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Morphology of the Reproductive Tract and Ovariole Histology of *Apanteles galleriae* (Hymenoptera: Braconidae) Reared on Two Host Species

Fevzi Uçkan, Ekrem Ergin, Selma Sinan and Olga Sak

Department of Biology, Faculty of Science-Art, Balıkesir University, Balıkesir, 10100, Turkey

Abstract: We investigated the morphology of the reproductive tract and ovariole histology of koinobiont solitary early instar larval endoparasitoid *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae) reared on two lepidopteran species, greater wax moth, *Galleria mellonella* (L.) and lesser wax moth, *Achoria grisella* Fabr. (both Pyralidae). The work did not reveal any apparent difference between the morphology of either males or females as well as histology of females reared on two different hosts. The internal organs of reproductive system in *A. galleriae* adults conform mostly to the basic plan displayed in other parasitoid species. However, the system is not typically hymenopteran in that ovarioles unite before calyx. The ovipositor anatomy is typical of terebrantes in general. Vesicula seminalis and accessory glands were found united in males. Our results may be beneficial for success in biological control applications as well as they might be of value in phylogenetic, evolutionary, and systematic studies.

Key words: *Apanteles galleriae*, parasitoid, reproductive tract, morphology, histology

Introduction

The exact form of reproductive organs provides potentially informative biological characters at several levels among higher taxa or even species within the Hymenoptera. However, information concerning the reproductive system of the braconids (Hymenoptera) including *Apanteles* species has been little investigated to date (LeRalec *et al.*, 1996). Approximately 8.5% of insects are parasitoids and Hymenoptera includes most of the parasitoid ($\approx 50,000$) species (Gaston, 1991). Hymenopteran species also include many biological control agents (Hokkanen and Lynch, 1995). Parasitoid reproduction is a critical feature in determining the effectiveness of an agent. Thus, knowledge about the characteristics of the reproductive system of that agent may be valuable. The impacts of developing in different hosts could also be significant for parasitoids attacking more than one species of host.

Apanteles galleriae Wilkinson is a koinobiont, solitary and early instar larval endoparasitoid of lepidopterous species (*Galleria mellonellae* L., *Achoria grisella* F., *Achoria innotata* Walker and *Vitula edmandsae* Packard) (Shimamori, 1987; Watanabe, 1987; Whitfield *et al.*, 2001) that can cause significant damage to comb in honeybee hives. Studies conducted on this species have been mainly focused on its biological characteristics and host-parasitoid interactions (Uçkan and Gülel, 2000 and 2001; Uçkan and Ergin, 2002 and 2003). Dindo *et al.* (1995) reported that they did not observe any apparent morphological or histological difference between the

reproduction system of *Brachymeria intermedia* (Nees) females when parasitoids were reared *in vitro* (fed on artificial diet) and *in vivo* (on *G. mellonella* pupa). Their results and the insufficient knowledge about the reproductive tract of parasitoid species led us to the idea to study the impacts of different host species on the reproductive tract of female and male *A. galleriae* adults reared on two different hosts. We aimed to show the general morphology of female and male reproductive tract, histology of female ovarioles and to determine if host induced differences occurred.

Materials and Methods

Stock cultures of the parasitoid and host species were established from adults collected at several beehives located in the vicinity of Rize, Turkey. Host exposure and parasitoid rearing were conducted in a rearing room at $25\pm 2^\circ\text{C}$, $60\pm 5\%$ relative humidity under a photoperiod of L12: D12. Seven males and females of early instar larvae of greater wax moth *G. mellonella* and lesser wax moth *A. grisella* were removed from host cultures, placed in separate five 2 L glass jars lined with cloth, allowed to mate, and provided with honeydew for feeding and oviposition. After host larva emergence (≈ 7 days), hosts were exposed to parasitization by placing five males and females (1 or 2 d old) parasitoids in each host jar. Laboratory colony of *A. galleriae* fed 50% honey solution was maintained on each host species for the observations.

Adults of 5 to 10-day-old *A. galleriae*, ages at which

females have the highest fecundity as reported by Uçkan and Gülel (2000), were collected from each rearing jar. Reproductive organs of males and females mounted on slides were dissected with the aid of dissection needles in order to examine and compare the reproductive tract morphologically. After staining with 0.01% methylene blue, the genitalia were examined and drawings were made with a Nikon stereoscopic microscope equipped with a camera lucida. For histological studies, reproductive organs of 5 to 10-day-old females were isolated and ovipositors were removed. Specimens were fixed in 10% ethyl alcohol solution, then, dehydrated by placing in filter papers because they were very small. Finally, they were embedded in paraffin wax and sectioned at 5 μ m. After staining with hematoxylin-eosin, sections were labeled and kept for drying. They were examined and visualized with a Nikon microscope. Morphological and histological comparisons were made with specimens taken from 10 male and female adults reared on each host species (*G. mellonella* and *A. grisella*).

Results

Visual observations did not reveal any apparent morphological differences between the reproductive system of either male or female adults reared on *G. mellonella* and *A. grisella*. Thus, figures concerning the morphology of each sex and ovariole histology are not illustrated separately for each host species.

Morphology: The female reproductive system of *A. galleriae* consists of a pair of ovaries (ov), two lateral oviducts (lo), a median oviduct (co), a pair of accessory glands (spg), spermatheca (spt), ovipositor (op) and a pair of valvula 3, valvifer 1 (vr₁), valvifer 2 (vr₂) (Fig. 1).

Each ovary is comprised of two ovarioles repeatedly folded on each other anteriorly and uniting posteriorly before calyx (Fig. 1). Ovaries open into a lateral oviduct via calyx (ca). Two lateral oviducts unite in the common oviduct that opens into the genital chamber via the gonopore. The opening of the genital chamber to the outside is the vulva. The ovipositor consists of two valvulae 1 (v₁) also named as lancets (lc) and a valvula 2 (v₂). Lancets, situated in a lateral cavity, are arched and extend out of the basal of valvula 2. Valvifers (vr₁ and vr₂), spermatheca accessory glands (spg) and ovaries are connected at the thickened basal of valvula 2. Valvula 3, valvifer 1 (vr₁) and valvifer 2 (vr₂) have some sensory hairs. The size of spermatheca (spt) is highly variable in different individuals. The glandular portion of the spermatheca forms an entity distinct from the storage portion. The distal end of the spermatheca is specialized for secretion and forms a relatively longer but thinner

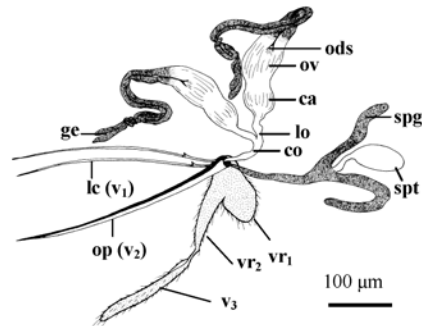


Fig. 1: Diagram of the female reproductive system of *Apanteles galleriae*. ge, germarium; ods, ovariole diverging site; ov, ovariole; ca, calyx; lo, lateral oviduct; co, median oviduct; spt, spermatheca; spg, spermathecal gland; lc (v₁), lancet; op (v₂), ovipositor; v₃, valvula 3; vr₁, valvifer 1; vr₂, valvifer 2

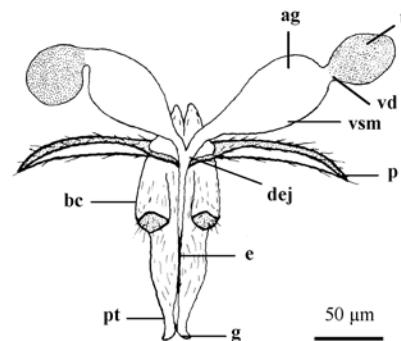


Fig. 2: Diagram of the male reproductive system of *Apanteles galleriae*. t, testes; vd, vasa deferentia; vsm, vesicular seminalis; ag, accessory gland; dej, ductus ejaculatorius; e, aedeagus; p, paramer; bc, basal sclerity; g, gonophore; pt, phallotreme

structure referred as spermathecal accessory glands (spg). These structures are united and open into the vulva (Fig. 1).

Internal organs of reproduction in male *A. galleriae* adults are in many respects similar to those of female. The essential parts of the male reproductive system include a pair of testes (t), a pair of lateral ducts, the Vasa deferentia (vd), corresponding to the lateral oviducts of the female, a pair of vesicula seminalis (vsm) united with accessory glands (ag) in a complex, big structure and the exit tube, ductus ejaculatorius (dej), functionally comparable with the median oviduct of the female. Two vesicula seminalis unite to connect with the ejaculatory ducts where two parameres (p) are segmented. The two mesomeres comprising ejaculatory ducts fuse to form the aedeagus

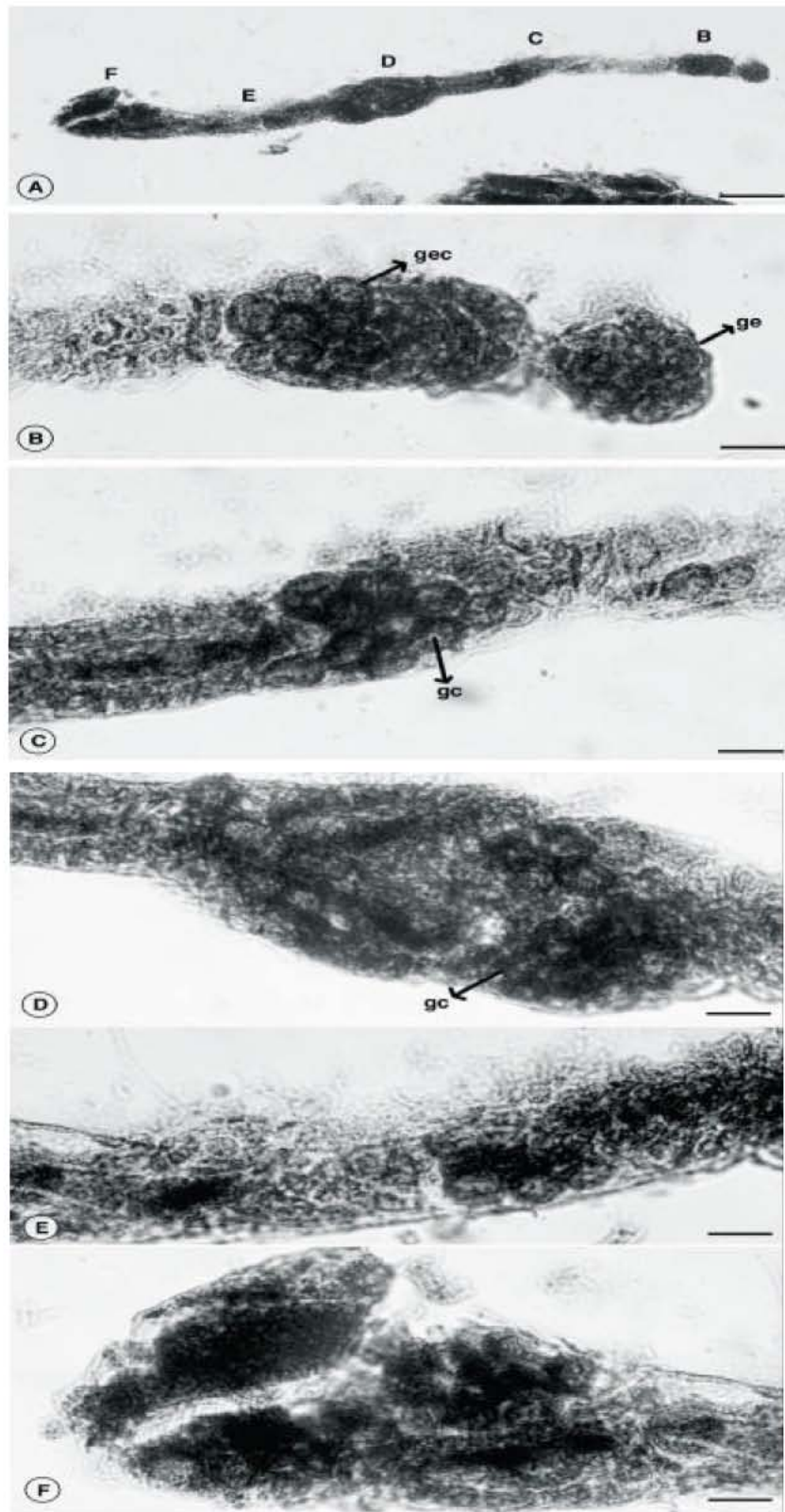


Fig. 3: (A) Longitudinal section of a female adult parasitoid ovariole. Scale bar indicates 40 μm . (B – F) An ovariole divided in five distinct regions lined parallel to the posterior-anterior axis. The apex of ovariole is towards the right of each figure. gec, growing egg cells; ge, gemarium; gc, growing cells. Scale bars indicate 8 μm

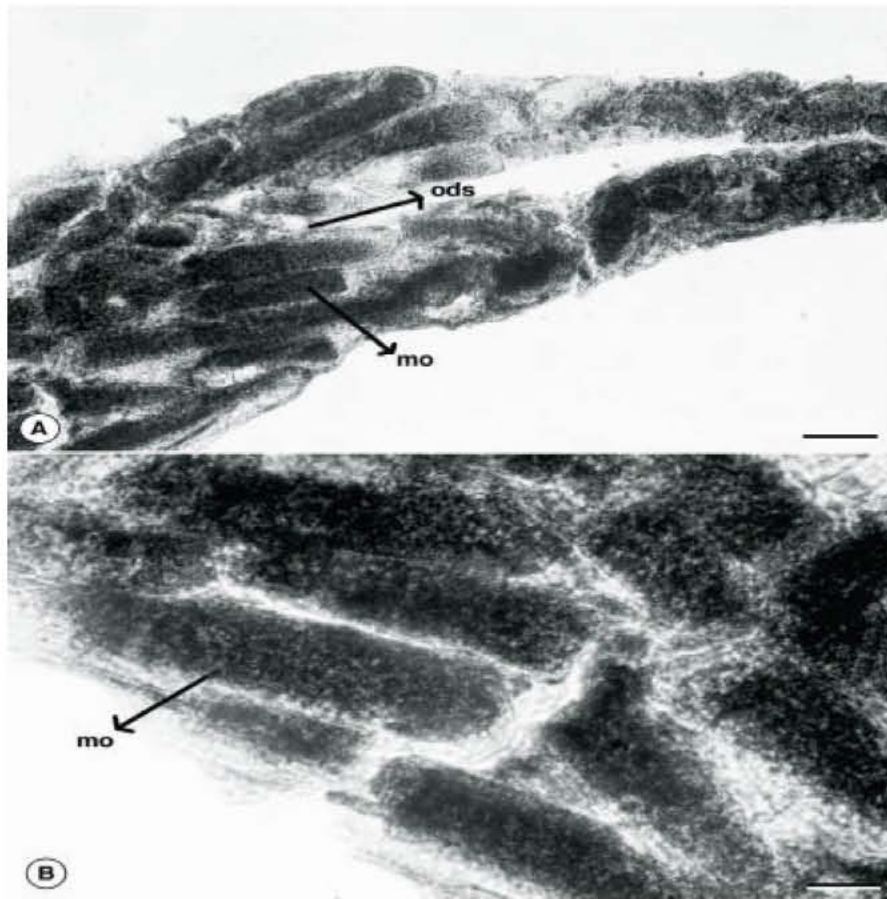


Fig. 4: (A) Longitudinal section of two ovarioles close to calyx. ods, ovariole diverging site; mo, mature oocyte. Scale bar indicates 20 μm . (B) Longitudinal section of an ovariole showing mature oocytes (mo). Scale bar indicates 8 μm

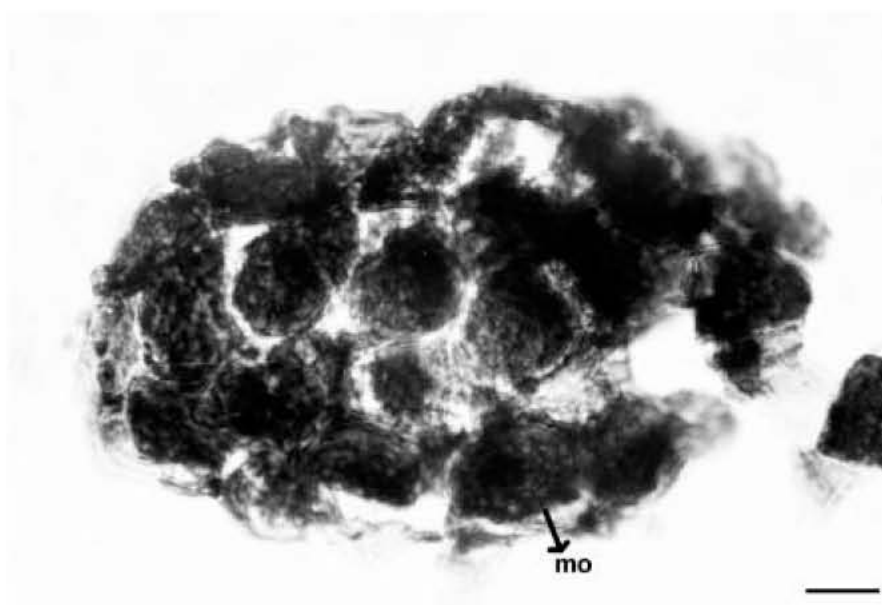


Fig. 5: A cross-section of an ovariole. mo, mature oocyte. Scale bar indicates 8 μm

(e). The distal end of the aedeagus is invaginated and connected with the gonophore (g). That part of gonophore is called phallotreme (pt). Paramers and basal sclerites (bc) have some hairs (Fig. 2).

Histology: The principal part of the ovariole is the egg tube that contains the germ cells and their derivatives. It is relatively denser probably as a result of cell accumulation at four regions toward the supporting stalk; pedicel that unites the egg tube with the lateral oviduct and it has no terminal filament located at the anterior (Fig. 3A). Histological structure of the ovariole is illustrated with five distinct photographs (Fig. 3B-F) by dividing the whole structure into five regions. The first condensed region at the anterior contains the germ cells and cytoblasts and it is called the germarium (ge). It is a spherical satellite-like structure filled with a mass of cells (Fig. 3B). Beyond the germarium is the second condensed region greater two times in length in which the egg cells begin to grow (gec) (Fig. 3B). The third condensed region is smaller and comprises 6 to 8 germ cells (gc) (Fig. 3C). The most condensed region is the fourth one in which the germ cells are considered to complete their development (Fig. 3D). Oocytes become successively larger increasing especially in length toward the posterior end of the tube as they are transported (Fig. 3E, F). Finally, a large number of oocytes accumulate in the exit passages before they are discharged. The two distinct ovarioles combining at the posterior end can also be seen. More than one mature oocyte (mo) is transported through calyx from each ovariole (Fig. 4A). The length and thickness of a cylindrical-shaped mature oocyte is about 35 and 11 μm , respectively (Fig. 4B). The cross section of the ovariole shows that it is wide enough to retain approximately 10 to 15 oocytes at the same time (Fig. 5). Oocytes are positioned vertically in an ovariole and ovariole is long enough to locate approximately 3 to 4 oocytes lined vertically behind each other.

Discussion

The basic structural and functional traits of Hymenopteran reproductive system such as testis shape, ovary type, the position and number of ovarioles and ovipositor steering mechanisms have been subject to many evolutionary, phylogenetic and systematic studies (Koeniger *et al.*, 1990; McDaniel and Boe, 1990; Quicke and Fitton, 1995). There are also morphological reproductive traits closely related with the host type in parasitic species (LeRalec *et al.*, 1996). However, our observations also did not reveal significant morphological (for both males and females) or histological (only for females) differences between the reproductive system of

adults reared on *G. mellonella* and *A. grisella*. Therefore, our results providing evidence for structural similarities of the reproductive system in *A. galleriae* males and females reared on two different host species indicate that fecundity does not change considerably depending on host type. This is in line with the situation observed by Uçkan and Gülel (2000) reporting no significant differences in fecundity of females when reared on two different species.

The female reproduction system of *A. galleriae* generally conforms to the structure given for insects (Snodgrass, 1935; Davey, 1965). However, some important structural variations were observed. Ovary structure of female *A. galleriae* differs from other ovary types given for most of insect species (Engelman, 1970; Chapman, 1998) in that ovarioles unite with each other before opening into calyx. It has also been reported that the anterior end of each lateral duct is generally somewhat expanded, forming a receptacle known as the calyx and the calyx is sometimes lengthened and enlarged in Lepidoptera, Hymenoptera and Diptera by the uniting of ovarioles before opening into calyx (Jervis and Kidd, 1996; Chapman, 1998). Although our results revealed that each ovary is formed of a pair of ovarioles united through calyx, the combining of two ovarioles or diverging of an ovariole into two filamentous structures in the phylogenetic process might have formed the structure. In many of the species, a slender, threadlike filament called terminal filament forms the anterior part of an ovariole, but ovarioles of *A. galleriae* do not possess this structure and each ovariole terminates with a satellite-like structure, called the germarium. Snodgrass (1935) also confirmed the absence of terminal filaments in some species. The highly developed spermatheca and spermathecal accessory glands and their connection to common oviduct at a different site are determined as other morphological distinctions for *A. galleriae*. Variations in the size of spermatheca may be related to the usage of spermatozoa in time. The fully developed accessory glands seem to serve the function of Dufour's gland. That female adult having a short ovipositor at the end of the abdomen resembles mostly the structure seen in Acuelata (Maschwitz and Klafft, 1986). Structural organization of valvulae 1, 2, 3 and valvifers 1, 2 conform to the basic plan displayed for hymenopterans (Edson and Vinson, 1979; Van Marle and Piek, 1986; Uçkan, 1999). But, lancets (v_1) situated in lateral cavity of ovipositor represent a little variation in position. Sensory hairs found on valvula 3 and both valvifers (vr_1 and vr_2) were also defined for other species (LeRalec *et al.*, 1996; Uçkan, 1999).

The structures of the internal reproductive organs in male *A. galleriae* are also similar to that of most hymenopteran

species. Accessory glands are not formed as diverticula from the vas deferens as in many cases, but, they are mesodermally derived ducts united with the vesicular seminalis (Davey, 1965).

Histological observations revealed that the developing oocytes ($\approx 11-33 \mu\text{m}$) and tropocytes in the ovarioles were significantly smaller in size than those of other hymenopteran species (Fleig *et al.*, 1991; Dindo *et al.*, 1995; Camargo-Mathias *et al.*, 1997). The relatively condensed regions observed through each ovariole might be an indicator of intense synthesis and transport activity. As it is in most other insect species, oocytes progressively increasing in size toward calyx were observed in each ovariole. However, studies conducted with electron microscope are needed to examine the level of cellular differentiation and changes at different maturation levels. More than one developing egg was found in each ovariole, up to a maximum of 10 to 15 eggs cross sectional and 3 to 4 eggs longitudinal per ovariole. This is in line with the observations made by Drost and Carde (1992) that each *B. intermedia* ovariole may contain more than one mature egg. This situation may also confirm the results why this species has a high level of fecundity in the first two weeks of maturation, which was reported by Uçkan and Gülel (2000).

Structural differences observed and mentioned above were generally encountered in the reproductive system among species in Hymenoptera and other insect orders. Many structural traits of the reproductive system give a good measure of rate of egg production and lifetime fecundity and are directly correlated with the biological control of host species by parasitoid species. In view of this fact, our results may be beneficial for success in biological control applications as well as phylogenetic, evolutionary, and systematic studies.

References

- Camargo-Mathias, M.I., E. Thiele and F.H. Caetano, 1997. Female reproductive system of ant species of the subfamily Ponerinae: Review and new data. *Sociobiol.*, 29: 307-321.
- Chapman, R.F., 1998. *The Insects: Structure and Function*. 4th Ed. Cambridge Univ. Press, Cambridge.
- Davey, K.G., 1965. The female reproductive tract. In: Davey, K.G. (Ed.), *Reproduction in the Insects*. Oliver and Boyd, Edinburgh and London, pp: 15-36.
- Dindo, M.L., G. Gardenghi, M. Grasso, 1995. Notes on the anatomy and histology of the female reproductive system of *Brachymeria intermedia* (Nees) (Hymenoptera; Chalcididae) reared *in vivo* and *in vitro*. *Boll. Ist. Ent. "G. Grandi" Univ. Bologna*, 50: 5-13.
- Drost, Y.C. and R.T. Carde, 1992. Influence of host deprivation on egg load and oviposition behavior of *Brachymeria intermedia*, a parasitoid of gypsy moth. *Physiol. Entomol.*, 17: 230-234.
- Edson, K.M. and S.B. Vinson, 1979. A comparative morphology of the venom apparatus of female Braconids (Hymenoptera; Braconidae). *Can. Entomol.*, 111: 1013-1024.
- Engelman, F., 1970. *The Physiology of Insect Reproduction*. Pergamon Press Inc., New York.
- Fleig, R., H.O. Gutzeit and W. Engels, 1991. Structural organization of ovarian follicle cells in the cotton bug (*Dysdercus intermedius*) and the honeybee (*Apis mellifera*). *Cell Tissue Res.*, 265: 297-305.
- Gaston, K.J., 1991. The magnitude of global insect species richness. *Conservation Biol.*, 5: 235-296.
- Hokkanen, H.M.T. and J.M. Lynch, 1995. *Biological control: benefits and risks*. Cambridge Univ. Press, New York.
- Jervis, M. and N. Kidd, 1996. *Insect Natural Enemies. Practical approaches to their study and evaluation*. Chapman and Hall, New York.
- Koeniger, G., M. Mardan and F. Ruttner, 1990. Male reproductive organs of *Apis dorsata*. *Apidologie*, 21: 161-164.
- LeRalec, A., J.M. Rabasse and E. Wajnberg, 1996. Comparative morphology of the ovipositor of some parasitic Hymenoptera in relation to characteristics of their hosts. *Can. Entomol.*, 128: 413-433.
- Maschwitz, U.W.J. and W. Klawns, 1986. Morphology and function of the venom apparatus of insects. In: W. Bucher, E.E. Buckley (Eds.), *Venomous Animals and Their Venoms* Academic Press, New York, pp: 1-60.
- McDaniel, B. and A. Boe, 1990. A new host record for *Eurytomocharis eragrostidis* Howard (Chalcidoidea: Eurytomidae). *Proc. Entomol. Soc. Wash.*, 92: 465-470.
- Quicke, D.L.J. and M.G. Fitton, 1995. Ovipositor steering mechanisms in parasitic wasps of the families Gasteruptionidae and Aulacidae (Hymenoptera). *Proc. R. Soc. London. B.*, 261: 99-103.
- Shimamori, K., 1987. On the biology of *Apanteles galleriae*, a parasite of the two species of wax moths. *Honeybee Sci.*, 8: 107-112.
- Snodgrass, R.E., 1935. *Principles of Insect Morphology*. McGraw-Hill Book Company, New York.
- Uçkan, F. 1999. The morphology of the venom apparatus and histology of venom gland of *Pimpla turionellae* (L.) (Hymenoptera; Ichneumonidae) females. *Tr. J. Zool.*, 23: 461-466.

- Uçkan, F. and A. Gülel, 2000. Effects of host species on some biological characteristics of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae). *Tr. J. Zool.*, 24: 105-113.
- Uçkan, F. and A. Gülel, 2001. The effects of cold storage on the adult longevity, fecundity and sex ratio of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae). *Tr. J. Zool.*, 25: 187-191.
- Uçkan, F. and E. Ergin, 2002. Effect of host diet on the immature developmental time, fecundity, sex ratio, adult longevity, and size of *Apanteles galleriae* (Hymenoptera: Braconidae). *Environ. Entomol.*, 31: 168-171.
- Uçkan, F. and E. Ergin, 2003. Temperature and food source effects on adult longevity of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae). *Environ. Entomol.*, 32: 441-446.
- Van Marle, J. and T. Piek, 1986. Morphology of the venom apparatus. In: T. Piek (Ed.), *Venoms of the Hymenoptera*, Academic Press, New York.
- Watanabe, C., 1987. Occurrence of *Apanteles galleriae* (Hymenoptera: Braconidae), a parasite of wax moth in Japan. *Kontyû*, 55: 165-168.
- Whitfield, J.B., S.A. Cameron, S.R. Ramirez, K. Roesch, S. Messinger, O.M. Taylor and D. Cole, 2001. Review of the *Apanteles* species (Hymenoptera: Braconidae) attacking lepidoptera in *Bombus* (*Fervidobombus*) (Hymenoptera: Apidae) colonies in the new world, with description of a new species from South Africa. *Ann. Entomol. Soc. Am.*, 94: 851-857.