

## SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Larval Ontogeny and Morphological Variations of *Psaroniocompsa incrustata* (Lutz) (Diptera: Simuliidae) in the State of Rio Grande do Norte, Northeastern BrazilADRIELE N B SILVA<sup>1</sup>, HERBET T A ANDRADE<sup>1</sup>, CLAUDIA M RÍOS-VELÁSQUEZ<sup>2</sup>, FELIPE A C PESSOA<sup>2</sup>, JANSSEN F MEDEIROS<sup>3</sup>

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Ontogenia Larval e Variações Morfológicas de *Psaroniocompsa incrustata* (Lutz) (Diptera: Simuliidae) do Rio Grande do Norte

RESUMO - *Psaroniocompsa incrustata* (Lutz) é uma espécie antropofílica amplamente distribuída na América Central e do Sul. No foco brasileiro de oncocercose é considerada vetora de *Onchocerca volvulus*, sendo praga em outras regiões do país. Este estudo teve por objetivos determinar o número de estádios larvais e descrever as variações morfológicas e teratologias de uma população de *P. incrustata* do rio Pium, Rio Grande do Norte. O número de estádios larvais dessa espécie foi determinado medindo o comprimento lateral da cápsula cefálica de 3.164 larvas. Os estádios larvais foram determinados usando distribuição de frequências, teste-t de Student, regra de Dyar e de crescimento de Crosby. Foram identificados oito estádios larvais para *P. incrustata*. Foi encontrada uma elevada taxa de teratologias (9,6 %) no hipostômio, variações nas serrações laterais e no processo látero-mandibular.

PALAVRAS-CHAVE: Estágio larval, teratologia, Nordeste do Brasil

ABSTRACT - *Psaroniocompsa incrustata* (Lutz) is an anthropophilic species widely distributed in Central and South America. It is the vector of *Onchocerca volvulus* in a Brazilian focus and has been considered a plague in several areas of this country. The objective of this study was to determine the number of larval instars and to describe the morphological variations and teratologies of a population of *P. incrustata* from the Pium river, Rio Grande do Norte State. The number of larval instars was determined measuring the head capsule lateral length of 3,164 larvae. The larval instars were determined using the measurement frequency distribution, Student's t-test, the Dyar and Crosby growth rules. Eight larval instars were determined for *P. incrustata*. A high rate of teratologies (9.6%) in the hypostomium and variations in the lateral serrations and the latero-mandibular process were found.

KEY WORDS: Larval instar, teratology

*Psaroniocompsa incrustata* (Lutz) is an anthropophilic species widely distributed on South America and in the island of Trinidad (Coscarón 1991). In Brazil, it is involved in the transmission of *Onchocerca volvulus* (Filarioidea: Onchocercidae) (Shelley *et al* 1997, Andreazze & Py-Daniel 1999). In the state of Rio Grande do Norte, *P. incrustata* is the only anthropophilic hematophagous species that is a nuisance to the local population (Almeida *et al* 1999).

In order to forecast and to develop successful control programs, the detailed knowledge of the insect life cycle and

its response to environmental factors are essential (Ross & Merritt 1978, Ross & Craig 1979, Baba & Takaoka 1991).

Traditionally, hard structures have been measured (antenna size, cephalic capsule lateral length and cephalic apodeme width) which are useful to define the number of larval instars (Dyar 1890). In simuliids, the measurement of the lateral length of the cephalic capsule has yielded more reliable results (McCreadie 1991, Alencar *et al* 2001).

There is a specific variation in the number of simuliid larval instars in univoltine and multivoltine species, ranging

from four to nine instars (Ross & Merritt 1978, Ross & Craig 1979, Colbo & Okaeme 1988, Peterson 1996).

Reports on the probable morphological variations among the larval instars are rare. These variations may be important for fauna survey and environmental impact studies, as identification keys are traditionally based on last-instars, often requiring dissection of the gill histoblasts. If a good stable character were found in any instar proposed as diagnostic, then the immature larval population could be identified and counted.

The presence of toxic materials in food very often interrupts normal metamorphosis and may produce different types of malformed individuals at any stage of insect life. Deformities can be defined as morphological features that depart from the normal configuration (Warwick 1988). Effects produced by breakage or abrasion are common phenomena of the mouthparts stressed by hard substrate surfaces, mineral particles and sclerotized organic material (Vermeulen 1995). Most of the chironomid larvae are directly exposed to contaminants in sediments throughout their development due to their benthic feeding habits. Results from some field studies indicate a relationship between increased incidence of malformations and toxic sediment stress (Warwick 1985, Nazarova *et al* 2001).

The objective of this study was to determine the number of instars and describe the probable morphological variations and teratologies in a population of *P. incrustata* from the Pium river, in the State of Rio Grande do Norte, Brazil.

## Material and Methods

**Study area.** The study was carried out on a stretch of the Pium river (05° 58' 54" S / 35° 11' 47" W), which is part of the Pirangi river basin, located on the south coast of the state of Rio Grande do Norte, Northeast Brazil (Fig 1AB). This basin is located in the morphological unity of coastal plains or Barreira group. A hot humid climate prevails with a pronounced dry season,

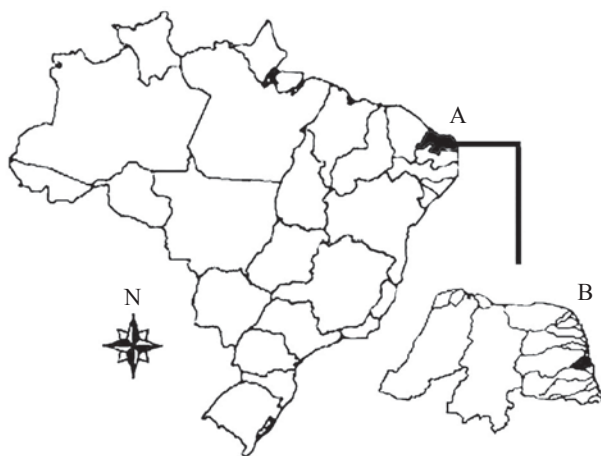


Fig 1 Map of the study area: A – Map of Brazil, showing the state of Rio Grande do Norte; B – Map of the state of Rio Grande do Norte, showing the Pirangi river basin.

which makes the vegetation somewhat semi-deciduous. The Valley of Pium river has a total area of 2,500 ha and is located in the eastern coast of the Nísia Floresta municipality (Borges 2002). The studied area of the Pium river presents a sandy streambed, with organic material resulted from the decomposition of the riparian forest.

**Sampling methods.** Collections were made on a stretch of the Pium river, situated in the Aconchego farm, located approximately 10 km apart from the head of the Pium Lake. Collections were made from April to July/2005, October to December/2005 and in January 2006. Immatures of black flies were collected from plant (Ceratophyllaceae) substrates located on the bed stream, drifting (leaves, branches) or on the river banks (Poaceae). The collected larvae were fixed in 80% ethanol and identified according to species published descriptions and keys (Shelley *et al* 1997, Almeida *et al* 1999, Pessoa *et al* 2005).

**Measuring methods.** In order to determine the larval instars, the lateral length of the cephalic capsule was used according to Gorayeb (1981) and Hamada (1989); the measures were taken from the lateral proximal part of the gena to the base of the cephalic fan stem. Larvae were placed on a petri dish containing sand on the bottom, and the side of the cephalic capsule was measured with the aid of a stereomicroscope.

Besides the biometric technique, each larval stage was defined by using the frequency distribution of the measurements taken and the morphological characteristics such as, gill histoblast development and the number of antennal segments (Ross & Merritt 1978). Craig & Batz (1982) reported that the antennae of first instars consist of a single article, with a basal article being added after the first molt. At later molts, the basal article becomes annulated.

The Student's t-test was used to verify whether there were significant differences among the means of the measurements of each larval instar (Cunha *et al* 1998, Alencar *et al* 2001, Alvan-Aguilar & Hamada 2003). The Crosby growth rule was used to verify the precision in the grouping of each instar (Craig 1975, Alencar *et al* 2001, Alvan-Aguilar & Hamada 2003). Linear regression analysis was applied among the means of the measurements against their respective instars to verify the geometric growth based on the Dyar's rule (Cunha *et al* 1998, Alvan-Aguilar & Hamada 2003, Andrade *et al* 2004). The growth rate between one instar and another was calculated using the formula:  $r = \ln(N_i / N_0) / t$ ; where  $r$  = growth rate,  $\ln$  = neperian logarithm,  $N_i$  = mean of the measurement of the next instar,  $N_0$  = mean of the measurement of the previous instar and  $t$  = number of intervals between one instar and another, which in this case was equal to one instar (Cunha *et al* 1998).

**Morphological study.** Some larvae from the second to the last instar were dissected and mounted on slides. Structures of the cephalic capsule and posterior cirlet rows were described and characterized. In addition to the observations of changes in the structures, teratologies were also detected, described and illustrated.

## Results and Discussion

All specimens collected in the Pium river were identified as *P. incrustata*, as reported in early studies on the same area (Medeiros *et al* 1999). A total of 3,164 larvae were measured and seven group sizes were established from the cephalic capsule lateral length. Since no first instars were found, we then considered the occurrence of eight instars for this species in the studied area (Fig 2). The number of instars presented by *P. incrustata* in Rio Grande do Norte was similar to the one inhabiting a stream in the southern region of Brazil, Paraná State (Cunha *et al* 1998). The absence of differences in larval development of other species, such as *Hemicnetha rubrithorax* (Lutz), was already reported in areas far apart, like Roraima and Minas Gerais, but that shared few environmental differences that could have affected the insect larval development (Alvan-Aguilar & Hamada 2003). The number of instars reported in here for *P. incrustata* is also similar to that presented by other black flies reported in Brazil (Gorayeb 1981, Andrade *et al* 2004).

The Student's t-test showed significant differences among

the means of each larval instar ( $P < 0.05$ ) (Table 1). The linear regression ( $\log_{10} y = 1.366 + 0.133x$ ,  $r^2 = 0.96$ ) of the means of the cephalic capsule lateral length against the larval instars indicated that there was no loss of any larval instar, showing a expected geometric growth of the population (Fig 3), based on the Dyar rule (Dyar 1890). However, our data did not corroborate the Crosby rule (Table 1), as the relationship of the percentage of the Brook ratio between the second and third instars was greater than 10% (-19.43%), indicating that the grouping of the means for each instar was not agreeable. Similar results were reported by Alencar *et al* (2001) and Alvan-Aguilar & Hamada (2003). The larval growth rate between the second and third instars was higher than between others. There was also a decrease in the larval growth rate, with an increase between the sixth and seventh instars. The decrease in growth rate during larval development can be explained by the need for reserves of nutrients that would be used by in the last instar, in preparation for pupation (silk production to spin the cocoon) and during the development of reproductive tissues (Ross & Merritt 1978, Alvan-Aguilar & Hamada 2003).

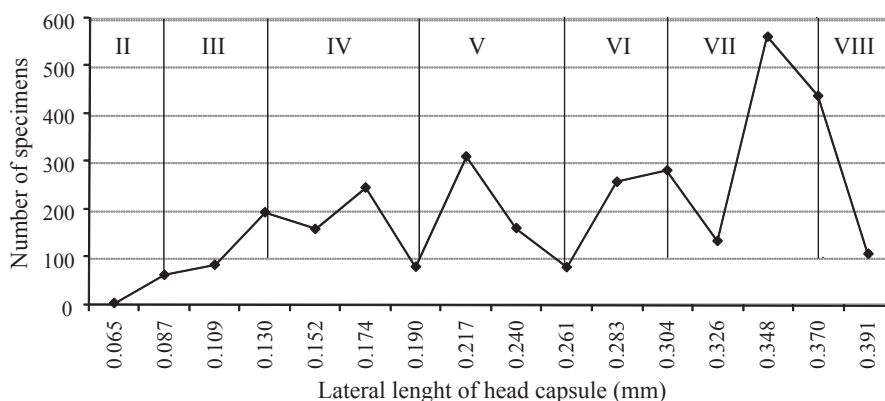


Fig 2 Frequency distribution of the cephalic capsule lateral length of *Psaroniocompsa incrustata* larvae, from the Pium river, Nísia Floresta municipality, Rio Grande do Norte, Brazil, from April 2005 to January 2006.

Table 1 Measurements of the lateral length of the larval cephalic capsule (mm) and larval instars of a population of *Psaroniocompsa incrustata* from the Pium river, Nísia Floresta municipality, Rio Grande do Norte, Brazil.

Instar	N	Class range (mm)	Mean $\pm$ SD	t - test	Brook ratio	Crosby ratio (%)	Growth rate (r)
II	4	0.065 – 0.068	0.067 $\pm$ 0.001				
III	341	0.087 – 0.130	0.117 $\pm$ 0.017	- 31.75*	1.75	- 19.43	0.55
IV	405	0.152 – 0.174	0.165 $\pm$ 0.111	- 90.78*	1.41	- 9.22	0.34
V	391	0.190 – 0.217	0.211 $\pm$ 0.011	- 96.00*	1.28	- 1.56	0.25
VI	497	0.240 – 0.283	0.266 $\pm$ 0.019	- 60.84*	1.26	3.17	0.23
VII	1523	0.304 – 0.391	0.347 $\pm$ 0.026	- 82.82*	1.30		0.26

N: number of larvae, SD: standard deviation: \* $P < 0.05$

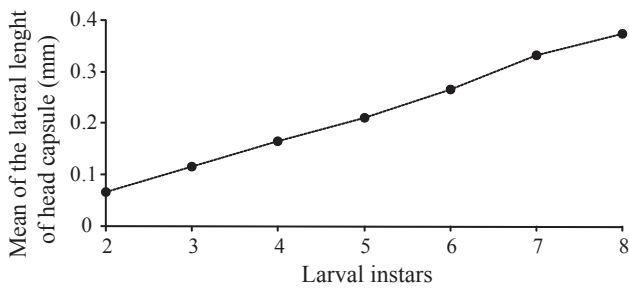


Fig 3 Linear regression of the cephalic capsule lateral length against larval instars of *Psaroniocompsa incrustata*, from the Pium river, Nísia Floresta municipality, Rio Grande do Norte, from April 2005 to January 2006.

**Description of *P. incrustata* instars.** The eggs and first-instar larvae were not described because they were not collected. In this study, 66 larvae were slide mounted and examined. In general, the mature larva of *P. incrustata* studied in the Pium river is very similar to that described by Shelley *et al* (1997) from a population from the north region of Brazil. All stages have positive head spot pattern, and simple or bifid setae randomly distributed in the head capsule. The mandibles may have one or two latero-mandibular processes, and this variation was observed for the first time. The postgenal cleft bridge is half to a third of the length of the hypostomium. The number of intermediate and lateral hypostomial teeth is 3+3 and 2+2, respectively for all instars, but some variations can occur in the last and penultimate instars. The number of lateral serrations of the hypostomium is smaller in earlier instars, and increases in late instars. Setae on the surface of the hypostomium are absent on the three first instars examined (second to fourth instars). The characters that varied in numbers observed here were structures of the hypostomium as lateral serrations, hypostomial setae and setae on the surface of hypostomium, labral fan, latero-mandibular process, posterior circling rows and the hooks of the rows of posterior circling (Table 2). The ventral posterior papillae are well developed in all instars, which may

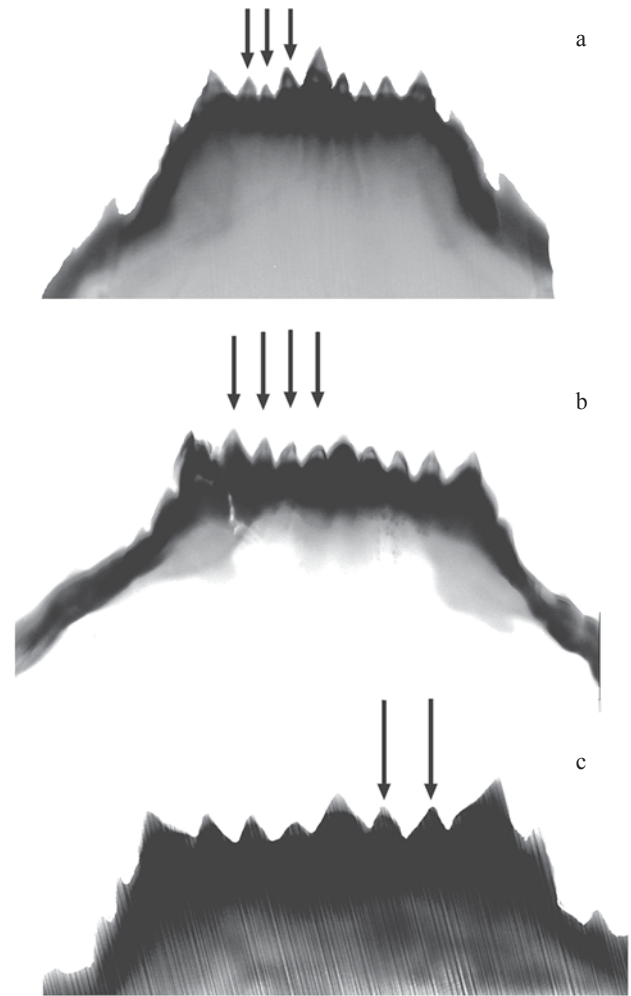


Fig 4 Distal part of the hypostomium of *Psaroniocompsa incrustata* larva. a – normal number of sublateral teeth; b – supranumerous sublateral teeth; c – subnumerous sublateral teeth.

Table 2 Variations in the structures (teratologies) of larval instars of *Psaroniocompsa incrustata* from the Pium river, Nísia Floresta municipality, Rio Grande do Norte, Brazil.

Character	Larval instar					
	2 <sup>th</sup> (N = 5)	3 <sup>th</sup> (N = 13)	4 <sup>th</sup> (N = 8)	5 <sup>th</sup> (N = 8)	6 <sup>th</sup> (N = 12)	7 <sup>th</sup> (N = 20)
Lateral serrations	1-2+1-2	1-2+1-2	1-4+1-4	2-4+2-4	2-5+2-5	4+4
Hypostomial setae	2+2	1-2+1-2	2-3+2-3	2-5+2-5	2-4+2-4	3-5+3-5
Setae on surface of hypostomium	-	-	-	- to 1-2+1-2	- to 1+1	- to 1+1 2+2
Labral fan rays	16 (14-18)	15.5 (13-18)	20.5 (16-25)	17.5 (16-19)	21.5 (19-24)	26.0 (25-27)
LMP	1	1 or 2	1 or 2	1 or 2	1 or 2	1 or 2
Posterior circling rows	39 (35-43)	41 (33-48)	49 (41-51)	48 (41-58)	54 (51-60)	58 (50-68)
Hooks of the rows of posterior circling	4 to 7	5 to 7	4 to 10	6 to 11	7 to 11	7 to 15

-: not observed



be useful for regional identification keys if associated with the positive head spot pattern on all instars of *P. incrustata* (Almeida *et al* 1999, Pessoa *et al* 2005).

Common teratologies in the hypostomium were found as sub or supernumerary sublateral teeth (Fig 4) in 9.6% of the specimens examined. Deformities of the larva mentum of Chironomidae have been attributed to pollutants such as oil tars, pesticides, organochlorines and heavy metals (Martinez *et al* 2002, Meregalli *et al* 2002, Swansburg *et al* 2002). The significance of the observed teratologies in *P. incrustata* larvae from the Pium river is unknown because the presence of pollutants was not measured.

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