L., *Miconia* Ruiz & Pav., *Hedyosmum* Sw., 1788 and even an orchid *Lepanthes* Sw., 1799 (Kuijt, 1988; Press & Graves, 1995). At the El Gullán Biological Station, we found *A. andina* parasitizing a tree, *Maytenus verticillata* (Ruiz & Pav) DC. family Celastraceae, which is a common tree in the area. Identifications of host plant and host tree were carried out by a Danilo Minga, a botanist from the Herbario Azuay in Cuenca, Ecuador, and were corroborated by comparison with images on the Missouri Botanical Garden web page (http://www.tropicos.org).

## DISCUSSION

With immature stages known for no more than 10% of *Catasticta* species, our understanding of the natural history of the genus is very limited, making any new contributions on the natural history, biology, host plant and ecological interactions very important. Here, we describe for first time the immature stages and larval host plant of *C. i. incerta*, a high Andean species restricted to Ecuador. The scarce existing knowledge of host plants of *Catasticta* shows a clear preference for mistletoes in the order Santalales and inside the families Loranthacea, Santalaceae and Viscaceae (Schultze, 1935; Braby & Nishida, 2010; Montero & Ortiz 2013; Padrón, *et al.,* 2020). There is only a single reliable report of a non-mistletoe host plant; the rare, east Colombian species *C. philothea* (Felder & Felder) feeds on *Axinaea macrophylla* (Melastomataceae) (Callaghan,

**Figure 3 (p. 69, facing page). A.** First instar lateral view; **B.** Second instar dorsal view; **C.** Third instar lateral view; **D.** Third instar dorsal view; **E.** Fourth instar dorsal view; **F.** Fourth instar lateral view; **G.** Fifth instar dorsal view; **H.** Fifth instar lateral view; **I.** Pupa lateral view; **J.** Pupa dorsal view. Scale bar: A and B 0.5 cm, from C to J 1 cm.


A
B
C
D
E
F
G
H
I
J

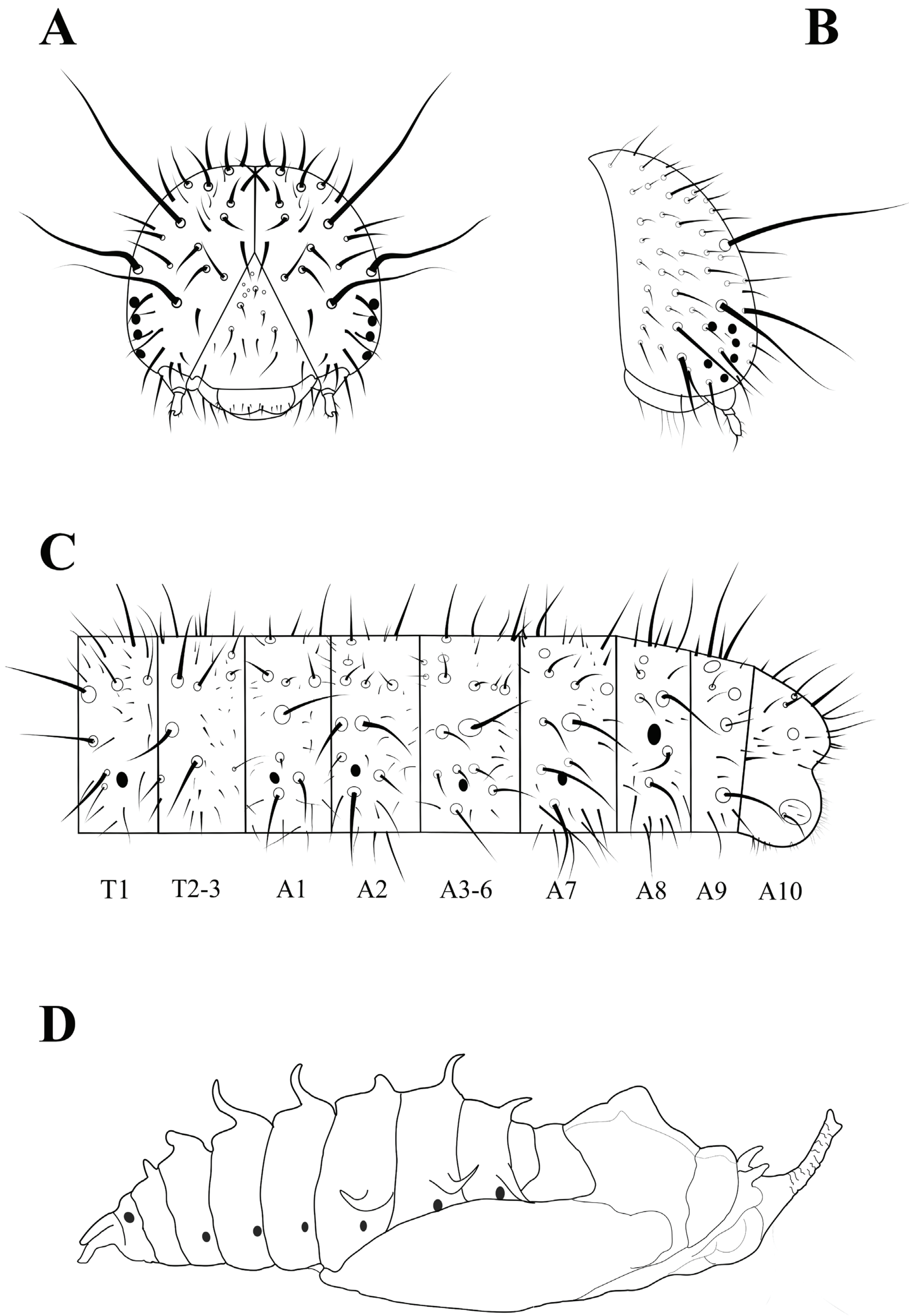



**Figure 4.** *Catasticta incerta incerta*, immature stage morphology. **A.** Head chaetotaxy of fifth instar, in anterior view; **B.** Head chaetotaxy of fifth instar, in lateral view. **C.** Setal maps of fifth instar; **D.** Pupa in lateral view.

2019). With the present publication the mistletoe-feeding trend in *Catasticta* is strengthened, making all the more remarkable the deviation from this group of plants recently reported by Callaghan (2019) for *C. philothea*.

We found that the pupa of *C. incerta* is overall similar to the pupa of *C. philothea*, but with a few differences: the pupa of *C. incerta* has an orange anterior projection with black at the top and a dark maroon longitudinal dorsal ridge, while, in contrast, the *C. philothea* pupa has a light reddish brown anterior projection and a light brown longitudinal dorsal ridge (Callaghan, 2019). All the middorsal projections in the pupa of *C. philothea* are spine-like and are present from segments A2 to A7 (A8, A9 and A10 have no projections) (Callaghan, 2019). However, *C. incerta* has middorsal projections from segments A2 to A8 (only A9 and A10 have no projections), and on segments A4 and A7 the middorsal projections are rounded (Fig. 4D).

One key aspect of the research reported here was the use of the Sock Enclosure method, for rearing *Catasticta* immature stages. One of the most serious impediments for successful rearing is the availability of a fresh supply of leaves of the host plant, which tend to desiccate very quickly when removed from the plant. The *in situ* rearing method also excludes predators and parasitoids, and prevents the larvae from wandering away from the host leaves on which they were located, a typical behavior of some *Catasticta* last instar larvae (Braby & Nishida, 2010). Because of their small size and camouflage, it is often difficult to otherwise relocate wandering larvae. In addition, this method maintains the environmental conditions (mainly temperature and humidity) that are appropriate for development, which are key factors for successfully rearing Lepidoptera immature stages.

Increasing descriptions of the immatures stages and identification of host plants in the last few years have provided a better understanding of the biology, host plant association and ecology of *Catasticta*. The addition of more data, especially from groups of mid- and high elevation Andean species, will be key to understanding the speciation process in *Catasticta*, helping to understand the origins of the remarkable diversity of this genus.

## ACKNOWLEDGEMENTS

We thank Keith Willmott and Curtis Callaghan for their helpful comments which substantially improved the quality and organization of this manuscript, Cristina Vásquez and Sara Durán for their assistance and participation in fieldwork activities. Furthermore, we would like to thank Fernando Juela from Ministerio del Ambiente in Cuenca for supporting our study by issuing the collecting permits (148-2017-DPAA/MA and 181-2018-DPAA/MA). We thank Danilo Minga, curator of the Herbario Azuay in Cuenca, Ecuador, for his help in the identification of plants. Finally, we are grateful to the Universidad del Azuay and its research funding program 2019 and 2020 - 0095.

## LITERATURE CITED

**Bollino, M., Greeney, H., Vitale, F.** 2002. On some hitherto unknown females of the genus *Catasticta* from Ecuador (South America) (Lepidoptera: Pieridae). *Lambillionea* 102(3): 267-272.

**Bollino, M., Padrón, P. S.** 2016. Description of a new subspecies of *Catasticta fulva* Joicey & Rosenberg, 1915, with notes on several other species in the genus (Lepidoptera: Pieridae). *Tropical Lepidoptera Research* 26(1): 1-5.

**Braby, M. F., Trueman, J. W. H.** 2006. Evolution of larval host plant associations and adaptive radiation in pierid butterflies. *Journal of Evolutionary Biology* 19(5): 1677-1690.

**Braby, M. F., Vila, R., Pierce, N. E.** 2006. Molecular phylogeny and systematics of the Pieridae (Lepidoptera: Papilionoidea): higher classification and biogeography. *Zoological Journal of the Linnean Society* 147(2): 239-275.

**Braby, M. F., Nishida, K.** (2010). The immature stages, larval food plants and biology of Neotropical mistletoe butterflies (Lepidoptera: Pieridae). II. The *Catasticta* group (Pierini: Aporiina). *Journal of Natural History* 44(29-30): 1831-1928.

**Callaghan, C.** 2019. Notes on the biology of a nonconformist *Catasticta* species from Colombia: *Catasticta philothea* (Lepidoptera: Pieridae). *Journal of the Lepidopterists' Society* 73(3): 203-207.

**Dognin, P**. 1888. Diagnoses de Lépidoptéres nouvelles de l´Equateur. *Le Naturaliste* 2(2): 4.

**Eitschberger U., Racheli T.** 1998. *Catasticta* studies (Lepidoptera: Pieridae). *Neue entomologische Nachtrichten* 41: 5-93.

**Ferrer-Paris, J. R., Sanchez-Mercado, A., Viloria, A. L., Donaldson, J.** 2013. Congruence and diversity of butterfly-host plant associations at higher taxonomic levels. *PLoS One* 8(5): 1-15.

**Kuijt, J.** 1988. Monograph of the Eremolepidaceae. *Systematic Botany Monographs* 18: 1-60.

**Kuijt, J., Hansen, B.** 2014. *Flowering plants. Eudicots: Santalales, Balanophorales*. Springer International Publishing. 225 pp.

**Lamas, G.** (Ed.). 2004. *Checklist: Part 4A. Hesperioidea - Papilionoidea*. *In*: Heppner, J. B. (Ed.), *Atlas of Neotropical Lepidoptera*. Volume 5A. Gainesville, Association for Tropical Lepidoptera; Scientific Publishers. 463 pp.

**Montero, F., Ortiz, M.** 2013. Aporte al conocimiento para la conservación de las mariposas (Hesperioidea y Papilionoidea) en el Páramo del Tablazo, Cundinamarca (Colombia). *Boletín científico. Museo de Historia natural. Universidad de Caldas* 17(2): 197-227.

**Nakahara, S., Macdonald, J. R., Delgado, F., Padrón, P. S.** 2018. Discovery of a rare and striking new pierid butterfly from Panama (Lepidoptera: Pieridae). *Zootaxa* 4527(2): 281-291.

**Padrón, P. S.** 2014. *Molecular Phylogeny and Biogeography of the Genus* Catasticta *Butler, 1870*. PhD Dissertation. Gainesville, University of Florida.

**Padrón, P. S.** 2016. *Una Mirada a la Biodiversidad de un Bosque Alto Andino*. Cuenca, Universidad del Azuay. 220 pp.

**Padrón, P. S., Vélez, A., Miorelli, N., Willmott, K. R.** 2020. Urban areas as refuges for endemic fauna: description of the immature stages of *Catasticta flisa duna* (Eitschberger & T. Racheli, 1998) (Lepidoptera: Pieridae) and its ecological interactions. *Neotropical Biodiversity* 6(1): 109-116.

**Press, M., Graves, J.** (Eds.). 1995. *Parasitic Plants*. London, New York, Chapman & Hall. 292 pp.

**Schultze-Rhonhof, A.** 1935. Ueber die ersten Stände von *Catasticta flisa* H. Schäff. *Deutsche entomologische Zeitschrift "Iris"* 49: 52-54.

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

**Wahlberg, N., Rota, J., Braby, M. F., Pierce, N. E., Wheat, C. W.** 2014. Revised systematics and higher classification of pierid butterflies (Lepidoptera: Pieridae) based on molecular data. *Zoologica Scripta* 43(6): 641-650