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Morphology and development of *Pteromalus cerealellae* (Ashmead) (Hymenoptera: Pteromalidae) on *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae)

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Abstract *Pteromalus cerealellae* (Ashmead) (Hymenoptera: Pteromalidae) is an ectoparasitoid of several stored-product insect pests. Very little information has been published on its biology and development in host larvae, which typically are concealed within seeds. We documented the development of *P. cerealellae* within fourth instar larvae of its concealed host, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) infesting cowpea seeds. The preimaginal life stages of the parasitoid were characterized for the first time using morphological structures revealed by microscopic techniques including scanning electron microscopy. *Pteromalus cerealellae* produces hymenopteriform eggs and larvae. Eggs hatch into 13-segmented first instar larvae with peripneustic condition of spiracles. The larvae have simple, tusk-like mandibles, whereas the mandibles of the pupae and the adults are of the conventional toothed types. Using statistical analyses of the sizes of the larval mandibles and head capsules in conjunction with reliable characters such as the number of exuviae on the body of parasitoid larvae, cuticular folding, and excretion of the meconium, we recorded four larval instars for *P. cerealellae.* The data showed significant positive correlations between larval mandible lengths and widths of larval head capsules, as well as between mandible lengths and larval instars, suggesting that mandible length is a good predictor of the number of instars in *P. cerealellae*. Developmental time from egg to adult emergence was ~12 d for females and ~11 days for males at 30 ± 1°C, 70 ± 5% r.h. and 12L:12D photoperiod.

Keywords *Pteromalus cerealellae* · *Callosobruchus maculatus* · Head capsule · Mandibles · Meconium · Peripneustic · Scanning electron microscopy

Introduction

Pteromalus cerealellae (Ashmead) (Hymenoptera: Pteromalidae) is a solitary ectoparasitoid of larvae of various stored-product insect pests. Native to West Africa (Southgate

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1978), it has been reported from many parts of the world, including United States (Wen and Brower 1994). The known hosts of *P. cerealellae* include *Sitotroga cerealella* Olivier (Lepidoptera: Gelechidae) and several stored-product beetles including bruchids (e.g., *Callosobruchus maculatus* (F.)), anobiids (e.g., *Lasioderma serricorne* (Fab.)), bostrichids (e.g., *Prostephanus truncatus* (Horn)), and grain weevils (*Sitophilus* spp.) (Ashmead 1902; Brower 1991). However, the cowpea bruchid, *C. maculatus* was reported as the most susceptible stored-product beetle host of *P. cerealellae* (Brower 1991).

Most studies on *P. cerealellae* have focused on its potential utilization for biological control of pests of stored products (Smith et al. 1995; Wen and Brower 1994; Mbata et al. 2004; Onagbola et al. 2007). Little information is available on the developmental biology of *P. cerealellae* (although see Noble 1932; Fulton 1933). Developmental biology studies including morphological characterization of the preimaginal life stages can be important for the identification of an insect to the species level before adult emergence, which can simplify the quantification of the impact of natural enemies in biological control programs (Bellows and Van Driesche 1999). Little is known about larval morphology in pteromalid wasps (Grassberger and Frank 2003; Rojas-Gómez and Bonet 2003), and we are not aware of any published studies to date on the morphological characterization of the immature stages of *P. cerealellae*. Furthermore, the number of larval instars produced by this species has not been conclusively determined.

The number of larval instars produced by an insect species is commonly determined by measuring the widths of the head capsules of the larvae and plotting the frequency distribution of the measurements followed by statistical analyses (Dyar 1890; Odebiyi and Bokonon-Ganta 1986; Löhr et al. 1989; Llácer et al. 2005). However, Löhr et al. (1989) cautioned against sole reliance on head capsule size for determination of number of larval instars due to possible overlap of the range of head capsule sizes of different instars. Consequently, it may be necessary to use additional structural characters such as measurements of body length and width, mandible length, and reliable characters such as presence of exuvia for accurate determination of larval instars (Wright 1986; Löhr et al. 1989; Llácer et al. 2005). This present study was designed to characterize the developmental biology and morphology of the preimaginal stages of *P. cerealellae*. Several morphological parameters including measurements of width of the head capsule, mandible size, and body length were used in conjunction with reliable characters to determine the number of larval instars produced by this parasitoid.

Materials and methods

Insects

The host insect, *Callosobruchus maculatus* was reared in our laboratory on cowpea seeds, *Vigna unguiculata* Walp (California Black Eyed variety) in 1-l wide-mouthed Mason glass jars. A fresh culture was started every five days by placing ~25 pairs of 3-day-old mated *C. maculatus* in a glass jar containing ~100 g of cowpea seeds held at 30 ± 1°C, 70 ± 5% r.h., and 12L:12D (Mbata et al. 2005; Onagbola et al. 2007). The beetles were allowed to lay eggs on the seeds for 24 h after which they were removed with an aspirator. The infested seeds were incubated at the conditions specified above until the larvae had reached the fourth instar stage, which were then provided to *P. cerealellae* for parasitization. Fourth instar larvae of *C. maculatus* were used as hosts in this study because previous experiments showed that *P. cerealellae* develops better on this instar (unpublished data).

The original culture of *P. cerealellae* was obtained from Fort Valley State University, Fort Valley, GA, USA (contact: Dr. George Mbata) where it has been reared continuously on *C. maculatus* for several years. The parasitoid was maintained in our laboratory by transferring about 30 adult pairs into a glass jar containing *C. maculatus*-infested cowpea seeds at a stage when most of the bruchid larvae were at the fourth larval instar (this occurred at ~15 days after infestation of cowpea seeds under our rearing conditions). The jars were held at the environmental conditions stated above for *C. maculatus*. Adult *P. cerealellae* were removed from the jars after five days of oviposition. Parasitized host larvae were incubated in a growth chamber at the above environmental conditions until the emergence of adult parasitoids.

Morphology and characterization of the preimaginal stages of *P. cerealellae*

To study the development of immature stages of *P. cerealellae* within the host, ~80 fourth instar larvae of *C. maculatus* (which were concealed within cowpea seeds) were exposed to 20 freshly emerged (2 days old) mated female *P. cerealellae* in plastic Petri dish (6-cm diameter) for 6 h. In order to obtain adequate parasitized hosts for the experiment the above set-up was replicated six times (i.e., a total of 120 female *P. cerealellae* were used). Cowpea seeds containing exposed host larvae were transferred into a growth chamber at the above environmental conditions until dissection. For dissection, cowpea seeds were first carefully cracked open to expose the concealed larvae of *C. maculatus*, which were then grouped into parasitized (dead) or unparasitized (alive). Parasitized larvae were first frozen for ~4 h to arrest development and then dissected to excise the developing stages of the parasitoid. Dissection of parasitized larvae were made in 6-h intervals from 6 h to 48 h (after exposure to female parasitoids) and, thereafter, in 12 h intervals until adult parasitoids started to emerge (~11 days after exposure). Parasitized host larvae were dissected in a drop of water under a 20× stereomicroscope scouting for preimaginal stages (eggs, larvae, prepupae and pupae) of the parasitoid. Excised preimaginal stages were immediately transferred into glass vials containing 50% ethyl alcohol. At least 30 parasitized hosts were dissected for each time interval.

Measurements were made of the length and width of eggs and larvae and the width of the head capsule of the larvae to the nearest 0.02 mm under a stereomicroscope (National Microscope, Model DC 3–420, Meiji, Japan) equipped with an ocular micrometer. Separation of the larval instars was done by statistical analyses of the head capsule measurements (width) combined with the use of reliable characters such as the number of exuviae on the body of parasitoid larvae, cuticular folding, and excretion of the meconium. In addition, the maximum lengths of the mandibles (m-length) of larvae (measured from the points of articulation with the head to the pointed tip) belonging to the different instar groupings (as determined from the analyses of the width of the head capsules), were measured to the nearest 0.02 μm under a compound microscope (National Microscope, Model ML2000, Meiji, Japan) at 40× magnification. Micrographs of all stages were taken under the above stereo and compound microscopes fitted with a Nikon Coolpix 4500 camera. Developmental time for each stage was recorded.

Histological methods and scanning electron microscopy

Preimaginal stages of *P. cerealellae* were further processed using clearing techniques similar to those described by Hansson (1996) and Kondo and Williams (2005). Immature specimens were

transferred from alcohol vials into heated 10% KOH (300°F) for ~5 min. They were then transferred individually into distilled water for ~2 min to remove excess alkali and then into a previously stained heated Essig's fluid (200°F) for ~3 min, and finally into 75% ethyl alcohol for ~2 min to remove excess stain. The specimens were then dehydrated for ~5 min in 99.9% ethyl alcohol. Specimens were cleared in clove oil with spatulated probes leaving them in the oil for ~30 min to further fix the stains. They were then individually removed from the clove oil, placed centrally on clean glass slide and mounted in Canada balsam. All mounted specimens were dried in Model 4 Precision oven (Thelco, Chicago, USA) set at 60°C for 4 weeks and later observed under the compound microscope for morphological details.

In addition, eggs of *P. cerealellae* were further processed by scanning electron microscopy (SEM). Preparation for SEM was modified from Sukontason et al. (2003). Eggs were removed from alcohol vials after 24 h and subjected to progressive dehydration in graded ethyl alcohol series of 70, 80, 90, 95 and 100% for 4 h in each alcohol concentration. The dehydrated specimens were mounted on aluminum stubs with double-faced silver-based adhesive and subjected to critical-point drying (Baker 2001). Specimens were sputter coated with gold/platinum mix using Pelco SC-7 (EMS 550X/250) Sputter coater and observed under the DSM 940 SEM (Zeiss, W. Germany) at 10 kV and 15 mm working distance.

Statistical analyses

Data obtained on days 2–8 after oviposition were used in the analyses of larval morphological characters because under our experimental conditions, occurrence of larvae started 36–48 h after oviposition while the earliest pupae were recorded on day 7. Data obtained from measurements of the width of the head capsules of larvae obtained on days 2–8 (after oviposition) were pooled and first subjected to student's *t*-test to test for differences in head capsule sizes of developing male and female larvae (determination of sex of larvae was made when they were in the second instar stage), and then analyzed by using a two-way factorial analysis of variance (ANOVA) (SAS 2003) followed by Tukey's HSD test ($P < 0.05$) to separate the means of the larval head capsules.

The data obtained from measurements of the mandible length (m-length) of the second through the fourth instar larvae were first subjected to *t*-test analysis to determine significant difference between m-lengths of male and female larvae. For each instar, m-length measurements were obtained from 10 individuals of each sex. Since no significant effect of sex was recorded on the m-length data, the m-length data for all the larvae were pooled and analyzed with one way ANOVA followed by Tukey's HSD test ($P < 0.05$) to determine significant variations in the mandible lengths of larvae of various instars. Pooled data were further analyzed by testing for correlation between m-lengths and number of larval instars as determined using measurements of width of larval head capsules. Data were not transformed prior to analysis since the assumption of normality was generally met.

Results

Description of the preimaginal stages of *P. cerealellae*

The egg

The description of immature insects and classification methods used in this paper were based on the methods of Clausen (1940) and Hagen (1964). Fully developed eggs of

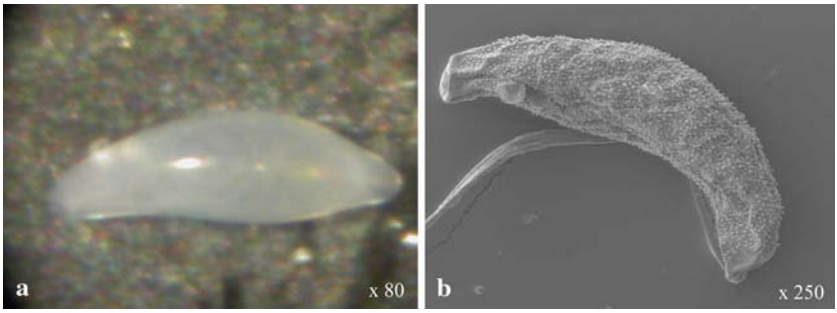

Fig. 1 Hymenopteriform eggs of *P. cerealellae* as observed at different magnifications. Egg with smooth chorion as seen under a stereomicroscope at 80× (**a**); Stalked and sculptured egg with warty tubercles on the surface of the chorion as observed under SEM at 250× (**b**)

P. cerealellae are typically hymenopteriform (i.e., they have pointed anterior and posterior tips with enlarged posterio-medial portion) in shape with thin transparent chorion (Fig. 1a). The chorion appears smooth when observed under the stereomicroscope at 80× but SEM revealed the chorion to be sculptured and of the stalked type with warty tubercles (Fig. 1b). The egg is approximately three times as long as wide, almost round at one end and narrow at the opposite end. The mean (±SE) length and width (at the widest part) of the egg are 0.54 ± 0.002 mm and 0.19 ± 0.002 mm, respectively (n = 15). A single egg is most often laid by female *P. cerealellae* per host, although a female may occasionally deposit more than one egg in a single host. The egg is nearly transparent when newly deposited (0–6 h) turning opaque-whitish 24–36 h after oviposition. The egg is not encapsulated by the host. Prior to hatching, the eggs become round and increased in size.

First instar

Most of the eggs hatched between 36 and 48 h after oviposition (parasitization) at 30 ± 1°C, 70 ± 5% r.h. and 12L:12D photoperiod, although viable eggs could still be detected four days after oviposition (Table 1). The neonate endophytic larva usually moves onto the body surface of the host to complete its development. The first instar has smooth, transparent cuticle that exposes its developing (whitish) gut systems (Fig. 2a). Shortly after hatching, the first instar measured 0.67 ± 0.02 mm in length and 0.44 ± 0.03 mm in width (Table 1) with the width of the head capsule ranging between 0.30 ± 0.02 mm and 0.32 ± 0.01 mm (Table 1). The head capsule of the larva is almost indistinct from the body when viewed at 20× magnification. The larva gradually turns yellowish as it feeds on the tissues of the dead host. The sex of the parasitoid could not be easily determined at this stage, which lasts ∼2 days. Observation of cleared first instar larvae under the compound microscope revealed that the body is made up of 13 segments with peripneustic condition (9 pairs consisting of 1 pair of mesothoracic and 8 pairs of abdominal) of spiracles. The crania of the larvae (Fig. 3a) are well developed, with antennal lobes and chitinized, tusk-like mandibles (M, Fig. 3b).

Second instar

The second instar resembles very much the previous instar having smooth cuticle but with a distinct head capsule (Fig. 2b), and occurs 2–6 days after oviposition (Table 2). The key

Table 1 Measurements (mean ± SE; in mm) of the body sizes of the immature stages of *P. cerealellae* at specified intervals after parasitization

Age (days) after parasitization	First instar			Second instar			Third instar			Prepupa			Pupa		
	N	Length (mean ± SE)	Width (mean ± SE)	*N*	Length (mean ± SE)	Width (mean ± SE)	*N*	Length (mean ± SE)	Width (mean ± SE)	*N*	Length (mean ± SE)	Width (mean ± SE)	*N*	Length (mean ± SE)	Width (mean ± SE)
2	7	0.67 ± 0.02	0.44 ± 0.03	1	0.71 ± 0.00	0.42 ± 0.00									
3A	7	1.10 ± 0.15	0.57 ± 0.04	1	1.61 ± 0.00	0.68 ± 0.00									
3B	2	1.20 ± 0.27	0.66 ± 0.02	3	1.82 ± 0.03	0.71 ± 0.00									
4A	2	1.20 ± 0.02	0.54 ± 0.00	11	2.25 ± 0.18	0.82 ± 0.04									
4B	1	1.22 ± 0.00	0.54 ± 0.00	8	2.44 ± 0.15	0.87 ± 0.04	3	3.05 ± 0.06	0.99 ± 0.05						
5A				11	2.17 ± 0.08	0.90 ± 0.02	11	2.81 ± 0.09	1.00 ± 0.03						
5B				5	2.22 ± 0.14	0.96 ± 0.05	6	3.05 ± 0.17	1.14 ± 0.04						
6A				6	2.14 ± 0.02	0.98 ± 0.02	9	2.99 ± 0.14	1.11 ± 0.02						
6B							9	2.90 ± 0.10	1.14 ± 0.06	3	2.84 ± 0.34	1.26 ± 0.04			
7A							5	2.52 ± 0.12	1.06 ± 0.04	1	2.54 ± 0.00	1.12 ± 0.00	8	2.58 ± 0.10	1.10 ± 0.05
7B							4	2.52 ± 0.23	1.05 ± 0.09				6	2.29 ± 0.13	0.92 ± 0.07
8A										2	2.27 ± 0.37	1.09 ± 0.06	10	2.53 ± 0.07	0.97 ± 0.03
8B							3	2.51 ± 0.02	0.95 ± 0.04	1	1.93 ± 0.00	0.81 ± 0.00	10	2.41 ± 0.10	0.94 ± 0.02
9A										1	2.44 ± 0.00	0.93 ± 0.00	13	2.39 ± 0.07	0.92 ± 0.02
9B										2	2.78 ± 0.01	1.04 ± 0.04	9	2.33 ± 0.08	0.88 ± 0.02
10A							1	1.88 ± 0.00	0.88 ± 0.00				11	2.65 ± 0.10	0.96 ± 0.02
10B							2	2.09 ± 0.04	0.87 ± 0.04				8	2.52 ± 0.08	0.91 ± 0.03

"A" indicates the first 12 h and "B" the last 12 h of each day

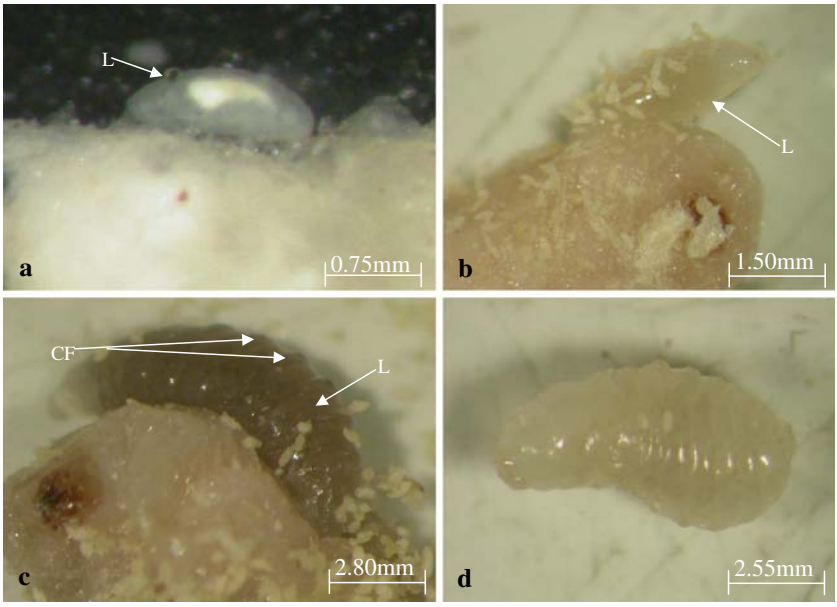

Fig 2 Larvae of *P. cerealellae* as observed under a stereomicroscope at 20×. Neonate, first instar (L) on *C. maculatus* host (**a**). Figure shows larva with smooth and transparent cuticle revealing its whitish gut systems; Second instar (L) on the host (**b**). Figure shows a larva feeding on the necrotic host with its head firmly attached to the body of the host; Third instar (L) actively feeding on the necrotic host (**c**). Figure shows a third instar with folded cuticle (CF) on the host; Prepupa (fourth instar) (**d**)

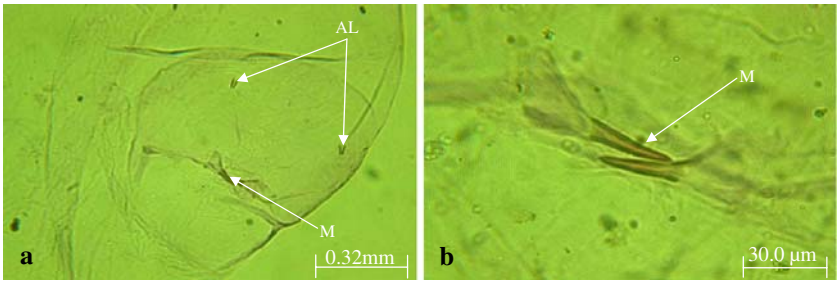

Fig 3 Photomicrographs of the head capsule of a cleared larval specimen of *P. cerealellae* as observed under a compound microscope. Figure shows the short antennal lobes (AL) and the simple, tusk-like mandibles (M) at 40× (**a**); The magnified tusk-like larval mandibles (M) at 100× (**b**)

characters which separate this instar from the previous instar include the distinct head capsule, yellowish-brown gut system, size, and presence of the exuviae of the first instar on its body. An early second instar (n = 1) (Table 1) was excised ~72 h after oviposition. The sex of the developing parasitoid can be determined at this stage using the tip of the abdomen, which is nearly pointed in females and blunt in males. The mean width of the head capsule is ~0.45 ± 0.03 and 0.59 ± 0.01 mm for males and females, respectively. The larva is robust and brownish possibly due to feeding on the necrotic host larva. The head of the larva remained sunk in the body of the host (probably because the mandibles are buried in host tissue) while other parts of the body are swayed around as it feeds on the

Table 2 Body sizes of male and female *P. cerealellae* larvae of different instars as categorized using Tukey HSD analysis of the widths of head capsules of larvae

Sex	Age after oviposition	BL	BW	HC	Instar grouping
Male	2	0.68 ± 0.02d	0.47 ± 0.01c	0.30 ± 0.02d (a)	1
	3	1.28 ± 0.17cd	0.59 ± 0.05c	0.32 ± 0.02d (a)	1
	4	1.58 ± 0.09c	0.66 ± 0.03c	0.45 ± 0.03c (b)	2
	5	2.09 ± 0.04b	0.90 ± 0.02b	0.59 ± 0.02b (b)	3
	6	2.42 ± 0.09a	1.02 ± 0.03a	0.68 ± 0.02a (b)	4
	7	2.27 ± 0.08ab	0.96 ± 0.03ab	0.67 ± 0.01ab (a)	3, 4
	8	2.40 ± 0.13ab	1.03 ± 0.07ab	0.80 ± 0.01a (a)	4
Female	2	0.67 ± 0.03d	0.41 ± 0.04e	0.32 ± 0.01d (a)	1
	3	1.43 ± 0.16c	0.65 ± 0.03d	0.35 ± 0.02d (a)	1
	4	2.75 ± 0.07b	0.93 ± 0.02c	0.59 ± 0.01c (a)	2
	5	2.89 ± 0.07ab	1.05 ± 0.02b	0.68 ± 0.01b (a)	3
	6	3.17 ± 0.06a	1.21 ± 0.03a	0.74 ± 0.01a (a)	4
	7	2.78 ± 0.10ab	1.16 ± 0.03ab	0.69 ± 0.03ab (a)	3, 4
	8	2.67 ± 0.11ab	1.00 ± 0.04bc	0.81 ± 0.00a (a)	4

BL = body length; BW = body width; HC = width of the head capsules. Means (±SE) within each column for the same sex followed by the same letters are not significantly different ($P < 0.05$). Similarly, for each column letters in parenthesis indicate significant difference between male and female larvae of the same instar (Student's *t*-test, $P < 0.05$)

host. The tracheal systems (tubes) are visible through the transparent cuticle. The cleared specimen of this instar was not morphologically different from the first instar. However, the mandibles appeared to be more chitinized in the second instar than in the first.

Third instar

The hymenopteriform third instar is present from days 5–11 after oviposition (Table 1). It is far more tracheated than the preceding instars and has cuticular folds (CF, Fig. 2c) showing that the cuticle of the third instar larvae is also transparent. Under the stereomicroscope, the larva appears brownish possibly due to meconium (the midgut waste materials). The parasitoid spends nearly 50% of the total larval developmental period at this stage. The structures on the head capsules are more visible in the third instar when observed at 20× magnification. The imaginal eyes develop late at this stage. The larva stops feeding and excretes the guts contents, the meconium to form the prepupae (Fig. 2d). As with the preceding instars, the mandibles of the third instar are simple, tusk-like and with no serrations on its blade (Fig. 3b).

Fourth instar (the prepupa)

Soon after the third instar begins to expel its meconium, its length shortens to form the prepupa, the fourth instar larva (Fig. 2d). Excretion of the meconium starts after the fifth day post oviposition and some third instar larvae had turned into prepupae by the end of the sixth day. The prepupal stage is characterized by narrowed thoracic region, widened

abdominal region and presence of the imaginal eyes (Fig. 2d). The caudad end of the prepupa is also rounded. The prepupal period is short and lasts between 12 and 24 h at 30°C. The prepupa has clear midgut. Formation of pupa begins towards the end of day 7 after oviposition.

The pupa

Pteromalus cerealellae, like other Hymenoptera, produces exarate pupae with clearly visible mouthparts, antennae and legs. Pupation occurs on the necrotic host larva and the pupa is not protected by any special cocoon. The newly formed pupa is whitish with no pigmentation. Within ~12 h of pupation, the pupal cuticle gets tanned and turns yellowish-brown. Eyes of the pupa become pigmented after ~24 h. The head and the dorsum of the thorax darken (become blackish) shortly thereafter. No morphological difference was observed between the different stages of pupal development. Emergence of the adult *P. cerealellae* occurs ~5 days after the start of pupation with males emerging ~24 h earlier than females.

Total developmental time

Eggs of *P. cerealellae* hatch into neonate first instar larvae within 2 days at the environmental conditions stated above. Total larval developmental period is between 5–9 d at 30°C. Male and female individuals spend ~4 and 5 days, respectively as pupae before eclosion. Thus, complete development of male *P. cerealellae* takes ~11 days with the females emerging ~1 day later.

Characterization and separation of larval instars

Two-way factorial ANOVA revealed significant effects of age (time period between oviposition and extraction of larva from the host; $F = 74.05$, $df = 6$, $P < 0.0001$) and sex ($F = 3.94$, $df = 1$, $P < 0.0493$) on the width of the head capsules of excised *P. cerealellae* larvae (Table 2). Further analysis using Tukey's HSD tests separated all excised larvae into four groups (instars). The width (mean ± SE) of the head capsules of the first instar ranged between 0.30 ± 0.02 and 0.35 ± 0.02 mm (Table 2). Student's *t*-test revealed that the width of the head capsules of female larvae were significantly greater than the head capsule sizes of counterpart male larvae for second (male: 0.45 ± 0.03; female: 0.59 ± 0.01; $t = 2.06$, $P < 0.0001$) and third instars (male: 0.59 ± 0.02; female: 0.68 ± 0.01; $t = 2.04$, $P = 0.0002$). However, no significant difference was detected between the head capsule sizes of male and female fourth instar larvae (male: 0.80 ± 0.01; female: 0.81 ± 0.00) (Table 2). This result suggests that both sexes of *P. cerealellae* develop through the same number of instars.

Two distinct types of mandibles were observed in *P. cerealellae*. The mandibles of the first through the fourth instar larvae are simple and tusk-like (M, Fig. 3a, b) while those of the pupae and adults are of the conventional toothed types. Student's *t*-test analysis of the mandible size (m-length) showed no significant difference between the sexes in the first ($F = 0.22$, $df = 1$, $P = 0.64$), second ($F = 0.55$, $df = 1$, $P = 0.47$) third ($F = 2.0$, $df = 1$, $P = 0.17$) and fourth ($F = 0.07$, $df = 1$, $P = 0.79$) larval stages (instars). However, the

m-length of the different instars were significantly different ($F = 232.8$, $df = 3$, $P < 0.0001$). The mean (±SE) m-lengths of first, second, third, and fourth instar larvae were 26.28 ± 0.24, 28.96 ± 0.75, 35.02 ± 0.18 and 42.16 ± 0.45 µm, respectively. Analysis of the pooled m-length data using Tukey's HSD test resulted in four larval groupings (Fig. 4), further confirming that *P. cerealellae* undergo four larval instars. Significant positive correlations were recorded between m-lengths and instar groupings (Fig. 5), as well as between m-lengths and widths of the head capsules (Fig. 6a, b), suggesting that larval mandible length is a good predictor of the number of instars in *P. cerealellae*.

Discussion

The morphology and biology of the family Pteromalidae is poorly known making it difficult to compare our results with those reported for other members of the family. However, many of the characteristics observed in the preimaginal stages of *P. cerealellae* are common among the Chalcidoidea super family. Fully developed eggs of *P. cerealellae* are hymenopteriform, which is typical of chalcidoids (Clausen 1940; Llácer et al. 2005; Kazimirova and Vallo 1999). The eggs appeared to have smooth and transparent chorion when observed under stereomicroscope. However, detailed observation of the eggs using SEM revealed the chorion to have warty tubercles. The presence of warty tubercles on the egg chorion has not been commonly observed in chalcidoids and may be a distinctive

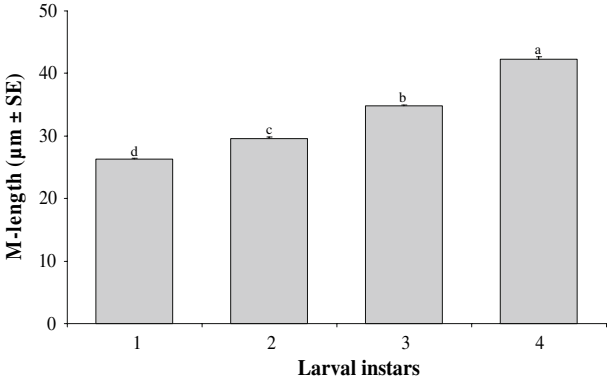

Fig 4 Significant differences in the mandible lengths (m-length) of the four larval instars of *P. cerealellae*. Figure shows mean (µm ± SE) m-length. Means having different letters are significantly different (Tukey's HSD test, $P < 0.05$)

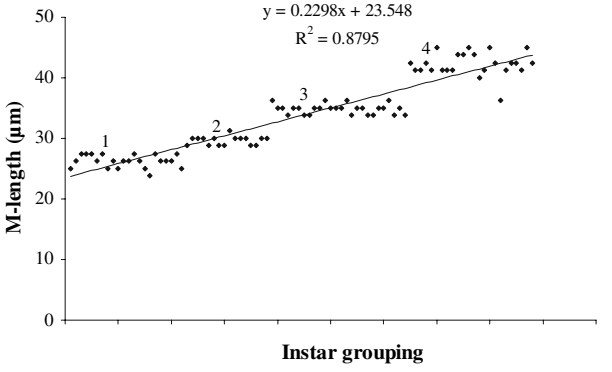

Fig 5 Significant positive correlation between mandible lengths (m-length) and instar groupings of *P. cerealellae* larvae. Numbers 1 to 4 indicate the four larval groupings

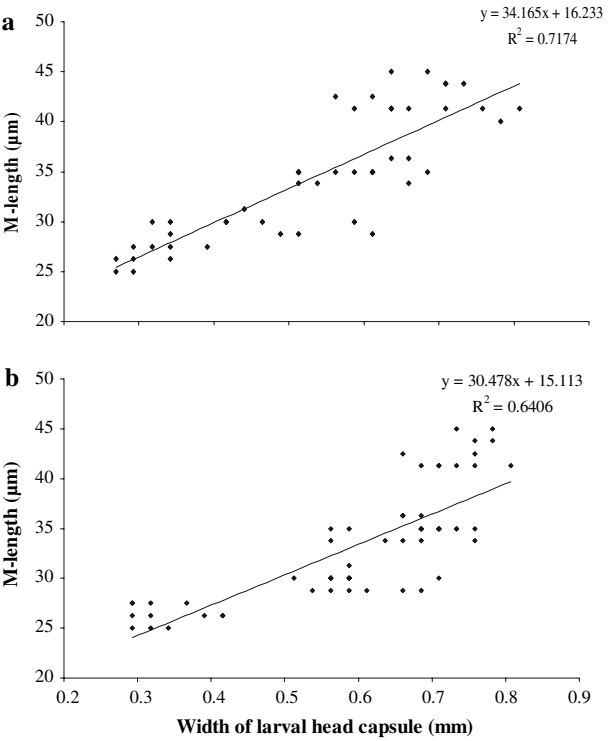

Fig 6 Significant positive correlation between mandible lengths (m-length) and head capsules sizes (widths) of *P. cerealellae* larvae: Male (**a**); Female (**b**)

feature of *P. cerealellae* and other pteromalids. The function of the warty tubercles is unclear, but may serve to aid movement of the egg during oviposition (Austin 1985) or to protect the chorion.

Female *P. cerealellae* typically lays a single egg per host, which can be deposited in any body region of the host. Upon hatching, the first instar larvae moved onto the surface of the host to complete its development, as reported for some other ectoparasitoids such as *Galeopsomyia fausta* LaSalle (Hymenoptera: Eulophidae) (Llácer et al. 2005). Four larval instars were observed in *P. cerealellae*. All instars are typically hymenopteriform with 13-segmented body and peripneustic conditions of spiracles, and have simple tusk-like mandibles similar to those described for *Spilomicrus hemipterus* Marshall (Hymenoptera: Diapriidae) (Hoffmeister 1989) and *Coptera occidentalis* Mues (Hymenoptera: Diapriidae) (Kazimirova and Vallo 1999).

Internal parasitoids typically undergo significant morphological changes as they develop through various instars, which could range considerably from 2 to 5 instars (Clausen 1940). For example, five larval instars were reported for *Encyrtus saliens* Prinsloo and Annecke (Hymenoptera: Encyrtidae) (Wright 1986) and *Epidinocarsis lopezi* (DeSantis) (Hymenoptera: Encyrtidae) (Odebiyi and Bokonon-Ganta 1986), four reported for *Encarsia formosa* (Hymenoptera: Aphelinidae) (Hu et al. 2002), and three for *C. occidentalis* (Kazimirova and Vallo 1999). The first instar is usually regarded as the most distinctive immature stage of parasitoids with diverse structures (Hagen 1964). However, no distinctive diverse structures were observed on the first instar of *P. cerealellae*. Separation of this instar from the second instar was based on the presence of a distinct head capsule in the second instar and the presence of the exuviae of the first

instar on the body of the second instar. Wright (1986) utilized the presence of distinct head capsule to discriminate the second from the first instars of *E. saliens*, while the presence of the exuviae was used by Löhr et al. (1989) to separate the second instar of *E. lopezi* from the first instar. The third instar larvae appeared approximately five days after oviposition and are highly tracheated with folded cuticle. Late third instar larvae excrete meconium to transform into prepupae (fourth instar). The prepupae of *P. cerealellae* have narrowed thoracic region, widened abdominal region and presence of imaginal eyes, typical of parasitoid prepupae (e.g., Kazimírova and Vallo 1999; Wilk and Kitayama 1981). No morphological difference was observed in the head capsule (crania) of the first, second and early third instars of *P. cerealellae*. However, bulging imaginal eyes appeared in the late third instar, as reported for *C. occidentalis* (Kazimírova and Vallo 1999). In addition, the levels of chitinization of the tusk-like mandibles increased in progressive instars.

Measurements of the widths of larval head capsules have been used by many authors to characterize larval instars in parasitoids (Dyar 1890; Odebiyi and Bokonon-Ganta 1986; Wen et al. 1995). In the current study, we observed considerable overlap in the range of the widths of the head capsules of third and fourth instars of *P. cerealellae*, as reported also for *E. lopezi* (Löhr et al. 1989). Due to this overlap, this character could not be used alone to separate the last two larval instars of *P. cerealellae*. Additional characters such as the length of the mandibles (m-length) (Tschinkel et al. 2003) and reliable non-structural characters were necessary to determine the number of larval instars in this parasitoid. Thus, our results suggest that the use of the width of head capsule alone to determine number of larval instars in parasitoids is unreliable and may explain some contrasting results reported by different authors for the same species. For instance, Wright (1986) reported that members of the parasitoid genus *Encyrtus* develop through five instar stages, whereas Löhr et al. (1989) reported only four instars for *E. lopezi.* Our results showing that the mandible lengths correlated positively not only to head capsule sizes but also to instar groupings suggest that mandible length, which has been traditionally used in morphometric studies (Kawano 2000; Manzoor and Akhtar 2006) may also be a reliable character for determining the number of instars in parasitoids.

Pupae of *P. cerealellae* are exarate and are not protected by any cocoon. Exarate pupae have also been reported in several other chalcidoids (Wright 1986; Llácer et al. 2005). The pupal stage of *P. cerealellae* has many of the features that characterize the imago, as reported for some other parasitoids (Schauff et al. 1998; Llácer et al. 2005). The mandibles of the pupae and adults are of the conventional toothed types, compared to the tusk-like type observed in the larvae. At the conditions of this study, life cycle was completed in about 11 days in males and 12 days in females. Male parasitoids are known to emerge earlier than conspecific females, probably to ensure sexual maturity upon female emergence (Thornhill and Alcock 1983; Doyon and Boivin 2006). In summary, our results showed that *P. cerealellae* undergoes four larval instars. It is hoped that this study will provide much needed information on the morphology and biology of immature stages of pteromalid wasps.

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