Morphometry of eggs and immatures of *Culex* (*Culex*) *saltanensis* Dyar (Diptera, Culicidae) obtained in the laboratory and on the field

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ABSTRACT. The objectives of this morphometric study of *Culex* (*Culex*) *saltanensis* Dyar, 1928 larvae were to observe the continuous growth of siphon and cephalic capsule length and width in each instar, and to propose a linear equation to differentiate instars according to the size of both structures. Larvae obtained in laboratory at 27 ± 1ºC, photoperiod 14L:12D were studied. Larvae of the forth instar were collected on the field and compared with same instar larvae reared in the laboratory. Egg size and pigmentation pattern, first instar continuous growth, and differentiation of larval stages measurements were studied. Egg pigmentation patterns and the shapes of raft eggs differentiated specimens of *C. saltanensis* from *Culex* (*Culex*) *quinquefasciatus* Say, 1823 found in polluted pond water.
KEY WORDS. Instar; larva; measurement.

RESUMO. **Morfometria de ovos e imaturos de *Culex* (*Culex*) *saltanensis* Dyar (Diptera, Culicidae) obtidos em laboratório e campo.** O objetivo dessa pesquisa foi realizar um estudo morfométrico de largura e comprimento de cápsula cefálica e de sifão em imaturos de *Culex* (*Culex*) *saltanensis* Dyar, 1928 para observar a existência de crescimento da larva sem troca de exúvia e propor uma equação linear para separação dos diferentes instares através do tamanho dessas estruturas. Os estudos morfométricos foram realizados em imaturos que tiveram desenvolvimento em laboratório a 27 ± 1ºC e fotoperíodo 14L:10E. Também coletou-se larvas de quarto instar no campo, para comparação com as do mesmo instar criadas em laboratório. O tamanho do ovo e o padrão de pigmentação também foi pesquisado. Verificou-se crescimento contínuo no primeiro instar, e foi possível separar os estádios larvais através das mensurações realizadas. Através do padrão de pigmentação e formato das jangadas de ovos, foi possível separar *C. saltanensis* de *Culex* (*Culex*) *quinquefasciatus*, Say, 1823 os quais coabitam em lagoas com água poluída.
PALAVRAS-CHAVE. Instar; larva; medida.

Culex (*Culex*) *saltanensis* Dyar, 1928, a mosquito that can be infected with *Plasmodium cathemerium* Hartman, 1927, is the primary vector of *Plasmodium juxtanucleare* Versiani & Gomes, 1941 (Lourenço-de-Oliveira & Castro 1991). Sibajev *et al.* (1993) described the trypanosomal species *Crithidia ricardoi*, which was isolated from *C. saltanensis*. This mosquito is typical of neotropical areas and can be found in northern Paraná, Brazil. Its larvae are found in industrial effluents and sewer sedimentation treatment ponds, and in manufactured breeding sites, competing with *Culex* (*Culex*) *quinquefasciatus* Say, 1823 (Lopes 1997a).

Culex saltanensis and eight other species of Culicidae, were first found in the state of Rio de Janeiro by Lourenço-de-Oliveira (1984). In Entre Rios and Corrientes, Argentina, Rossi (1995) found *C. saltanensis* for the first time while capturing adult Culicidae. In a study on the coexistence of Culicidae species in artificial breeding sites in northern Paraná, Lopes (1997b) found larvae of *Culex bahamensis* Dyar & Knab, 1906 in water reservoirs and in troughs. These specimens were then identified as *C. bahamensis*, but now they are known as *C. saltanensis* (Lopes, personal information). Therefore, the morphometric knowledge of this mosquito's different stages of development is important because of its colonization potential in urban areas and because it competes with *C. quinquefasciatus*.

Micrometric oculars were used to conduct the anatomic measurement of variations in form and to establish standard measurement means of structures in a given stage of animal life. Anatomic measurements are also useful to separate cryptic species based on anatomical structures, allowing for a better understanding of the morpho-physiological and ecological adaptations of organisms to the environment.

Studies based on anatomic measurements of Culicidae are rare. Nonetheless, NAYAR & SAUERMAN (1970) measured adults to evaluate the effect of larvae density, diet variations, and salinity on morphology. JUPP (1987) measured both immature and adult Culicidae to separate individuals of the same species of *Culex* (*Culex*) *pipiens*, from two sites.

This study used mean anatomical measurements to understand the intraspecific morphometric variations of eggs and immature forms of *C. saltanensis*. Mean measurements were used to define patterns for identification of instars and patterns of size and features of eggs, and to assess numerical variations of instars in relation to the *Culex Linnaeus*, 1758 genus pattern.

MATERIAL AND METHODS

Culex saltanensis were reared in a 50 X 40 X 40 cm cage, where pupas were introduced every 15 days. The pupas were obtained from eggs collected in the effluent treatment ponds of a soft drink factory in Londrina, Paraná, Brazil. Adults were fed daily on a sugar solution at 12% and on quail blood [*Coturnix japonica*, Tinamidae (Temminck & Schlegel, 1849)]. *C. japonica* was kept immobilized in a cage (16 x 10 x 6 cm) with 1.5 cm wire mesh. The cage was put inside the insect breeder three times a week, after twilight, for about three hours.

Eggs obtained in the laboratory were either individually placed in 40 mL flasks or kept altogether in 600 mL polyethylene pots, and were stored in an acclimatized chamber (BOD) at 27 ± 1ºC and 14L:10D photoperiod. Chamber conditions were similar to the summer weather in Londrina. Larvae were fed daily with 20 mg of "Dog Show", a kind of puppy food, ground into one-millimeter particles.

Measurements of anatomical structures in different instars were normally taken one day after the cuticle changed, except for the first instar. In this case, measurements were taken soon after hatching and were repeated 48 hours later. In the spring and summer of 1999, 50 immature specimens and eggs laid in the laboratory, in addition to 50 larvae collected in the field 48 hours after hatching were used for each measurement. The larvae collected on the field and in the laboratory that had started the measurement series were immersed in alcohol (70%) and were placed between glass slides containing Hoyer liquid. A stereoscopic microscope with a 10 x micrometric ocular and 6.3 x objective lenses were used for measuring the larvae.

Siphon length of immature specimens was measured from base to apex, whereas siphon width was measured at the base, at the insertion, and at the eighth abdominal segment (Fig. 1). Cephalic capsule length was measured from one head extremity to the other, and width was measured between the two eye extremities (Fig. 1). A digital electronic micrometer caliper (STARRETT, series 727) was used to transform all measures into millimeters.

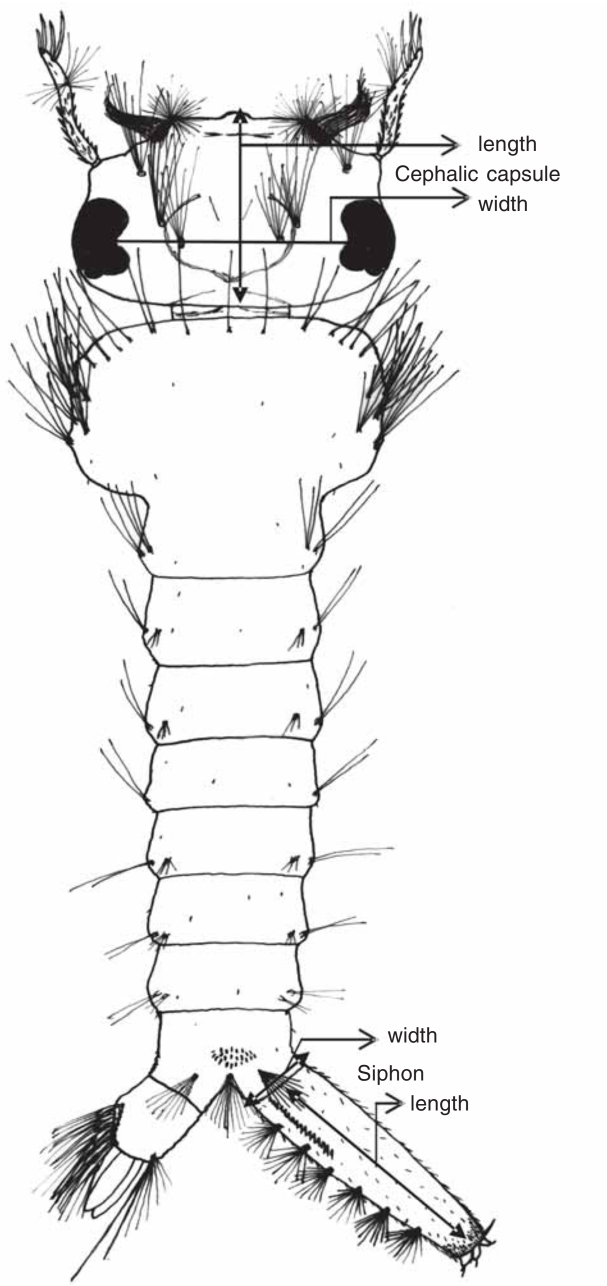

Figure 1. Larvae of *C. saltanensis* showing structures and extension where anatomical measurements were made

Forth instar larvae collected on the field were anatomically compared to immature specimens of 4th instar bred in the laboratory. Recently hatched 1st instar larvae in laboratory were compared to late 1st instar larvae after 48 hours.

Laboratory and field egg length was assessed before larvae hatching, whereas opercula were measured after hatching.

Culex saltanensis and *C. quinquefasciatus* occurred simultaneously and constantly on the field breeding site. Therefore, it became necessary to separate the two species, during the egg phase, to observe morphological aspects such as raft egg and chorion pigmentation.

Data were submitted to calculations of the arithmetic mean, standard deviation, analysis of variance complemented by the Tukey test at 5% significance level, and linear regression (VANZOLINI 1993).

Some of the biological material used for this study is stored in the Laboratório de Entomologia do Centro de Ciências Biológicas at the Universidade Estadual de Londrina, Paraná.

RESULTS AND DISCUSSION

The length and opercula size of eggs laid on the field and in the laboratory did not differ (Tabs I and V).

Although *C. saltanensis* can have several hosts, LOURENÇO-DE-OLIVEIRA & HEYDEN (1986) consider it mainly ornithophilous. *C. japonica* (quail) blood, the only feed for females bred in laboratories, did not affect egg size pattern when compared to females coming from fields where they had wide access to different blood sources. Therefore, we may assume that either blood type does not affect egg size, or *C. saltanensis* is strongly ornithophilous in northern Paraná. Consequently, blood type did not explain the absence of differences in egg size, and further studies are needed.

Larval growth of *C. saltanensis* occurred without exuviate change in the first instar, at 27 ± 1ºC and 14L:10D photoperiod. This observation was made after comparing recently hatched, 1st instar immature specimens 48 hours later (Tab. II). Immature specimens differed in siphon length and cephalic capsule size (Tabs II and V). Anatomical structures means were larger for late 1st instar larvae (48 hours) (Tab. II).

According to SERVICE (1993), cuticle deposition rate, in some species, depends on temperature and ultraviolet light. In each instar, the continuous larval growth of mosquitoes can be observed by the progressive increases in body size, in areas covered with thin cuticle layers (CLEMENTS 1992).

The 4th instar immature specimens obtained on the field and in the laboratory had measurable differences in siphon width and in width and length of the cephalic capsule. In all structures, the larvae obtained in the laboratory had the highest means but siphon length. (Tab. III).

Larger mean sizes among laboratory larvae (Tabs III and V) may have been influenced by the food ("Dog Show") given

Table I. Length (mm) of *C. saltanensis* eggs and opercula from laboratory and field.

Structures	Field (n = 50)				Laboratory (n = 50)			
	Max.	Min.	Average	S.D.	Max.	Min.	Average	S.D.
Egg length	0.733	0.671	0.709	0.013	0.796	0.577	0.706	0.054
Opercula length	0.234	0.156	0.199	0.019	0.234	0.156	0.202	0.014

Table II. Length and width (mm) of siphon and cephalic capsules of recent (0 hours) and late hatching (after 48 hours) of 1st instar larvae of *C. saltanensis*, n=50.

Structures	Recent hatching (0 hours)				Late hatching (48 hours)			
	Max.	Min.	Average	S.D.	Max.	Min.	Average	S.D.
Siphon length	0.156	0.078	0.115	0.018	0.343	0.140	0.236	0.038
Siphon width	0.094	0.047	0.068	0.012	0.125	0.062	0.093	0.015
Cephalic capsule length	0.218	0.109	0.168	0.031	0.250	0.156	0.219	0.019
Cephalic capsule width	0.281	0.187	0.243	0.020	0.328	0.203	0.288	0.027

Table III. Length and width (mm) of siphon and cephalic capsules of 4th instar *C. saltanensis* larvae obtained in the laboratory and on the field, n = 50.

Structures	Field				Laboratory			
	Max.	Min.	Average	S.D.	Max.	Min.	Average	S.D.
Siphon length	1.482	1.248	1.372	0.058	1.482	1.248	1.366	0.050
Siphon width	0.359	0.234	0.297	0.028	0.406	0.250	0.336	0.042
Cephalic capsule length	0.702	0.468	0.593	0.046	0.733	0.515	0.661	0.050
Cephalic capsule width	1.404	0.905	1.095	0.069	1.217	1.014	1.143	0.041

to immature specimens. The dog food provided contained most of the 10 aminoacids and lipids ideally needed for larval development. Without these nutrients, the larvae would not have survived the third instar, particularly due to the lack of cholesterol, cephalin, and lecithin, which are particularly important for them (Consoli & Lourenço-de-Oliveira 1998).

When in natural environments, larvae feed indistinctively on the habitat microplankton made of rotifers, bacteria, fungi spores, and other particles of organic matter. Nevertheless, occasional nutritional deficiency may either extend development time of immature specimens, or increase mortality during the adult transition phase (Bergo *et al.* 1990).

Larvae comparison of first, second, third, and forth instars showed a linear increase in size for all analyzed structures (Fig. 2). Morphometry is, therefore, an efficient method to determine larvae developmental stages (Tab. IV). Morphometric

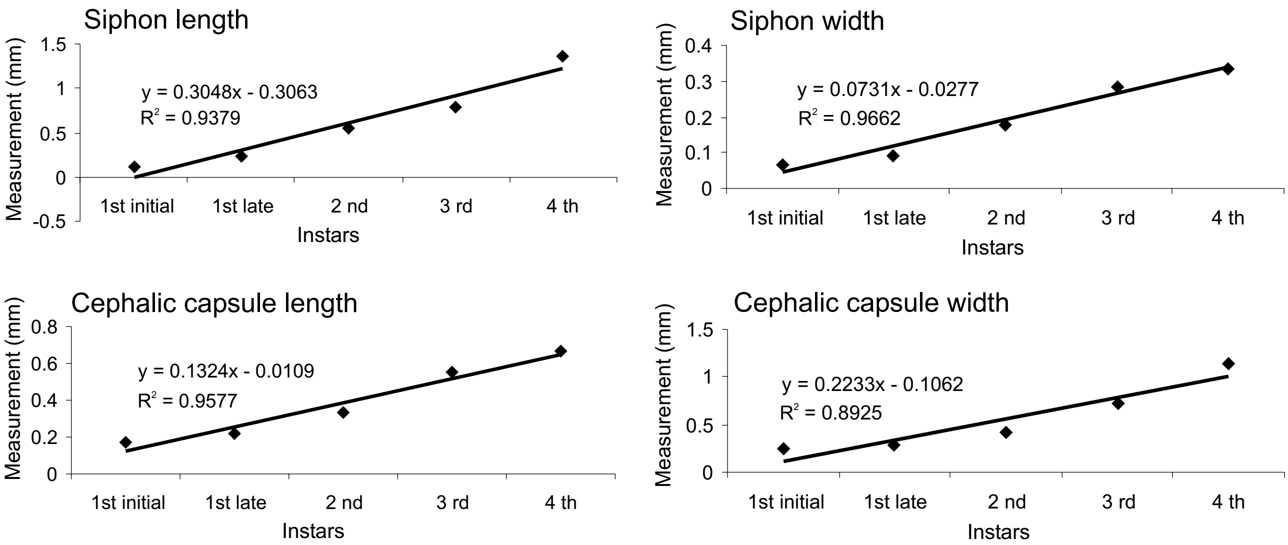

Figure 2. Anatomical structure of *C. saltanensis* obtained in the laboratory, 24 hours after instar change, and linear correlation.

Table IV. Length and width (in mm) of *C. saltanensis* siphon and cephalic capsule in all immature instars bred in the laboratory, n = 50.

Instars		Structures			
		Siphon length	Siphon width	Cephalic capsule length	Cephalic capsule width
	Max.	0.34	0.13	0.25	0.33
1st (48 h)	Min.	0.14	0.06	0.16	0.20
	Average	0.24	0.09	0.22	0.29
	S.D.	0.04	0.02	0.02	0.03
	Max.	0.62	0.27	0.39	0.50
2nd	Min.	0.47	0.13	0.27	0.31
	Average	0.54	0.18	0.33	0.42
	S.D.	0.04	0.03	0.03	0.04
	Max.	0.86	0.33	0.62	0.78
3rd	Min.	0.70	0.23	0.47	0.62
	Average	0.78	0.29	0.55	0.72
	S.D.	0.03	0.03	0.03	0.04
	Max.	1.48	0.41	0.73	1.22
4th	Min.	1.25	0.25	0.52	1.01
	Average	1.37	0.34	0.66	1.14
	S.D.	0.05	0.04	0.05	0.04

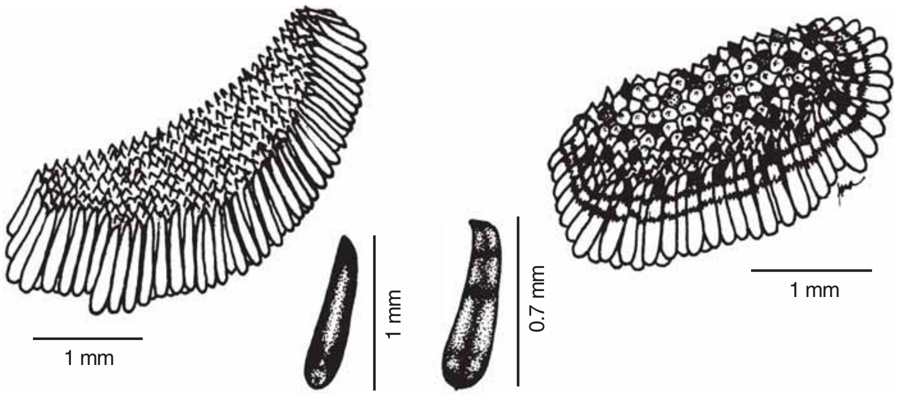

Figure 3. Comparison of egg rafts and general egg aspect *C. quinquefasciatus* (left) and *C. saltanensis* (right).

Table V. Measurements (mm) of anatomical structures of *C. saltanensis*, from the egg phase (eggs and opercula length) to the larval last instar (siphon length, siphon width, cephalic capsule length and width).

Larval and egg stages	Siphon length	Siphon width	Cephalic capsule length	Cephalic capsule width	Egg length	Opercula length
Laboratory 4th instar	1.3658* A	0.3358 A	0.6664 A	1.1426 A		
Field 4th instar	1.3704 A	0.2968 B	0.5930 B	1.0954 B		
Laboratory 3rd instar	0.7816 B	0.2854 B	0.5480 C	0.7208 C		
Laboratory 2nd instar	0.5422 C	0.1792 C	0.3298 D	0.4238 D		
Laboratory Late 1st instar	0.2362 D	0.0904 D	0.2190 E	0.2878 E		
Recent hatching 1st instar	0.1146 E	0.0676 D	0.1688 F	0.2428 F		
Field eggs					0.7090 A	0.1988 A
Laboratory eggs					0.7038 A	0.2020 A
DMS	0.0362	0.0299	0.0364	0.0389	0.0588	0.0129
CV %	2.52	7.30	4.43	3.05	5.71	4.41

*Original data. Means followed by the same letter in the column do not differ, according to the Tukey test at 5% significance level.

differences among parts with thicker sclerotization, such as the cephalic capsule and the breathing siphon, were due to the increase in size immediately after ecdysis. This is a manifestation of insect growth (CLEMENTS 1992), which has also occurred in our study.

When all analyzed structures were compared within each instar, the Tukey test showed the same siphon length only among the fourth instar field and laboratory larvae (Tab. V). Siphon width structures showed no differences among paired larvae of first instar recently hatched, first instar that hatched late, fourth instar obtained on the field, and third instar obtained in the laboratory. Larvae cephalic capsule differed in length and width in all instars (Tab. V). Second and 3rd instar larvae differed in all of the structures analyzed (Tab. IV).

Cephalic capsule measures showed significant differences in all phases of larval development and may be considered the most important parameter for the separation of larval instars.

In this study, the species *C. quinquefasciatus* and *C. saltanensis* had to be separated from each other, preferably in the egg stage, because they were frequently found in the same habitat. Differently from *C. quinquefasciatus, C. saltanensis* eggs had a metallic pigmentation normally halfway the egg length and the larger overall egg width. On the other hand, *C. quinquefasciatus* chorion is flat and more filiform than *C. saltanenis* (Fig. 3). *C. saltanensis* eggs develop rafts that are more oval than rafts developed by *C. quinquefasciatus*, which are more filiform (Fig. 3).

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