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The preimaginal stages and development of *Spalangia cameroni* Perkins (Hymenoptera: Pteromalidae) on *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

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ABSTRACT

The development and morphology of the immature phases of *Spalangia cameroni* Perkins, 1910 (Hymenoptera, Pteromalidae) are described from a laboratory rearing culture maintained on *Ceratitis capitata* (Wiedemann, 1824) (Diptera, Tephritidae), using microscopic techniques, including light and scanning electron microscopy. The surface of the chorion of the egg is smooth and the micropyle occurs at the anterior end. The immature larvae are similar to the mature larva, differing mainly in the size of the head capsule and mandibles. The mature larva displays tubercules on the body segments as well as a pleurostoma and superior and inferior mandibular processes. On completion of its larval development, an adecticous and exarate pupa is produced. The mandibles of the pupa, as for the adult, are toothed. Three larval instars are recorded based on statistical analyses of the sizes of the larval mandibles and head capsules, in combination with such characters as the number of exuviae and excretion of the meconium. There are significant positive correlations between mandible length and width of larval head capsule with the number of instars, thus indicating that the mandible length and width of larval capsule are good predictors of the number of instars in this parasitoid. Developmental time from egg to adult emergence was ~33–34 days for females and ~28–29 days for males at 21–26 °C, 55–85 RH and a L16:D8 photoperiod. Our results show that the eggs and different instars of *S. cameroni* can be unambiguously identified only by SEM. Therefore, characterization of the immature stages of *Spalangia* species using SEM should be done before subsequent routine identifications using a binocular microscope or stereomicroscope.

1. Introduction

Spalangia cameroni Perkins, 1910 (Hymenoptera, Pteromalidae) is a solitary primary ectoparasitoid of the pupae of various Diptera pests. Currently it is one of the parasitoids most used worldwide for biological control of *Musca domestica* Linnaeus, 1758 – the "housefly" – and *Stomoxys calcitrans* (Linnaeus, 1758) – the "stable fly" – species that are harmful in the intensive (confined) raising of livestock and birds (Novartis Animal Health Inc., Perkins Ltda.; Protecnet (2009)). In this respect, it is being used in countries such as Denmark, the United States, Australia, Costa Rica and Colombia in inundative releases, sometimes reaching parasitoidism rates of 40% (Geden and Hogsette, 2006; Steenberg et al., 2001). In Spain, the species has been bred since 2003 on a semi-massive basis using *Ceratitis capitata* (Wiedemann, 1824), the "Mediterranean Fruit Fly", as host with a view to testing its usefulness as a biological weapon against this Diptera.

As indicated above, most studies addressing *S. cameroni* have focused on its potential use for biological control of Diptera pests. Little attention has been paid to its developmental biology (although see Gerling and Legner, 1968). Developmental biology studies, including morphological characterization of the preimaginal stages, can be important for the identification of an insect at species level before adult emergence and can simplify quantification of the impact of natural enemies in biological control programs (Bellows and Van Driesche, 1999; Llácer et al., 2005; Onagbola and Fadamiro, 2007). Little is known about larval morphology in pteromalid wasps (Grassberger and Frank, 2003; Rojas-Gómez and Bonet, 2003; Onagbola and Fadamiro, 2007; Tormos et al., 2007). The present study addresses the characterization of the developmental biology and morphology of the preimaginal stages of this species because the characterization and description offered by Gerling and Legner (1968) are brief and lacking in detail. Descriptions of the preimaginal morphology of another species of this genus, *S. endius* Walker, 1839 have been provided by Handschin (1934) and Zhang and Zhang (1990). Nevertheless, these descriptions are also extre-

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Table 1
Measurements (mean ± S.E.M., in mm) of the body sizes of the instars and pupae of *S. cameroni* at specific intervals after parasitization (mature larva: third instar + prepupa[a]).

Age (days)	First instar			Second instar			Mature larva			Pupa	
After parasitization	*N*	Length (mean ± S.E.M.)	Width (mean ± S.E.M.)	*N*	Length (mean ± S.E.M.)	Width (mean ± S.E.M.)	*N*	Length (mean ± S.E.M.)	Width (mean ± S.E.M.)	*N*	Length (mean ± S.E.M.)
3	4	0.31 ± 0.02	0.10 ± 0.00								
4	5	0.57 ± 0.10	0.23 ± 0.05								
5	2	0.65 ± 0.00	0.27 ± 0.03								
6	8	0.86 ± 0.30	0.39 ± 0.15								
7	2	0.92 ± 0.07	0.47 ± 0.01	13	1.31 ± 0.66	0.66 ± 0.37					
8				4	1.10 ± 0.20	0.52 ± 0.14					
9				10	1.61 ± 0.75	0.75 ± 0.36					
10				1	1.62 ± 0.00	0.75 ± 0.00	6	2.31 ± 0.66	1.08 ± 0.31		
11							10	2.42 ± 0.59	1.19 ± 0.27		
12							7	2.48 ± 0.52	1.14 ± 0.28		
13							11	2.64 ± 0.39	1.22 ± 0.21		
14							3	2.11 ± 0.82	1.12 ± 0.30		
15							5	2.17 ± 0.62	1.02 ± 0.28		
16							7[a]	2.23 ± 0.64	1.08 ± 0.33		
17							5[a]	2.29 ± 0.64	1.12 ± 0.31		
17/33–34										20	2.01 ± 0.26

mely brief and cannot be used for comparative purposes. In this study morphological parameters, including measurements of the width of the head capsule and mandible length and plotting of the frequency distribution of these data, followed by statistical analyses were used in conjunction with reliable characters, such as the presence of exuvia, excretion of the meconium, and the initiation of the prepupal period (especially the initiation of the "pronymph") to determine the number of instars (Muesebeck and Parker, 1933; Löhr et al., 1989; Llácer et al., 2005; Onagbola and Fadamiro, 2007; Wright, 1986,). Characterization of the developmental biology of this parasitoid on *C. capitata* is of particular relevance because this is the first time it has been reared on this host since being recorded as a parasitoid (Falcó et al., 2006).

2. Materials and methods

2.1. Insects

The stages of the preimaginal phases and data on the developmental biology of *S. cameroni* were obtained by rearing of the parasitoid at the Instituto Valenciano de Investigaciones Agrarias (València, Spain), in a climatic chamber (Sanyo MLR350) at 21–26 °C, 55–85% RH, and a L16:D8 photoperiod. Pupae of the Mediterranean Fruit Fly were used as hosts, and the parasitoid, confined in a plastic cage (30 cm × 20 cm × 20 cm) with ventilation, were fed with honey impregnated on strips of blotting paper, plus sugar and water. These conditions were used throughout the study, except that a RH of 95% was used (following Gerling and Legner, 1968) in the experiments aimed at determining the moment of eclosion and the presence of different moults.

2.2. Development, morphology and characterization of the preimaginal stages

To study the development of the immature stages, 10 recently parasitized pupae of *C. capitata* were dissected to completely expose the eggs, and were placed in a chamber with 95% RH. Periodic observations were made of the development through eclosion and production of exuviae. The slowing down of movement and expulsion of the meconium were

Table 2
Length (in μm) of the mandible and width (in mm) of the head capsule of the instars of *S. cameroni* at specific intervals after parasitization (mature larva: third instar + prepupa[a]). Intervals of presence of non-melanized (NM) and melanized (M) pupae.

Age (days)	First instar				Second instar				Third instar + prepupa[a]				Pupae			
After parasitization	*N*	Length (mean ± S.E.M.)	*N*	Width (mean ± S.E.M.)	*N*	Length (mean ± S.E.M.)	*N*	Width (mean ± S.E.M.)	*N*	Length (mean ± S.E.M.)	*N*	Width (mean ± S.E.M.)	*N* NM	♂♂ (M)	♀♀ (M)	
3	4	29.75 ± 0.95	6	0.14 ± 0.05												
4	5	35.00 ± 2.54	5	0.14 ± 0.05												
5	2	38.00 ± 0.00	7	0.14 ± 0.03												
6	8	44.50 ± 2.07	3	0.14 ± 0.00												
7	2	47.00 ± 1.41	7	0.15 ± 0.00	13	70.53 ± 3.12	1	0.31 ± 0.00								
8					4	70.00 ± 2.44	6	0.31 ± 0.00								
9					10	77.90 ± 3.21	5	0.31 ± 0.00								
10					1	80.00 ± 0.00	8	0.32 ± 0.00	6	96.66 ± 1.86	1	0.40 ± 0.00				
11									10	97.20 ± 3.61	5	0.41 ± 0.00				
12									7	102.00 ± 6.95	5	0.41 ± 0.01				
13									11	107.45 ± 8.59	6	0.43 ± 0.03				
14									3	110.00 ± 13.07	4	0.41 ± 0.01				
15									5	114.60 ± 9.52	4	0.41 ± 0.01				
16									7[a]	111.00 ± 8.08	5	0.41 ± 0.01				
17									5[a]	114.00 ± 8.03	5	0.40 ± 0.01				
17–27													20	20		
28–29														8	12	0
33–34														0	0	8