as follows: 2 mi. N Nixon, Gonzales County (Raun, 1959); west-southwest to 7 mi. E Lytle, Atascosa County (Blair, 1952); south-southwest to 2 mi. NE Bustamante, Zapata County (Baumgardner and Schmidly, 1981); and east-southeast to Sauz Ranch, Willacy County (Schmidly and Hendricks, 1976).

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## Literature Cited

Allen, J. A. 1891. On a collection of mammals from southern Texas and northeastern Mexico. Bull. Amer. Mus. Nat. Hist., 3:219-228.

Bailey, V. 1905. Biological survey of Texas. N. Amer. Fauna, 25:1-222.

Baumgardner, G. D., and D. J. Schmidly. 1981. Systematics of the southern races of two species of kangaroo rats (Dipodomys compactus and D. ordii). Occas. Papers Mus., Texas Tech Univ., 73:1-27.

Blair, W. F. 1952. Mammals of the Tamaulipan biotic province in Texas. Texas J. Sci., 4:230-250.

Hall, E. R. 1981. The mammals of North America. John Wiley and Sons, New York, 2nd ed., 1;xv+600+*90*.

Rand, McNally and Co. 1887. New enlarged scale railroad and county map, Texas. Rand, McNally and Co., Chicago.

Raun, G. G. 1959. A new Texas locality for *Dipodomys ordi*. J. Mamm., 40:146-147.

Schmidly, D. J., and F. S. Hendricks. 1976. Systematics of the southern races of Ord's kangaroo rat, *Dipodomys ordii*. Bull. Southern California Acad. Sci., 75:225-237.

Setzer, H. W. 1949. Subspeciation in the kangaroo rat, Dipodomys ordii. Univ. Kansas Publ., Mus. Nat. Hist., 1:473-573.

State Department of Highways and Public Transportation. 1980. General highway maps of Texas. State Department of Highways and Public Transportation, Austin.

# THE STANDARD AND NOR-STAINED KARYOTYPE OF *RIVULUS AGILAE* (RIVULIDAE: TELEOSTEI)

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During a recent herpetological collection trip to South America by members of our department, a small (total length less than two centimeters) specimen of the aplocheiloid fish *Rivulus agilae* was inadvertently returned to College Station in the water used to maintain live specimens of turtles. The fish was brought to our laboratory, and we were able to obtain surprisingly good chromosome preparations, which were then stained with silver nitrate to determine the number and chromosomal locations of the nucleolar organizer regions (NORs). Herein, we document the standard karyotype and chromosomal NORs of *R. agilae*, describe the procedures used to obtain chromosomes from such a small specimen, and reference other studies that have shown that chromosomal NOR phenotypes appear to be effective taxonomic and systematic characters in fishes. Our primary intent in this note is to stimulate further chromosome research into the diverse and cytogenetically variable aplocheiloid fishes of the New World tropics.

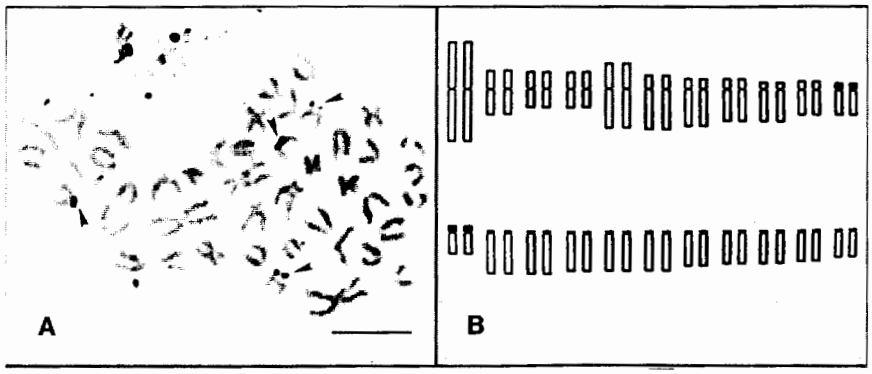


FIGURE 1. (A) Silver-stained metaphases of *Rivulus agilae* (chromosomal NORs are indicated by arrowheads; bar equals 5 μm). (B) Computer-generated idiogram of the *Rivulus agilae* NOR karyotype (NORs are indicated by darkened areas).

The specimen of *R. agilae* was discovered in water obtained from a small ditch near the Hotel Le Grillardin in Matoury (Guyane Province), French Guyana, on 13 June 1987. Upon return to Texas, the individual was injected with 0.1 cubic centimeters per gram body weight of a 0.5 percent colchicine solution and maintained under aerated conditions for 50 minutes. The gills were removed, placed into fresh hypotonic solution (0.4 percent KCl) for 30 minutes (two changes), and then fixed (several changes) in freshly prepared methanol:acetic acid, 3:1. Metaphases were obtained using the slide warmer procedure of Kligerman and Bloom (1977) as modified by Rayburn and Gold (1982) and using fresh 50 percent acetic acid to dissociate cells. NOR staining was accomplished using the controlled silver nitrate procedure of Howell and Black (1980) as modified by Gold and Ellison (1983). Chromosomes stained with silver (and counterstained with Giemsa) were photographed in bright-field using Kodak Technical Pan 2415 film (ASA 40) developed in Diafine (Acufine). Determinations of NOR band positions and size, and of relative size and centromere position of NOR-bearing and other chromosomes, were made off positive prints using a digitizer, a small laboratory computer, and the BANDSCAN program described in Gold and Amemiya (1986).

Fourteen good metaphases were examined from the specimen of *R. agilae*. A representative silver-stained metaphase and a computer-generated diploid idiogram of the metaphase are shown in Figure 1. More than 90 percent of the metaphases had 2N=44 chromosomes, which is assumed to be the chromosome number of the species. The *R. agilae* karyotype is noticeably asymmetric (*sensu* Stebbins, 1971) and contains several "marker" chromosomes. These include an exceptionally large metacentric pair, a medium- to large-sized submetacentric pair, and three pairs of metacentric chromosomes, one of which is measurably larger than the other two (Fig. 1B). The remaining chromosomes are all medium- to small-sized subtelo- or telocentrics (acrocentrics). The arm number (NF) of *R. agilae* is estimated to be 54. In most silver-stained metaphases, a maximum of four (two pair) NOR-bearing chromosomes was observed (Fig. 1A). In all cases, the NORs were located terminally on the short arms of small acrocentric chromosomes.

Recent studies in our laboratory (Gold and Amemiya, 1986; Amemiya et al., 1987; Amemiya and Gold, 1988) have shown that chromosomal NORs are effective taxonomic and systematic markers in fishes, particularly when morphologically similar types (differing in NOR phenotypes) are found sympatrically. Chromosomal NORs represent the sites for the 18S and 28S ribosomal RNA genes (Ritossa and Spiegelman, 1965; Wallace and Birnstiel, 1966), and hence identify homologous genes that if located on morphologically different chromosomes serve to identify the past occurrence of chromosomal rearrange-

ments. Intraspecific NOR variants occur, but are qualitatively different from the types of variants observed between species (Gold and Amemiya, 1986). In cyprinid fishes, we have been able to unequivocally identify 15 of the 24 taxa examined solely on the basis of chromosomal NOR phenotypes (Amemiya and Gold, 1988).

The fish suborder Aplocheiloidei (*sensu* Parenti, 1981), which includes the tropical families Rivulidae (New World) and Aplocheilidae (Old World), contains more than 500 nominal species (more than 40 nominal genera) and is one of the most taxonomically and systematically troublesome fish groups in the world. Standard karyotypes are known for species in both families (particularly the Aplocheilidae) and indicate the past occurrence of numerous chromosomal rearrangements (Scheel, 1972). The inability to homologize chromosomes or chromosomal regions across species, however, has somewhat precluded efforts to use the chromosomal information effectively in a taxonomic or systematic context. The use of NOR and other types of chromosome banding (Gold et al., 1986, for example) on fish chromosomes should overcome the homologization problem, and the tropical aplocheiloid fishes should represent a prime group on which to use the recent developments in fish chromosome staining.

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## Literature Cited

Amemiya, C. T., and J. R. Gold. 1988. Chromosomal NORs as taxonomic and systematic characters in North American cyprinid fishes. Genetica, 76:81-90.

Amemiya, C. T., S. W. Kelsch, F. S. Hendricks, and J. R. Gold. 1987. The karyotype of the Mexican blindcat, *Prietella phreatophila* Carranza. Copeia, 1987: 1024-1028.

Gold, J. R., and C. T. Amemiya. 1986. Cytogenetic studies in North American minnows (Cyprinidae). XII. Patterns of chromosomal NOR variation among fourteen species. Canadian J. Zool., 64:1869-1877.

Gold, J. R., and J. R. Ellison. 1983. Silver-staining for NORs of vertebrate chromosomes. Stain Technol., 58:51-55.

Gold, J. R., C. T. Amemiya, and J. R. Ellison. 1986. Chromosomal heterochromatin differentiation in North American cyprinid fishes. Cytologia, 51:557-566.

Howell, W. M., and D. A. Black. 1980. Controlled silver-staining of nucleolus organizer regions with a colloidal developer: a 1-step method. Experientia, 31:260-262.

Kligerman, A. D., and S. E. Bloom. 1977. Rapid chromosome preparations from solid tissues of fishes. J. Fish. Res. Board Canada, 34:266-269.

Parenti, L. R. 1981. A phylogenetic and biogeographic analysis of cyprinodontiform fishes (Teleostei, Atherinomorpha). Bull. Amer. Mus. Nat. Hist., 168:335-557.

Rayburn, A. L., and J. R. Gold. 1982. A procedure for obtaining mitotic chromosomes from maize. Maydica, 27:113-121.

Ritossa, F. M., and S. Spiegelman. 1965. Localization of DNA complementary to ribosomal RNA in the nucleolus organizer region of *Drosophila melanogaster*. Proc. Nat. Acad. Sci., 53:737-745.

Scheel, J. J. 1972. Rivuline karyotypes and their evolution (Rivulinae, Cyprinodontidae, Pisces). Zeit. Zool. Syst. Evol., 10:180-209.

Stebbins, G. L. 1971. Chromosomal evolution in higher plants. Edward Arnold Publ., Ltd., London, viii+216 pp.

Wallace, H., and M. L. Birnstiel. 1966. Ribosomal cistrons and the nucleolus organizer. Biochim. Biophys. Acta, 114:296-310.