IMMATURE STAGES OF FOPIUS ARISANUS (HYMENOPTERA: BRACONIDAE) IN BACTROCERA DORSALIS (DIPTERA: TEPHRITIDAE)

Authors: Rocha, Kathy L., Mangine, Thomas, Harris, E. J., and Lawrence, P. O.

Source: Florida Entomologist, 87(2) : 164-168

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/0015-4040(2004)087[0164:ISOFAH]2.0.CO;2

IMMATURE STAGES OF *FOPIUS ARISANUS* (HYMENOPTERA: BRACONIDAE) IN *BACTROCERA DORSALIS* (DIPTERA: TEPHRITIDAE)

KATHY L. ROCHA[1], THOMAS MANGINE[2], E. J. HARRIS[2] AND P. O. LAWRENCE[1]
[1]Department of Entomology and Nematology, P.O. Box 110620, Building 970,Natural Area Drive
University of Florida, Gainesville, FL 32611-0620, USA

[2]United States Department of Agriculture, Agriculture Research Service
2727 Woodlawn Drive, Honolulu, HI 96822-1842, USA

ABSTRACT

We describe all immature stages, particularly the previously undescribed instars, of *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae), an egg-pupal parasitoid of tephritid fruit flies. This is essential for quality control in mass rearing programs and for physiological studies of host-parasite interactions. *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) eggs were parasitized for 24 h and serial collections of hosts were made every 24 h until adults emerged. Immature wasps were dissected from hosts and their mouthhooks and body dimensions measured. Scatter plots of the above measurements and scanning electron microscopy indicated that there are three instars. This contrasts with the four instars previously reported. There appears to be no true fourth instar because the stage immediately following the second instar is indistinguishable from that preceding the prepupal stage.

Key Words: Braconid wasp, tephritid fruit fly host, egg-pupal parasitoid, biological control

RESUMEN

Nosotros describimos todas los estadíos inmaduros, particularmente los estadios no descritos anteriormente, de *Fopius arisanus* (Sonan) (Hymenoptera: Braconidae), un parasitoide del huevo-pupal de las moscas de la frutas de la familia Tephritidae. Esto es esencial para el control de cualidad en los programas de cria masiva y para estudios fisiológicos de la interacción entre hospedero y parasitoide. Los huevos de *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) fueron parasitados por 24 horas y hicieron colecciones en serie de los hospederos cada 24 horas hasta que los adultos emergieron. Las avispas inmaduras fueron disectadas de sus hospederos y los ganchos bocales y las dimensiones de cuerpo fueron medidos. Las diagramas de dispersión de las medidas mencionadas y imagenes tomados por el microscopio electrónico (SEM) indicaron que habian tres estadíos. Esto es contrario de los cuatro estadíos reportados anteriormente. Parece que no hay un cuatro estadío verdadero por que el estadío siguiente inmediatamente al segundo estadío es indistinguible del que precede al estadío prepupal.

Fopius arisanus (Sonan) is a parasitoid of many tephritid fruit fly species (Diptera: Tephritidae) including the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Vargas & Ramadan 2000). It is one of the most effective biological control agents of tephritids in Hawaii (Harris & Okamoto 1991) and also parasitizes some New World tephritids such as *Anastrepha suspensa* (Loew) in the laboratory (Lawrence et al. 2000).

Previous reports based on mouthhook dimensions indicated that *F. arisanus* has four instars (Ibrahm et al. 1992). However, there were no illustrations of the larval morphology to facilitate the identification of each instar. Other reports have provided diagrams of the egg, first and fourth instars (Palacio et al. 1992), and the pupa that were useful for their identification, but gave no diagrams of the second or third instars. The goal of this study was to confirm the morphologies of the first and last instars and to describe the previously undescribed intermediate instars of *F. arisanus*.

MATERIALS AND METHODS

Rearing of Parasites

Bactrocera dorsalis eggs were inserted into holes punched into the rind of *Carica papaya* L. and given to adult wasp females aged 10-25 d at a ratio of 20:1 at 75-80°C and 40-50% R.H. under constant light for 24 h. Twenty-four hour sequential collections of hosts were made for 21 d when adult wasps began to emerge. The experiment was duplicated.

Light Microscopy

Fopius arisanus eggs, early instars [1-7 days post parasitism (dpp)], and the heads of late in-

stars (9-14 dpp) were dissected from hosts and placed in fluoromount-G or TE buffer (10 mM Tris, 1 mM EDTA). Other instars (7-9 dpp) were cleared in cellosolve [ethylene glycol monoethyl ether (Carbide and Carbon Chemicals, New York)] for 10 min and mounted with euparal (Barbosa 1974).

Mouthhooks of *F. arisanus* (10 individuals × 2 per time point) and body lengths were measured. The means and standard errors of all measurements were calculated and plotted against one another. The resulting number of aggregations indicated the number of instars according to Dyar's (1890) rule that the "width of the head of a larva in its successive stages follow a regular geometrical progression." Although the rule is applied primarily to lepidopteran larval head capsules, we found that these measurements provided a reliable indicator of instars when used in combination with sequential dissections and other morphological factors.

Scanning Electron Microscopy (SEM)

All larvae were placed in Trump's fixative (1% glutaraldehyde and 4% formaldehyde in phosphate buffer) overnight, washed with 0.1 M cacodylate buffer (3 × 10 min), then fixed in 1% osmium tetroxide for three days. After 3 × 10 min washes in deionized water, the samples were dehydrated in a graded series of ethanol, then incubated in hexamethyldisilazane (HMDS) for 2 × 15 min and air dried (Nation 1983). The larvae were then sputter coated with gold and observed on a Hitachi S-570 scanning electron microscope at 20 kV.

Results

Fopius arisanus eggs measured 300 ±(SE) 11.0 µm (range 250-350 µm) long and 55 ±(SE) 3.0 µm (range 50-75 µm) wide. The egg stage lasted 1-2 d and eggs were observed 0-2 dpp. Scatter plots of mouthhook widths vs. mouthhook lengths (Fig. 1a) and body lengths vs. mouthhook widths (Fig. 1b) show two distinct aggregations of points, the first occurring between 2-8 dpp and the second between 9-14 dpp. The mouthhook, cephalic, and overall morphologies of these two groups correspond to those previously described as first (Fig. 2) and last (fourth) instars, respectively (Ibrahm et al. 1992; Palacio et al. 1992). An instar with overall body size and morphology, differing from the first and last instars, occurred between 7-9 dpp and had no sclerotized mouthhooks (Fig. 3a). This time period coincides with the gap between the two mouthhook size aggregations of the first and last instar and no doubt represents the second instar. Further analysis of the integument of this putative second instar (Fig. 3b) indicated that the integument is distinct from that of the subsequent (last) instar (Figs. 4 and 5).

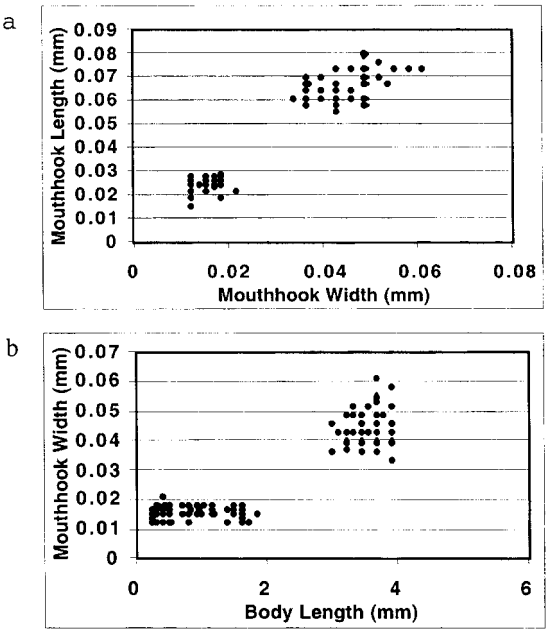

Fig. 1. Scatter plots of (a) mouthhook widths vs mouthhook lengths and (b) body lengths vs mouthhook widths to show distinct aggregations representative of larval instars of *Fopius arisanus* based on Dyar's (1890) rule.

Larval lengths from the tip of the head capsule to the tip of the last abdominal segment were 0.848 ± 0.06 mm (range 0.250-1.84 mm) for first instar, 2.56 ± 0.14 mm (range 1.50-3.22 mm) for second instar, and 3.35 ± 0.10 mm (range 2.99-3.91 mm) for third instar.

The duration of the first, second, and third stadia were eight, two, and six days, respectively. Mouthhook dimensions of the first instar (Fig. 2) were 16 ± 1.0 µm × 24 ± 1.0 µm, second instars had no sclerotized mouthhooks, and third instars had

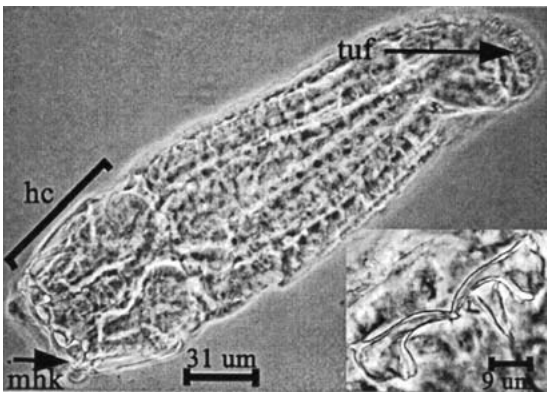

Fig. 2. Light micrographs of first instar (3 dpp) *Fopius arisanus* to show sclerotized head capsule (hc), sclerotized mouthhooks (mhk), and posterior tuft of setae (tuf). Inset = enlargement of mouthhooks.

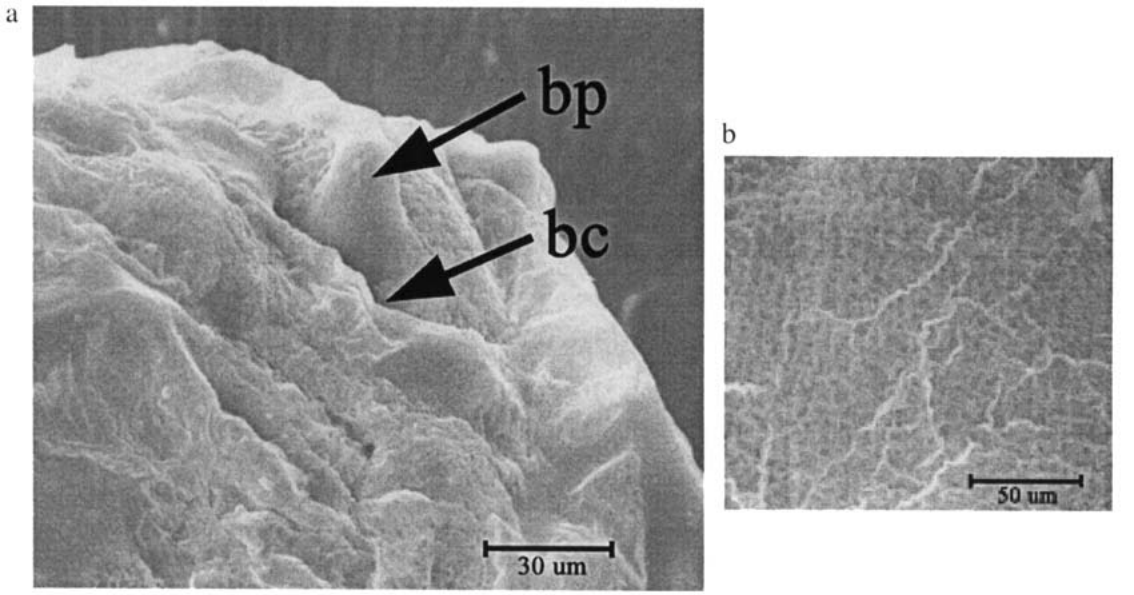

Fig. 3. Scanning electron micrograph of a second instar *Fopius arisanus* (8 dpp) to show (a) cephalic region lacking sclerotized mouthhooks and (b) lack of spines on the integument. bc = buccal cavity; bp = buccal papilla.

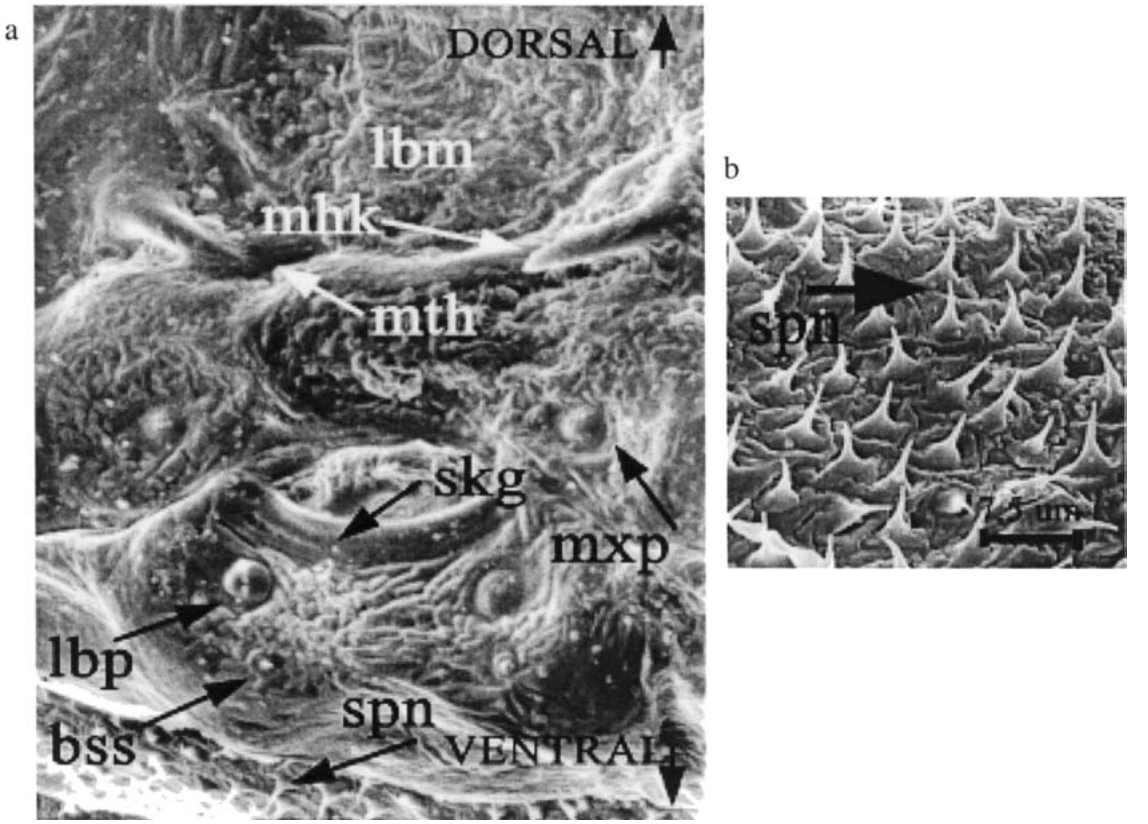

Fig. 4. Scanning electron micrographs of early third instar *Fopius arisanus* (9 dpp) to show cephalic region (a) and spines that cover integument (b). bss = basiconic sensillum; lbm = labrum; lbp = labial palp; mhk = mouthhook; mth = mouth; mxp = maxillary palp; skg = silk gland; spn = spines.

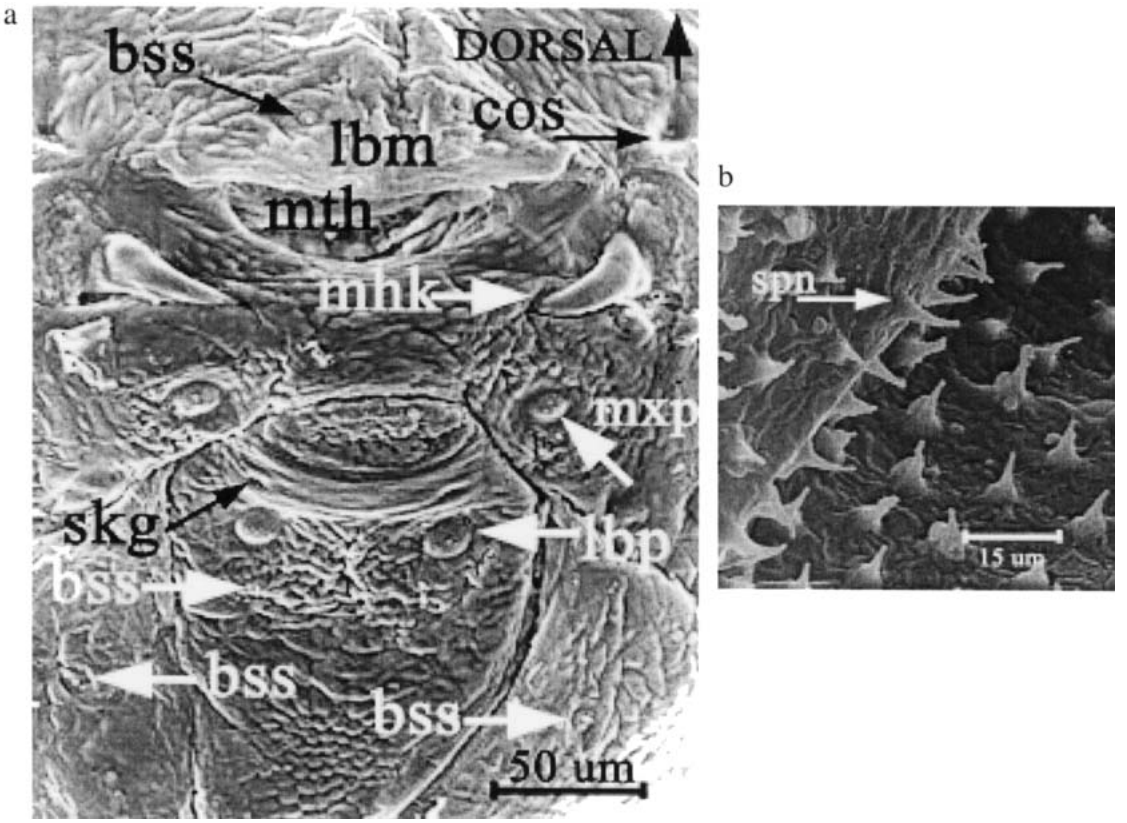

Fig. 5. Scanning electron micrographs of a late third instar (13 dpp) *Fopius arisanus*. (a) cephalic region to show mouthparts. (b) spines that cover integument. bss = basiconic sensillum; cos = coeloconic sensillum; lbm = labrum; lbp = labial palp; mhk = mouthhook; mth = mouth; mxp = maxillary palp; skg = silk gland; spn = spines.

mouthhooks 63 ± 3.0 µm × 42 ± 2.0 µm (3.94× that of first instars.) The distribution of the sensory papillae surrounding the mouthparts of early and late third instars is similar (Figs. 4 and 5).

Discussion

Based on our direct observations of sequentially dissected samples and morphology of *F. arisanus* larvae, we believe that this parasitoid has three instars and a prepupal stage (Fig. 6) because antennal elongation was evident at 13 dpp. While the third instar may have molted to a fourth instar of similar morphology, there was no distinct aggregation of mouthhook dimensions to suggest an increase in size that is normally expected following a larval molt. Although Ibrahm et al. (1992) and Palacio et al. (1992) reported a fourth instar, our SEM evidence indicates that the mouthhooks and cephalic region of the early third instar which occurred immediately after (9-11 dpp) the second instar (7-9 dpp) are similar in morphology and dimension to those of the stage (late third instar) immediately preceding the prepupal stage (13-14 dpp).

Size and duration of parasitoid instars vary with the size, age, and quality of the host in which they are reared (Lawrence et al. 1976; Lawrence 1990). In addition we have observed size differences with different methods of fixation and mounting (P. O. Lawrence, pers. obs.). Consequently, we focused on sclerotized structures such as larval mouthhooks and head capsules because they are reliable characters for identification. Nevertheless, measurements of soft tissues such as body length, in relation to those of sclerotized structures may prove useful for identification. Our larval body measurements vary greatly from those reported by Palacio et al. (1992) and Ibrahm et al. (1992), even though the host species are the same (*B. dorsalis*). This further underscores the unreliability of soft tissue measurements for identifying larval instars of parasitoids in general and *F. arisanus* in particular.

Evaluation of the integuments of the early and late third instars (according to our definition) as well as the pharate pupa, revealed no clear morphological differences. There were no distinctions between mouthhook sizes, integument, antennal and labial sclerites, or distribution of cephalic

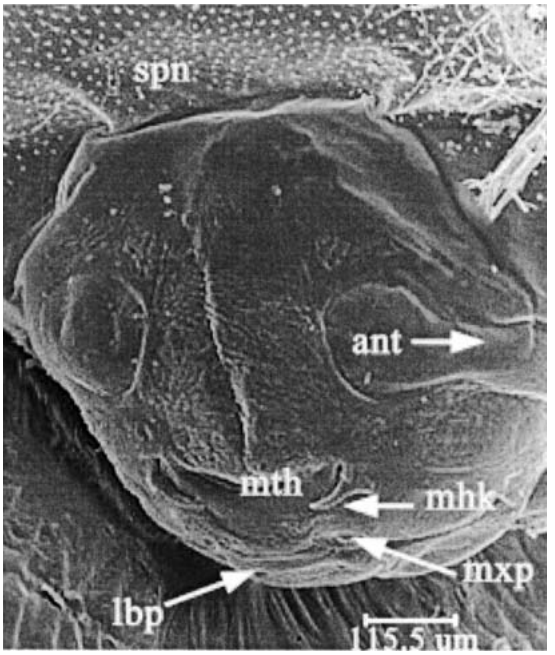

Fig. 6. Scanning electron micrograph of a prepupal *Fopius arisanus* (13 dpp) to show differentiation of antennae (ant). lbp = labial palp; mth = mouth; mhk = mouthhook; mxp = maxillary palp; spn = spines.

sensilla between the early and late third instars. Only direct observation of the molting of second or third instars can definitively distinguish the third from a presumed fourth instar. However, our goal was to establish identification criteria that are useful during dissections for quality control in mass rearing facilities. We believe that standardized sequential sampling along with these morphologies that are also visible under the light microscope will suffice.

Acknowledgments

We thank Donna Williams from the Microbiology and Cell Science Department at the University of Florida for her assistance with SEM and L. Matos and Y. Hashimoto for reviewing the manuscript. Also, financial support from the National Science Foundation (IBN 9986076) to P. O. Lawrence is gratefully acknowledged. This article results from research conducted under CRIS project ENY 03507 and is a Florida Agriculture Journal Series No. R-09948.

References Cited

Barbosa, P. 1974. Manual of Basic Techniques in Insect Histology. Autumn Publishers. Amherst, Massachusetts. p. 89.

Dyar, H. G. 1890. The number of molts of lepidopterous larvae. Psyche 5: 420-422.

Harris, E. J., and R. Y. Okamoto. 1991. A method for rearing *Biosteres arisanus* (Hymenoptera: Braconidae) in the laboratory. J. Econ. Entomol. 84: 417-422.

Ibrahm, A. G., I. P. Palacio, and I. Rohani. 1992. The life cycle of *Biosteres arisanus*, with reference to adult reproductive capacity on eggs of oriental fruitfly. Malaysia Appl. Biol. 21: 63-69.

Lawrence, P. O., R. M. Baranowski, and P. D. Greany. 1976. Effect of host age on development of *Biosteres* (=*Opius*) *longicaudatus*, a parasitoid of the Caribbean fruit fly, *Anastrepha suspensa*. The Florida Entomol. 59: 33-39.

Lawrence, P. O. 1990. The biochemical and physiological effects of insect hosts on the development and ecology of their insect parasites: an overview. Arch. Insect Biochem. Physiol. 13: 217-228.

Lawrence, P. O., E. J. Harris, and R. C. Bautista. 2000. Development and reproductive biology of the egg-pupal parasite, *Fopius arisanus* in *Anastrepha suspensa*, a new tephritid host. pp. 739-748. *In* K. H. Tan [ed.], Area-Wide Control of Fruit Flies and Other Insect Pests. Penerbit Universiti Sains Malaysia. 780 pp.

Nation, J. L. 1983. A new method using hexamethyldisilazane for preparation of soft insect tissues for scanning electron microscopy. Stain Technology 58: 347-351.

Palacio, I. P., R. Ibrahim, and A. G. Ibrahim. 1992. Identification of the immatures and male adults of the opiine parasitoids (*Biosteres* spp.) of the Oriental fruit fly, *Bactrocera dorsalis* (Hendel). The Philippine Entomol. 8: 1124-1146.

Vargas, R. I., and M. M. Ramadan. 2000. Comparisons of demographic parameters: six parasitoids (Hymenoptera: Braconidae) and their fruit fly (Diptera: Tephritidae) hosts, pp. 733-737. *In* K. H. Tan [ed.], Area-Wide Control of Fruit Flies and Other Insect Pests. Penerbit Universiti Sains Malaysia. 780 pp.