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# HIGH RESOLUTION OF FISH CHROMOSOMES: THE G-BANDED KARYOTYPE OF *NOTHOBRANCHIUS RACHOWI*

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## ABSTRACT

G-banded prometaphase chromosomes of the cyprinodontid fish *Nothobranchius rachowi* were produced at a level of resolution previously unattained in the study of chromosome structure in fishes. Minimal exposure to colchicine, with respect to both dosage and treatment time, reduced the effects of this mitotic inhibitor on chromosome contraction. Extended hypotonic exposure aided in dispersal of the elongated prometaphase chromosomes. Mild trypsin treatment followed by dilute Giemsa staining was used to produce G-banding. Ideographic representation of the chromosome bands is presented.

## INTRODUCTION

The study of fish chromosomes is in transition from the use of conventional methods which rely on centromeric position for classification toward the application of modern differential staining techniques (C-, G-, Q- and R-banding). These techniques, introduced by mammalian cytogeneticists, have revolutionized cytogenetics (Caspersson *et al.* 1970, Pardue and Gall 1970, Dutrillaux and Lejeune 1971, Sumner *et al.* 1971, Wang and Federoff 1972); for example, G-banding techniques have provided accurate detection of chromosome rearrangements and allowed precise "pairing" of homologous chromosomes (Therman *et al.* 1974, Arrighi *et al.* 1976). The comparison of G-banding patterns has also proved to be useful in studies of the evolution of species (Mascarello *et al.* 1974, Bickham 1981).

In many fishes chromosomes are minute and numerous. Often they are of similar size and centromeric location. Modern staining techniques that allow differentiation of specific regions along chromosome arms would be valuable in the study of such chromosomes, however, only a few reports describe successful G-banding of fish chromosomes (Hafez 1979, Kirpichnikov 1981, Blaxhall 1983b, Liu 1983).

Recently, high resolution techniques have been developed that yield sufficient numbers of cells in the early stages of mitosis (prophase and prometaphase) for detailed analysis (Yunis 1976, Yunis *et al.* 1978). These methods synchronize peripheral blood cultures by blocking the lymphocytes in S-phase and then releasing the block. Subsequent harvest is timed to obtain the maximum number of cells possessing elongated chromosomes. Although peripheral blood culture has been demonstrated for fishes, high resolution banding has not been reported (Heckman and Brubaker 1970, Ojima *et al.* 1970, Hartley and Horne 1983, Blaxhall 1983a).

The killifish, *Nothobranchius rachowi* (Microcyprini: Cyprinodontidae), has been successfully introduced as a model for the *in vivo* assessment of genotoxic substances in water (Van der Hoeven *et al.* 1981, 1982). *N. rachowi* possesses a low number (2n = 16) of relatively large chromosomes. These characteristics render it advantageous for studies of chromosome aberrations and sister chromatid exchanges. Although sister chromatid exchanges in this fish have been described (Van der Hoeven *et al.* 1982), banding of the chromosomes has not been reported. G-banding of the chromosomes of *N. rachowi* would allow definitive localization of structural abnormalities and changes, such as breaks and gaps.

In this study, simple modifications of conventional cytogenetic protocols were utilized to produce a high resolution G-banded karyotype for *N. rachowi.*

## MATERIALS AND METHODS

Five large female specimens of *N. rachowi* were obtained commercially and maintained in a well-aerated aquarium throughout the experiment. A one-tenth percent solution of colchicine (GIBCO) was administered intraperitoneally at a dose of 0.075 ml per 5 g body weight 4 hours prior to death. Gills were dissected and macerated in 4 ml of 0.075 percent potassium chloride. The suspensions of gill epithelial cells were transferred to centrifuge tubes for a total hypotonic treatment of 40 minutes. After centrifugation the cellular pellet was gently dispersed in 5 ml of cold fresh fixative (3:1, methanol:acetic acid). This step was repeated four times. The cells were finally suspended in 0.25 ml of fixative, dropped from a height of one meter onto cold slides, and air-dried. These techniques are modifications of standard harvest procedures utilized by Clark and Mathis (1982).

Slides were aged for 2 weeks and stained by the GTG technique (G-banding by trypsin treatment followed by Giemsa staining) (Wang and Federoff 1972). Two-minute exposure to 0.025 percent trypsin (GIBCO) at room temperature yielded distrinct banding patterns. Trypsin treatment was arrested by three washes in phosphate-buffered saline (pH 7.0). Chromosomes were stained in 2 percent Giemsa diluted with Sorensen's buffer (pH 6.8) for 10 minutes.

Chromosomes were examined and photographed using a Zeiss Photomicroscope III and Kodak Technical Pan film 2415. Agfa Brovira BEH III 5 paper was utilized to print the chromosomes. This combination of materials produces a high contrast photograph that enhances discrimination of chromosome bands.

## RESULTS AND DISCUSSION

The modal diploid chromosome number as determined from the examination of 120 metaphase cells was 16. This is in accordance with the observations of Post (1964), Gyldenholm and Scheel (1971), and Van der Hoeven *et al.* (1981). Table I indicates standard classification of the chromosomes by centromeric index (long arm/short arm) and percent total complement length. Classification was based on the criteria established by Levan *et al.* (1964). Terms suggested by the Denver Study Group (1960) which are still in general use have also been included in Table I.

TABLE 1. *Classification of chromosomes by arm ratio (long arm/short arm) and percent total complement length (% TCL). Terminology for chromosome designations is from Levan* et al. *(1964) and the Denver Study Group (1960) (in parentheses).*

| Chromosome Number | % TCL | Arm Ratio | Chromosome Designation |
|---|---|---|---|
| 1 | 18.66 | 1.04 | m (Metacentric) |
| 2 | 16.86 | 1.11 | m (Metacentric) |
| 3 | 15.70 | 2.21 | sm (Submetacentric) |
| 4 | 14.67 | 1.11 | m (Metacentric) |
| 5 | 12.48 | 2.13 | sm (Submetacentric) |
| 6 | 10.55 | 1.34 | m (Metacentric) |
| 7 | 6.69 | 1.36 | m (Metacentric) |
| 8 | 4.39 | 8.50 | t (Acrocentric) |

Twenty well-spread prometaphase cells were examined for G-banding patterns. Production of these elongated chromosomes was facilitated by low dosage and exposure to colchicine. Chromosome contraction effects were thereby minimized while still inhibiting spindle fiber formation. Although the number of cells observed in mitosis was depressed, chromosomes were sufficiently elongated for high resolution banding. This condition also increases overlapping of chromosomes which necessitated prolonged hypotonic treatment to ensure dispersal. In spite of the minimal colchicine treatment, numerous metaphase cells were observed on each slide preparation.

Mild trypsin treatment and dilute staining by Giemsa were found to be essential to avoid obscuring or eliminating bands. Aging of slides for two weeks prior to trypsin treatment enhanced the consistency of banding in contradiction to the report by Blaxhall (1983b) that aging was contraindicated for reproducible trypsin banding. Blaxhall (1983b) also found the effects of trypsin to be too variable and preferred the ASG (acetic/saline/Giemsa) banding method of Sumner *et al.* (1971).

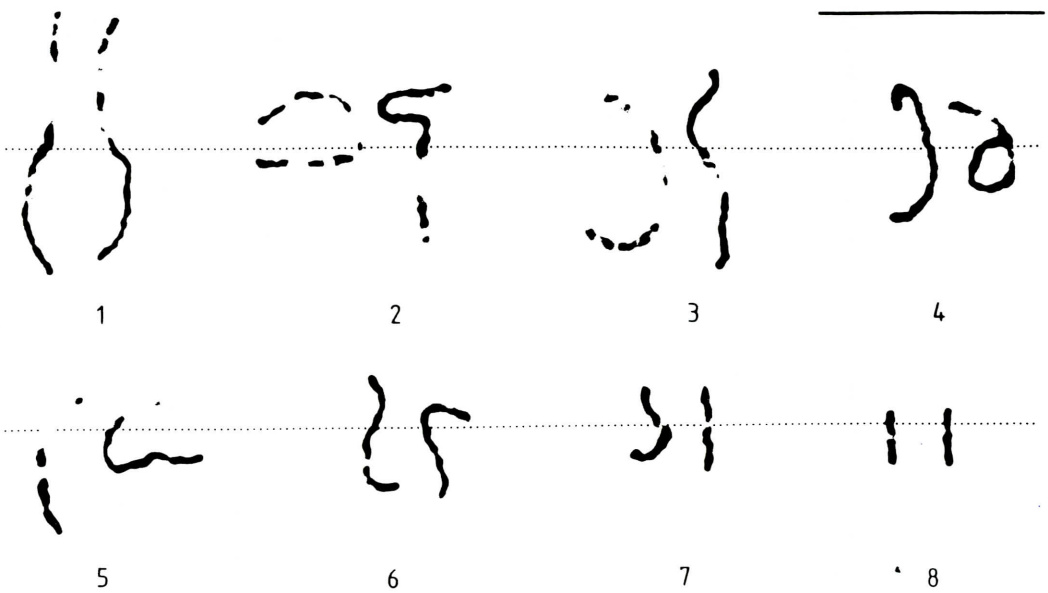


FIG. 1. *High resolution G-banded karyotype of* Nothobranchius rachowi. *Heavily stained regions were discerned by comparison to similar regions on the chromosomes of other cells (bar represents 10 μm).*

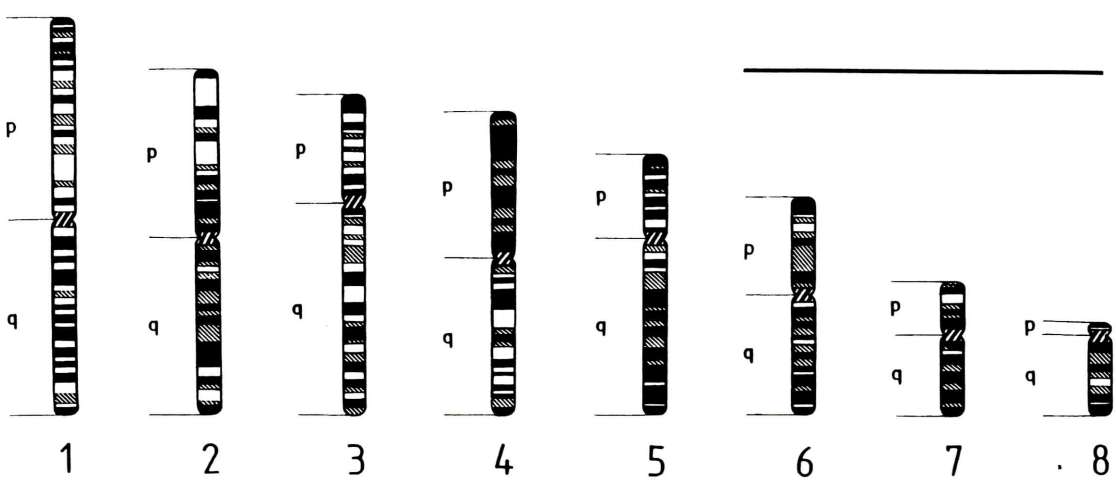


FIG. 2. *Ideogram representing the 163 band haploid karyotype of* Nothobranchius rachowi. *The long arm of each chromosome is designated by the letter q and the short arm by p (bar represents 10 μm).*

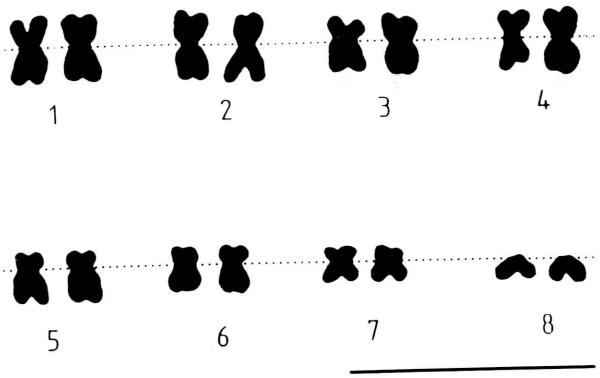


FIG. 3. *Standard metaphase karyotype of* Nothobranchius rachowi *(bar represents 10 μm).*

Approximately 163 positively stained G-bands were distinguishable per haploid set of prometaphase chromosomes. An ideogram of the most stable G-banding is shown in Figure 1. Representative GTG-banded chromosomes have been enlarged to the size of the ideogram for comparison of the bands depicted with those present on a photomicrograph of actual chromosomes (Figure 2). A non-banded standard karyotype is shown in Figure 3 to demonstrate the greater degree of contraction present in karyotypes prepared from metaphase chromosomes.

Features such as relative width of bands, staining intensity, and distances from centromeres have been shown to vary with the stage of chromosome condensation and with the banding techniques employed (Oliver and Francke 1978). Homologous chromosomes or even chromosome arms may contract differentially during prophase. The sequence of bands was found to be the most constant feature of chromosome banding. Consequently, the ideogram is based on relative banding patterns rather than on intensity or measurement. However, an attempt was made to indicate relative width of bands among the 20 karyotypes analyzed.

A simple black and white ideogram was used to show the position and relative width of stable bands. Variable regions are indicated by shading with parallel lines. Low intensity bands were often present within these variable regions. A few chromosomes displayed additional subbands that have not been included in the ideogram. The centromeric region is depicted on each chromosome by darker shading. These regions in the ideogram do not represent specifically banded structures. The conservative nature of this description should allow expansion of the ideogram as more bands are recognized.

Recently, Delany and Bloom (1984) have discussed the applicability of G-banding methods to the study of fish chromosomes in a report of chromosomal replication banding patterns (replicon clustering) in the rainbow trout, *Salmo gairdneri.* These authors attribute their failure to G-band fish chromosomes to the inherent nature of the fish genome. Furthermore, they suggest that "traditional" banding techniques, such as G-banding, are of limited use for differentiating fish chromosomes and advocate development of alternate techniques such as replication banding. The investigators later detract from their argument by describing variability in replication bands and suggesting caution be exercised when applying this method to karyotypic analyses. Delany and Bloom (1984) offer their results as support for the theory advanced by Holmquist *et al.* (1982) that the chromosomes of lower chordates, including fishes, exhibit replicon clustering but not G-banding.

The results reported here and in previous successful G-banding studies do not support this hypothesis. Reports of difficulty and failure to produce G-banding in fishes appear to have resulted from improper treatment of chromosomes (Kligerman and Bloom 1977, Rishi 1979). For example, Kligerman and Bloom (1977) were unable to demonstrate G-banding in the central mudminnow, *Umbra limi,* reporting that the chromosomes were uniformly stained or bloated in appearance. These results are indicative of inappropriate trypsin exposure, being under- or overtreated, respectively. With further investigation and modifications, replication banding may become a useful technique for the identification of fish chromosomes. At the present time, however, it would be unwise to abandon a powerful analytic technique like G-banding at such an early stage of application.

Methods utilized by mammalian cytogeneticists for obtaining large numbers of mitotic cells, such as lymphocyte and tissue culture, should be incorporated in prospective cytogenetic studies of fishes. Cell synchronization techniques could then be applied to accumulate cells in the earlier stages of division, thereby avoiding the compromise of total mitotic yield in order to obtain elongated prometaphase chromosomes. Other differential staining methods such as R-banding (complementary to G-banding) should also be investigated.

This study provides the first demonstration of high resolution G-banding of fish chromosomes. Exploitation of high resolution techniques combined with differential staining should refine future comparative cytogenetic studies of fishes. It is hoped that the information in this report will also be useful to genetic toxicologists for the precise localization of chromosomal aberrations in *N. rachowi.*

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# THE VASCULAR PLANTS OF TENNESSEE: A TAXONOMIC AND GEOGRAPHIC GUIDE TO THE FLORISTIC LITERATURE

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## ABSTRACT

A summary of the Tennessee floristic literature is presented. In general, articles were included if they: 1) were primarily about Tennessee and reported new state and/or county records, or 2) summarized Tennessee distributional records, or 3) provided original baseline field work within the state. Additional efforts were made to include both early historical accounts and various unpublished studies, such as theses and dissertations. Taxonomic and geographic indices are provided to increase the useability of the bibliography.

## INTRODUCTION

During the past twenty-five years, there has been a dramatic increase in the number of publications concerned with the distribution of vascular plants in Tennessee. Over 200 documents reporting almost as many new state records have been published since 1960 (Table 1). In the same time period, the Generic Flora of the Southeastern United States Project (Wood, 1983) has completed reviews of 130 families and 475 genera. This project has been instrumental in summarizing important changes in nomenclature and species distributions for Tennessee. During the past decade, a diversity of organizations, both public and private, have stimulated interest in the conservation of rare plants which has resulted in new field work in all parts of the state.

In the absence of a suitable compilation of the state flora, it is most appropriate to have at least a current guide to floristic literature. Interestingly, there have been no previous attempts to summarize the Tennessee floristic literature. Several bibliographies have dealt with the Great Smoky Mountains National Park (White, 1982) and the Southern Appalachian region (DeYoung *et al.,* 1982; Evans, 1971; Evans *et al.,* 1981; Wofford & White, 1981), but none have addressed the state as a whole. Two shorter bibliographies include a number of Tennessee publications but the emphasis was on the historical (Andre, 1971) and vegetational aspects (Egler, 1961). Corgan (1977) presents a brief summary of technical and scientific journals published in Tennessee prior to 1862.

The purpose of this paper is to summarize the Tennessee floristic literature. Guidelines for the inclusion/exclusion of certain references were developed to complement other primary works. Publications were included if they were: 1) primarily about Tennessee floristics and presented new distributional records, or 2) systematic treatments of taxa primarily distributed in Tennessee (eg. *Leavenworthia),* or 3) ecological studies of places in Tennessee that recorded the occurrence of many woody and herbaceous taxa, or 4) unpublished documents that are basically taxonomic or floristic in nature and concern taxa that occur in Tennessee, or 5) works considered to be noteworthy for Tennessee from a historic point of view.

Several general categories of publications were purposefully excluded from the bibliography. Unpublished notes and letters currently housed at the Great Smoky Mountains National Park were excluded since these have been listed in detail recently (White, 1982). Most systematic revisions were excluded because they are currently being reviewed through the Generic Flora Project. Also, Wofford & White (1981) have recently presented a thorough index to the systematic literature for the Southern Appalachian region.