Rev. Brasil. Genet. 15, 4, 821-829 (1992)
(Brazil. J. Genetics)

ANALYSIS OF THE rDNA OF *Chironomus sancticaroli* (DIPTERA, CHIRONOMIDAE)

Miguel Angelo Martins Moreira, Luiz Antônio Ferreira da Silva,
Jurce Portella Bezerra and Orílio Leoncini

ABSTRACT

Chironomus sancticaroli is a South-American chironomid. In order to compare its rDNA with that of other chironomids, genomic DNA was analysed and a rDNA repeat unit was cloned. We compared the data with that from *Chironomus thummi* (from Europe) and *C. tentans* (from Europe and North-America). rDNA units did not show length variation in the repeat units (8.5 Kb). Physical map data showed variation in the 28S coding region, when compared with *C. thummi*. These small differences are probably related to expansion segments located between conserved regions. New restriction sites for HindIII and HaeIII, in internal transcribed spacer and 28S coding regions, respectively, are reported.

INTRODUCTION

rDNA sequences are valuable for estimating evolutionary and phylogenetic divergence, by comparing restriction maps and repeat units sequence data of different species. Data on the rDNA organization of chironomids are available for European, North-American, and Australian species, though comparable information on South-American species is lacking. Polytenic chromosomes of *Chironomus sancticaroli* have been described by Freitas *et al.* (1985). Two nucleoli were found in this species: one in chromosome I and the other in chromosome IV (the smallest of the complement). In this report, we present data on the genetic characterization and molecular cloning of the rDNA sequences of this species, and a comparison with similar data on species from other continents.

Instituto de Biologia, Departamento de Genética, Universidade Federal do Rio de Janeiro, Caixa Postal 68011, 21944-970 Rio de Janeiro, RJ, Brasil. Send the correspondence to O.L.

MATERIAL AND METHODS

Species identification - Specimens of *C. sancticaroli* were identified following the taxonomic criteria of Strixino and Strixino (1981). Specimens were bred in our laboratory from eggs collected in the state of Rio de Janeiro, Brazil.

Genomic DNA isolation - A sample of 35 adult individuals was studied. They were split into seven samples of five individuals and each sample was homogenized in 1.5 ml Eppendorf tubes. Nuclei were lysed with SDS, as described by Coen *et al.* (1982), and lysed suspensions were treated sequentially with one volume of phenol, one volume of phenol-chloroform (1:1), and one volume of chlorophorm. DNA was later precipitated with two volumes of ethanol, washed in ethanol 70%, air dryed, and dissolved in T.E. buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8).

Lambda EMBL-4 DNA isolation - EMBL-4 is a substitution vector that accepts inserts ranging from 9 to 23 Kb (Frishauf *et al.*, 1983). Phage DNA was isolated as described by Sambrook *et al.* (1989).

Plasmid DNA - Plasmid DNA isolation was carried out by the lysis alkaline method, as described by Sambrook *et al.* (1989).

Cloning - Ligation was carried out with T4 DNA ligase. The genomic DNA was partially digested with EcoRI (0.5 units/microgram of DNA for one hour). Vector DNA was totally digested with EcoRI. Ligation was carried out at a 4:1 ratio of vector to genomic DNA.

DNA Labeling and hybridization - Probes were labelled by nick-translation using the BRL Nick Translation kit. [α^{32}P]dATP was the labelled nucleotide. Hybridization was carried out as described by Sambrook *et al.* (1989).

Transfection - *Escherichia coli* Q358 were prepared for transfection as indicated by Mandel and Higa (1970).

Screening - Screening was carried out as described by Benton and Davis (1977), using the probe pDm238, containing a rDNA repeat unit of *Drosophila melanogaster* inserted at the EcoRI site of pBR322 (Roiha *et al.*, 1981).

Sub-cloning - An insert of a positive amplified phage was sub-cloned at the EcoRI site of pBR325. This plasmid, which is 5.99 Kb in size, codes for resistance to Ampicillin, Chloramphenicol, and Tetracycline; the EcoRI site being located in the Chloramphenicol resistance gene.

Restriction enzymes - We used: BamHI (Sigma), ClaI (BRL), EcoRI (Sigma, Fiocruz), HaeIII (Pharmacia), HindIII (Pharmacia, Sigma), PstI (Sigma), and SmaI (BRL). Reactions were carried out as described by suppliers.

Physical mapping - Single and double digestions were used for most enzymes. For HaeIII, the method used was based on terminal labeling. The repeat unit cloned in pBR325 produced two fragments of 2.2 and 6.3 Kb by a double digestion with EcoRI/PstI.

These fragments were isolated by eletroelution, and partially digested with 0.1, 0.2 and 0.5 HaeIII units/microgram of DNA, electrophoresed in a 1.5% agarose gel, transfered to nylon membranes, and hybridized with a 1.1 Kb HindIII fragment, containing the 3'end of the 2.2 Kb EcoRI/PstI fragment, and the 5 'end of the 6.3 Kb EcoRI/PstI fragment (Figure 4).

RESULTS

Hybridizations of an EcoRI digest of genomic DNA with pDm238 detected a 8.5 Kb fragment. Digestions with HindIII showed a 7.4 Kb fragment (Figure 1). Whereas the 1.1 Kb fragment, representing the rest of the unit, was not observed, this was probably due to its location within or partially within the spacer region, as demonstrated by the physical mapping.

On the basis of these data, a partial EcoRI digestion of genomic DNA was carried out in order to obtain 17 kb fragments, containing two rDNA repeats. This sequence size lies within the insert size range accepted by phage Lambda EMBL-4 vector (see Material and Methods). Ligation products were then transfected in *E. coli* Q358, and recombinants

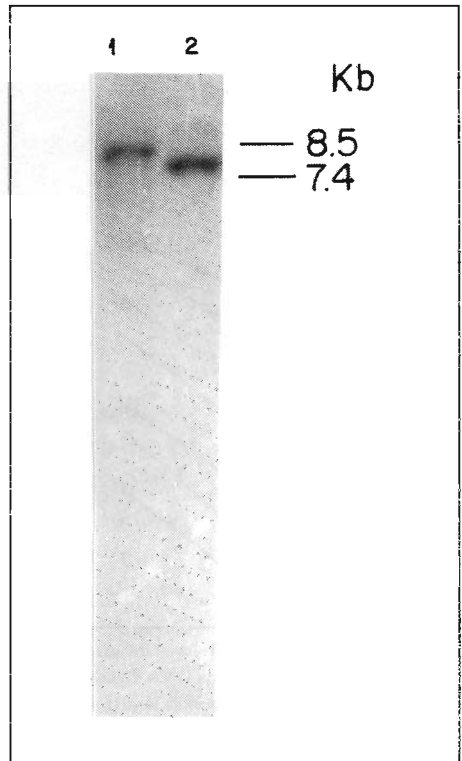

Figure 1 - Autoradiograms of *C. sancticaroli* genomic DNA digested with restriction endonucleases, electrophoresed in 0.7% agarose gel, transfered to nylon filters, and hybridized with pDm238. Following hybridization the filters were washed twice with 2X SSPE and 1% SDS at room temperature for 20 minutes, and twice with 1X SSPE, 1% SDS at 60°C for 20 minutes. 1 - Digestion with EcoRI; 2 - Digestion with HindIII.

were selected in *E. coli* Q359 to eliminate non-recombinant phages. Screening was carried out in *E. coli* Q358, and positive recombinants were obtained. Following digestion with EcoRI, the DNA of three recombinant phages showed a 8.5 Kb band homologous to rDNA. This fragment was inserted in the EcoRI sites of pBR325; the recombinant plasmid recovered was named pCs01.

Physical mapping was carried out with single and double digestions. The resulting fragments are shown in Figure 2 and Table I.

In order to verify the localization of the 18S and 28S regions, a double digestion of pCs01 was carried out with PstI/EcoRI, producing fragments of 2.2 Kb and 6.3 kb,

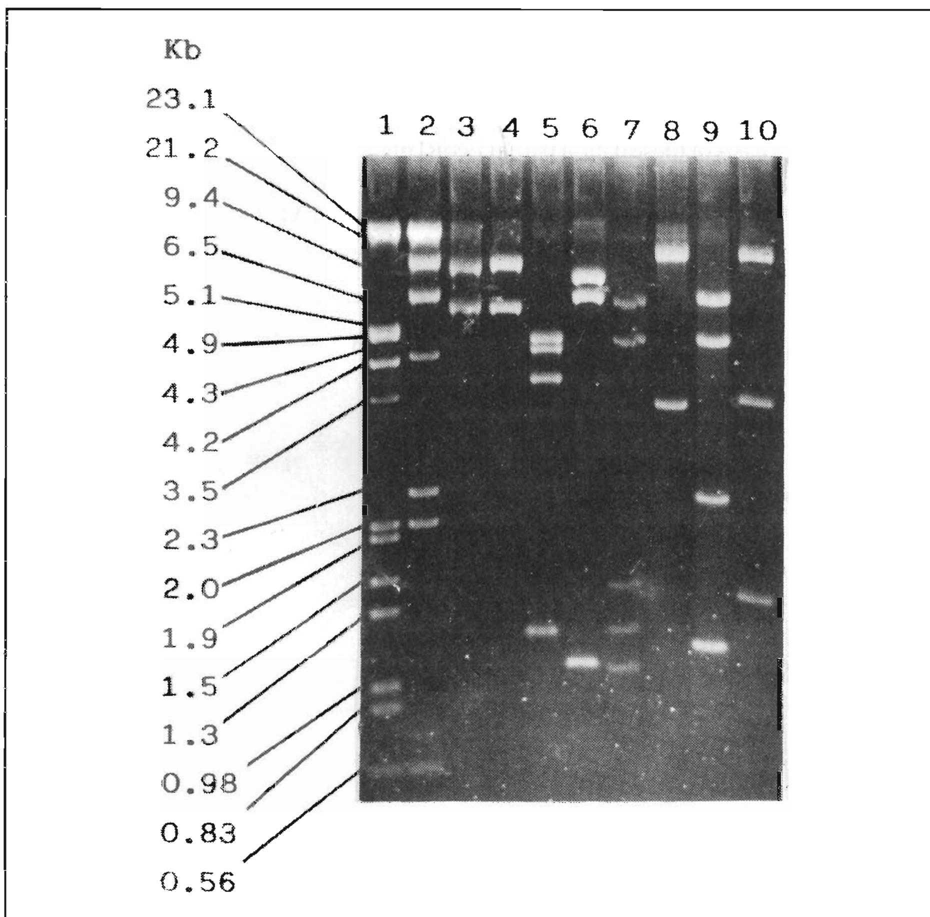

Figure 2 - Agarose gel (0.7%) showed digesten fragments for the physical map. 1 - Lambda DNA digested with EcoRI and HindIII; 2 - Lambda DNA digested with HindIII; 3 - pCs01 digested with EcoRI; 4 - pCs01 digested with ClaI; 5 - pCs01 digested with EcoRI and ClaI; 6 - pCs01 digested with HindIII; 7 - pCs01 digested with EcoRI and HindIII; 8 - pCs01 digested with PstI; 9 - pCs01 digesed with EcoRI and PstI; 10 - pCs01 digested with PstI and SmaI.

Table I - Length of the fragments presented in Figure 2, for the physical map of pCs01.

Enzymes	Fragments (kb)
Eco RI	8.5; 6.0
ClaI	8.5; 5.7; 0.2
ClaI/EcoRI	4.7; 4.5; 3.7; 1.2; 0.2
HindIII	6.2; 7.0; 1.1
HindIII/EcoRI	5.8; 4.7; 1.5; 1.2; 1.1
PstI	11.1; 3.4
PstI/EcoRI	6.3; 4.8; 2.2; 1.1
SmaI/PstI	9.6; 3.4; 1.5

corresponding to the insert. They were isolated by electroelution, electrophoresed and hybridized with probes containing the 18S or 28S regions of *Xenopus laevis* (Meunier-Rotival *et al.*, 1979). Our results showed that the fragments of 2.2 Kb were homologous to the 18S region and, the 6.3 Kb fragment to the 28S (Figure 3).

In order to map HaeIII sites, the above isolated fragments (2.2 Kb and 6.3 Kb) were partially digested with HaeIII, electrophoresed, transfered to a nylon membrane and hybridized to the 1.1 Kb HindIII fragment. This fragment lacks HaeIII sites, and overlaps the 3'and 5'extremities of the fragmetns of 2.2 and 6.3 Kb respectively. Distances between bands allowed us to estimate distances between the HaeIII sites in each fragment (Figure 4).

The physical map of the cloned sequence was compared with data from other chironomids (Figure 5).

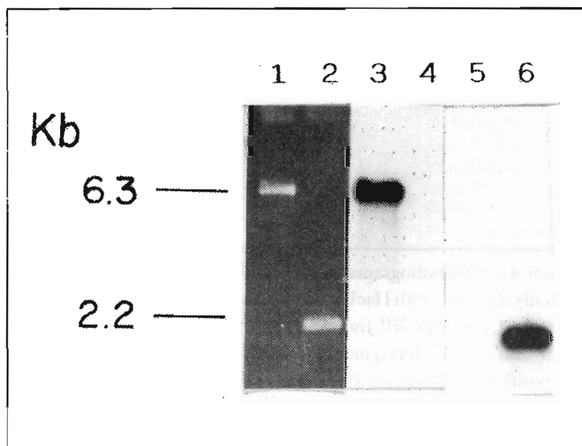

Figure 3 - Identification of 18S and 28S regions in pCs01. Autoradiograms from hybridizations with the 18S and 28S regions isolated from the *Xenopus laevis* rDNA recombinants HM456 and HM123 respectively (Meunier-Rotival *et al.*, 1979). The filters were washed twice with 2X SSPE, SDS 0.5% for 20 minutes each. Following hybridization with the 18S probe the filters were dehybridized and hybridized with the 28S probe. Lanes 1 and 2 - gel with the fragmetns EcoRI/PstI isolated from pCs01. Lanes 3 and 4 - hybridization with 28S probe. Lanes 5 and 6 - hybridization with 18S probe.

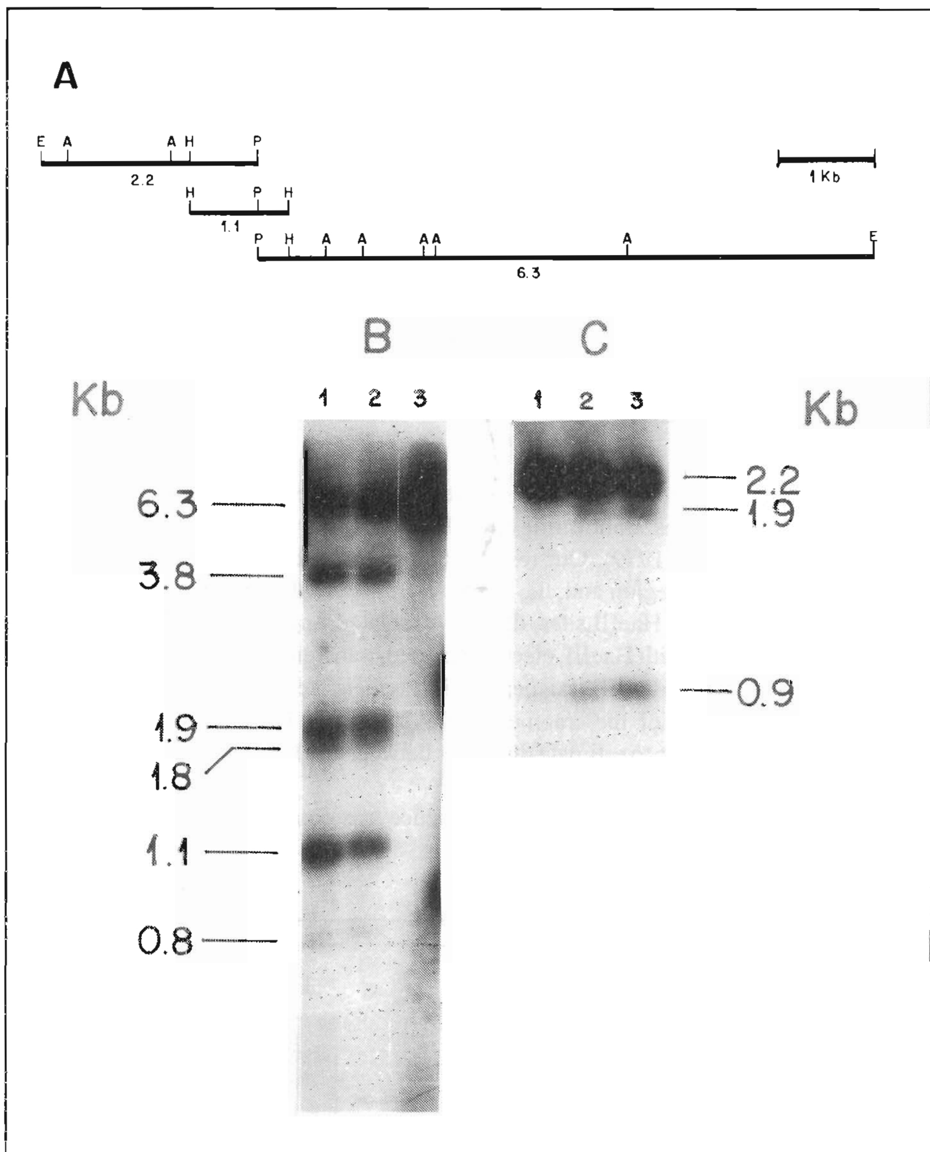

Figure 4 - Autoradiograms of the EcoRI/PstI 2.2 and 6.3 Kb fragment, corresponding to the pCs01 insert, partially digested with HaeIII, electrophoresed on a 1.5% agarose gel, transfered to nylon filters and hybridized with the 1.1 Kb HindIII fragment from pCs01. This fragment overlaps the 3' end of the 2.2 Kb fragment and the 5' end of 6.3 Kb fragment. A - Schematic representation of the 6.3 and 2.2 Kb EcoRI/PstI fragments, and the position of the 1.1 Kb HindIII fragment: A - HaeIII; E - EcoRI; H - HindIII; P - PstI. B - Autoradiogram of the 6.3 Kb fragment partially digested with 0.5 (lane 1), 0.2 (lane 2), and 0.1 (lane 3) units of HaeIII per microgram of DNA. C - Autoradiogram of the 2.2 Kb fragment partially digested with 0.1 (lane 1); 0.2 (lane 2) and 0.5 (lane 3) units of HaeIII per microgram of DNA.

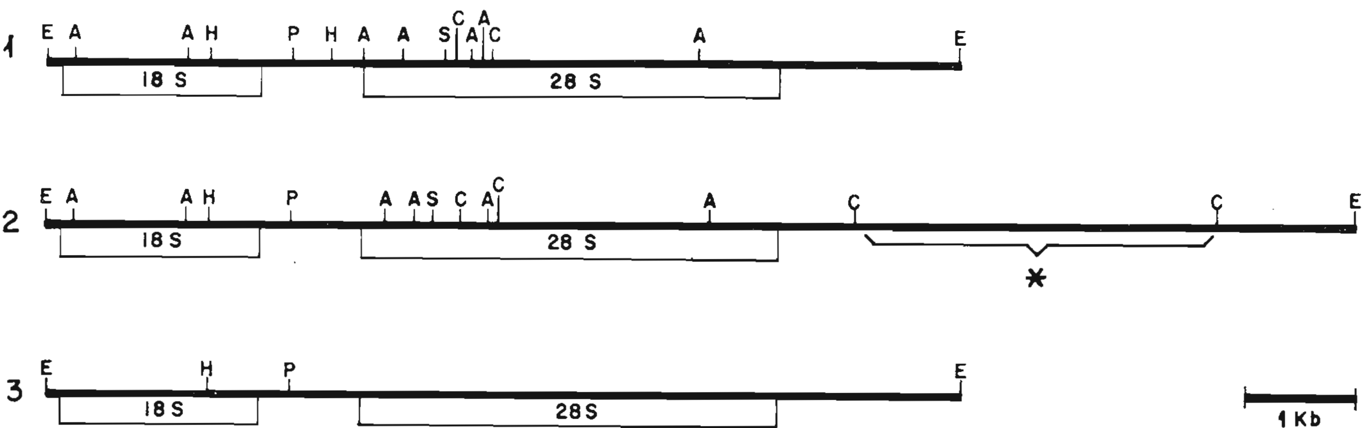

Figure 5 - Physical maps of: 1 - *Chironomus sancticaroli* rDNA cloned unit (pCs01); 2 - *C. thummi* (Israelewski and Schmidt, 1982); 3 - *C. tentans* (Degelmam *et al.*, 1979). A - HaeIII; C - ClaI; E - EcoRI; H - HindIII; P - PstI; S - SmaI; * 120 bp ClaI repetitive sequences.

DISCUSSION

Analysis of genomic DNA showed no variation in the length of the rDNA repeats of *C. sancticaroli*. A single band without a smear above or below the fragment produced by EcoRI or PstI digestions was found. A similar uniformity of these repeat units has been described for other chironomids like *C. tentans, C. pallidivittatus* (Degelmann *et al.*, 1979) and *C. tepperi* (Israelewski and Schmidt, 1982). However *C. thummi* and *C. melanotus* (Degelmann *et al.*, 1979), showed variation in repeat lenght, probably related to the presence of a large amount of heterochromatin in these species (Degelmann *et al.*, 1979). As *C. sancticaroli* contains a very small amount of hererochromatin (Freitas *et al.*, 1985), our results are consistent with the interpretation of Degelmann *et al.* The variability observed in *C. thummi* is found in the 120 bp repetitive sequences of the intergenic spacer. These sequences are related to transposition events, and are also present in other genomic regions (Schmidt, 1984; Hankeln and Schmidt, 1989).

A comparison of the physical map of *C. sancticatoli* with those of *C. thumi* and *C. tentans*, shows a high level of evolutive conservation. In the 18S region, the localization of EcoRI and HindIII sites are identical in the three species, as are HaeIII sites in *C. sancticatoli* and *C. thummi*. The PstI site, located in the internal transcribed spacer, is also identical in the three species. Within the 28s region, there are small distance differences between restriction sites, when comparing *C. sancticaroli* with *C. thummi*. A new HindIII site, probably in the internal transcribed spacer, and another for HaeIII, between the ClaI sites, are present in *C. sancticaroli*. These differences are probably related to expansion segments, or short sequences located between conserved regions (Gerbi, 1985; Mindell, 1990).

A more detailed analysis of the 28S region and the intergenic spacer may offer interesting data for evolutionary comparisons in this widely distributed genus.

ACKNOWLEDGMENTS

Publication supported by CNPq, FAPERJ, CEPG (UFRJ), FUJB and CAPES. We thank Dr. Hector Seuanez Abreu for reviewing the manuscript, and Dr. Itamar R.G. Ruiz for the probes HM123 and HM456.

RESUMO

Chironomus sancticaroli é um quironomídio sulamericano. Com a finalidade de comparar seu rDNA com o de outros chironomídios, analisamos o DNA genômico e clonamos uma unidade de repetição. Os dados obtidos foram comparados com os dados de *Chironomus thummi* e *C. tentans*. As unidades de rDNA não mostraram variação no seu comprimento (8,5 Kb). O mapa físico mostrou variações na região 28S, quando comparados aos dados de *C. thummi* estas pequenas diferenças podem estar relacionadas a segmentos de

expansão. Sítios de clivagem para Hind III (ausentes em *C. thummi* e *C. tentans*) e Hae III (ausente em *C. thummi*), foram encontradas no espaçador transcrito interno e na região 28 S, respectivamente.

REFERENCES

Benton, W.D. and Davis, R.W. (1977). Screening of Lambda gt recombinants by hybridization to single plaques in situ. *Science 196*: 180-182.

Coen, E.S., Thoday, J.M. and Dover, G. (1982). Rate of turnover of structural variants in the rDNA genes family of *Drosophila melanogaster. Nature 295*: 564-568.

Degelmann, A., Royer, H.D. and Hollenberg, C. (1979). Characterization of the ribosomal RNA genes of *Chironomus tentans* and some closely related species. *Chromosoma 71*: 263-281.

Freitas, F., Floeter-Winter, L.M. and Leoncini, O. (1985). Cytological and cytochemical characterization of the politene chromosomes of *Chironomus sancticaroli. Brazil. J. Genet. 8*: 47-60.

Frishauf, A.M., Lehrach, H., Pourstka, A. and Murray, N. (1983). Lambda replacement vectors carrying polykinker sequences. *J. Mol. Biol. 170*: 827-842.

Gerbi, S.A. (1985). Evolution of ribosomal DNA. In: *Molecular Evolutionary Genetics* (MacIntyre, R.J., ed.). Plenum Press.

Hankeln, T. and Schmidt, E.R. (1989). Cotransposition of highly repetitive DNA element with flanking sequences in the genome of the midge *Chironomus thummi. J. Mol. Evol. 26*: 311-319.

Israelewski, N. and Schmidt, E.R. (1982). Spacer size in ribosomal DNA of *Chironomus thummi* is due to a 120 bp repeat homologous to a predominantly centromeric repeat sequence. *Nuclei. Acid. Res. 10*: 7689-7700.

Mandel, M. and Higa, A. (1970). Calcium dependent bacteriophage DNA infection. *J. Molec. Biol. 53*: 159-162.

Mindell, D.P. (1990). Ribosomal RNA in vertebrates: Evolution and phylogenetic applications. *Ann. Rev. Ecol. System. 21*: 541-566.

Meunier-Rotival, M., Cortatas, J., Macaya, G. and Bernardi, G. (1979). Isolation and organization of calf ribosomal DNA. *Nucleic Acid. Res. 6*: 2109-2123.

Roiha, H., Miller, J.R., Woods, L.C. and Glover, D.M. (1981). Arrangements and rearrangements of sequences flanking the two types of rDNA insertion in *Drosophila melanogaster. Nature 290*: 749-753.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). *Molecular Cloning. A laboratory manual.* 2nd edition. Cold Spring Harbor Laboratory.

Schmidt, E.R. (1984). Cluster and interspersed repetitive DNA sequence family of *Chironomus. J. Mol. Evol. 178*: 1-15.

Strixino, S.t. and Strixino, G. (1981). Nova espécie do gênero *Chironomus* Meigen do sul do Brasil (Diptera: Chironomyidae). *Rev. Bras. Ent. 25*: 333-340.

(Received January 9, 1992)