IMMATURE STAGES OF *ACTINOTE ZIKANI* (NYMPHALIDAE: HELICONIINAE), A CRITICALLY ENDANGERED BUTTERFLY FROM SOUTHEASTERN BRAZIL

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Abstract – The early stages, food plant, and behavior of the endangered species *Actinote zikani* D'Almeida, 1951, are described from southeastern Brazil, Paranapiacaba, São Paulo. The only recorded host plant is a liana, *Mikania obsoleta* (Asteraceae). Eggs and larvae are grouped, and general morphology and behavior are similar to those of other species of *Actinote*. All immature stages are described in detail and compared with other *Actinote* species.

Resumo – Os estágios imaturos, a planta hospedeira e o comportamento da espécie ameaçada *Actinote zikani* D'Almeida, 1951, são descritos de material proveniente de Paranapiacaba, São Paulo, Sudeste do Brasil. A única planta hospedeira registrada é a trepadeira *Mikania obsoleta* (Asteraceae). Os ovos e as larvas são agrupados, e a morfologia geral e comportamento são muito similares aos de outras espécies conhecidas de *Actinote*. Todos os estágios imaturos são descritos em detalhes e comparados àqueles de outras espécies conhecidas de *Actinote*.

Key words: Acraeini, life history, *Mikania obsoleta*, conservation biology

Butterflies in the genus *Actinote* Hübner, [1819] (*sensu* Silva-Brandão *et al*. 2008) are usually associated with open habitats and forest edges, with several species being locally abundant in secondary forests and other anthropic environments (Francini 1989, 1992; Paluch 2006; Francini & Freitas 2010). However, there are some species which are rare and local, e.g., in southern Brazil there are at least two endangered species, namely *Actinote quadra* (Schaus, 1902) and *A. zikani* D'Almeida, 1951 (MMA 2003; Francini *et al*. 2005; Freitas & Brown 2008a, 2008b; Freitas *et al*. 2009b). *Actinote zikani* (Fig. 1F) has been recorded from only three localities of high altitude forests on the slopes of Serra do Mar, São Paulo State, southeastern Brazil (Francini *et al*. 2005). The species was described based on material collected in 1941–1942 from two forest sites at the summits of the Serra do Mar mountain range (D'Almeida 1951) and remained unrecorded for almost 50 years until being rediscovered in 1991 at a third location near the type localities (Francini *et al*. 2005).

The species is bivoltine with adults flying in March and November. The only known larval host plant is a liana, *Mikania obsoleta* (Vell.) G. M. Barroso (Asteraceae) (Figs. 1D, E). Some data on population biology and basic natural history of *A. zikani* were described by Francini *et al*. (2005) from southeastern Brazil, Paranapiacaba, São Paulo; however, the immature stages were only very briefly described. Field studies showed that population size can strongly fluctuate from year to year, with the species being extremely common in some years and then virtually absent for several years (Francini *et al*. 2005). In fact, after being common from 1991 to 1994 in Paranapiacaba (Francini *et al*. 2005), *A. zikani* was not observed in the field from 1997 to 2005, being recorded again in March 2006.

Based on the combination of 1) restricted area of occupancy, 2) few known populations and sites, 3) small total population size, and 4) extreme fluctuations in number of individuals through time, *A. zikani* was classified as 'critically endangered' in the most recent Brazilian list of endangered species (Freitas & Brown 2008b).

The present paper provides a detailed description of the early stages, as well as larval and adult behavior of *A. zikani*, comparing them with those of other *Actinote* species.

STUDY AREA AND METHODS

Adults and immature stages of *A. zikani* were studied from material collected in Santo André Municipality, São Paulo State, near the village of Paranapiacaba. The study site is located east of Paranapiacaba along a 2500 m long road in the region where the peak reaches 1200 m (46º18'16"W, 23º47'18"S) (Figs. 1A–C). A detailed description of the study area with a map can be found in Francini *et al*. (2005).

Field work was carried out between 1991 and 1994 with 48 field trips to the study area and a total of 161 hours of fieldwork. Adults were observed using binoculars and various aspects of their behavior were photographed. When necessary, they were collected by insect nets. Eggs (Fig. 2B) were collected and brought to laboratory. Larvae were reared in glass jars with the top covered by nylon screens. A piece of cotton moistened with rain water was used to maintain adequate humidity. Head capsules and pupal shells were conserved, and eggs, larvae and pupae were fixed in Kahle solution (Triplehorn & Johnson 2005). Specimens were deposited in the Museu de Zoologia da Universidade de São Paulo and Museu de Zoologia da Unicamp, Campinas, São Paulo State, Brazil. All measurements were made using a stereomicroscope fitted with a calibrated micrometric ocular. Egg measurements consisted of height and diameter, and for the head capsule the distance between the most external ocelli of stemmata, as in Freitas *et al*. (2009a, 2009b). Scanning electron microscopy (SEM) was conducted using a

JEOL® JSM-5800 microscope, and samples were prepared in accordance with the following protocol: critical point drying using Bal-tec® - CPD030 equipment, with the sample attached with double-sided tape to aluminum stubs, followed by gold/palladium coating with a Bal-tec® - SCD050 sputter coater.

Fig. 1: Habitat, host plant and natural history of *Actinote zikani*. A) General view of the study site. Arrows pointing at specific study site of higher mountains; B) General view of the habitat at Paranapiacaba showing one of the two groups of towers; C) Habitat at Paranapiacaba; D) Young individual (sapling) of *Mikania obsoleta*; E) Flowering individual of *M. obsoleta*; F) Male visiting on flowers.

RESULTS

Host plant and oviposition. We recorded 13 species of *Mikania* in the study site. Some species were abundant and some were less abundant or rare. We were able to locate the host plant, *Mikania obsoleta*, on the eighth trip (April 1st 1991) by observing two females in oviposition around noon. Contrary to most species of *Mikania*, which grow in open gaps and forest edges, *M. obsoleta* was observed growing only inside the forest, mainly in areas of wet soil near and at the edges of small creeks (being partially covered by the stream in the rainy season). However, the plant is easily recognized by its halberd-shaped leaves. It grows around tree trunks climbing up to 6 m high. In 1991 and 1993 *M. obsoleta* flowered between October and November, coinciding with the flight period of *A. zikani*, but no *A. zikani* was observed visiting its flowers. The growth of *M. obsoleta*, as measured by the number of new leaves, was relatively slow when compared with other *Mikania* species in the area (e.g. *M. hirsutissima*) (R. B. Francini unpublished results).

The oviposition of *A. zikani* is much like that observed for other *Actinote* species. Prior to oviposition, the female flew around the host plant and landed briefly several times on several leaves. Later she landed on the underside of a leaf and remained inactive for about 1 to 5 minutes and then started to oviposit (Fig. 2A). The ovipositon lasted up to one hour (n = 2 observations). We collected three egg masses on April 1st containing 269, 273, and 520 (mean = 354; SD = 143.77) eggs respectively, with egg density varying from 11 to 21.5 eggs per cm^2. In total, five oviposition events were observed in April

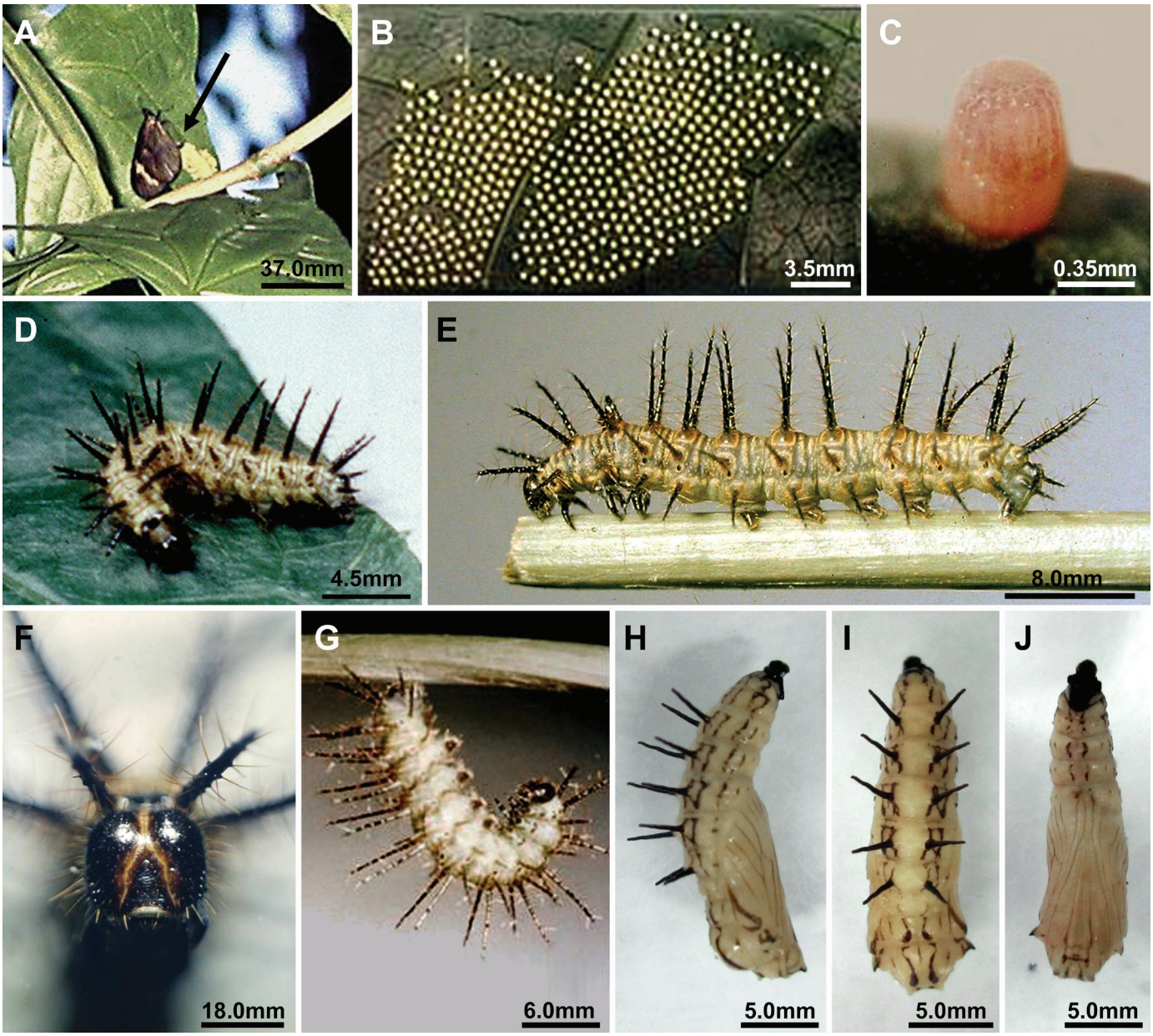

Fig. 2: Immature stages of *Actinote zikani*. A) Female laying eggs on the underside of the host plant (dark arrow); B) An egg mass consisting of 520 eggs; C) Close-up of an egg (latero-dorsal view); D) Penultimate instar (latero-dorsal view); E) Last instar (lateral view); F) Head of last instar (frontal view); G) Prepupa (lateral view); H–J) Pupa (H, lateral; I, dorsalJ,ventral view).

1991, two of which occurred on a single leaf, i.e., two females were ovipositing together at the same time (female voucher #F-2337 and F-2338 in the RBF collection).

On November 16th 1991, a field-collected female was submitted to stress conditions in the laboratory by putting her in a one liter glass jar with a piece of the host plant under a 150 W incandescent lamp. Generally this situation elicits oviposition behavior as described in Francini (1989). Under these conditions oviposition took a little over five hours. The female vibrated her wings during the entire ovipositional process. On November 30th, the eggs (voucher #F-2361) with larvae on the point of hatching (black head capsules formed) were found dead, probably due to dehydration. In 1993 only one egg mass was observed; however, later the entire mass disappeared, probably due to predation. Despite the search conducted in the study area in 1994 no eggs were found and no ovipositing females observed.

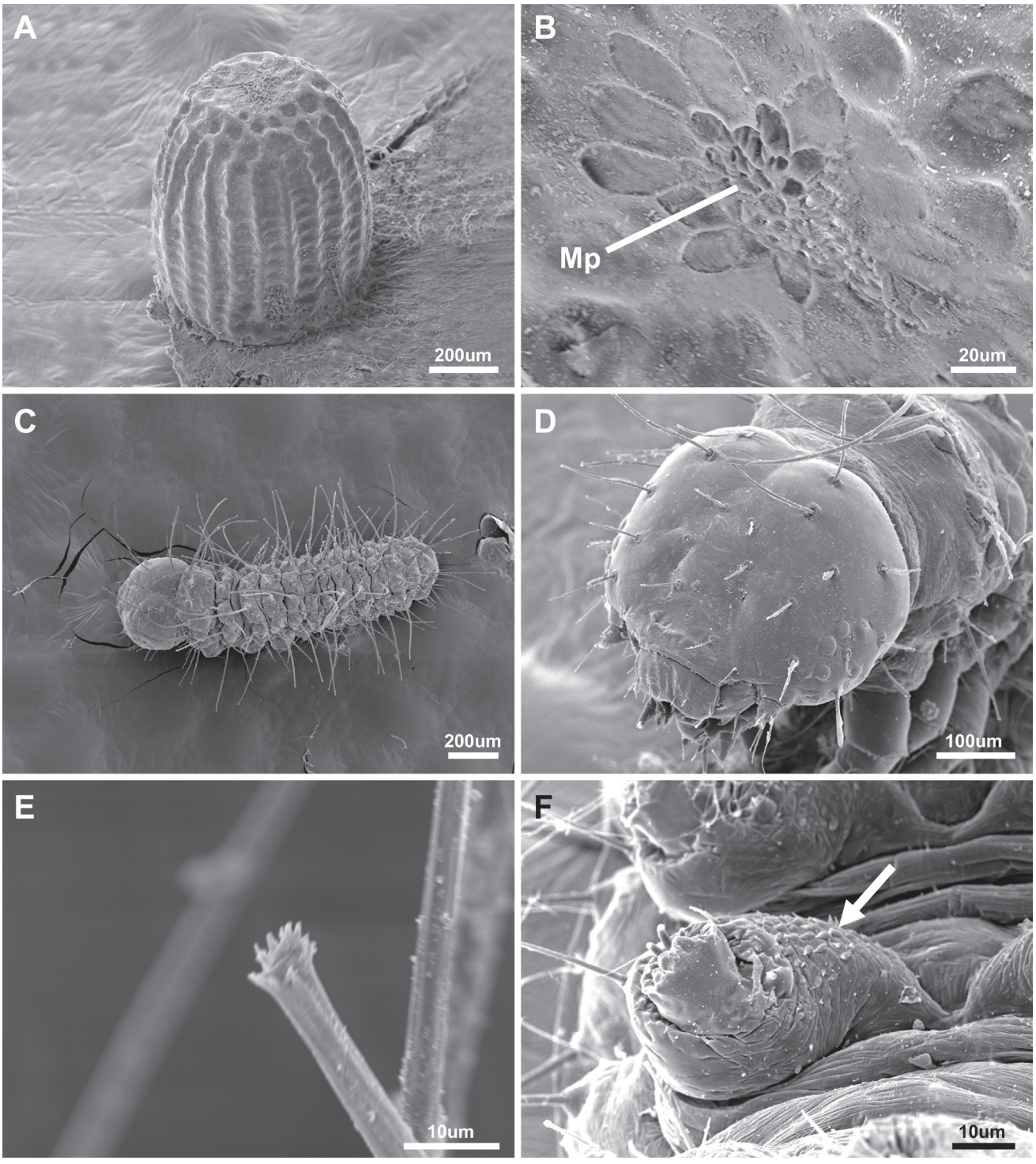

Fig. 3: Scanning electron microscopy of egg and first instar of *Actinote zikani*. A) Egg (latero-dorsal view); B) Egg, detail of micropyle region; C) First instar (dorsal view); D) Head (latero-frontal view); E) Setal apex in detail; F) Microtrichia on proleg ventral region (arrow). Mp = micropyle region.

Larval behavior and natural history: Newly hatched larvae first consumed the chorion, and then began to feed on leaf tissue after 3–5 hours. The first instar fed on the underside leaf tissue by scraping the leaf surface while later instars fed on the entire leaf. Frass pellets were glued onto the leaf by silk, not falling to the ground. Larvae were consistently gregarious and all activities occurred at the same time, such as feeding, resting, or moving between leaves. The egg stage duration was 15 days. The larval stage of the individuals reared from field-collected eggs in April 1991 lasted 73 days, with no significant difference between the mean duration of the larval periods for males (72.5 days, SD = 2.76, n = 16) and females (73.6 days, SD = 2.68, n = 12) (t = – 1.0414, p = 0.30, DF= 26). The duration of the pupal stage was 17.2 days for males (SD = 1.41; n = 16) and 16.9 days for females (SD = 0.94; n = 12) (differences not significant, H = 0.002; p > 0.95; DF = 1). The mean total duration of the immature stage under laboratory conditions was 106 days (SD = 3.92 days, n = 28).

Descriptions of Immature stages:

Egg (Figs. 2C, 3A–B). Light yellow when first laid, changing to red after ca. two hours (Fig. 2C); barrel shaped, with 19–21 vertical ribs and ca. 16–17 weakly marked horizontal ribs; rosette cells elongated surrounding the micropyle, aeropyles seldom observed and restricted to the basal region of the vertical ribs; mean height 0.61 mm (range 0.60–0.62 mm, SD = 0.11, n = 7), mean diameter 0.49 mm (range 0.47–0.51 mm, SD = 0.01, n = 29).

First instar (Figs. 3C–F, 4, 5A). Head brown, smooth, without scoli, mean width 3.12 mm (range 3.00–3.20 mm, SD = 0.101 mm, n = 11); body pale cream, without scoli and with long pale setae (especially the dorsal and sub-dorsal setae, which are longer than the segment height) (Fig. 3E) arising from slightly sclerotized pinacula; prothoracic and anal plates weakly sclerotized; prolegs brown, with microtrichia on ventral region (Fig. 3F). Microscopic detail of head in Fig. 3D and head chaetotaxy as in Fig. 4. Body chaetotaxy as in Fig. 5A. D2 is missing on the prothoracic shield, and in the sub-dorsal setae, most individuals presented only two setae, although a very few individuals presented one additional SD, usually on only one side of the body. The same occurred in the sub-ventral group on segment T1, with most individuals presenting three setae but a few others presenting only two sub-ventral setae. The ventral setae are not present on segments A7, A8 and A9, but are present from segments A1 to A6, although they are not shown in Fig. 5A.

Intermediate instars (Fig. 2D). The intermediate instars (2 to 4) have a strong yellowish background color contrasting with black scoli, giving a strong aposematic appearance. Head capsule width: 3rd instar 0.90 mm (n = 1); 4th instar 1.50 mm (n = 2).

Last instar (Figs. 2E–G, 5B). Head pale black and pilose, without scoli, spines or chalazae; width 2.50 mm (n = 1); body greenish yellow, pale ventrally, covered with long, dark brown scoli bearing brown setae; a conspicuous dark plate on prothorax; legs black, prolegs with a conspicuous brown lateral plate; anal plate black with pale areas. Length: 35 mm (n = 1). Scoli distribution as in Fig. 5B. Prepupa pale yellow with more homogeneous coloration (Fig. 2G).

Pupa (Figs. 2H–J). General profile elongated; pale greenish-yellow after molting, turning white with dark brown markings on wing cases and abdomen after a few hours; abdominal segments A5 to A8 mobile, with a series of five pairs of long subdorsal black spines from segments A2 to A6. Length 18–20 mm (n = 4).

DISCUSSION

Natural History and Immature stages

The immature stages of *A. zikani* are in most respects quite similar in morphology and behavior to those described of other species of *Actinote* (Francini 1989, 1992; Paluch *et al*. 1999, 2001; Freitas *et al*. 2009a, 2009b, 2010; Francini & Freitas 2010). Eggs are laid in large clusters which are larger than those reported for *A. quadra*, but not as large as those reported for *Actinote pellenea*, which has some clutches exceeding 1000 eggs (Francini 1989; Freitas *et al*. 2009b, 2010; Francini & Freitas 2010). The observation of two oviposition events on the same leaf is an apparently common pattern among certain species of *Actinote*, being observed in *A. quadra*, *A. pellenea*, *A. parapheles*, *A. carycina*, *A. brylla*, *A. pyrrha* and *A. melanisans* (Francini 1989; Francini & Freitas 2010; Freitas *et al*. 2010 and personal observations of the authors).

The absence of D2 on the prothoracic shield in the first instar differs from other known *Actinote* species (Paluch *et al.* 1999, 2001; Freitas et al. 2010), while the presence of usually two sub-dorsal setae is found in other *Actinote*.

The last instar of *A. zikani* has long scoli compared with other species of *Actinote* where immature stages are known and it is similar to those of *A. melanisans* Oberthür, 1917 and *A. discrepans* D'Almeida, 1935 (RBF, unpublished data). The pupa has no marked differences in general coloration and shape when compared to other *Actinote* species.

The larvae reared in the laboratory completed their life cycles in about three and half months, what can be considered exceptionally short for an *Actinote* species (except for *A. pellenea pellenea* that can complete its life cycle in about two

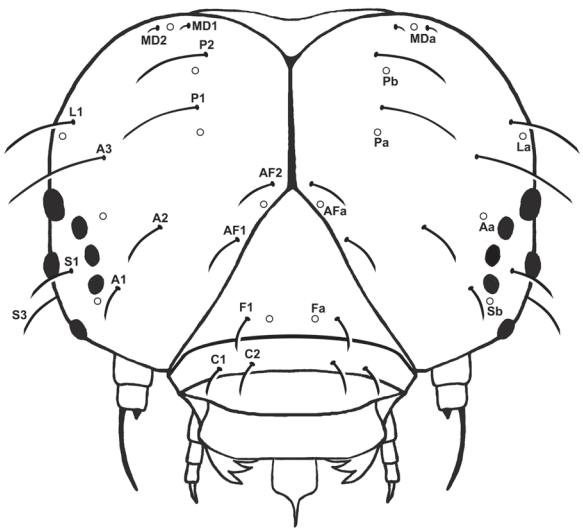

Fig 4: Head chaetotaxy of first instar *Actinote zikani*.

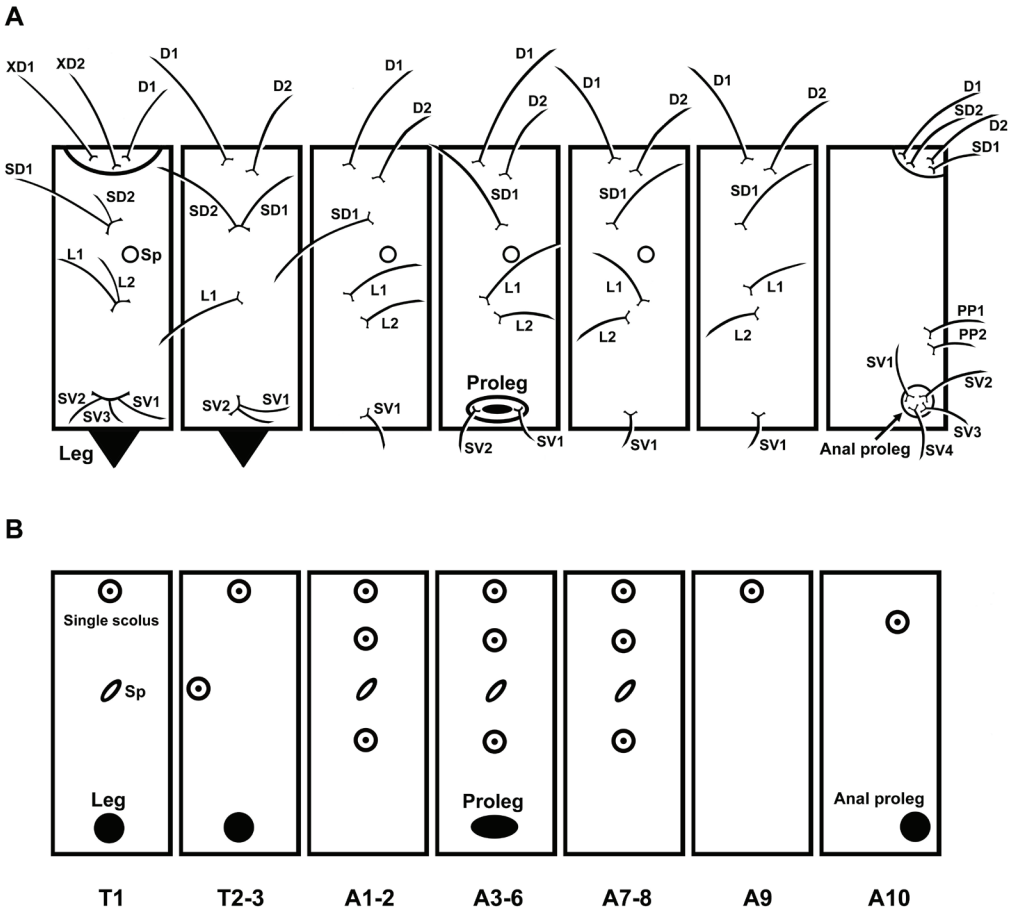

Fig 5: Larval body diagrams of *Actinote zikani*. A) First instar body chaetotaxy; B) Last instar scoli distribution. SP = spiracle.

months in the summer, Francini 1989). This means that adults in the laboratory emerged in July, almost four months before the flight period in the field, which occurs in November (Francini *et al*. 2005). Even considering that larvae were reared in a much warmer environment, the effects were much more pronounced than those observed in *A. quadra* for example, whose larvae reared in a laboratory with warmer temperatures emerged in synchrony with adults in nature (Freitas *et al*. 2009b). Some multivoltine species, such as *A. pellenea* and *A. brylla* are known to accelerate their development in response to warmer temperatures (Francini 1989) and it would be interesting to investigate if this occurs in additional montane species other than *A. zikani*.

Conservation of *A. zikani*

Francini *et al*. (2005) made several recommendations for the conservation of this endangered species. For example, there is an urgent need to discover additional populations of this species in the Serra do Mar region, since there is only one known site inhabited by *A. zikani*.

Between 2005 and 2007, RBF and AVLF searched for *A. zikani* and its host plant *M. obsoleta* in four localities with habitats similar to those where this species was present, from Paranapiacaba to Ubatuba (45º5'17"W, 23º19'32"S), and including the type locality in Salesópolis (see Francini *et al*. 2005). The host plant, *M. obsoleta*, was recorded in all four sites, but *A. zikani* was not observed. Also, *A. zikani* has never been recorded from 1.5 km west to the study site (Paranapiacaba Biological Reserve, 46º18'40"W, 23º46'45"S), where *M. obsoleta* is locally abundant and where females could potentially arrive by flight.

Thus, the cause of the rareness and highly-localized distribution of *A. zikani* remains unknown. From this and previous studies, *A. zikani* seems to be quite sensitive to environmental or microhabitat conditions, and the butterfly probably requires particular microclimatic conditions near the host plants where the immature stages can complete their development. The distribution and abundance of natural enemies of *A. zikani* could also be a potential cause for the restricted distribution seen in the latter species, but this needs further investigation. The reported monophagy of *A. zikani* on *M. obsoleta* also cannot explain the rarity of the former, since several other well known more widespread species of *Actinote* are also found to be restricted to a single host species (Francini 1989). Besides protecting the habitat and its surrounding environment, further field studies are necessary to better understand the habitat and resource requirements of this enigmatic butterfly species.

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