

GENERAL NOTES

CHROMOSOMAL NOR PHENOTYPES OF TWO SPECIES OF NORTH AMERICAN CYPRINID FISHES (CYPRINIDAE: TELEOSTEI)

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Studies in our laboratory over the past several years have focused on identifying the nucleolar organizer region (NOR)-bearing chromosomes of North American cyprinid fishes (Amemiya and Gold, 1988, 1990; Amemiya et al., 1992). To date, chromosomal NOR phenotypes have been documented for more than 100 North American cyprinid species (Amemiya et al., 1992; Powers and Gold, 1992; Li and Gold, 1992; Jenkin and Gold, unpublished data), including 62 species currently placed in the putatively monophyletic assemblage referred to as the "notropins" by Coburn and Cavender (1992). Our studies have demonstrated that chromosomal NOR phenotypes vary among North American cyprinids and often can be utilized as character states in the inference of phylogenetic hypotheses of species relationships.

In this note, we document the chromosome numbers and chromosomal NOR phenotypes of two cyprinid species currently placed in the "untidy" genus *Notropis*-*N. amnis* and *N. atrocaudalis*. The former is a small, not easily obtained species found in the Mississippi River drainage and in medium to large rivers in central Texas (Lee et al., 1980). Historically, *N. amnis* has been considered to have closest affinities to some of the species formerly placed in the nominal subgenus of the genus *Hybopsis* (Hubbs, 1951; Clemmer, 1971). Mayden (1989) placed *N. amnis* in his revised genus *Hybopsis*, which he considered to be part of a large polytomy basal to a putatively monophyletic assemblage he referred to as the "*Notropis*"-like shiners. Coburn and Cavender (1992) placed *N. amnis* in a revised *Hybopsis*, but considered the latter to be a subgenus in their revised genus *Notropis*. Coburn and Cavender (1992) included *Notropis* in their notropin clade. The systematic relationships of *N. atrocaudalis*, a cyprinid inhabiting the lower Brazos River drainage of eastern Texas and the Calcasieu and Red river drainages of southwestern Louisiana and southeastern Oklahoma, respectively (Lee et al., 1980), are essentially unknown. Mayden (1989) tentatively placed *N. atrocaudalis* in the large polytomy basal to his "*Notropis*"-like shiner clade. This polytomy also included several species of "*Notropis*" (for example, *N. stramineus* and *N. topeka*) for which relationships were essentially unknown.

The purpose of this study was to determine if the chromosomal NOR phenotypes of *N. amnis* and *N. atrocaudalis* could be used to evaluate their relationships within North American Cyprinidae. Specimens of both species were obtained by seine from natural populations as follows: *N. amnis* (Neches River near Crockett, Cherokee Co., Texas), and *N. atrocaudalis* (Sabine River near Carthage, Panola Co., Texas). All specimens were transported live to College Station and maintained in well-aerated aquaria until sacrificed. Metaphase chromosomes were obtained using methods outlined in Gold (1984). AgNOR banding followed methods in Howell and Black (1980), and bright-field microscopy followed methods in Gold and Amemiya (1986).

Summary data of the AgNOR-stained material are shown in Table 1, and AgNOR-stained metaphases from each species are shown in Figure 1. All specimens possessed $2N = 50$ chromosomes, as do most North American cyprinids (Gold et al., 1980; Amemiya et al., 1992; Gold et al., unpublished data). The chromosome number of *N. amnis* is reported here for the first time. *Notropis amnis* possessed two pair of NOR-bearing chromosomes: one

TABLE 1. Summary of AgNOR-stained material examined.

	<i>Notropis amnis</i>	<i>Notropis atrocaudalis</i>
Number of specimens examined	5	3
Number of metaphases examined	33	39
Number of (haploid) NOR chromosomes	2	1
NOR chromosome phenotype*	<i>D,F</i>	<i>D</i>

* NOR chromosome phenotype (after Gold and Amemiya, 1986; Amemiya and Gold, 1988): *D*, NOR terminal on the short arm of a medium-sized submetacentric chromosome; *F*, NOR terminal on the short arm of a large-sized acro- or subtelocentric chromosome.

pair exhibited a *D* NOR phenotype (NOR terminal on the short arm of a medium-sized submetacentric chromosome), whereas the other exhibited an *F* NOR phenotype (NOR terminal on the short arm of a large-sized acro- or subtelocentric chromosome). *Notropis atrocaudalis* possessed only a single pair of NOR-bearing chromosomes of the *D* NOR phenotype.

The occurrence of *D* and *D,F* NOR chromosome phenotypes in *N. atrocaudalis* and *N. amnis* suggests that both species properly belong in the genus *Notropis* as defined by Coburn and Cavender (1992). Of the 30 species of *Notropis* (*sensu* Coburn and Cavender, 1992) thus far examined for chromosomal NORs, 18 possess a single pair of NOR chromosomes of the *D* phenotype, four species possess *D* and *F* NOR chromosomes, and eight species possess NOR chromosomes that have other than *D* or *F* NOR phenotypes (Amemiya, 1987; Li and Gold, 1991; Gold et al., 1990; Amemiya et al., 1992). Further, either *D* or *D,F* NOR chromosome phenotypes have been found in all of the major subgroups within *Notropis*. These include the subgenera *Alburnops* (one of two species examined possesses a *D* NOR phenotype), *Hydropholox* (all three species examined possess a *D* NOR phenotype), *Notropis* (one of six species examined possesses a *D* NOR phenotype), and *Pteronotropis* (two of three species examined possess a *D,F* NOR phenotype), and the *N. dorsalis* (four of five species examined possess either *D* or *D,F* NOR phenotypes), *N. texanus* (all four species examined possess either *D* or *D,F* NOR phenotypes), and *N. volucellus* (all three species examined possess a *D* NOR phenotype) species-groups. In addition, *D* NOR phenotypes have been found in four species (*N. baileyi*, *N. greeni*, *N. stramineus*, and *N. topeka*) currently assigned to *Notropis*, but the relationships of which within the genus are not fully resolved.

Among other notropins (32 species examined for chromosomal NORs), *D* NOR chromosomes have been found only in the genus *Luxilus* (Amemiya, 1987; Li and Gold, 1992; Powers and Gold, 1992; Amemiya et al., 1992). Two *D* NOR chromosomes were found in *Luxilus*: one (referred to as *D*₂) was hypothesized to be derived within *Luxilus*, whereas the other (referred to as *D*₁) was found only in the three species of the *L. zonatus* species-group (Powers and Gold, 1992). Interestingly, Coburn and Cavender (1992) have noted that the scale morphology of members of the *L. zonatus* species-group is quite similar to that of two species (*N. leuciodus* and *N. nubilus*) of the subgenus *Hydropholox*, suggesting that the affinities of the *L. zonatus* species-group may reside within the genus *Notropis*.

Outside of the notropins, *D* NOR chromosomes have been found in three of 15 species examined from the "chub" clade, and in four of 22 species examined from the "western" clade (Amemiya, 1987; Gold et al., 1990; Amemiya et al., 1992; Gold et al., 1992; Jenkin and Gold, unpublished data). In each case, however, the species with *D* NOR chromosomes

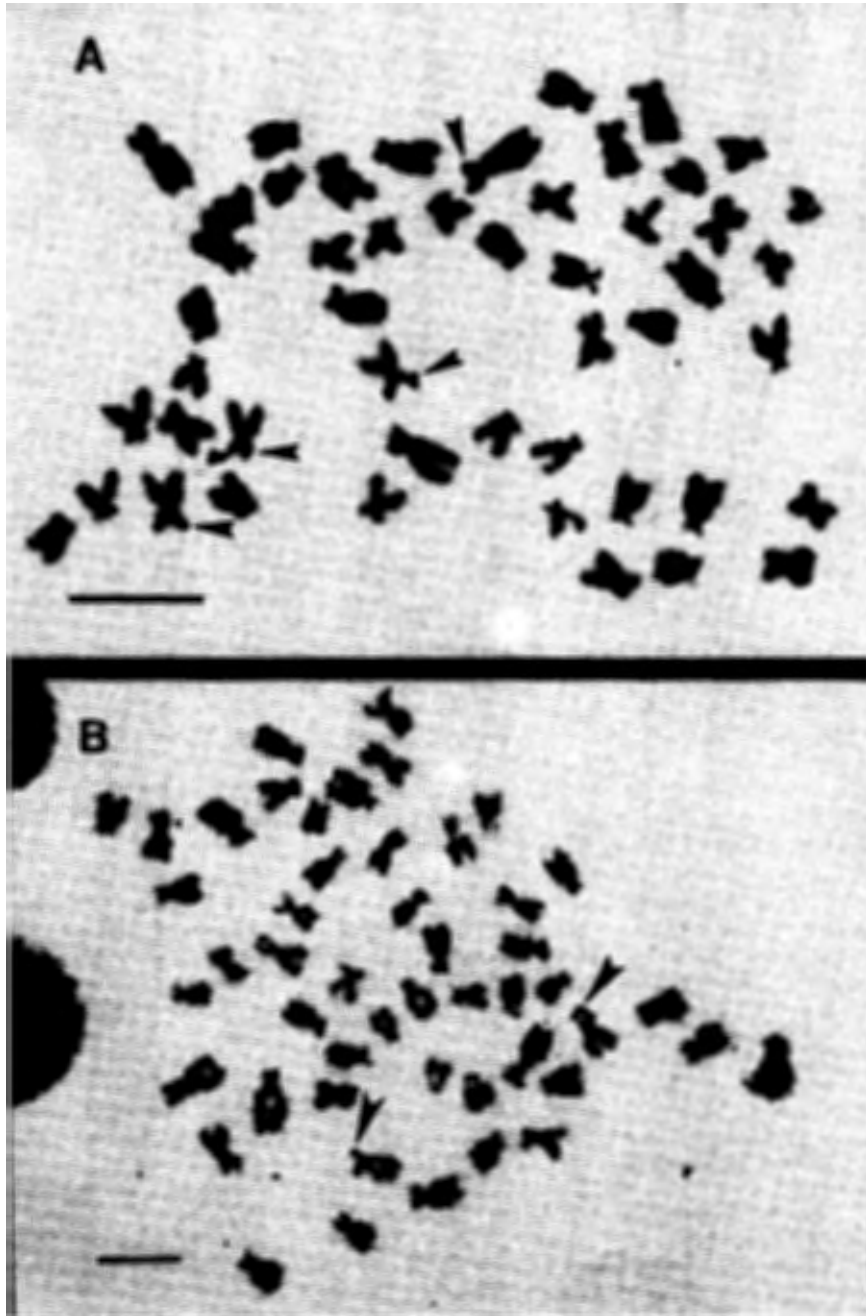


FIGURE 1. AgNOR-stained metaphases of a) *Notropis amnis*, and b) *Notropis atrocaudalis*. Chromosomal NORs are indicated by arrowheads. Bars are the equivalent of 5 μm .

possessed other NOR chromosomes and the *D* NOR chromosomes were hypothesized to be derived within particular lineages.

Gold and Li (1991) hypothesized that a single pair of *F'* NOR chromosomes (defined as a NOR situated terminally on the short arm of a large-sized acro- or subtelocentric chromosome, which also is the largest chromosome in the complement) was plesiomorphic for the "*Notropis*"-like shiners. If true, this suggests the hypothesis that a single pair of *D* NOR chromosomes may be derived and represent a synapomorphy for most of the lineages hypothesized by Coburn and Cavender (1992) as belonging in the genus *Notropis*. These lineages would include the subgenera *Alburnops*, *Hydrophlox*, *Hybopsis*, and *Pteronotropis*, the *N. dorsalis*, *N. texanus*, and *N. volucellus* species-groups, and several species (*N. atrocaudalis*, *N. baileyi*, *N. greenei*, *N. stramineus*, and *N. topeka*) for which relationships are not as yet well defined. This hypothesis differs from that of Mayden (1989), who proposed that *N. atrocaudalis*, his genus *Hybopsis* (which included *N. amnis* and the *N. dorsalis* species-group), *N. stramineus*, and *N. topeka* formed part of the large polytomy basal to the "*Notropis*"-like shiners.

Our hypothesis that a single pair of *D* NOR chromosomes may represent a synapomorphy for *Notropis* suggests that species of *Notropis* possessing *F'* NORs (or their apparent derivatives) may not belong in *Notropis*. This group includes four species (*N. amabilis*, *N. atherinoides*, *N. jemezianus*, and *N. shumardi*) currently assigned to the subgenus *Notropis* and one species (*N. potteri*) currently assigned to the subgenus *Alburnops*. A major caveat to our hypothesis, however, is that notropin complements contain several pair of medium-sized submetacentric chromosomes (that is, *D* chromosomes) and relatively few of the *D* NOR chromosomes have been rigorously tested for homology. Of obvious interest for the future will be tests of homology of *D* NOR chromosomes using alternative banding techniques (for example, G-banding).

One final point to note is that *D,F* NOR chromosome phenotypes have now been found in five species (*N. amnis*, *N. petersoni*, *N. sabiniae*, *N. signipinnis*, and *N. welaka*) in *Notropis* (Amemiya and Gold, 1990; Gold et al., 1990; this paper). Whether the *D,F* NOR phenotype represents a synapomorphy uniting these five species is problematic, in part because neither the *D* nor *F* NOR chromosomes in the five species have been tested for homology, and in part because the five species are currently placed (based on morphological data) in four different subgroups within *Notropis*.

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LITERATURE CITED

- Amemiya, C. T. 1987. Cytogenetic and cytosystematic studies on the nucleolus organizer regions of North American cyprinid fishes. Unpublished Ph.D. dissertation, Texas A&M Univ., College Station, 271 pp.
- Amemiya, C. T., and J. R. Gold. 1988. Chromosomal NORs as taxonomic and systematic characters in North American cyprinid fishes. *Genetica*, 76:81-90
- . 1990. Cytogenetic studies in North American minnows (Cyprinidae). XVII. Chromosomal NOR phenotypes of 12 species with comments on cytosystematic relationships among 50 species. *Hereditas*, 112:231-247.
- Amemiya, C. T., P. K. Powers, and J. R. Gold. 1992. Chromosomal evolution in the North American cyprinids. In *Systematics, historical ecology, and North American freshwater fishes* (R. L. Mayden, ed.), Stanford Univ. Press, Palo Alto, California, in press.

- Clemmer, G. H. 1971. The systematics and biology of the *Hybopsis amblops* complex. Unpublished Ph.D. dissertation, Tulane Univ., New Orleans, Louisiana, 155 pp.
- Coburn, M. M., and T. M. Cavender. 1992. Interrelationships of North American cyprinid fishes. In Systematics, historical ecology, and North American freshwater fishes (R. L. Mayden, ed.), Stanford Univ. Press, Palo Alto, California, in press.
- Gold, J. R. 1984. Silver-staining and heteromorphism of chromosomal nucleolus organizer regions in North American cyprinid fishes. *Copeia*, 1984: 133-139.
- 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 Y. C. Li. 1991. Trypsin G-banding of North American cyprinid chromosomes: phylogenetic considerations, implications for fish chromosome structure, and chromosomal polymorphism. *Cytologia*, 56:199-208.
- Gold, J. R., J. D. Jenkin, and P. K. Powers. 1990. Cytogenetic studies in North American minnows (Cyprinidae). XVIII. Chromosomal NOR variation among eight species. *Cytologia*, 55:483-492.
- Gold, J. R., W. J. Karel, and M. R. Strand. 1980. Chromosome formulae of North American fishes. *Progr. Fish-Cult.*, 42:10-23.
- Gold, J. R., Y. C. Li, M. C. Birkner, and J. D. Jenkin. 1992. Chromosomal NOR karyotypes and genome sizes in *Dionda* (Osteichthys: Cyprinidae) from Texas and New Mexico. *Southwestern Nat.*, in press.
- Howell, W. M., and D. A. Black. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia*, 31:260-262.
- Hubbs, C. L. 1951. *Notropis amnis*, a new cyprinid fish of the Mississippi fauna, with two subspecies. *Occas. Papers Mus. Zool., Univ. Michigan*, 530:1-31.
- Lee, D. S., C. R. Gilbert, C. H. Hocutt, R. E. Jenkins, D. E. McAllister, and J. R. Stauffer, Jr. 1980. Atlas of North American freshwater fishes. Publ. North Carolina Biol. Surv., North Carolina State Mus. Nat. Hist., Raleigh, 1980-12:x + 1-867.
- Li, Y., and J. R. Gold. 1991. Standard and NOR-stained karyotypes of three species of North American cyprinid fishes. *Texas J. Sci.*, 43:207-211.
- . 1992. Cytogenetic studies in North American minnows (Cyprinidae). XXII. Chromosomal NORs in the genus *Pimephales*. *Canadian J. Zool.*, in press.
- Mayden R. L. 1989. Phylogenetic studies of North American minnows, with emphasis on the genus *Cyprinella* (Teleostei: Cypriniformes). Misc. Publ. Mus. Nat. Hist., Univ. Kansas, 80:1-189.
- Powers, P. K., and J. R. Gold. 1992. Cytogenetic studies in North American minnows (Cyprinidae). XX. Chromosomal NOR variation in the genus *Luxilus*. *Copeia*, in press.

TWO ADDITIONS TO THE FLEA (SIPHONAPTERA:
HYSTRICHOPSYLLIDAE, LEPTOPSYLLIDAE) FAUNA OF TEXAS

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When the *Fleas of Texas* was published more than four decades ago (Eads, 1950), 39 species were listed for the state. At that time, Eads considered Texas to have a rich flea fauna, even though he regarded his work as only preliminary. Since then, several species have been added to the state fauna (see Eads and Dalquest, 1954; Morlan and Prince, 1954;