Morphology and Development of Immature Stage of *Diadromus collaris* (Hymenoptera: Ichneumonidae), an Important Endoparasitoid of *Plutella xylostella* (Lepidoptera: Plutellidae)

WEI ZHAO,[1] SHUAI DONG,[1] MIN SHI,[1] AND XUE-XIN CHEN[1,2]

Ann. Entomol. Soc. Am. 107(1): 234–241 (2014); DOI: http://dx.doi.org/10.1603/AN12132

ABSTRACT *Diadromus collaris* (Gravenhorst) is an important pupal parasitoid of the diamondback moth, *Plutella xylostella* (L.), a major insect pest of cruciferous vegetables worldwide. We studied the development of immature stage of *D. collaris* by dissecting parasitized hosts in the laboratory at 25 ± 1°C and 50–80% relative humidity. The results show that all immature stages complete their development within the same host in 11≈12 d. The egg is hymenopteriform and appears to be anhydropic. There are four larval instars. The first instar is transparent with a sclerotized rectangular chitinous head capsule and distinct mandibles. Head capsule of the second instar turns into more isosceles trapezium-shaped. The third instar looks similar to the second instar but proportionally much larger. The sclerization and dimension of the fourth instar increases significantly. The pupa is a typical exarate form and lacks a cocoon. All life history was fully documented with detailed photomicrographs. Our study will be useful for understanding the physiological interactions between *D. collaris* and *P. xylostella*.

KEY WORDS *Diadromus collaris*, development, parasitoid, *Plutella xylostella*, Ichneumonidae

Diadromus collaris (Gravenhorst) (Hymenoptera: Ichneumonidae) is an important pupal endoparasitoid of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), one of the most destructive pests of cruciferous vegetables worldwide (Waterhouse and Norris 1987, Talekar and Shelton 1993). It has been recorded in many parts of the world as one of the major biological control agents of *P. xylostella* (Waterhouse and Norris 1987, Mustata 1992, Wakisaka et al. 1992, Ali and Karim 1995, He et al. 1996, Chauhan et al. 1997, Kfir 1997, Liu et al. 2000). The first decade of this century has seen an increase in the studies of this parasitoid. Liu et al. (2001, 2002) studied its biology and the intraspecific variability of different geographic populations. Wang and Liu (2002) reported its host age preference and suitability. Moreover, venom gland structure and effects of parasitism on the fat body and hemocytes of host were also reported (Li et al. 2006a,b, 2007).

Successful parasitism of hosts by parasitoids depends on not only gene products that the adult wasp injects at oviposition like venom and polydnavirus, but also that the offspring produces during the course of development including embryonic and larval stages such as teratocytes in some parasitoid–host relationships (Pfister–Wilhelm and Lanzrein 1996, Bonvin et al. 2004, Pennacchio and Strand 2006, Falabella et al. 2009). The diversity of tactics parasitoids have evolved to exploit host resources share the goal of synchronizing host nutrient availability with key phases in offspring development (Pennacchio and Strand 2006, Falabella et al. 2007, Bai et al. 2009). Venom seems to be the major factor of *D. collaris* in host immune suppression and regulation (Li et al. 2006a,b, 2007). However, although some aspects of its biology and final instar larva have been studied (Given 1944, Liu et al. 2001), the knowledge of the immature stages is still not enough to elucidate how *D. collaris* alters its host development and immune defenses to ensure its offspring growth inside host larvae. This study describes the development of immature stage of *D. collaris* with a detailed description of external morphology, which will benefit the study on the physiological relationship of *P. xylostella* and *D. collaris*.

Materials and Methods

Insect Collection and Rearing. A colony of *D. collaris* was established from parasitized *P. xylostella* pupae collected from cabbage (*Brassica oleracea* L.) fields in the suburbs of Wuhan, Hubei Province, China. Both *P. xylostella* and *D. collaris* colonies were raised on cabbage in an environmental chamber at 25 ± 1°C, 60–65% relative humidity (RH), and a photoperiod of 14:10 (L:D) h. Adult wasps were fed with 20% honey-water solution and propagated using *P. xylostella* pupae.

[1] Ministry of Agriculture Key Laboratory of Agricultural Entomology, Institute of Insect Sciences, Zhejiang University, Hangzhou 310058, China.

[2] Corresponding author, e-mail: xxchen@zju.edu.cn.

0013-8746/14/0234–0241$04.00/0

Table 1. Sizes of immature stages of *D. collaris*

Stage	Duration	Body length (mm ± SD)	Body width (mm ± SD)	Sample size
Embyro, 0–1 h	—	0.750 ± 0.020	0.172 ± 0.005	3
Embyro, 12–13 h	—	0.700 ± 0.012	0.165 ± 0.006	3
Embyro, 24–25 h	—	0.738 ± 0.010	0.170 ± 0.002	3
L1	20–24 h	0.981 ± 0.292	0.292 ± 0.064	15
L2	12–16 h	1.395 ± 0.226	0.484 ± 0.047	12
L3	8–12 h	1.753 ± 0.250	0.651 ± 0.071	8
L4	44–48 h	3.436 ± 0.919	1.027 ± 0.215	37
Prepupa	20–24 h	4.640 ± 0.360	1.057 ± 0.070	30
Pupa	128–132 h	4.712 ± 0.355	0.878 ± 0.086	102

Development of *D. collaris*. Newly eclosed male and female (1:1) wasps were collected and placed in plastic containers. Two days postemergence, mated female wasps were used for parasitization experiment. Fresh host pupae (0–1 d) were exposed to female wasps in a transparent vial. After a host pupa was parasitized once, the parasitized host pupa containing the parasitoid egg was transferred to a plastic tube kept in the environmental chamber. Developmental time was counted from the moment the parasitoid egg was laid into the host.

To follow development, the parasitoid eggs and larvae were dissected out from host larvae in phosphate-buffered saline (18.6 mM $NaH_2PO_4 \cdot H_2O$, 84.1 mM $NaH_2PO_4 \cdot 2H_2O$, and 1.75 M NaCl, pH 7.4) with the aid of a dissecting microscope (M125, Leica, Biberach, Germany) at 3- and 4-h intervals for embryonic development and the following stages, respectively, until all stages of parasitoid development were recorded (Table 1).

For each time point, three eggs, larvae, or pupae were observed and measured. Length measurements were taken from the anterior to the posterior end of eggs, larvae, and pupae. Width measurements were taken from the anterior of the thorax of larvae and pupae, and the widest portion of eggs and head capsules.

All stages of the wasp were photographed using a stereomicroscope (MZ16A, Leica) with an attached digital camera in conjunction with AutoMontage5.0 software (Synoptics Group, Cambridge, United Kingdom) and an inverted phase contrast microscope (DM IRB, Leica) equipped with an image manager (IM1000, Leica).

Fig. 1. *D. collaris* eggs. (A–C) Eggs under subdivision 1–6 h after oviposition. (D–E) Eggs in gastrulation 9–10 h after oviposition. (F–H) Eggs in segmentation 14–20 h after oviposition. (I) Embryo ready for ecdysis ≈ 24 h after oviposition. Scale bar = 0.05 mm. (Online figure in color.)

Results

Egg and Embryo. Newly deposited eggs were hymenopteriform, elongate-oval, creamy white, and broader at the cephalic end (Fig. 1A). Approximately 1–2 h after oviposition, the egg began subdivision (Fig. 1B and C). A group of cells grew out from both the anterior and posterior poles of the egg and spread over the surface of the embryo, forming a complete outer membrane, the serosal membrane. This lasted 5–6 h followed by gastrulation (Fig. 1D and E), which was complete in 2–3 h. The embryo then underwent segmentation (Fig. 1F). About 14 h after oviposition, the gut became visible (Fig. 1F). Then another 5 h later, the cephalic capsule, body segments, and digestive system became distinguishable (Fig. 1G and H). Approximately 24 h after oviposition, the first instar was visible through the transparent chorion (Fig. 1I). During embryonic development, there was no significant difference among different stages in width or length.

Larvae. Dissection and counting the number of exuvia revealed that *D. collaris* had four instars. Exuvia of the first three instars were thin, transparent, and still had intact head capsules bearing mandibles, whereas exuvium of the fourth instar was thicker and more opaque consisting of only mouthparts including mandibles and body cuticle (Fig. 2B–E). Mandible shape and size also differed in each instar (Fig. 2F). The larvae were ordinarily oriented on the same axis as the host with their heads directed anteriorly. Each instar

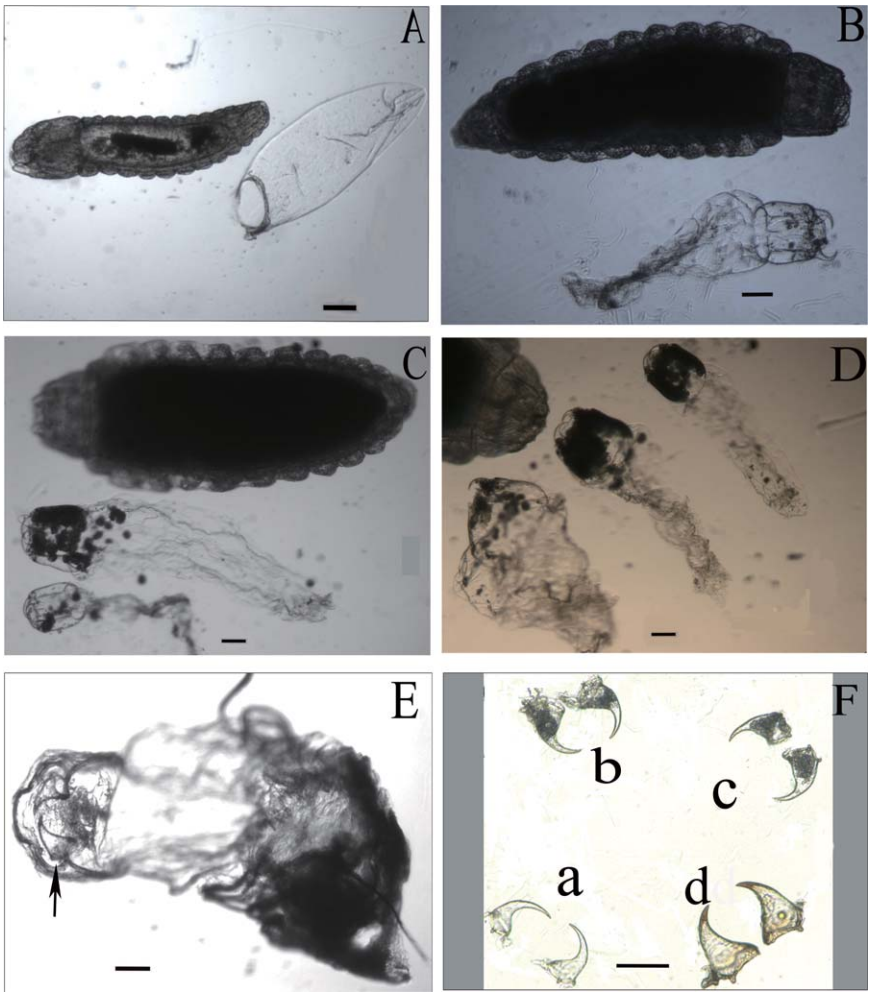

Fig. 2. Exuvia of *D. collaris* larvae. (A) First-instar larva and the rest egg membrane. (B) Second-instar larva and exuvium of first instar. (C) Third-instar larva and exuvia of last two instars. (D) Head of fourth-instar larva and exuvia of last three instars. (E) Exuvium of fourth instar; arrow, mandibles. (F) Mandibles of each instar exuvium. a, first instar; b, second instar; c, third instar; d, fourth instar. Scale bars = 0.1 mm. (Online figure in color.)

lasted a different period of time (Fig. 3). The first instar was caudate-mandibulate, whereas the second to fourth instars were hymenopteriform. All instars completed their development inside the same host.

First Instar. Upon hatching the chorion was ruptured at the anterior end with the rest egg membrane left behind (Fig. 2A). The first instar (≈24 h) was caudate-mandibulate with 13 segments and a distinct sclerotized rectangular head capsule of 0.20 ± 0.018 mm in width (Fig. 4A and B). The falcate mandibles were fully articulated. At the early stage, the larva was translucent and the cephalic capsule was almost as wide as the following several segments. As the larva developed, its body grew quickly and segmentation became more distinguishable. At the late stage, the segments behind the head exceeded the cephalic capsule in width and the tracheal system turned visible through the integument.

Second Instar. The second instar appeared at the third day after oviposition, lasting ≈16 h. Head capsule increased into 0.20 ± 0.018 mm in width and turned into more isosceles trapezium-shaped (Fig. 4C). When the body size of larvae was increasing, the gut occupied more and more volume of the body and squirmed frequently. The midgut and the V–shaped hindgut both end blindly but abutting one another. The tracheal system became more clearly visible, surrounding the gut.

Third Instar. The third instar was still spindle shaped, similar to the second instar but proportionally much larger (Fig. 4D). The posterior part of the sclerotized head capsule (0.39 ± 0.019 mm) was tucked under the first thoracic segment as body segments were increasing in size. Mandibles were also not as obvious as in the earlier instars. The larva became more opaque with gut increasing in pro-

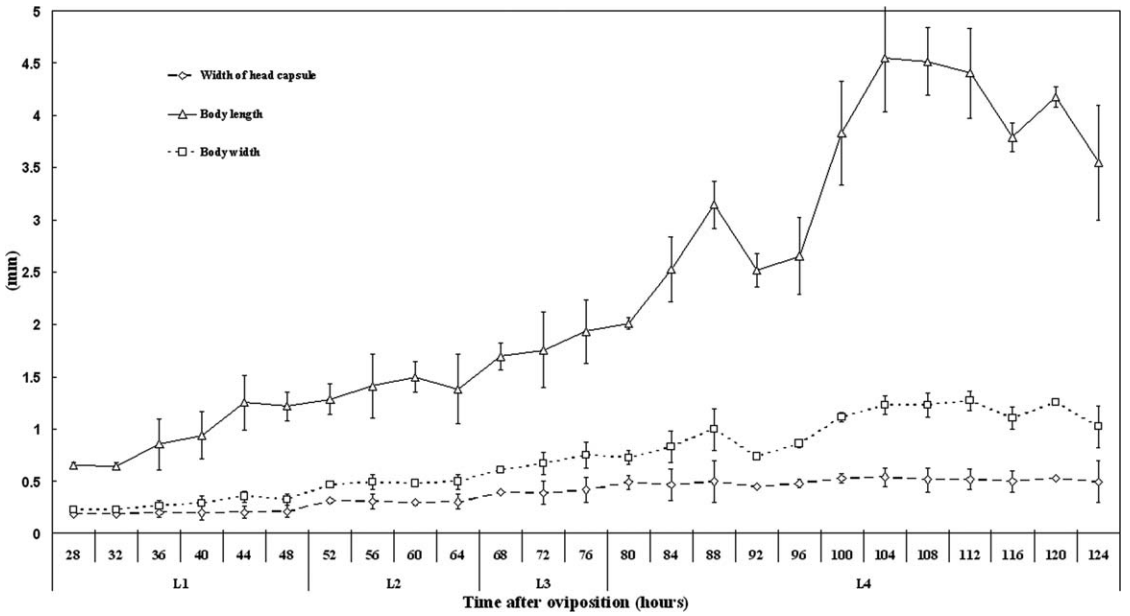

Fig. 3. Sizes of different instars of *D. collaris* larvae.

portion. The developmental time of the third instar was only 12 h.

Fourth Instar. The fourth instar lasted ≈48 h, nearly as long as the combined duration of the last three instars. The head of the fourth instar larva hardly changed its size as the larva developed, which averaged 0.50 ± 0.03 mm in width, but the sclerization and dimension of the fourth instar increased significantly. As growing, the body segments increased to such a size that the head became proportionately smaller and finally almost enveloped by the first thoracic segment (Fig. 4F). Many white globules appeared as bands of small white spots and scattered beneath the cuticle of abdominal segments, larger and more obvious at the later stage (Fig. 4F–H). Body color turned from yellowish green to dark green as development proceeded (Fig. 4E–H). Simultaneously the thoracic segments gradually differentiated. At the later stage, surface of thoracic segments became rough with wrinkles and somewhat uplifted, which could be easily distinguished from ab-

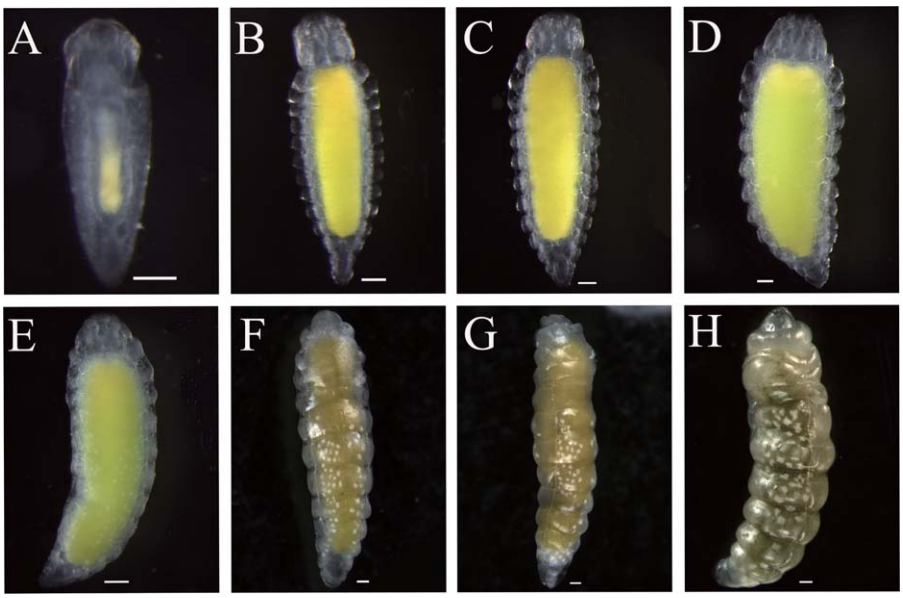

Fig. 4. *D. collaris* larvae. (A, B) First-instar larva. (C) Second-instar larva. (D) Third-instar larva. (E–H) Fourth-instar larva. Scale bars = 0.2 mm. (Online figure in color.)

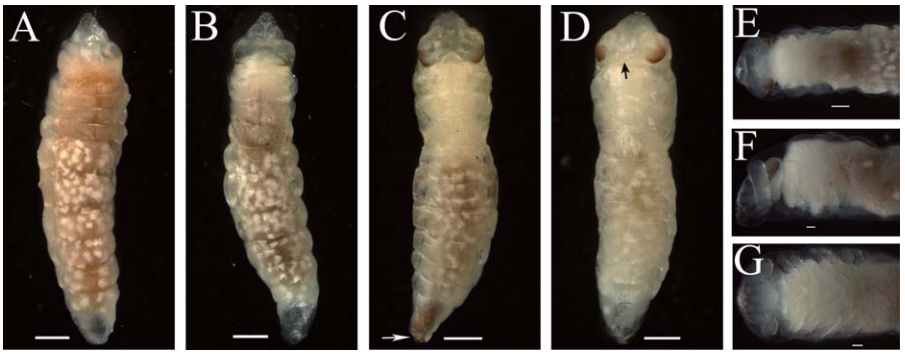

Fig. 5. *D. collaris* prepupae. (A) Newly formed prepupa. (B) Prepupa at the early phase. (C) Prepupa voiding its meconium; arrow indicating meconium. (D) Prepupa ready for molting; arrow indicating dorsal ocelli. (E–G) Head and thoracic segments in dorsal and lateral and ventral view. Scale bars = 0.5 mm (A–D) and 0.1 mm (E–G). (Online figure in color.)

dominal segments (Fig. 4F–H). Head capsule surface also turned rough with many sculptures (Fig. 4H). Approximately 48 h later, the parasitoid larvae had consumed nearly all the tissues of the host except the cuticle, and were completely opaque (Fig. 4H).

Prepupa and Pupa. *Prepupa.* In the prepupal stage, the parasitoid was still larviform, sculptured, shortened, and constricted, but with some pupal morphology indicated. The gut was filled with brown meconium visible through the cuticle of the abdomen (Fig. 5A). The midgut became connected to the hindgut at this stadium. In the later prepupal phase, the prepupa voided the meconium (Fig. 5B and C) and the body turned to ivory-white (Fig. 5D). At this stage, the compound eyes and three dorsal ocelli became visible and pigmented (Fig. 5D). As the prepupa approached ecdysis, the pupal characteristics such as antennae, and legs showed through the integument (Fig. 5E–G).

Pupa. The pupa was a typical exarate form and lacked a cocoon. The exuvium of the fourth instar and the meconium could be observed attaching to the end of the abdomen (Fig. 6A, B, and D). Initially, only eyes and ocelli were pigmented and the white granules in the larval phase were still detectable (Fig. 7A, F, and K). Then, the color of eyes darkened and the white granules disappeared (Fig. 7B, G, and L). The mandibles and mesoscutum became pigmented (Fig. 7C, H, and M), and pigmentation of the remaining parts of the thorax, petiole, and legs followed (Fig. 7D, I, and N). The postabdomen and antennae were the last body parts becoming pigmented (Fig. 7E, J, and O). It was also at this final stage of pupal development that wing venation and setae development occurred. Six days after pupation, the adult made a circular cut at the cephalic end of host integument using its mandibles, and then emerged, leaving behind the brown host integument, which could be easily differentiated from the white integument of unparasitized hosts (Fig. 6C).

Discussion

Flanders (1942) described two functional types of egg chorion for parasitic wasps—anhydropic and hydropic. Anhydropic eggs have enough yolk for subse-

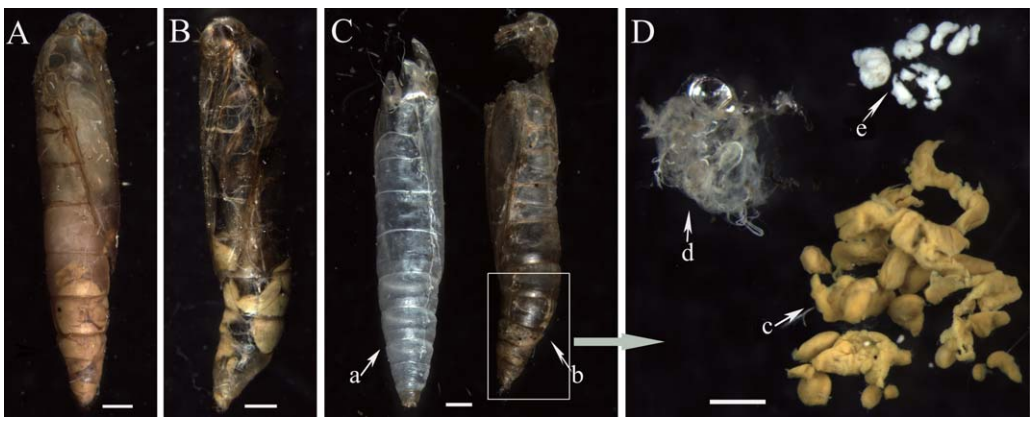

Fig. 6. Host pupae parasitized by *D. collaris.* (A) Host pupa 5 d after parasitism. (B) Host pupa 10 d after parasitism. (C) Exuvium of *P. xylostella* pupae. a, unparasitized; b, parasitized. (D) Remnants in posterior segments of host exuvium. c, meconium; d, ecdysis of fourth instar; e, white granules. Scale bars = 0.5 mm. (Online figure in color.)

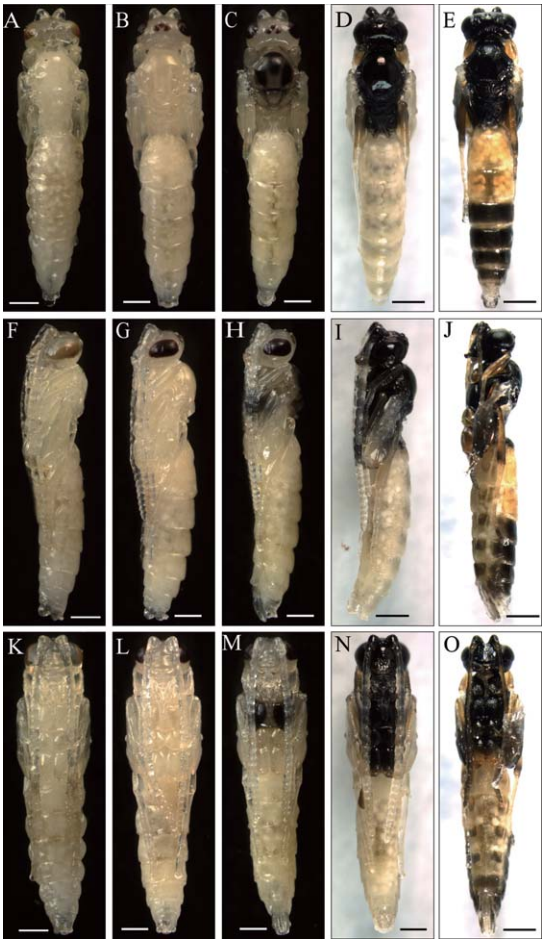

Fig. 7. *D. collaris* pupae in dorsal and lateral and ventral view. (A, F, K) Pupa at the first stage. (B, G, L) Pupa at the second stage. (C, H, M) Pupa at the third stage. (D, I, N) Pupa at the fourth stage. (E, J, O) Pupa at the fifth stage. Scale bars = 0.5 mm. (Online figure in color.)

quent embryonic development, whereas hydropic eggs do not and absorb nutrients from the host through the chorion (Ferkovich and Dillard 1986). Hydropic eggs occur widely in endoparasitic Hymenoptera. Once laid inside the host, hydropic eggs usually swell greatly over a period of hours to days (Quicke 1997). Two important larval endoparasitoids of *P. xylostella* both have hydropic eggs. *Cotesia vestalis* (Haliday) eggs doubled in length and increased almost sixfold in width after 48 h (Yu et al. 2008), whereas length measurements (micropyle end to nonmicropyle end) of *Diadegma semiclausum* (Hellén) egg, as ratios, increased from 1.13 ± 0.01 to 1.67 ± 0.03 after 1≈1.5 h (Huang et al. 2009). Our study revealed that no obvious increase or expansion of *D. collaris* eggs in length or width occurred during embryonic development, indicating that they are anhydropic. However, it should be further confirmed by examining the ultrastructure of egg chorion and determining if the egg consists of yolk before laid.

The ichneumonoid parasitoids usually have five larval instars (He et al. 1996). However, the number of instars is reduced in many groups (Quicke 1997). Hagen (1964) suggested that reduced instar number was nearly always associated with endoparasitism. Our study revealed that there were four larval instars in *D. collaris*, and all the exuvia of each instar were discovered by careful dissection.

Morphological characteristics of immature instars, especially the cephalic structures of the final instar, play an important complementary role in recognition, identification, and classification of Ichneumonidae (Short 1978, Gillespie and Finlayson 1983, Wahl 1993, Wahl and Gauld 1998). Given (1944) described the final instar and its head-capsule of *D. collaris*. Our study agreed basically with the previous description but provided more intuitive photographs of its all life history (Fig. 4F–H). These results will facilitate the measurement of the interaction between *D. collaris* and *P. xylostella*, which requires the recognition of its immature stage to different developmental stages.

D. collaris pupa was a typical exarate form and lacked a cocoon. Cuticle construction of parasitized host pupa serves as a protection for the vulnerable parasitoid pupa (Fig. 6A and B). It is consistent with the results of Given (1944). Similar phenomenon was reported in several other endoparasitoids (Gillespie and Finlayson 1983, Wahl 1986, Tormos et al. 1999). Interestingly, most of the parasitoid pupae without cocoons were discovered in species whose final larvae emerged from host pupae not larvae.

The successive pigmentation changes of *D. collaris* in body color are similar to those of *Chelonus inanitus* (L.) (Albert et al. 1994) and *D. semiclausum* (Huang et al. 2009). The white granules along the abdominal sides first appeared at fourth-instar larval stage and disappeared at pupal stage, which are believed to be urate cells of the fat body. The same observation was reported for *D. semiclausum*, *Chelonus blackburni* (Cameron), and *Peristenus digoneutis* (Loan) (Jackson et al. 1978, Carignan et al. 1995, Huang et al. 2009,). They were finally ejected at the posterior segments of host pupal exuvium (Fig. 6D).

Liu et al. (2001) reported that *D. collaris* took 11≈12 d to develop from oviposition to adult emergence at 25°C. Our results confirmed this conclusion. For pupal endoparasitoids, there are potential risks from putrefaction of unconsumed host tissue. Therefore, these endoparasitoids tend to develop rapidly or at least to complete their larval development quickly. This will reduce the effects of any decline in host quality that will occur naturally or as a result of infection by microorganisms (Quicke 1997). For two larval endoparasitoids, both *D. semiclausum* and *C. vestalis* spend ≈2 d on embryonic development, then take 5–6 d and 7–8 d, respectively, to become the final instar at 22 ± 2 and 25 ± 1°C (Yu et al. 2008, Huang et al. 2009). However, as a pupal endoparasitoid, *D. collaris* completed embryonic development in 1 d. Only 3 d later, *D. collaris* larva had consumed most of the host tissue and reached the last instar. The final 7–8 d were spent

on internal development from older fourth-instar larva to emergence. The above difference may reflect the different developmental tactics evolved between larval and pupal endoparasitoids.

Teratocytes, a special kind of cells derived from the embryonic membrane of parasitoids at egg hatching, play several important roles (trophic, immunosuppression, and secretory) in the parasitoid–host interaction (Falabella et al. 2009). Our results showed that no teratocytes were released when the first instar larva of *D. collaris* eclosed. Therefore, whether the products the *D. collaris* larva produces itself are essential for successful parasitism or how the parasitoid redirects host nutritional resources to coincide with key phases in offspring development need further study.

In conclusion, our study revealed the entire development of all immature stages of *D. collaris*, and thus provided a solid foundation for further studies on physiological interactions between *D. collaris* and *P. xylostella.*

Acknowledgments

Funding for this study was provided jointly by the 973 Program (2013 CB127600), and the State Key Program of National Natural Science Foundation of China (31230068).

References Cited

Albert, U., T. Wyler, R. Pfister–Wilhelm, A. Gruber, P. Stettler, P. Heiniger, E. Kurt, D. Schumperli, and B. Lanzrein. 1994. Polydnavirus of the parasitic wasp *Chelonus inanitus* (Braconidae) characterization, genome organization and time point of replication. J. Gen. Virol. 75: 3353–3363.

Ali, M. I., and M. A. Karim. 1995. Host range, abundance and natural enemies of diamondback moth in Bangladesh. Bangladesh J. Entomol. 5: 25–32.

Bai, S. F., D. Z. Cai, X. Li, and X. X. Chen. 2009. Parasitic castration of *Plutella xylostella* larvae induced by polydnaviruses and venom of *Cotesia vestalis* and *Diadegma semiclausum.* Arch. Insect Biochem. Physiol. 70: 30–43.

Bonvin, M., D. Kojic, F. Blank, M. Annaheim, I. Wehrle, S. Wyder, M. Kaeslin, and B. Lanzrein. 2004. Stage-dependent expression of *Chelonus inanitus* polydnavirus genes in the host and the parasitoid. J. Insect Physiol. 50: 1015–1026.

Carignan, S., G. Borivin, and R. K. Stewart. 1995. Developmental biology and morphology of *Peristenus digoneutis* Loan (Hymenoptera: Braconidae: Euphorinae). Biol. Control 5: 553–560.

Chauhan, U., O. P. Bhalla, and K. C. Sharma. 1997. Biology and seasonality of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) and its parasitoids on cabbage and cauliflower. Pest Manag. Hortic. Ecosyst. 3: 7–12.

Falabella, P., L. Riviello, P. Caccialupi, T. Rossodivita, M. T. Valente, M. L. De Stradis, A. Tranfaglia, P. Varricchio, S. Gigliotti, F. Graziani, et al. 2007. A gamma-glutamyl transpeptidase of *Aphidius ervi* venom induces apoptosis in the ovaries of host aphids. Insect Biochem. Mol. Biol. 37: 453–465.

Falabella, P., L. Riviello, M. L. De Stradis, C. Stigliano, P. Varricchio, A. Grimaldi, M. de Eguileor, F. Graziani, S. Gigliotti, and F. Pennacchio. 2009. Aphidius ervi teratocytes release an extracellular enolase. Insect Biochem. Mol. Biol. 39: 801–813.

Ferkovich, S. M., and C. R. Dillard. 1986. A study of uptake of radiolabeled host proteins and synthesis during development of eggs of the endoparasitoid, *Microplitis croceipes* (Cresson) (Braconidae). Insect Biochem. 16: 337–345.

Flanders, S. E. 1942. Oosorption and ovulation in relation to oviposition in the parasitic Hymenoptera. Ann. Entomol. Soc. Am. 35: 251–266.

Gillespie, D. R., and T. Finlayson. 1983. Classification of final-instar larvae of the ichneumoninae (Hymenoptera: Ichneumonidae). Mem. Entomol. Soc. Can. 115: 5–81.

Given, B. B. 1944. The anatomy of the final larval instar of *Diadromus* (*Thyraella*) *collaris* Grav. (Ichneumonidae), with notes on structural changes through the prepupal and pupal stages. Trans. R. Soc. N. Z. 74: 297–301.

Hagen, K. S. 1964. Developmental stages of parasites, pp. 168–246. *In* P. DeBach (eds.), Biological Control of Insect Pests and Weeds. Chapman & Hall, London, United Kingdom.

He, J. H., X. X. Chen, and Y. Ma. 1996. Economic insect fauna of China, Hymenoptera:Ichneumonidae, vol. 51. Science Press, Beijing, China.

Huang, F., M. Shi, X. X. Chen, G. Y. Ye, and J. H. He. 2009. External morphology and development of immature stages of *Diadegma semiclausum* (Hymenoptera: Ichneumonidae), an important endoparasitoid of *Plutella xylostella* (Lepidoptera: Plutellidae). Ann. Entomol. Soc. Am. 102: 532–538.

Jackson, C. G., J. S. Delph, and E. G. Neemann. 1978. Development, longevity and fecundity of *Chelonus blackburni* (Hymenoptera: Braconidae) as a parasite of *Pectinophara gossypiella* (Lep.: Gelechiidae). Biocontrol 23: 35–42.

Kfir, R. 1997. Parasitoids of *Plutella xylostella* (Lepidoptera: Plutellidae) in South Africa: an annotated list. Entomophaga 42: 517–523.

Li, W. D., F. Huang, Y. F. Chen, and X. X. Chen. 2006a. Immunosuppression effects of venom of pupal endoparasitoid wasp, *Diadromus collaris* (Gravenhorst) on its host, *Plutella xylostella* pupae. Acta Entomol. Sin. 49: 206–212.

Li, W. D., R. X. Yu, X. X. Chen, and J. H. He. 2006b. Venom gland of the ichneumonid *Diadromus collaris:* morphology, ultrastructure and age-related changes. Insect Sci. 13: 137–143.

Li, W. D., M. Shi, and X. X. Chen. 2007. Effects of parasitism by *Diadromus collaris* (Hymenoptera: Ichneumonidae) on morphology and ultrastructure of fat body and adipocytes of host *Plutella xylostella* (Lepidoptera: Plutellidae) pupae. Acta Entomol. Sin. 50: 662–666.

Liu, S. S., X. G. Wang, S. J. Guo, J. H. He, and Z. H. Shi. 2000. Seasonal abundance of the parasitoid complex associated with the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) in Hangzhou, China. Bull. Entomol. Res. 90: 221–231.

Liu, S. S., X. G. Wang, Z. H. Shi, and F. B. Gebremeskel. 2001. The biology of *Diadromus collaris* (Hymenoptera: Ichneumonidae), a pupal parasitoid of *Plutella xylostella* (Lepidoptera: Plutellidae), and its interactions with *Oomyzus sokolowskii* (Hymenoptera: Eulophidae). Bull. Entomol. Res. 91: 461–469.

Liu, S. S., F. B. Gebremeskel, and Z. H. Shi. 2002. Reproductive compatibility and variation in survival and sex ratio between two geographic populations of *Diadromus collaris,* a pupal parasitoid of the diamondback moth, *Plutella xylostella.* Biocontrol 47: 625–643.

Mustata, G. 1992. Role of parasitoid complex in limiting the population of diamondback moth in Moldavia, Romania, pp. 203–211. *In* N. S. Talekar (ed.), Diamondback Moth and Other Crucifer Pests. Proceedings of the Second International Workshop. AVRDC, Taiwan.

Pennacchio, F., and M. R. Strand. 2006. Evolution developmental strategies in parasitic Hymenoptera. Annu. Rev. Entomol. 51: 233–58.

Pfister–Wilhelm, R., and B. Lanzrein. 1996. Precocious induction of metamorphosis in *Spodoptera littoralis* (Noctuidae) by the parasitic wasp *Chelonus inanitus* (Braconidae): identification of the parasitoid larva as the key regulatory element and the host corpora allata as the main targets. Arch. Insect Biochem. Physiol. 32: 511–525.

Quicke, L.J.D. 1997. Parasitic wasps. Chapman & Hall, London, United Kingdom.

Short, J.R.T. 1978. The final larval instars of the Ichneumonidae. Mem. Am. Entomol. Inst. No. 25.

Tormos, J., J. D. Asis, and J. Selfa. 1999. Description of the final instar larva of *Perithous scurra* with comments on its morphological characters (Hymenoptera: Ichneumonidae, Pimplinae, Delomeristini). Fla. Entomol. 82: 333–339.

Talekar, N. S., and A. M. Shelton. 1993. Biology, ecology, and management of the diamondback moth. Annu. Rev. Entomol. 38: 275–301.

Wahl, D. B. 1986. Larval structures of oxytorines and their significance for the higher classification of some Ichneumonidae (Hymenoptera). Syst. Entomol. 11: 117–127.

Wahl, D. B. 1993. Cladistics of the Ichneumonid subfamily Labeninae (Hymenoptera: Ichneumonidae). Entomol. Gen. 18: 91–105.

Wahl, D. B., and I. D. Gauld. 1998. The cladistics and higher classification of the Pimpliformes (Hymenoptera: Ichneumonidae). Syst. Entomol. 23: 265–298.

Wakisaka, S., R. Tsukuda, and F. Nakasuji. 1992. Effects of natural enemies, rainfall, temperature and host plants on survival and reproduction of the diamondback moth, pp. 15–26. *In* N. S. Talekar (ed.), Diamondback Moth and Other Crucifer Pests. Proceedings of the Second International Workshop. AVRDC, Taiwan.

Wang, X. G., and S. S. Liu. 2002. Effects of host age on the performance of *Diadromus collaris*, a pupal parasitoid of *Plutella xylostella.* Biocontrol 47: 293–307.

Waterhouse, D. F., and K. R. Norris. 1987. Biological control: pacific prospects. Inkata Press, Melbourne, Australia.

Yu, R. X., M. Shi, F. Huang, and X. X. Chen. 2008. Immature development of *Cotesia vestalis* (Hymenoptera: Braconidae), an endoparasitoid of *Plutella xylostella* (Lepidoptera: Plutellidae). Ann. Entomol. Soc. Am. 101: 189–196.

Received 27 October 2012; accepted 12 July 2013.