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 simuliiids, 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 simuliiid 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, 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: $r = \frac{\ln (N_1 / N_0)}{t}$; where $r = \text{growth rate}$, $\ln = \text{neperian logarithm}$, $N_1 = \text{mean of the measurement of the next instar}$, $N_0 = \text{mean of the measurement of the previous instar}$ and $t = \text{number of intervals between one instar and another}$, 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 circket 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.](image-url)

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

N: number of larvae, SD: standard deviation: *P < 0.05
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 circket rows and the hooks of the rows of posterior circket (Table 2). The ventral posterior papillae are well developed in all instars, which may

| Character | Larval instar |
|-----------|---------------|
| Lateral serrations | 2\(^{\text{nd}}\) (N = 5) | 3\(^{\text{rd}}\) (N = 13) | 4\(^{\text{th}}\) (N = 8) | 5\(^{\text{th}}\) (N = 8) | 6\(^{\text{th}}\) (N = 12) | 7\(^{\text{th}}\) (N = 20) |
| 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\(^{\text{nd}}\) | 2+2 | 1-2+1-2 | 2-3+2-3 | 2-5+2-5 | 2-4+2-4 | 3-5+3-5 |
| 3\(^{\text{rd}}\) | 1-2+1-2 | 2-3+2-3 | 2-5+2-5 | 2-4+2-4 | 3-5+3-5 |
| 4\(^{\text{th}}\) | 1-4+1-4 | 2-4+2-4 | 2-5+2-5 | 2-4+2-4 | 3-5+3-5 |
| 5\(^{\text{th}}\) | 1-2+1-2 | 2-4+2-4 | 2-5+2-5 | 2-4+2-4 | 3-5+3-5 |
| 6\(^{\text{th}}\) | 1+2 | 2-4+2-4 | 2-5+2-5 | 2-4+2-4 | 3-5+3-5 |
| 7\(^{\text{th}}\) | 1+2 | 2-4+2-4 | 2-5+2-5 | 2-4+2-4 | 3-5+3-5 |
| Setae on surface of hypostomium | 4\(^{\text{th}}\) | 5+4 | 2-4+2-4 | 2-5+2-5 | 2-4+2-4 | 3-5+3-5 |
| Labral fan rays | 5\(^{\text{th}}\) | 15 (13-18) | 20 (16-25) | 17.5 (16-19) | 21.5 (19-24) | 26.0 (25-27) |
| 6\(^{\text{th}}\) | 16 (14-18) | 15.5 (13-18) | 20.5 (16-25) | 17.5 (16-19) | 21.5 (19-24) | 26.0 (25-27) |
| 7\(^{\text{th}}\) | 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 circket 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 circket | 4 to 7 | 5 to 7 | 4 to 10 | 6 to 11 | 7 to 11 | 7 to 15 |

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.
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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References

Alencar Y B, Hamada N, Magni-Darwich S (2001) Morphometric comparison of *Simulium perflavum* larvae (Diptera: Simuliidae) in relation to season and gender in Central Amazônia, Brazil. Mem Inst Oswaldo Cruz 96: 785-789.

Almeida H T, Py-Daniel V, Torres A V S (1999) Simuliidae (Diptera: Culicomorpha) no Nordeste brasileiro. Entomol Vect 6: 323–337.

Alvan-Aguilar M A, Hamada N (2003) Larval biometry of *Simulium rubrithorax* (Diptera: Simuliidae) and size comparison between populations in the states of Minas Gerais and Roraima, Brazil. Mem Inst Oswaldo Cruz 98: 507-511.

Andrade H T A, Strixino S T, Py-Daniel V, Medeiros J F (2004) Determinação dos estádios larvais de *Hemicentra brachyclada* (Lutz & Pinto) (Diptera, Simuliidae). Entomol Vect 11: 541-550.

Andreatze R, Py-Daniel V (1999) Atividade hematofaga mensal e infeção natural de *Psaroniocompsa incrustata* (Lutz, 1910) (Diptera, Culicomorpha, Simuliidae) vetor de *Onchocerca volvulus* (Leuckart, 1893) em Xitei/Xidea, área indígena Yanomami, Roraima, Brasil. Entomol Vect 6: 415-440.

Baba M, Takaoka H (1991) Larval instars and growth pattern of a univoltine black fly, *Prosimulium kiotoense* (Diptera: Simuliidae), in Kyushu, Japan. J Med Entomol 28: 214-218.

Borges A N (2002) Implicações ambientais na bacia hidrográfica do Rio Pium (RN) decorrentes das diversas formas de uso e ocupação do solo. MSc dissertation, Universidade Federal do Rio Grande do Norte, Natal, 128p.

Colbo M H, Okaeme A N (1988) The larval instars of *Cnepho ornithophilus* (Diptera: Simuliidae), a black fly with a variable molting pattern. Can J Zool 66: 2084-2089.

Coscarón S (1991) Fauna de agua dulce de la República Argentina. FECIC. Bs. Aires. Insecta, Diptera, Simuliidae 38, Fasc. II, 295p.

Craig D A (1975) The larvae of Tahitian Simuliidae (Diptera: Nematocera). J Med Entomol 12: 463-476.

Craig D A, Batz H (1982) Innervation and fine structure of antennal sensilla of Simuliidae larvae (Diptera: Culicomorpha). Can J Zool 60: 696-711.

Cunha M C I, Coscarón S, Bassi R M A (1998) Determinación de los estadios larvales de *Simulium* (Diptera, Simuliidae) de Paraná, Brasil. Acta Biol Par 27: 57-66.

Dyar H G (1890) The number of molts of lepidopterous larvae. Psyche 5: 420-422.

Gorayeb I S (1981) Comportamento de ovoposição e ciclo evolutivo de *Simulium fulvinotum* Cerq. & Mello 1968 (Diptera, Nematocera). Acta Amaz 11: 595-604.

Hamada N (1989) Aspectos biocológicos de larvas de *Simulium goeldii* Cerqueira & Nunes de Mello, 1967, com referências a larvas de *Simulium rorotense* Floch & Abonnenc, 1946 (Diptera: Simuliidae) na Reserva Florestal Adolfo Ducke, Amazônia Central. MSc dissertation, Instituto Nacional de Pesquisas da Amazônia, Manaus, 106p.

Martinez E A, Moore B C, Schaumloffel J, Dasgupta N (2002) The potential association between menta deformities and trace elements in Chironomidae (Diptera) taken from a heavy metal contaminated river. Arch. Environ. Contam Toxicol 42: 286-291.

Mccreadie J W, Colbo M H (1992) Spatial-distribution patterns of larval cytotypes of *The Simulium venustum/verecundum complex* (Diptera, Simuliidae) on the Avalon Peninsula, Newfoundland: factors associated with cytotype abundance and composition. Can J Zool 70: 1389-1396.

Medeiros J F, Andrade H T A, Guerrero J C H (1999) Larval preference of different colors at artificial substrate for *Psaroniocompsa incrustata* (Diptera: Simuliidae) in breeding grounds at river Pium, Rio Grande do Norte state, Brazil. Mem Inst Oswaldo Cruz 94: 849-850.

Meregalli G, Bettinetti R, Pluymers L, Vermeulen A C, Rossaro B, Ollevier F (2002) Mouthpart deformities and nucleo activity in field-collected *Chironomus riparius* larvae. Arch Environ Contam Toxicol 42: 405-9.

Nazarova L B, Govorkova L K, Sabirov R M, Latypova V Z (2001) Morphological deformations of chironomid larvae on assessment of Kuybyshev water reservoir ecological state/ Environment. Radioecol App Ecol 7: 22-27.

Pessoa F A C, Ríos-Velásquez C M, Py-Daniel V (2005) Simuliidae, p.355-391 In Merritt R W, Cummins K W (eds) An introduction to the aquatic insects of North America. Kendall/Hunt Plub. Dubuque, Iowa, 862p.

Ross D H, Craig D A (1979) The seven larval instars of *Prosimulium mixtum* Syme and Davis and *P. fuscum* Syme and Davis (Diptera: Simuliidae). Can J Zool 57: 290-299.

Ross D H, Merritt R W (1978) The larval instars and population
dynamics of five species of black flies (Diptera: Simuliidae) and their responses to select environmental factors. Can J Zool 56: 1633-1642.

Shelley A J, Lowry C A, Maia-Herzog M, Luna Dias A P A, Moraes M A P (1997) Biosystematic studies on the Simuliidae (Diptera) of the Amazonia onchocerciasis focus of Brazil. Bull British Mus Nat Hist, Ent series 66: 1-121.

Swansburg E O, Fairchild W L, Fryer B J, Ciborowski J J (2002) Mouthpart deformities and community composition of Chironomidae (Diptera) larvae downstream of metal mines in New Brunswick, Canada. Environ Toxicol Chem 21: 2675-84.

Vermeulen A C (1995) Elaboration of chironomid deformities as bioindicators of toxic sediment stress: the potential application of mixture toxicity concepts. Ann Zool Fenn 32: 265-285.

Warwick W F (1985) Morphological abnormalities in Chironomidae (Diptera) larvae as measures of toxic stress in freshwater ecosystems: indexing antennal deformities in Chironomus Meigen. Can J Fish Aqu Sci 42: 1881-1914.

Warwick W F (1988) Morphological deformities in Chironomidae (Diptera) larvae as bioindicators of toxic stress, p.281-320. In Evans M S (ed) Toxic contaminants and ecosystem health. A Great Lake focus. Wiley and Sons, New York, 602p.

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