*Journal of Research on the Lepidoptera* 31(1-2):1-11, 1992

# Biology and behavior of the neotropical butterfly *Eunica bechina* (Nymphalidae) with special reference to larval defence against ant predation.

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**Abstract.** This paper describes the biology and behavior of *Eunica bechina* (Nymphalidae). Eggs are laid singly on *Caryocar brasiliense* (Caryocaraceae), a plant that bears extrafloral nectaries. Most of the eggs are laid on young leaves, on which the caterpillars preferably feed. Pupation occurs off the host plant. The fifth instar larvae and pupae are like those of *Nica flavilla* and *Temenis laothoe*, suggesting that the genus is among the Callicorini. First to fourth instar larvae construct frass chains, where they rest immune from attacks by foraging ants that climb on *Caryocar* for its nectary secretions. While feeding on leaves, however, caterpillars may be killed and removed by foraging ants. When attacked by ants, *Eunica* caterpillars may repel their aggressors by regurgitating and/or bleeding. Ants receiving these fluids exhibited strong disturbance and conspicuously cleaned their mandibles and head. Dropping off the plant and hanging on the end of a drag line was also observed in *Eunica* larvae after successive bites from the ants. We suggest that frass chains are probably related to defence against "walking" predators, especially ants, who have difficulty in attacking the caterpillars at these refuges.

**Key Words:** *Eunica bechina*, Eurytelinae, *Caryocar brasiliense*, cerrado vegetation, ant predation, extrafloral nectaries, herbivores, defensive behavior, frass chains

The genus *Eunica* Hübner (1819), includes 45 species and 24 additional subspecies distributed throughout the Neotropical region, the majority in the Andean Region and the Amazon Basin (Jenkins, 1990). The genus has an uncertain systematic position within the Nymphalidae (Otero, 1990) and few larvae and hostplants are known (Barcant, 1970; DeVries 1986, 1987; Ackery, 1988; Jenkins, 1990, Oliveira & Freitas, 1991). *Eunica bechina magnipunctata* Talbot 1928 occurs in the cerrados (savanna-like vegetation) of Central and Southeast Brazil (Jenkins, 1990; Oliveira & Freitas, 1991). Larvae of *E. bechina* feed on leaves of *Caryocar brasiliense* Camb. (Caryocaraceae), a plant bearing extrafloral nectaries and frequently visited by ants (Oliveira & Oliveira-Filho, 1991; Oliveira & Brandão, 1991). Early instar larvae construct frass chains (Oliveira & Freitas, 1991), a behavior also observed in other Nymphalidae, especially among the Eurytelinae and Charaxinae (Muyshondt, 1973a, b, c 1974, 1976; Muyshondt & Muyshondt, 1976; Casagrande & Mielke, 1985; DeVries, 1987; Aiello, 1991). Immature stages are still undescribed for many genera and species of Nymphalidae; studies of their morphology and behavior could help to understand the relationship among members of this family of butterflies. The present study describes the early stages of *E. bechina*. We also provide data on the natural history of immatures and adults, as well as on larval behavior and its relation to ant predation on the host plants.

## Study sites and methods

The study was carried out in a cerrado area in Itirapina (21°15′S, 47°49′W), São Paulo, SE Brazil during 1987, 1991 and 1992. The vegetation consists of a scrub of shrubs and trees, which is the cerrado *sensu stricto* of Goodland (1971). Average annual rainfall and temperature are ca. 1400 mm and 21°C respectively (Setzer, 1949).

A total of 27 shrubs of *Caryocar brasiliense* (35-150 cm tall) were censused to determine the preference for oviposition sites by *Eunica bechina*. The eggs were collected and the larvae were reared in plastic boxes containing leaves of *Caryocar*. Boxes were cleaned and the leaves replaced daily. Egg size is given as height and diameter; the head capsule size is the distance between the two groups of ocelli; size of cephalic horns was also measured.

The behavior of *Eunica* caterpillars and visiting ants, as well as their responses to one another, were investigated through natural and provoked encounters on *Caryocar* shrubs. Encounters were provoked by removing the caterpillars from their frass chains and placing them in the proximity of different ant species. Larvae of diferent sizes were placed on leaves or buds of ant-occupied shrubs. After the ants had encountered the caterpillar, the behavioral interactions between them were registered in observation sessions lasting 15 - 30 min. A detailed account of the ant fauna associated with *Caryocar brasiliense* is given in Oliveira & Brandão (1991).

## Results

### Descriptions of early stages

Egg (Fig. 1A): yellowish, conical, and flattened at the top, with 12 to 14 longitudinal ridges and 10 to 12 transverse ridges. Average height 0.76 mm ($\sigma$=0.03 mm, n=15); average diameter 0.72 mm ($\sigma$=0.06 mm, n=15). Larvae hatch 5 days after oviposition (n=5).

First instar larva (Fig. 1B): head translucent brown, body translucent yellow changing to pale red after feeding (due to visible intestinal contents), legs and prolegs translucent yellow; maximum length 3 mm; average width of head capsule 0.42 mm ($\sigma$=0.02 mm, n=16), average duration 2.6 days ($\sigma$=0.54 days, n=47). The distribution of setae in the first instar larva is given in Fig 2A.

Second instar larva (Fig. 1C): head black with two short stubby horns; body pale brown with short conical scoli; maximum length 6 mm; average width of head capsule 0.67 mm ($\sigma$=0.06, n=30); average length of the horn 0.33 mm ($\sigma$=0.05 mm, n=30), average duration 1.5 days ($\sigma$=0.59 days, n=46).

Third instar larva (Fig. 1D): Head black with white warts and with two long diverging horns armed with accessory spines in the middle and ending distally in a knob crowned with short spines; body dark brown with several scoli; maximum length 12 mm; average width of head capsule 1.26 mm ($\sigma$=0.06

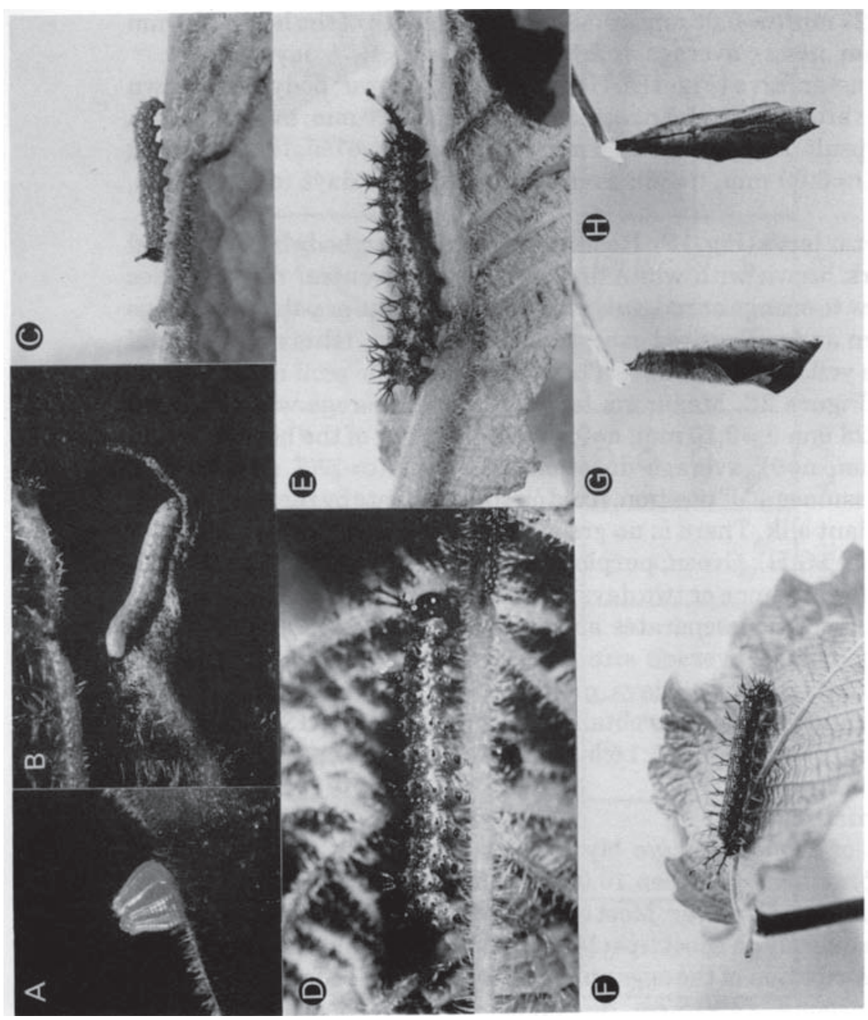


Figure 1. Immature stages of *Eunica bechina*. A, Egg. B, First instar larva. C, Second instar larva. D, Third instar larva. E, Fourth instar larva. F, Fifth instar larva. G, H, Pupa (lateral, ventral).

mm, n=43); average length of the horn 2.36 mm (σ=0.20 mm, n=43); average duration 2.4 days (σ=0.75 days, n=44).

Fourth instar larva (Fig. 1E): Head as in third instar; body dark brown with a pale brown lateral stripe; maximum length 20 mm; average width of head capsule 1.95 mm (σ=0.06 mm, n=36); average length of the horn 4.56 mm (σ=0.09 mm, n=36); average duration 3.7 days (σ=1.47 days, n=33).

Fifth instar larva (Fig. 1F): Head as in fourth instar; body brown, dorsal region dark brown with white lines and stripes, ventral region varies from yellow to orange or red, sublateral stripe orange or pale yellow, legs dark brown and prolegs red except the anal prolegs, (shiny black), scoli black with yellow and red dots. The placement of the scoli in the body is shown in Figure 2B. Maximum length 40 mm; average width of head capsule 3.28 mm (σ=0.10 mm, n=9); average length of the horn 6.62 mm (σ=0.30 mm, n=9); average duration 6.33 days (σ=1.77 days, n=12). Prepupa assumes a "J" position, fixed on the substrate by the anal prolegs and abundant silk. There is no great change in color.

Pupa (Fig. 1G,H): Green, purple or yellowish, changing to brown and gray or green after one or two days; spiracula inconspicuous light brown; a dorsal indentation separates abdomen from thorax. Abdominal segments are mobile; average size 2.2 cm (σ=0.15 cm, n=11), average duration 8.7 days (σ=1.35 days, n=18).

The sex ratio of the adults obtained in the laboratory (13 males and 9 females), can be considered 1:1 (chi square test; $\chi^2$ =0.72, p >0.20; D.F.=1).

**Natural history**

Females of *Eunica bechina* lay their eggs singly on small shrubs of *Caryocar brasiliense* between 10.00 and 13.00 hours. A total of 141 eggs were censused on *Caryocar*. Most eggs were found on young leaves (87%), and less frequently on shoot tips (10%), petioles (1%), and stems (1%). The vertical distribution of the eggs on the host plant varied from 3 to 150 cm above the ground ($\bar{x}$=60.5 cm, σ=44.8 cm, n=141). The caterpillars eat part of the egg shell after hatching and feed preferentially on young leaves of *C. brasiliense*. Although *E. bechina* larvae were seen on *Caryocar* from September to January (rainy season), the highest infestation level occurred between September and October when the majority of the leaves are still young, soft, and red in color.

First to fourth instar larvae of *E. bechina* construct frass chains, on the tip of which they rest (Figure 3A). When disturbed the caterpillars may jump off the leaf suspending themselves from silk threads. Pupation usually occurs off the host plant, on neighbouring shrubs. Adults of *E. bechina* are easily seen in the field throughout the year flying about 3 m high. Agonistic behavior and chases between males of *E. bechina* are frequently observed, suggesting a kind of territoriality. The males were seen feeding on sap oozing from tree wounds, and they probably also feed on decaying fruits and mud puddles (K. S. Brown Jr., personal communication), like other "fruit feeding nymphalids" (DeVries, 1988).

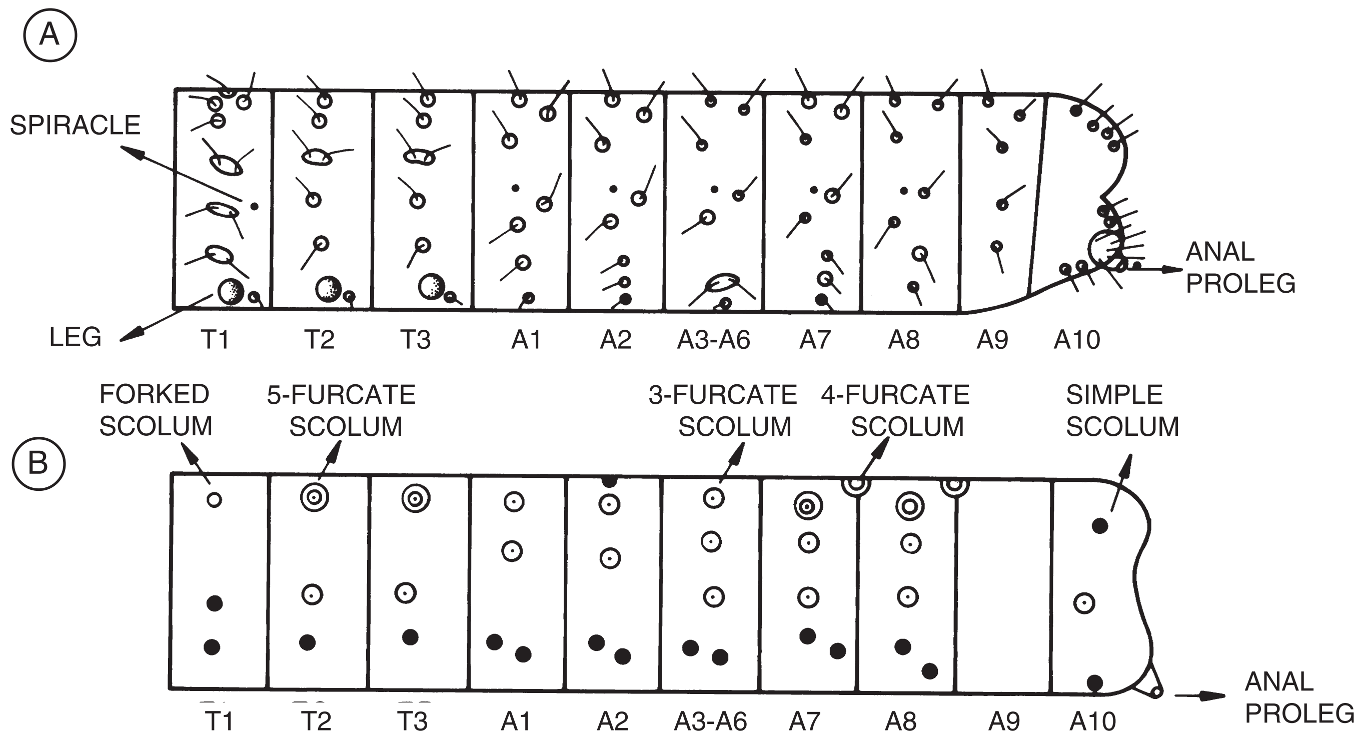


Figure 2. A, Chaetotaxy of first instar larva of *Eunica bechina*. B, Distribution of scoli in a fifth instar larva. The subdorsal 5-furcate scolum in the segment T3 is a bit larger than that of T2. The simple dorsal scolum in the segment A2 is generally absent. Many larvae may present the dorsal scoli in the segments A7 and A8 as 5-furcate.

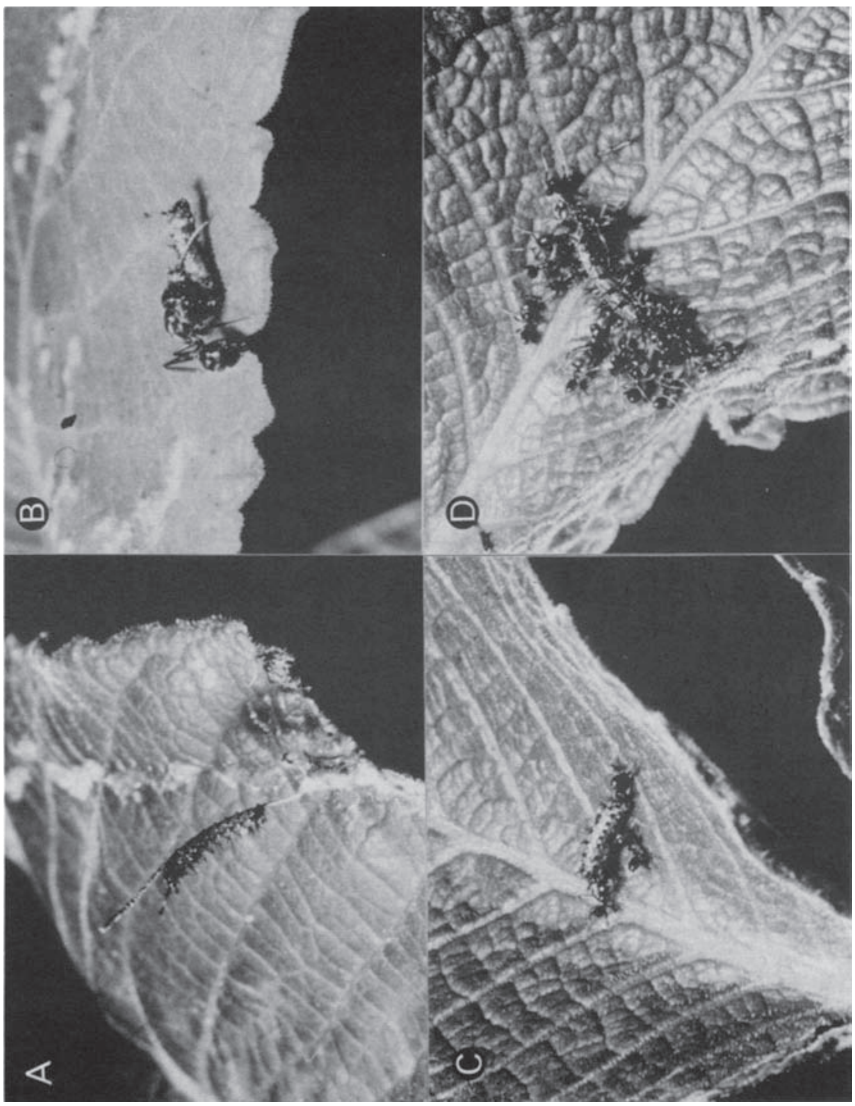


Figure 3. A, Third instar larva of *Eunica bechina* resting on a frass chain. B, Worker *Camponotus* aff. *blandus* retrieving a third instar larva. C, D, Workers of *Azteca* sp. attacking a third instar larva of *Eunica*, before and after recruitment of nestmates.

**Interactions between ants and caterpillars**

When not on their frass chains, *E. bechina* caterpillars may interact aggressively with the ants that climb on *Caryocar* attracted to its extrafloral nectary secretions. Behavioral interactions between *Eunica* caterpillars and ants are summarized in Table 1. In all, 47 ant × caterpillars encounters were provoked on *Caryocar* shrubs; in 36 of these the larvae were attacked by foraging ants. Such attacks resulted in the death and removal of the caterpillar from the plant in 20 instances. Two *Camponotus* species (*C. crassus* and *C.* aff. *blandus*) and one species of *Azteca* were most aggressive towards caterpillars. Unlike *Camponotus* which are large enough to subdue and carry the caterpillar alone to their nests (Fig. 3B), the small *Azteca* ants recruited tens of nestmates to help with these tasks (Fig. 3C, D).

Besides the 7 species tested against *E. bechina* larvae (Table 1), we also observed 6 other species attacking the caterpillars in the field (but without quantification), including 2 species of *Crematogaster*, 2 of *Pheidole*, one of *Pseudomyrmex* (*pallidus* group) and *Ectatomma tuberculatum* (Olivier).

When bitten by the ants, *Eunica* caterpillars frequently regurgitate and/or bleed, a behavior shown to effectively repel their aggressors who may end up abandoning the larvae. After successive bites on the larvae, attacking ants frequently exhibit strong disturbance behavior and vigorously clean their mandibles, antennae and head.

Seven larvae dropped off the plant after successive bites from the ants (Table 1). In three instances the caterpillars suspended themselves on the end of a silken line for approximately 20 min before climbing back to the leaf. Four other larvae dropped directly to the ground and hid among the leaf litter.

Table 1. Behavioral interactions between larvae of *Eunica bechina* and ants on shrubs of *Caryocar brasiliense*. Results are based on 15-30 min of observation after provoked encounters between *Eunica* larvae and ants.

| Ant species | N° of ant × larvae encounters | N° of larvae attacked | N° of larvae removed | N° of larvae jumping of the leaf |
|---|---|---|---|---|
| *Camponotus crassus* Mayr | 15 | 10 | 6 | 3 |
| *C.* aff. *blandus* (Fr. Smith) | 10 | 10 | 7 | 1 |
| *C. rufipes* (Fabricius) | 1 | 1 | 1 | 0 |
| *C. renggeri* Emery | 2 | 2 | 0 | 1 |
| *C.* aff. *cingulatus* Mayr | 3 | 0 | 0 | 0 |
| *Azteca* sp. | 5 | 5 | 5 | 0 |
| *Zacryptocerus pusillus* (Klug) | 11 | 8 | 1 | 2 |
| Total | 47 | 36 | 20 | 7 |

## Discussion

### General biology

This is the first detailed description of the biology and behavior of *Eunica* immatures (see also Jenkins, 1990).

The distribution of setae in the first instar larvae is very similar to the "primitive" pattern of Nymphalidae (Nakanishi, 1988). The distribution pattern of the scoli of the fifth instar larvae and pupae is like that of *Nica flavilla* and *Temenis laothoe* (Muyshondt, 1973b, c); however, the spine distribution pattern in *N. flavilla* is quite distinctive. The pupae of *Eunica* suggest that the genus is among the Callicorini, as stated by Otero (1990), who places *Eunica* in the most advanced branch of Callicorini, paraphyletic with *Temenis* and *Nica*. However, Otero's results are based on only eight characters of adult morphology (apparently with high consistency). On the other hand, Harvey (1991) proposes that *Eunica* is related with *Myscelia, Catonephele, Nessaea, Cybdelis* and *Libythina* (this one very close to Eunica) and the paleotropical genus *Sallya* (see also Jenkins, 1990 and Otero, 1990). Further study of immatures of other Eurytelinae genera may help solve some systematic problems in this group, as has been done for the Ithomiinae (K. S. Brown & A. V. L. Freitas, in preparation).

### Defence against ants

Ants are the most frequent visitors to the extrafloral nectaries of *Caryocar brasiliense* in the cerrado (Oliveira & Oliveira-Filho, 1991; Oliveira & Brandão, 1991). Foraging ants may encounter *Eunica* caterpillars on leaves and occasionally remove them from *Caryocar*. All ant genera observed attacking *E. bechina* caterpillars are known to tend Lycaenidae and Riodinidae larvae, Homoptera, and to visit extrafloral nectaries (see DeVries, 1991; Oliveira & Brandão, 1991). *Azteca* ants, however, were also observed killing *Thisbe irenea* (Riodinidae) caterpillars (DeVries, 1991). We noted that some caterpillars can overcome predation or injury by ants through an array of behavioral mechanisms (see also Heads & Lawton, 1985; Costa et al. 1992).

The behavior of suspending themselves by silken threads is common in *E. bechina* caterpillars and appears to be widespread among the Lepidoptera (see also DeVries, 1987 and several citations therein). Dropping and suspending on the end of a drag line is a technique known to be employed by arthropods who live in close proximity to aggressive ants (Robinson & Valerio, 1977; Oliveira & Sazima, 1984, 1985; Heads & Lawton, 1985).

Regurgitation is also common in butterflies (see Brower, 1984). Just after attacking the caterpillars, ants receiving this fluid exhibited strong disturbance (walking erratically and shaking the body) and conspicuously cleaned their mandibles and head. Ant deterrence can also occur from bleeding by the injured caterpillars, as also noted by Heads & Lawton (1985) for some herbivores of bracken fern (*Pteridium aquilinum*). Rearing up the body, curling and wriggling vigorously (beat reflex) can intimidate or temporarily expel some predators from the plant. For some ants, however, the beat reflex may stimulate additional attacks (Malicky, 1970). These behaviors are very common among butterfly larvae, except for some Lycaenoideae (Malicky, 1970), and seem to be more effective in late instar caterpillars due to their larger size in relation to the ants (see also Heads & Lawton, 1985).

The frass chains constructed by the larvae may diminish their predation/ removal by ants, since the latter were never observed climbing on this structure. The behavior of resting or taking refuge on frass chains is analogous to that exhibited by some Heliconiini larvae, which rest at the end of tendrils or on "island-like" leaf segments (Benson et al., 1976; Bentley & Benson, 1988). Frass chains are observed in several other larvae of Nymphalidae butterflies feeding on various plant families. This trait is especially common among the Charaxinae and Limenitidinae (*sensu* Harvey, 1991), a fact supporting the idea that this structure permits the utilization by *Eunica* of a plant often occupied by ants. This behavior needs to be studied in other genera of Nymphalidae such as *Hamadryas* and *Anaea*, whose larvae commonly feed on plants bearing extrafloral nectaries. Although the primary role of frass chains has not been tested so far, we suggest that it is related to defence against "walking" predators, specially ants, that would have difficulty in attacking the caterpillars on their chains.

*Acknowledgements.* We thank Dr. K. S. Brown Jr., C. F. Klitzke, K. Fiedler and P. J. DeVries for discussions and helpful comments on the manuscript. R. B. Francini helped with the photographs. S. Geraldi and E. Z. Borghi made the line drawings. Financial support to P. S. Oliveira was provided by grants from the CNPq (no.300101/ 90-2 and 400692/92-9), FAPESP (no. 90/2775-6) and FAEP/UNICAMP (no. 634/ 91). A. V. L. Freitas also acknowledges a fellowship from CAPES.

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