Rev. Brasil. Genet. 14, 4, 1085-1090 (1991)
(Brazil. J. Genetics)

METHODOLOGY

EXTRACTION AND USE OF THE CEPHALIC KIDNEY FOR CHROMOSOME STUDIES IN SMALL FISH

Orlando Moreira-Filho and Luiz A.C. Bertollo

ABSTRACT

The kidney of teleostean fish is divided into two portions: an anterior one (cephalic kidney) and a posterior one. The present communication describes an easy method for the extraction of the cephalic kidney of small fish and demonstrates the advantages of obtaining chromosome preparations from this material.

INTRODUCTION

The last few years have seen a marked advance in fish cytogenetics thanks to the improvement of chromosome preparation techniques. Jim and Toledo (1973) and Foresti (1974) presented the first cytogenetic results obtained for fresh-water fish in Brazil using the branchial epithelium and portions of the intestine as starting material for metaphase preparation by the squash technique. Bertollo (1978) adapted the air-drying technique of Egozcue (1971) and Cestari (1973) using branchiae and kidney.

When the kidney started to be used as the main source of metaphases after other methodological adaptations, fish cytogenetics overcame one of the obstacles standing in the way of chromosome studies in this group.

In fish, the kidney also has a hematopoietic function and, according to Hibiya (1982), its shape varies from species to species. In teleosteans, the kidney is subdivided

Universidade Federal de São Carlos, Via Washington Luiz, Km 235, Caixa Postal 676, 13560 São Carlos, SP, Brasil.

into two portions: the cephalic kidney, or anterior portion, and the kidney body corresponding to the posterior portion of the organ.

The objectives of the present paper were to perform a comparative qualitative analysis of chromosome preparations obtained from these two kidney portions and to describe a methodology for cephalic kidney extraction from small fish.

METHODOLOGY AND DISCUSSION

In small fish, the cephalic kidney is of considerably reduced size and therefore of difficult extraction. Thus, we devised a methodology that would facilitate cephalic kidney extraction according to the following procedures (Figure 1):

1. The animal is positioned with the ventral portion up and a transversal cut is made at the base of the isthmus (A), followed by a longitudinal cut from the base to the anus (B).
2. The sectioned parts are pulled apart and secured with pins to a styrofoam base to expose the region of the digestive tube (C).
3. The region between the pharynx and esophagus is sectioned and the viscera are pulled toward the posterior part of the body to expose the cephalic kidney (D, arrow).
4. Removal of the viscera is completed to also expose the posterior kidney (E, larger arrow).
5. The desired portions are removed with a fine-tipped dissection forceps.

The above procedure can be made easier by anesthetizing the fish with benzocaine. Benzocaine (300 mg) is dissolved in 3 ml absolute alcohol and mixed into two liters of water. The animal is submerged in this solution for four to five minutes, a time usually sufficient to complete the process of anesthesia. The concentration of this solution can be increased or decreased according to the susceptibility of the species under study.

Embryologically, the cephalic kidney of fish derives from the pronephron and usually consists of lymphoid tissue, whereas the kidney body derives from the mesonephron and is composed of many nephrons, interstitial tissue and fibers (Hibiya, 1982). The differential composition of these materials can be observed in transversal histological sections of the cephalic and posterior kidney (Figure 2).

Test chromosome preparations were obtained separately from cephalic and posterior kidney material by the air-drying technique of Egozcue (1971) and Cestari (1973), adapted for fish by Bertollo (1978). The advantage of using the cephalic kidney is clearly visible in terms of metaphase number and quality of the preparations obtained as they are free from the tissue fibers often observed in preparations from the posterior kidney (Figure 3). In our laboratory we have also used the cephalic kidney in short-term

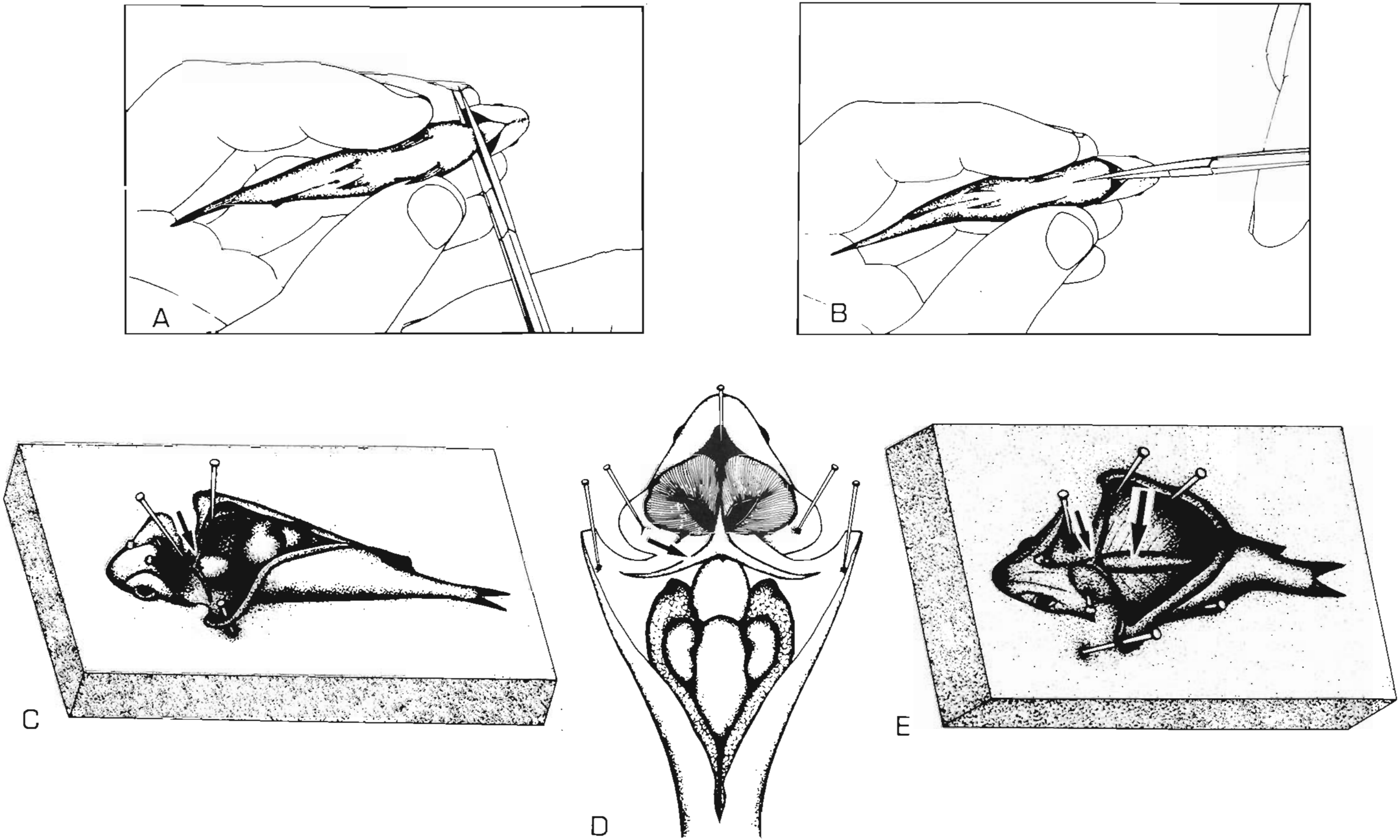

Figure 1 - Procedures for the exposure and extraction of the cephalic and/or posterior kidney of small-sized fish (see the text for a description). The smaller arrows indicate the cephalic kidney, and the large arrow the posterior kidney.

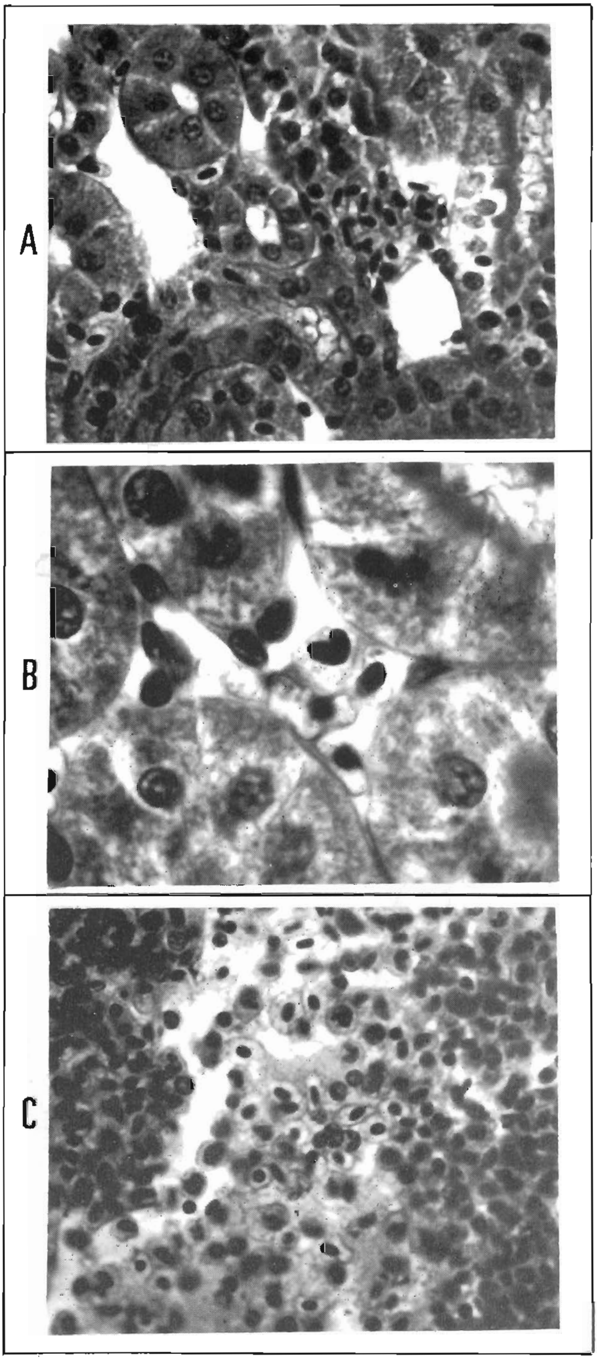

Figure 2 - Transversal six μm sections of *Astyanax scabripinnis* kidney stained with hematoxylin and eosin. The histological differentiation between the cephalic and posterior kidney portions can be observed. A and B, posterior kidney (420X and 1,400X, respectively); C, cephalic kidney (1,100X).

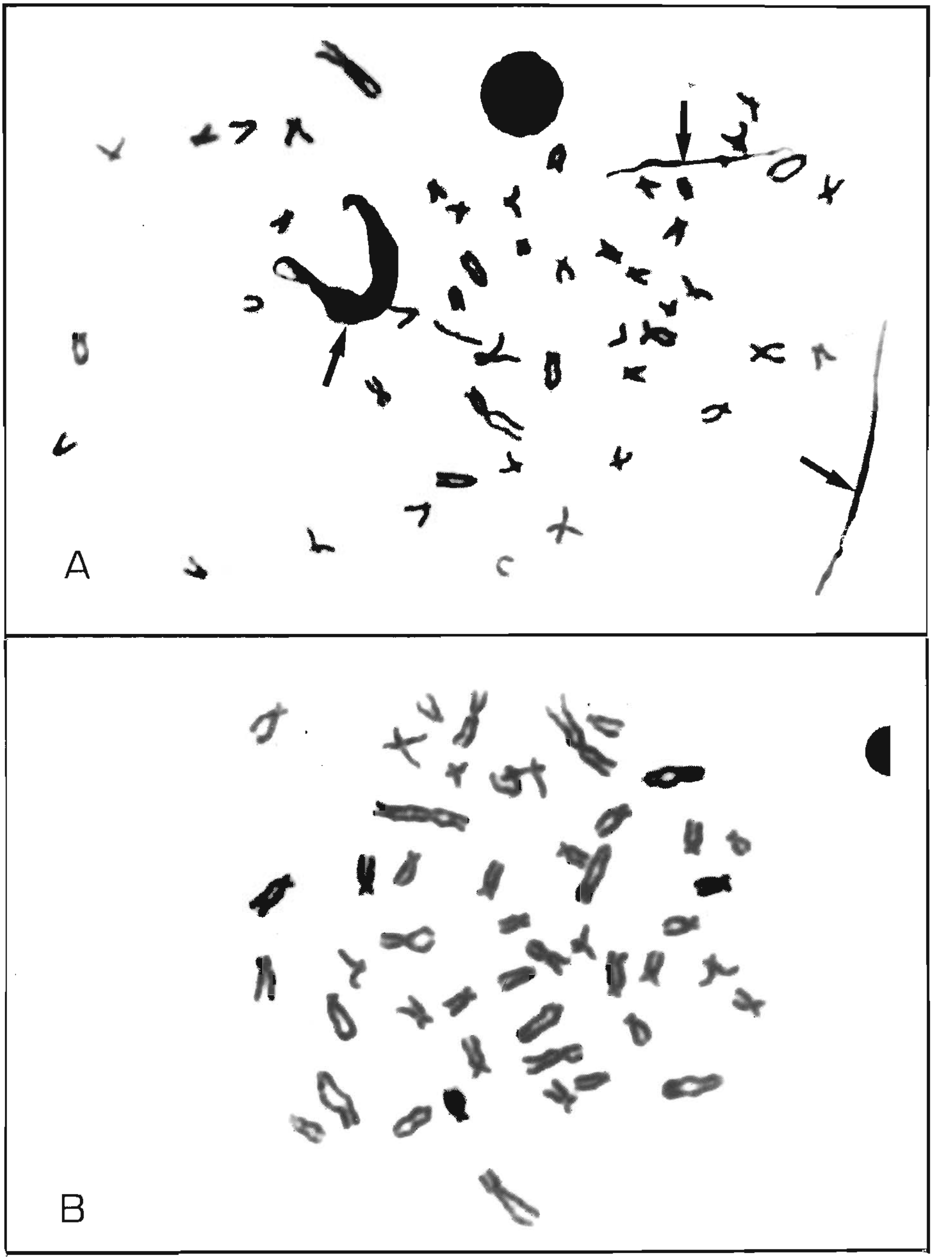

Figure 3 - Metaphases obtained from material of the posterior kidney (A) and of the cephalic kidney (B) of small-sized fish (*Astyanax scabripinnis*). Observe the presence of fibers (arrows) among the chromosomes in the material from the posterior kidney.

cultures (Fenocchio *et al.*, in press), with excellent results demonstrating that the material also responds very efficiently to this technique.

On this basis, the use of the cephalic kidney permits obtaining good-quality metaphases for chromosome studies in fish. In medium- to large-sized species, the extraction of the cephalic kidney is made easier by the relatively large size of the organ. However, in small species the extraction may be quite difficult. The methodology described here facilitates extraction since the cephalic kidney is directly exposed at the site of the initial incisions for dissection.

The South American fish fauna is quite rich in small species and is practically unexplored from a chromosomal viewpoint because of the difficult manipulation of this material. We believe that the technique presented here may contribute to the cytogenetic study of this important fraction of the Brazilian fish fauna.

ACKNOWLEDGMENTS

Publication supported by FAPESP.

RESUMO

Nos peixes teleósteos o rim é dividido em duas porções: porção anterior (rim cefálico) e porção posterior. Este trabalho descreve um método de fácil extração do rim cefálico em peixes de pequeno porte e demonstra as vantagens na obtenção de preparações cromossômicas a partir deste material.

REFERENCES

Bertollo, L.A.C. (1978). Estudos citogenéticos no gênero *Hoplias* Gill, 1903 (Pisces, Erythrinidae). Doctoral Thesis, Departamento de Genética e Matemática Aplicada à Biologia, FMRP-USP, Ribeirão Preto.

Cestari, A.N. (1973). Métodos de estudo dos cromossomos de vertebrados. In: *Exercícios Práticos de Genética*: 29-31, (Azevedo, J.L. de and Costa, S.O.P., eds.). Companhia Editora Nacional - Editora da Universidade de São Paulo, São Paulo.

Egozcue, J. (1971). Estudios cromosómicos. Preparaciones directas. In: *Tecnicas en Citogenetica*: 15-37. Editorial Espaxs, Barcelona.

Fenocchio, A.S., Venere, P.C., Gobbo, A.C., Dias, A.L. and Bertollo, L.A.C. Short term culture from solid tissues of fishes. *Caryologia* (in press).

Foresti, F. (1974). Técnicas para obtenção de cromossomos de pequenos peixes. *Cienc. Cult. 26*: 248.

Hibiya, T. (1982). Kidney. In: *An Atlas of Fish Histology and Pathological Features*: 94-104. Gustaw Fisher Verlog, Tokyo.

Jim, S.M. and Toledo, V. (1973). Citogenética de *Astyanax fasciatus* e *Astyanax bimaculatus* (Characidae, Tetragonopterinae). *Cienc. Cult. 27*: 1122-1124.

(Received March 1, 1991)