IMMATURE STAGES OF THE BUTTERFLY *ACTINOTE CONSPICUA* (NYMPHALIDAE: HELICONIINAE: ACRAEINI)

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Abstract – The early stages, food plant and behavior of *Actinote conspicua* Jordan, 1913, are described from Campos do Jordão, São Paulo, Brazil. Immature stage morphological characters are compared to those of other *Actinote* species.

Resumo – Os estágios imaturos, a planta hospedeira e o comportamento de *Actinote conspicua* Jordan, 1913, foram descritos de material proveniente de Campos do Jordão, São Paulo, Brazil. Os caracteres morfológicos dos imaturos foram comparados àqueles encontrados em outras espécies de *Actinote*.

Key words: Acraeini, *Actinote*, *Actinote conspicua*, life-cycle, Nymphalidae

The genus *Actinote* Hübner, [1819] (*sensu* Silva-Brandão *et al*., 2008) (Nymphalidae: Heliconiinae) contains about 35 described species of Neotropical butterflies, with at least 23 present in southeastern Brazil and 12 in the Andes (Lamas, 2004; Paluch, 2006; Paluch *et al*., 2006; Silva-Brandão *et al*., 2008; Neild, 2008; Willmott *et al*., 2009). Due to intra-specific variation, considerable similarity among species and mimicry, adults of *Actinote* (especially the females) are often difficult to distinguish (D'Almeida, 1935, 1958; Penz & Francini, 1996). Conversely, the immatures of most species (especially larvae) are quite distinctive, and potentially of great value for species discrimination (Francini, 1989; Francini *et al*., 2004).

The larvae of all known species of *Actinote* are gregarious and feed on species of Asteraceae (Francini, 1989, 1992; Brown, 1992; Paluch *et al*., 1999, 2001; Silva-Brandão *et al*., 2008; Freitas *et al*., 2009, 2010). All life stages contain cyanogenic compounds and the adults have a short lifespan (three to seven days on average), occurring in a few (usually one to three) discrete generations throughout the year (Brown & Francini, 1990; Francini *et al*., 2005).

Adults of several species of *Actinote* are particularly difficult to identify, especially in the orange-yellow mimicry group (Francini *et al*., 2004). It was D'Almeida (1958) who first noticed that *Actinote* caterpillars are diagnostic for some species in southeastern Brazil, showing that larval morphology is of great importance for *Actinote* systematics. In recent years, several descriptions of *Actinote* early stages have been published (Paluch *et al*., 1999, 2001; Freitas *et al*., 2009a,b), but there are still many gaps to be filled, especially concerning the Andean species (Freitas *et al*., 2009a).

In southeastern and south Brazil, there is a complex of seven highly similar species of *Actinote* whose males are characterized by a dark orange and dark brown striped pattern, which forms the "orangish red mimicry complex" or "*alalia* mimicry complex" (Francini, 1989; Brown & Francini, 1990; Penz & Francini, 1996). Of these seven species, four comprise a clade of univoltine species characteristic of forested areas in high mountains, usually associated with elevations above 1000 m (Penz & Francini, 1996; Francini, 1990). Several of the species in this clade have often been misidentified, usually as *Actinote alalia* (C. Felder & R. Felder, 1860), including the largest species in this group, *Actinote conspicua* Jordan, 1913.

The present paper provides a description of the early stages, larval and adult behavior of *A. conspicua*, which are compared with those of other *Actinote* species.

STUDY SITES AND METHODS

Adults and immatures of *A. conspicua* were studied in the field, on the slopes of the Serra da Mantiqueira in six different sites in three states in southeastern Brazil: (1) São Paulo, Alto da Serra, Campos do Jordão (22°46'9"S 45°36'57"W), ca. 1600 m; (2) São Paulo, Pico do Itapeva, Pindamonhangaba (22°45'52"S 45°31'20"W), ca. 2000 m; (3) São Paulo, road to São Francisco dos Campos do Jordão, Piquete (22°34'8"S 45°13'36"W), ca. 1500 m; (4) Minas Gerais, trail to Pico Chapéu do Bispo, Monte Verde (22°52'57"S 46°1'25"W), ca. 1600 m; (5) Minas Gerais-São Paulo border, road to Pedra do Cume, Extrema (22°54'10"S 46°20'44"W), ca 1600 m; and (6) Minas Gerais-Rio de Janeiro border, road to Agulhas Negras, Resende (22°22'25"S 44°45'15"W), ca. 1600 - 1800m. Adults, immatures and host plants were observed along trails in well preserved subtropical wet montane forest in the above sites (Figs. 1A-B). Larvae were reared in the laboratory in plastic containers, which were cleaned daily, with fresh plant material provided every two or three days (following Freitas *et al*., 2009a,b). Immatures were fixed in Kahle solution (Triplehorn & Johnson, 2005), and deposited at the Museu de Zoologia, Universidade Estadual de Campinas (Unicamp), Campinas, São Paulo, Brazil. All measurements were made using a stereomicroscope fitted with a calibrated micrometric ocular. Egg sizes are height and diameter, and head capsule size is the distance between the most external ocelli (as in Freitas, 2007).

RESULTS

Adult behavior and natural history: Adults were only observed on sunny days, disappearing quickly if weather conditions

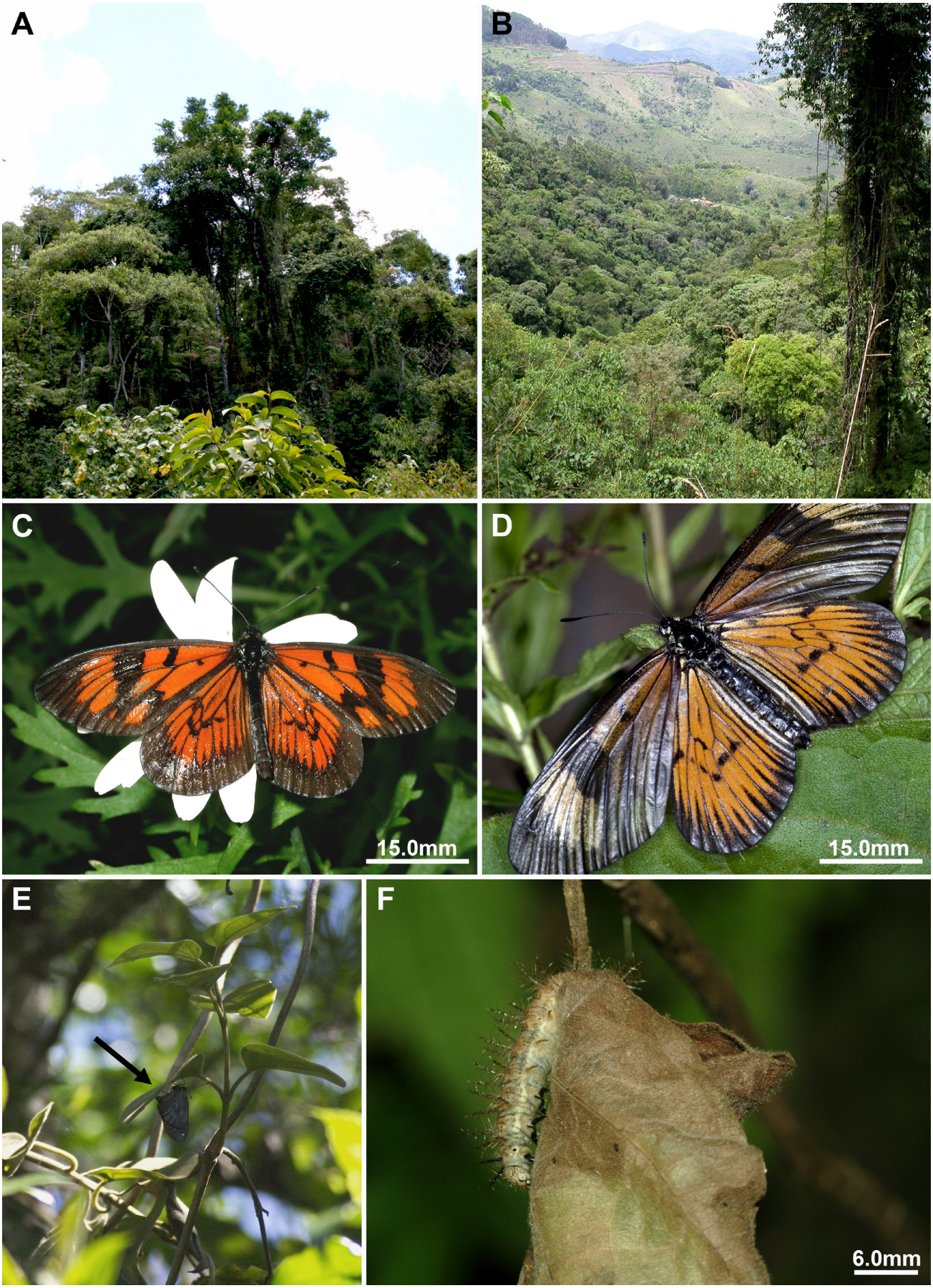

Fig. 1: A - B) Two views of the habitat of *Actinote conspicua* in Alto da Serra, Campos do Jordão (22°46'9"S 45°36'57"W), ca. 1600 m elevation; C) Adult male (dorsal view); D) Adult female (dorsal view); E) Female laying eggs on *Mikania hirsutissima* (dark arrow); F) Last instar in a leaf shelter.

became cloudy. Males began to fly around 10:30 hr, usually flying 5-7 m high, and on slopes they were observed flying above the canopy on mountain slopes. Territorial behavior was never observed in this species. Females are seldom observed, and courtship behavior or copulation were not observed. All known populations of *A. conspicua* were recorded in preserved subtropical wet montane forest above 1400 m altitude, where the climate approaches temperate conditions, including cold winters with frequent frosts and temperature frequently falling below 0°C. Males were observed feeding on flowers of *Croton urucurana* Baill. (Euphorbiaceae), *Chromolaena punctulata* (D.C.) R. King & H. Robins, and on an unidentified species of Fabaceae. Males were sometimes observed feeding on mud. Adults are univoltine in all known populations, with only one flight period during the warmer months, from late November to early February. Adults of *A. conspicua* are sexually dimorphic, with males having a deep orange coloration (Fig. 1C), and females being much more translucent and sometimes having the subapical spot on the forewing light cream (Fig. 1D). In most study sites individuals were never abundant, with usually 10 - 50 individuals observed in a typical day of field work (4 - 5 hours of observation), most of which were males.

Host plant and oviposition: The reported host plants for *A. conspicua* are the vines *Mikania hirsutissima* D.C. and more rarely *Mikania pilosa* Baker (Asteraceae). Ovipositions were observed on leaves from 5-7 m above the ground (n = 7), on plants growing near forest edges (Fig. 1E). Eggs were laid in clusters (Fig. 2A), and three ovipositions from Campos de Jordão had 406, 470 and 580 eggs. In one oviposition some eggs were laid on top of other eggs. Of the three observed ovipositions, the first (with 406 eggs) included 48 sterile eggs. Sterile eggs are recognized because they remain yellow, while fertilized eggs change color to red after a few days (see Freitas *et al*., 2009b). In January 2005 an oviposition sequence was observed in Piquete, São Paulo; oviposition started at 09:16 hr, and during 5 hours and 46 minutes the female laid 56 eggs at intervals of 2 seconds to 8.3 minutes; being disturbed only when the wind velocity reached 5 - 6 m/s, when the oviposition process was interrupted and the eggs were collected.

Larval behavior and natural history: Newly hatched larvae first consumed the egg chorion, and then, after several hours, began to feed on leaf tissue. Larvae were strongly gregarious, and all activities occurred at the same time, such as feeding, resting, or moving between leaves. First instar larvae scratched the leaf surface while feeding only on the superficial tissue, while last instar larvae were observed consuming the entire leaf, creating holes in the leaves. In the Alto da Serra de Campos do Jordão, last instars were found dispersed in the field, and several larvae (n = 7) were observed singly or in pairs taking refuge inside rolled dried leaves of the host plants (Figs. 1F and 2G). Whether these shelters were constructed by larvae or they are just opportunistically using naturally curled, dry leaves was not elucidated.

Natural enemies: One egg cluster was found to have damaged eggs at the edge of the cluster, very similar to the damage caused by stinkbugs. One pupa reared from a field collected larvae died after the emergence of a larva of a tachinid fly parasitoid (Diptera: Tachinidae). Predation on adults was not observed.

Immature stages: All descriptions of immature were based on material collected in Serra de Campos do Jordão (the site 1 – see methods).

Egg (Figs. 2A-B). Light yellow when first laid, changing gradually to pinkish during the first 24 hours (Fig. 2B); barrel shaped, with a conspicuous concavity in the micropilar area, with 13 - 16 vertical ribs and several (~16 - 17) weakly marked horizontal ribs; mean height 0.74 mm (range 0.73 - 0.75 mm, SD = 0.0095, n = 4), mean diameter 0.51 mm (range 0.48 - 0.53 mm, SD = 0.0216, n = 4).

First instar (Figs. 2C). Head brown, smooth, without scoli, mean width 0.314 mm (range 0.30 - 0.33 mm, SD = 0.0126 mm, n = 10); body pale cream, without scoli and with long pale setae arising from sclerotized insertions; legs pale brown, prolegs pale, anal plate pale brown. Prothoracic plate pale and difficult to observe under stereomicroscope. Body chaetotaxy as in Fig. 3A. Maximum reported length 3.0 mm. All larvae were killed and preserved in the first instar due to lack of additional food for subsequent stages.

Penultimate instar (Figs. 2D). Head pale brown mottled with several small brown irregular markings, smooth with thin pale setae and without scoli, spines or chalazae, width 2.3 mm (n = 1); body pale yellowish brown dorsally, pale cream ventrally, covered with long pale brown scoli mottled with black dots and with long brown setae; those scoli on T1 - T3 and A8 - A10 predominantly or entirely black; legs black, anal prolegs pale cream; anal plate pale cream. Maximum length: 30 mm (n = 1).

Last instar (Figs. 2E-F). Head pale cream mottled with several small brown irregular markings, smooth with thin pale setae and without scoli, spines or chalazae, mean width 3.14 mm (range 3.01 - 3.24 mm, SD = 0.073 mm, n = 4); body pale brown dorsally, pale cream ventrally, covered with long white scoli mottled with black dots and bearing brown setae, except for the subdorsal scoli on T1 which are entire black; legs black, anal prolegs pale cream; anal plate pale cream. Maximum length: 40 mm (n = 4). Scoli distribution as in Fig. 3B. Prepupa changes color, becoming pale cream with a more homogeneous coloration (Fig. 2H).

Pupa (Fig. 2I-K). General profile elongated, ground color pale cream with dark brown markings in wing cases and abdomen; abdominal segments mobile, with a series of five pairs of subdorsal black spines from segments A2 to A6. Length 20 - 21 mm (n = 2).

DISCUSSION

As reported in all other known *Actinote*, eggs of *A. conspicua* are laid in tight clusters and are initally yellow, changing to a pinkish tone after few days (Francini, 1989, 1992; Freitas *et al.*, 2009a,b; Francini & Freitas, 2010). The eggs of *A. conspicua*, however, are quite distinct by presenting a conspicuous concavity in the micropylar area, very similar to the condition observed in *A. dalmeidai* (Francini, 1992; Penz & Francini, 1996). This condition was only observed in these two species, and based on the possible close relationship of these two species (Silva-Brandão *et al*., 2008) this condition is apparently homologous for both species.

First and last instars are both mostly similar to those described for other species of *Actinote* in morphology and behavior (Francini, 1989; Brown, 1992; Paluch *et al*., 1999, 2001; Freitas *et al*., 2009a,b). The first instar of *A. conspicua*, however, differs from those of the other known species of *Actinote* by the pale body setae and pale prothoracic plate; all other known *Actinote* have dark body setae, and a conspicuous dark plate on the prothorax (Paluch *et al*., 1999, 2001; Freitas *et al*., 2009a,b). The last instar head capsule, which is pale cream mottled with dark irregular markings, is very similar to that of *A. parapheles* Jordan, 1913 (D'Almeida, 1935; Francini, 1989); all other known *Actinote* have dark head capsules, varying from reddish brown to black (D'Almeida, 1935; Francini, 1989; Paluch *et al*. 1999, 2001; Freitas *et al*., 2009a,b). The light

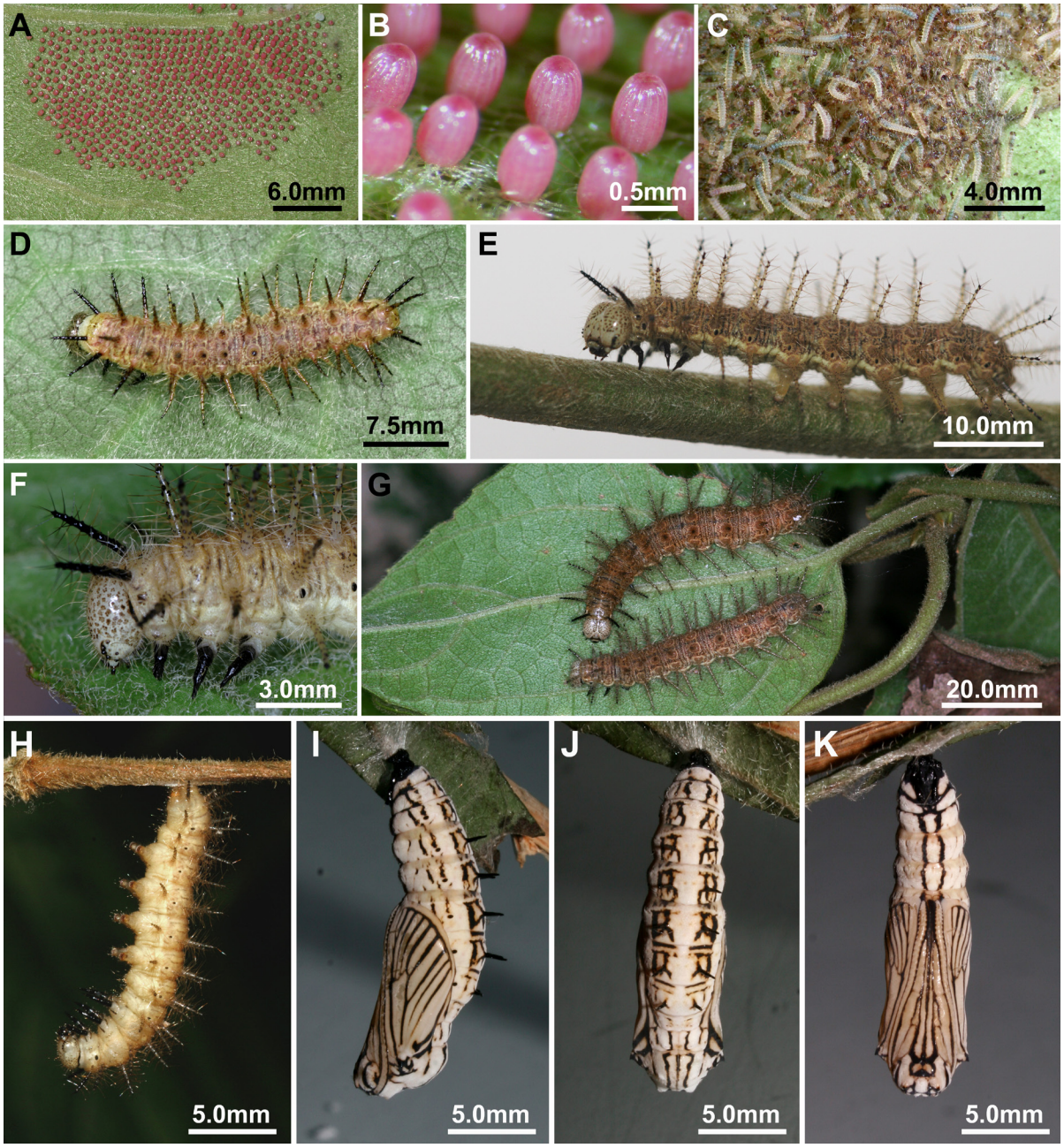

Fig. 2: Life stages of *Actinote conspicua*. A) Egg cluster; B) Eggs in close view; C) Aggregation of first instar; D) Penultimate instar; E) Last instar; F) Close view of last instar head capsule and thoracic scoli; G) Two last instars; H) Prepupa; I) Pupa (lateral view); J) Pupa (dorsal view); K) Pupa (ventral view).

brown body color of the last instar *A. conspicua* is quite unique among known *Actinote* larvae (Francini, 1989; Freitas *et al*., 2009a,b). The behavior of taking refuge in dead leaves in the last instar has never before been observed in any other species of *Actinote*. Larvae of *A. dalmeidai* Francini, 1996 were reported to construct shelters with silk and new leaves in the first instar (as *Actinote* sp2 in Francini, 1992), but these shelters are very different in structure from those observed for *A. conspicua*.

The pupa is very similar to those of most known *Actinote* in general shape and color pattern, and having five pairs of subdorsal tubercles on the abdomen resemble all other known species of *Actinote* (Francini, 1989; Paluch *et al*., 1999, 2001; Freitas *et al*., 2009a,b) except for *A. canutia* (Hopffer, 1874) and *A. mamita* (Burmeister, 1861), which have six pairs tubercles.

The univoltinism of *A. conspicua* appears to have a single origin among the Neotropical *Actinote*, being restricted to five species of "orangish red" *Actinote* which form a clade of species restricted to the high mountains of southern Brazil (Silva-Brandão *et al*., 2008) (*A. bonita* Penz, 1996, *A. alalia*, *A. conspicua*, *A. dalmeidai*, and an undescribed species (AVLF in prep.)). This "orangish red" pattern, however, is not restricted to this clade, being present also in the bivoltine *A. quadra* (Schaus, 1902), *A. catarina* Penz, 1996 and *A. surima* (Schaus, 1902) in the Atlantic Forest, and in *A. rufina* Oberthür, 1917 in the Andes (Penz & Francini, 1996; Paluch *et al*., 2006).

As we increase our knowledge of *Actinote* immature stages, new and interesting patterns emerge and we improve our understanding of this diverse group's ecology and evolution.

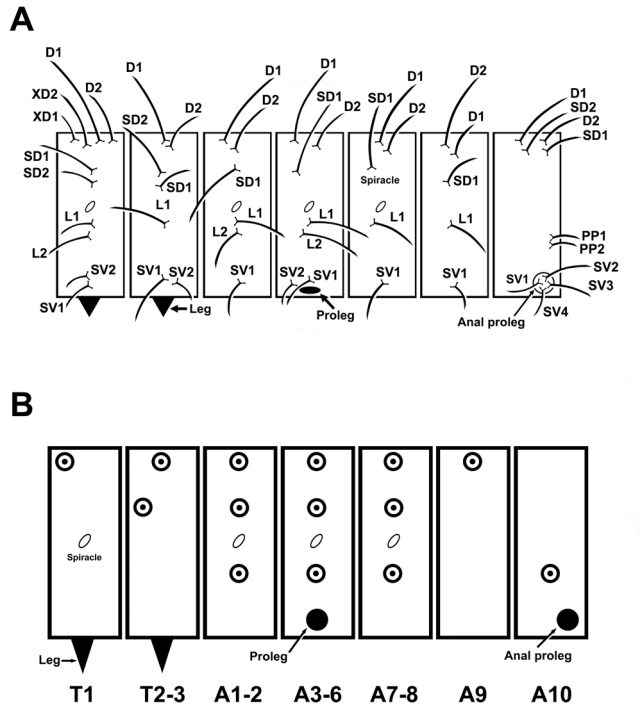

Fig 3: Larval body diagrams of *Actinote conspicua*. A) First instar body chaetotaxy; B) Last instar scoli distribution.

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