Life history descriptions of *Adelpha naxia naxia* and *A. nea nea* (Nymphalidae) from Costa Rica

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Running head: *Adelpha naxia* and *A. nea* immatures

ABSTRACT.

Butterflies in the genus *Adelpha* Hübner (Nymphalidae) are a challenging group to identify owing to their convergence in wing patterns among species. Immature stage biology including morphology and hostplant relationships have helped clarify relationships in this group and will likely continue to do so. Here we report descriptions of the immature stages of two species, *Adelpha naxia naxia* (C. Felder & R. Felder, 1867) and *Adelpha nea nea* (Hewitson, 1847), based on recent fieldwork on the Osa peninsula of Costa Rica. We observed *A. naxia naxia* caterpillars feeding on *Vitex cooperi* Standl. (Lamiaceae), and the immature stage morphology corroborates its position in recent phylogenetic analyses. The food plant of *A. nea nea* is *Micropholis melinoniana* Pierre (Sapotaceae), representing the first record of this host family for *Adelpha* and Neotropical nymphalids. *Adelpha nea nea* also appears unique in always developing through six larval instars. In addition to descriptions and discussion of immature stage biology and food plant interactions, adult observations are also provided.

Keywords: immature stages, latex, Limenitidinae, vein cutting, vein trenching.

Butterflies in the genus *Adelpha* are widespread and conspicuous throughout the Neotropics, with species found in a diverse range of habitats extending from the United States to Uruguay (Willmott 2003b). Convergence in wing pattern among species (Ebel et al. 2015; Willmott 2003b) makes species delimitations based on adult morphology difficult, and misidentifications have increased the confusion through the description of numerous synonyms. Work by Willmott (2003b) gave a comprehensive summary of the present knowledge of *Adelpha* systematics and clarified the redundant names. In combination with subsequent work (Prudic et al. 2008; Willmott & Hall 2013), the genus contains more than 90 species and more than 120 additional subspecies.

Immature stages have been useful in helping to clarify *Adelpha* species groups and understand taxonomy in the face of mimetic similarity among adults (Aiello 1984, 1991; Otero & Aiello 1996). Using immature stage morphology, Aiello and others (Aiello 1984, 1991; Otero & Aiello 1996) identified seven or more species groups within *Adelpha*. Based on his studies of adults and immatures, Willmott (2003a) rearranged *Adelpha* into six provisional groups, after finding some of the previous groups to be polyphyletic. Recent molecular work on the phylogeny of *Adelpha* corroborates some of these species groups (e.g. *A. alala*, *A. serpa*, *A. phylaca*) (Ebel et al. 2015) and allows further exploration of the traits associated with hypothesized clades.

Despite the series of studies focused on this genus thus far, there remains much to be discovered. Willmott (2003b) and Aiello (2006) called for publishing any information on *Adelpha*, and on life history stages in particular, in order to clarify species boundaries, determine larval host breadth, and elucidate mimicry patterns. During recent fieldwork in Costa Rica we discovered the immature stages and larval food plants for two *Adelpha* species with previously unknown life histories, *A. naxia* (C. Felder & R. Felder, 1867) and *A. nea* (Hewitson, 1847). In this paper we describe the life histories of those two species, and discuss their immature stage characters as related to identification and systematic relationships, as well as larval behavior and food plant interactions, and also provide observations on adult behavior.

Materials & Methods

Observations were made in the vicinity of Barrio Guadalupe (08.61801,-83.48025), near the town of La Palma, in the Osa Peninsula, from June to August 2016. In general, forest edges, and ridges in primary forest were searched for larvae and adults. *Adelpha naxia naxia* were collected in secondary forest habitat and edges, whereas *Adelpha nea nea* were collected along the sides of a narrow dirt road that follows a ridge in primary forest. Larval food plants were identified by Orlando Vargas and Reinaldo Aguilar F., as well as by referencing the Organization for Tropical Studies online plant database.

Larvae were reared individually in plastic bags under ambient conditions. Bags were hung along a line so that leaves would approximate natural positions, and to expose leaf margins for larvae to build frass chains. Larval bags were cleaned daily with toilet paper. Larval and pupa 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 a ring flash (Nikon SB-R200 Speedlight).

Larval descriptions were made based on photos taken during rearing. Morphological terminology follows that described and used by Willmott (2003b) and Aiello (2006). We use the taxonomic arrangement of Willmott(2003b). Reported head size is based on measurements of head capsules. Head capsule drawings and measurements were made with a Leica S8 APO stereomicroscope with an attached Leica DFC295 camera. Head capsule width was measured at the widest part (generally near the most dorsal stemmata) using Leica Application Suite version 3.8.

Results

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

Food plant and oviposition. The larval food plant of *Adelpha naxia naxia* was found to be *Vitex cooperi* Standl. (family Lamiaceae, formerly placed in Verbenaceae). *Vitex cooperi* is found from sea level to 600m in secondary habitats and along edges in tropical moist to wet forests, and is native from Guatemala to Panama (Croat 1978). The range of *A. naxia* extends beyond that of *V. cooperi*, being found from Mexico to as far south as Bolivia and the Amazon basin of Brazil. Eggs were laid singly on the upper surfaces of leaves at the leaf margin or tip, from one to four meters above the ground. Plants containing eggs were mature trees in open habitats. It should be noted that DeVries' host records for *A. naxia* feeding on *Vitex cooperi* and *Piper arieianum* C.DC. (Piperaceae) reported in Aiello (1984) were errors (see Appendix C of Willmott 2003b), and actually pertain to *A. heraclea* (C. Felder & R. Felder, 1867).

Vitex cooperi is also a host for *Adelpha heraclea heraclea* (C. Felder & R. Felder, 1867) in Costa Rica (CER pers. obs, DeVries 1985(86)), and eggs and larvae of *A. h. heraclea* were found on *V. cooperi* at the study site. Both *A. n. naxia* and *A. h. heraclea* were found intermingled on the same trees, but not on the same leaf. *Adelpha h. heraclea* immatures were found in much higher abundance than *A. n. naxia* when collected haphazardly from the three main trees where immatures were found.

Egg. Fig. 1. Duration unobserved. Like other *Adelpha* the egg is pale greenish, 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. 1. Duration: 4 days, n = 1. Head: 0.63 mm, n = 1. Very similar to the first instar of other *Adelpha* species. The head is rounded with short setae, it is pale brown dorsally and laterally, and has a dark frons. The body is pale green after first feeding and takes on a brown hue by the end of the stadium. The body tapers noticeably toward the posterior, is darker in between segments, and is adorned with numerous tubercles and short setae.

Second Instar. Fig. 1. Duration unobserved. Head: 0.86 mm, n = 1. The head is brown dorsally and medially and has a black frons. The body is dark brown with shades of black between segments, and is covered with yellow-white bumps and short setae. Developing subdorsal scoli are most pronounced on T2 and A2.

Third Instar. Fig. 1. Duration: 6 days, n = 1. Head: $\bar{x}$(xbar) = 1.19 mm, s.d. = 0.22, n = 3. The head is entirely brown-black with tubercles and developing chalazae that are both light colored. The body is dark brown to black, darkest between segments. Short, dull brown-orange setae cover the body. A pair of pronounced subdorsal scoli are present on T2 with a slightly raised transverse ridge connecting them. Short subdorsal scoli are also present on T3 and abdominal segments A2-A8 and A10.

Fourth Instar. Fig. 1. Duration: x̅(xbar) = 4.4 days, s.d. = 0.60, n = 5. Head: x̅(xbar) = 2.07 mm, s.d. = 0.10, n = 4). The head is similar to that of the previous instar but the chalazae are better developed and the colors contrast less. The body is dark brown to black, and is textured with yellow-white bumps. Only subdorsal scoli are present. The pair of subdorsal scoli on T2 are most prominent, with spines developing along their length. The scoli on T3 and A3-A6 have spines radiating directly off body in groups of 3-5. The scoli on A7-A8 are short with radiating spines at their tip, and A10 has short scoli.

Fifth Instar. Fig. 1. Duration: x̅(xbar) = 7.7 days, s.d. = 1.0, n = 6. Head: x̅(xbar) = 3.27 mm, s.d. = 0.07, n = 5. The head is dark brown to black and the frons has vertical pale-brown streaks laterally. Some variation is seen in the width of the streaks on the frons, and they also vary from off-white to dark brown. Chalazae on the head are triangular, dark brown with black tips, and arranged in 3 series: 6 posterior, 4 medial, 2 anterior (Fig. 2). The body is dark brown with shades of black and is covered in small pale yellow dots. A subspiracular yellow-green spot is present on A2, and a similarly colored subspiracular streak is present on A7-A8. The body becomes pale brown the day before pupation. The body is adorned with prominent subdorsal scoli and reduced subspiracular scoli. Subdorsal scoli on T2 are relatively long, cylindrical, notably thick and arced anteriorly. The T2 scoli are dark brown, with many spines along their length. These spines are orange-brown on the posterior side and dark brown on the anterior side. A slightly raised transverse ridge connects the T2 scoli at their base and is nearly black on its anterior side. Subdorsal scoli on T3 are conical and pale-tan with a dark brown tip and 3-5 spines along their length. Scoli on A2 are conical and thicker than the T3 scoli, and show variation between individuals. The A2 scoli range from dark brown to tan, and have orange-brown spines along their length and radiating from the tip. Subdorsal scoli on A3-A6 are the shortest present. They are pale tan and conical with 3-5 spines radiating at the tip. The A7-A8scoli resemble A3-A6 in shape, but are longer and dark brown at the crown where the spines arise. Variation was seen in one individual with the A2 scoli paler, resembling the A7-A8 scoli. All spines arising from the subdorsal scoli are orange-brown and tipped with black, and vary in size and thickness. Subspiracular scoli on A3-A7 are somewhat reduced, consisting of a crown of 1-3 spines.

Larval Behavior. Larvae were observed feeding in a manner typical for the genus. 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, eating around the vein to expose it. After the vein was exposed the larvae silked together pieces of frass to extend the vein. They then rested on this frass chain when not feeding, either in a straight position or the front-curved position (as described by Aiello 1984) with the head and thorax lifted off the substrate. 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 to their frass chain when the leaf was disturbed. This behavior continued through the fourth instar. During the fifth stadium, the larvae no longer created or used frass chains, and ate the entire leaf. Larvae rested at the base of the leaf on the upper or lower surface in the front-arched-rear-up position (Aiello 1984), in which both the anterior and posterior portions of the body were raised and arched, or in a straight position along the main vein. Pupation did not occur at a specific place on the leaf, and larvae often attached themselves to the side of the plastic rearing bag.

Pupa. Fig. 1 and Fig. 3. Duration: $\overline{x}$(xbar) = 7.1 days, s.d. = 0.30, n = 9. The pupa is pearly white with copper tints, and has dark sutures, spots, and streaks that are highlighted with orange-brown. The pupa darkens the day before adult eclosion. As is characteristic for *Adelpha*, the head bears a pair of apical projections (appearing as "head horns"). In *A. n. naxia* these projections are widely separated at the base (as in *A. h. heraclea*), and shaped like small laterally pointing triangles. The thorax rises abruptly posterior to the head and forms a keel posteriorly along the dorsal midline. On T2 the dorsal keel leads into a projection that forms a slight hump directed posteriorly. A dorsal projection, curved sharply to the anterior, is also present on A2, and is slightly larger than that on T2. The dorsal projections on T2 and A1 are moderately sized for the genus. Posterior to A2 each abdominal segment has a dorsal keel that is smaller than that of the preceding segment. Segments A3 and A4 have a pair of dorsolateral tubercles that are irregularly shaped and dark in color. These partly create a rough broken line from A2 merging with the dark colored spiracles. The abdomen also has two ventral series of dark spots, one ventrolateral and one ventral, with the ventrolateral series smaller than the ventral series. Segment A9 has a short lateral spine and a pair of curved black ridges ventrally. The cremaster is pale, tipped with black. The wing pad is unmarked except for brown along wing veins. Pupation occurred on the surface of the leaf or on the rearing bag.

Adult. Fig. 4a. Based on our field observations adult male *A. naxia naxia* were found flying from early morning to early afternoon (2-3pm). In the morning males were observed basking very low or on the ground in direct sunlight, and flying lazily compared to their afternoon flight.

Adelpha n. naxia male perching behavior appeared unique compared to other *Adelpha* males in Costa Rica. *Adelpha n. naxia* males perched as low as 1 m and higher, with the highest observed perch being ~8m. In contrast, our observations of *A. serpa celerio* (H. W. Bates, 1864), *A. erymanthis erymanthis* (Godman and Salvin, 1884), *A. salmoneus salmonides* (Hall, 1938) and *A. n. naxia's* sister species *A. h. heraclea* (Ebel et al. 2015), indicate that these species typically perch high (usually observed 5m and higher) and defend their perches with direct fast flight. Furthermore, the flight of *A. n. naxia* males while defending their perches was especially fast and erratic with seemingly higher accelerations compared to the aforementioned species.

Adelpha nea nea Hewitson, 1847

Food plant and oviposition. Immatures of *Adelpha nea nea* were found eating leaves of *Micropholis melinoniana* Pierre (Sapotaceae). This tree ranges from Brazil to Mexico in moist to wet lowland tropical forests, at low to medium elevations, and the damaged parts dripped a white latex (Condit et al. 2011; Gentry 1993). Immatures were found on a single tree. The host plant grew on a ridge in primary forest at the edge of a road that created a gap through which sun could reach during the middle of the day. Eggs were laid singly on mature trees and on old or damaged leaves. Eggs were placed along the leaf edge or at damaged portions of the leaf.

Egg. Fig.5. Duration unobserved. Like other *Adelpha* eggs, pale green and round with hexagonal pits that have a seta at each angle. *Adelpha nea nea* eggs are relatively large for *Adelpha*, similar to other members of the *serpa* group.

First Instar. Fig.5. Duration: $\bar{x}$(xbar) = 4.3 days, s.d. = 0.50, n = 4. Head: $\bar{x}$(xbar) = 0.85 mm, s.d. = 0.38, n = 4. Similar to other species of *Adelpha*. After hatching, the larva is green-grey and bears small pale bumps along the body, with short setae on the head. The head is light brown with a black frons. By the end of this instar the body is light brown with small bumps where scoli will be in later instars.

Second Instar. Fig. 5. Duration: $\bar{x}$(xbar) = 5.4 days, s.d. = 0.80, n = 7. Head: $\bar{x}$(xbar) = 1.12 mm, s.d. = 0.57, n = 3. The larva is brown with shades of black, blending in with the frass and leaf debris, and the body tapers slightly to the posterior. The head is brown with a black frons, with the black extending dorsally to the rudimentary m1 chalazae. The subdorsal scoli begin to show in this instar and are distinct from the other miscellaneous lighter colored bumps by the end of this stage.

Third Instar. Fig.5. Duration: $\bar{x}$(xbar) = 5.3 days, s.d. = 0.80, n = 6. Head: $\bar{x}$(xbar) = 1.50 mm, s.d. = 0.62, n = 5. This instar is very similar to the previousone, with the following changes. The scoli are more developed this instar, with subdorsal scoli on T2, A2, A4 and A7 most pronounced. Both supra- and subspiracular scoli appear along the body. The larva is brownand contrasts with lighter colored scoli,and there are darker black patches present as well as more apparent black bands between segments.

Fourth Instar. Fig.5. Duration: $\bar{x}$(xbar) = 5.2 days, s.d. = 1.0, n = 6. Head: $\bar{x}$(xbar) = 2.03 mm, s.d. = 0.83, n = 5. The body is brown with shades of darker brown and light brown mottling on segments, and light brown between segments. The head is the same light brown as the body, but has darker vertical stripes down the frons, and small distinct patches laterally between the first and second rows of developing chalazae. Thick short subdorsal scoli are present on T1, T2, A2, A4, A7, and A8. The subdorsal scoli terminate in an irregular spreading crown of spines. A9 and A10 have a pair of thick dorsal scoli.

Fifth Instar. Fig.5. Duration: x̅(xbar) = 6 days, s.d. = 0.89, n = 6. Head: x̅(xbar) = 2.85 mm, s.d. = 1.16, n = 5. The body coloration in this instar is a combination of dark and tan patches with purple-brown showing between segments. The head is patterned similar to the previous instar but with chalazae more developed. The chalazae are relatively pale compared with the head. The subdorsal scoli are brown, and are generally long and cylindrical with spines arising at irregular intervals along their entire length and clustered near the tip. Subdorsal scoli are well developed on all segments except A1. The presence of well-developed subdorsal scoli on A9 in *A. nea nea* is distinct from other *serpa* group species. The prolegs are relatively pale, as are the scoli arising dorsal to them.

Sixth Instar. Fig.5. Duration: x̅(xbar) = 10.2 days, s.d. = 0.98, n = 6. Head capsule: x̅(xbar) = 3.94 mm, s.d. = 1.61, n = 4. Distinct in color from the previous instars. This instar is predominantly mottled pale green and dark green with pink to purple bands between segments. The larva changes to yellow-brown the day before pupating. The head is pale tan with distinct brown vertical stripes along the frons reaching from the tip of the m1 chalazae to the bottom of the frons. The lateral part of the head is dark brown near the anterior and medial rows of chalazae. The chalazae are pale brown with m1 having a brown anterior face. The chalazae are elongated and triangular in shape, and arranged in 3 series: 7 posterior, 4 medial, 2 anterior (Fig. 2). Subdorsal scoli are the best developed and present on each segment except T1 and A1. Subdorsal scoli are generally relatively long and cylindrical with intermittent spines, and end in an asymmetric fan of flattened spines. Subdorsal scoli are shortest on A5, A6, A9, and A10. The subdorsal scoli are brown and have dark green patches at the base, except forA9 and A10 which are pale green. Supraspiracular scoli on T1 are cylindrical and pale brown with intermittent spines along the length and curved slightly dorsally. Short supraspiracular scoli are found T3, A2-A7, are pale green and have 2-6 spines radiating at the tip. Supraspiracular scoli on T2 are slightly shorter than the subdorsal scoli, and are pale brown. Reduced subspiracular spines are found on A2-A8, and these pale green spines project directly from the body in groups of 2-4 spines. Prolegs are tan.

Larval behavior. Upon hatching, larvae alternated between eating the remains of the egg and wandering over the leaf. Compared to other newly hatched *Adelpha* (CER pers. obs.), *A. nea nea* wandered over more area and for a longer time before beginning to eat at the border of the leaf, generally at points of leaf damage, where a frass chain was made. To feed, larvae first made an arc or U-shaped cut basal to where the frass chain would be made, effectively making an island of leaf to consume. The cut was generally perpendicular to the secondary, inter-secondary and tertiary veins (which are parallel in this host plant), but did not cross the main vein of the leaf (Fig. 6). Once this cut was made they commenced eating distal to the cut and worked toward the leaf tip or leaf margin in an overall basal-to-apical fashion.

First to fifth instars made frass chains off the main or a secondary vein, and created a mass of silked-together frass and leaf bits. The mass was constructed at the base of the frass chain on top of the leaf, or hanging below the leaf (Fig.6). Early instars rested on the middle or tip of the frass chain, with the body either straight or with the anterior portion raised ("front-curved position" of Aiello 1984). If disturbed while feeding, larvae retreated to the frass chain and either rested in the front-curved position or began adjusting the mass. If further disturbed by touch they would thrash about and head-butt the source of the disturbance. Early instars rested on frass chains during molts, but molting to the sixth larval stadium took place on the surface of the leaf.

During the sixth stadium the larva 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-with-spines-out position (in which the larva is curled to the side in a C or J shape with scoli pointing outward all around) typical of other *Adelpha* (Aiello 1984). Pupation occurred on the underside of the leaf or on the rearing bag.

Pupa. Fig.7. Duration: $\bar{x}$=9 days, s.d. = 0, n=6. Typical of species in the *serpa* group. Pupa is shimmering silver after first day, with dark sutures and orange-brown highlights. The pupa turns black the day before eclosion. The head has short, sickle-shaped lateral projections that are widely separated at the base. The thorax rises abruptly behind the head and has a dorsal keel that merges into the larger dorsal projection on T2. The T2 projection slopes gradually from the head and then drops off abruptly to T3, and has a rounded apex. There is also a dorsal projection on A2 that is somewhat square when viewed laterally. This projection is curved anteriorly at the apex, and arises abruptly on the anterior side. On the posterior side the projection slopes somewhat gradually into A3 where a dorsal keel continues with rounded apices on A3-A6. The keel on A7 is squared off and slopes abruptly into A8. The T2 and A2 projections greatly resemble those of *A. serpa*. There are short dorsolateral projections present on T2, T3, and A2-A4. Those on T2-T3 each make a narrow transverse ridge. The projections on A2 are rounded bumps, and those on A3-A4 are conical tubercles with sharp tips. On the abdomen these tubercles are dark colored, forming the beginning of a dark dorsolateral stripe that runs dorsal to the spiracles along the length of the abdomen. Beginning at A4 there is a dark ventrolateral stripe that runs to the end of the abdomen. The ventral portion of A8/9 has dark curved ridges and the cremaster is dark brown.

Adult. Fig. 4b. The known range of *A. nea nea* is Costa Rica to Venezuela and southern Peru, Amazonian Brazil and the Guianas. Adult morphology of the *A. nea nea* collected in Northeastern Costa Rica and reared in Southwestern Costa Rica matches descriptions by Willmott (2003b) for the subspecies *A. nea nea* and reflects previous knowledge of range and variation. Individuals from Costa Rica that we have studied do not resemble the distinctive *A. nea sentia* (Godman and Salvin, 1884) to the North (potentially Guatemala and Nicaragua to Mexico, Willmott 2003b) and indicate no gradation into *A. nea sentia*. The subspecies are distinguished by *A. nea nea* being entirely orange in cells Cu2-Cu1 and Cu1-M3 of the DFW postdiscal (Willmott 2003b), and this is true of all Costa Rica specimens observed so far both in the north (n = 4) and south (n = 4) of Costa Rica. There is variation in the DFW postdiscal band of *A. nea nea*, across its range and in Costa Rica, with cells 2a-Cu2 being either entirely orange, or orange and white, and the orange sometimes extending to the anal margin.

Adelpha nea nea is a rare species in Costa Rica, and in general throughout its range (Willmott 2003b). Adults were observed only four times in ten months of fieldwork, all instances at La Selva Biological Station in Sarapiqui, never at Guadalupe where immatures were found. Adult males were observed perching from 3-5 m at the edges of secondary forest and at 1m in a gap in primary forest, in both the wet and dry season (June n=2, January n=2). No territorial perching behavior was observed, but possibly it does occur in the canopy (~20 m). Males landed on ripe to over-ripe fruits of fig trees (*Ficus* sp., Moraceae), as well as puddling at water droplets on top of leaves of *Heliconia* L. (Heliconiaceae).

Discussion

Immature stage morphology and systematics. The immature morphology described here agrees with previous systematic hypotheses and strongly corroborates recent phylogenetic analyses. *Adelpha naxia* and *A. heraclea* are hypothesized to be within the same species group (*capucinus* group) based on shared adult characters of the male genitalia and ventral hind wing pattern (Willmott 2003b). However, adult characters in *Adelpha* are known to be misleading (Aiello 1984; Moss 1933), and Aiello (1984) heavily emphasizes the utility of using immatures to determine relatedness among species. A recent molecular analysis (Ebel et al. 2015) recovered *A. naxia* and *A. heraclea* as sister species, and this is supported by several immature stage characters. First, these species both use *Vitex cooperi* as a larval host in Costa Rica, and Aiello (1984) notes that species that feed on *Vitex* seem more specialized in their foodplant choice than are most *Adelpha* species. Second, the pupal morphology is very similar, being pearly white with black spots and streaks and very similar in shape (Fig.3). Across the genus at large both pupal morphology and foodplant use are indicators of close evolutionary relationships (Aiello 1984; Willmott 2003b). In addition, although larval coloration is exceptionally different in the ultimate larval stadium between the two species, both exhibit the longest subdorsal scoli at T2/A2 and A7/8, while noticeably lacking supraspiracular scoli, as do other members of this group, including *A. malea fundania* (Fruhstorfer, 1915) (07-SRNP-58380 & 59259) and *A. zina lacina* (Butler, 1872) (05-SRNP-2674) (Janzen & Hallwachs 2009). Overall, integrating molecular data with information from immature stages provides the best resolution of species relationships in the genus.

Larvae of *A. naxia naxia* and *A. heraclea heraclea* closely resemble each other in early instar morphology, but may be clearly distinguished in later stages. Larvae can be identified to species beginning with the third stadium. At this stage *A. n. naxia* is significantly darker brown, the head becomes uniformly black/brown, the T2 scoli are significantly more pronounced than all other scoli and are connected by a low prominent ridge. In contrast, third instar *A. h. heraclea* are light brown and the scoli on T2,T3, and A2 are equally pronounced. Larvae are clearly different by the ultimate stadium, with *A. n. naxia* being dark brown and black, whereas *A. h. heraclea* is dull green and pink. The pupae are most readily differentiated in that *A. n. naxia* has the lateral head projections more triangular whereas in *A. h. heraclea* they are rounded (Fig. 3) (although described as 'laterally pointing triangles' by Willmott 2003b, this more accurately describes those of *A. n. naxia* when being compared). This is a difference similar to that seen between pupae of *A. phylaca pseudaethalia* (Hall, 1938) and *A. messana messana* (C. & R. Felder, 1867) (CER and RIH pers. obs.; Aiello 2006). Additionally, lateral wing projections on the T2-T3 junction are rounded in *A. n. naxia* and pointed in*A. h. heraclea*, along with differences in placement and shape of black spots and streaks (Fig.3).

Results presented here also corroborate the placement of *A. nea* within the *serpa* group. The *serpa* group is the most strongly supported of the species groups based on characters of the male genitalia, wing pattern, pupal coloration, and by being non-Rubiaceae feeders (Aiello 1984; Willmott 2003b). *Adelpha nea* is included in the *serpa* group based on adult characters (Willmott 2003b). *Adelpha nea*'s adult morphology also makes it unique within the group, with a unique male valva, and the DFW postdiscal band of subspecies *A. nea nea* entirely orange in cells Cu2-

Cu1 and Cu1-M3 (Willmott 2003b) (Fig. 4). The immature morphology of *A. n. nea* also strongly corroborates its position in the *serpa* group. The relatively large egg, and the shape and shimmering silver coloration of the pupa (Fig. 7), resemble other *serpa* group species (e.g. *A. serpa celerio*, *A. paraena massilia* (C. Felder & R. Felder, 1867), *A. radiata aiellae* Willmott & Hall, 1999) (CER & RIH pers. obs.). The presence of conical tubercles on T2, T3 and A2-A4 is similar to other derived *serpa* group species (Willmott 2003b). The sixth instar is very similar to the ultimate instar of other *serpa* group species, (e.g. *A. serpa celerio*, *A. paraena massilia, A. radiata aiellae*) in the arrangement of scoli and pattern of flattened spines on the scoli. *Adelpha nea nea*'s ultimate instar is especially similar to that of *A. radiata aiellae* (CER & RIH pers. obs.) being very similar in color pattern and in the asymmetric arrangement of spines at the apex of the scoli. However, *A. nea nea* is clearly distinguished by the prominent subdorsal scoli on A9 which *A. radiata aiellae* lacks.

Implications of Sapotaceae feeding. Much of the natural history of *Adelpha nea nea* observed here appears to be strongly related to its unique sapotaceous food plant. Various species within the plant family Sapotaceae are known to produce latex (Gentry 1993) which functions as a potent defense against herbivores (Agrawal & Konno 2009). This includes the larval food plant of *A. n. nea*, *Micropholis melinoniana*, which produces visible latex from leaves and stems when torn or cut. To our knowledge this is the first record of Sapotaceae being used as a host for the genus *Adelpha*, or any Neotropical member of the family Nymphalidae (Beccaloni et al. 2008; Robinson et al. 2010).

Our observations on the egg placement and feeding pattern in *A. nea nea* indicate that these are likely adaptations to overcome the latex defense of its host *Micropholis melinoniana*.

Eggs were placed on older damaged leaves adjacent to fresh undamaged leaves, and young larvae found in the field were on previously damaged leaves. During rearing the larvae had equal access to old damaged leaves and young leaves, and preferentially fed on the older damaged leaves. Field observations also indicated that the older leaves have reduced latex (C.E.R pers. obs.). Thus the observed female oviposition and larval preference for older leaves likely reduced latex ingestion and increases larval survival.

An additional reason for selection of damaged leaves could be the feeding difficulty associated with the durability of this food plant. A healthy third instar *A. nea nea* was given a young undamaged leaf with an entire margin to feed on in place of its original damaged leaf. This individual died the following day with no damage seen to the leaf and no obvious cause of death other than starvation. These observations suggest the small early-instar was unable to chew through the thick border of *M. melinoniana* leaves, and latex was not the only defense. This is important to consider for future rearing of *Adelpha*, as giving larvae leaves with whole margins may result in unwanted fatalities. An alternate interpretation is that the larva rejected the host leaf because of secondary defenses other than latex in the young leaf. However this does not seem to be the case because the leaf was from the same tree as all other leaves fed to the larvae reared in this study.

The basal-to-apical feeding pattern we described here for *A. nea nea* (Fig. 6) appears to be unique among *Adelpha*, and likely reduces latex consumption. *Adelpha* species that we have studied, like *A. n. naxia*, feed by removing apical (or marginal) leaf tissue from both sides of the frass chain first, and working their way back toward the base (or midvein) of the leaf. In contrast, the basal-to-apical feeding pattern of *A. n. nea* is likely a mechanism for severing the lateral veins that contain latex, so that the larvae can then feed distal to the cut without encountering latex. Other insects feeding on latex-defended plants, such as milkweeds (*Asclepias* L. Apocynaceae), have evolved this behavioral defense, where they sever the veins of the leaf to stop the flow of latex before feeding (Dussourd 1993; Dussourd & Eisner 1987).

The presence of six larval stadia and the long development time in *A. nea nea* appear to be unique in *Adelpha* and are likely additional adaptations to its sapotaceous host. All *A. nea nea* larvae reared on *M. melinoniana* passed through six larval stadia. This is in contrast to the five instars typical of other *Adelpha* species, although Aiello (1984) observed *A. basiloides* (Bates, 1865) occasionally molting to sixth instar. Aiello (1984) noted that the sixth instar of *A. basiloides* occurred only in individuals feeding on *Amaioua corymbosa* Kunth (Rubiaceae), and that this occurred in only a portion of individuals. In addition to the number of instars, the total development time of *A. n. nea* appears to be ~10 days longer when compared to another *serpa* group species (*A. serpa celerio*) reared on the Osa Peninsula (C.E.R. pers. obs.). Aiello (1984) observed that *A. basiloides* individuals showed an extended development time when feeding on *Amaioua corymbosa* whether they went through five or six instars. Together these observations suggest variation in development time in *Adelpha* may represent individual responses to food plant quality or defenses. Thus the sixth instar and extended development time in *A. nea nea* may be specific to *M. melinoniana*, and likely help *A. n. nea* to feed on this well-defended food plant.

The discovery of novel larval hostplant families and life histories in a country such as Costa Rica where the butterfly fauna has been intensively studied (DeVries 1987, 1997; Janzen & Hallwachs 2009) indicates that there remains much to be discovered about *Adelpha*. For example while adults of *A. nea nea* were observed at La Selva Biological Station the immatures remain unknown there, and according to botanist Orlando Vargas (pers. com.) the food plant *M. melinoniana* has not been found on the station property. The presence of adult *A. n. nea* could mean that the host remains to be found there or very close by, or that *A. n. nea* uses an additional unknown host, or perhaps it is transient at LSBS, showing up when it is especially abundant elsewhere. However, the fresh condition of individuals observed at LSBS suggests it is resident, making it most likely that it uses a different but as yet unknown host there. It would be typical for an *Adelpha* species to use hosts from multiple species, genera and even families (Aiello 1984; Janzen & Hallwachs 2009; Willmott 2003b). This is especially true for species within the *serpa* group who feed on the greatest variety of plant families (Aiello 1984; Janzen & Hallwachs 2009; Willmott 2003b). With the discovery of this potential additional host we may be able to determine whether six instars is a general trait for *A. n. nea*, or whether it is due to the foodplant. Observations recorded here indicate that we are still just developing our understanding of *Adelpha* host breadth.

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Explanation of Figures

Figure 1. Immature stages of *Adelpha naxia naxia*. A. Egg. B. First instar. C. Second instar. D. Third instar. D. Fourth instar. F. Fifth instar. G. Pupa, lateral view.

Figure 2. Ultimate instar head capsule drawings of *A. naxia naxia* (A) and *A. nea nea* (B). Each scale bar represents one millimeter.

Figure 3. Comparison of pupae of *A. naxia naxia* (A & C) and *A. heraclea heraclea* (B & D) from the Osa peninsula*.*

Figure 4. Adult images of *A. naxia naxia* (A) and *A. nea nea* (B) 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 nea nea*. A. Egg. B. First instar. C. Second instar. D. Third instar. E. Fourth instar. F. Fifth instar. G. Sixth instar.

Figure 6. Feeding damage typical of *A. nea nea*. Images illustrate feeding pattern and structures made by *A. nea nea*. In the top image (A) the larva is feeding on the portion of the leaf distal to the cut in the typical basal-to-apical manner, consuming tissue distal to where secondary, intersecondary and tertiary veins have been cut. In the bottom image (B) nearly all the leaf distal to the cut has been consumed.

Figure 7. Pupal images of *Adelpha nea nea*. Dorsal (A), lateral (B), and ventral (C) views are shown. The lateral image was taken the first day of pupation before silver coloration formed.