Life history descriptions of *Adelpha attica attica*, *Adelpha epione agilla*, and *Adelpha jordani* from an eastern Ecuador lowland forest

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Abstract: The diverse Neotropical butterfly genus *Adelpha* Hübner (Nymphalidae) has long been recognized by tropical lepidopterists as a challenging group to classify owing to wing pattern similarity among sympatric species. The study of immature stages and food plants has historically played an important role in helping clarify *Adelpha* systematics and continues to offer insight into their adaptive radiation. Here we describe the immature stages of three species for the first time, *Adelpha attica attica* (C. Felder & R. Felder, 1867), *Adelpha epione agilla* (Fruhstorfer, 1907) and *Adelpha jordani* (Fruhstorfer, 1913), based on fieldwork in lowland forest of eastern Ecuador. We observed caterpillars of *A. attica attica* and *A. epione agilla* feeding on *Chomelia tenuiflora* Benth. (Rubiaceae), whereas those of *Adelpha jordani* fed on *Calatola costaricensis* Standl. (Icacinaceae). The immature morphology of these three species generally supports their position in recent phylogenetic analyses, and helps define species groups. In addition, continued study of the immature stage biology and food plant interactions promises to help provide a better understanding of the evolution of *Adelpha* host relationships.

Descripciones de la historia de vida de *Adelpha attica attica*, *Adelpha epione agilla*, y *Adelpha jordani* en un bosque de tierras bajas al este de Ecuador

Resumen: El diverso género de mariposas neotropicales *Adelpha* Hübner (Nymphalidae) ha sido reconocido por los investigadores de lepidópteros tropicales como un grupo difícil de clasificar debido a la similitud del patrón de alas entre especies simpátricas. El estudio de los estadios inmaduros y las plantas hospederas históricamente ha desempeñado un rol importante en ayudar a clarificar la sistemática de *Adelpha*. En el presente estudio describimos por primera vez los estadios tempranos de tres especies, *Adelpha attica attica* (C. Felder & R. Felder, 1867), *Adelpha epione agilla* (Fruhstorfer, 1907) y *Adelpha jordani* (Fruhstorfer, 1913), basados en el trabajo de campo en un bosque de tierras bajas al este de Ecuador. Las orugas de *Adelpha a. attica* y *A. epione agilla* fueron observados alimentándose de *Chomelia tenuiflora* Benth. (Rubiaceae), mientras que las de *Adelpha jordani* se alimenta de *Calatola costaricensis* Standl. (Icacinaceae). La morfología de los estadios inmaduros de estas tres especies corrobora, en cierta medida, su posición filogenética basada en análisis recientes, y ayuda a definir su ubicación en los grupos de especies. Adicionalmente, el continuar estudiando la biología de los estadios tempranos y las interacciones con las plantas hospederas ayudará a tener un mejor entendimiento de la evolución en las relaciones de *Adelpha* con sus plantas hospederas.

Keywords: Icacinaceae, Rubiaceae, mimicry, insect-plant interactions.

Palabras clave: Icacinaceae, Rubiaceae, mimetismo, interacciones insecto-planta.

INTRODUCTION

In 1926 the Reverend A. Miles Moss remarked that the genus *Adelpha* was thought to have about half the diversity in the Amazon as in Central America (Moss, 1926). To investigate this odd pattern further and clarify the identity of the *Adelpha* at Pará, Brazil, which had confusingly similar wing patterns, he began a study of the immature stages. In his first six months focused on *Adelpha* immatures he discovered larvae and food plants for 11 species (Moss, 1926). He documented several similar adults that had very different immature stages. This led to him conclude that *Adelpha* were much more diverse than previously thought (Moss, 1926). Moss's inference from his rearing was correct, and we now know *Adelpha* to be the second most diverse nymphalid genus in the Neotropics with over 90 species in the genus (Willmott, 2003b; Willmott & Hall, 2013).

Moss's work documented a striking pattern of superficial wing pattern similarity in *Adelpha* that illustrated that immature stages might be the key to identifying species relationships within the genus. More than fifty years after Moss's (1926)

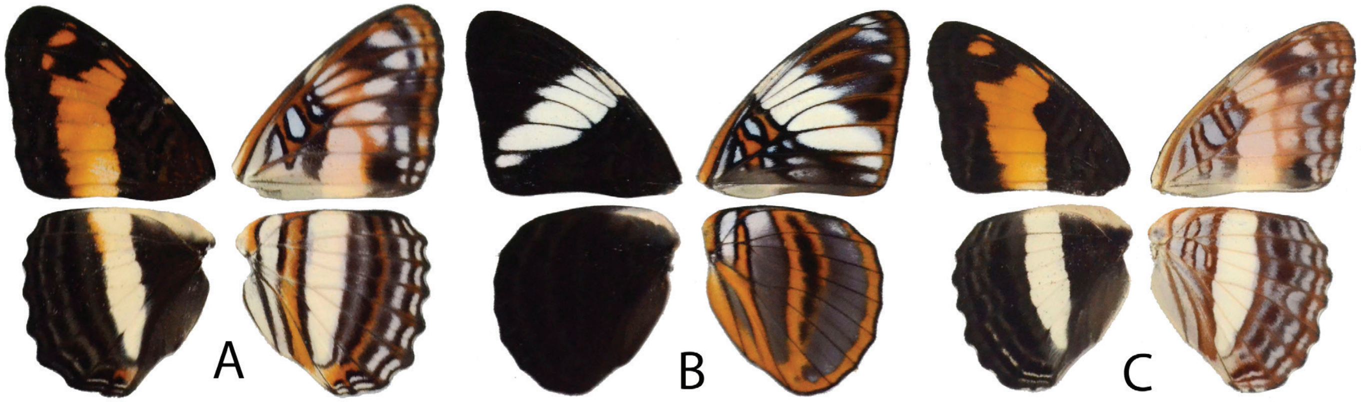

Figure 1. Adult images of *A. attica attica* (A), *A. epione agilla* (B) and *A. jordani* (C) reared in this study. Dorsal view is on the left of each image and ventral view is on the right.

work, Aiello (1984) approached the "*Adelpha* mess" (Aiello, 1984: 37) by summarizing morphology for all known immature stages. Aiello (1984) recognized several species groups, and expanded on the pattern found by Moss (1926): each species group had adults that resembled adults in other species groups. Aiello (1984) hypothesized that Rubiaceae-feeding larvae were protected models, with species feeding on other plants being mimics. More recently, detailed life history information has been published for a number of *Adelpha*, adding significantly to knowledge of the immature stage biology of the genus (Aiello, 1991; Otero & Aiello, 1996; Freitas *et al.*, 2001; Aiello, 2006; Freitas, 2006; Oliveira-Neto *et al.,* 2016; Rush & Hill, 2017; Janzen & Hallwachs 2019).

Recent works detailing morphological and biogeographic patterns, and testing systematic hypotheses have further elucidated relationships and mimicry in *Adelpha* (Willmott, 2003a,b; Ebel *et al.*, 2015). Willmott's (2003a,b) analysis of adult wing patterns, genitalia and immature stages confirmed many of the species groups, but left some species unassigned based on character combinations that did not clearly fit with other species. Furthermore, Willmott's (2003b) biogeographic data confirmed many cases of unrelated sympatric species converging onto a few dorsal wing patterns. A recent molecular analysis by Ebel *et al*. (2015) found that some species groups (*alala*, *serpa* and *phylaca*) are monophyletic, but the rest are paraphyletic. The paraphyly of species groups in the Ebel *et al.* (2015) analysis and fact that not all species were included in that analysis, combined with several species that Willmott (2003a) was not able to assign to species groups, indicate there is still scope for immature stages to help delineate species groups and evolutionary patterns within this complex genus, as Moss' original observations attest.

To provide data to help clarify systematic patterns and the evolution of *Adelpha*, we report observations from three months of intensive fieldwork focused on rearing *Adelpha* in eastern lowland Ecuador. In total, we reared 11 *Adelpha* species, including discovery of immature stages and food plants for *A. attica*, *A. epione*, and *A. jordani* (adults illustrated in Fig. 1), three species for which no information has previously been published. We describe what is known of the life history, ecology and the adult behavior of these species, as well as discussing their immature stages and phylogenetic relationships.

MATERIALS AND METHODS

Observations were made from August to October 2017 at the Yasuní Scientific Station (-0.674167, -76.397222, 220-250 m), Orellana Province, eastern Ecuador. We searched for immature stages and adults along forest trails and gaps, and edges of roads and rivers in primary forest. All potential food plants, especially those belonging to the family Rubiaceae, were searched for eggs and larvae. A major cue that helped in finding larvae was the typical leaf damage of the genus, in which the leaf tissue adjacent to the central, or a secondary vein, was eaten and the vein extended with pieces of frass silked together to create the frass chain. *Adelpha* females tend to lay eggs on the leaf upper surface at the leaf tip, or near a major secondary vein, and generally avoid very new growth. Larvae are often found on seedlings and immature plants from near the ground to 2-3 m, but we also searched higher on mature plants with binoculars. Larval food plants were identified by Pablo Alvia and Milton Zambrano, as well as by referencing the Tropicos online plant database (Tropicos.org, 2018).

Larvae were reared individually in plastic bags under ambient conditions. Larval bags were hung so that leaves would approximate natural positions. Bags were cleaned daily with toilet paper. Larval and pupal durations were recorded daily. Head capsules and pupal exuviae were collected for later description. Photographs of each stage were taken using a Nikon D7000, and Micro Nikkor 105mm lens fitted with extension tubes (Kenko) and ring flash (Nikon SB-R200 Speedlight).

Larval descriptions were made based on notes and photos taken during rearing. Morphological terminology follows that described and used by Aiello (1984), Willmott (2003b) and Rush & Hill (2017). We used the taxonomic arrangement of Willmott (2003a,b). Reported head size is based on measurements of head capsules. Head capsule measurements were made with a Leica MZ75 stereomicroscope. Head capsule width was measured at the widest part, using a microscope eyepiece reticle with a total scale of 12mm. Head capsule drawings were made with Wild M3 stereomicroscope with an attached Wild TYP 308700 camera. Immatures in different stages were preserved in 100% ethanol or RNAlater (Ambion, Inc.) for molecular work.

RESULTS

Adelpha attica attica C. Felder & R. Felder, 1867

Egg. Fig. 2A. Duration: 5.0 days, n=1. Like other *Adelpha*, the egg is yellowish green and round with hexagonal pits that have a seta at each angle.

First Instar. Fig. 2B. Duration: 4.0 days, n=1. Head: 0.60 mm, n=1. The head is pale brown dorsally and laterally and has a dark frons. The head is rounded with short setae. The body is pale green after the first feeding and takes on a brown hue by the end of the stadium.

Second Instar. Fig. 2C. Duration: mean=4.0 days, n=2. Head: mean=0.96 mm, s.d.=0.05, n=5. The body is greenish brown and is covered with light brown bumps and short setae. The whole head is dark brown to black and is ornamented with rudimentary chalazae. The subdorsal scoli begin to show in this instar.

Third Instar. Fig. 2D. Duration: mean=4.0 days, s.d.=0.63, n=6. Head: mean=1.4 mm, s.d.=0.07, n=6. The head is entirely dark brown with tubercles and developing chalazae that are both light colored. The body is light brown dorsally and dark brown laterally. Short, dull brown-orange setae cover the body. The most pronounced subdorsal scoli appear on T2, T3, A2, A7 and A8. Subspiracular scoli are present along the body.

Fourth Instar. Fig. 2E. Duration: mean=5.0 days, s.d.=0.58, n=7. Head: mean=2.3 mm, s.d.=0.07, n=7. The body is brown-orange dorsally and dark brown laterally. The head is similar to that of the previous instar but the chalazae are better developed and the colors show less contrast. Several subspiracular scoli show beige patches at their base. The subdorsal scoli on T2, T3, A2, A7 and A8 continue being the most pronounced, with spines developing along their length. The scoli on A3-A6 have spines radiating at their tip. The scoli on A1 and A10 are short with spines radiating directly off the body in groups of 3-5. The subdorsal scoli on T2 and T3 have a slightly raised transverse ridge connecting the subdorsal scoli on each segment.

Fifth Instar. Fig. 2F. Duration: mean=6.6 days, s.d.=0.88, n=9. Head: mean=3.7 mm, s.d.=0.15, n=7. The dorsal body coloration is a combination of black and green patches with pink-purple showing between segments; laterally, the body is white with black and oblique stripes. The larva changes to yellow-brown the day before pupating. The head is light brown laterally and the frons is black with beige vertical stripes reaching from the tip of the m1 chalaza to the bottom of the frons. The chalazae are light brown with m1 having a dark brown color. The chalazae are elongated and triangular in shape, and arranged in 3 series: 6 posterior, 4 medial and 2 anterior (Fig. 3A). The subdorsal scoli are cylindrical and thin with spines arising at irregular intervals along their length, and darker colored at the tip. The subdorsal scoli on A2 are the most developed for the entire body, followed by T2-T3 and A7-A8. The subdorsal scoli on A3-A6 are short, whereas those on A1 are very reduced; despite the difference in their size, all of these scoli have spines radiating from the tip. The A10 subdorsal scoli are pale, somewhat matching the lateral body coloration. The supraspiracular scoli on T2 are pale yellow with brown spines and are slightly shorter than the subdorsal scoli on the same segment. Short supraspiracular scoli are found on T3, and are pale yellow with spines radiating at the tip. Short subspiracular scoli are found on A1-A8, and these pale green scoli project ventrally and terminate in groups of 3-5 spines. Thoracic legs are light brown and prolegs are colored similar to the pink-purple dorsal patches.

Pupa. Fig. 2G and Fig. 4A, B. Duration: mean=9.1 days, s.d.=0.35, n=8. The pupa is pearly white to coppery brown with dark sutures and orange-brown highlights. The head has flat projections that are produced medially, creating an arc toward each other, and laterally they are very pointed and slightly recurved and darker at the tip. The thoracic projection is pointed and directed posteriorly. The abdominal projection is curved anteriorly making a hook-shape, and is slightly larger than the thoracic projection. The abdominal projection begins on A2 and continues to the segment A3. Each abdominal segment posterior of A3 has a dorsal keel which is squarish in each segment when viewed laterally. There are short dorsolateral projections present on A2-A4 (Fig. 4A). The projections on A2 and A3 are brown rounded bumps flecked with white, with A4's a conical tubercle that is darker colored. The pupa turns dark the day before eclosion, except for the cremaster that remains pale.

Food plant and oviposition: *Chomelia tenuiflora* Benth. (Rubiaceae) was found to be the larval food plant of *Adelpha attica attica*, the same plant as for *Adelpha epione agilla*. The habitat of this plant is areas with small trees or shrubs and its range is from Mexico to Bolivia, including Venezuela, French Guiana, Guyana and Brazil (Tropicos.org, 2018). In Ecuador, this plant is widely distributed under 100 m in *terra firme* forest and on poorly drained soils (Pérez *et al*., 2014). Both *A. a. attica* and *A. e. agilla* were found intermingled on the same trees, but never on the same leaf. *Adelpha e. agilla* immatures were found more often than *A. a. attica* on the food plant. The larval food plants used by *A. a. attica* were found at the edge of the Río Tiputini on a mud beach and inside the forest but close to gaps. *Adelpha a. attica* immatures were found on mature trees between 1.2 to 3.2 m above the ground in shady areas. At midday, a female was observed laying a single egg on the lower surface of the food plant leaf near the main vein. Oviposition occurred very quickly at 3-4 m.

Larval behavior. Upon hatching, the larva ate the remains of the egg and began eating around either the central or secondary vein, to expose it. Pieces of frass were silked together to extend the vein and create the frass chain. The larva rested on this frass chain with the body either straight or with the anterior portion raised ("front-curved position" of Aiello, 1984). A barricade was created between the leaf and the extended vein, at the base of frass chain, with frass and bits of leaf silked together. Like other *Adelpha*, the molt to the final instar took place on the surface of the leaf and the larvae did not create or use frass chains during the ultimate instar. Larvae rested on the top or underside of the leaf with the body straight or in a front-arched-rear-up position, and were not observed in the curled position. Pupation in captivity occurred on the underside of the leaf, on food plant stems or on the rearing bag.

Adult. According to Willmott (2003b), *A. attica attica* occurs east of the Andes from southern Venezuela to Bolivia and the central Amazon in Brazil. This subspecies is common in primary lowland rain forest up to 1000 m in eastern Ecuador (Willmott, 2003b), but in the study area at 300 m it was rare. Adults were observed at the edge of the Tiputini River, at a mud beach, and in gaps inside the forest.

Adelpha epione agilla Fruhstorfer, 1907

Egg. Fig. 5A. Duration: mean=4.5 days, s.d.=0.71, n=2. The egg is yellowish green like other species of *Adelpha*, round with hexagonal pits, and with a single seta arising from each angle of the hexagon. Eggs are laid singly, up to several per plant.

First Instar. Fig. 5B. Duration: mean=4.5 days, s.d.=0.71, n=2. Head: mean=0.53 mm, s.d.=0.01, n=2. Similar to other *Adelpha* species. The head is rounded with short setae, it is light brown dorsally and laterally

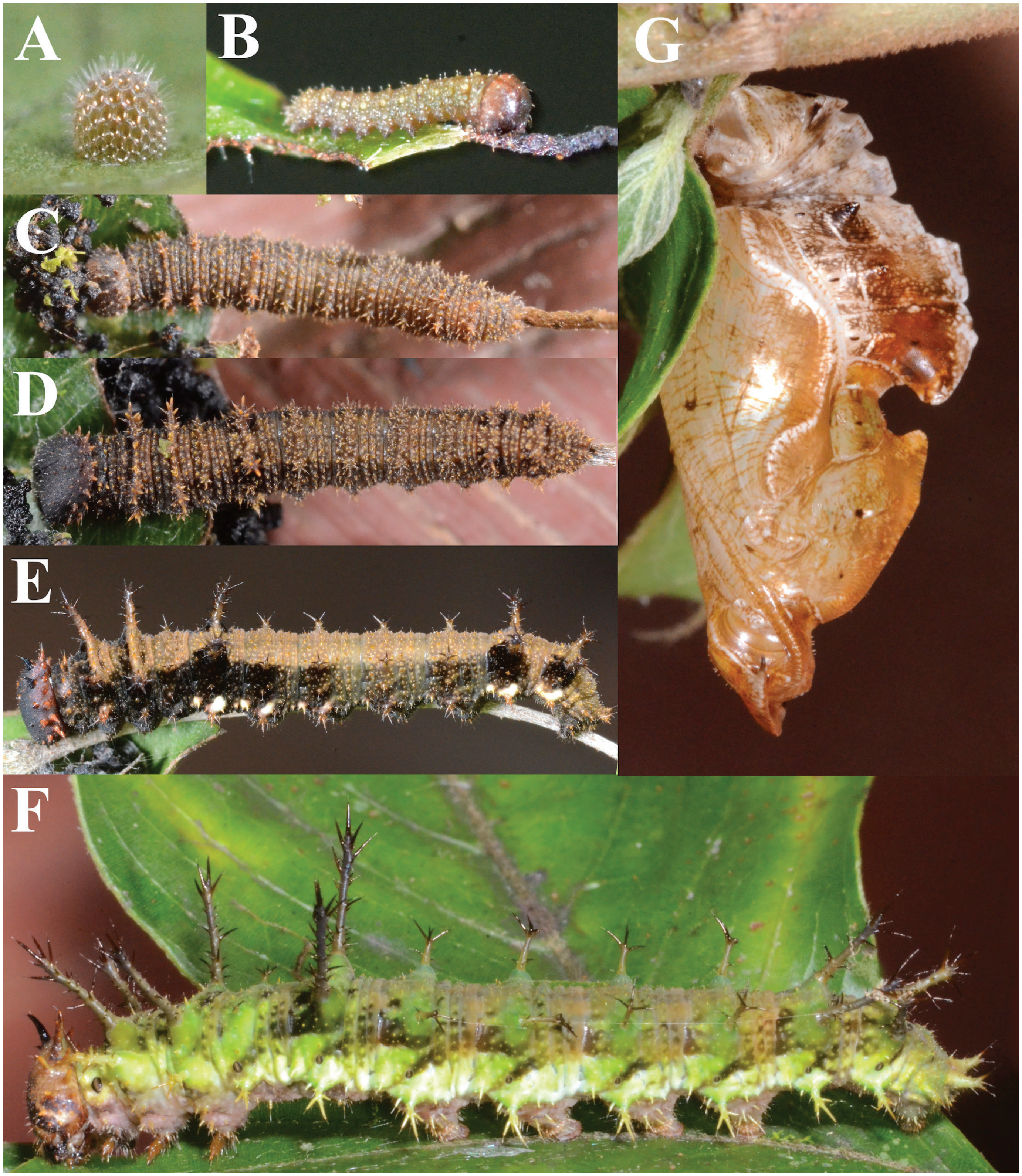

Figure 2. Immature stages of *Adelpha attica attica*. A. Egg. B. First instar. C. Second instar. D. Third instar. E. Fourth instar. F. Fifth instar. G. Pupa, lateral view.

with a dark frons. The body is pale brown after first feeding and takes on a greenish brown tone by the end of the instar. The body is adorned with numerous tubercles and short setae.

Second Instar. Fig. 5C. Duration: mean=4.6 days, s.d.=0.89, n=5. Head: mean=0.76 mm, s.d.=0.05, n=4. The head is brown dorsally and has a dark frons. The rudimentary chalazae and tubercles appear in the head with a contrasting light color. The body is brown and is covered with orange bumps and short setae. The pale subdorsal scoli are developing and the pair located on T2 are the most pronounced for the entire body.

Third Instar. Fig. 5D. Duration: mean=4.1 days, s.d.=0.78, n=9. Head: mean=1.2 mm, s.d.=0.04, n=7. This instar is similar to the previous one, with the following changes. The head capsule is more red-brown in this instar. The scoli show further development, with subdorsal scoli

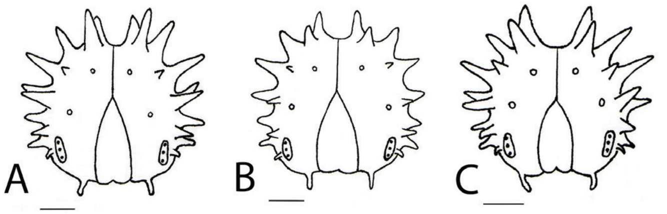

Figure 3. Ultimate instar head capsules of *A. attica attica* (A), *A. epione agilla* (B), and *A. jordani* (C). Scale bar = 1 mm.

on T2, A2 and A7 most pronounced. The pair of subdorsal scoli on T2 have a slightly raised transverse ridge connecting them, and subdorsal scoli on A2 are very close together. Subspiracular scoli appear along the body. The body is dark green-brown dorsally with pale brown tubercles, and laterally there are contrasting pale oblique stripes.

Fourth Instar. Fig. 5E. Duration: mean=5.0 days, s.d.=0.68, n=14. Head: mean=2.7 mm, s.d.=0.03, n=9. The head is similar to that of the previous instar but the chalazae are better developed and the color contrasts less. The body is brown with orange mottling on and between segments. Laterally there are patches of dark brown and orange oblique stripes. The subdorsal scoli on T2 and A7 are the most prominent, with spines developing along their length. The subdorsal scoli on A2, A8 and A10 are short with spines radiating at their tip. The pair of subdorsal scoli on A2 are conical at their base. The subdorsal scoli on T3, A1 and A3-A6 are very reduced with spines radiating directly from the body. The subdorsal scoli on T2 and T3 have a slightly raised transverse ridge of short spines connecting the pair on each segment. There is a green stripe under the spiracle on segment A8, that continues onto A9 and A10.

Fifth instar. Fig. 5F. Duration: mean=7.1 days, s.d.=1.2, n=13. Head: mean=3.6 mm, s.d.=0.27, n=8. The head is dark brown to black and the frons has a pair of weakly contrasting vertical light brown streaks. Chalazae on the head are triangular, light brown and arranged in 3 series: 6 posterior, 4 medial, 2 anterior (Fig. 3B). The body is brown with strongly contrasting patches of dark brown laterally on A3-A4, and on A7-A9 dorsally. There are pale brown oblique stripes laterally along the abdomen. Some individuals maintained the green stripe under the spiracle on segments A7-A10, whereas in others the stripe was brown and faded or absent during this instar. The subdorsal scoli on T2 are the longest, followed by A7 and A10. Subdorsal scoli on A2 and A8 are shorter with those on the remaining segments very reduced. Supra- and subspiracular scoli are also very reduced to absent. The T2 subdorsal scoli are dark brown, cylindrical, thin, and arced anteriorly. The spines along the length of the T2 scoli are arranged radially and are orange-brown at the tip. A slightly raised transverse ridge connects the T2 scoli at their base, and the ridge is black on its anterior side. The subdorsal scoli on T3 and A3-A4 have spines radiating at their tip in groups of 3-5. The subdorsal scoli on A2 are conical and fused together at their base, with spines radiating from the tip. Subdorsal scoli on A5-A6 and A9 are the shortest present on the whole body. The A8 scoli are cylindrical and paler at the base, with spines arising from the crown. The subdorsal scoli on A10 are slightly arced anteriorly. All spines arising from the subdorsal scoli are orange-brown to pale brown and vary in size and thickness. Subspiracular scoli on A3-A7 have a crown of 1-3 spines.

Pupa. Fig. 4C, D and Fig. 5G. Duration: mean=8.8 days, s.d.=0.38, n=13. The pupa is dark brown with green and light brown tints. The head horns (apical projections) are small triangles and slightly curved to the sides with little separation at the base. The thoracic projection is reduced to a slight hump. The abdominal projection is larger than the thoracic projection, curved anteriorly and hook-shaped when viewed laterally. Segments A3 and A4 have a pair of dorsolateral tubercles that are irregularly shaped and dark colored (Fig. 4C). The ventral keel on

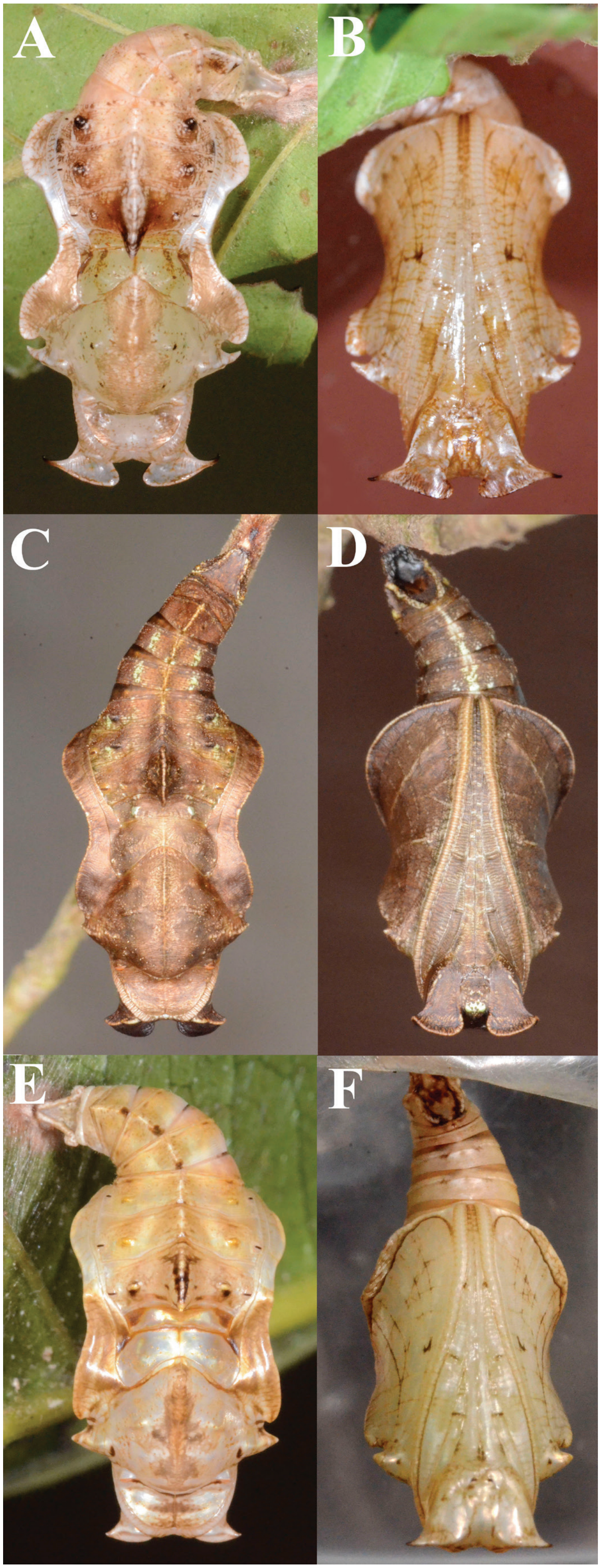

Figure 4. Pupal images of *A. attica attica* (A, B), *A. epione agilla* (C, D) and *A. jordani* (E, F) reared in this study. Dorsal view is on the left of each image and ventral view is on the right.

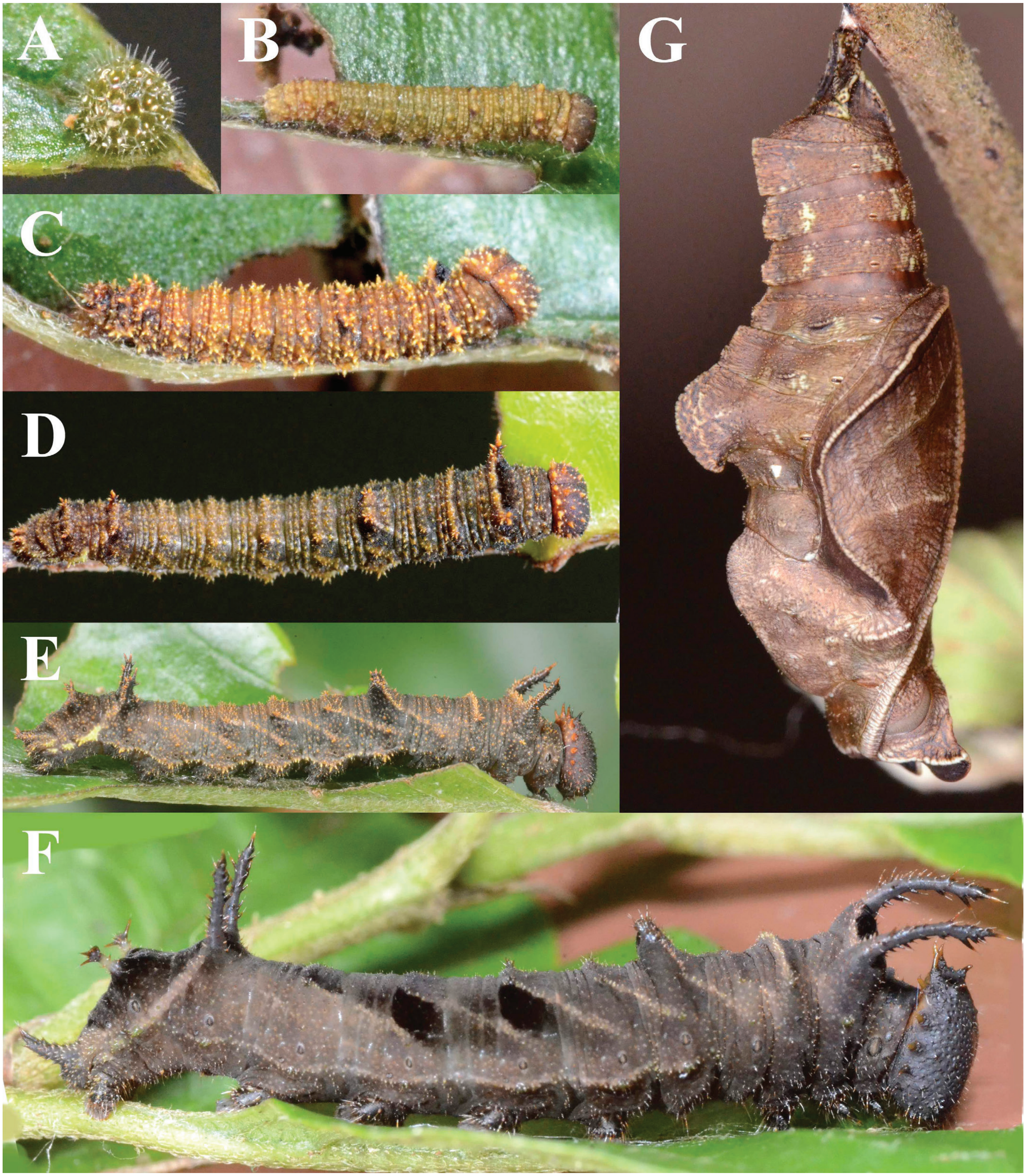

Figure 5. Immature stages of *Adelpha epione agilla*. A. Egg. B. First instar. C. Second instar. D. Third instar. E. Fourth instar. F. Fifth instar. G. Pupa, lateral view.

A7 is squared off and slopes abruptly into A8. Beginning at A5 there is a green-yellow ventral stripe that runs longitudinally to the end of the abdomen (Fig. 4D). The ventral portion of A8/9 has green-yellow curved ridges and the cremaster is dark brown.

Food plant and oviposition. The immature stages of *Adelpha epione agilla* were found eating leaves of *Chomelia tenuiflora* Benth. (Rubiaceae). Eggs were laid singly on the upper surfaces of leaves at an intact leaf margin or tip, as well as along damaged portions of leaves. Eggs were placed on mature trees from 1-3 m above the ground, in areas that were shaded or that directly received sun. A single female was observed ovipositing at midday on a sunny day. She laid eggs singly at 3.5-4 m, on the upper side of leaves at the leaf tip. Eggs were laid quickly by landing mid-leaf, feeling for the leaf edge with her abdomen and backing down the leaf, feeling for the tip.

Larval Behavior. Once hatched, larvae ate the remains of the egg and began eating at the border of the leaf either at the central vein or at a secondary vein. After the vein was exposed, the larvae silked together pieces of frass to extend the vein. The larvae rested on this frass chain when not feeding, either in a straight position or the front-curved position (as described by Aiello, 1984). At the base of the frass chain, bits of leaf and frass were silked together creating a barricade between the leaf and the extended vein. This barricade sat on top of the leaf or hung below. Larvae quickly retreated from the frass chain to the leaf when disturbed. During the fifth instar, the larvae no longer created or used frass chains and ate the entire leaf. Fifth instars rested at the base of the leaf on the upper or lower surface in the curled position (Aiello, 1984), in which the larva curls to one side into a "C", or in the front-arched-rear-up position. Pupation in captivity occurred at any place on the leaf, branch or on the rearing bag.

Adult. The known range of *A. epione agilla* is Colombia to Bolivia, east of the Andes. Although individuals have been found in flat lowland rain forest as low as 200 m (Willmott, 2003b), this taxon was relatively rare at the study site but consistently found at the edge of the Tiputini River and adjacent open clearings inside the forest.

Adelpha jordani Fruhstorfer, 1913

First Instar. Fig. 6A. Duration not observed. Head: mean=0.48 mm, n=3. Like other *Adelpha*, the body is covered with small pale bumps and short setae. The body is pale green after first feeding and tapers noticeably toward the posterior. The head bears setae, it is pale brown dorsally and laterally and has a dark frons.

Second instar. Fig. 6B. Duration: mean=3.0 days, n=3. Head: mean=0.78 mm, s.d.=0.04, n=4. The body is greenish brown and bears stubby scoli and tiny spots. The pair of subdorsal scoli on T2 are slightly larger than others. The head has a spiny appearance with tubercles and rudimentary chalazae, both light colored. The head is light brown dorsally and has a dark frons.

Third instar. Fig. 6C. Duration: mean=3.1 days, s.d.=0.38, n=7. Head: mean=1.2 mm, s.d.=0.04, n=9. The body is dark green to brown, slightly darker at each end, and the body color contrasts with lighter colored scoli and setae. The subdorsal scoli on T2 are the most pronounced along the body, with a slightly raised transverse dorsal ridge. Short subdorsal scoli are also present on T3 and A2-A8. The head is very similar to the previous instar but the chalazae are better developed.

Fourth Instar. Fig. 6D. Duration: mean=3.1 days, s.d.=0.30, n=11. Head: mean=1.9 mm, s.d.=0.07, n=10. The head is light brown dorsally and has a dark frons with light brown vertical stripes that reach from the m1 chalazae to the middle of the frons. The chalazae are mostly light brown with m1 and m2 dark colored. The body is dark brown to black with paler tubercles and a dorsal olive green or brown "saddle" patch. The "saddle" extends from abdominal segment 2 or 3 through segment 6. The subdorsal scoli on T2 are the most prominent for the entire body, followed by T3 and A7-A8, with spines radiating along the length of all these scoli. The subdorsal scoli on A3-A6 have spines radiating at their tip. The A1 and A10 subdorsal scoli are short with spines radiating directly off the body in groups of 3-5. Several subspiracular scoli show beige patches at their base.

Fifth Instar. Fig. 6E. Duration: mean=4.5 days, s.d.=0.52, n=12. Head: mean=3.2 mm, s.d.=0.0.61, n=12. The body is dark brown to black and covered with tan bumps resulting in a mottled appearance. There is an olive green to brown "saddle" patch dorsally, that extends from segment A2 or A3 to A6. The head is patterned similar to the previous instar but with chalazae more developed. The chalazae are elongated and triangular in shape, and arranged in 3 series: 6 posterior, 4 medial, 2 anterior (Fig. 3C). The body is adorned with prominent subdorsal scoli and reduced subspiracular scoli. The T2 scoli are relatively long, cylindrical and slightly arced anteriorly. The subdorsal scoli on T2-T3 and A2-A8 are dark apically and light green basally and have well developed spines along their length. The subdorsal scoli on A1 and A10 are shorter with spines radiating at their tip in groups of 3-5. The supraspiracular scoli on T2 are pale brown colored with spines radiating at the tip, and shorter than the subdorsal scoli on the same segment. Subspiracular scoli on A2-A8 are reduced and have a crown of 1-3 spines.

Pupa. Fig. 4E, F and Fig. 6F. Duration: mean=8.0 days, s.d.=0.39, n=14. The pupa is pearly white with copper tints and has brown lines and mottled patches. The pupa darkens the day before adult eclosion. The apical projections of the head are shaped like tiny asymmetrical leaves. The dorsal thoracic projection is pointed and directed posteriorly. The abdominal projection is slightly larger than the thoracic projection, like other *Adelpha*, and is pointed and directed anteriorly. Abdominal segments from A7 to A3 have ventral keels with squared shaped when viewed laterally. Segments A2-A4 have a pair of dorsolateral tubercles that have flattened cone shape (Fig. 4E). Some individuals have these tubercles dark colored. Segment A9 has a pair of curved black ridges ventrally. The cremaster is pale.

Food plant. The larval food plant of *Adelpha jordani* was found to be *Calatola costaricensis* Standl. (Icacinaceae). The plant was first identified as *C. venezuelana* Pittier, but we use the name *C. costaricencis* since *C. venezuelana* is recognized as a synonym (Tropicos.org, 2018). *Calatola* is a poorly understood genus because of its dioecious condition and its small flowers (De Stefano *et al.*, 2013). *Calatola costaricensis* has a distribution from Mexico to South America (Colombia, Venezuela, Ecuador, Brazil, Peru and Bolivia) (Tropicos.org, 2018). In Ecuador *C. costaricensis* is found from sea level to 1500m in the coastal, Andean and Amazonian regions of this country (Jorgensen & León-Yánez, 1999). *Calatola costaricensis* exhibits ample morphological variation and possibly includes more than one species (e.g., *C. venezuelana*, Vera-Caletti & Wendt, 2001). Larvae were collected in a low-lying area of primary forest habitat that is flooded by the Río Tiputini. *Adelpha jordani* was found on saplings and mature trees from 1.4 to 2.4 m above the ground in shaded areas. The information on the food plant and immature stages reported by Moss (1933) for "*A. jordani*" actually applies to *Adelpha heraclea* (C. Felder & R. Felder, 1867).

Larval behavior. The larvae fed on the leaf margin or tip leaving either the central or secondary vein intact. Eventually, the larvae extended the vein by addition of frass with silk. Once the frass chain was created, the larvae rested on it when not feeding, either in the straight position or the front-curved position (Aiello 1984). During the first instar, molting took place on the frass chain. This species was not observed constructing a barricade with frass, only bits of leaf were silked together, suspended beneath the base of the frass chain. After the larvae molted to the final instar, they abandoned the frass

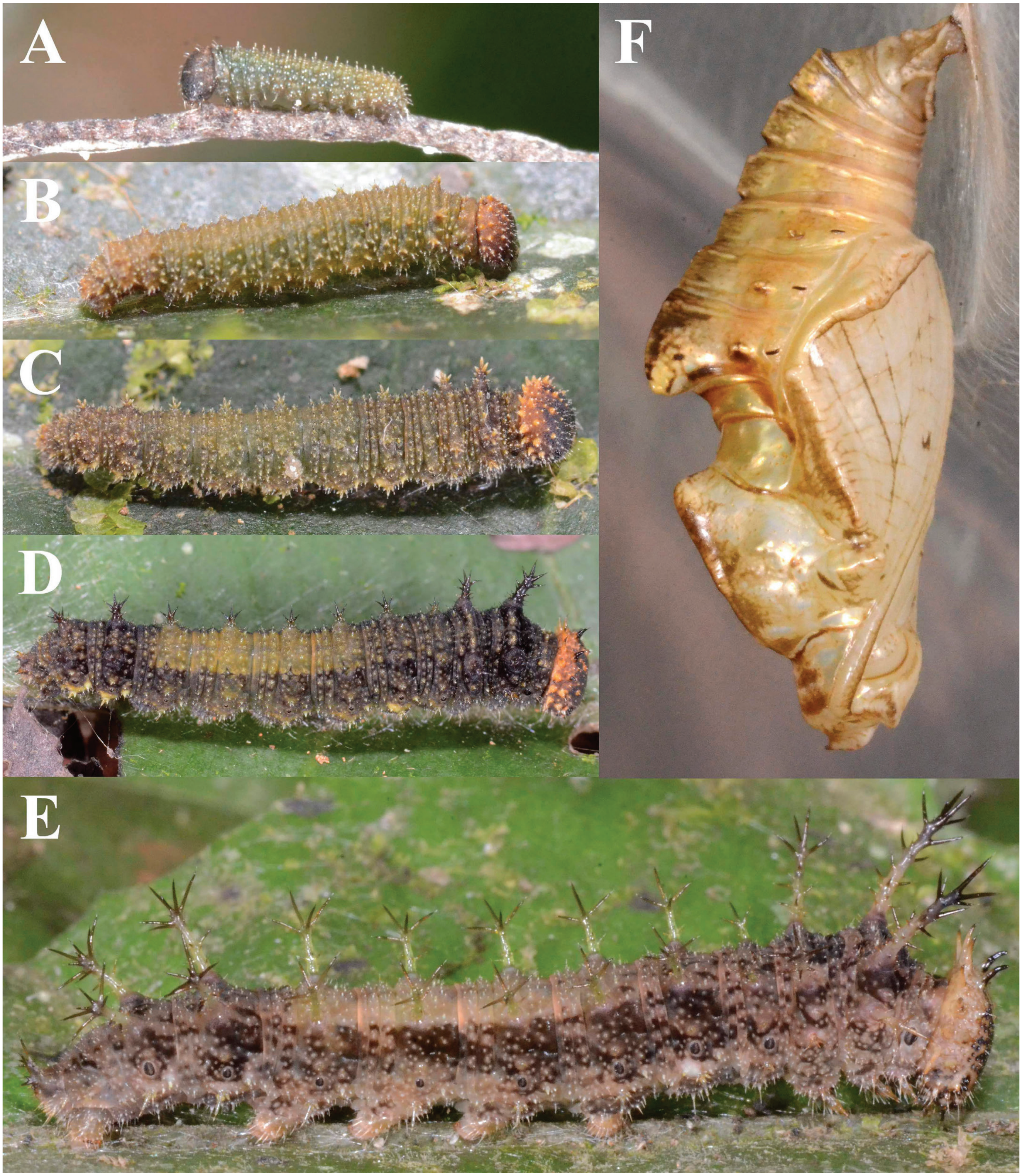

Figure 6. Immature stages of *Adelpha jordani*. A. First instar. B. Second instar. C. Third instar. D. Fourth instar. E. Fifth instar. F. Pupa, lateral view.

chain and rested on the upper or lower surface of the leaf in a straight position, curled position or in the front-arched-rear-up position (Aiello, 1984). Pupation occurred on the underside of the leaf or on the rearing bag.

Adult. *Adelpha jordani* has been recorded from eastern Ecuador to Bolivia between 250-1200 m, according to Willmott (2003b). It occurs in eastern Ecuador in a variety of habitats, from primary to selectively logged forest, along rivers and on ridge tops (Willmott, 2003b). In the study area, it was observed inside the forest, but in low lying, potentially flooded, areas near the Tiputini River. Adult males were observed flying very low and lazily in the morning.

DISCUSSION

The immature stage morphology of three *Adelpha* species is described and published here for the first time. Although Moss (1933) reported that *A. jordani* feeds on *Vitex* (Verbenaceae), his voucher specimens in the Natural History Museum, London, United Kingdom, show this species to be *A. heraclea* (Willmott, 2003b). This error was presumably due to the wing pattern similarities between these species and poor knowledge of distinguishing characteristics at that time. To help clarify relationships within this confusing group of butterflies, as many characters of the adult and immature stages should be studied as possible. For example, the immature stages of *A. serpa serpa* from southern Brazil (Oliveiro-Neto *et al.*, 2016) are remarkably different in comparison to those of *A. serpa celerio* from Costa Rica (Janzen & Hallwachs, 2019), suggesting that these taxa are not conspecific. By describing here the life history of three *Adelpha* species we hope to contribute to an improved understanding of the taxonomy and relationships in this genus.

The immature stage morphology of the three species described here is consistent with previous systematic hypotheses and phylogenetic analyses (Willmott, 2003a; Ebel *et al*., 2015). *Adelpha jordani* is hypothesized to be closely related to *A. zina* based on similar wing pattern and genitalia (Willmott, 2003b). The similarities found in the pupal and larval morphology between these two species and the fact that both species use the genus *Calatola* (Icacinaceae) as larval food plant (Janzen & Hallwachs, 2019), support this hypothesis. In the ultimate larval instar, both species exhibit a dark brown larval coloration. Some individuals have a pale patch dorsally on segments A3-A6. All the scoli are pale with darker tips, and they terminate in a rosette of thin spines. The subdorsal scoli on T2 are the longest, with those on T3, A7 and A8 being about half that length, and the remaining scoli are even shorter. The pupae of these species are metallic gold, with short dorsal projections on T2 and A2; and small, slightly laterally pointed head horns (see Figs. 4E, F; Janzen & Hallwachs, 2019).

The placement of *A. epione* and *A. ethelda* as closely related species (Ebel *et al*., 2015) is also corroborated by the descriptions presented here. These two species share a number of distinctive characters in the wing pattern and male genitalia (Willmott 2003b), and they both feed on Rubiaceae. DeVries (1987) reports that *A. ethelda sophax* uses *Sabicea* as a food plant in Costa Rica, and the taxon has also been observed feeding on *Chomelia spinosa* Jacq. and *Chomelia microloba* Donn. Sm. in Costa Rica (R.I.H. pers. obs.; Janzen & Hallwachs, 2019, respectively). Our observations indicate *A. epione agilla* uses *Chomelia tenuiflora*. DeVries (1987) stated that the mature larval coloration and the head capsule chalazae of *A. ethelda* were similar to those of *A. cytherea*, but this is only apparent in the broadest sense. Images of the immature stages of *A. ethelda sophax* (17-SRNP-32415 to 17-SRNP-32417) provided by Janzen & Hallwachs (2019) show that species to be very similar to *A. epione*, and, interestingly, the last instar larvae of *A. epione* and *A. ethelda* are clearly distinguished from any other *Adelpha* larvae. The subdorsal scolus on T3 is reduced to a few short spines in both species. *Adelpha epione* has well developed T2, A2 and A7-8 subdorsal scoli, and the remaining scoli are very reduced; whereas, *A. ethelda* has the subdorsal scoli on A7 short, and those on A8 are reduced to a few short spines. The subdorsal scoli on A2 are conical and fused together at their base in both species. The head capsule of *A. epione* and *A. ethelda* has short m1 chalazae and m2 reduced to a small bump. The pupa of both species has the T2 dorsal projection reduced to a slight hump, and the head horns are small triangles bent laterally in both species (see Figs. 4C, D and Janzen & Hallwachs, 2019).

A recent molecular analysis found *A. attica* and *A. leucerioides* to be closely related (Ebel *et al.*. 2015). However, *A. leucerioides* is believed to be closely related to *A. leuceria* based on wing pattern (Willmott, 2003b) making it very likely that *A. attica* is the sister species of both *A. leuceria* and *A. leucerioides*. Since there are no life history descriptions of *A. leucerioides*, we expected that the immature stage characters of *A. attica* and *A. leuceria* might be similar. However, both the larval and pupal morphology of these two species are quite different. The only published information on the immature stages of *A. leuceria* is based on personal communication from A. Muyshondt in Willmott (2003b), with no voucher specimen examined, but assuming that the species has been correctly identified, the following comparison can be made. The *A. leuceria* last instar is mottled with dark colors and has a broad lateral band on A4-6. The larvae of *A. attica* are mottled with black and green along the body and pink-purple between segments; and there is a lateral thin, white band on segments A2-A7, with black and oblique stripes. The subdorsal scolus on T3 of *A. leuceria* is short, whereas *A. attica* has this scolus well developed. The longest subdorsal scoli of *A. leuceria* are thick, whereas in *A. attica* they are thin. The head capsule of *A. leuceria* is brown with paler vertical stripes through chalazae a1 and a3, while *A. attica* is light brown laterally with black frons and beige vertical stripes from the m1 chalaza to the bottom of the frons. The size of the m1 and m2 chalazae between these two species are very similar. They both have pronounced, dark m1 and m2 chalazae that are reduced to a small bump. The pupae of these two species have a pointed thoracic dorsal projection on T2 and the abdominal projection is curved anteriorly and hook-shaped. However, the dorsal T2 projection is smaller in *A. leuceria* than in *A. attica*. The head horns of *A. leuceria* in dorsal view are long, half-moon shaped and curve outwards, whereas in *A. attica* they are small, asymmetrical leaf-shaped and curve outwards (see Figures 4A, B and Willmott 2003b).

The placement of *A. jordani*, *A epione* and *A. attica* into their respective species groups is difficult because of paraphyly of the candidate species groups and still incomplete understanding of phylogenetic relationships, or because of conflicting morphological characters present in these species. *Adelpha jordani*, *A. zina* and *A. milleri* were hypothesized to be within the *cocala*-group (Willmott, 2003a). Since *A. jordani* and *A. zina* appear to be sister species, *A. milleri* is therefore perhaps the sister species to *A. jordani* and *A. zina* (Willmott, 2003b), and in fact *CoI* barcode data support a close relationship between *A. milleri* and *A. zina* at least (Willmott *et al*., unpublished data). These three species have the hypothesized synapomorphic character of the *cocala*-group of lacking sclerotized bands on the corpus bursae in the female genitalia

(Willmott 2003a). However, the monophyly of the *cocala*-group as defined by Willmott (2003a) now seems very unlikely in light of more recent molecular data (Ebel *et al.*, 2015) and the few morphological characters used to define the group. The male genitalia of *A. epione* and *A. ethelda* have dorsally pointing spines at the distal tip of the valvae and a relatively elongate clunicula, which strongly resemble *A. melona*, *A. iphiclus* and relatives of the latter species. In addition, some wing patterns present in *A. epione* and *A. ethelda* might confirm a relatively primitive position in the genus. However, the fusion of many of the principal character elements on the VFW makes it difficult to place *A. epione* and *A. ethelda* within the genus (Willmott, 2003b). The hypothesized close relation between *A. attica* and *A. leucerioides* could not be confirmed with the results presented here because there is no immature morphology information for *A. leucerioides* and the only described immature stages of *A. leuceria* are not similar to those of *A. attica.* In addition, the genitalia and the wing pattern of *A. attica* do not help elucidate to which species group it belongs. The entirely fused VFW postdiscal series of this species is a character typical of the *phylaca*-group, but the shape of the corpus bursae and its sclerotized bands on the female genitalia suggest that *A. attica* belongs neither in the *phylaca*-group nor the *cocala*-group.

In conclusion, we demonstrate here that it is feasible to make a substantial contribution to knowledge of the highly complex immature stage biology of *Adelpha* with short, but intensive, periods of field work. Similar efforts in other areas of the Neotropics could relatively quickly help to fill in the gaps of our knowledge of *Adelpha* immature stages, contributing to refining the taxonomy of the genus as well as our understanding of its evolution and ecology.

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