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Morphology and Development of a Termite Endoparasitoid *Misotermes mindeni* (Diptera: Phoridae)

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ABSTRACT The morphology and developmental stages of *Misotermes mindeni* Disney & Neoh (Diptera: Phoridae), a newly described endoparasitoid of the fungus-growing termite *Macrotermes gilvus* (Hagen) (Termitidae: Macrotermitinae) were studied, and biometric descriptors of eggs, larvae, and pupae were recorded. The larvae of *M. mindeni* exhibit three larval stages. Larvae complete their first instar development in fourth larval instars and major presoldiers, whereas the second and third instars develop entirely in major soldiers' head capsule and abdomen, respectively. The second instar can be readily differentiated from the first by the presence of a posterior spiracular base and more defined body segments, and the third instar can be discriminated from the second by the presence of respiratory horns and a well-developed posterior spiracular base. Differentiation of the larval instars is further supported by morphometric measurements. The first instar moves freely within the host's body cavity and head capsule, whereas the last two instars remain in the host's head capsule and abdomen, respectively. Termite developmental stages were used as a model to determine the developmental time of *M. mindeni* larvae. Duration between the first and second instars was 19.00 ± 2.28 d and between second and third instars was 36.88 ± 5.17 d. It took the third instar, 0.53 ± 0.08 d to reach pupation. The pupal stage lasted for an average of 13.51 ± 0.74 d. Mean adult longevity was 1.47 ± 0.57 d and 3.00 ± 0.98 d for females and males, respectively. Longevity of males was significantly longer than that of females.

KEY WORDS dipteran, larval parasitoid, immature stages, longevity

The most primitive larval feeding habit of phorids was either fungivory or saprophagy (Disney 1994). Many termitophilous phorids have been reported to have fungivorous larvae. For example, Disney and Kistner (1989) found larvae of *Dohrniphora diminuens* Schmitz feeding on termite fungus comb. Saprophages, however, essentially feed on excrement or dead or decaying organic material (Askew 1971, Disney 1994). Well-documented polyphagous saprophage species include *Megaselia scalaris* (Loew), *Megaselia giraudii* (Egger), and *Megaselia rufipes* (F.) (Disney 1994). *M. scalaris* also has been reported to be a facultative parasite of humans due to its habit of feeding on the host's necrotic or living tissues, which can cause myiasis (Disney 2008). To date, numerous species from the family Phoridae have been reported to have specialized predatory or parasitoid larvae that usually are associated with termites, ants, bees, millipedes, and molluscs (Askew 1971, Disney and Kistner 1997). Two principal mechanisms that may cause the evolution from fungivore or saprophage to predator or parasitoid are interspecific competition of the larval phorids and specific olfactory cue attraction emanating from intact or wounded, but still-alive, organisms (Disney 1994).

Misotermes mindeni Disney & Neoh (Diptera: Phoridae) is a solitary endoparasitoid of the termite *Macrotermes gilvus* (Hagen) (Termitidae: Macrotermitinae). Disney et al. (2009) first described it from the soldier caste of *M. gilvus* from Malaysia. According to Neoh and Lee (2010), the fly larva develops entirely in the soldier's head capsule and then moves to the termite abdomen when the fly is about to pupate. To create a dry microenvironment for pupation, the fly larva uses its spiracles to apply sideways pressure to the soldier's abdomen, causing the body fluid to ooze out of the abdomen. The host dies of trauma or dehydration after pupation occurs.

Knowledge about the life history of *M. mindeni* and its interaction with the host during larval development are crucial as they may prove useful for mass culturing and for assessing their mechanism of parasitism. This study was designed to provide basic information about the development of *M. mindeni* in its presumptive host as well as morphological descriptions of various life stages of *M. mindeni*.

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Materials and Methods

Specimen Collection and Maintenance. Parasitized termites were collected from 15 *M. gilvus* colonies at the Minden Campus of Universiti Sains Malaysia, Penang, Malaysia (5° 21′ N, 100° 18′ E). Identification of the parasitized termites was based on Neoh and Lee (2010), in which both parasitized major soldiers and presoldiers possessed a rounded head capsule with remarkably short mandibles. Parasitized fourth larval instars (L4s) could be readily recognized by the presence of a brown dot on the head capsule, thorax, or abdomen. To collect parasitized L4, major soldiers, and presoldiers, previously surveyed infected *M. gilvus* mounds (unpublished data) were excavated by digging a trench around the base of the nest. Sideways pressure was applied and the entire mound casing was removed cautiously to prevent injury to parasitized termites. Parasitized termites often were found in an isolated concealed chamber at the inner part of the outermost layer of the mound (Neoh and Lee 2010). Termites were collected and maintained in a plastic container (18.0 by 12.0 by 6.0 cm) containing moistened vermiculate in an incubator (Incucell, MMM Medcenter Einrichtungen GmbH, München, Germany) at constant temperature (28 ± 1°C) and 90% RH in complete darkness. Fungus comb and major and minor workers were kept along with the parasitized termites to ensure that the latter were fed.

Morphological and Morphometric Studies of Immature Stages of *M. mindeni.* *Eggs.* After pupation, 34 male–female pairs were placed into individual plastic petri dish (5.5 cm in diameter and 1.5 cm in depth). They were then provided with a 10% sucrose solution. Oviposition began during the first day after eclosion, and numbers of eggs laid per female (n = 24) were counted every 24 h until the flies died. The eggs were observed under an SZ61 stereomicroscope (Olympus, Tokyo, Japan) equipped with IC Imaging Standard V2.1 (The Imaging Source Europe, Bremen, Germany), and length and width of 70 eggs per mating pair were measured using Analysis Image Processing software (Soft Imaging System GmbH, Münster, Germany).

Larvae. Parasitized major soldiers (n = 43), major presoldiers (n = 25), and L4s (n = 25) were dissected in a saline solution, and we inspected all *M. mindeni* larvae found in these termites. The periphery of the host's head capsule was incised carefully to minimize injuries to larva, and then the entire casing was removed. The larva was removed from the head capsule by using fine forceps. The lateral sides of the abdomen were dissected cautiously, and the larva then was forced out of abdomen using light pressure. Only a single larval parasitoid was found inside each host. Measurements were made immediately after dissection. The morphological characteristics of the *M. mindeni* larvae obtained from different stages of parasitized termites were observed under a stereomicroscope, and the following measurements were taken (Fig. 1): 1) body length (L); 2) body width (W); 3) width of posterior spiracles (P); 4) length of mandible (ML); and 5) width of mandible (MW). The larval instar stages were differentiated based on morphological characteristics and these morphometric measurements.

Pupae. In total, 85 pupae that were produced from laboratory-reared parasitized termites were measured for length (from the tip to the distal part of the pupa) and width (measured across the widest part of the pupa) under a stereomicroscope.

Development of *M. mindeni.* *Larvae.* Because laboratory culture of *M. mindeni* larvae is nearly impossible, we used termite development stages as a model to determine the amount of time required for *M. mindeni* larvae to develop. We recorded the duration of *M. mindeni* between the first and second instars based on the number of days required for the parasitized host to develop from L4 to major soldier, whereas the duration between the second and third instars was determined when the larva was found in the major soldier's head until it moved to the major soldier's abdomen. The presence of third instar in the major soldier's abdomen was indicated by the abdomen enlargement and sluggish movement of the major soldier. The developmental time between third instar and pupation was observed hourly and recorded once the pupa formed.

Pupae. Parasitized termites were checked daily until pupae appeared in the abdomens of dead major soldiers. The pupae were removed and confined individually in vials (2.5 cm in diameter and 5.0 cm in length), the mouths of which were capped with screen mesh to allow ventilation and to prevent escape of the flies upon emergence. To provide humidity during pupation, moistened soil was placed in the bottom of the vials and they were kept in the incubator (see above). Pupal development was observed until adult eclosion occurred.

Adult Flies. To measure adult longevity, two flies of the same sex (n = 32 flies for each sex) were placed in a plastic petri dish and provided with a 10% sucrose solution. All replicates were maintained in the same incubator at 28 ± 1°C and 90% RH. The flies were checked daily for mortality.

Statistical Analysis. We used discriminant analysis to classify individual larvae into groups based on measured body parts. Student's t-test was used to compare longevity between males and females. All analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL).

Results and Discussion

Morphology and Morphometric Studies of Immature Stages of *M. mindeni.* *Eggs.* Newly deposited *M. mindeni* eggs were white and elongated but rounded at one end, with the other end tapering to a sharp point (Fig. 2). The eggs were small, at 0.44 ± 0.02 mm (n = 70; range, 0.39–0.47 mm) in length and 0.12 ± 0.01 mm (n = 70; range, 0.10–0.15 mm) in width (Table 1). Minute eggs are advantageous as they can be inad-

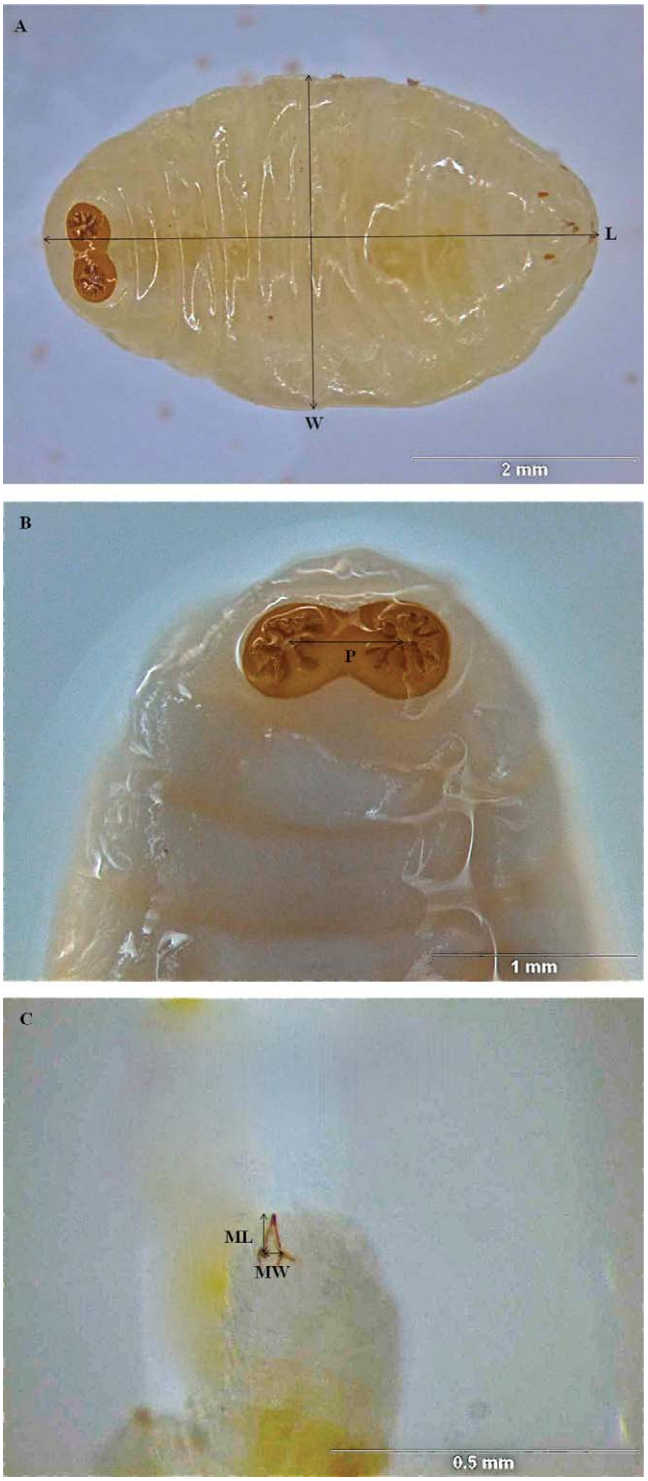

Fig. 1. Diagram of measured parts of *M. mindeni* larva. (A) L, body length and W, body width. (B) P, width of posterior spiracles. (C) ML, length of mandible and MW, width of mandible. (Online figure in color.)

Fig. 2. Egg of *M. mindeni*.

vertently ingested by the host (Stireman et al. 2006). Female *M. mindeni* laid large numbers of eggs (mean, 271 ± 157 eggs per female; range, 64–794 eggs per female), which suggests that they may use an indirect oviposition strategy, i.e., they lay eggs away from the host (Stireman et al. 2006). In fact, the majority of the female dipteran parasitoids never oviposit directly on the host (Feener and Brown 1997). Stireman et al. (2006) reported that females of the tribes Goniini, Blondeliini, Tachinini, Dexiinin, and Polideini, which lay 500–8,000 eggs, are indirect egg layers. Depositing a large numbers of eggs provides a greater chance that some will encounter the target hosts (Stireman et al. 2006). Furthermore, host defense, which might create a barrier to oviposition, encourages the use of indirect oviposition strategies (Stireman et al. 2006). *M. mindeni* were observed being attacked by the termites once the flies encountered them under laboratory conditions (unpublished data).

Larvae. Table 2 provides measurements of each of the larval characteristics and comparisons of the morphological characteristics among the different larval instars. We identified three larval developmental stages. All instars were whitish and had a translucent integument. The first instar (Fig. 3A) exhibited an elongated subcylindrical body with indistinct segmentation. The second instar (Fig. 3B) had oval-shaped body and could be distinguished readily from the first instar by the presence of a posterior spiracular base and more defined body segments. The third instar (Fig. 3C) resembled the second instar, but it had respiratory horns and a well-developed posterior spiracular base.

In addition to observing morphological traits, morphometric measurement could be a tool for distinguishing among the larval instars (Thyssen and Linhares 2007). When we used discriminant analysis to evaluate the morphometric measurements, we found that the first discriminant variable (Fig. 4) explained 95.2% of the total variance (eigenvalue = 32.108) and separated the larvae found in L4s and major presoldiers from the larvae found in major soldiers' head capsules and abdomens. The second discriminant variable, which explained 4.4% of the total variance (eigenvalue = 1.492), segregated the larvae found in major soldiers' head capsule from larvae found in major soldiers' abdomens. The variables, body length, body width, and width of posterior spiracles were most useful in classifying the larvae into the instar stages.

As the larvae developed, body width of the larval instars increased five-fold, from a mean of 0.59 ± 0.23 mm (n = 50; range, 0.32–1.17 mm) for first instar to a mean of 2.96 ± 0.26 mm (n = 18; range, 2.51–3.50 mm) for the third instar (Table 2). The mean body length of larval instars increased four-fold from 1.22 ± 0.38 mm (n = 50; range, 0.61–2.18 mm) for the first instar to 4.31 ± 0.36 mm (n = 18; range, 3.56–4.93 mm) for the third instar (Table 2). However, Fig. 4 shows that it was difficult to distinguish between second and third instars by using these morphometric measurements as individual variation in body size might be due to host endocrine activity (Stern 2003). Thus, morphological characteristics, i.e., respiratory horns and a well-developed posterior spiracular base provide a more reliable means to differentiate between these two instar stages.

M. mindeni larvae completed the first instar stage in L4s and major presoldiers, whereas the second and third instars developed entirely in the major soldier's head capsule and abdomen, respectively. On several occasions, we observed a first instar larva moving freely within the host's body cavity and head capsule. How the larval parasitoid comes into contact with its host remains unknown, but several possibilities exist. First, the egg may be ingested by the target host and subsequently hatch in the host's intestine. The larva then may penetrate the intestinal wall and enter into the host's hemolymph. A second possibility is that the hatched larva penetrates the host's abdominal wall and enters into the host's hemolymph.

Table 1. Measurements of eggs and pupae of *M. mindeni*

Stage	*n*	Length (mm)		Width (mm)	
		Mean ± SD	Range	Mean ± SD	Range
Egg	70	0.44 ± 0.02	0.39–0.47	0.12 ± 0.01	0.10–0.15
Pupa	85	3.84 ± 0.22	3.44–4.76	2.63 ± 0.17	2.29–2.98

Pupae. The puparium of *M. mindeni* was typically coarctate and barrel shaped. At first it was red with black posterior spiracles. After ≈24 h, it turned to black and became heavily sclerotized by hardening of the larval integument of the last larval instar (Fig. 5A). It had a single eclosion plate, from which a short tubular respiratory horn protruded (Fig. 5B), and a detachable anterior cup (Fig. 5C), from which the adult fly emerges. The pupal respiratory horns generally protruded through the weakened spots on the eclosion plates after the puparium formed. Table 1 provides the measurement of a puparium. These morphological characteristics are somewhat similar to the puparium of *Misotermes exenterans* Schmitz, a termite parasitoid of the same host species (Schmitz 1938, cited in Disney 1994).

Development of *M. mindeni.* In this study, all eggs deposited by the females failed to hatch under our controlled laboratory conditions. We postulate that egg hatchability requires host contact. Several researchers reported that eggs deposited by females of the tachinid tribe Goniini only hatched after being ingested by a feeding host, as the combination of salivary juice, mechanical rupture, and high pH in the host's gut is essential to stimulate egg hatching (Feener and Brown 1997, Stireman et al. 2006). Otherwise, the eggs remain dormant in the environment. For *M. mindeni*, however, the host contact mechanism still remains a mystery. An unfavorable physical environment is another possible explanation for egg hatching failure. Temperature is known to play a significant role (Baba and Takaoka 1992, Yoshimura et al. 2006), and determining the environmental conditions conducive to egg hatching is an important future experimental study.

Table 3 lists the mean time interval between the appearance of the first instar until appearance of third instar and from third instar to pupation. In addition, the time interval between pupation and adult emergence was on average 13.51 ± 0.74 d (n = 30; range, 12–15 d). Data about the time required for development of immature stages of phorids is limited in the literature. Moreover, it is difficult to compare the developmental time of immature stages of *M. mindeni* with that of other species due to different laboratory rearing conditions. However, the time required for larval development of *M. mindeni* was slower than that of all other reports for phorids reared at the same temperature (Bohart and Gressitt 1951, Tumrasvin et al. 1977). The pupation period found in the current study was comparable to that reported by Neoh and Lee (2010).

Adult *M. mindeni* are short lived, which is also true for some other phorid flies (e.g., *Pseudacteon lito-*

Table 2. Measurements (mean ± SD, millimeters) and comparisons of morphological characteristics of larvae of *M. mindeni* (ranges in parentheses)

Larval instar	*n*	Body length	Body width	Mandible length	Mandible width	Posterior spiracular width	Respiratory horns	Segmentation	Posterior spiracles	Posterior spiracular base	Mandibles
First instar	50	1.22 ± 0.38 (0.61–2.18)	0.59 ± 0.23 (0.32–1.17)	0.07 ± 0.01 (0.04–0.12)	0.05 ± 0.01 (0.03–0.09)	0.17 ± 0.04 (0.09–0.27)	No	Yes (less distinct)	Yes	No	Yes
Second instar	25	3.25 ± 0.54 (2.14–3.97)	2.42 ± 0.34 (1.96–2.93)	0.08 ± 0.02 (0.05–0.10)	0.05 ± 0.15 (0.03–0.07)	0.50 ± 0.05 (0.34–0.56)	No	Yes (eight segments)	Yes	Yes (less-developed)	Yes
Third instar	18	4.31 ± 0.36 (3.56–4.93)	2.96 ± 0.26 (2.51–3.50)	0.10 ± 0.02 (0.07–0.15)	0.09 ± 0.02 (0.07–0.15)	0.51 ± 0.03 (0.42–0.56)	Yes	Yes (eight segments)	Yes	Yes (well-developed)	Yes

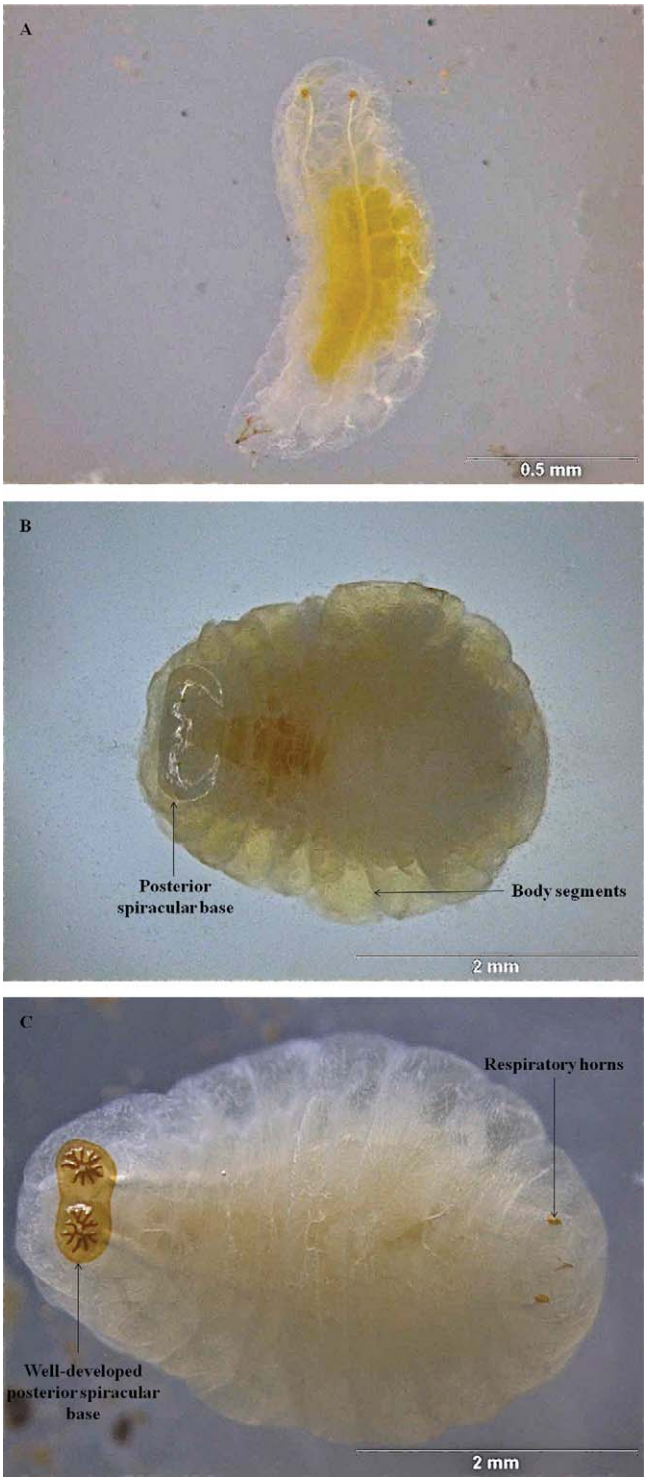

Fig. 3. Larvae of *M. mindeni*. (A) First instar. (B) Second instar: It can be readily distinguished from the first instar by the presence of a posterior spiracular base and more defined body segments. (C) Third instar can be readily differentiated from the second instar by the presence of respiratory horns and a well-developed posterior spiracular base. (Online figure in color.)

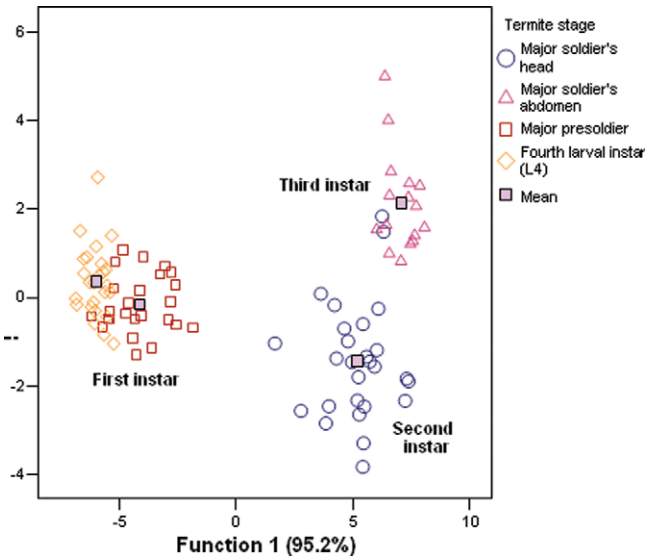

Fig. 4. Canonical discriminant functions of larval instars of *M. mindeni.* (Online figure in color.)

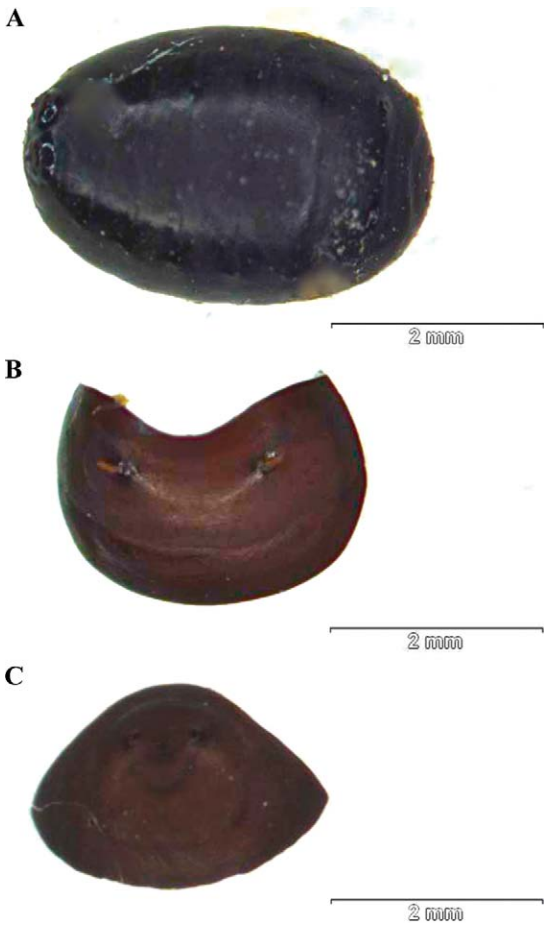

Fig. 5. Pupa of *M. mindeni.* (A) Whole puparium. (B) Pupal respiratory horns protrude through the eclosion plate. (C) Detached anterior cap. (Online figure in color.)

ralis Borgmeier; Porter et al. 1995). The life span of adult females ranged from 1 to 3 d, with an average of 1.47 ± 0.57 d ($n = 32$). In comparison, adult males lived longer than females, with an average of 3.00 ± 0.98 d ($n = 32$, range 2–5 d). There was a significant difference between the longevity of females and males at 28 ± 1°C and 90% RH ($t = -7.629$, df = 50, $P < 0.01$). Our results largely agree with those of Reznick (1985), who reported that mating and reproductive activity could result in the reduction of parasitoid life span due to energy consumption during mating and allocation of nutritional resources to reproductive tasks.

In conclusion, this study provides information about morphology and development of all stages of a new phorid fly species, *M. mindeni.* These results may prove useful for assessing the mechanism of parasitism of *M. mindeni.* Further exploration is warranted because questions remain about the host contact mechanism, their feeding behavior, and their mating behavior.

Table 3. Development duration of the specific stages of *M. mindeni* and adult stage longevity under laboratory conditions (28 ± 1°C, 90% RH)

Stage	*n*	Duration (d)	
		Mean ± SD	Range
Larva			
First instar to second instar	6	19.00 ± 2.28	16.00–23.00
Second instar to third instar	67	36.88 ± 5.17	29.00–50.00
Third instar to pupa	40	0.53 ± 0.08	0.33–0.63
Pupa to adult	30	13.51 ± 0.74	12.00–15.00
Adult			
Female	32	1.47 ± 0.57	1.00–3.00
Male	32	3.00 ± 0.98	2.00–5.00

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