Notes on the last instar larva and pupa of *Hemiargus hanno* (Stoll) (Lepidoptera, Lycaenidae, Polyommatinae)[1]

Marcelo Duarte[2, 3]
Gisele Luziane de Almeida[2, 4]
Mirna Martins Casagrande[2, 3]
Olaf H.H. Mielke[2, 3]

ABSTRACT. Larvae of *Hemiargus hanno* (Stoll, 1790) (Lepidoptera, Lycaenidae, Polyommatinae) are for the first time reported to be attended under field conditions by ants of the formicine genus *Brachymyrmex* Mayr, 1868 (Myrmelachistini). Structural features of the last instar larva and pupa of *H. hanno* are described and illustrated with the aid of scanning electron microscopy (SEM).

KEY WORDS. Lycaenidae, Polyommatinae, *Hemiargus*, *Brachymyrmex*, immature stages, scanning electron microscopy, morphology, myrmecophily.

The polyommatine lycaenids are found in all major biogeographical areas of the world, with the highest diversity of species reported for North America, Europe and Asia (ELIOT 1973). These butterflies have been considered one of the poorest studied groups within the family Lycaenidae. Several aspects of the biology, behaviour, morphology, taxonomy and phylogenetic/biogeographic affinities of the Neotropical Polyommatinae still remain obscure or completely unknown (NABOKOV 1945; ELIOT 1973).

Hemiargus hanno (Stoll, 1790) is a common lycaenid butterfly in many open weedy areas, pastures, and fields, exhibiting a marked sexual dimorphism on the dorsal wing color pattern (Figs 1, 2). It is widely distributed from southern Texas and Central America to Argentina in South America (LEWIS 1983; SMITH *et al.* 1994; VARGA 2000). According to BÁLINT & JOHNSON (1995), *H. hanno* has been observed flying synchronically and in sympatry with *H. bogotana* (Draudt, 1921) in some Bolivian mountain habitats. The adult exoskeletal morphology of *H. hanno* has been studied in some detail by DUARTE *et al.* (2001 and unpublished data).

Despite the fact that *H. hanno* is generally considered an abundant species throughout its geographical range, it still exists a scarcity of data on its biology and ecology compared to the common North American Reakirt's blue, *Hemiargus isola* (Reakirt, 1866), which has been the subject of several studies (see BURNS & JOHNSON 1971; BALLMER & PRATT 1988; WAGNER 1993, 1995; WAGNER & KURINA 1997; WAGNER & MARTÍNEZ-DEL-RIO 1997).

---

1) Contribution number 1241 of the Departamento de Zoologia, Universidade Federal do Paraná. Partially supported by grants from FUNPAR/UFPR.

2) Departamento de Zoologia, Universidade Federal do Paraná. Caixa Postal 19020, 81531-990 Curitiba, Paraná, Brasil. E-mail: lycaenidae@ig.com.br

3) Fellowship from CNPq.

4) Fellowship from CAPES.

Egg, larvae and pupa of *H. hanno* were superficially described by D'ALMEIDA (1933) and HASKIN (1933). No illustration was provided. These authors gave little attention to the structural characters, which are known to effectively help in the identification of the lycaenid immature stages (BALLMER & PRATT 1988). With respect to the most suitable larval foodplants for *H. hanno*, D'ALMEIDA (1933) and BROWN (1992) cited *Indigofera anil* L. (synonym of *I. suffruticosa* Mill.) (Fabaceae), and SCOTT (1986) listed three other legume species available for Latin American populations: *Crotalaria verrucosa* L. (Fabaceae), *Mimosa pudica* L. (Mimosaceae) and *Phaseolus* sp. (Fabaceae). BARCANT (1970) *apud* FIEDLER (1991: 196) also recorded larval foodplants in the family Oxalidaceae. Preferences for protein-rich plant parts (flower buds or inflorescences) have been noted not only for *H. hanno* larvae (personal observation), but for other myrmecophilous and non-myrmecophilous species as well (ROBBINS & AIELLO 1982; COTTRELL 1984: 24).

Some authors have suggested that *H. hanno* larvae are associated with ants in their microhabitat (FIEDLER 1991; SMITH *et al.* 1994). As far as we know, however, no ant taxon has been accurately identified in association with the *H. hanno* larvae, as well as the myrmecophilous organs normally found in lycaenid larvae and pupae have not yet been illustrated for this species. In this paper, we report the ants, identified to genus level, attending *H. hanno* larvae in nature. We also describe and illustrate, using SEM photographs, some structural features of the last instar larva and pupa. We believe that further such works regarding immature descriptions of Neotropical lycaenids should be encouraged in order to broaden our understanding of the evolution of these butterflies.

MATERIALS AND METHODS

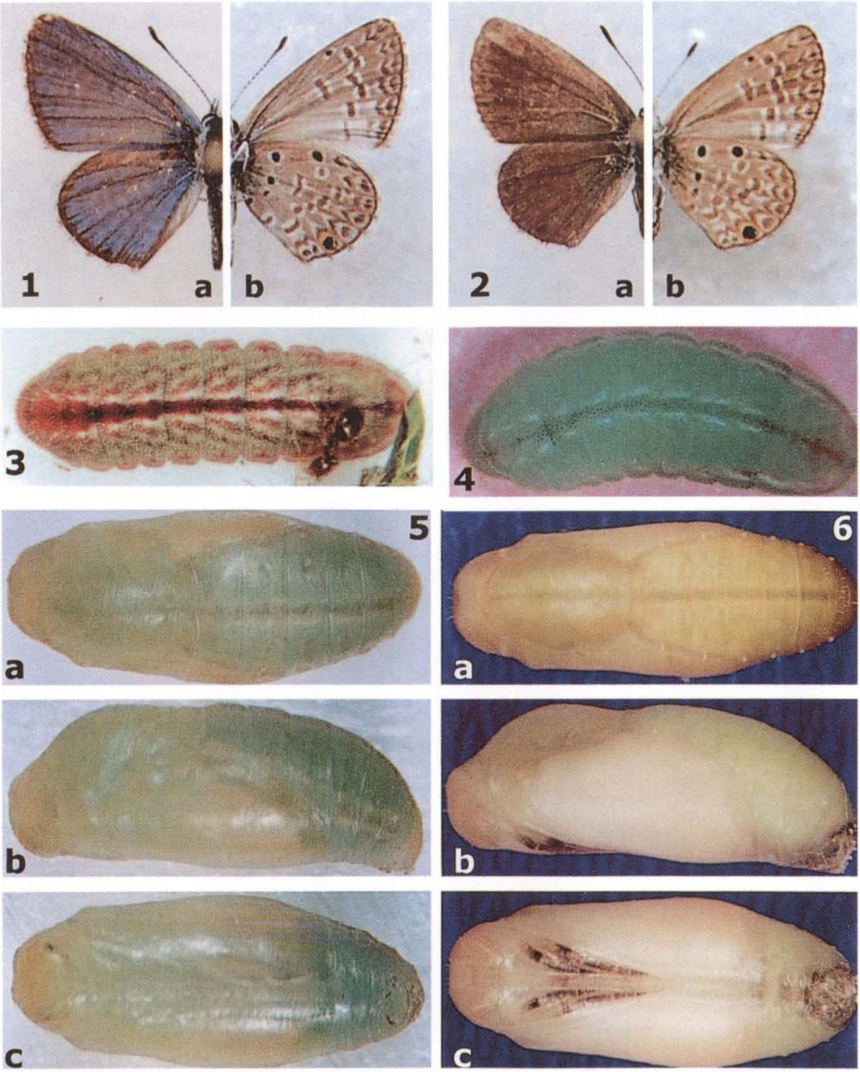
Collecting site and laboratory rearing

Larvae and pupae were respectively obtained from the field and laboratory rearings. Field trips were carried out near the town of Guapimirim (22° 32'S and 42° 59'W, elevation 50 meters), in the state of Rio de Janeiro, southeastern Brazil, where the growing real estate exploration during the last 10 years has resulted in major environmental changes. The area is located approximately 90 km northeastern of the city of Rio de Janeiro and a few kilometers from "Serra dos Órgãos" National Park (Teresópolis). The vegetation is characterized by an herbaceous physiognomy with many heliophilous species.

Flowers and young shoots of the legume foodplant *Mimosa pudica* L. (Mimosaceae) were searched for larvae and pupae. In the laboratory larvae were fed daily fresh flower buds of *Calliandra tweediei* Benth. (Mimosaceae). This plant was chosen as food because *M. pudica* was not found around Curitiba (Paraná State, Brazil) where the rearings took place. Attempts to raise *M. pudica* in the greenhouse have not been successful. Larvae and pupae were kept in clear plastic containers in a growth chamber at 25°C and under 12 hours photophase.

SEM techniques

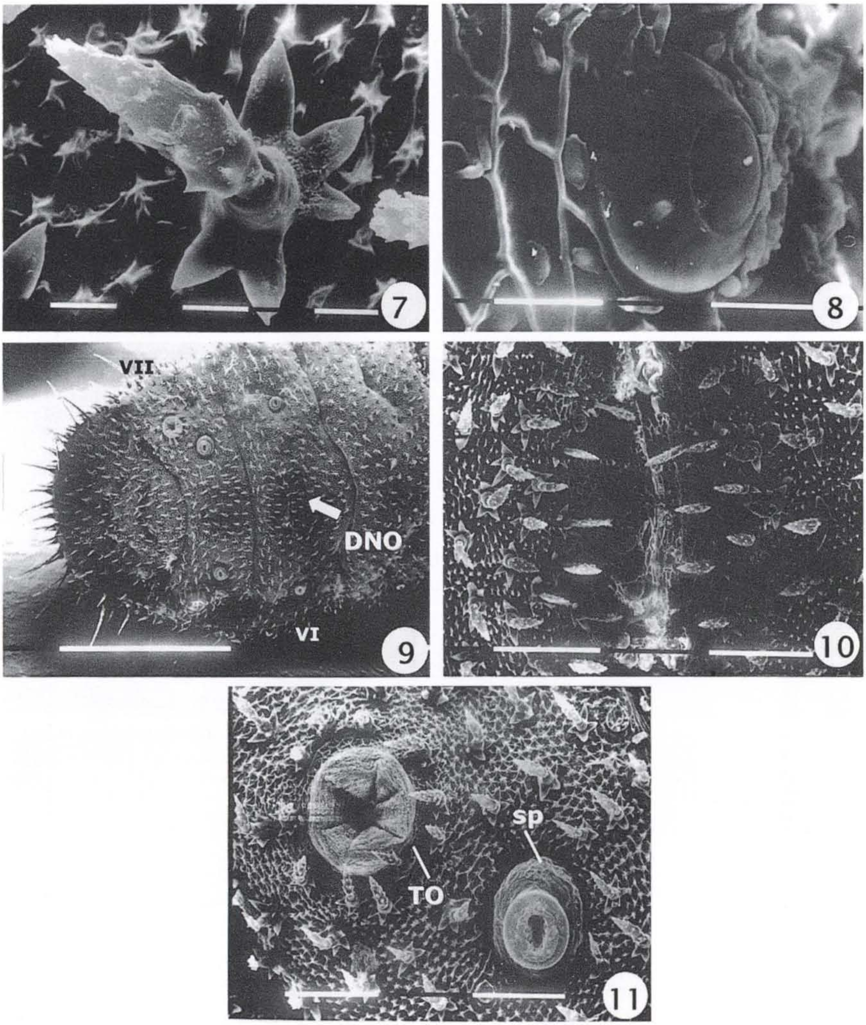
Specimens for SEM were critical point dried, mounted and sputter-coated before being photographed using a Philips SEM 505 (for more details on this methodology, see BONATTO & CARVALHO 1996).



Figs 1-6. *Hemiargus hanno*. (1) male and (2) female, (a) dorsal and (b) ventral view; (3) fourth instar larva in association with a *Brachymyrmex* sp. ant; (4) fourth instar larva few days before pupating; (5-6) different color patterns of the pupa: (5) at the beginning of the pupal stage; (6) few days before the adult emergence; (a) dorsal, (b) lateral and (c) ventral view.

Voucher specimens

Larvae, pupae and adults of *H. hanno* were deposited in the private collection of the first author. The ants were identified by Dr. Carlos Roberto F. Brandão, and were deposited in the following collections: Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZSP), and Coleção de Entomologia Padre Jesus Santiago Moure, Departamento de Zoologia, Universidade Federal do Paraná, Paraná, Brazil (DZUP).



Figs 7-11. *Hemiargus hanno*, fourth instar larva. (7) Stellate based seta, scale bar = 10 µm; (8) pore cupola organ (PCO), scale bar = 10 µm; (9) abdominal segments, arrow points the dorsal nectary organ (DNO) on the sixth abdominal segment, scale bar = 1 mm; (10) a detail of the DNO opening, scale bar = 0,1 mm; (11) seventh abdominal segment, detail of a retracted tentacle organ (TO) encircled by stellate based setae and a spiracle (sp), scale bar = 0,1 mm.

RESULTS AND DISCUSSION

Myrmecophily in *Hemiargus hanno* (Fig. 3)

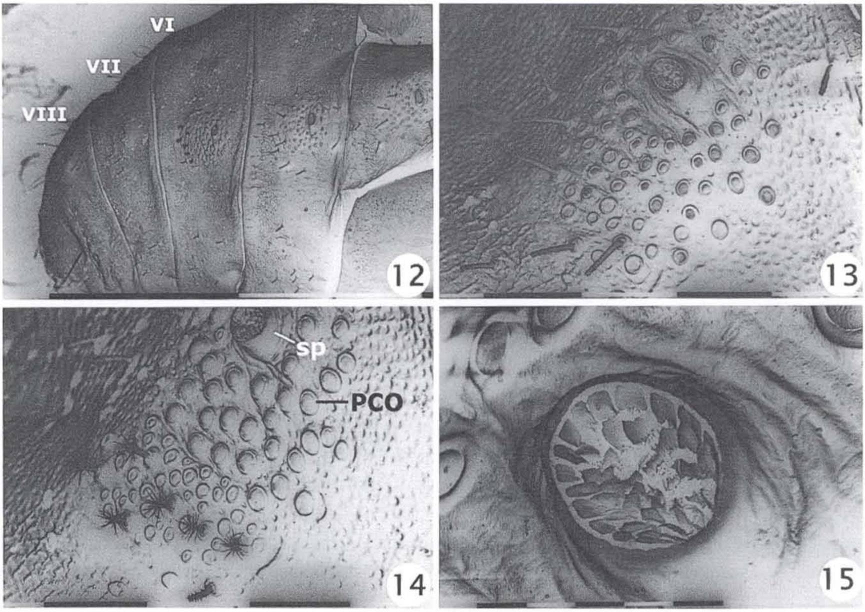
In the study site, larvae of *H. hanno* were observed being attended by ants of the formicine genus *Brachymyrmex* Mayr, 1868 (Myrmelachistini). This association apparently starts with the second instar larvae. WAGNER & KURINA (1997) reported the same behaviour between *H. isola* larvae and a tending ant species. Although no pupae of *H. hanno* were found during our field trips, in the laboratory one or more workers of *Brachymyrmex* sp. intensively palpated the pupae when placed together in the same container. Further studies should be carried out to confirm if under natural conditions ants tend pupae of *H. hanno*. The presence of some structural characters in the pupal skin that seem to be involved in chemical and/or tactile communication with ants (see description below) suggests that pupae are indeed tended by ants. It would also be interesting to test under laboratory conditions whether or not the larval and pupal development of *H. hanno* is affected by the ant tending behaviour (see reviews in ROBBINS 1991; FIEDLER & HÖLLDOBLER 1992; WAGNER 1993; FIEDLER & SAAM 1994, 1995). Our preliminary experimental data show that larvae can survive without ants in laboratory, and that there is no apparent effect of the ant tending behaviour on the pupal size. However, quantitative analyses were not yet performed.

Last instar larva (Figs 3, 4, 7-11)

Contrary to D'ALMEIDA (1933) who described five larval instars for *H. hanno*, we reported only four instars (Figs 3, 4, 7-11). DOWNEY & ALLYN (1979) also reported only four larval instars for *Leptotes cassius* (Cramer, 1775) (Lycaenidae, Polyommatinae) instead of the five described by D'ALMEIDA (1933). It is important to mention that for other lycaenid butterflies the number of instars has been shown to vary in response to certain environmental conditions under which either females oviposit or larvae are reared (SAKAI & MASAKI 1965; ENDO *et al.* 1985; BALLMER & PRATT 1989; M. DUARTE, unpublished data).

The most striking morphological feature of the larval body is the presence of several stellate based setae covering the thorax and abdomen (Fig. 7). These setae are regularly distributed around the myrmecophilous organs on the seventh and eighth abdominal segments (Figs 9-11).

Three types of myrmecophilous organs are found in the last instar larva of *H. hanno*: pore cupola organs (PCOs; Fig. 8), dorsal nectary organ (DNO; Figs 9, 10) and tentacle organs (TOs; Figs 9, 11). PCOs are wart-like glandular structures that have been observed both in lycaenid larvae and pupae. All lycaenid larvae ever investigated possess these organs (FIEDLER 1991; M. DUARTE, unpublished data). The DNO is located on the dorsum of the seventh abdominal segment, and is more restricted to some genera within the Lycaenidae (see review in FIEDLER 1991). This organ is associated not only with the stellate based setae, as mentioned above, but also with an increased number of PCOs around its opening (Fig. 10). Droplets of an aqueous solution were secreted by the DNO when larvae of *H. hanno* were stimulated by the antennae of *Brachymyrmex* sp. ants. This liquid probably represents an important nutritional source to the ants, although its chemical composition

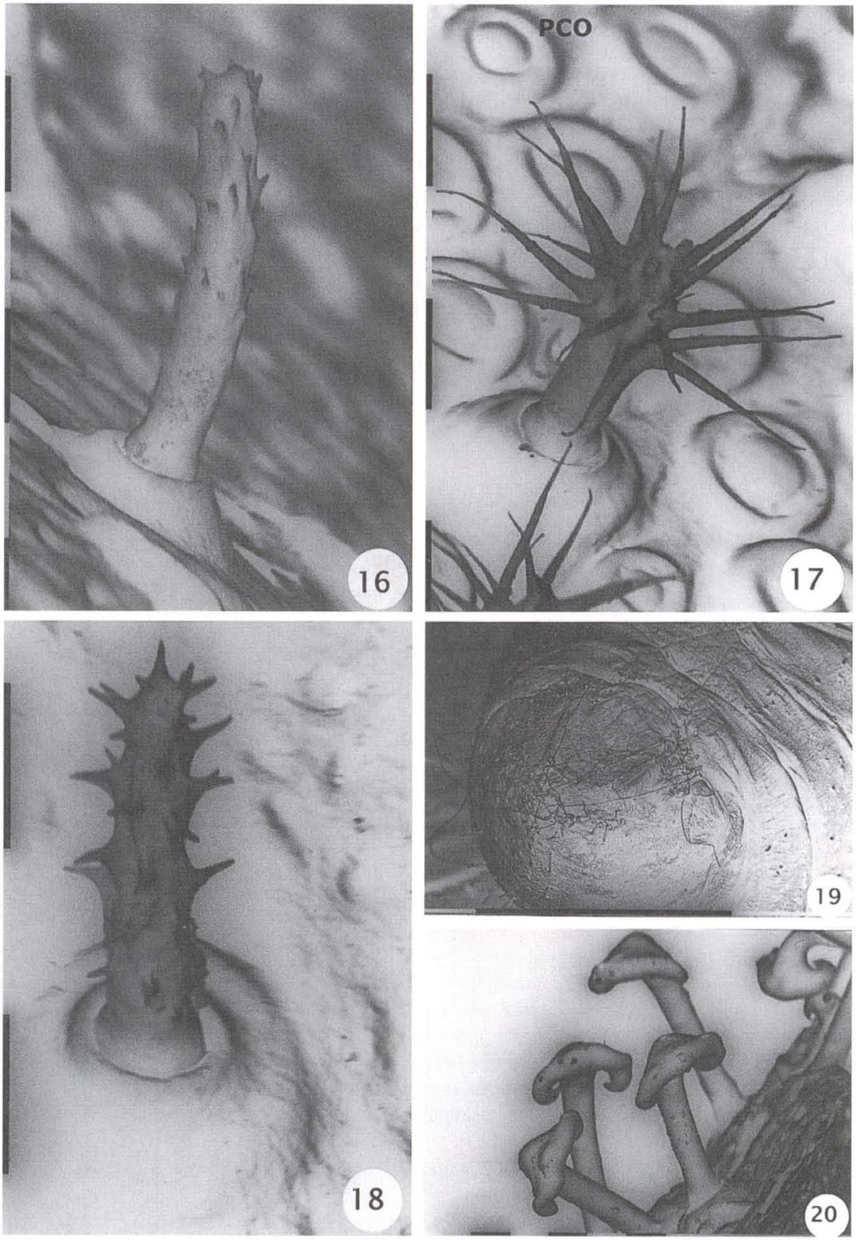


Figs 12-15. *Hemiargus hanno*, pupa. (12) Lateral view of the abdominal segments, scale bar = 1 mm; (13) fifth abdominal segment, detail of the structures associated with the spiracle, scale bar = 0,1 mm; (14) sixth abdominal segment, spiracle (sp) and pore cupola organ (PCO), scale bar = 0,1 mm; (15) an abdominal spiracle, scale bar = 10 µm.

is still poorly known (KITCHING 1983; PIERCE 1989). Finally, the TOs are a pair of eversible epidermal tentacles located on the dorsum of the eighth abdominal segment and posteriorly to the spiracle. Similarly to the DNO, the TOs are encircled by stellate based setae (Figs 9, 11). These tentacles are everted when palpated by ants, showing a corona of spiny setae. The functions of the TOs have been reviewed in FIEDLER (1991).

Pupa (Figs 5, 6, 12-20)

Most of the information herein added to D'ALMEIDA's (1933) description actually refers to the pupal integument, which contains some specialized cuticular characters. Figures 5 and 6 show the general coloration of the pupa at the beginning of the stage and few days before the emergence of the adult. There is a general resemblance of *H. hanno* pupae to other polyommatine species (D'ALMEIDA 1933; HASKIN 1933; LAWRENCE & DOWNEY 1966; DOWNEY & ALLYN 1979), i.e. they are very small, obtect, long and slender. Lumina of the pupal spiracles are decorated with a coral-like sculpturing (Fig. 15). WRIGHT (1983) pointed out that the interspecific variation of the spiracle sculpturing should be more thoroughly studied to elucidate some uncertainties in the taxonomy of lycaenid butterflies. There are four different types of setae on the pupal integument. One of them is longer, with apical spine-like projections, and relatively abundant over the entire body surface except



Figs 16-20. *Hemiargus hanno*, pupa. (16-18) Different types of setae of the pupal integument, scale bars = 10 µm; (16) a long seta with short spicules; (17) a dendritic setae; (18) a setae with stronger spicules; (19) ventral surface of the tenth abdominal segment, scale bar = 0,1 mm; (20) mushroom-like setae (cremastral hooks) around the posterior part of the anal opening, scale bar = 10 µm.

on the wing, head and appendage cases (Fig. 16). The other types of setae are less numerous and confined to some areas of the pupal integument. Two types of setae occur in close proximity to the spiracles on the sixth abdominal segment (Figs 12, 14): one is termed dendritic setae due to their long, fine, lateral spicules (Fig. 17), and according to BALLMER & PRATT (1988), these setae are supposed to secrete ant-attractive chemicals; the other type is very similar to the aforementioned abundant setae found on the pupal integument, differing only by the reduced size and presence of stronger spicules (Fig. 18). The last type of setae (mushroom-like cremastral hooks) is found on the ventral surface of the tenth segment. These setae are prominently clustered in a "C" shaped band around the posterior part of the anal slit (Figs 19, 20). Finally, supporting our hypothesis of myrmecophily during the pupal stage, a great number of pore cupola organs (PCOs), similar to those found in the last instar larva, were found on the abdominal segments, always very close to the spiracles (Figs 12-15, 17).

ACKNOWLEDGEMENTS. We would like to thank Carlos Roberto F. Brandão for the identification of the ants, Daura R. Eiras-Stofella, Matilde Machado and Vera Regina F. Pionteke for helping with the SEM techniques, and Astrid Caldas, Gabriel A. R. Melo, Luciane Marinoni and Robert K. Robbins for comments and suggestions to the manuscript.

REFERENCES

BÁLINT, Z. & K. JOHNSON. 1995. Neotropical polyommatine diversity and affinities. I. Relationships of the higher taxa (Lepidoptera: Lycaenidae). **Acta zool. hung. 41** (3): 211-235.

BALLMER, G.R. & G.F. PRATT. 1988. A survey of the last instar larvae of the Lycaenidae of California. **Jour. Res. Lepid. 27** (1): 1-81.

————. 1989. Instar number and larval development in *Lycaena phlaeas hypophlaeas* (Boisduval) (Lycaenidae). **Jour. Lepid. Soc. 43** (1): 59-65.

BONATTO, S.R. & C.J.B. CARVALHO. 1996. Análise morfológica das formas imaturas de *Sarconesia chlorogaster* (Wiedemann) (Diptera, Calliphoridae, Toxotarsinae). **Revta bras. Zool. 13** (3): 707-726.

BROWN JR., K.S. 1992. Borboletas da Serra do Japi: diversidade, hábitats, recursos alimentares e variação temporal, p. 142-187. *In*: L.P.C. MORELLATO (ed.). **História natural da Serra do Japi: ecologia e preservação de uma área florestal no Sudeste do Brasil**. Campinas, São Paulo, Editora da UNICAMP/ FAPESP, 321p.

BURNS, J.M. & F.M. JOHNSON. 1971. Esterase polymorphism in the butterfly *Hemiargus isola* stability in a variable environment. **Proc. Nat. Acad. Sci. USA 68** (1): 34-37.

COTTRELL, C. B. 1984. Aphytophagy in butterflies: its relationship to myrmecophily. **Zool. Jour. Linnean Soc. 79**: 1-57.

D'ALMEIDA, R.F. 1933. Les chenilles des genres *Hemiargus* et *Leptotes* (Lép. Lycaenidae). **Lambillionea 33** (11): 230-236.

DOWNEY, J.C. & C.A. ALLYN. 1979. Morphology and biology of the immature stages of *Leptotes cassius theonus* (Lucas) (Lepid.: Lycaenidae). **Bull. Allyn Mus. 55**: 1-27.

DUARTE, M.; M.M. CASAGRANDE & O.H.H. MIELKE. 2001. Morfologia externa do adulto de *Hemiargus hanno* (Stoll) (Lepidoptera, Lycaenidae, Polyommatinae, Polyommatini). I. Cabeça. **Revta bras. Zool. 18** (1): 225-238.

ELIOT, J.N. 1973. The higher classification of the Lycaenidae (Lepidoptera): a tentative arrangement. **Bull. Brit. Mus. (Nat. Hist.), Ent. 28** (6): 371-505.

ENDO, K.; Y. MARUYAMA & K. SASAKI. 1985. Environmental factors controlling seasonal morph determination in the small copper butterfly, *Lycaena phlaeas daimio* Seitz. **Jour. Insect Physiol. 31** (7): 525-532.

FIEDLER, K. 1991. Systematic, evolutionary, and ecological implications of myrmecophily within Lycaenidae (Insecta: Lepidoptera: Papilionoidea). **Bonn. Zool. Monogr. 31**: 1-210.

FIEDLER, K. & B. HÖLLDOBLER. 1992. Ants and *Polyommatus icarus* immatures (Lycaenidae) – sex-related developmental benefits and costs of ant attendance. **Oecologia 91**: 468-473.

FIEDLER, K. & C. SAAM. 1994. Does ant-attendance influence development in 5 European Lycaenidae butterfly species? (Lepidoptera). **Nota Lepid. 17** (1/2): 5-24.

————. 1995. Ant benefits from attending facultatively myrmecophilus Lycaenidae caterpillars: evidence from a survival study. **Oecologia 104**: 316-322.

HASKIN, J.R. 1933. The life histories of *Eurema demoditas*, *Lycaena theonus* and *L. hanno* (Lepid.: Pieridae, Lycaenidae). **Ent. News 44**: 153-156.

KITCHING, R.L. 1983. Myrmecophilous organs of the larvae and pupa of the lycaenid butterfly *Jalmenus evagoras* (Donovan). **Jour. Nat. Hist. 17**: 471-481.

LAWRENCE, D. & J.C. DOWNEY. 1966. Morphology of the immature stages of *Everes comyntas* Godart (Lycaenidae). **Jour. Res. Lepid. 5** (2): 61-96.

LEWIS, H.L. 1973. **Butterflies of the world**. London, Lionel Leventhal Limited, 312p.

NABOKOV, V. 1945. Notes on neotropical Plebejinae (Lepidoptera, Lycaenidae). **Psyche 52** (1-2): 1-61.

PIERCE, N.E. 1989. Butterfly-ant mutualisms, pp. 299-324. *In*: P.J. GRUBB & J.B. WHITTAKER (eds). **Toward a more exact ecology**. Oxford, Blacwell.

ROBBINS, R.K. 1991. Cost and evolution of a facultative mutualism between ants and lycaenid larvae (Lepidoptera). **Oikos 62**: 363-369.

ROBBINS, R.K. & A. AIELLO. 1982. Foodplant and oviposition records for Panamanian Lycaenidae and Riodinidae. **J. Lepid. Soc. 36** (2): 65-75.

SAKAI, T. & S. MASAKI. 1965. Photoperiod as a factor causing seasonal forms in *Lycaena phlaeas daimio* Seitz. **Kontyû 33** (3): 275-283.

SCOTT, J.A. 1986. **The butterflies of North America: A natural history and field guide**. Stanford University, CA. 583p.

SMITH, D.S.; L. MILLER & J.Y. MILLER. 1994. **The butterflies of the West Indies and South Florida**. New York, Oxford Univ. Press Inc., 264p.

VARGA, A.E. 2000. **Mariposas Argentinas. Guía práctica e ilustrada para la identificación de las principales mariposas diurnas y nocturnas de la Provincia de Buenos Aires. Método y técnicas para la cría, colección y preservación de mariposas**. Buenos Aires, Edited by the author, 148p.

WAGNER, D. 1993. Species-specific effects of tending ants on the development of lycaenid butterfly larvae. **Oecologia 96**: 276-281.

————. 1995. Pupation site choice of a North American lycaenid butterfly: the benefits of entering ant nests. **Ecol. Entomol. 20**: 384-392.

WAGNER, D. & L. KURINA. 1997. The influence of ants and water availability on oviposition behaviour and survivorship of a facultatively ant-tended herbivore. **Ecol. Entomol. 22**: 352-360.

WAGNER, D. & C. MARTÍNEZ-DEL-RIO. 1997. Experimental tests of the mechanism for ant-enhanced growth in an ant-tended lycaenid butterfly. **Oecologia 112**: 424-429.

WRIGHT, D.M. 1983. Life history and morpholgy of the immature stages of the bog copper butterfly *Lycaena epixanthe* (Bsd. & Le C.) (Lepidoptera: Lycaenidae). **Jour. Res. Lepid. 22** (1): 47-100.

Recebido em 29.VI.2001; aceito em 19.X.2001.