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## The life cycle of *Orthemis ferruginea* (Fabricius, 1775) (Odonata: Libellulidae)

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**Abstract:** The complete life cycle of *O. ferruginea* is described for the first time, represent the first complete life cycle described for an odonate in Mexico. The 17 larval instars obtained are described and illustrated in detail, from prolarva through F-0. Two egg batches of different females were obtained in the field and were subsequently reared in the laboratory. Eggs and larvae of the batches were raised under 26°C controlled temperature conditions until they reached instars F-6 and F-5. An extra collection of wild organisms was made in order to complete the life cycle from F-5. Only four of the wild larvae managed to complete the last five missing larval instars at 30°C. Larvae of the youngest instars (F-15 to F-8) were fed nauplii of *Artemia franciscana*, while F-7 to F-0 were fed larvae of Culicidae and Chironomidae. Larval life cycle from F-0 to F-16 lasted average of 186 days.

**Keywords:** Anisoptera, dragonfly, larvae, rearing, temperature, feeding, instars, description, morphology

### Introduction

Descriptions of life cycles of aquatic macroinvertebrates are highly relevant due to potential application in various branches of biology and ecology, conservation, restoration, and biomonitoring of freshwater bodies, as well as in the understanding of evolutionary processes that have shaped these life cycles. On the other hand, a taxonomic crisis in species identification has increased in recent years (Ebach *et al.*, 2011; Evenhuis, 2007), and insect groups are no exception (Audisio, 2017). In Odonata, the issue is that the vast majority of species have been described based on mature adults, mainly males, although descriptions of females and larvae have recently increased. However, knowledge about life cycles of odonates has not advanced equally relative to other areas including taxonomy, systematics, evolution, and ecology, with research focusing mainly on descriptions of the last instar larva prior to adult emergence (González-Soriano & Novelo-Gutiérrez, 2014; Johnson, 1991). Currently, 6335 odonate species have been described worldwide (Paulson & Schorr, 2021). From these, the life cycle of only 122 species is known, i.e., only 2% of species have a general description of larval development. In addition, not all descriptions comprise all instars or record all morphological changes that take place over the course of development (Corbet, 2002). In Mexico, knowledge of life cycles of odonates is virtually nil, with only a single unpublished description for the life cycle for *Perithemis tenera* (Say, 1840) (Ramos-García, 2016 as *P. mooma*).

*Orthemis ferruginea* was described under *Libellula ferruginea* by Fabricius in 1775 and placed to *Orthemis* by Fabricius in the same year. During many years adults of *O. ferruginea* and *O. discolor* were confused as a single species, however De Marmels (1988) established that the species in North America is *O. ferruginea* and the species with distribution in South America is *O. discolor*. Donnelly (1995) did not find structural characteristics to separate the three morphs (*ferruginea*, *discolor* and the form of the Antilles), while Paulson (1998), proposed that differences in the vulvar lamina are the structures indicated for the separation of the species. So far, now there is no problem in the identification of adults of *O. ferruginea* and *O. discolor*, while the information available for larvae only includes the descriptions of the last instar larvae of both species. The last instar larva of *O. ferruginea* was first characterized by Needham in 1904, but Calvert (1928), Geijskes (1934), and Roldán & Arango (1996) described what they believed it was *O. ferruginea* larvae, but now it is known that may actually be the descriptions of *O. discolor*, and only the Needham description matches the cor-

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rect characterization of *O. ferruginea* (Carrillo-Lara & Novelo-Gutiérrez, 2018). An important aspect of the description of life cycles is that it allows to know the variation throughout development, as well as to lay the foundations for future morphological, physiological, and behavioral studies (Stoks & Córdoba-Aguilar, 2012) in which ontogeny experiments can collaborate to deepen this knowledge.

## Material and methods

### Egg Collection

Copulating adults of *O. ferruginea* were collected to ensure that the eggs produced belong to this species. Females were stimulated to oviposit within plastic containers with water and a piece of filter paper at the bottom for attachment of eggs; afterwards, eggs were transported to the laboratory for further conditioning. Two batches of eggs were obtained, one in June and another in July 2015. Adult couples were preserved and deposited in the "Dr. Miguel Angel Morón Ríos" Entomological Collection at Instituto de Ecología, A.C. (IEXA).

### Life-Cycle Experiment

Egg containers were placed in plastic tanks with 10 L of tap water and a plastic grid inside to hold egg containers. Temperature was controlled using aquarium heaters immersed at the bottom of the tank. As larvae hatched, these were individually placed in acrylic containers (2.5 cm in diameter) with filter paper at the bottom; this facilitated ease of care and access to individual exuviae after each molt. Larger larvae were placed in glass tanks containing a small amount of litter to be used as support for instars when molting. The first egg batch collected (Batch I) was initially reared at room temperature (18 °C); however, since this led to high mortality in the population, the remaining larvae were reared at 26 °C. Batch II was reared at 26 °C from day 1, but larvae survived only to F-5, making it necessary to collect wild larvae to complete the cycle. Wild larvae were reared at 30 °C. Food was provided daily to all organisms. Soon after hatching, the F-15 instar was fed 0.25 ml of nauplii from *Artemia franciscana* Kellogg, 1906,  $\approx$  28-30 prey per day. The instars from F-14 to F-12 had doubled food ration to 0.5 ml,  $\approx$  30 to 50 prey. From instar F-11 to F-9, the food was administered at two times at 08:30 am and at 03:00 pm with a 1 ml ration ( $\approx$ 80-100 preys). A mixed diet comprised of Chironomidae ( $\approx$ 10-12/p) and Culicidae larvae ( $\approx$ 10-12/p), as well as brine shrimp adults ( $\approx$  20/p), was administered from stage F-8. The same amount of food of each type was administered according to the availability of each one of them to each larvae, respecting the same feeding schedule as the previous instars. Water with no salts or chlorine was supplied and replaced regularly in each container to avoid contamination.

### Life-Cycle Ranges

Each stage of the experiment was monitored daily in order to obtain accurate records molting times. For each individual larva, the exact day when the exuvia was found was recorded, thus marking the beginning of a new larval instar. For each larval instar, average number of days taken to advance from one instar to the next was calculated, including ranges (minimum and maximum).

## Larval Description

Larvae that died during development and exuviae of each molt were preserved in 80% ethanol (Carvalho & Werneck-De-Carvalho, 2005). Individual larvae were measured under a stereomicroscope (Leica MZ6) with an eyepiece grid of 100 units, calibrated with a 1 mm micrometer. During measurements, exuviae were immersed in 80% alcohol. For eggs and first 11 instar larvae (F-16 to F-6), photographs were taken of structures and their morphological changes (Nikon Eclipse 80i light microscope), assisted with a well slide, glycerin, antibacterial gel, and coverslips. For subsequent instars, we used a stereo microscope fitted with a Nikon H550S-DSUS3 camera. Characterization of ornamentation and description of eggs were conducted using a QUANTA FEG 250 scanning electron microscope (SEM). We used the technique proposed by Ivey *et al.* (1988) to capture high-quality images. Each anatomical region (head, thorax, and abdomen) was observed, as well as structures belonging to each region. General traits of odonates and characters unique to family Libellulidae were observed in each tagma guided by illustrations in the works of Novelo-Gutiérrez and González-Soriano (1991), Ramírez (2010), and Arango & Roldán (1983). Mandibles and labium were described according to the terminologies of Watson (1956) and Corbet (1953), respectively.

## Results

Prolarvae hatched eight days after egg collection for both batches, despite being kept under different temperatures. A total of 1526 larvae were counted for Batch I (Table 1); however, most of them did not survive past instar F-15, and the same occurred with the 506 larvae obtained from Batch II. Once instars survived past F-15, larvae advanced smoothly to subsequent instars; for this reason, F-15 was called a “critical” instar.

**Table 1. Number of larvae of *Orthemis ferruginea* obtained for each treatment.**

	Breeding temperature (° C)	Number of larvae	Number of survivals	Instar reached
Batch I	18 to 26	1 526	5	F-6
Batch II	26	506	7	F-5
Wild batch	30	56	4	F-0

## Development Ranges

Batch I showed a total development duration of 135 days, from day of hatching (03 March 2015) to last molt recorded from F-6 to F-5 (14 November 2015); this is equivalent to four and a half months of development, although without having completed the cycle (Table 1). A period of 8–16 days passed from time of egg collection to first hatched prolarvae. The prolarva (F-16) instar lasted only one to three minutes, so that larvae belonging to instars F-16 and F-15 were obtained within 24 hours. Average duration of instar F-15 was  $17 \pm 9$  (SD) days, but the range of instar duration was 1–29 days, with a higher range frequency of 12 to 14 days. It was noted that from instars F-13 to F-6, average number of days increased as larval development progressed, as well as the minimum and maximum number of days in each instar (duration range). For instance, instars F-13 and F-12 had a similar average duration of  $4 \pm 2$  (SD) days, with ranges of 1–12 days for F-13 and 2–10 days for F-12, while for subsequent instars both average duration and respective range increased (Table 2).

**Table 2. Duration (average days + SD) of each instar in the life cycle of *Orthemis ferruginea*.**

Instar	F-0	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9	F-10	F-11	F-12	F-13	F-14	F-15	F-16	Egg
Batch I ×							23	16.5	15.4	11.7	11.3	9.4	4.4	4.2	10.3	17.2	1–3 min- utes	11.5
SD							9.8	4.8	3.9	2.1	2	2.8	2	2.5	8.3	9.2		2.30
Min*							9	10	10	7	9	4	2	1	3	1		8
Max**							36	21	22	14	14	15	10	12	25	29		15
Batch II ×						13.3	14.8	17.2	13.3	11	9.8	7.2	5.4	4.8	5.7	6	1–3 min- utes	42.5
SD						2.5	5.4	7	3.8	3.4	4.1	2.3	2.3	2.1	2.3	2.5		20
Min						9	7	3	4	3	2	2	1	1	2	2		8
Max						17	26	40	27	27	42	20	22	16	15	16		70
Wild ×	11	18.3	8.8	13.2	12.3													
SD	6	29.8	3.4	9	3.3													
Min	8	2	6	7	6													
Max	20	58	13	26	12													

\* Min: minimum number of days it took an individual to move to the next instar.

\*\* Max: maximum number of days it took an individual to move to the next instar.

Batch II showed a total duration of 149 days, although larval cycle was not completed either, covering instars F-16 to F-5 (Table 2). This period can be summarized as five months and 13 days, from 23 July 2015 (hatching day) to 3 January 2016 (passage of last larva from F-5 through F-4). Instar duration ranges for Batch II were wider than for Batch I in some cases, from a minimum of one to two days up to a maximum of 42 days in F-10. In both batches, duration range for early instars was narrower, and became broader from instars F-11 and F-10. This also influenced instar size, since as duration of instars increased, instars underwent a far more vigorous growth relative to earlier instars. Only four out of the 56 wild larvae could be reared to mature adult stage. Primary cause of death during larval development was related to food, as not all larvae consumed the food supplied (chironomids and adults of *Artemia franciscana*). Conditions considered for larvae rearing chambers allowed us to obtain five instars beyond those reached with larvae reared from eggs, making it possible to complete the whole larval cycle comprising 17 instars. Duration of this larval period was 57 days from instar F-4 through F-0, observed from 15 February to 11 April 2016. Maturation period from teneral adult to adult displaying of diagnostic characteristics of the species was approximately one week. Ranges, in days, for eggs and larvae stages are summarized in Table 2. For example, for instar F-4, minimum and maximum number of days elapsed were seven and 26, respectively, with a frequency of seven days, i.e., the largest number of larvae molted again seven days after the previous molt. The highest number of days per instar was recorded for F-1, which took 58 days before molting occurred.

## Description of the life cycle of *Orthemis ferruginea*

### Egg

**Measurements:**  $n=100$ . Average length  $\pm$  SD:  $0.39 \pm 0.02$  mm; average width  $\pm$  SD:  $0.23 \pm 0.04$  mm.

**Description:** Oval, wrapped in a gelatinous matrix. Endocorion smooth (Figure 1A), without ornamentation or any other pattern, micropile with two micropillar pores. Translucent at the time of laying, turning pale yellow after a few hours (Figure 1B), and becoming amber in color as incubation period elapses. On the fourth day of incubation, a small dark dot on each side of the embryo, which appear to be the compound eyes, could be seen by naked eye. At the time of hatching, the longitudinal suture from pole to pole is visible on the eggshell (Figure 1C).

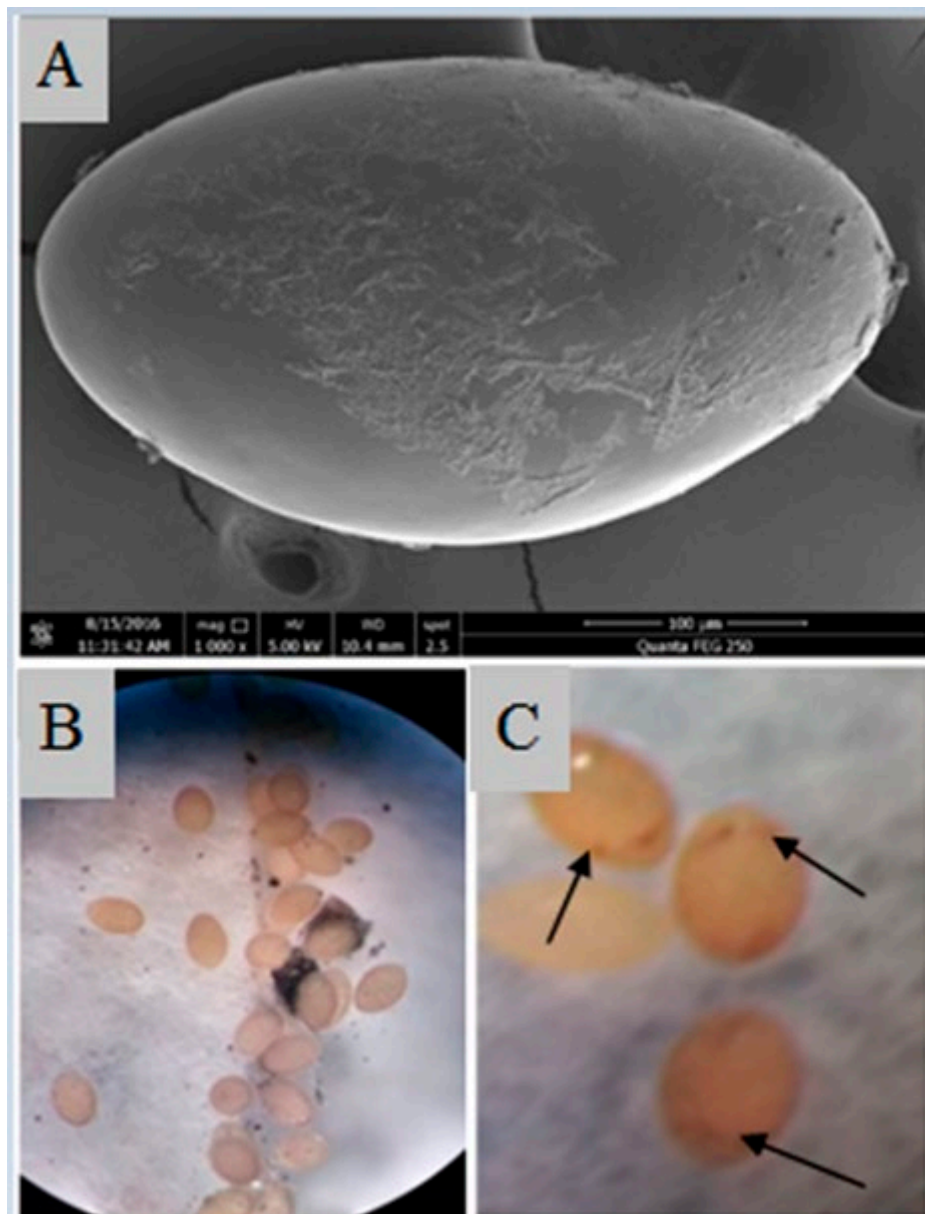


Figure 1. A) Above, egg of *Orthemis ferruginea* observed under Scanning Electron Microscope (SEM); B) left below, segmented eggs; right below, C) eggs with embryo visible after four days of incubation, the arrows point to the eyes on the embryo.

**F-16 Prolarva**

**General description:** Average length  $\pm$  SD:  $0.25 \pm 0.03$  mm; maximum body width:  $0.4 \pm 0.02$  mm. This is the first larval instar (Figure 2). Coloration creamy pale, integument translucent. Cephalic portion with a pointed horn-shaped structure that seems to involve antennae and mouthparts. Compound eyes visible as a dark brown spot on each side of head. Three pairs of legs are kept ventrally closed in relation to the body.



**Figure 2.** *Orthemis ferruginea* prolarva, the left arrow points to the prolarva leaving the exuvia and the right arrow indicates the exuvia of the F-16 stage.

**F-15**

**Head.** Wider than long, rounded; compound eyes small, not protruding laterally (Figure 3A). Cephalic lobes rounded, not bulging; occiput straight. Anterior margin of head without setae. Labrum smooth, margins with a light brown line. Antennae 3-segmented, scape short with an apical bristle in dorsal view, pedicel longer than scape, scape barrel-shaped, flagellum cylindrical, longer than scape and pedicel together, scape and flagellum beset with abundant tubercles, with a few thin, long setae on flagellum (Figure 4A). Labium: Premental setae lacking, ligula slightly crenulated with two central protuberances. Labial palp lacking setae, its distal margin with six crenations, internal margin straight (Figure 5A). Movable hook short, tapering distally to a fine tip.



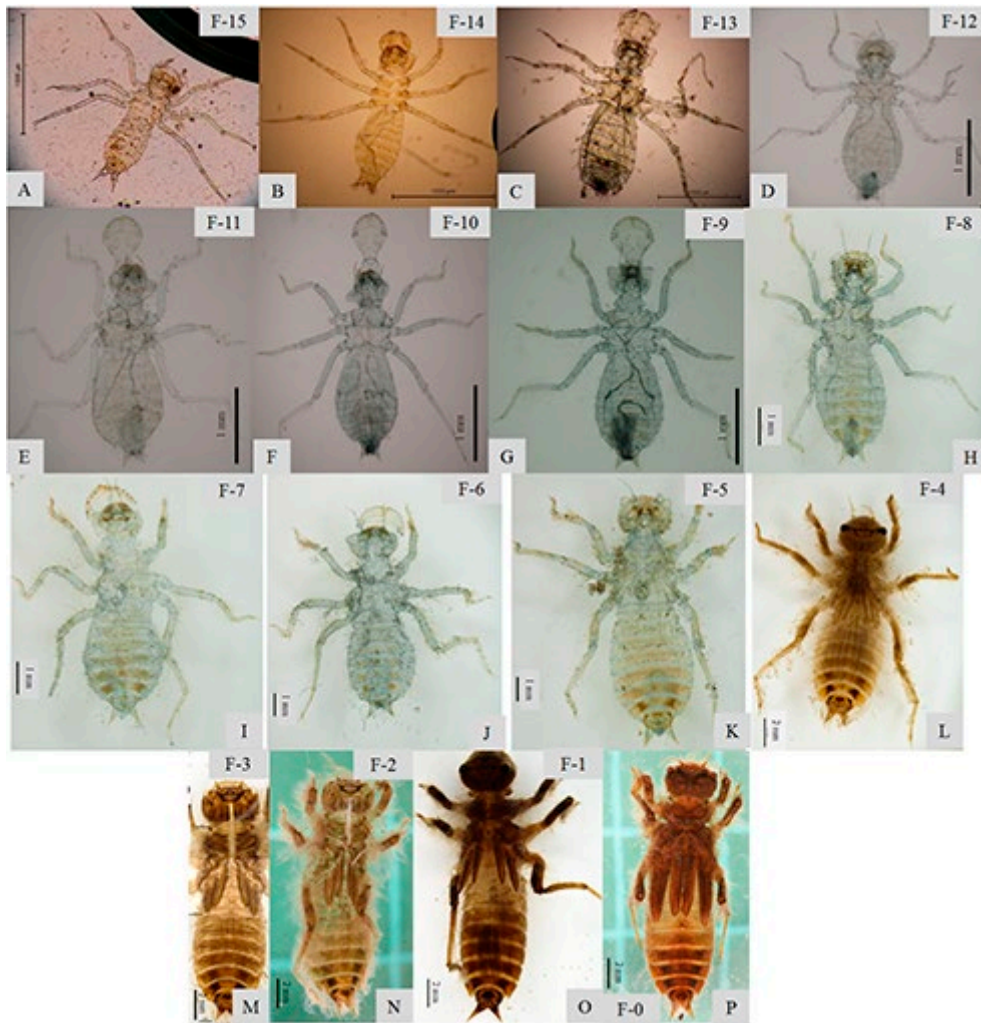


Figure 3. Details of the morphology of the whole body in dorsal view of *O. ferruginea* in the 16 larval stages subsequent to the prolarva. A) F-15; B) F-14; C) F-13; D) F-12; E) F-12; F) F-10; G) F-9; H) F-8; I) F-7; J) F-6; K) F-5; L) F-4; M) F-3; N) F-2; O) F-2; P) F-0.

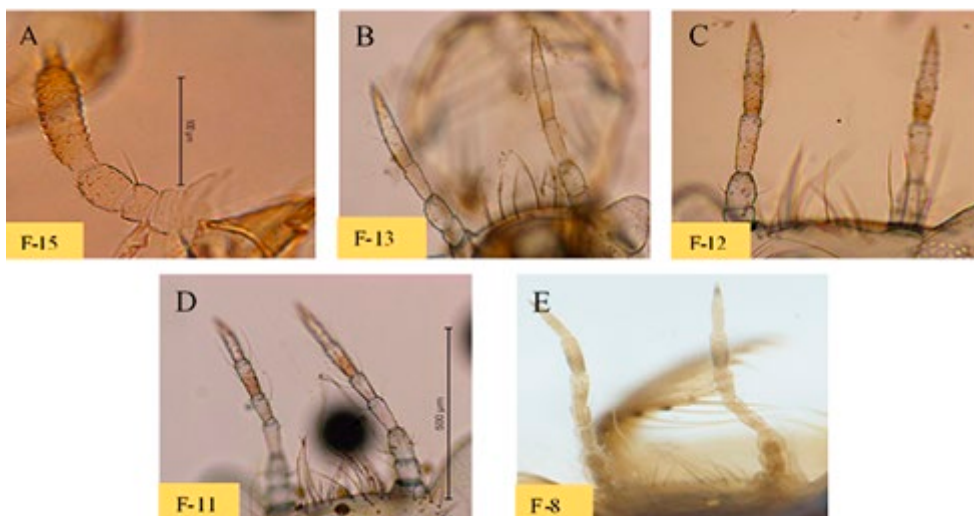
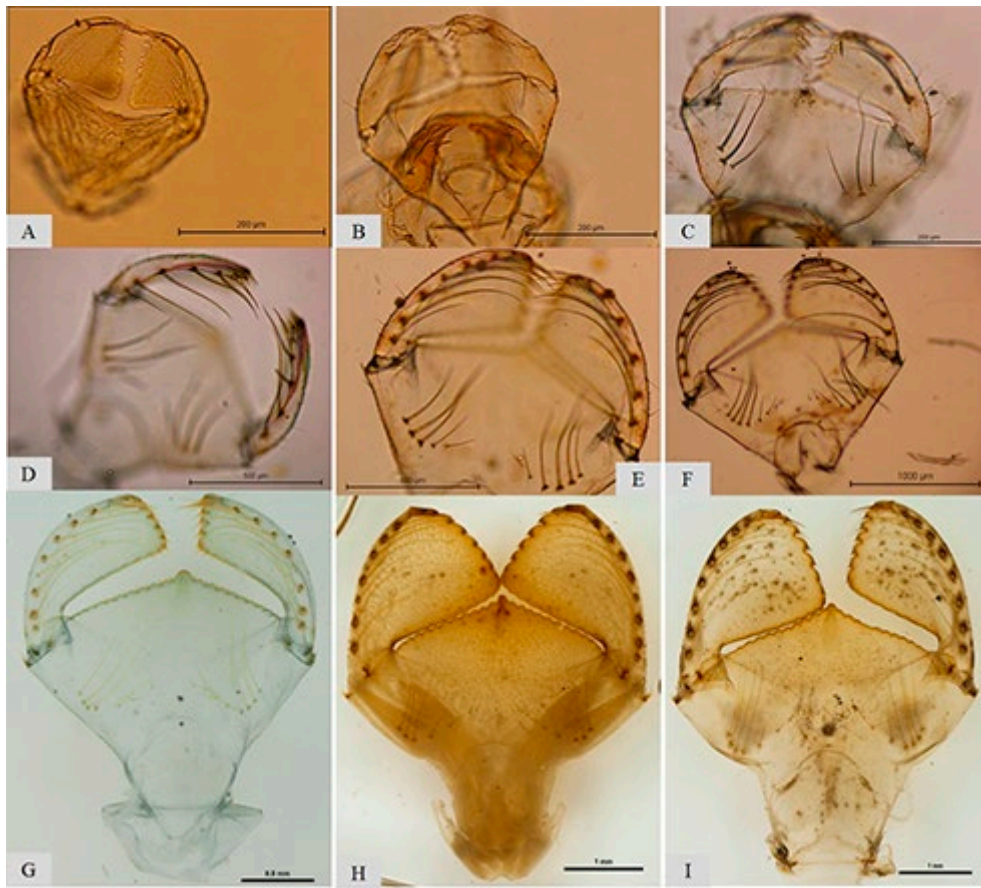


Figure 4. Antennae divisions throughout the life cycle, A) F-15 3-segmented; B) F-13 4-segmented; C) F-12 5-segmented; D) F-11 6-segmented; E) F-8 7-segmented.



**Figure 5.** Details of the labium showing the prementum, labial palpi and ligula, including setae, crenations and crenulations; A) F-15, B) F-14, C) F-13, D) F-10, E) F-9, F) F-7, G) F-6, H) F-4, I) F-3.

*Thorax.* Longer than wide, pronotum shorter than meso- and metanotum. A pair of long dark setae on middorsal thorax. Legs long, spidery, translucent, with a faint of amber rings, covered moderately with long, thin setae. Tarsi with only one tarsomere (Figure 7A), proportions as shown in Table 3.

*Abdomen.* Elongate, more or less parallel-sided, wider on S7–8. S1–9 with a pair of long, curved setae on middorsal portion. Posterior margins of terga S6–9 darker, with a row of long, thin, white setae overlapping next segment. Caudal appendages large, sharply-pointed, about as long as 1/3 of abdomen total length. Epiproct and paraprocts with short, thick setae. Epiproct directed dorsally. Paraprocts long but shorter than epiproct.



**Table 3. Legs dimensions of *Orthemis ferruginea* (mm  $\pm$  SD).**

Instar	<i>n</i>	Prolegs					
		Femora	Tibiae	Tarsi without claw	Tarsomeres		
					Total	1°	2°
F-15	40	0.23 $\pm$ 0.03	0.23 $\pm$ 0.02	0.16 $\pm$ 0.01			
F-14	37	0.28 $\pm$ 0.07	0.29 $\pm$ 0.02	0.17 $\pm$ 0.02			
F-13	39	0.36 $\pm$ 0.04	0.38 $\pm$ 0.04	0.20 $\pm$ 0.02			
F-12	48	0.49 $\pm$ 0.07	0.51 $\pm$ 0.05	0.21 $\pm$ 0.03			
F-11	37	0.61 $\pm$ 0.07	0.65 $\pm$ 0.06	0.25 $\pm$ 0.03	0.07 $\pm$ 0.03	0.18 $\pm$ 0.02	
F-10	36	0.73 $\pm$ 0.09	0.80 $\pm$ 0.04	0.31 $\pm$ 0.03	0.1 $\pm$ 0.01	0.21 $\pm$ 0.02	
F-9	28	0.88 $\pm$ 0.15	1.00 $\pm$ 0.08	0.38 $\pm$ 0.04	0.15 $\pm$ 0.02	0.23 $\pm$ 0.02	
F-8	28	1.05 $\pm$ 0.08	1.15 $\pm$ 0.05	0.47 $\pm$ 0.04	0.2 $\pm$ 0.02	0.13 $\pm$ 0.01	0.14 $\pm$ 0.01
F-7	27	1.24 $\pm$ 0.24	1.41 $\pm$ 0.22	0.55 $\pm$ 0.12	0.21 $\pm$ 0.05	0.16 $\pm$ 0.04	0.18 $\pm$ 0.03
F-6	26	1.50 $\pm$ 0.16	1.78 $\pm$ 0.16	0.76 $\pm$ 0.08	0.26 $\pm$ 0.03	0.19 $\pm$ 0.02	0.31 $\pm$ 0.02
F-5	25	1.84 $\pm$ 0.17	2.11 $\pm$ 0.26	0.94 $\pm$ 0.10	0.35 $\pm$ 0.04	0.28 $\pm$ 0.03	0.31 $\pm$ 0.03
F-4	12	2.23 $\pm$ 0.0	2.48 $\pm$ 0.01	1.05 $\pm$ 0.05	0.35 $\pm$ 0.02	0.30 $\pm$ 0.01	0.40 $\pm$ 0.01
F-3	4	2.37 $\pm$ 0.08	3.33 $\pm$ 0.0	1.56 $\pm$ 0.08	0.44 $\pm$ 0.02	0.44 $\pm$ 0.01	0.66 $\pm$ 0.01
F-2	4	2.36 $\pm$ 0.43	1.52 $\pm$ 0.03	1.56 $\pm$ 0.1	0.44 $\pm$ 0.01	0.44 $\pm$ 0.01	0.66 $\pm$ 0.01
F-1	4	2.75 $\pm$ 0.01	2.90 $\pm$ 0.01	1.63 $\pm$ 0.1	0.46 $\pm$ 0.02	0.44 $\pm$ 0.01	0.11 $\pm$ 0.02
F-0	4	2.81 $\pm$ 0.01	3.56 $\pm$ 0.01	1.99 $\pm$ 0.1	0.50 $\pm$ 0.01	0.63 $\pm$ 0.02	0.86 $\pm$ 0.01

		Mesolegs					
		Femora	Tibiae	Tarsi without claw	Tarsomeres		
Instar	<i>n</i>			Total	1°	2°	3°
F-15	40	0.26 ± 0.03	0.26 ± 0.02	0.19 ± 0.01			
F-14	37	0.32 ± 0.07	0.32 ± 0.07	0.20 ± 0.01			
F-13	39	0.41 ± 0.04	0.42 ± 0.04	0.23 ± 0.02			
F-12	48	0.54 ± 0.07	0.56 ±0.06	0.25 ±0.03			
F-11	37	0.67 ± 0.08	0.70 ± 0.05	0.28 ± 0.05	0.09 ± 0.02	0.19 ± 0.03	
F-10	36	0.77 ± 0.08	0.86 ± 0.07	0.36 ± 0.04	0.13 ± 0.02	0.23 ± 0.02	
F-9	28	0.83 ± 0.02	1.07 ± 0.08	0.44 ± 0.03	0.17 ± 0.01	0.27 ± 0.02	
F-8	28	1.12 ± 0.05	1.20 ± 0.07	0.53 ± 0.05	0.22 ± 0.02	0.15 ± 0.02	0.16 ± 0.02
F-7	27	1.37 ± 0.23	1.53 ± 0.21	0.66 ± 0.08	0.25 ± 0.03	0.19 ± 0.03	0.22 ± 0.02
F-6	26	1.63 ± 0.14	1.87 ± 0.17	0.78 ± 0.08	0.27 ± 0.03	0.22 ± 0.01	0.29 ± 0.02
F-5	25	1.91 ± 0.21	2.15 ± 0.26	1.00 ± 0.11	0.4 ± 0.04	0.2 ± 0.04	0.4 ± 0.03
F-4	12	2.31 ± 0.04	2.23 ± 0.0	1.09 ± 0.01	0.41 ± 0.02	0.32 ± 0.01	0.36 ± 0.01
F-3	4	2.54 ± 0.08	3.42 ± 0.17	1.42 ± 0.01	0.45 ± 0.02	0.43 ± 0.01	0.54 ± 0.01
F-2	4	2.63 ± 0.01	3.50 ± 0.03	1.49 ± 0.01	0.50 ± 0.01	0.44 ± 0.01	0.55 ± 0.01
F-1	4	2.75 ± 0.01	3.53 ± 0.01	1.9 ± 0.47	0.55 ± 0.03	0.55 ± 0.02	0.80 ± 0.01
F-0	4	3.13 ± 0.01	3.75 ± 0.01	1.93 ± 0.06	0.55 ± 0.02	0.55 ± 0.02	0.83 ± 0.02

		Metalegs					
Instar	<i>n</i>	Femora	Tibiae	Tarsi without claw	Tarsomeres		
				Total	1°	2°	3°
F-15	40	0.28 ± 0.03	0.29 ± 0.02	0.24 ± 0.01			
F-14	37	0.38 ± 0.06	0.38 ± 0.03	0.26 ± 0.02			
F-13	39	0.50 ± 0.05	0.53 ± 0.05	0.32 ± 0.03			
F-12	48	0.66 ± 0.10	0.72 ± 0.10	0.39 ± 0.04			
F-11	37	0.81 ± 0.09	0.91 ± 0.08	0.47 ± 0.09	0.16 ±0.01	0.31 ± 0.03	
F-10	36	0.94 ± 0.11	1.08 ± 0.10	0.62 ± 0.12	0.22 ± 0.05	0.4 ± 0.05	
F-9	28	1.14 ± 0.09	1.36 ± 0.10	0.75 ± 0.09	0.28 ± 0.05	0.47 ± 0.05	
F-8	28	1.32 ± 0.09	1.59 ± 0.10	0.96±0.14	0.34±0.05	0.25±0.05	0.37±0.05
F-7	27	1.61 ± 0.15	1.98 ± 0.17	1.2±0.10	0.45±0.05	0.3±0.03	0.45±0.02
F-6	26	1.96 ± 0.18	2.39 ± 0.25	1.4±0.18	0.45±0.05	0.45±0.05	0.5± 0.05
F-5	25	2.27 ± 0.23	2.62 ± 0.53	2.03±0.24	0.6±0.05	0.6±0.08	0.83±0.10
F-4	12	2.39 ± 0.04	2.84 ±0.01	2.29±0.1	0.72±0.02	0.72±0.01	0.85±0.01
F-3	4	3.27 ± 0.08	4.58 ± 0.17	2.37±0.01	0.77±0.02	0.74±0.01	0.86±0.2
F-2	4	3.40 ± 0.03	4.06 ± 0.01	2.48±0.01	0.8±0.01	0.8±0.01	0.88±0.2
F-1	4	3.57 ± 0.03	4.02 ± 0.03	2.57±0.01	0.81±0.01	0.8±0.01	0.96±0.01
F-0	4	4.33 ± 0.04	5.49 ± 0.01	2.84±1.25	0.83±0.05	0.83±0.05	1.18±0.3

**F-14**

*Head.* As in previous instar, except for a few setae on vertex and on cephalic lobes just behind compound eyes (Figure 3B). Antennal flagellum longer. A longitudinal row of long, thin setae from anterior margin of frons to occipital margin on both sides of head, crossing above compound eyes. Labium: Prementum trapezoid; one long, thin seta on each side of midline. Ligula with eight tiny crenulations, four on each side of midline. Labial palp with a long palpal seta (Figure 5B); distal margin with five crenulations, each crenulation with a short, thick setae. Movable hook as in previous instar.

*Thorax.* Same as in F-15, except: Mesonotum and metanotum with a pair of long, dark setae on each side of midline. Leg proportions as in Table 3. Lateral surface of protibiae with a spiniform tubercle followed by a row of six or seven fine, long setae in its proximal part; mesial surface with a row of thin spiniform setae ending in a pair of dark, hook-shaped setae on distal portion. Mesofemora with five to six spines and a few setae. Mesotibiae same as protibiae. Tarsi as in F-15.

*Abdomen.* Same as in F-15, except: Posterior margin of sternum S9 with a row of long, thin setae reaching basal half of sternum S10.

**F-13**

*Head.* Eyes project above the head for the first time. Higher number of setae in the same position as compared to previous instar (Figure 3C). Frons with long, thin, transverse setae. Antennae 4-segmented, scape pale, pedicel dark basally, both barrel-shaped and subequal in length, 3<sup>rd</sup> antennomere cylindrical, 4<sup>th</sup> antennomere fusiform, pale at middle, darker at both ends, longest (Figure 4B). Prementum with three long setae on each side of midline. Ligula with 11 crenulations on distal margin, five on each side of the larger central crenulation; between each crenulation there is a small spiniform seta. Labial palp with 3–4 short palpal setae. (Figure 5C). Movable hook as in previous instar.

*Thorax.* Pro- and mesonotum with a pair of short spiniform setae on each side of midline, metanotum with a pair of darker, longer, thinner setae in central portion that reach posterior margin of S2. Legs (Table 3): Profemur shorter than protibia, with short spiniform setae on dorso-distal region. Lateral surface of protibia with a spiniform tubercle followed by a row of 6–7 fine, long setae in its proximal part; mesal surface with a row of darker, fine setae ending with a pair of hook-shaped setae on distal portion. Mesofemora with 5–6 spiniform setae and a few hair-like setae; remainder of mesoleg as foreleg. Metatibiae with a double row of long, spiniform setae, the last pair curved. Metatarsi with large, dark, ventral setae.

*Abdomen.* S1–3 and S10 narrower than S4–9. S1–3 with a pair of short spiniform setae, a long, thin seta on each side of midline. S4–9 with dark, spiniform setae on midline. Epiproct and paraprocts with short, fine setae. Paraprocts longer and darker, margins with short and long setae regularly spaced.

**F-12**

*Head.* Posterior lobes of head well defined (Figure 3D). Antennae 5-segmented, scape short and square, pedicel barrel-shaped, with a ring of short, thin setae, antennomeres 3–5 granulate, antennomeres 3–4 shorter than 5<sup>th</sup> antennomere, 4<sup>th</sup> antennomere with an apical ring of short, delicate setae, 5<sup>th</sup> antennomere as long as previous two together, with very thin setae (Figure 4C). In ventral view, lateral surface of mandibles covered with long, fine setae; mandibular formula: L 1234 0 ab / R 1234 y abd, *a* and *b* rudimentary in left mandible, *a*, *b*, and *d* as small points in right mandible. Labium: five premental setae on each side of midline, the external four longer, second seta longest, fifth seta shortest. Ligula as in F-13, but central crenulation with five tiny crenulations on its margin. Labial palp with four long palpal setae; distal margin of labial palp and movable hook as in previous instar. Maxilla: Galeolacinia with three teeth, two ventral teeth and a dorsal one.

*Thorax.* Notum and legs proportioned as in previous instar (Table 3). All femora noticeably more setose than in previous instar. Protibiae with a row of long, thin setae along lateral margin, and two



spines on each side of midline; protarsi, in ventral view, with two rows of four setae on each side of midline, the basal setae short, thick, spiniform, remaining three setae tridentate. Meso- and metatibiae, with a ventral pair of longitudinal rows of thin, dark setae along midline; meso- and metatarsi with a ventral pair of longitudinal rows with four simple spiniform setae. Tarsi 2-segmented, basal tarsomere shorter than distal tarsomere, with short setae; pretarsal claws long and thin (Figure 7B).

*Abdomen.* As in previous instar, except: Paraprocts with a basal ring of spiniform setae.

### F-11

*Head.* Anterior margin of frons with long, bristle-like setae (Figure 3E). Antennae 6-segmented, scape short and square, pedicel barrel-shaped, with a ring of four long setae at midlength, antennomeres 3-6 cylindrical, granulose, 3 and 4 with long, delicate setae on apical portion, 5 and 6 longer, beset with long setae, 6 distally acuminate (Figure 4D). A tuft of long, bristle-like setae on each cephalic lobe. Labium: Prementum longer than in F-12. Premental setae as in F-12. Ligula with 15 crenulations, seven on each side of the largest central crenulation, a spiniform setae between each crenulation, margin of central crenulation with seven tiny serrations of same size. Labial palp as in F-12; distal margin with six crenations, the first five dorsal crenations with a submarginal spiniform seta, the sixth crenation largest, with three short spiniform setae, the middle one slightly longer. Movable hook as in previous instar. Mandibular formula: R 123+4 y a b d / L 1234 0 a b, b>d>a (Figure 6A). Maxillae: Galeolacinia with four teeth, two ventral and two dorsal (Fig. 6C).

*Thorax.* Prothorax and mesothorax with a tuft of four long, thin setae on each side of midline. Metathorax with one long, thick, dark brown seta on each side of the midline. Wing rudiments appear for the first time, barely visible on meso- and metathorax (Figure 8A). Leg proportions as shown in Table 3. First pair of legs as in F-12. Mesofemora with 5–6 short, dorsal, spiniform setae, and very few thin setae. Mesotibiae as in F-12. Tarsi as in F-12; metatarsi longer than pro- and mesotarsi.

*Abdomen.* S4–9 with a row of 4–5 long setae on each side of midline on posterior margin, the two mesial setae longer, spiniform, and dark brown.

### F-10

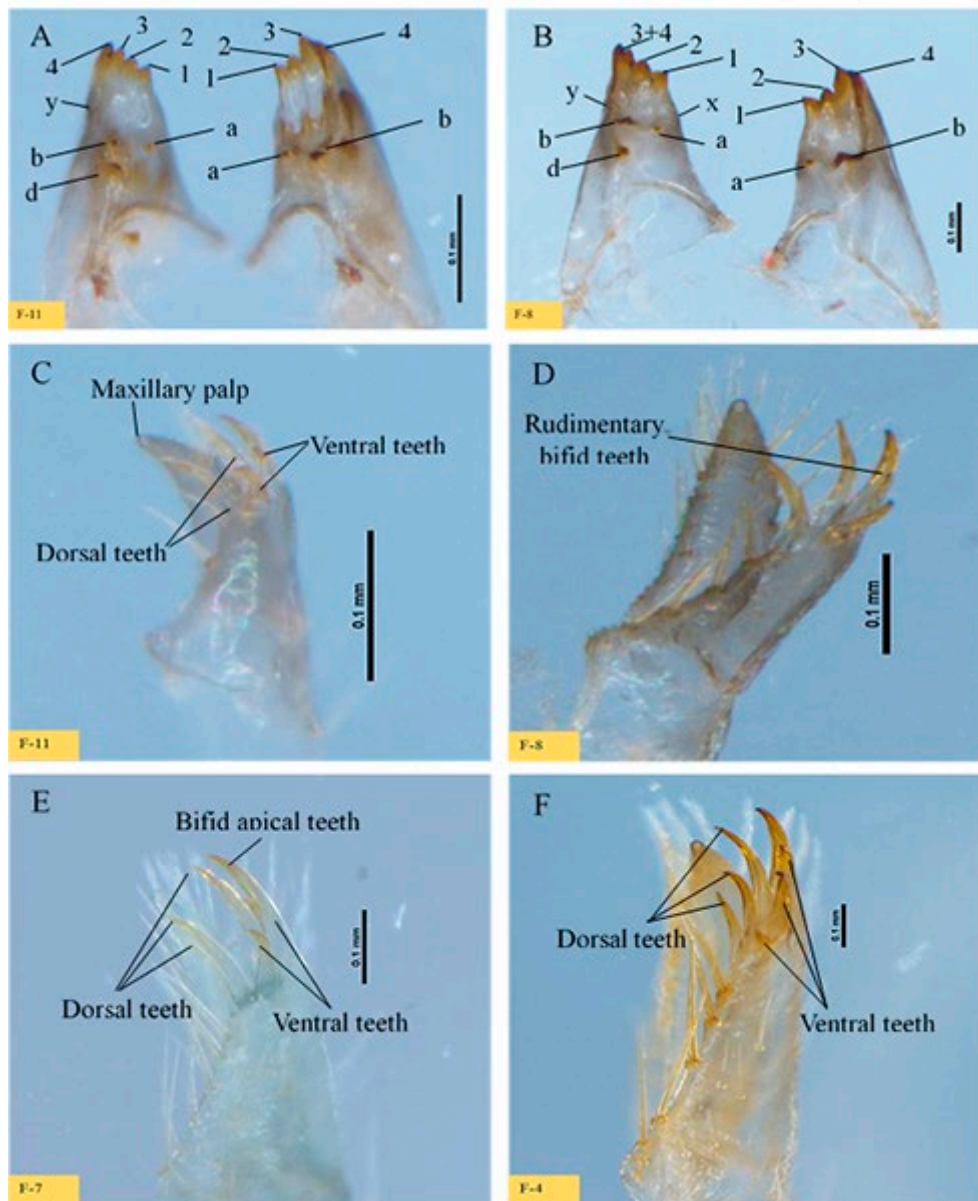
*Head.* As in F-11 except: A higher number of setae on frons, vertex and occiput (Figure 3F). Labium: Prementum with 5–6 setae on each side of midline. Ligula as in F-11. Labial palpi with five setae (Figure 5D).

*Thorax.* Pronotum with long and thin setae on lateral margins; meso- and metanotum as in previous instar. Wing sheaths divergent, larger and surpassing posterior margin of metathorax (Figure 8B). Legs: as in previous instar (Table 3) but becoming more setose.

*Abdomen.* As in previous instar. Epiproct darker apically.

### F-9

*Head.* (Figure 3G). The antennae in F-10 and F-9 has the same number of antennomeres as in F-11, with the exception scape cylindrical, smaller, pedicel almost spherical covered by long, very fine setae throughout its surface, 3<sup>rd</sup> antennomere longest, slightly narrowed towards base and apex, 4th antennomere shorter than 3<sup>rd</sup> and 5<sup>th</sup> antennomeres, 5th and 6th antennomeres covered by fine setae, darkened as in F-10, 6th antennomere granulose (Figure 3). Labium: Ligula with 17 crenulations, eight crenulations on each side of central one; the tiny serrulations on central crenulation slightly flattened (Figure 5E). Prementum with six long, thick, premental setae, the four most lateral ones longer than remaining two. Ligula as in F-10. Labial palp with six palpal setae; seven crenations on distal margin of palp, the six well-defined dorsal crenations of same size, a pair of spiniform setae on each crenation, the seventh and more ventral crenation largest, with four spiniform setae.



**Figure 6.** A) Left and right mandibles of F-11; B) Left and right mandibles of the F-8 with the mandibular formula fully-developed; C) F-11 maxilla showing two ventral teeth and two dorsal teeth; D) F-8 maxilla showing a small tooth at the base of the apical tooth; E) F-7 maxilla with two ventral teeth and three dorsal teeth; F) F-4 maxilla fully-developed with three ventral teeth and three dorsal teeth.

*Thorax.* Prothorax with 3–4 long, robust, dark brown setae on ventral margin. Anterior and posterior wing sheaths converging, posterior ones with a tuft of long, robust, dark brown setae on ventral margin (Figure 8C). Legs: Latero-distal surface of profemora with two long and thin setae followed by shorter and more randomly distributed setae; mesial surface entirely covered by long and fine setae. Protibiae with two rows of long and thin setae on lateral surface, thicker in the middle part; mesial surface with two rows of setae, distal end with three, short, tridentate setae. Protarsi with delicate setae on lateral surface, spiniform setae on mesodistal area. Mesofemora same as profemora. Mesotibiae with a pair of bidentate setae on distal margin. Metalegs same as mesolegs.

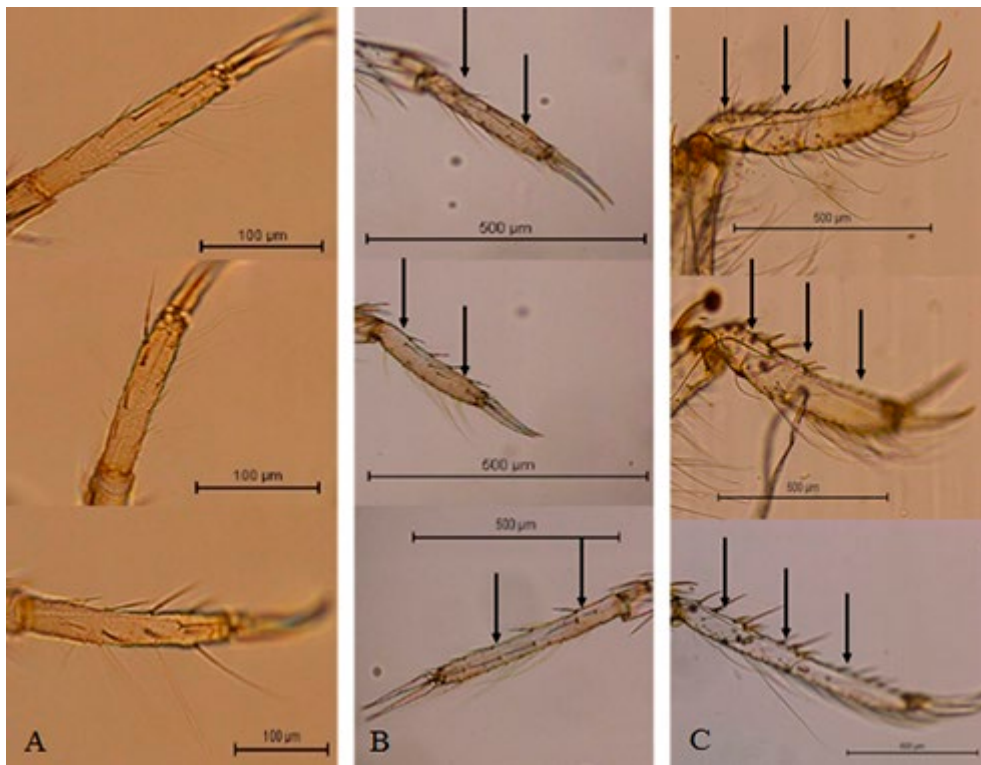
*Abdomen.* As in F-10.

**F-8**

**Head.** (Figure 3H). Antennae 7-segmented (Figure 4E). Cephalic lobes with very long, dark setae intermingled with shorter and stouter setae, longer setae reach anterior margin of prothorax. Labium: Premental setae as in F-9. Ligula with 21 or 23 crenulations, 10 or 11 crenulations on each side of midline, serrations on central crenulation completely fused. Labial palp same as F-9. Mandibles: R 123 + 4 xy abd / L 1234 0 ab, b > d > a. Maxillae: as F-11, except for a bifid rudiment in apical tooth (Fig. 6D).

**Thorax.** Propleuron with a row of setae on anterior margin; pronotum with short, dark setae on midline that increase in size towards lateral margins; a transverse tuft of long, robust setae on middle area; four small setae on each side of midline, a pair of erect setae on central part; posterior margin with three long, thin, dark brown setae directed towards mesothorax. Anterior wing sheaths 0.16 mm length, with basal, very short, dark setae; posterior wing sheaths 0.22 mm length (Figure 8D). A patch of long, thin setae covering apical part of posterior wing sheath. Tarsi with three tarsomeres, basal tarsomere longest, distal tarsomere slightly longer than middle tarsomere (Table 3) (Figure 7C).

**Abdomen.** A brown pattern becomes evident on middle portion of S6–9.



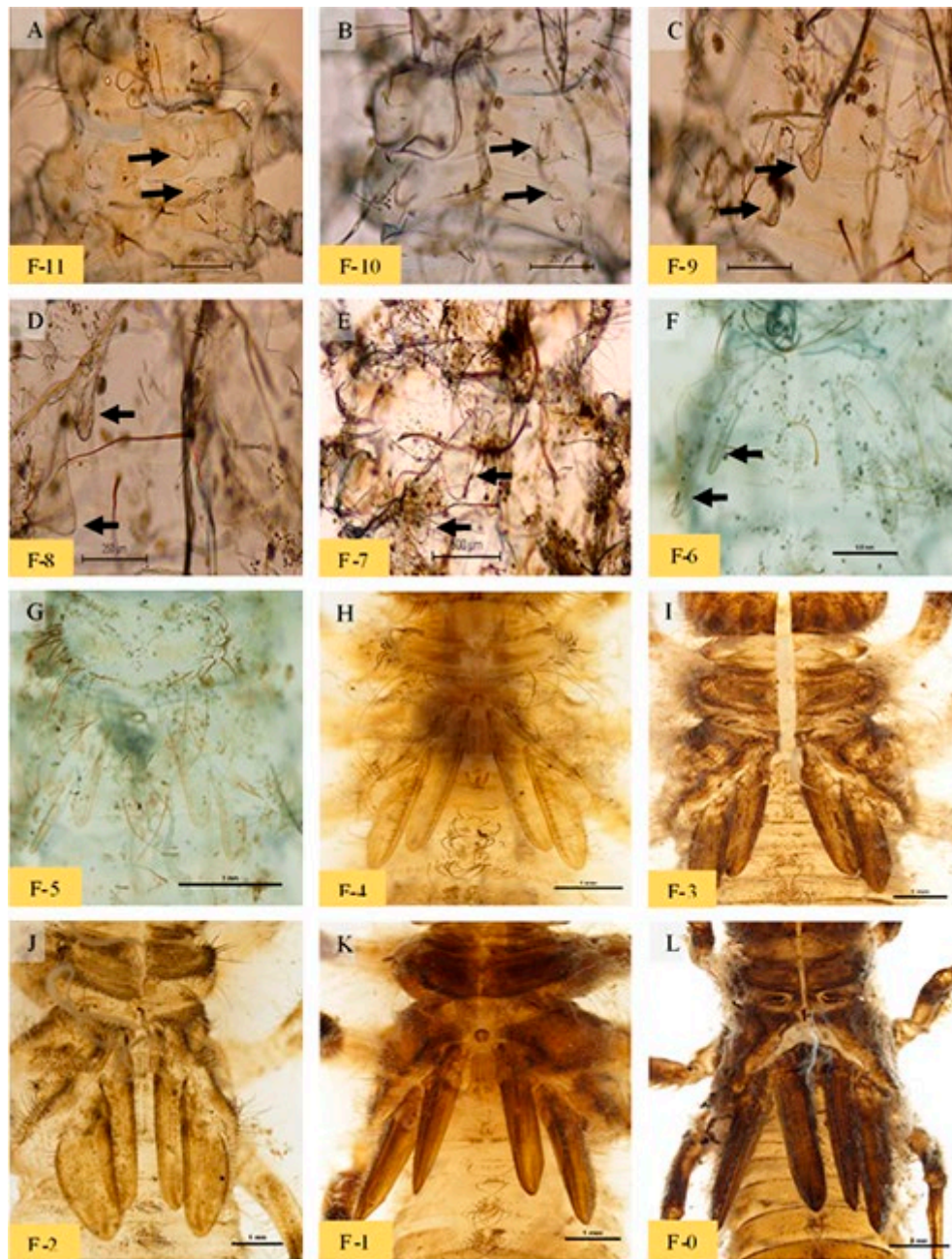
**Figure 7.** Divisions of the tarsi; A) Tarsi unisegmented from F-15 to F-11; B) tarsi bi-segmented from F-12 to F-9; C) tarsi tri-segmented from F-8 to F-0.

**F-7**

**Head:** (Figure 3I). Prementum with 8–9 setae (Figure 5F). Ligula as in F-8. Labial palp with six palpal setae; distal margin with seven, well-differentiated crenations; internal margin with 6–7 spini-form setae. Maxillae: Galeolacinia with five teeth, two ventral and three dorsal teeth, ventroapical tooth bidentate (Figure 6E).



*Thorax*. Pronotum as in previous instar. Anterior and posterior wing sheaths 0.58 mm length, (Figure 8E). A patch of long, thin setae covering anterior wing sheaths (Figure 8E). Legs: as previous instar (Table 3).



**Figure 8.** Details of wing sheaths since their appearance in F-11 as wing buds and until the last instar, the arrows point to the divergent wing sheaths.

*Abdomen*. There is a higher number of long, thin setae throughout abdomen. Setae cover lateral margins of all segments; ventral setae on S9 covering S10 and half of anal pyramid. S1–10 with a row of spiniform, short setae on distal margins.



**F-6**

*Head.* Occiput with long, thin, spiniform setae reaching anterior margin of pronotum (Figure 3J). Labium: Prementum with 10 setae on each side of midline. Labial palp with eight palpal setae, distal margin with five crenations, first four dorsal crenations with two spiniform setae of different sizes each, fifth crenation with three short spiniform setae (Figure 5G). In dorsal view, base of palp's articulation with a pair of very small spiniform setae. Maxillae: Galeolacinia as in previous instar.

*Thorax.* Pronotum as in previous instar. Wing sheaths convergent (Figure 8F), with short spiniform setae on margins. A patch of long, thin setae on each side of metanotum covering dorsal margin of posterior wing sheaths.

*Abdomen.* A posterolateral spine appears on S8–9. Epiproct and paraprocts of same size, with light brown sharply pointed apex. Cerci become apparent, very short and hard to see because epiproct covers them.

**F-5**

*Head.* Antennal scape trapezoid, a row of fine, long setae on apical region directed towards pedicel, pedicel cylindrical with long, fine setae throughout, flagellomeres covered by long, thin setae. Three tufts of 4–5 setae on vertex: one anterior, one median, and one posterior (Figure 3K). Ventral margin of compound eyes with long, very fine, whitish setae. Cephalic lobes same as previous instar, but with short, spiniform setae on midline. Labium: Prementum with 8–9 long setae on each side of midline. Ligula: with 23 crenulations, 11 on each side of the central crenulation (Figure 5G), dorsal area of large central crenulation swollen, rough. In ventral view, base of prementum with a tuft of very long, fine, straight setae on each side. Labial palp with eight long setae; distal margin with eight crenations, first four dorsal crenations with three spiniform setae, remaining with two spiniform setae.

*Thorax.* Pronotum with a rectangular shield of setae in the medially but leaving a bare space in the center, anterior margin with spiniform setae, lateral margins with long, thin setae, same as those on posterior margin, both projected towards the back of body (Figure 8G). Anterior wing sheaths reaching posterior margin of metanotum (Figure 8G). Legs: as in F-6 (Table 3).

*Abdomen.* As in F-6 except: S9–10 with a pair of spots on midline darker than those on previous segments. Anal pyramid as in F-6.

**F-4**

*Head.* Vertex with a rectangular, dark brown spot. Occiput with three dark bare spots, one on midline, one on each side of central one (Figure 3L). Labium: Prementum with 9–10 long setae. Labial palp same as F-5. (Figure 5H). Maxillae: Galeolacinia with six teeth, three ventral and three dorsal teeth, ventroapical tooth bidentate (Figure 6F).

*Thorax.* Anterior and posterior wing sheaths reach basal half and posterior margin of S3, respectively (Figure 8H). Legs as in F-5.

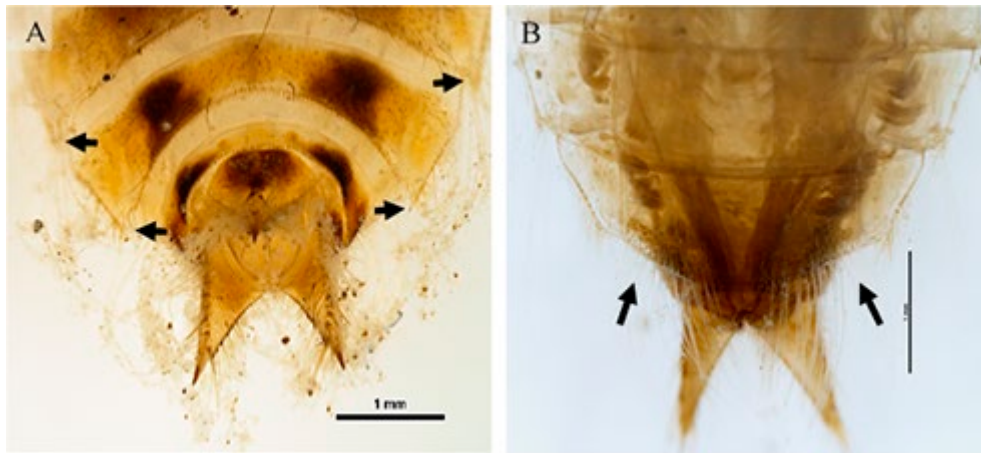
*Abdomen.* As in F-5. Pattern of spots in S9–10 darker, S8 becomes darker as in S9 and S10. Epiproct with a small dorsal, dark brown spot.

**F-3**

*Head.* Vertex darker than in previous instars. Occiput with 9 visible spots, one in midline of head and four to each side of the first (Figure 3M). Labium: prementum with 11 long setae on each side of the midline (Figure 5I).

*Thorax.* (Figure 8I). Legs more setose on dorsal surface than in previous instars. Fore- and mesolegs with distal tarsomere longest, basal tarsomere in metalegs longest (Table 3).

*Abdomen.* S8–10 with a dark brown spot on each side of midline (Figure 9A), remaining of these segments light brown. S6–10 darker than S1–5, in ventral view, a row of long setae on distal margin of S10 with the same length as paraprocts (Figure 9B).



**Figure 9.** A) Dorsal view of S9–10 and anal pyramid showing dark spots, arrows indicate lateral spines on S8–9; B) Ventral view of S9–10 and anal pyramid, the arrows indicate the detail of long and fine setae in S9 that reach the tip of paraprocts.

## F-2

*Head.* Antennal scape with a lateral brown spot, scape and pedicel darker than flagellomeres, covered entirely by fine setae. In dorsal view, a trapezoid dark spot on vertex reaching anterior margin of head towards base of eyes. Occiput spots like in previous instar. A vertical dark line on each side of head just below the eyes is accompanied by a pair of vertically oriented parallel rows of dots (Figure 3N).

*Thorax.* Pronotal disc oval, anterior margin covered by short, spiniform setae, lateral margins bulging, protruding as a crest, covered by spiniform setae and some long, thin setae, posterior margin equal to anterior one; with a crest-shaped structure on lateral margins of pronotum (Figure 8J).

*Abdomen.* Same as in F-3, except: S7 darker.

## F-1

*Head.* (Figure 3O). Labium: Lateral margins of prementum thickened, dark. Ligula and labial palp as in F-2.

*Thorax.* Anterior and posterior wing sheaths reaching basal half and posterior margin of S4, respectively (Figure 8K). Legs more setose than in previous instars (Table 3).

*Abdomen.* Same as in F-2, except: S5-6 and anal pyramid darker.

## F-0

*Head.* (Figure 3p). Labium: 11 long setae on each side of prementum. Eight palpal setae; distal and ventral margins of palp as in F-1.

*Thorax.* Anterior and posterior wing sheaths reaching basal half and posterior margin of S5 (Figure 8L).

*Abdomen.* Same as in F-1. A detailed description of this instar is given in Carrillo-Lara & Novelo-Gutiérrez (2018).

## Discussion

The present work increases the knowledge of life cycles of Mexican odonates to two species, and to 45 species for the family Libellulidae worldwide. According to Corbet (2002), life cycles of 44 species of Libellulidae have been described; however, not all studies included detailed descriptions of the morphological characters of each larval instar, and indeed some reports only indicated duration ranges among instars.

### Total duration

The number of days that some members of the family Libellulidae take to complete their life cycle ranges from a minimum of 109 days (about three and a half months) in species such as *Trithemis festiva* (Rambur, 1842) (Kumar, 1972), to a maximum of 235 days (nearly eight months) in *Orthetrum brunneum* (Fonscolombe, 1837) (Kumar, 1971), followed by *Orthemis ferruginea*, the species studied in this work, with an average of 186 days (just over six months) from instar F-16 (prolarva) to F-0. In this study, we observed 17 larval instars in *O. ferruginea*, one in addition to those recorded in the compilation by Corbet (2002), where the minimum number for the family Libellulidae is eight for *Crocothemis erythraea* (Brullé, 1832) and the maximum is 16 for the genus *Orthetrum*. Initially, we speculated that the total duration of the life cycle of *O. ferruginea* would be four months, considering its preference for ephemeral environments and the fatal consequences that the organisms living in them could face, such as mortality before completing their life cycle because of dry condition. An additional fact we considered was that these organisms fly practically all year round. In this work, under controlled laboratory conditions (26-30°C), in rearing chambers and with provided food, our initial hypothesis of the time needed to complete the life cycle in this species was not supported. Mathavan (1990) conducted experiments with two dragonfly species, *Brachytemis contaminata* (Fabricius 1793) and *Orthetrum sabina* (Drury, 1770), rearing the former at 27°C and the latter at 37°C. In both species, the 10°C increase resulted in an additional larval instar; this suggests that temperature might be the main factor influencing the number of instars observed in *O. ferruginea*. Bick (1941) and Painter *et al.* (1996) studied the life cycle of *Erythemis simplicicollis* (Say, 1839). In the first study Bick pointed out that the complete life cycle (13 instars) was completed in 109 days, contrasting with the 59 days that took the Painter's *et al.* experiment. The difference in life cycle length may be related to rearing conditions and diet offered during development.

### Duration of individual instars

Average number of days per instar was highly variable in *O. ferruginea* and differed from the patterns described for other odonate species. However, instar the time elapsed from collection of eggs to hatching of first prolarvae fell within the period already documented for other libellulid species,

i.e., from four days on average for *Pantala flavescens* (Fabricius 1798) up to 12 days for *Pseudorthemis zonata* (Burmeister, 1839) (Shimura, 2006). Eggs of *Orthetrum japonicum* (Wain, 1858), *O. triangulare* (Selys, 1878), and *Lyriothemis pachygastra* (Selys, 1878) required eight days to hatch (Shimura, 2006), sharing this feature with *O. ferruginea*. Prolarvae (F-16) of *O. ferruginea* took one to three minutes to attain larval instar 2 (F-15), a relatively short time compared to other species of Libellulidae, ranging from 2.5 minutes in *P. flavescens* to 11 minutes in *L. pachygastra* and *O. triangulare* (Shimura, 2006). For the rest of the instars, no such close matches were observed in the duration of each instar compared to those described for this family. In the majority of species with a known duration of each larval instar there is a noticeable increase in the length of each instar in number of days as larval development progresses; accordingly, early instars last from a few days to just over one week, while instars more advanced in development may double this duration instars. The same pattern was observed in 11<sup>th</sup> instar (F-4) for Batch I and 12<sup>th</sup> instar (F-5) for Batch II in *O. ferruginea*. However, instars F-13 to F-17 displayed a very similar duration, even being shorter than some of the previous instars. This may be explained by the different temperature conditions to which lab-reared larvae were exposed, during egg stage (26°C), and for wild larvae during F-13 and subsequently (30°C). Seemingly, this 4°C difference may have caused the reduction of the duration of each larval instar in wild larvae.

Considering the above, the controlled conditions (temperature and food supply) during incubation of eggs and rearing of larvae were the key factors affecting life cycle duration. Surface and bottom temperature in the dam at the time of copula always 35°C, while controlled temperature in the lab never exceeded 30°C. Larvae rearing in the laboratory was initially planned to take place at room temperature, i.e., between 18°C and 20°C; however, mortality in Batch I for instar F-15 was too high, with only 26 individuals surviving out of the 1528 larvae that originally hatched. Consequently, the rearing experiment continued under a controlled temperature of 26°C to prevent further deaths from low temperatures. Along with other factors, low temperatures during incubation of eggs and the rearing of larvae lead to higher mortality and longer duration of embryonic and larval development instars in other studies (Stoks & Córdoba-Aguilar, 2012; Ward, 1992). Many studies addressing embryonic development of eggs in aquatic insects have been carried out under controlled constant temperature. We believe that hatching of eggs would have occurred earlier if daily variations in temperature more closely resembled those in their natural habitats (Ward, 1992). Eggs collected in Batch II were maintained under a controlled temperature of 26°C since day 1, thus preventing a high mortality rate in instar F-15. This temperature was optimal for larval survival; however, no individual managed to complete the larval cycle. Both batches took over four months to reach instars F-5 and F-4, probably affected by temperature again. Wild larvae were kept at a constant temperature of 30°C and completed the remainder of their larval cycle in two months. However, their food intake may have been insufficient for them to survive, resulting in just four out of 62 collected larvae reaching the adult stage. Both egg batches (I and II) took eight days to hatch from the date of collection. Other studies reported that time elapsed from collection to hatching of prolarva ranges from seven to 22 days in dragonfly species inhabiting tropical or subtropical areas (India and southern United States of America), and up to 57 and 58 days in temperate areas, as in the case of species living in northern United States (Bick, 1941; Krull, 1929; Kumar, 1971, 1972; Nevin, 1980). Size of food supplied during rearing of larvae was apparently optimal for growth; the small size of *Artemia franciscana* nauplii allowed proper consumption by small instar larvae. Also, size of chironomid larvae and culicids (five to six dipteran larvae per container) suited the more advanced instars of both batches and wild larvae (Blois, 1985). Some studies suggest that size of predators and prey is positively correlated Córdoba-Aguilar & Lee, 1994). It is well known that some members of the order Anisoptera are generalist consumers, and the broad range of prey that they can consume probably enables them to survive under various conditions, including laboratory environments (Anderson & Cummins, 1979; Pritchard, 1964). Although food quality is not a major factor in the development of *O. ferruginea*, it seems that feeding frequency and food availability do influence larval development. Córdoba-Aguilar and Lee (1994) report that *O. ferruginea* consumed an average of 25 mosquito larvae of different larval instars in five to 15 minutes; therefore, five or six larvae twice per day may lead to impaired instar development and cause an increase in the



number of days throughout development. As in *E. simplicicollis*, the amount of days that Bick (1941) reported was the double of Painter *et al.* 1996 mentioned in their work. The difference between both works was the quantity of preys that they offered of each organism. Environmental conditions affect instar development and larval mortality rates, thus influencing fitness in the adult stage; similarly, food shortage exerts a negative impact on the life cycle of odonates. In other odonate species, it has been observed that field-reared larvae under high population densities or low food availability show delayed emergence, with a body mass index far lower than well-fed larvae (Anholt, 1990). Some living conditions of larvae reduce body mass and storage of energy used for emergence, which have been associated with survival of maturing adults (Anholt, 1991). This is related to the fact that poor energy storage during larval stage will lead to alterations of defense mechanisms such as flight, as well as of mating fitness of sexually mature males (Stoks & Córdoba-Aguilar, 2012). These variables not only define the behavior of larvae to effectively face any challenges and survive, but also determine shape and size of individuals, enabling them to better adapt to their environment and, therefore, survive under these conditions (Ward, 1992).

### Description of larvae

With regards to the characteristics of each stage, appearance or development of structures in each tagma differ with each instar or group of instars. Egg traits are identical to those of other members of the family, with eggs always longer than wide (oval). However, *O. ferruginea* lays smaller eggs, similar in size to those of *Erythrodiplax minuscula* (Rambur, 1842) and *Ladona deplanta* (Rambur, 1842) (Ivey *et al.*, 1988). Conventional preparation and mounting technique for observation under a scanning electron microscope (SEM) revealed no apparent pattern in egg exochorion; endochorion was observed as a smooth layer throughout the egg, devoid of any other type of structure. Characteristics of the prolarval stage are poorly known and insufficient to perform a morphological or morphometric comparison; however, the protruding “horn-like” structure observed in this study is also present in other Libellulidae prolarvae (Shimura, 2006). Studies on other members of the family Libellulidae have shown seven final antennal segments in species like *Erythemis simplicicollis* (Selys, 1839) (Bick, 1941), *Orthetrum brunneum* (Fonscolombe, 1837) (Kumar, 1971), *Trithemis festiva* (Rambur, 1842) (Kumar, 1972), *Sympetrum vicinum* (Hagen, 1861) (Nevin, 1890), and *Diplacodes bipunctata* (Brauer, 1865) (Rowe, 1992). When the prolarva hatches it has three antennomeres, which are maintained until instars F-14 or F-13. In all these species, including *O. ferruginea*, the instar four final antennomeres are added one at time until instar F-8, and that number is maintained until adult emergence. In the last instar, *O. ferruginea* had a total of 11 setae on prementum and eight on each labial palp. *T. festiva* shares the number of premental setae with *O. ferruginea*; *Orthetrum brunneum* shows 22 and *D. bipunctata* 12 to 16. There are six to nine palpal setae in *E. simplicicollis*, *O. brunneum*, *T. festiva* and *S. vicinum*, and nine to 11 in *D. bipunctata*. Another change noted throughout the development of *O. ferruginea* was the division of the tarsus from one into three tarsomeres. In general, the tarsus in other Libellulidae species remains as a single structure from the second larval instar (F-15) through F-13; however, it can also divide into two tarsomeres in F-14 or more advanced instars such as F-10. In most Libellulidae species, the third and last tarsal division occurs between instars F-12 and F-11. In *O. ferruginea*, the tarsus was divided into two tarsomeres up until F-12 or F-13, and the third tarsomeres was added in later instars. Development of wing primordia is another distinctive feature in the description of life cycles. Most studies describing full cycles for the family state that wing primordia are apparent from F-10 in *E. simplicicollis*, *Diplacodes haematodes*, (Burmeister, 1839) (Hawking & New, 1996), *D. bipunctata*, *S. obtrusum*, and *O. brunneum*. Other reports indicate that wing primordia are first evident from the sixth larval stage as small protuberances that will evolve to be wing sheaths in *T. festiva* and *S. vicinum*, coinciding with *O. ferruginea*. Length of anterior wing sheaths is used as a diagnostic trait. These reached mid-length of abdominal S5 in F-0 of *O. ferruginea*, like in some other Libellulidae species, although in others they reached posterior margin of S6.

Within the genus *Orthemis* it is possible to make comparisons of only some structures with those in *Orthemis nodiplaga* Karsch, 1981 (Rodrigues Capítulo & Muzón, 1990). Meristic information for the different instars show that larvae of *O. ferruginea* are larger overall and that proportions reflect a non-cylindrical body. Some other morphological characteristics can be highlighted in different tagmata, such as absence of incisor 2 in the mandibular formula of *O. ferruginea*, contrary to what was mentioned by Watson (1956). It should be clarified that this can only be compared in the last instar of both species but not for the younger instars. A detailed comparison of the last instar can be consulted in Carrillo-Lara and Novelo-Gutiérrez (2018). For very young instars, setae on palps begin with one in *O. ferruginea* (F-15) and two in *O. nodiplaga* (F-10). There are three in the prementum *O. nodiplaga* (F-9) and one in *O. ferruginea* (F-14); these two instars are comparable because with 10 instars reported for *O. nodiplaga* (Rodrigues-Capítulo & Muzón, 1990) both represent the second instar. Wing pads appear in F-7 in *O. nodiplaga* and in F-11 in *O. ferruginea*. Furthermore, the first division of tarsomeres in the three pairs of legs occurs in F-7 and the second division in F-5 in *O. nodiplaga*, while in *O. ferruginea* it takes place in F-11 and F-8 respectively although sometimes third tarsomeres already present in the three pre-emergence instars in both species.

**Table 4. Wing sheaths length of *Orthemis ferruginea* (mm).**

Instar	F-11	F-10	F-9	F-8	F-7	F-6	F-5	F-4	F-3	F-2	F-1	F-0
Anterior	0.05	0.13	0.34	0.16	0.58	0.77	1.49	2.87	3.21	3.79	3.46	7.5
Posterior	0.05	0.18	0.17	0.22	0.58	0.69	1.34	2.59	3.00	3.21	3.21	7.47

**Table 5. Average length dimensions of body and tagmata of *Orthemis ferruginea* (mm ± SD).**

Instar		F-15	F-14	F-13	F-12	F-11	F-10	F-9	F-8	F-7	F-6	F-5	F-4	F-3	F-2	F-1	F-0
	<i>n</i>	40	37	39	48	37	36	28	28	27	26	25	12	4	4	4	4
<b>Total length</b>	X	1.28	1.53	1.97	2.54	3.08	3.69	4.64	5.49	7.09	8.3	11.57	12.89	17.29	18.54	21.54	23.92
	SD	0.05	0.16	0.14	0.25	0.22	0.26	0.29	0.22	0.75	0.26	0.46	0.21	0.25	0.16	0.16	0.59
<b>Head</b>																	
Width	X	0.32	0.37	0.49	0.63	0.74	0.89	1.11	1.34	1.71	1.92	2.61	3.44	3.63	3.67	4.75	5.17
	SD	0.01	0.04	0.03	0.06	0.05	0.05	0.07	0.07	0.19	0.08	0.12	0.08	0.08	0.16	0.17	0.19
Length	X	0.25	0.28	0.37	0.47	0.58	0.66	0.84	1.08	1.26	1.33	1.95	2.5	2.56	3.13	3.13	3.54
	SD	0.02	0.03	0.03	0.07	0.07	0.05	0.12	0.06	0.08	0.03	0.09	0.11	0.01	0.08	0.08	0.16
<b>Thorax</b>																	
Width	X	0.33	0.4	0.51	0.69	0.85	0.99	1.33	1.56	2.1	2.45	3.3	3.37	3.79	4.17	5.79	5.75
	SD	0.02	0.03	0.03	0.07	0.09	0.09	0.09	0.07	0.25	0.15	0.25	0.08	0.08	0.01	0.08	0.1
Length	X	0.27	0.33	0.43	0.55	0.67	0.86	1.22	1.29	1.73	2.08	3.01	3.5	3.67	4.54	5.37	6.29
	SD	0.02	0.03	0.05	0.06	0.08	0.12	0.13	0.1	0.3	0.08	0.3	0.08	0.14	0.08	0.08	0.44
<b>Abdomen</b>																	
Width	X	0.34	0.43	0.66	0.87	1.15	1.34	1.66	1.94	2.49	2.84	3.74	4.92	5.04	5.29	6.09	6.25
	SD	0.02	0.05	0.06	0.22	0.11	0.13	0.14	0.07	0.29	0.15	0.19	0.11	0.08	0.08	0.17	0.1
Length	X	0.58	0.7	0.93	1.17	1.45	1.7	2.0	2.48	3.23	3.86	5.13	5.81	8.25	8.25	11.17	11.67
	SD	0.04	0.06	0.12	0.22	0.14	0.16	0.2	0.18	0.45	0.29	0.35	0.1	0.17	0.17	0.14	0.01

**Table 6. Main changes throughout the life cycle of *Orthemis ferruginea*.**

Instar	Head								Thorax					Abdomen			
	#AS	PRS	#LiCr	LPS	#LPCr	MaxT		Mandibular formula	BMT	WP		Tarsomeres			LS	Cercus	ACP
						V	D			A	P	1°	2°	3°			
F-15	3	0	0	0	5							x					
F-14		1	8	1													
F-13	4	3	5	3 or 4	6												
F-12	5	5		4				L 1 2 3 4 0 a b / R 1 2 3 4 0 a b d, a=b=d									
F-11	6		6 or 7			2	2	L 1 2 3 4 0 a b / R 1 2 3+4 0 a b d, b>d>a		R	R		x				
F-10		5 or 6	7 or 8	5													
F-9		6	8	6	7												
F-8	7							L 1 2 3 4 0 a b / R 1 2 3+4 x y a b d, b>d>a.						x			x
F-7		8 or 9				2	3		x								
F-6				8											x		
F-5			11		8												
F-4		9 or 10				3	3			1/2 S3	S3					x	
F-3		11															
F-2																	
F-1										1/2 S4	S4						
F-0										1/2 S5	S5						

#AS: number of antennomeres

MaxT: maxillary teeth; V: ventral D: dorsal

PRS: premental setae

BMT: bidental maxillary tooth

#LiCr: number of crenulations on ligula

WP: wing pads; A: anterior P: posterior

LPS: labial palp setae

LS: lateral spines on S8-9

#LPCr: number of labial palp crenulations

ACP: abdominal color pattern of S8-10

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## References

- Anderson, N. H. & Cummins, K. W. (1979). Influences of diet on the life histories of aquatic insects. *Journal of the Fisheries Research Board of Canada*, 36: 335–342. <https://doi.org/10.1139/f79-052>
- Anholt, B. R. (1990). An experimental separation of interference and exploitative competition in a larval damselfly. *Ecology*, 71: 1483–1493. <https://doi.org/10.2307/1938285>
- Anholt, B. R. (1991). Measuring selection on a population of damselflies with a manipulated phenotype. *Evolution*, 45: 1091–1106. <https://doi.org/10.1111/j.1558-5646.1991.tb04377.x>
- Arango, M. C. & Roldán, G. (1983). Odonatos inmaduros del Departamento de Antioquia en diferentes pisos altitudinales. *Actualidades Biológicas*, 12: 91–105.
- Audisio, P. (2017). Insect taxonomy, biodiversity research and the new taxonomic impediments. *Fragmenta Entomologica*, 49: 121–124. <https://doi.org/10.4081/fe.2017.252>
- Bick, G. H. (1941). Life-history of the dragonfly, *Erythemis simplicicollis* (Say). *Annals of the Entomological Society of America*, 34: 215–230. <https://doi.org/10.1093/aesa/34.1.215>
- Blois, C. (1985). The larval diet of three anisopteran (Odonata) species. *Freshwater Biology*, 15: 505–514. <https://doi.org/10.1111/j.1365-2427.1985.tb00220.x>
- Carrillo-Lara, D. E. & Novelo-Gutiérrez, R. (2018). Description of the larva of *Orthemis ferruginea* (Fabricius, 1775) (Odonata: Libellulidae). *Zootaxa*, 4455 3: 547–554. <https://doi.org/10.11646/zootaxa.4455.3.10>
- Carvalho, A. D. L. & Werneck-De- Carvalho, P. C. (2005). Descrição da larva de *Orthemis cultriformis* Calvert, 1899 (Insecta, Odonata, Libellulidae). *Arquivos do Museu Nacional*, 63: 267–273.
- Corbet, P. S. (1953). A terminology for the labium of larval Odonata. *The Entomologist*, 86: 191–196.
- Corbet, P. S. (2002). Instars and growth ratios of odonata: A review. *International Journal of Odonatology*, 5: 45–73. <https://doi.org/10.1080/13887890.2002.9748176>
- Córdoba-Aguilar, A. & Lee, M. (1994). Prey Selection by *Orthemis ferruginea* (Fabricius) larvae (Odonata: Libellulidae) over mosquito instars. *Folia Entomologica Mexicana*, 91: 23–30.
- De Marmels J. (1988). Odonata del Estado Táchira. *Revista Científica Unet*, 2: 91–111.
- Donnelly, T. W. (1995). *Orthemis ferruginea*—an adventure in Caribbean biogeography. *Argia*, 7(4), 9–12.
- Ebach, M. C., Valdecasas, A. G. & Wheeler, Q. D. (2011). Impediments to taxonomy and users of taxonomy: accessibility and impact evaluation. *Cladistics*, 27: 550–557. <https://doi.org/10.1111/j.1096-0031.2011.00348.x>
- Evenhuis, N. L. (2007). Comments on “Helping solve the ‘other’ taxonomic impediment: Completing the Eight Steps to Total Enlightenment and Taxonomic Nirvana.” *Zootaxa*, 12: 3–12. <https://doi.org/10.11646/zootaxa.1494.1.3>
- González-Soriano, E. & Novelo-Gutiérrez, R. (2014). Biodiversidad de Odonata en México. *Revista Mexicana de Biodiversidad*, 85: 243–251. <https://doi.org/10.7550/rmb.34716>
- Hawking, J. H. & New, T. R. (1996). The development of dragonfly larvae (Odonata: Anisoptera) from two streams in north-eastern Victoria, Australia. *Hydrobiologia*, 317. <https://doi.org/10.1007/BF00013722>
- Ivey, R. K., Bailey, J. C., Stark, B. P. & Lentz, D. L. (1988). A preliminary report of egg chorion features in dragonflies (Anisoptera). *Odonatologica*, 17: 393–399.
- Johnson, D. M. (1991). Behavioral ecology of larval damselflies and dragonflies. *Tree*, 6: 8–13. doi: [https://doi.org/10.1016/0169-5347\(91\)90140-S](https://doi.org/10.1016/0169-5347(91)90140-S)
- Krull, W. H. (1929). The rearing of dragonflies from eggs. *Annals of the Entomological Society of America*, 22: 651–658. <https://doi.org/10.1093/aesa/22.4.651>
- Kumar, A. (1971). The larval stages of *Orthetrum brunneum brunneum* (Fonscolombe) with a description of the last instar larva of *Orthetrum taeniolum* (Schneider) (Odonata: Libellulidae). *Journal of Natural History*, 5: 121–132. <https://doi.org/10.1080/00222933.1971.10309717>
- Kumar, A. (1972). Studies on the life history of *Trithemis festiva* (Rambur, 1842) (Odonata: Libellulidae). *Odonatologica*, 1: 103–112.
- Mathavan, S. (1990). Effect of temperature on the bio-energetics of the larvae of *Brachythemis contaminata* (Fabricius) and *Orthetrum sabina* (Drury) (Anisoptera : Libellulidae). *Odonatologica*, 19: 153–165.

- Needham, J.G. (1904). New dragon-fly nymphs in the United States National Museum. Proceedings of the United States National Museum, 27, 685–720. <https://doi.org/10.5479/si.00963801.27-1371.685>
- Nevin, F.R. (1890). Larval development of *Sympetrum vicinum* (Odonata: Libellulidae: Sympetrini). *Transactions of the American Entomological Society*, 55: 79–102.
- Novelo-Gutiérrez, R. & González-Soriano, E. (1991). Odonata de la Reserva de la Biosfera de La Michilía, Durango, México. Parte II. Náyades. *Folia Entomológica Mexicana*, 81:107–164.
- Painter M. K., K. Tennessen & T. D. Richardson (1996). Effects of repeated applications of *Bacillus thuringiensis israelensis* on the mosquito predator *Erythemis simplicicollis* (Odonata: Libellulidae) from hatching to final instar. *Environmental Entomology* 25 (1): 184–191. <https://doi.org/10.1093/ee/25.1.184>
- Paulson D. (1998). The distribution and relative abundance of the sibling species *Orthemis ferruginea* (Fabricius, 1775) and *O. discolor* (Burmesister, 1839) in North and Middle America (Anisoptera: Libellulidae). *International Journal of Odonatology*, 1 (1): 89–93.
- Paulson D. & M. Schorr (2021). World Odonata List. <https://www.pugetsound.edu/academics/academic-resources/slater-museum/biodiversity-resources/dragonflies/world-odonata-list2/> last revision: 10 September 2021.
- Pritchard, G. (1964). The prey of dragonfly larvae (Odonata; Anisoptera) in ponds in Northern Alberta. *Canadian Journal of Zoology* 42: 785–800. <https://doi.org/10.1139/z64-076>
- Ramírez, A. (2010). Odonata. *Revista de Biología Tropical*, 58: 97–136.
- Ramos-García, L. 2016. Ciclo de vida de *Perithemis mooma* Kirby, 1889. Master Thesis, INECOL, A. C. Xalapa, Veracruz, Mexico.
- Rodrigues-Capítulo, A. & Muzón, J. (1990). The larval instars of *Orthemis nodiplaga* Karsch, 1981 from Argentina (Anisoptera: Libellulidae). *Odonatologica* 19 (3): 283–291.
- Rowe, R. J. (1992). Larval development in *Diplacodes bipunctata* (Brauer) (Odonata: Libellulidae). *Journal of Australian Entomological Society*, 31: 351–355. <https://doi.org/10.1111/j.1440-6055.1992.tb00525.x>
- Shimura, S. (2006). Eggs of dragonflies recorded in Kinki District (1) Zygoptera and Anisozygoptera. *Sympetrum Hyogo*, 9: 3–14. <https://doi.org/10.2108/zs170051>
- Stoks, R. & Córdoba-Aguilar, A. (2012). Evolutionary Ecology of Odonata: A Complex Life Cycle Perspective. *Annual Review of Entomology*, 57, 249–265. <https://doi.org/10.1146/annurev-ento-120710-100557>
- Ward, J. V (1992). Aquatic insect ecology. 1. Ecology and habitat. John Wiley & Sons, Inc., New York, USA.
- Watson, M. C. (1956). The utilization of mandibular armature in taxonomic studies of anisopterous nymphs. *Transactions of American Entomological Society*, 81:155–209.