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RESEARCH

Biological characteristics of *Palmistichus elaeisis* Delvare & LaSalle (Hymenoptera: Eulophidae) on refrigerated pupae of *Anticarsia gemmatalis* Hubner (Lepidoptera: Noctuidae)

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Mass rearing of parasitoids is a fundamental step for biological control programs. The biological characteristics of *Palmistichus elaeisis* Delvare & LaSalle (Hymenoptera: Eulophidae) were evaluated in pupae of *Anticarsia gemmatalis* Hubner (Lepidoptera: Noctuidae) stored at low temperatures. Twenty four hours-old pupae of *A. gemmatalis* were stored at 12 °C for 0, 1, 3, 6, 9, and 12 d and then exposed to parasitism by *P. elaeisis* females. The life span of *P. elaeisis* was affected and ranged from 19 to 24 d on the pupae of the host. Parasitism reached 100% of the host pupae after storage in all periods at 12 °C, and adults emerged from 20.00 to 54.54% of them. The progeny of *P. elaeisis* ranged from 71 to 198 and had an inverse relationship with the increase in the storage period. Pupae of *A. gemmatalis* may be stored for up to 6 d at 12 °C and subsequently used in rearing of *P. elaeisis*.

Key words: Biological control, parasitism, parasitoid rearing, temperature.

INTRODUCTION

Palmistichus elaeisis Delvare & LaSalle, 1993 (Hymenoptera: Eulophidae) is a parasitoid with potential for use in the control of Lepidoptera of economic importance (Pereira et al., 2010), which has been reported in insects of the families Arctiidae (Zaché et al., 2012a), Bombycidae (Pereira et al., 2009), Crambidae (Bittencourt and Berti-Filho, 2004; Chichera et al., 2012), Noctuidae (Bittencourt and Berti-Filho, 1999; 2004; Andrade et al., 2010), and Lymantriidae (Zaché et al., 2012b).

The use of parasitoids depends on selection of a suitable host for mass rearing (Bittencourt and Berti-Filho, 1999; Paron and Berti-Filho, 2000; Pereira et al., 2010; Pastori et al., 2012). Large-scale rearing of parasitoids is fundamental for the implementation of biological control programs and the lack of artificial diets requires the use of a large number of preferred hosts or alternatives for production (Pratissoli et al., 2003; Milward-de-Azevedo et al., 2004).

Natural enemies maybe reared on alternative hosts with low production cost and that do not reduce their efficiency of control on the natural host (Pratissolli et al., 2005; Zanuncio et al., 2008). *Anticarsia gemmatalis* Hubner, 1818 (Lepidoptera: Noctuidae) has a short life cycle, it can be reared on artificial diet, and the pupae are hosts for development of *P. elaeisis* (Bittencourt and Berti-Filho, 1999; 2004; Andrade et al., 2010; Pereira et al., 2010). However, the conservation of this host at low temperatures has not yet been studied and for this reason the present study was developed. In order to investigate the reproductive capacity of parasitoids on pupae stored at low temperatures, it is important to synchronize both parasitoid and host demands while seeking mass production of these natural enemies for release in the field (Pereira et al., 2009).

The objective of this study was to evaluate the biological characteristics of *P. elaeisis* on *A. gemmatalis* pupae stored at 12 °C for different periods of time.

MATERIALS AND METHODS

Experiments were performed at the laboratory of Controle Biológico de Insetos in Departamento de Biologia Animal of Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais, Brazil, with the following steps:

Rearing of insects

Rearing of *A. gemmatalis* was initiated with eggs from the rearing stock, maintained on artificial diet in the laboratory of Controle Biológico de Insetos in Departamento de Biologia Animal of Universidade Federal de Viçosa. These eggs were placed on moistened filter paper in

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10.0 cm diameter × 2.5 cm height Petri dishes. Newly-hatched larvae were transferred with a fine brush to plastic containers containing artificial diet. The plastic containers with newly-hatched larvae were maintained in a room at 25 ± 2 ºC, 70 ± 10% relative humidity (RH) and 12:12 h photoperiod until transformation into pupae.

Pupae of *A. gemmatalis* were placed in 20 × 20 × 20 cm creen cages, lined on the sides with bond paper as substrate for oviposition, and the emerging adults were fed with a 10% honey in water of the plastic containers (3.0 cm in diameter × 4.0 cm in height), through an inserted cotton wick, a methodology adapted from Greene et al. (1976).

Rearing of adults of *P. elaeisis* were kept in jars labeled 12.5 cm diameter × 17.0 cm height, closed with fine cloth and contained plastic containers (3.0 cm diameter × 4.0 cm height) and parasitoids feeding with droplets of honey. Pupae of *A. gemmatalis* or *Thyrinteina arnobia* (Stoll, 1972) (Lepidoptera: Geometridae) aging 24 to 72 h were exposed to parasitism for 24 h at a temperature of 25 ± 2 ºC, 70 ± 10% RH, and 12:12 h photoperiod, a methodology adapted from Pereira et al. (2010).

Experimental design

Twenty four hours-old pupae of *A. gemmatalis* were weighed 0.196 ± 3.61 g (reducing the effects of host biomass variation) and stored at 12 ºC (temperature base) as proposed by Magrini et al. (1996). The host pupae were stored by 1, 3, 6, 9, or 12 d and in the control host pupae of the same age were used, but without storage at 12 ºC. Each pupa was exposed to parasitism by six females of 72 h-old *P. elaeisis* in 14 cm × 2.2 cm glass tubes plugged with cotton, for 24 h at 25 ± 2 ºC, 70 ± 10% RH, and 12:12 h photoperiod, after which, they were removed from the tubes. The egg to adult period life cycle duration parasitism percentage [discounting natural mortality (Abbott, 1925)], emergence percentage of the progeny, number of parasitoids emerged per host pupa, offspring longevity and sex ratio were determined. Parasitoids were sexed by morphology of their antennae and abdomens (Delvare and LaSalle, 1993).

The treatments were 0, 1, 3, 6, 9 or 12 d storage periods. Ten replicates were used in a completely randomized design, where each replicate was a pupa of *A. gemmatalis*. The data on life cycle duration, progeny of *P. elaeisis* emerged per pupa of *A. gemmatalis*, sex ratio and longevity of female parasitoids were subjected to ANOVA at 5% probability and regression analysis. Selection of the equation that best fit to the data was performed using polynomial models, based on the coefficient of determination (R^2), the significance of the regression coefficients (β_i) and regression testing by the F-test (up to 5% probability).

Data of parasitism percentage and emergence of *P. elaeisis* were subjected to analysis of generalized linear models with binomial distribution ($P \leq 0.05$) using the R Statistical System (Ihaka and Gentleman, 1996). This analysis was performed with the original non-parametric data, but data a represented in percentage to facilitate viewing.

RESULTS

Palmistichus elaeisis parasitized 100% of *A. gemmatalis* pupae, with adult emerging from 20 to 54.54% of the host pupae subjected to the different storage periods at 12 ºC, respectively ($\chi^2 = 6.977$, $P = 0.008$) (Figure 1).

The duration of the pre-reproductive period (egg to adult) of *P. elaeisis* on pupae of *A. gemmatalis* after storage for 0 to 12 d at 12 ºC was affected and ranged from 19 to 24 d, respectively ($R^2_{Treat} = 0.4279$; $F = 6.1489$; $P = 0.0202$; $df = 26$) (Figure 2).

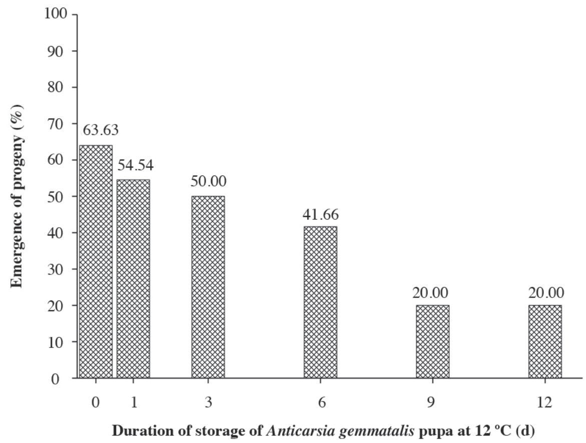

Figure 1. Percentage of *Anticarsia gemmatalis* pupae (Lepidoptera: Noctuidae) with emergence of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) after storage for 0, 1, 3, 6, 9, or 12 d at 12 ºC, 70 ± 10% RH, and 12:12 h photoperiod ($\chi^2 = 6.977$; $P = 0.008$).

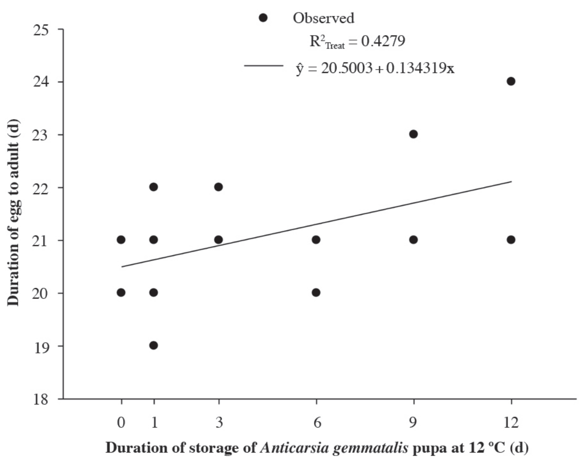

Figure 2. Duration of egg to adult (days) of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) on pupae of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) after storage for 0, 1, 3, 6, 9, or 12 d at 12 ºC, 70 ± 10% RH, and 12:12 h photoperiod. ($F = 6.1489$, $P = 0.0202$).

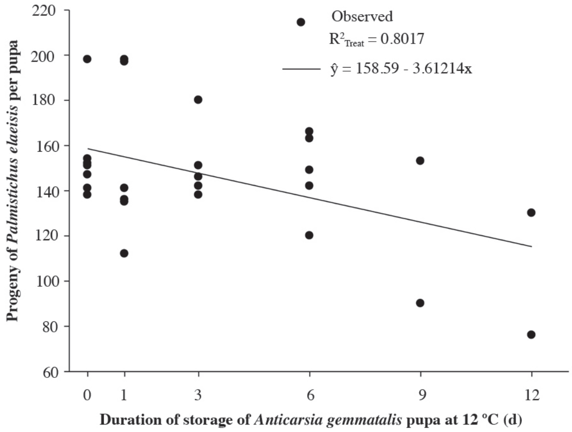

Figure 3. Progeny of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) per pupa of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) after storage for 0, 1, 3, 6, 9, or 12 d at 12 ºC, 70 ± 10% RH, and 12:12 h photoperiod (F = 7.3937, P = 0.0117).

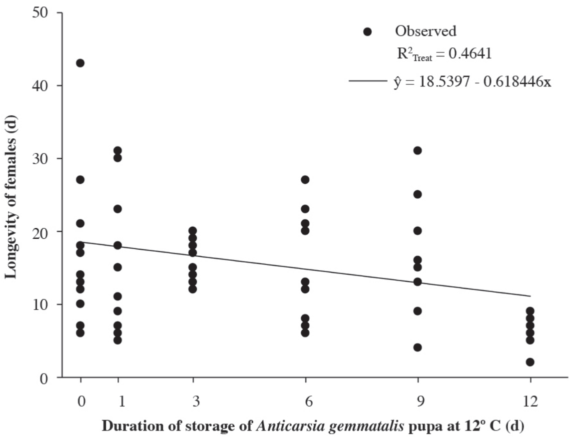

Figure 4. Longevity of *Palmistichus elaeisis* females (Hymenoptera: Eulophidae) on emerged pupae of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) after storage for 0, 1, 3, 6, 9, or 12 d at 12 ºC, 70 ± 10% RH, and 12:12 h photoperiod (F = 10.8649, P = 0.0014).

Progeny of *P. elaeisis* per pupae of *A. gemmatalis* ranged 71 to 198 and presented an inverse relationship with the increase in storage period (R^2_{Treat} = 0.8017; F = 7.3937; P = 0.0117; df = 26) (Figure 3). The sex ratio of *P. elaeisis* was similar for the 0 to 12 d storage periods of *A. gemmatalis* pupae at 12 ºC, with means of 0.94 to 0.95 (P > 0.05), respectively.

Longevity of *P. elaeisis* females emerged from pupae of *A. gemmatalis* decreased with the increase in storage period of these pupae at 12 °C (R^2_{Treat} = 0.4641; F = 10.8649; P = 0.0014; df = 89) (Figure 4).

DISCUSSION

Palmistichus elaeisis developed on pupae of *A. gemmatalis* after being stored at 12 ºC for different periods of time. The similarity in duration of the life cycle of *P. elaeisis* on pupae of *A. gemmatalis* not refrigerated or stored for up to 6 d at 12 °C indicates that host pupae at these conditions are suitable for the development of this parasitoid.

Pupae can maintain physiological and/or suitable nutritional conditions for parasitoids a certain period, but this varies with the host species and/or parasitoid (Pereira et al., 2009; 2010; Pastori et al., 2012). *Muscidifurax uniraptor* Kogan & Legner (Hymenoptera: Pteromalidae) had a lower reproductive capacity on pupae of *Musca domestica* (Linnaues, 1758) (Diptera: Muscidae) after storage for 1 to 2 d when compared to those not refrigerated. However, reproduction of *M. uniraptor* on *M. domestica* pupae stored for a longer period of time was greater than or similar to the control (Thomazini and Berti-Filho, 2000), as observed herein for *P. elaeisis* in pupae of *A. gemmatalis*.

Insect pupae possess an immune response against immature parasites, but are not able to maintain this defense mechanism (encapsulation rate and toxin production) active for a long time due to high metabolic costs (Schmidt et al., 2001; Schmid-Hempel, 2005; Andrade et al., 2010). The increased period of development of *P. elaeisis* on pupae of *A. gemmatalis* after storage at 12 °C for 9 to 12 d may be due to inadequate quality for the development of the juvenile parasitoid. This is due to the fact that refrigeration for long periods can injure the cells of pupae, which compromises their sequence of metabolic reactions and therefore its nutritional quality (Milward-de-Azevedo et al., 2004; Pereira et al., 2009).

The high rates of parasitism and emergence of *P. elaeisis* on *A. gemmatalis* pupae after storage at 12 ºC, mainly until the sixth day, confirm that the conservation of hosts at low temperatures is useful for parasitoid production programs (Pratissoli et al., 2003; Milward-de-Azevedo et al., 2004; Pratissoli et al., 2005; Zanuncio et al., 2008).

Palmistichus elaeisis produced progeny on pupae of *A. gemmatalis* after storage at 12 °C during all time periods. However, there was a trend for a decreased reproductive capacity of *P. elaeisis* with an increase in the storage period at 12 °C. The smaller progeny of this parasitoid on *A. gemmatalis* pupae after 9 and 12 d of storage at 12 °C may be related to morphological and physiological changes, even a reduced metabolism (Chapman, 1998; Pereira et al., 2009; Andrade et al., 2010). The effects of these morphological and physiological changes in the progeny of parasitoids are unknown, but it may be possible to determine the degree of susceptibility to the development of these natural enemies (Pfannenstiel et al., 1996).

The sex ratio of *P. elaeisis* was greater than 93%, which is important in mass rearing systems, laboratory experiments and selection of individuals for release in the field. The predominance of females over males can increase the number of individuals produced in the next generation and can positively contribute to the control of agricultural pests after field releases (Uçkan and Gulel, 2002; Amalin et al., 2005).

The longevity of *P. elaeisis* females that emerged from pupae of *A. gemmatalis* presented a decrease when increasing the storage period of these pupae at 12 °C. Nevertheless, females of *P. elaeisis* lived long enough to ensure their reproduction, as the pre-oviposition and oviposition periods of this parasite in pupae of *A. gemmatalis* are 1.00 ± 0.75 and 16.6 ± 0.75 d, respectively (Bittencourt and Berti-Filho, 1999). This is important because in mass rearing of parasitoids, survivability is one of the requirements for insect control (Van-Lenteren, 2000).

The use of parasitoids for biological control programs depends on development and reproductive success of the natural enemy on pupae of the preferred and/or alternative host with suitable nutritional conditions and low production cost. *Palmistichus elaeisis* develops on pupae of *A. gemmatalis* stored at 12 °C and this is important for programmed rearing in the lab in order to synchronize the emergence of the parasitoid with the presence of the stage of development of the target host, as well as appropriating the mass rearing efficiency and program for release of *P. elaeisis*.

CONCLUSIONS

Pupae of *Anticarsia gemmatalis* stored at 12 ºC for up to 6 d were suitable for rearing of *Palmistichus elaeisis* since they favored a greater progeny, with minor variations between the percentage of progeny, emergence and egg to adult duration.

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LITERATURE CITED

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18:265-267.

Amalin, D.M., J.E. Pena, and R.E. Duncan. 2005. Effects of host age, female parasitoid age, and host plant on parasitism of *Ceratogramma etiennei* (Hymenoptera: Trichogrammatidae). Florida Entomologist 88:77-82.

Andrade, G.S., J.E. Serrão, J.C. Zanuncio, T.V. Zanuncio, G.L.D. Leite, and R.A. Polanczyk. 2010. Immunity of an alternative host can be overcome by higher densities of its parasitoids *Palmistichus elaeisis* and *Trichospilus diatraeae*. Plos One 05:1-7.

Bittencourt, M.A.L., e E. Berti-Filho. 1999. Preferência de *Palmistichus elaeisis* por pupas de diferentes lepidópteros pragas. Scientia Agricola 56:1281-1283.

Bittencourt, M.A.L., e E. Berti-Filho. 2004. Desenvolvimento dos estágios imaturos de *Palmistichus elaeisis* Delvare & LaSalle (Hymenoptera, Eulophidae) em pupas de Lepidoptera. Revista Brasileira de Entomologia 48:65-68.

Chapman, R.F. 1998. The insects: structure and function. 4th ed. 788 p. Cambridge University Press, New York, USA.

Chichera, R.A., F.F. Pereira, S.O. Kassab, R.H. Barbosa, P.L. Pastori, e C. Rossoni. 2012. Capacidade de busca e reprodução de *Trichospilus diatraeae* e *Palmistichus elaeisis* (Hymenoptera: Eulophidae) em pupas de *Diatraea saccharalis* (Lepidoptera: Crambidae). Interciência 37:852-856.

Delvare, E.G, and J. LaSalle. 1993. A new genus of Tetrastichinae (Hymenoptera: Eulophidae) from the Neotropical region, with the description of a new species parasitic on key pests of oil palm. Journal of Natural History 27:435-444.

Greene, G.L., N.C. Leppla, and W.A. Dickerson. 1976. Velvetbe an caterpillar. A rearing procedure and artificial medium. Journal of Economic Entomology 69:447-448.

Ihaka, R., and R.A. Gentleman. 1996. A language for data analysis and graphics. Journal of Computational and Graphical Statistics 5:299-314.

Magrini, E.A., S. Silveira-Neto, J.R.P. Parra, P.S.M. Botelho, e M.L. Haddad.1996. Biologia e exigências térmicas de *Anticarsia gemmatalis* Hübner em laboratório. Anais da Sociedade Entomológica Brasileira 25:513-519.

Milward-de-Azevedo, E.M.V., I. Serafin, E.M. Piranda, e C.C. Gulias-Gomes. 2004. Desempenho reprodutivo de *Nasonia vitripennis* Walker (Hymenoptera: Pteromalidae) em pupas crio conservadas de *Chrysomia megacephala* Fabricius (Diptera: Calliphoridae): avaliação preliminar. Ciência Rural 34:207-211.

Paron, M.R., and E. Berti-Filho. 2000. Capacidade reprodutiva de *Trichospilus diatraeae* (Hymenoptera: Eulophidae) em pupas de diferentes hospedeiros (Lepidoptera). Scientia Agricola 57:355-358.

Pastori, P.L., F.F. Pereira, G.S. Andrade, R.O. Silva, J.C. Zanuncio, and A.I.A. Pereira. 2012. Reproduction of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) in pupae of two lepidopterans defoliators of eucalypt. Revista Colombiana de Entomología 38:91-93.

Pereira, F.F., J.C. Zanuncio, P.L. Pastori, A.R.P. Pedrosa, e H.N. Oliveira. 2010. Parasitismo de *Palmistichus elaeisis* (Hymenoptera: Eulophidae) em hospedeiro alternativo sobre plantas de eucalipto em semi-campo. Revista Ciência Agronômica 41:715-720.

Pereira, F.F., J.C. Zanuncio, J.E. Serrão, H.N. Oliveira, K. Favero, e E.A.L.V. Grance. 2009. Progênie de *Palmistichus elaeisis* Delvare & LaSalle (Hymenoptera: Eulophidae) parasitando pupas de *Bombyx mori* L. (Lepidoptera: Bombycidae) de diferentes idades. Neotropical Entomology 38:660-664.

Pfannenstiel, R.S., H.W. Browning, and J.W. Smith-Junior. 1996. Suitability of Mexican rice borer (Lepidoptera: Pyralidae) as a host for *Pediobius furvus* (Hymenoptera: Eulophidae). Environmental Entomology 25:672-676.

Pratissoli, D., U.R. Vianna, H.N. Oliveira, e F.F. Pereira. 2003. Efeito do armazenamento de ovos de *Anagasta kuehniella* (Lepidoptera: Pyralidae) nas características biológicas de três espécies de *Trichogramma* (Hymenoptera: Trichogrammatidae). Ceres 50:95-105.

Pratissoli, D., U.R. Vianna, H.B. Zago, e P.L. Pastori. 2005. Capacidade de dispesão de *Trichogramma* em tomateiro estaqueado. Pesquisa Agropecuária Brasileira 40:613-616.

Schmid-Hempel, P. 2005. Evolutionary ecology of insect immune defenses. Annual Review of Entomology 50:529-551.

Schmidt, O., V. Theopold, and M.R. Strand. 2001. Innate immunity and its evasion and suppression by Hymenoptera endoparasitoid. BioEssays 23:344-351.

Thomazini, M.J., e E. Berti-Filho. 2000. Influência da densidade e idade de pupas da mosca doméstica no parasitismo por *Muscidifurax uniraptor* (Hymenoptera: Pteromalidae). Revista de Agricultura 75:339-348.

Uçkan, F., and A. Gulel. 2002. Age-related fecundity and sex ratio variation in *Apanteles galleriae* (Braconidae) and host effect on fecundity and sex ratio of its hyperparasitoid *Dibrachys boarmiae* (Hym., Pteromalidae). Journal of Applied Entomology 126:534-537.

Van-Lenteren, J.C. 2000. Controle de qualidade de agentes de controle biológico produzidos massalmente: conhecimento, desenvolvimento e diretrizes. p. 21-40. In Bueno, V.H.P. (ed.) Controle biológico de pragas: produção massal e controle de qualidade. Universidade Federal de Lavras (UFLA), Lavras, Minas Gerais, Brasil.

Zaché, B., R.R.C. Zaché, N.M. Souza, M.H.F.A.D. Pogetto, and C.F. Wilcken. 2012b. Evaluation of *Trichospilus diatraeae* (Hymenoptera: Eulophidae) as parasitoid of the Eucalyptus defoliator *Eupseudosoma aberrans* Schaus, 1905 (Lepidoptera: Arctiidae). Biocontrol Science and Technology 22:363-366.

Zaché, B., R.R.C. Zaché, and C.F. Wilcken. 2012a. Evaluation of *Palmistichus elaeisis* Delvare & LaSalle (Hymenoptera: Eulophidae) as parasitoid of the *Sarsina violascens* Herrich-Schaeffer (Lepidoptera: Lymantriidae). Journal of Plant Studies 1:85-89.

Zanuncio, J.C., F.F. Pereira, G.C. Jacques, M.T., Tavares, and J.E. Serrão. 2008. *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae), a new alternative host to rear the pupae parasitoid *Palmistichus elaeisis* Delvare & LaSalle (Hymenoptera: Eulophidae). The Coleopterists Bulletin 62:64-66.