IMMATURE STAGES AND NATURAL HISTORY OF THE THREATENED BUTTERFLY *ACTINOTE QUADRA* (NYMPHALIDAE: HELICONIINAE: ACRAEINI)

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Abstract – The early stages, food plant and behavior of *Actinote quadra* (Schaus, 1902), a threatened, high-elevation species from southeastern Brazil, are described from Campos do Jordão, São Paulo. Immature stage morphological characters are compared to those of other *Actinote* species.

Resumo – Os estágios imaturos, a planta hospedeira e o comportamento de *Actinote quadra* (Schaus, 1902), uma espécie ameaçada que ocorre nas montanhas altas do Sudeste do Brasil, foram descritos em Campos do Jordão, São Paulo. Os caracteres morfológicos dos imaturos foram comparados àqueles encontrados em outras espécies de *Actinote*.

Key words: Acraeini, *Actinote*, *Actinote quadra*, life-cycle, Nymphalidae.

INTRODUCTION

Members of the genus *Actinote* Hübner, [1819] (*sensu* Silva-Brandão *et al*. 2008) (Nymphalidae: Heliconiinae) are common in most montane habitats in the Neotropics, being seasonally abundant in wet forests above 800 m altitude (Francini 1989, 1992). The genus contains about 35 described species (Penz & Francini 1996, Francini et al. 2004, Lamas 2004, Paluch 2006, Paluch *et al*. 2006, Silva-Brandão *et al*. 2008, Neild 2008, Willmott *et al*. 2009). Although most species are locally common and associated with secondary habitats and open vegetation (Francini 1989), at least two species are considered threatened in Brazil: *Actinote zikani* D'Almeida, 1951, and *Actinote quadra* (Schaus, 1902) (Francini et al. 2005, Freitas & Brown 2008a, b).

The known localities for *A. quadra* include 12 sites in southeastern Brazil, in São Paulo, Minas Gerais and Rio de Janeiro (Francini 1992, Paluch 2006, Freitas & Brown 2008a) (Fig. 1), and the species usually occurs in wet montane forests in elevations of 1200-1600m. Field surveys and data from collections show that most populations of *A. quadra* occur in low densities, contrary to most species in the genus *Actinote*. Field records by AVLF and RBF are of few individuals per collecting trip (usually one to five), and series in most collections are correspondingly small (Paluch 2006: 290). Together, the naturally low population density and short adult flight period of *A. quadra* have resulted in very little knowledge of this species' biology. These features partially justified the recent inclusion of *A. quadra* in the Brazilian list of threatened butterflies as a vulnerable species (MMA 2003, Freitas & Brown 2008a).

Knowledge of the biology of threatened species is obviously essential for conservation programs (Lewinsohn *et al*. 2005). The present paper improves our knowledge of *Actinote quadra* by describing its early stages, larval host plants, and adult and larval behavior.

STUDY SITES AND METHODS

Adults and immatures of *A. quadra* were studied in the field, in a dense population on the slopes of the Serra da Mantiqueira in Campos do Jordão, São Paulo, southeastern Brazil (22°46'9"S 45°36'57"W), ca. 1600 m elevation (Fig. 2a, b). Adults, immatures and host plants were observed along a 1000 m trail in well preserved montane vegetation during 16 field days in November-December 2006, 2007, 2008 and March-April 2007, 2008, 2009. 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 1991). Dry head capsules and pupal castings were conserved in glass vials. Immatures were fixed in Kahle solution (Borror & DeLong 1971), and all the samples (preserved eggs and larvae, head capsules and pupal castings) were deposited at the Museu de Zoologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil (Unicamp). All measurements were made using a stereomicroscope fitted with a calibrated micrometric ocular.

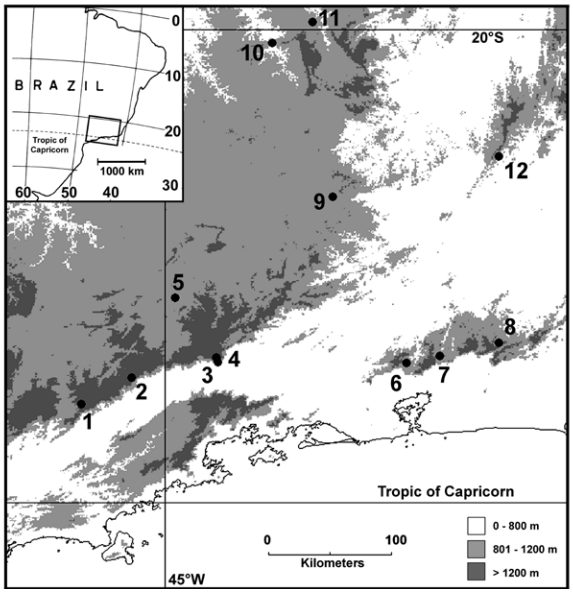

Fig. 1. Map showing 12 known localities for *Actinote quadra* in Southeastern Brazil; 1. Campos do Jordão, SP; 2. Piquete, SP; 3. Itatiaia (sede), RJ; 4. Itatiaia (Maromba), RJ; 5. Caxambu, MG; 6. Petrópolis, RJ; 7. Teresópolis, RJ; 8. Nova Friburgo, RJ; 9. Barbacena, MG; 10. Ibirité (Mata do Fecho), MG; 11. Belo Horizonte (Parque das Mangabeiras), MG; 12. Rosário da Limeira (Iracambi), MG. Abbreviations for States: MG = Minas Gerais; RJ = Rio de Janeiro; SP = São Paulo.

Egg size measurements included height and diameter, and head capsule width refers to the distance between the most external ocelli (as in Freitas 2007).

RESULTS

Adult behavior: The first sighting of *A. quadra* in the study area occurred on November 3, 2006, around 11:00. Later on the same day and during the following week, several adults were observed flying around in a sunlit spot near a waterfall, and in four other sunlit sites along the road. In these sunlit sites, males were observed perching and promenading in a typical territorial behavior from 11:00 to 13:30 when there was intense sunlight. Perching sites were usually the upper surface of large leaves in the sunlight from 1 to 10 m high (Fig. 2d, e), with most perching sites on leaves above 2m high. Perching males were observed chasing any conspecific males that approached their perching sites, and less intensively pursuing individuals of other medium-sized species, including other species of *Actinote* present in the area and several Nymphalidae and Pieridae. These territories were usually 10 to 50 meters far from the more dense aggregation of host plants and immatures, but on four occasions females were observed flying in these territories and being chased by males. In one of these chases the male reached the female and grabbed her with his legs, upon which the pair fell to the ground and had a successful copulation (Fig. 3b). During copulation, females always hold the males, both when perched and in flight. Females were otherwise usually observed near the host plants, and were much rarer than males. Most observations of females were of individuals ovipositing or searching for plants. Males were seldom observed feeding, and the few nectar sources observed were flowers of *Croton urucurana* Baill. (Euphorbiaceae), *Chromolaena punctulata* (DC.) R. King & H. Robins and *Senecio bonariensis* Hook. & Arn. (Asteraceae). Adults are bivoltine, with flight periods of about two months; the first generation occurs in March/April and the second in November/December. Adults of *A. quadra* are sexually dimorphic, with males predominantly orange with black contours (Fig. 2d, e) and females predominantly black with cream areas (Fig. 3a). In the study site, individuals were abundant during the flight periods, with 100 - 300 individuals easily observed in few hours of field work (most of which were males as stated above).

Host plant and oviposition: Oviposition was first observed and photographed on November 12, 2006 (Fig. 3c). Eggs were deposited on the underside of a leaf of *Ophryosporus regnellii* Baker (Asteraceae), a medium sized evergreen shrub (1-3m tall) that is very common along the road edges in the study site (Fig. 2c). Eggs are laid in clusters of 63 to 357 (mean = 190.3 eggs, SD = 71.81 eggs, n = 33 clusters), always under the leaf. Ovipositions were mostly irregular in shape, and the observed density was of 1 egg/mm^2 (SD = 0.28 egg/mm2, n = 25). Of the 33 observed ovipositions, 25 presented sterile eggs varying in number from one to 14. Sterile eggs are recognized because they remain yellow, while fertilized eggs change color to red after a few days (Fig. 4a). We have documented one case of double oviposition, where two females oviposited on the same leaf (Fig. 3d).

Larval behavior and natural history. Larvae exited the egg shell laterally, and consumed part of the egg chorion. Larvae of early instars were strongly gregarious with synchronous activity, including feeding, resting, and moving between leaves. Final instars were found dispersed in the field, suggesting that they become solitary just before pupation. First instars scraped the leaf surface and fed only on the superficial tissue (Fig. 3f), while last instar were observed consuming the entire leaf, creating holes in the leaves as they fed. Based on eggs collected in November, the larval period in the laboratory lasted 78-84 days (with eight to nine instars), followed by 11-12 days as pupa, with adults emerging in beginning of March, when adults were again observed flying in nature.

Natural enemies. Several egg clusters were found to have damaged eggs at the edge of the cluster, and nymphs of the stinkbug *Stiretrus* sp. (Hemiptera: Pentatomidae) were actually observed sucking peripheral eggs (Fig. 3e). Pupae of two field collected larvae died after the emergence of a larva of a tachinid fly parasitoid (Diptera: Tachinidae). The only predation of adults observed was that of a female observed trapped in an orb-weaver spider web.

Immature stages

Egg (Figs. 3d, 4a, b). Light cream when first laid (Fig. 3c), changing gradually to reddish during the first 36 hours (Fig. 4a, b); barrel shaped with 18 - 21 longitudinal ribs and ca. 18 - 19 weakly marked transverse ribs; mean height 0.689 mm (range 0.59 – 0.75 mm, SD = 0.0403, n = 50), mean diameter 0.529 mm (range 0.48 - 0.59 mm, SD = 0.0235, n = 50). Duration 14 days, in the laboratory, with all larvae emerging at the same time.

First instar (Figs. 3f, 4c, d). Head black, smooth, without scoli, mean width 0.389 mm (range 0.38 - 0.4 mm, SD = 0.011 mm, n = 7); body light cream, without scoli and with long black setae arising from dark sclerotized chalazae; a conspicuous dorsal black prothoracic plate present; legs black, prolegs with lateral dark grey plate, anal plate black. Maximum length 3.5 mm. Duration 10 days.

Second instar (Fig. 4e). Head dark brown, smooth, without scoli, mean width 0.57 mm (range 0.52 – 0.58 mm, SD = 0.021 mm, n = 10); body light cream, with short very light brown scoli; legs black, prolegs light cream, anal plate light brown. Maximum length 4.1mm. Duration 8-9 days.

Intermediate instars (Fig. 4f-h). The number and size of the intermediate instars (third to penultimate) are both variable, and because of this, these will not be described separately in detail. In the laboratory, larvae presented 8-9 instars (based on two clusters reared), all very similar in color pattern. Head dark brown, smooth, without scoli; body dark brown dorsally, greenish yellow ventrally, covered with long dark brown scoli; legs black, prolegs yellow and anal prolegs light brown, becoming gradually darker in later instars; anal plate dark brown. Larvae grown from 6.0 mm in third instar to 20 mm in penultimate instar. Duration of the instars vary from 7 to 20 days.

Last instar (Fig. 5a-d). Head dark brown with medium brown areas in frontal view, smooth with thin dark setae and without scoli, spines or chalazae, mean width 2.69 mm (range 2.38 – 2.85 mm, SD = 0.164 mm, n = 6); body dark brown dorsally, greenish yellow ventrally, covered with long black scoli with long brown setae; legs black, prolegs yellow and anal prolegs dark brown; anal plate dark brown. Larvae become lighter when fully grown, three to four days before pupation (Fig. 5c, d). Maximum length: 40 mm. Duration 19-21 days.

Pupa (Fig. 5e-g). General profile elongated, ground color white with dark brown markings on wing cases and abdomen; abdominal segments mobile, with a series of five pairs of subdorsal black tubercles from segments A2 to A6. Length 24 - 26 mm (n = 5). Duration 11-12 days.

DISCUSSION

Natural history and immatures. In general, the behavior and appearance of *A. quadra* immatures are similar to those described for other species of *Actinote* (DeVries 1987, Francini

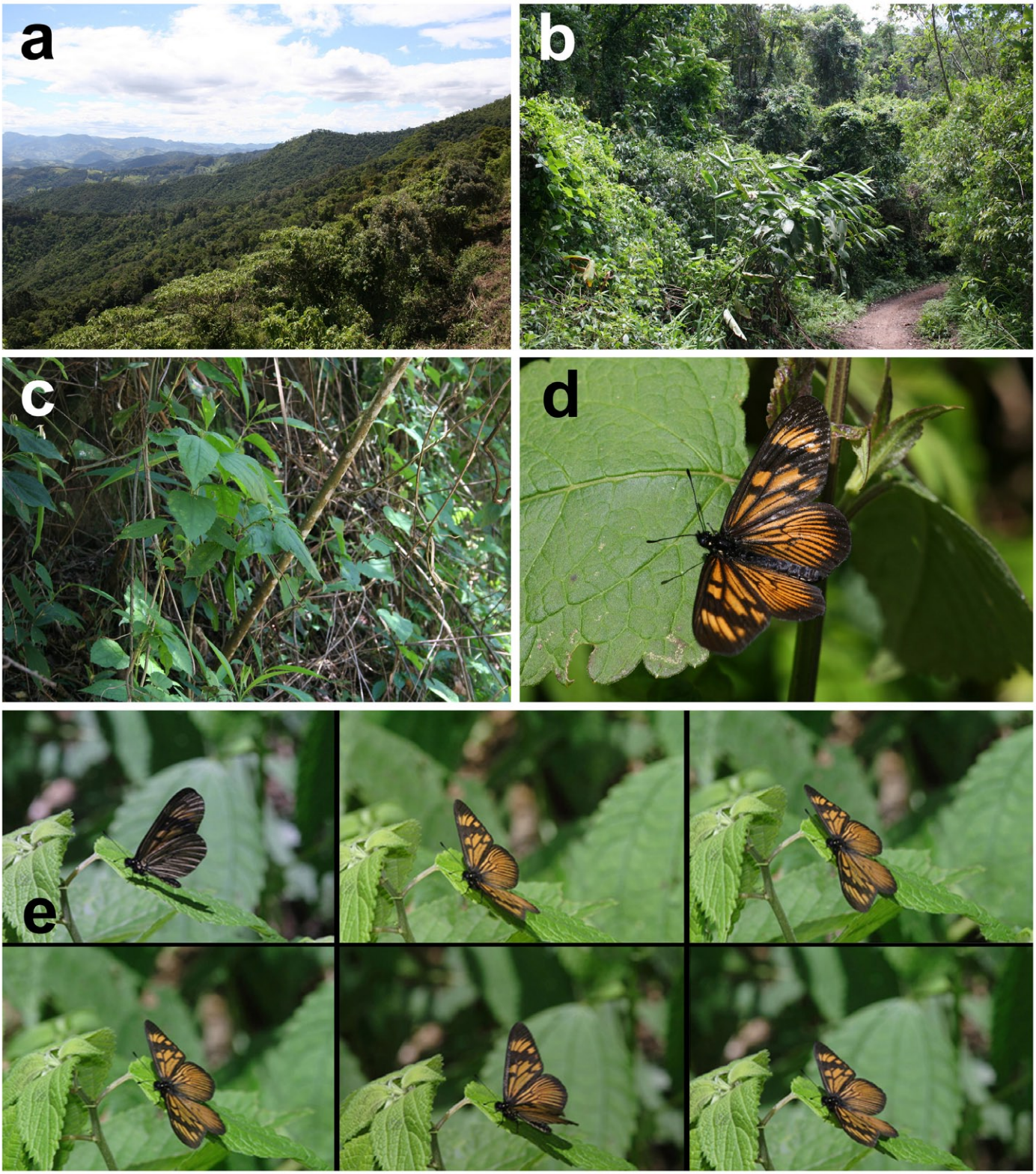

Fig. 2. Habitat and Natural History of *Actinote quadra;* a) General habitat of *A. quadra*; b) trail where immatures and adults were observed; c) close view of the larval host plant *Ophryosporus regnellii*; d) adult male landing above leaf; e) six sequential pictures of a male *A. quadra* opening and closing wings while perching.

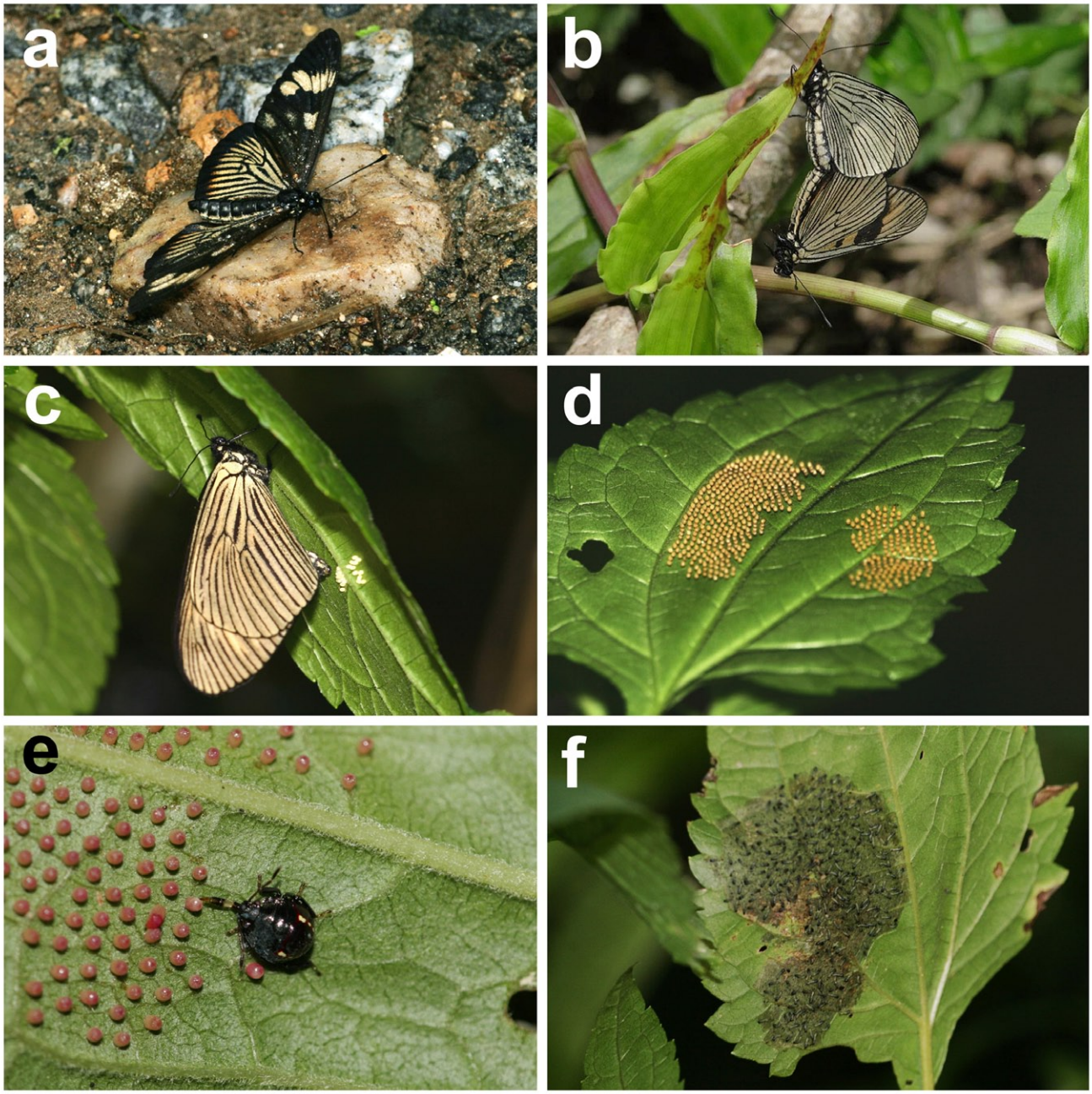

Fig. 3. Adults and immatures of *Actinote quadra*; a) female basking on the soil surface on early morning; b) couple in copula; c) female ovipositing on *O. regnellii*; d) double oviposition; e) egg predation by the stinkbug *Stiretrus* sp.; f) group of first instar feeding.

1989, Brown 1992, Paluch *et al*. 1999, 2001, Freitas *et al*. 2009). Eggs and larvae are grouped as described for all known Acraeini (Freitas *et al*. 2009). The observed ovipositions of *A. quadra* are asymmetrical, thus differing from other species of *Actinote* such as *A. pellenea pellenea* Hübner, [1821] and *A. parapheles* Jordan,1913, whose ovipositions are approximately symmetrical in shape (Francini 1989). Both the average and the maximum number of eggs per oviposition in *A. quadra* are lower than those reported for all 10 other species of *Actinote* for which there are data from southeastern Brazil (Francini 1989, Paluch *et al*. 1999, 2001). In *A. pellenea* for example, ovipositions average more than 400 eggs, with some ovipositions exceeding 1,000 eggs (Francini 1989, Francini & Freitas in press.). First and last instars are similar to those described for other species of *Actinote* in both general look and scoli distribution (Francini 1989, Paluch *et al*. 1999, 2001, Freitas *et al.* 2009). The color pattern of last instars resembles that of *A. zikani* (R. B. Francini, unpublished), but the scoli are relatively shorter. The pupa, with five pairs of subdorsal tubercles on the abdomen, is similar to those of most species of *Actinote*, except *A. canutia* (Hopffer, 1874) and *A. mamita* (Burmeister, 1861), which have six pairs of subdorsal tubercles (Francini 1989, Paluch *et al*. 1999, 2001, Freitas *et al*. 2009). The pupal tubercles are relatively short, similar to those of *A. surima* (Schaus, 1902), *A. carycina* Jordan, 1913 and *A. p. pellenea* (Paluch et al. 1999, 2001, and pers. obs.). The sexual dimorphism of *A. quadra* adults is remarkable and very similar to that present in *A. rufina* Oberthür, 1917 (see Freitas *et al*. 2009), although these two

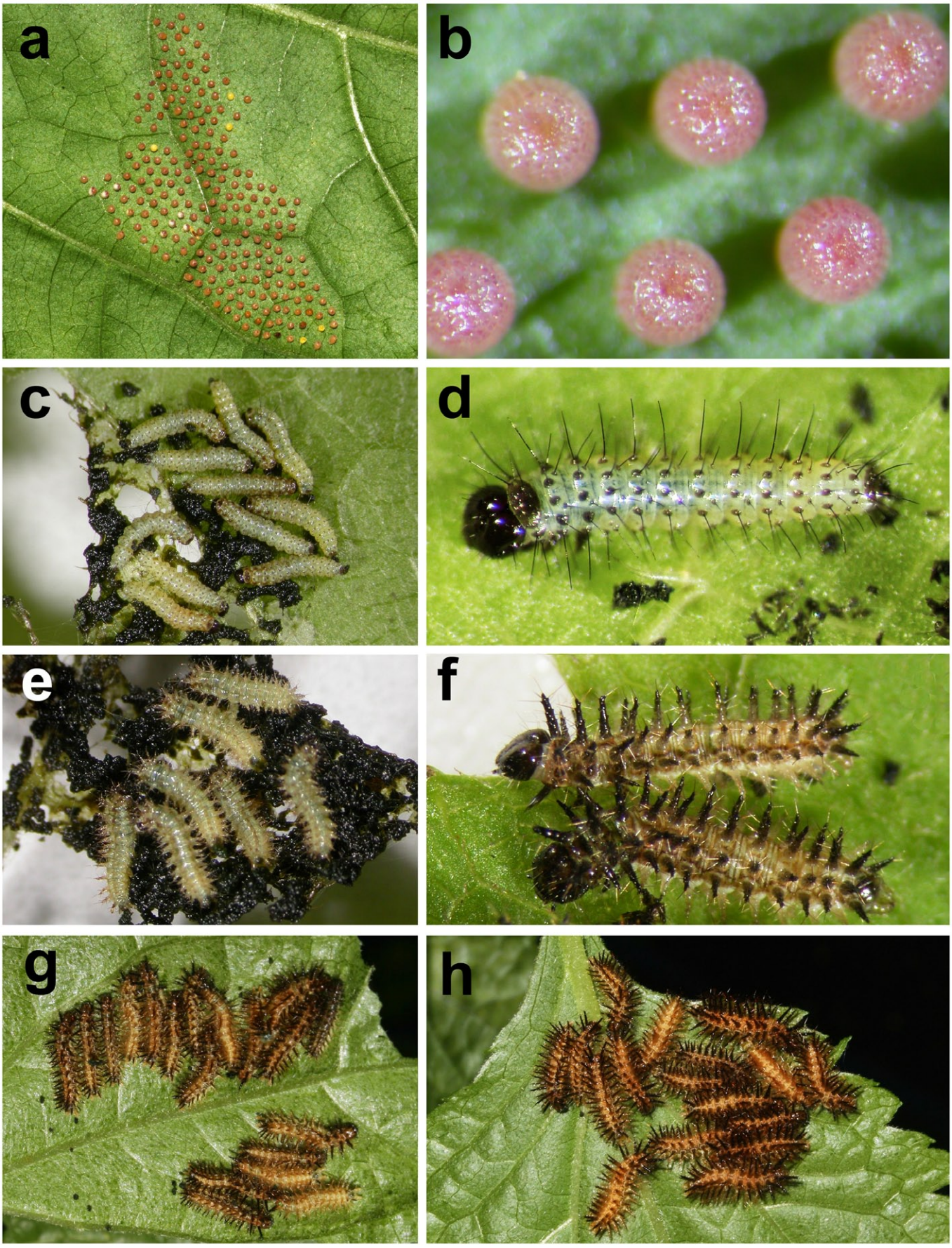

Fig. 4. Immatures of *Actinote quadra*; a) egg cluster four days after oviposition; note yellow sterile eggs ; b) close view of eggs; c) group of first instar; d) close view of first instar; e) second instar; f) third instar; g) group of forth instar; h) group of fifth instar.

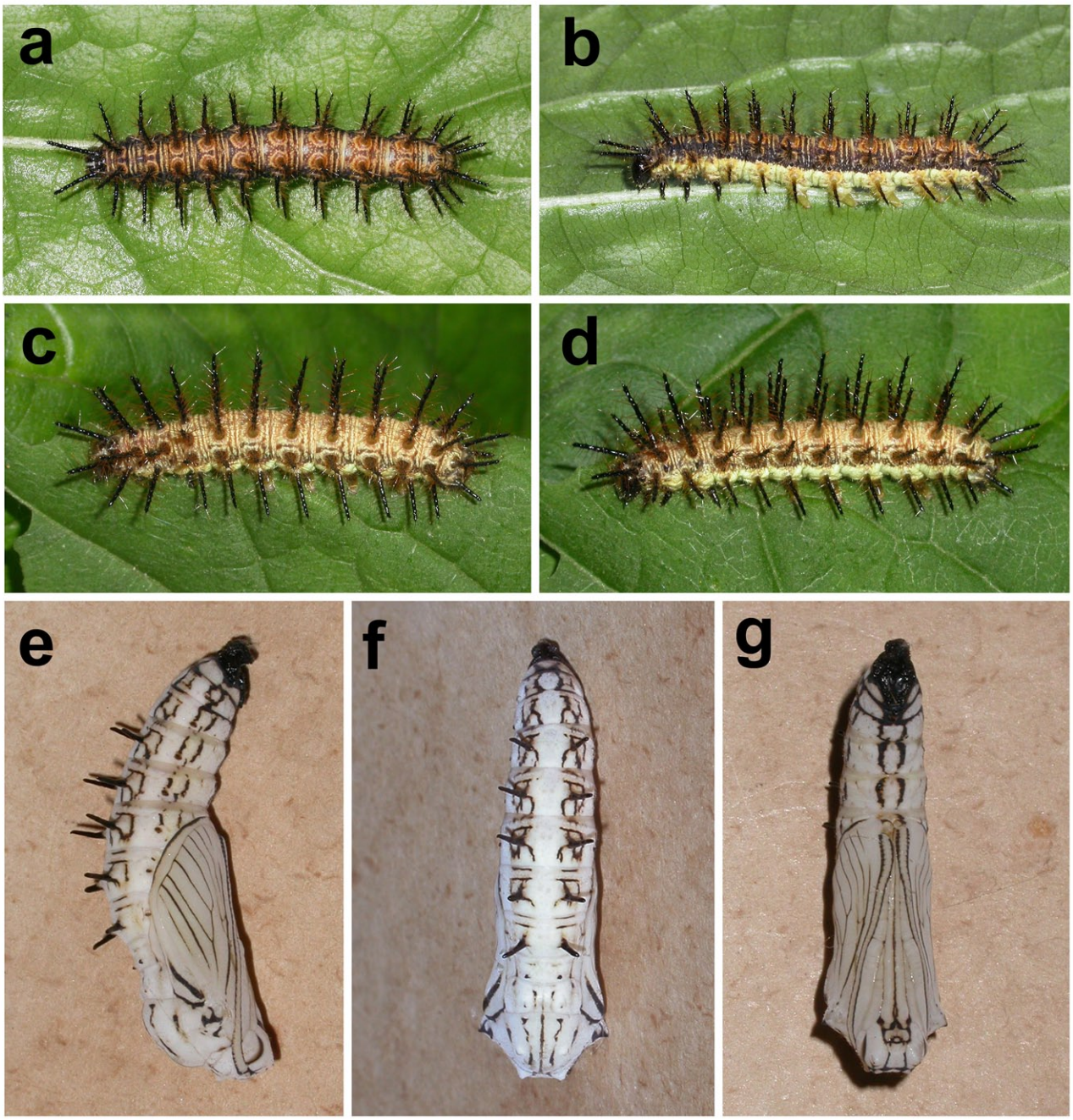

Fig. 5. Immatures of *Actinote quadra*; a, b) early last instar (dorsal, lateral); c, d) late last instar (dorsal, lateral); e-g) pupa (lateral, dorsal, ventral).

species are not closely related (Silva-Brandão *et al*. in prep.).

Conservation strategies. In the most recent evaluation, the conservation status of *A. quadra* was assessed as Vulnerable (Freitas & Brown 2008a), the threatened category with the lowest implied risk of extinction based on the current IUCN criteria (IUCN 2001). All of the 12 known populations of *A. quadra* that have been visited in the last 15 years still persist, though the present paper represents the only published observations of abundance for any of these. Most field observations of *A. quadra* are of only a few individuals (pers. obs., Brown, Francini, pers. comm.), contrasting with the high density of adults reported for other *Actinote* species (Francini 1989). However, population sizes of *A. zikani*, for example, are known to vary extensively from year to year, with periods of high adult density alternating with several years with no records (Francini *et al*. 2005), and it could be that some populations of *A. quadra* follow the same pattern.

Since some populations of *A. quadra* already reside in protected areas, rather than creating new reserves, research efforts should be focused on discovering new sites for this species in the areas between the known populations, coupled with additional ecological studies in known colonies, to better understand population size, temporal fluctuations and the ranges of each population unit. At the present the known populations appear to be isolated from each other, even if there exists large areas of apparently similar suitable habitat between some of them, such as the extensive slopes of the Mantiqueira Mountain Range. It also would be interesting to know if *A. quadra* occurs

south of Petrópolis and Teresópolis in the Serra do Mar, such as in the region of the Serra da Bocaina National Park. The Serra da Bocaina region has large areas of suitable habitat, and another three species of *Actinote* that usually fly together with *A. quadra* have already been recorded there, namely *A. alalia* (C. Felder & R. Felder, 1860), *A. bonita* Penz, 1996 and *A. conspicua* Jordan, 1913. Improved distributional and biological data are essential for a more informed assessment of the species' threat status and for the development of effective conservation strategies.

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REFERENCES CITED

Borror, D. J. & D. M. DeLong
1971. An Introduction to the Study of Insects. Holt, Rinehart and Winston. New York. 812pp.

Brown Jr., K. S.
1992. Borboletas da Serra do Japi: diversidade, habitats, recursos alimentares e variação temporal. In Morellato, L.P.C.O., ed, História natural da Serra do Japi. Unicamp, Fapesp, Campinas, SP, PP. 142-187.

DeVries, P. J.
1987. The Butterflies of Costa Rica and their Natural History. Princeton University Press, New Jersey. xxii + 317pp.

Francini, R. B.
1989. Biologia e ecologia das borboletas *Actinote* (Lepidoptera: Nymphalidae, Acraeinae) do sudeste do Brasil. Ms Thesis. Universidade Estadual de Campinas, Campinas. xiv + 236pp.

Francini, R. B.
1992. Ecologia das taxocenoses de *Actinote* (Lepidoptera: Nymphalidae) em Asteraceae (Angiosperma, Magnoliatae) no sudeste do Brasil: subsídios para conservação. PhD Thesis. Unicamp, Campinas. vii + 194pp.

Francini, R. B., A. V. L. Freitas & C. Penz
2004. Two new species of *Actinote* (Lepidoptera, Nymphalidae) from Southeastern Brazil. Zootaxa 719: 1-10.

Francini, R. B., A. V. L. Freitas & K. S. Brown Jr.
2005. Rediscovery of *Actinote zikani* (D'Almeida) (Nymphalidae, Heliconiinae, Acraeini): Natural history, population biology and conservation of an endangered butterfly in SE Brazil. Journal of the Lepidopterists' Society 59: 134–142.

Freitas, A. V. L.
1991. Variação morfológica, ciclo de vida e sistemática de *Tegosa claudina* (Eschscholtz) (Lepidoptera: Nymphalidae, Melitaeinae) no Estado de São Paulo. Revista Brasileira de Entomologia 35: 301-306.

Freitas, A. V. L.
2007. A new species of *Moneuptychia* Forster (Lepidoptera: Satyrinae: Euptychiina) from the highlands of Southeastern Brazil. Neotropical Entomology 36: 919-925.

Freitas, A. V. L. & K. S. Brown Jr.
2008a. *Actinote quadra.* In Machado, A. B. M., G. M. M. Drummond & A. P. Paglia (Eds.), Livro vermelho da fauna brasileira ameaçada de extinção, Brasília, DF: MMA; Belo Horizonte, MG: Fundação Biodiversitas pp. 402-403.

Freitas, A. V. L. & K. S. Brown Jr.
2008b. *Actinote zikani.* In Machado, A. B. M., G. M. M. Drummond & A. P. Paglia (Eds.), Livro vermelho da fauna brasileira ameaçada de extinção, Brasília, DF: MMA; Belo Horizonte, MG: Fundação Biodiversitas pp. 403-404.

Freitas, A. V. L., L. A. Kaminski, R. G. Matos & K. L. Silva-Brandão
2009. Immature stages of the Andean butterfly *Actinote rufina* (Nymphalidae: Heliconiinae: Acraeini). Tropical Lepidoptera Research, 19(1): 18-21.

IUCN
2001. IUCN Red List Categories and Criteria: Version 3.1. IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK. ii + 30 pp. http://www.redlist.org/info/categories_criteria.html.

Lamas, G.
2004. Checklist: Part 4A. Hesperioidea-Papilionoidea. In Heppner, J.B., ed, Atlas of Neotropical Lepidoptera, Vol 5A. Association for Tropical Lepidoptera/Scientific Publishers, Gainesville, p xxxvi + 439 pp.

Lewinsohn, T. M., A. V. L. Freitas & P. I. Prado
2005. Conservation of terrestrial invertebrates and their habitats in Brazil. Conservation Biology, 19(3): 640-645.

MMA
2003. Anexo à Instrução Normativa n° 3, de 27 de maio de 2003, do Ministério do Meio Ambiente. Lista das Espécies da Fauna Brasileira Ameaçadas de Extinção. http://www.ibama.gov.br/fauna/downloads/lista%20spp.pdf

Neild, A. F. E.
2008. The Butterflies of Venezuela, Part 2: Nymphalidae II (Acraeinae, Libytheinae, Nymphalinae, Ithomiinae, Morphinae). Greenwich, London, Meridian Publications. 272pp.

Paluch, M.
2006. Revisão das espécies de *Actinote* Hübner, [1819] (Lepidoptera, Nymphalidae, Heliconiinae, Acraeini). PhD Thesis. Universidade Federal do Paraná, Curitiba. xix + 410pp.

Paluch, M., M. M. Casagrande & O. H. H. Mielke
1999. Estágios imaturos de *Actinote surima* (Schaus) (Lepidoptera, Nymphalidae, Acraeinae). Revista Brasileira de Zoologia, 16: 129-140.

Paluch, M., M. M. Casagrande & O. H. H. Mielke
2001. Estágios imaturos de *Actinote carycina* Jordan (Lepidoptera, Nymphalidae, acraeinae Revista Brasileira de Zoologia, 18: 883-896.

Paluch, M., M. M. Casagrande & O. H. H. Mielke
2006. Três espécies e duas subespécies novas de *Actinote* Hübner (Nymphalidae, Heliconiinae, Acraeini). Revista Brasileira de Zoologia, 23: 764-778.

Penz, C., & R. B. Francini
1996. New species of *Actinote* Hübner (Nymphalidae: Acraeinae) from Southeastern Brazil. Journal of the Lepidopterists' Society, 50: 309-320.

Silva-Brandão, K. L., N. Wahlberg, R. B. Francini, A. M. L. Azeredo-Espin, K. S. Brown Jr., M. Paluch, D. C. Lees & A. V. L. Freitas
2008. Phylogenetic relationships of butterflies of the tribe Acraeini (Lepidoptera, Nymphalidae, Heliconiinae) and the evolution of host plant use. Molecular Phylogenetics and Evolution 46: 515-531.

Willmott, K. R., A. V. L. Freitas, J. P. W. Hall, K. L. Silva-Brandão & M. Paluch
2009. A new species of *Actinote* Hübner from the Eastern Andes of Ecuador (Lepidoptera: Nymphalidae: Heliconiinae). Proceedings of the Entomological Society of Washington, 111: 47-56.