
**TITULO: CYTOGENETICS OF THE FRESHWATER CYCLOPOID
MESOCYCLOPS LONGISETUS LONGISETUS (CRUSTACEA,
COPEPODA) FROM SÃO CARLOS, SÃO PAULO, BRAZIL.**

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Biota Neotropica v4 (n2) – <http://www.biotaneotropica.org.br/v4n2/pt/abstract?short-communication+bn03604022004>

Date Received : 05/01/04

Revised: 09/03/2004

Accepted: 09/21/2004

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Abstract

A cytogenetic study was carried out on the early embryonic stages of the cyclopoid species *Mesocyclops longisetus longisetus* (Crustacea, Copepoda) from freshwater environments of the São Carlos area of south-east Brazil. Chromatin was staining using acetic orcein solution. The species showed $2n = 14$ chromosomes and presented chromatin diminution in the 4th embryonic cleavage. The eliminated chromatin was concentrated in the equatorial region of the embryonic cell. The results were compared with data for other cyclopoid species of temperate regions.

Key words: *Cyclopoida*, *Mesocyclops longisetus longisetus*, *cytogenetics*, *chromatin diminution*, *chromosomal number*.

Resumo

Realizou-se estudos citogenéticos das fases iniciais da ovogênese da espécie de Cyclopoida *Mesocyclops longisetus longisetus* (Crustacea, Copepoda) de corpos de água doce de São Carlos, SP, Brasil. A cromatina foi corada com solução de orceína. A espécie apresentou $2n=14$ cromossomos e também foi verificado o fenômeno de diminuição de cromatina na 4^a clivagem da ovogênese. A cromatina eliminada concentrou-se na região equatorial das células. Estes resultados são comparados com outras espécies de Cyclopoida de regiões temperadas e também com a mesma espécie registrada na América do Norte. A importância do estudo citogenético para a taxonomia é discutida.

Palavras-chave: *Cyclopoida*, *Mesocyclops longisetus longisetus*, *citogenética*, *diminuição de cromatina*.

1. Introduction

Cytogenetic studies are important to understand better the biology and evolution of organisms. Copepod taxonomists have utilized such studies to separate morphologically close populations and species (Wyngaard & Chinnapa, 1982). Cytotaxonomy was proposed by Chinnapa & Victor (1979) as a tool in the differentiation of North American and European cyclopoid species. These authors concluded that some morphologically similar species from Europe and North America have different chromosomal numbers. Similar studies were carried out by Grishanin & Akif'ev (1999) for discriminating two cyclopoid populations from Europe that had different chromosome numbers but similar morphologies. Besides chromosomal number, the presence or absence of chromatin diminution, a phenomenon that occurs in some species of Copepoda, can be a useful characteristic in the taxonomy of cyclopoid species (Doward & Wyngaard, 1997).

Cytogenetic studies on copepod populations have been concentrated in the northern hemisphere, especially the United States and Europe, while in the southern hemisphere, such studies are nonexistent. The present study represents a contribution to cytogenetic studies on copepods, involving the cyclopoid *Mesocyclops longisetus longisetus* (Crustacea, Copepoda) from São Paulo State, Brazil.

2. Material and Methods

The organisms were collected with a 68µm net in artificial ponds at São Carlos (22° 01' S and 47° 89' W) São Paulo, Brazil. The species was identified using morphological characters, such as the last segment of the antennule (Fig. 1A) and the seminal receptacle (Fig. 1B), as proposed by Dussart (1987) for *Mesocyclops longisetus* sub-species.

Ovigerous females were separated, and the egg sacs removed with a needle, without killing the mothers. The eggs sacs were put in 1.5mL microcentrifuge Eppendorf tubes in a 5:1 solution of distilled water colchicine (0,025%) for 30 minutes. The fixation and staining procedures followed those utilized by Grishanin et al. (1996) and Grishanin & Akif'ev (1999): the egg sacs were fixed in a 3:1 solution of ethanol:acetic acid for 15 minutes, and transferred to fresh solution for one hour at 4°C. The staining was carried out with orcein 1% in a 1:1 solution of acetic acid:lactic acid, immersing the egg sacs for the duration of one hour. Stained egg sacs were squashed under a coverslip on microscope slides, and observed using optical microscopy; images were captured with a digital recording system.

3. Results

Figures 2a, b and c show pictures and schematic drawings of the copepod chromosomes, presenting a value

of where chromosome number is $2n = 14$. Figures 3a, b and c demonstrate the occurrence of chromatin diminution; Fig. 3a shows the chromosomes before the 4th cleavage of the egg cell; Fig. 3b shows the 4th cleavage where the chromatin diminution occurs with eliminated chromatin in the equatorial region of the cell, and Fig. 3c shows the egg cells after 4th cleavage, presenting short chromosomes.

4. Discussion

Wyngaard & Rasch (2000), reviewing the occurrence of chromatin diminution in cyclopoid species, stated that this phenomenon has been recorded for two species of the genus *Mesocyclops*, *M. edax* and *M. longisetus* (registered in North American freshwaters bodies), while it has not been observed in *M. ruttneri*.

Comparing the chromatin diminution phenomenon in *Mesocyclops longisetus* from North American specimens with the *M. longisetus longisetus* specimens from Brazil, it can be noted that the diminution was found to occur in different embryonic cleavage stages. In the *Mesocyclops* from Brazil, the chromatin diminution occurred in the 4th egg cleavage (16 cells) while in the *Mesocyclops* from North America, the chromatin diminution occurred in the 6th egg cleavage (64 cells) (Doward & Wyngaard 1997). These authors considered that the embryonic stage for chromatin diminution is constant for a given species, with rare exceptions and, therefore, can be species specific.

Wyngaard (2000) reported that utilization of chromatin diminution as a taxonomic feature was proposed by Einsle (1962) to resolve identification problems for the European cyclopoid species *Cyclops furcifer*, *C. heberti* and *C. singularis*. Thus, the difference in the cleavage stage between North American and Brazilian *Mesocyclops longisetus* could be strong indication that different species, and not sub-species, are involved, however, further studies are needed.

Cytogenetic data is useful for differentiation between closely related morphologically similar species. An example of such a study is that of Grishanin & Akif'ev, (1999) for *Cyclops strennus strennus* from Russia and *C. strennus strennus* from Germany. According to these authors chromosome numbers were different in two cases i.e., with *Cyclops strennus strennus* from Russia had $2n = 24$ and *C. strennus strennus* from Germany $2n = 22$. This difference would account for genetic isolation of the two populations through interbreeding disability. The comparison of chromosome number between Brazilian and North American *Mesocyclops longisetus* is not yet possible due to the lack of information on the chromosome number of North American species. However, based on the difference in the cleavage stage for chromatin diminution, here is supportive evidence that the two populations may belong to two different species, as observed with *Cyclops strennus*. Further stud-

Figure 1. *Mesocyclops longisetus longisetus* from south-east Brazilian fresh water: (A) 17th antennule segment, (B) genital segment and seminal receptacle. Bar is 100 μ m.

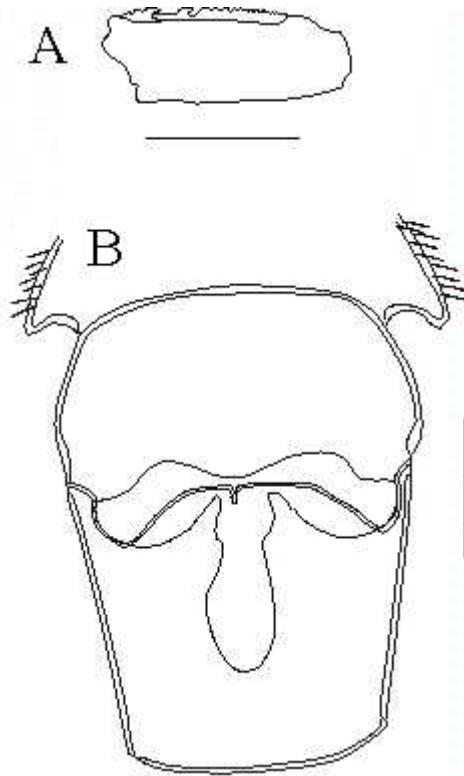


Figure 2. *Mesocyclops longisetus longisetus* egg cell: (A) Picture of the chromosomes; (B) Draw of chromosomes presented in the picture; (C) Chromosomes pair wised.

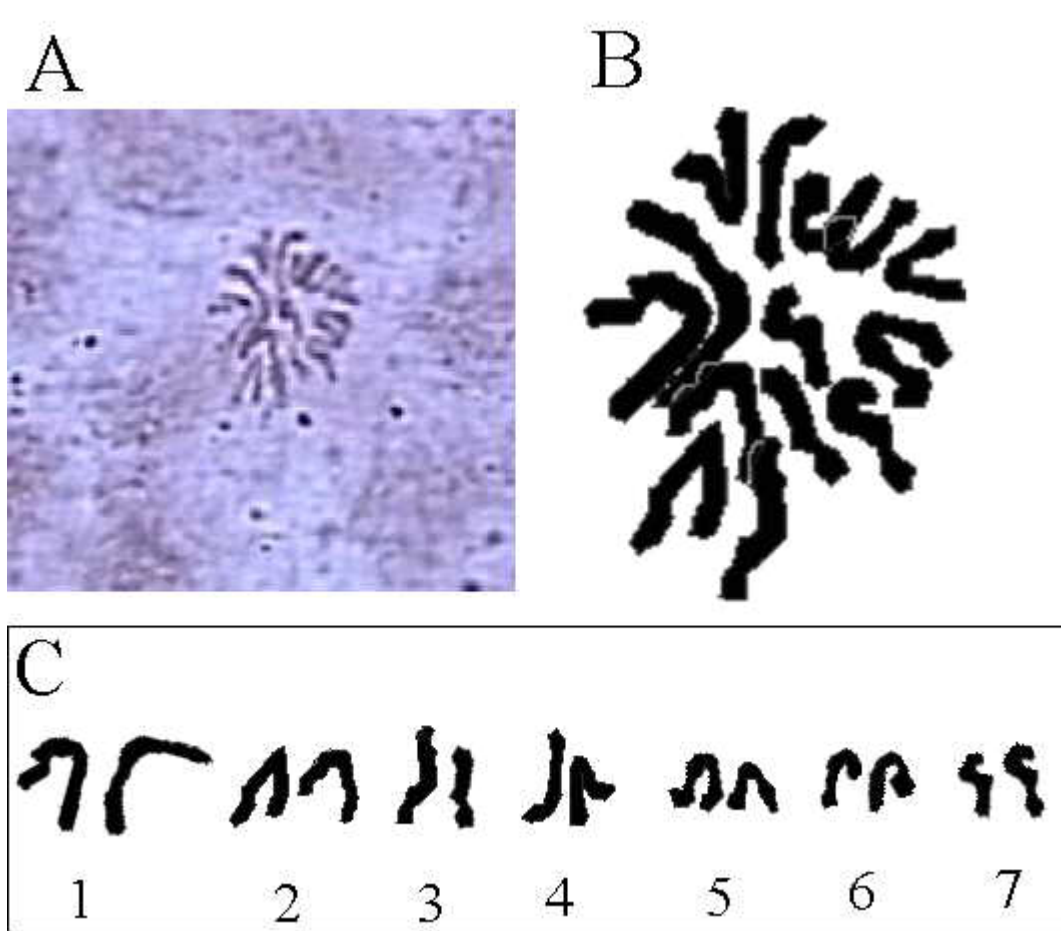
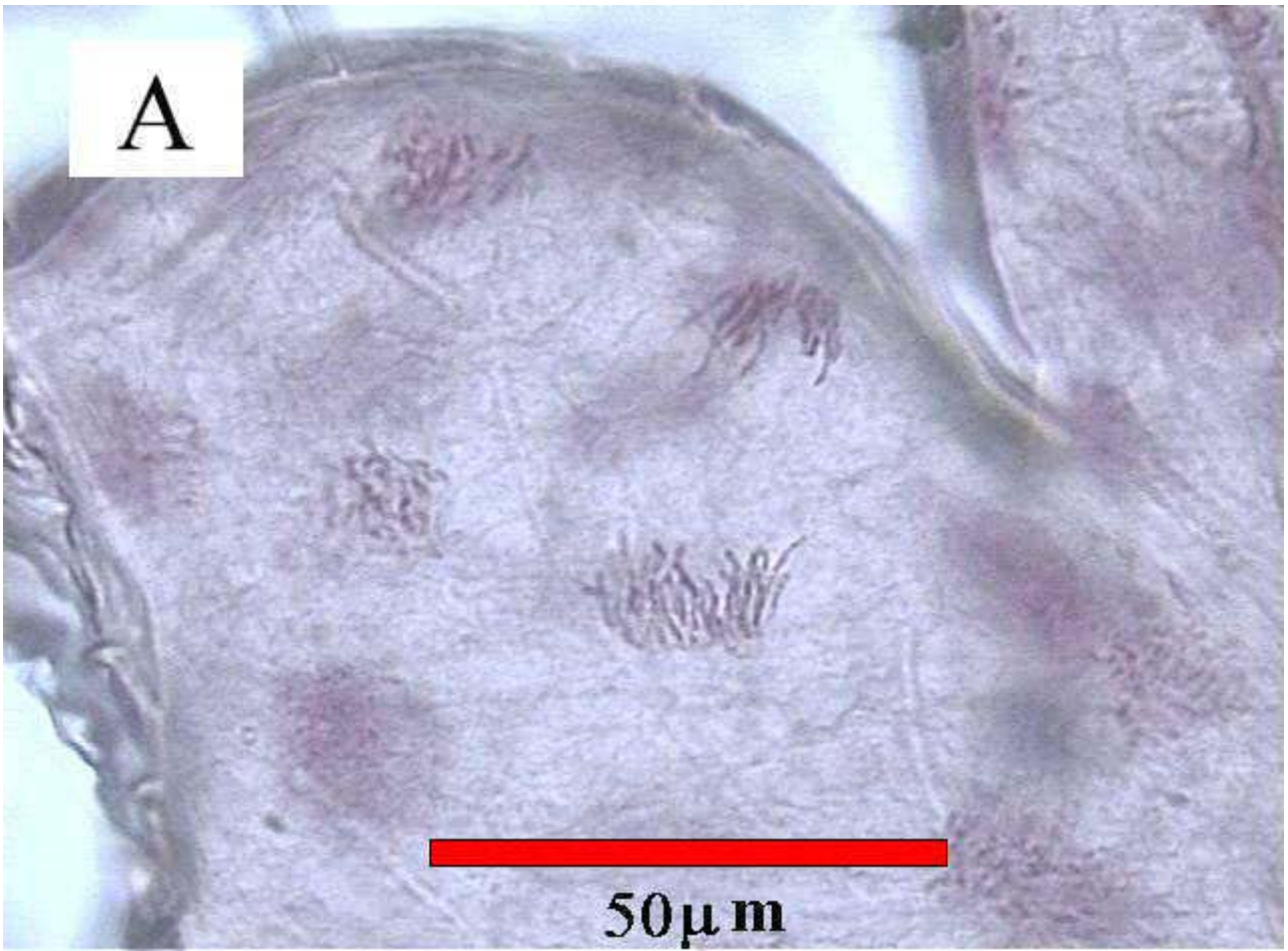
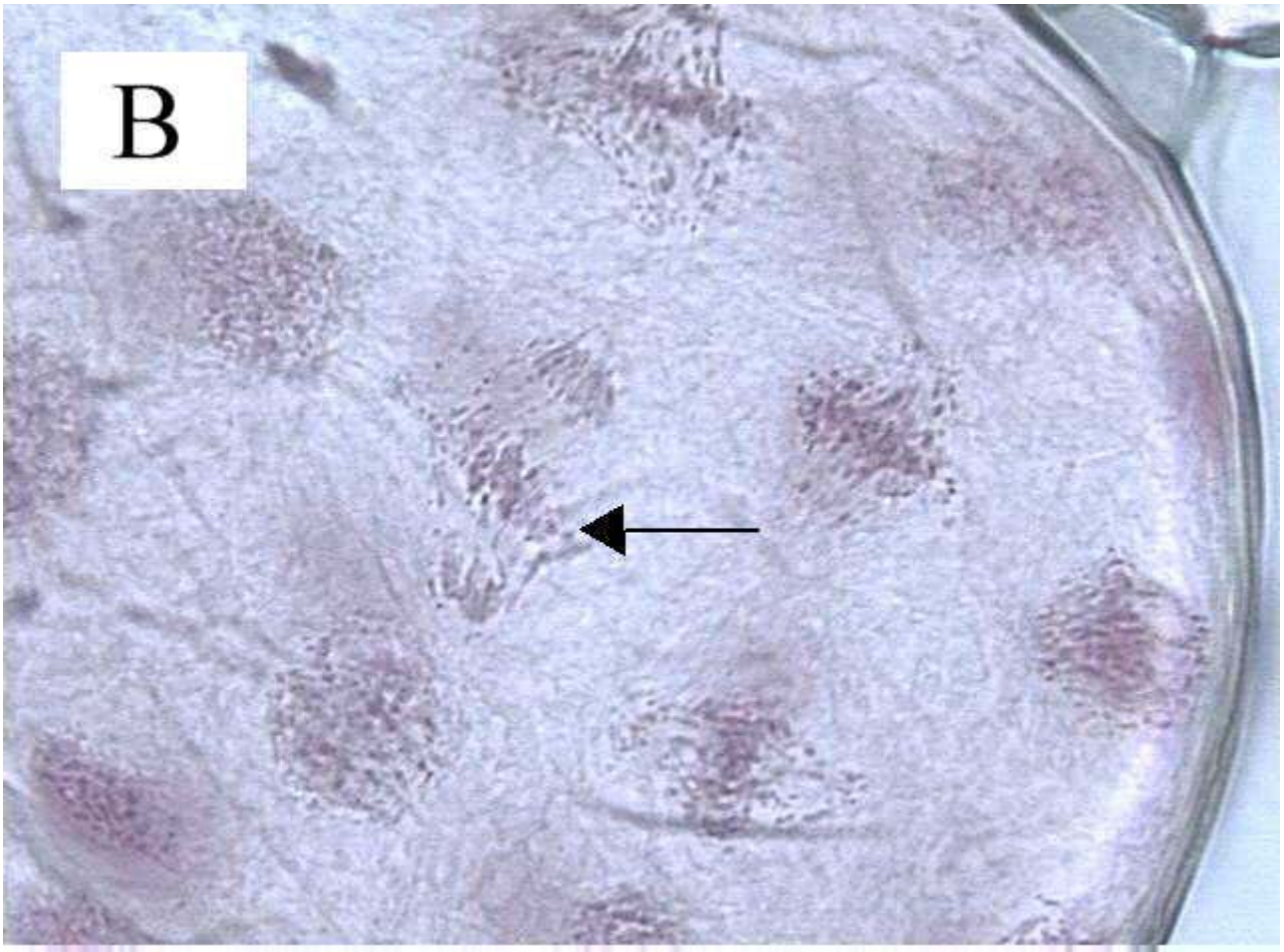


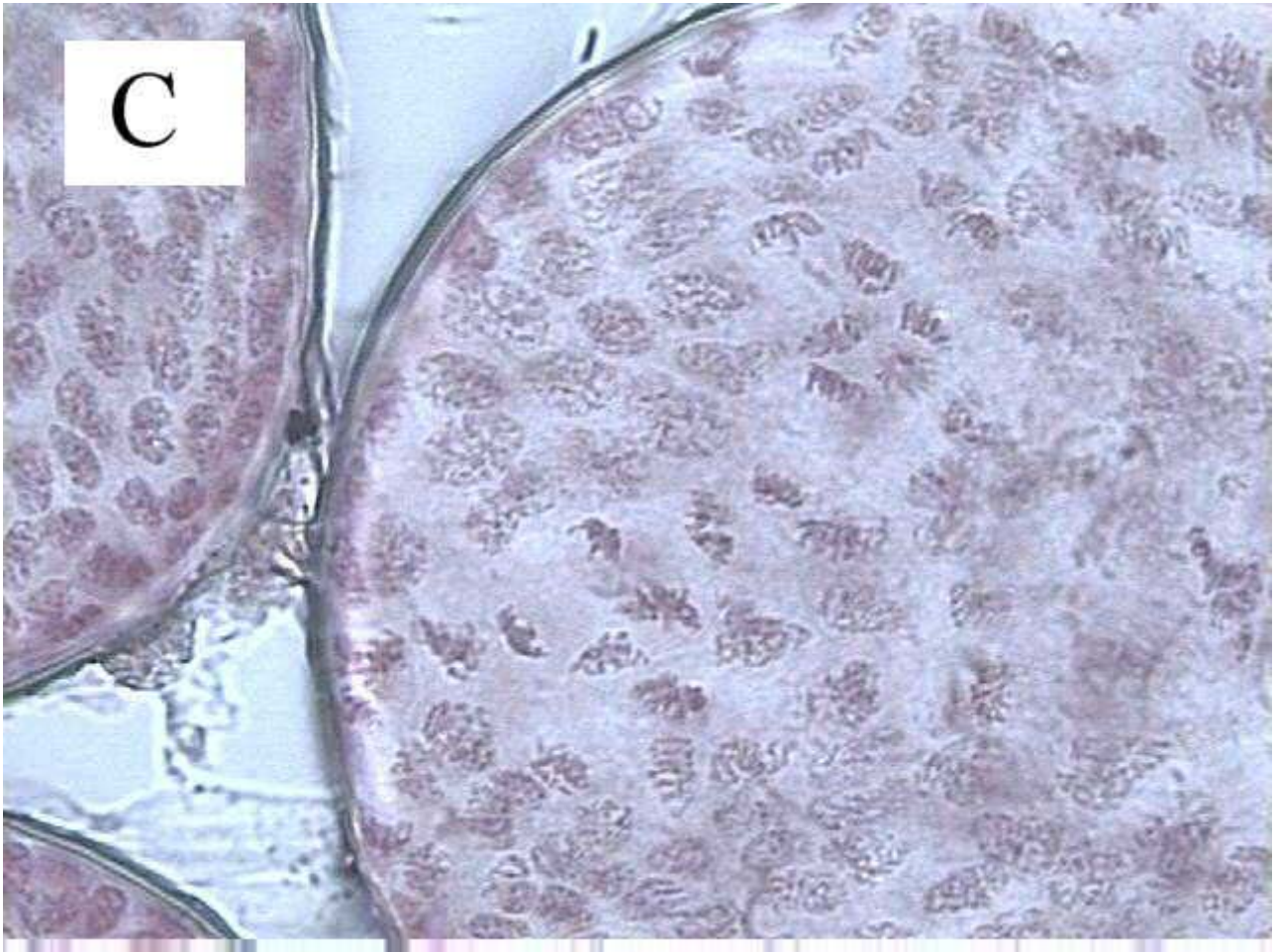
Figure 3. *Mesocyclops longisetus longisetus* chromatin diminution: (A) Chromosomes before 4th cleavage;



(B) 4th cleavage with chromatin diminution, setae indicates chromosome decrease in the equatorial area;



(C) shorter chromosomes after 4th cleavage.



ies are needed to elucidate their status.

5. Acknowledgements

We express thanks to Dr. Grace Wyngaard and Dr. Andrey Grishanin from James Madison University (VA, USA). To Dr. Orlando Moreira Filho from Universidade Federal de São Carlos and to FAPESP for financial support. This work is part of BIOTA/FAPESP: The Virtual Institute of Biodiversity (www.biota.org.br).

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Title: Cytogenetics of the freshwater cyclopoid *Mesocyclops longisetus longisetus* (Crustacea, Copepoda) from São Carlos, São Paulo, Brazil.

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Biota Neotropica, Vol. 4 (number 2): 2004
<http://www.biotaneotropica.org.br/v4n2/pt/abstract?short-communication+bn03604022004>

Date Received : 05/01/04 - Revised: 09/03/2004
Accepted: 09/21/2004

ISSN 1676-0611