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Aspects of the biology of *Cotesia alius* (Muesebeck, 1958) (Hymenoptera: Braconidae: Microgastrinae) on *Opsiphanes invirae amplificatus* Stichel (Lepidoptera: Nymphalidae) in Rio Grande do Sul, Brazil

Aspectos da biologia de Cotesia alius (Muesebeck, 1958) (Hymenoptera: Braconidae: Microgastrinae) em Opsiphanes invirae amplificatus Stichel (Lepidoptera: Nymphalidae) no Rio Grande do Sul

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Abstract

The species *Cotesia alius* (Muesebeck, 1958) (Hymenoptera: Braconidae: Microgastrinae) is a larvae parasitoid of *Opsiphanes invirae amplificatus* Stichel (1904) (Lepidoptera: Nymphalidae). Hymenopteran parasitoids larvae and pupae are recorded acting as defoliators of palm. Concerning the scientific and economic importance of these interespecific relationship, and aiming to increase the knowledge to natural and conservative biological control, the present study records in the State of Rio Grande do Sul State, Brazil, some data on the biology of this *C. alius* as larval parasitoid of O. *invirae amplificatus* Stichel (1904). Based on 35 postures were recorded 4581 cocoons. On 16 postures (48%) observed all cocoons were open and empty, indicating that all parasitoids emerged (Normal). In the 19 remaining postures (52%) (Altered), 134 cocoons (6%) were closed with *C. alius intacts* in six postures (40%) (Intacts) or remains (fragments) (44%), and in Eulophidae intact within three postures (16%). These data can be used in mass rearing of parasitoids, and biological control of defoliator's palm.

Keywords: Biological control. Natural enemy. Parasitoids. Parasitoids wasps.

Resumo

A espécie Cotesia alius *(Muesebeck, 1958) (Braconidae: Microgastrinae) é parasitoide de larvas de* Opsiphanes invirae amplificatus *Stichel (1904) (Lepidoptera: Nymphalidae). Representantes de Hymenoptera têm sido registrados como parasitoides de larvas e pupas de desfolhadores de palmeiras. Diante da importância científica e econômica desse tipo de relação interespecífica, visando à ampliação de conhecimentos relacionados ao controle biológico natural e conservativo, este trabalho registra no estado do Rio Grande do Sul, aspectos biológicos da ocorrência de parasitismo por* C. alius *(Muesebeck, 1958) em larvas de* O. invirae amplificatus *Stichel (1904). Com base em 35 posturas, foram registrados 4.581 casulos. Em 16 posturas (48%), todos os casulos observados estavam abertos e vazios, indicando que todos os parasitoides emergiram (normal). Nas 19 posturas restantes (52%) (Alterados), 134 casúlos (6%) estavam fechados com* C. alius *intactas em seis posturas (40%) e com restos (fragmentos) (44%), e com* Eulophidae íntactos *dentro em três posturas (16%). Esses dados podem ser utilizados na criação massal de parasitoides e no controle biológico de desfolhadores de palmeiras.*

***Palavras-chave**: Controle biológico. Inimigo natural. Parasitoides. Vespas parasitoides.*

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Introduction

The species *Opsiphanes invirae* (Huebner, 1818) is commonly found between the Brazilian States of Rio de Janeiro — Southeastern Region — and Rio Grande do Sul — Southern Region — (Ferreira, Lima, Santana, Moura & Souza 1998; Silva, Gonçalves, Galvão, Gonçalves, Gomes, Silva, et al., 1968). This species is considered a pest of the "açaí" palm tree (*Euterpe oleraceae*) in Eastern Brazilian Amazonia (Northern Region) (Souza & Lemos, 2007). In Rio Grande do Sul, this subspecies was registered by Link and Alvarez Filho (1979) and Link, Biezanko, Carvalho and Tarragó (1980) [treated as *Opsiphanes invirae amplificatus* Stichel (1904) by those authors]; more recently, Lamas (2004) also reported the occurrence of this subspecies in Paraguay.

Species of Hymenoptera, especially members of Braconidae, (Penteado-Dias, 1987; Rodríguez, Fariñas, Díaz, Silva-Acuña & Piña, 2006) have been recorded as larval endoparasitoids, acting therefore as biocontrol agents of *Brassolis sophorae* L., a pest in coconut crops (Ferreira, et al. 1998). The present study reporter data on the biology of *Cotesia alius* M. parasitizing larvae of *O. invirae amplificatus* S. (1904), on Rio Grande do Sul State, Brazil. These data can be used in mass rearing of parasitoids, sex allocation analysis and biological control of defoliator's palm.

Five species of Braconidae have been recorded as larval endoparasitoids of *Opsiphanes*: *Apanteles biezankoi* (Blanchard, 1954), *Apanteles opsiphanis* (Schrottky, 1909), *Cotesia* sp., *Cotesia alius* (Muesebeck, 1958) (Penteado-Dias, 1987; De Santis, 1989; Mason, 1981), *Rhysipolis* sp. (Briceño-Vergara, 1978, 1997; Costa Lima, 1950, 1962; De Santis, 1980; Mexzón, 1997; Sauer, 1946; Silva, et al., 1968; Penteado-Dias, 1987; Mason, 1981; Rodríguez, et al. 2006). While Peigler (1994) registered the hyperparasitism of *Horismenus floridanus* (Ashmead, 1888) (Eulophidae: Entedoninae) *ex Cotesia anisotae* (Muesebeck, 1921) (Hymenoptera: Braconidae) in *Anisota* spp. in the state of Texas, USA, similar relationship as described by (Gil-Santana, et al., 2008), first record of species belonging to these genera (*Cotesia*/*Horismenus*) and parasitism on *Automeris melanops* in the state of Rio de Janeiro, Brazil.

The species *C. alius* (Figure 1) has its occurrence reported in the Tropics, at South America (Venezuela, Argentina and Brazil) (De Santis, 1980, 1989; Mason, 1981; Penteado-Dias, 1987). Although, *C. alius* endoparasitoid of *Opsiphanes* has been reported in Brazil for São Paulo State in host larvae *Opsiphanes cassiae lucullus* (Fruhstorfer, 1907) (Penteado-Dias, 1987). The species *C. alius* larvae kills at the end of last instar and form their white cocoons in regular masses, arranged and disposed under the host. The larvae of gregarius species all emerge from the host in a short time through many different cuts and make a common mass of cocoons. The shape and arrangement of the cocoon mass varies greatly interspecifically, but is relatively constant in respect to each species. The characteristics of the cocoons mass are very useful for identifying reared series (Mason, 1981).

The study of biological aspects is important for the understanding of the taxonomy and systematics of the subspecies involved, concerning the scientific and economic importance of these interespecific relationship, in a study on biological control, regulation of egg number per host to maximize the reproductive success in the gregarious parasitoid, with the objective of to research biological control data. The study present data about the cocoons and average cocoons number, by posture, and the emergency time elapsed, the adults' longevity and sexual rate.

Materials and methods

The insects was collected between March 2006 and March 2007; were counted 35 larvae of *O. invirae amplificatus*. This survey was carried out on native and exotic Palms in the Universidade Federal de Santa Maria (UFSM) campus. **The sampling sites** Avenida Roraima (53° 42' 57.67' W; 29° 42' 19.52' S) and Jardim da Escola Agrícola (53° 43' 05.98' W; 29° 43' 17.62' S). The larvae of *O. invirae amplificatus* were found in Palmaceae: *Syagrus romanzoffianum* (Cham.) Becc. (Gerivá); *Livistona chinensis* (N. J. Jacquim) R. Brown (Chinese Range); *Roystonea regia* (HBK) O. F. Cook (Cuban Real Palm); *Archontophoenix cunninghamiana* Wendland & Drude (Australian Real Palm).

The postures collected were stored in paper envelopes vegetable, properly labeled and transported to the Laboratório de Biologia Evolutiva do Departamento de Biologia – UFSM, where the count was made. The larvae collected were created in nontoxic plastic trays (30 cm × 40 cm), covered with transparent PVC film perforated and kept in an were kept in acclimatized chamber as

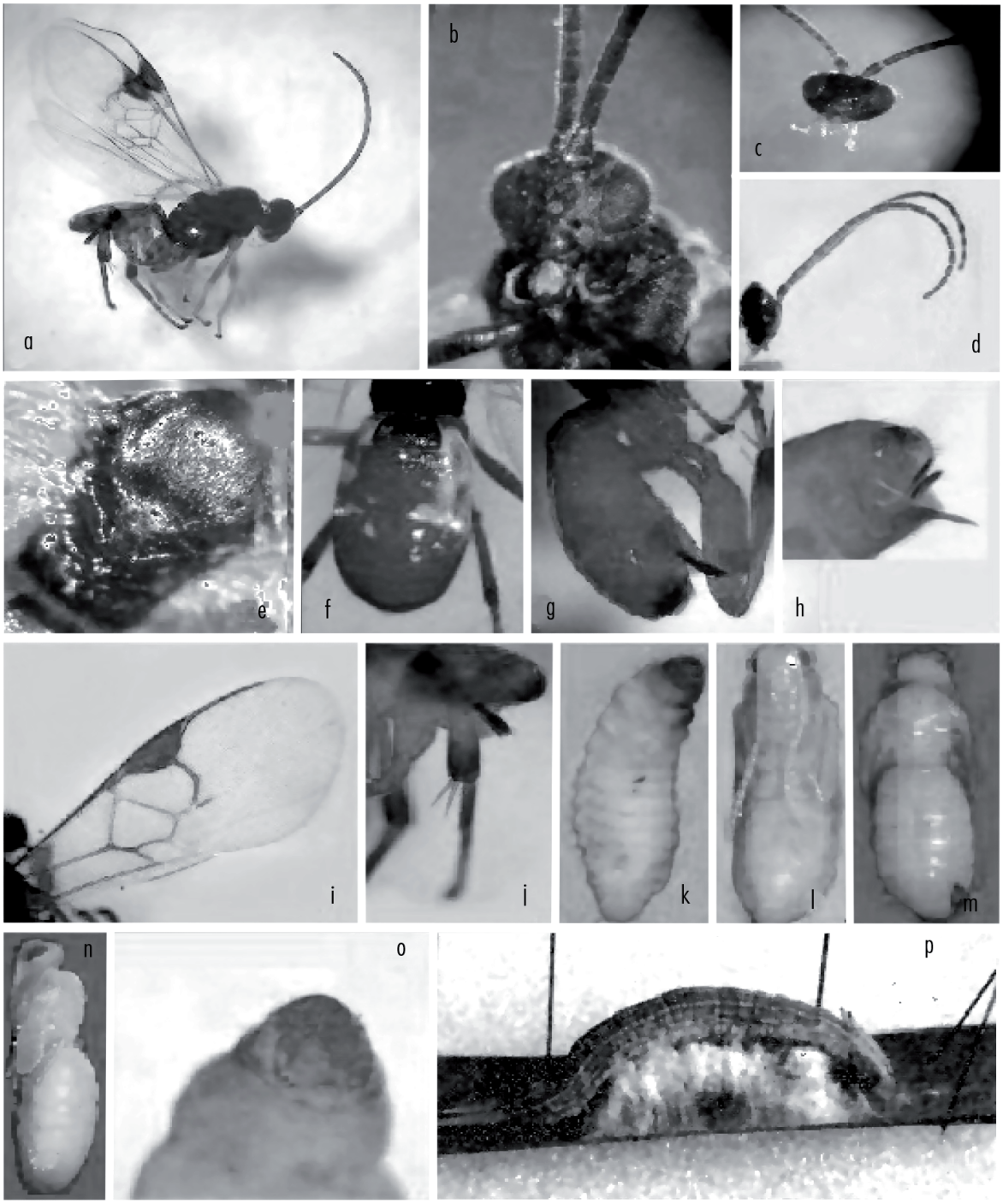

Figure 1 - a) Side view of *Cotesia alius* female; b) View of the labrum, mandibles and labial palps of the mouth parts; c) Front view of the face; d) Side view of the antenna; e) Dorsal view of the chest; f) Dorsal view of abdomen; g) View of the forewing; h) Details of the spurs of the tibia (internal and external); i) Larvae of *C. alius*; j) Front view of the pupa of *C. alius*; k) Dorsal view of pupa of *C. alius*; l) Side view of the pupa of *C. alius*; m) Front view of head of larvae of *C. alius*; n) View from the cocoons of *C. alius* arranged regular secured with silk threads on each other in the form of palisades on the host

Source: Research data.

standard procedure (25 ± 1 °C; 70% UR; photoperiod of 14L;10D) (Parra, Botelho, Corrêa-Ferreira, Bento, 2002).

The daily diet of the larvae was performed using the leaves *Syagrus romanzoffianum*, previously washed with a solution of sodium hypochlorite at 1.0%. The inspection and cleaning of the trays were also daily. Upon reaching the final stage larvae were individually placed in transparent nontoxic plastic pots (9 cm × 6 cm), where they remained until the outbreak of the parasitoids and observed daily until the emergence of the hiperparasitoids, which were preserved in 70% ethanol.

Exemplary of the specimens were deposited in the collection of the Laboratório de Biologia Evolutiva of Universidade Federal de Santa Maria (UFSM), without number tipping.

All hiperparasitoids were identified by Dr. Christer Hansson (Lund University, Sweden) and Dr. Valmir Antonio Costa (Instituto Biológico de Campinas, Brazil) (Salgado-Neto & Di Mare, 2010).

Results

Based on 35 analyzed postures the specimens of *C. alius* emerged first from the cocoons mass (Normal – 48%), posteriorly the hiperparasitoids (Eulophidae – 16%). The cocoon mass were always under the caterpillar (host) living or decomposed, observed (Table 1) some Altered cocoons (52%) others attacked (Remains -44%) by predatory ants (*Solenopsis* sp. and *Paratrechina* sp.). Based on 7 larvae parasitized for *C. alius,* due parthenogenesis in Braconidae cannot count the eggs thus take into account the number of cocoons may correspond to the number of eggs laid by the females 73% relative to males 27% (Table 2). These data are interesting for analysis of natural biological control.

Postures of *C. alius*, each one referring to a larva of *O. invirae amplificatus* (Table 1), from nature, 4581 cocoons were registered, whose average was 130.88 ± 49.02 cocoons/posture. Also on seven larvae parasitized for *C. alius* were recently captured (Table 2), with a total of 789 cocoons (112.71 ± 31.21 cocoons/posture), it was found that 104 cocoons (13%), split into two postures of *C. alius* were parasitized by Eulophidae and 685 (87%) emerged from cocoons 172 (27.0%) males and 477 (73.0%) females of *C. alius*, whose estimated sex ratio (males/total) was 0.26.

The average number of parasitoids that emerged per larvae was 97.7 ranging from 73 to 171. Observed 54 males and females, obtained from egg masses collected in the field and observed until death last individual. Daily mortality was recorded, at which time they were removed and sexed. The average longevity and maximum longevity of males (6.74 ± 4.8; 17 days, and females (6.41 ± 4.9, 17 days) did not differ statistically by Student's t-test calculated mean a standard deviation.

The cumulative mortality (Figure 2) reached 100% on the seventeenth day for males and females. The females (50%) died between the third and fourth day, while males between the fourth and fifth days. The cumulative mortality in both sexes is similar to from the sixth day. However, survival of females until the fifth day is greater than that of males.

We identified four species of hiperparasitoids (Eulophidae) exploiting the coccon mass of *C. alius* collected in the field, two of the Entedoninae subfamily (*Horismenus opsiphanis* Schrottky, 1909 and *Horismenus* sp. nov.) and two of Tetrastichinae subfamily (*Oomyzus sokolowskii* Kurdjumov, 1912 and *Aprostocetus* sp. nov.).

Discussion

Taking into account that the number of cocoons is the number of eggs laid per host-larva, the average observed this study is higher than that observed by Penteado-Dias (1987) in *C. alius*, whose average number of eggs laid was 87, obtained an average of 64% females. *O. invirae* is not considered pests, because the caterpillars live isolated and are controlled naturally by several parasitoids (Otero, 1986). Regulation of egg number per host to maximize the reproductive success in the gregarious parasitoid, adaptive patterns of progeny and sex allocation observed by Ikawa and Okabe (1985) in Braconidae. The data suggest that the natural biological control is done by *C. alius* efficient since all caterpillars *O. invirae amplificatus* collected in nature were parasitized.

The molecular analysis (DNA barcoding) support the hypothesis that there are least four species of wasp (*Cotesia* spp.), parasitizing *Opsiphanes* and having extremely similar but distinct (*O. cassina* and *O. quiteria*).These caterpillars eat the same food plant and occur in the same microhabitat but are easily distinguished morphologically (*O. tamarindi* and *O. bogotanus*). Species complex of *Cotesia* can distinguish between these

Table 1 - Number of cocoons and average number of coccons per postures of *Cotesia alius* (Muesebeck, 1958) observed in 35 postures collected in the field. Santa Maria, Rio Grande do Sul, Brazil

Larvae parasitized		Number of cocoons	Number of cocoons open and empty	Average number of cocoons /postures (Average ± s.d.)
Normal	16	2204 (48%)	2204	142,25 ± 36,57
Altered	19	2377 (52%)	1766 (75%)	
With *Cotesia alius* intact within	6	944 (40%)	810 (86%)	157,33 ± 56,43
With remains *Cotesia alius* within	10	1042 (44%)	956 (92%)	105,42 ± 45,00
With Eulophidaeintact in	3	391 (16%)	350 (90%)	130,00 ± 45,06
Total	35	4581		130,88 ± 49,02

Source: Research data.

Table 2 - Total number of coccons, number of coccons with *Cotesia alius*, number of coccons parasitized, number of males and females and total sex ratio from seven postures collected in the wild and kept in laboratory conditions (25 ºC ± 1 ºC; humidity 70%; 14 hours/light). Santa Maria, Rio Grande do Sul, Brazil

Larvae parasitized	Total of cocoons	Number of individuals *Cotesia alius*	Number of cocoons Hiperparasitized for Eulophidae	Males	Females
1	171	171	0	51	108
2	99	42	57	7	32
3	104	104	0	16	84
4	73	73	0	14	54
5	125	78	47	18	54
6	123	123	0	16	101
7	94	94	0	50	44
Total	789	685 (87%)	104 (13%)	172 (27%)	477 (73%)
Average ± S.D.	112,71 ± 31,21	97,86 ± 41,16		24,57 ± 18,05	68,14 ± 29,46

Source: Research data.

two species of caterpillars, they represent the extreme end of host specificity (Smith, et al. 2008).

With the discovery of the larvae of *O. invirae amplificatus* serving as hosts for *C. alius in Santa Maria (Rio Grande do Sul State)* this is the first report of these similar species and subspecies relationships from *C. alius* complex in the state of Rio Grande do Sul. Because there four distinct species of Eulophidae exploring the mass of cocoons of *C. alius*, collected in the field, probably there are four subspecies of *C. alius* very close working together on the hosts *O. invirae* and *O. invirae amplificatus.*

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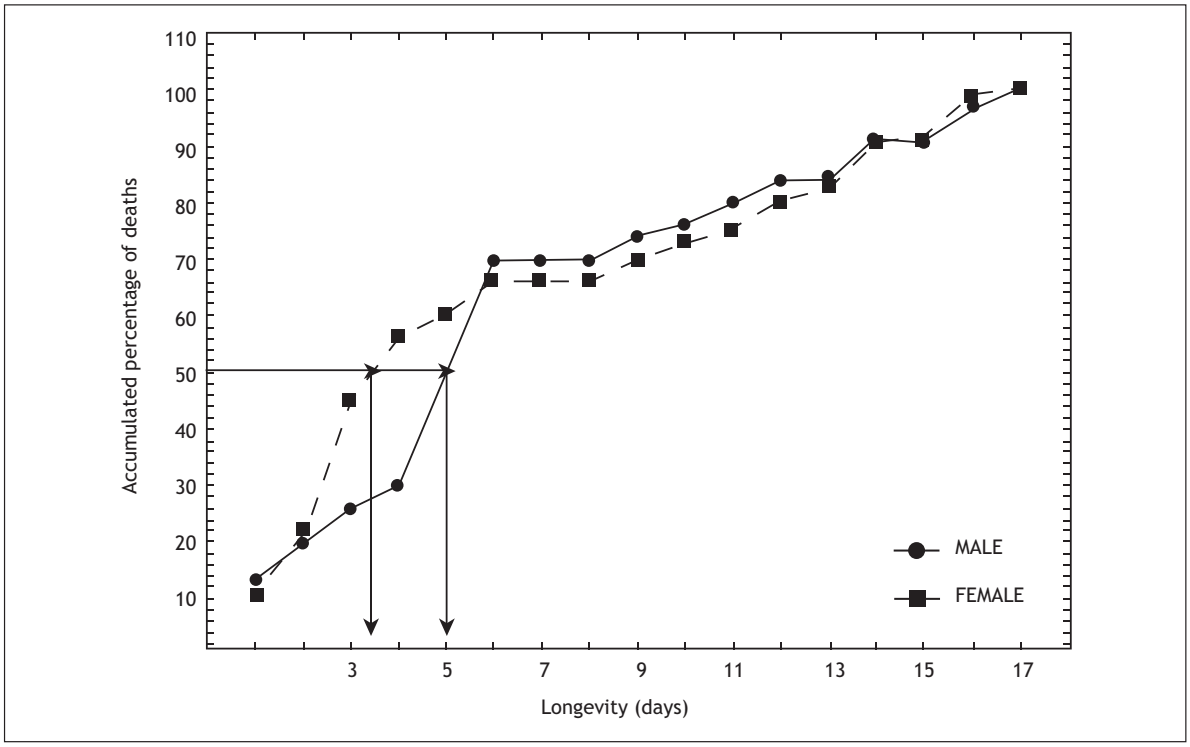

Figure 2 - Cumulative mortality of adults of *Cotesia alius* in laboratory conditions (25 ± 1 °C; photoperiod of 14 hours/light)
Source: Research data.

University, Sweden) for their assistance for parasitoids identification.

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