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Chromosomal NOR Phenotypes of Seven Species of North American Cyprinidae, with Comments on Cytosystematic Relationships of the *Notropis volucellus* Species-group, *Opsopoeodus emiliae*, and the Genus *Pteronotropis*

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Chromosomal NOR phenotypes are documented for seven species of North American cyprinid fishes: *Notropis buchanani, N. maculatus, N. stramineus, N. volucellus, Pteronotropis hubbsi, P. signipinnis,* and *P. welaka.* All seven species possessed 2n = 50 chromosomes. Five of the species were found to possess a single pair of NOR-bearing chromosomes. In four of these (*N. buchanani, N. maculatus, N. stramineus,* and *N. volucellus*), the single NOR was located terminally on the short arm of a medium-sized submetacentric chromosome; whereas in the fifth (*P. hubbsi*), the single NOR was located terminally on the short arm of a large-sized acro-/subtelocentric chromosome. *Pteronotropis signipinnis* and *P. welaka* each possessed two pair of NOR-bearing chromosomes: one pair had a NOR located terminally on the short arm of a medium-sized submetacentric chromosome; whereas the other pair had a NOR located terminally on the short arm of a large-sized acro-/subtelocentric chromosome. Consideration of these and previous NOR data in North American cyprinids suggest that: 1) the *N. volucellus* species-group as presently constituted may not be monophyletic; 2) the species *Opsopoeodus emiliae* may belong in an assemblage which includes, among others, at least four species of the genus *Cyprinella*; 3) *O. emiliae* may not be closely related to *N. maculatus*; and 4) *P. hubbsi* may belong in an assemblage which includes *P. signipinnis* and *P. welaka,* and hence may be a valid member of the genus *Pteronotropis.*

OVER the last few years, there has been a renewed interest in the systematics of North American cyprinid fishes. This has been sparked, in large part, by the extensive researches of Coburn (1982), Cavender and Coburn (1986), and Mayden (1989). These researchers have surveyed independently a large number of North American cyprinid species for a variety of morphological (including osteological) characters and from these data inferred hypotheses of species relationships using a Hennigian or cladistic approach. Cyprinids represent the dominant freshwater fish group in North America in terms of number of species (Lee et al., 1980; Nelson, 1984), and, as might be expected for a large group of morphologically similar, small forms, have had a long and storied taxonomic and systematic history. Many of the problems encountered in cyprinid systematics are exemplified by the troublesome "genus" *Notropis* in which many of the morphological characters used in cyprinid taxonomy and systematics apparently have been modified repeatedly during the evolution of the group (Gilbert and Bailey, 1962; Swift, 1970). Discussions of many of the systematic and taxonomic problems within and among North American cyprinids may be found in Coburn (1982) and Mayden (1989). The importance of the investigations of Coburn, Cavender, and Mayden has been the generation of phylogenetic hypotheses which now can be tested using comparable treatments of alternative data bases.

Studies in our laboratory over the last decade have focused on North American cyprinid karyology (Gold and Avise, 1977; Gold et al., 1981; Amemiya and Gold, 1987a). The contribution of karyology to fish systematics in general, however, has been severely limited by the general inability to consistently produce quality chromosome spreads from fish tissues and by the problem that most fish complements contain a relatively large number of comparatively small chromosomes (Gold, 1979). Differential chromosome banding, although used successfully in

mammalian cytosystematics (Dutrillaux et al., 1982; Baker et al., 1983; Rogers et al., 1984), has not been readily applied in fishes (Amemiya and Gold, 1988; Gold et al., 1988). Recently, however, we have developed reliable methods for obtaining suitable harvests of fish chromosomes (Amemiya et al., 1984) and for differentially staining the nucleolus organizer regions or NORs (Gold and Ellison, 1983; Amemiya and Gold, 1986, 1987b). These methods have been employed to examine inter- and intraspecific patterns of chromosomal NOR variation among North American cyprinids, and to test phylogenetic hypotheses of cyprinid relationships inferred from morphological data (Gold and Amemiya, 1986; Amemiya and Gold, 1988; Gold et al., 1988). Although problems of homology of NOR-bearing chromosomes across taxa still remain (Amemiya and Gold, 1988), our studies have demonstrated that: 1) interspecific differences in chromosomal NOR phenotypes among North American cyprinids are informative taxonomically; and 2) systematic or phylogenetic hypotheses inferred from the interspecific NOR chromosome differences can be used to test hypotheses of relationships inferred from morphological or other data bases.

In this paper, we document the chromosomal NOR phenotypes from seven North American cyprinid species whose NOR karyotypes have not been reported previously. The species include *N. buchanani*, *N. maculatus*, *N. stramineus*, *N. volucellus*, *P. hubbsi*, *P. signipinnis*, and *P. welaka*. Three of the species (*N. buchanani*, *N. maculatus*, and *N. volucellus*) were placed by Mayden (1989) into the putatively monophyletic *N. volucellus* species-group along with 5–6 other species including *O. emiliae*, an alleged close relative of *N. maculatus* (Gilbert and Bailey, 1972; Dimmick, 1987; Mayden, 1989), whose NOR karyotype has been examined previously (Gold, 1984; Amemiya and Gold, 1988). The placement of *O. emiliae* into the *N. volucellus* species-group and the close relationship between *N. maculatus* and *O. emiliae* have been questioned by Cavender and Coburn (1986). Three of the other species examined (*P. hubbsi*, *P. signipinnis*, and *P. welaka*) may belong to the genus *Pteronotropis* (Coburn and Cavender, pers. comm.), although the inclusion of *P. hubbsi* and *P. welaka* in *Pteronotropis* (and their alleged close relationship to each other, see Bailey and Robison, 1978) has been questioned by Dimmick (1987). The relationships of the last species examined, *N. stramineus*, are essentially unknown, although Snelson (1971) suggested possible affinities between *N. stramineus* and members of the *N. procne* species-group. The systematic implications of the chromosomal NOR data relative to these species are discussed.

Materials and Methods

The specimens examined in this study were collected by seine from natural populations. The species (collection localities) were as follows: *N. buchanani* (Brazos River, Robertson Co., Texas); *N. maculatus* (Pearl River, St. Tammany Par., Louisiana); *N. stramineus* (Guadalupe River, Comal Co., Texas); *N. volucellus* (Brazos River, Robertson Co., Texas; Cahaba River, Bibb Co., Alabama; Navasota River, Brazos Co., Texas; Thompson Creek, W. Feliciana Par., Louisiana); *P. hubbsi* (Big Cypress Bayou, Harrison Co., Texas); and *P. signipinnis* and *P. welaka* (Talisheek Creek, St. Tammany Par., Louisiana). All individuals were returned live to our laboratory in College Station, Texas, and maintained in well-aerated aquaria. Voucher specimens for all but two of the samples of *N. volucellus* were deposited in the Texas Cooperative Wildlife Collections (TCWC) at Texas A&M University. TCWC catalogue numbers are as follows: *N. buchanani* (6815.1), *N. maculatus* (6816.1), *N. stramineus* (6811.1), *N. volucellus*—Brazos R. (6810.1) and Cahaba R. (6809.1), *P. hubbsi* (6813.1), *P. signipinnis* (6812.1), and *P. welaka* (6814.1). Only single specimens of *N. volucellus* from the Navasota River and Thompson Creek were examined, and both were too badly damaged for deposition.

Metaphase chromosomes were prepared either directly from solid tissues (Gold, 1984) or from cultured fibroblasts (Amemiya et al., 1984). AgNOR-banding was accomplished via the controlled silver technique of Howell and Black (1980) as modified by Gold and Ellison (1983). Chromomycin A_3 (CMA) NOR-staining followed Amemiya and Gold (1987b). The AgNOR method presumably differentiates only those NORs which were active metabolically during the preceding interphase (Howell, 1982); whereas CMA presumably differentiates NORs regardless of prior metabolic activity (Schmid, 1982; Amemiya and Gold, 1986). R-banding of heat-denatured chromosomal preparations (RHG technique) followed the protocol of Bernheim and Berger (1981). Bright field and fluorescence photomicroscopy followed procedures outlined in Amemiya and Gold (1986).

TABLE 1. SUMMARY OF NOR-STAINED MATERIAL EXAMINED FOR EIGHT SPECIES OF NORTH AMERICAN CYPRINIDAE.

Taxon	Number of specimens examined	Number of metaphases examined	Number of (haploid) NOR chromosomes	NOR chromosome phenotypes*
Notropis buchanani	5	112	1	D
Notropis longirostris†	2	48	1	D
Notropis maculatus	3	50	1	D
Notropis stramineus	1	20	1	D
Notropis volucellus	5	140	1	D
Pteronotropis hubbsi	5	200	1	F
Pteronotropis signipinnis	4	125	2	D, F
Pteronotropis welaka	4	140	2	D, F

* NOR chromosome phenotypes: D, terminal on the short arm of a medium-sized submetacentric; F, terminal on the short arm of a large-sized acro-/subtelocentric.
† Data are from Gold and Amemiya (1986).

The letter designations used for the NOR chromosome phenotypes were developed by Gold and Amemiya (1986) and Amemiya and Gold (1988), and are based on the position of the NOR on the chromosome (terminal, subterminal, etc.), the centromere position of that chromosome (median, submedian, etc.), and the relative size of the chromosome within the complement. Given the similarity in size of most North American cyprinid chromosomes, the last criterion was somewhat difficult to assess. Determinations of NOR-band position(s) 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).

We have used the nomenclatorial revisions of Mayden (1989), with two exceptions. The changes from previous taxonomic concepts (relative to taxa discussed in this paper) are: (1) the elevation of the *Notropis* subgenera *Pteronotropis, Cyprinella,* and *Luxilus* to generic status; and (2) the placement of *Hybopsis aestivalis* and *H. storeriana* into the genera *Extrarius* and *Macrhybopsis,* respectively. The exceptions are: (1) the retention of *N. longirostris* in *Notropis* as opposed to placement in *Hybopsis;* and (2) the placement of *N. emiliae* into the genus *Opsopoeodus.* The reason for the former is that Coburn (pers. comm.) has evidence which suggests that the *N. dorsalis* species-group (sensu Mayden, 1989), which includes *N. longirostris,* may be related to other species of *Notropis* and *Ericymba buccata.* This raises several questions about the appropriate placement of the *N. dorsalis* species-group and suggests that a conservative approach is warranted. The reason for the latter is that several lines of evidence, including morphological (Cavender and Coburn, 1986) and allozymic (Mayden, pers. comm.), suggest that *O. emiliae, Pimephales,* and *Cyprinella* form a monophyletic lineage. On this basis, it seems warranted to place the species in *Opsopoeodus* (Campos and Hubbs, 1973) and remove it from *Notropis.*

RESULTS AND DISCUSSION

The NOR chromosome data from the seven species examined are presented in Table 1; representative NOR-stained metaphases of each species are shown in Figures 1 and 2. All individuals from all seven species possessed $2n = 50$ chromosomes, as has been the case for most North American cyprinids surveyed to date (Gold et al., 1980; Amemiya and Gold, 1987a; Gold et al., 1988). The chromosome numbers of *N. buchanani, N. maculatus, Pteronotropis hubbsi,* and *P. welaka* are reported for the first time.

Five of the seven species examined were found to possess a single pair of NOR-bearing chromosomes (Table 1). In four of these (*N. buchanani, N. maculatus, N. stramineus,* and *N. volucellus*), the single NOR was located terminally on the short arm of a medium-sized submetacentric chromosome (NOR phenotype D); whereas in the fifth (*P. hubbsi*), the single NOR was located terminally on the short arm of a large-sized acro-/subtelocentric chromosome (NOR phenotype F). The other two species, *P. signipinnis* and *P. welaka,* each possessed two pairs of NOR-bearing chromosomes: on one pair the NOR was located terminally on the short arm of a medium-sized submetacentric chromosome (NOR phenotype D), and on the other the NOR

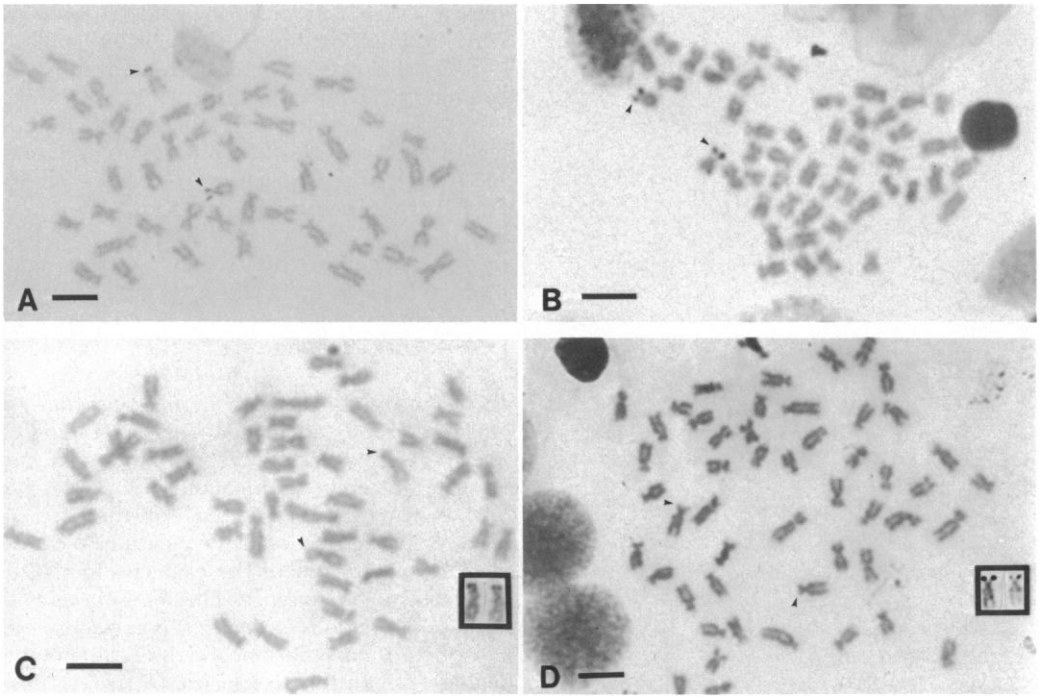

Fig. 1. Representative NOR karyotypes of (A) *Notropis buchanani,* (B) *N. maculatus,* (C) *N. stramineus,* and (D) *N. volucellus.* The metaphases in (A) and (B) were silver-stained; the metaphases in (C) and (D) were undifferentially stained with Giemsa then subsequently stained with silver (NOR-banded chromosomes are shown in the boxed inserts). Chromosomal NORs are indicated by arrowheads. Bars are the equivalent of 5 μm.

was located terminally on the short arm of a large-sized acro-/subtelocentric chromosome (NOR phenotype F). The computer-generated idiograms for the seven species are shown in Figure 3 and are intended only to demonstrate the NOR position and relative size and centromere position(s) of NOR-bearing and other chromosomes within the complements of each species.

Intraspecific NOR heteromorphisms were observed in approx. 13% of all individuals examined regardless of species. These heteromorphisms included either amplifications of a given NOR site or what are termed "activity" heteromorphisms. The former are defined phenotypically by a significant increase in size of a silver- or CMA-stained NOR; whereas the latter are defined by the absence of silver-staining at a given NOR site (Gold and Amemiya, 1986; Amemiya and Gold, 1988). These types of intraspecific NOR heteromorphisms appear common in fishes (Foresti et al., 1981; Moreira-Filho et al., 1984), and photographed examples of both types in cyprinids may be found in Gold (1984), Gold and Amemiya (1986), and Gold et al. (1988). Although frequent, these intraspecific NOR heteromorphisms differ qualitatively from the types of NOR differences found among cyprinid species (i.e., changes in the number, position, and chromosomal types of NORs), and reinforce our observations (Gold and Amemiya, 1986; Amemiya and Gold, 1988) that interspecies NOR differences are valid taxonomic and systematic characters.

Cytosystematic considerations.—As generally discussed by Amemiya and Gold (1988), the central difficulty in using chromosomal NORs for systematic or phylogenetic inference stems from the dual problems of establishing homologies of NOR-bearing chromosomes among various taxa and of determining character transformations. NOR-banding alone does not provide the resolution necessary for determining whether a given NOR chromosome phenotype (e.g., D) found in different species represents

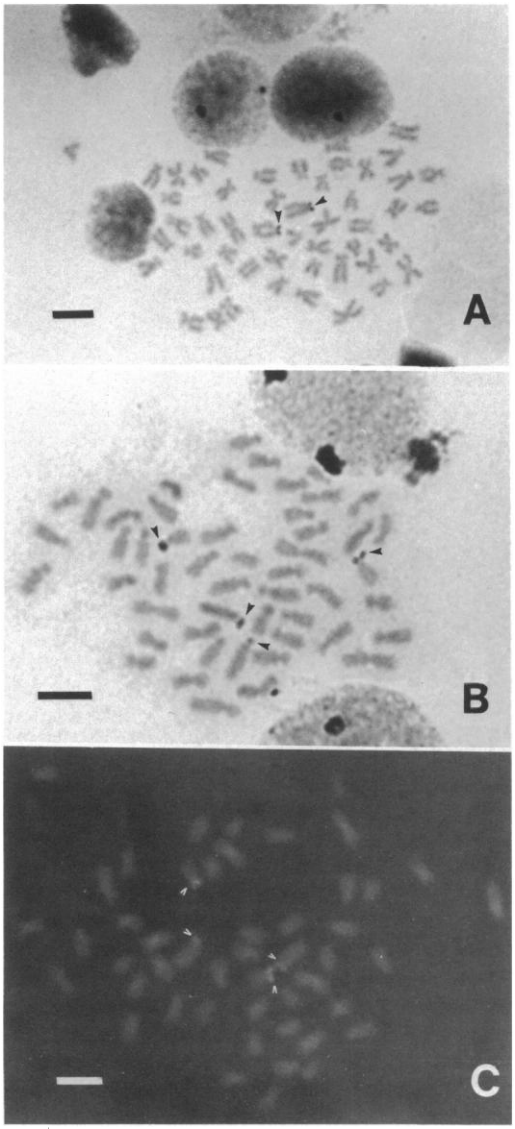

Fig. 2. NOR-stained metaphases of (A) *Pteronotropis hubbsi*, (B) *P. signipinnis*, and (C) *P. welaka*. The metaphases in (A) and (B) were silver-stained; the metaphase in (C) was CMA-stained. Chromosomal NORs are indicated by arrowheads. Bars are the equivalent of 5 μm.

the same homologous character, nor does NOR-banding provide direct evidence for the transformation of one NOR chromosome phenotype to another. Nonetheless, the overall similarities in NOR chromosome phenotypes yield phenetic relationships which are not discordant with present concepts of North American cyprinid relationships, and we have identified a few NOR chromosome homologies through the application of other types of metaphase chromosome banding (Gold and Amemiya, 1986; Amemiya, 1987; Amemiya and Gold, 1988). In the following, the different NOR chromosome conditions are treated as separate character states. As examples from the present data set (Table 1), *N. buchanani*, *N. maculatus*, *N. stramineus*, and *N. volucellus* each have a D NOR character state; whereas *P. hubbsi* has an F NOR character state. *Pteronotropis signipinnis* and *P. welaka* have a D, F NOR character state.

The N. volucellus species-group and O. emiliae.— Mayden (1989) identified the *N. volucellus* species-group as a putatively monophyletic assemblage of eight nominal species of *Notropis* (plus one undescribed species) on the basis of derived characteristics of the palatine. We now have examined four of these species for NOR chromosome phenotypes. Three (*N. buchanani*, *N. maculatus*, and *N. volucellus*) possess a single pair of NOR chromosomes of the D phenotype (Table 1). The fourth species, *O. emiliae*, possesses a single pair of NOR chromosomes of the E′ phenotype which is defined as a NOR situated subterminally (interstitially) on the short arm of a large submetacentric chromosome which also is the largest chromosome in the complement (Gold, 1984; Gold and Amemiya, 1986). As outgroups (Watrous and Wheeler, 1981) to this assemblage, we have chosen to employ *N. stramineus* and *N. longirostris*, two species representing distinct lineages which are currently placed by Mayden (1989) in positions basal to almost all of the recognized lineages within *Notropis* (sensu). Both *N. stramineus* and *N. longirostris* possess a single pair of NOR chromosomes of the D phenotype (Table 1; Gold and Amemiya, 1986). With the tentative assumption that the D NOR chromosome is homologous among these species, the simplest phylogenetic inference is that the single D NOR character state as found in *N. buchanani*, *N. maculatus*, and *N. volucellus* is symplesiomorphic and uninformative. The single E′ NOR chromosome in *O. emiliae* would then be considered autapomorphic and informative only to the extent that *O. emiliae* represents a discrete species. Gold and Amemiya (1986), Amemiya (1987), and Amemiya and Gold (1988), however, demonstrated through C-banding that the E′ NOR chromosome in *O. emiliae* is putatively homologous to (and easily derived by a single paracentric in-

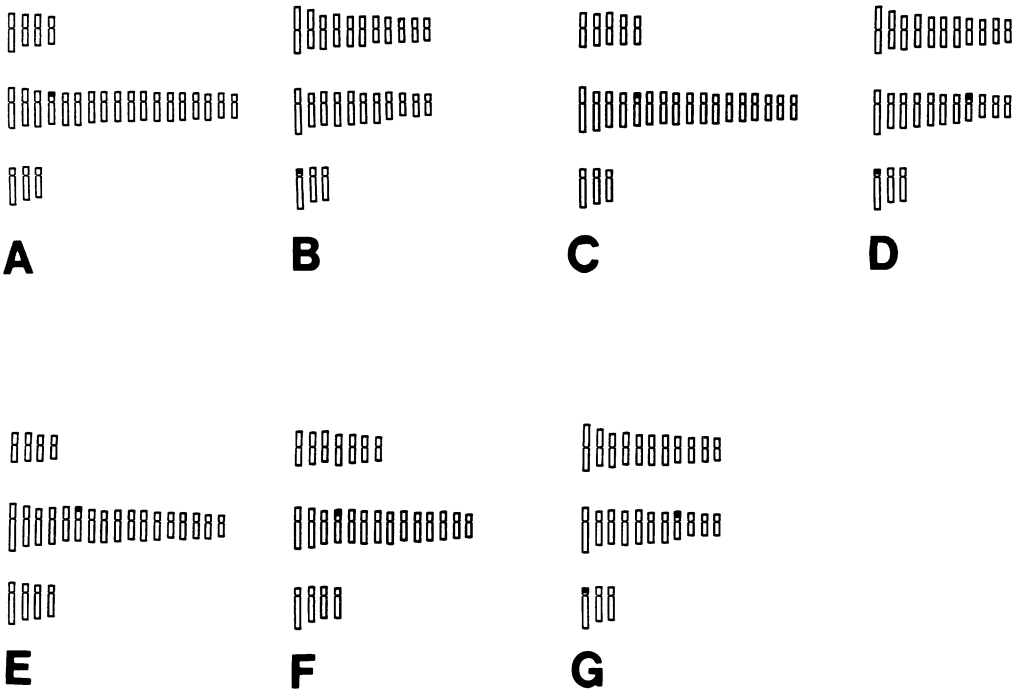

Fig. 3. Haploid idiograms of NOR karyotypes of all taxa examined in this study. Chromosomes are arranged into rows in each karyotype on the basis of centromere position (after Levan et al., 1964): metacentric chromosomes are in the top row, submetacentric chromosomes are in the middle row, and acrocentric (subtelocentric) chromosomes are in the bottom row. Within rows, chromosomes are arranged according to relative size. NORs are indicated by darkened areas. (A) *Notropis buchanani,* (B) *Pteronotropis hubbsi,* (C) *N. maculatus,* (D) *P. signipinnis,* (E) *N. stramineus,* (F) *N. volucellus,* and (G) *P. welaka.*

version from) the C′ NOR chromosome found in the following North American cyprinid species: *Hybognathus nuchalis, N. braytoni, L. chrysocephalus,* and four species of *Cyprinella* (*C. lepida, C. lutrensis, C. spiloptera,* and *C. venusta*). [Note: Gold and Amemiya (1986) originally described the single pair of NOR chromosomes in *N. potteri* (subgenus *Alburnops*) and *N. shumardi* (subgenus *Notropis*) as being of the C′ NOR phenotype. Examination of additional specimens of both species (Amemiya, 1987; Amemiya and Gold, 1988) has shown that the NOR chromosomes of both species are of the F′ NOR phenotype.] The C′ NOR chromosome is defined by a NOR situated terminally on the short arm of a large submetacentric chromosome which also is the largest chromosome in the complement, and when compared to the E′ NOR chromosome in terms of relative size, centromere position, and diagnostic C-band pattern differs only in the position (terminal vs subterminal) of the NOR (Amemiya and Gold, 1988). From the outgroup perspective using *N. stramineus* and *N. longirostris,* the phylogenetic inferences are that: (1) the *N. volucellus* species-group as hypothesized by Mayden (1989) is not monophyletic with respect to *O. emiliae*; and (2) *O. emiliae* belongs in a derived, monophyletic clade which is comprised of several cyprinid lineages.

Our hypothesis that *O. emiliae* belongs in an assemblage which includes at least some members of the genus *Cyprinella* is in agreement with the suggestions of Cavender and Coburn (1986) who have hypothesized on morphological bases that *O. emiliae* has phylogenetic affinities with *Cyprinella* but not with *N. maculatus* or other members of Mayden's *N. volucellus* species-group. Dimmick (1987) examined allozyme variation at 21 putative gene loci for several species of North American Cyprinidae including *O. emiliae, N. maculatus,* and two species of *Cyprinella* (*C. camura* and *C. venusta*). Both an unweighted pair group method using arithmetic averages (UPGMA) phenogram (based on Rogers' [1972] genetic distance) and a consen-

sus tree (constructed using Swofford's [1985] phylogenetic analysis using parsimony [PAUP] program) suggested that both *O. emiliae* and *N. maculatus* may belong in an assemblage which includes the two species of *Cyprinella,* at least in relation to the other cyprinid species examined (Dimmick, 1987). Alternatively, Cavender and Coburn's (1986) hypothesis was that *O. emiliae* was closely related to the genus *Pimephales,* and that a clade including *O. emiliae* and *Pimephales* represented the sister group to *Cyprinella.* We have now examined NOR chromosomes from three species of *Pimephales* (*P. notatus, P. promelas,* and *P. vigilax*), all of which possess a single pair of NOR chromosomes with the NOR situated terminally on the short arm of a medium to large submetacentric chromosome (Gold and Amemiya, 1986; Gold and Shipley, unpubl.). The NOR chromosome in all three species, however, is definitely not the largest chromosome in any complement, meaning that the NOR chromosome data do not support the inclusion of *Pimephales* within Cavender and Coburn's (1986) putative assemblage which includes *O. emiliae* and members of the genus *Cyprinella.*

The last question raised by the chromosome data regards the hypothesized close relationship between *O. emiliae* and *N. maculatus.* Gilbert and Bailey (1972) were the first to suggest a close relationship between these two species following a phenetic treatment of three diagnostic morphological characters. Mayden (1989) interpreted these characters as synapomorphies and proposed a sister group relationship between the two species within the *N. volucellus* species-group. Dimmick's (1987) allozyme data also supported a relationship in that the two species clustered in the UPGMA phenogram before clustering with any of the other species of *Notropis* (*s.l.*) examined. However, the node which united *O. emiliae* and *N. maculatus* was located at approx. 0.375 (Roger's) distance units and was only 0.015 distance units from the node which united *O. emiliae* and *N. maculatus* with the two species from *Cyprinella.* It seems likely that the difference between these nodes may not be statistically significant, and that *O. emiliae, N. maculatus,* and the two species of *Cyprinella* form an unresolved trichotomy. Such an unresolved trichotomy was in fact suggested by a consensus tree constructed using PAUP (Dimmick, 1987), indicating that *O. emiliae* and *N. maculatus* are no more closely related to one another than either is to *Cyprinella.*

The outgroup comparison with *N. stramineus* and *N. longirostris* (above) tentatively suggests that the D NOR chromosome phenotype as found in *N. maculatus* is the plesiomorphic condition for much of the assemblage which used to be recognized as *Notropis.* Further evidence for this latter hypothesis stems from our findings (Amemiya and Gold, 1988; Gold et al., 1988; Jenkin and Gold, unpubl.) that a single pair of NOR chromosomes of the D phenotype is found in several different, putatively monophyletic assemblages within North American Cyprinidae including the genus *Luxilus* (*L. pilsbryi*), the *Notropis* subgenera *Hydrophlox* (*N. baileyi* and *N. nubilus*) and *Alburnops* (*N. girardi*), the *N. texanus* species-group (*N. texanus*), and the "black-chin" (sensu Coburn, 1982) species-group (*N. boops*). What remains is to test this hypothesis by examining the homologies of the D NOR chromosomes by alternative banding patterns. At present, however, the chromosome data do not corroborate many currently hypothesized relationships: a close relationship between *O. emiliae* and *N. maculatus* is not supported; *Pimephales* may not be a sister group to *Cyprinella;* and *O. emiliae* should not be part of the *N. volucellus* species-group sensu Mayden (1989).

Although not analyzed using parsimony analysis in conjunction with other characters, our current view is that the C′ NOR chromosome possibly represents a chromosomal synapomorphy potentially uniting at least four different North American cyprinid lineages. Thus far, C-banding patterns (Amemiya, 1987; Amemiya and Gold, 1988; Zoch and Gold, unpubl.) have indicated homology of C′ NOR chromosomes in *N. braytoni* (a species whose affinities are essentially unknown), *Hybognathus nuchalis,* and the four species of *Cyprinella* listed previously. A C′ NOR chromosome (not examined for C-bands) also has been found (Amemiya, 1987) in one specimen of *L. chrysocephalus* from the Blue River in Oklahoma, although this finding has yet to be confirmed by additional sampling. As noted previously, the E′ NOR chromosome in *O. emiliae* is essentially homologous to the C′ NOR chromosome in C-band pattern (Amemiya and Gold, 1988), and was presumably derived from a C′ NOR chromosome by a small paracentric inversion in the short arm. Accordingly, and based on the premise that *Cyprinella* is monophyletic (Mayden, 1989), *O. emiliae* would appear to be related phylogenetically to *Cyprinella,* although the relationship could be quite distant. In addition, this view would also suggest that the other NOR chromosome phenotypes thus far observed in *Cyprinella* (Amemiya, 1987;

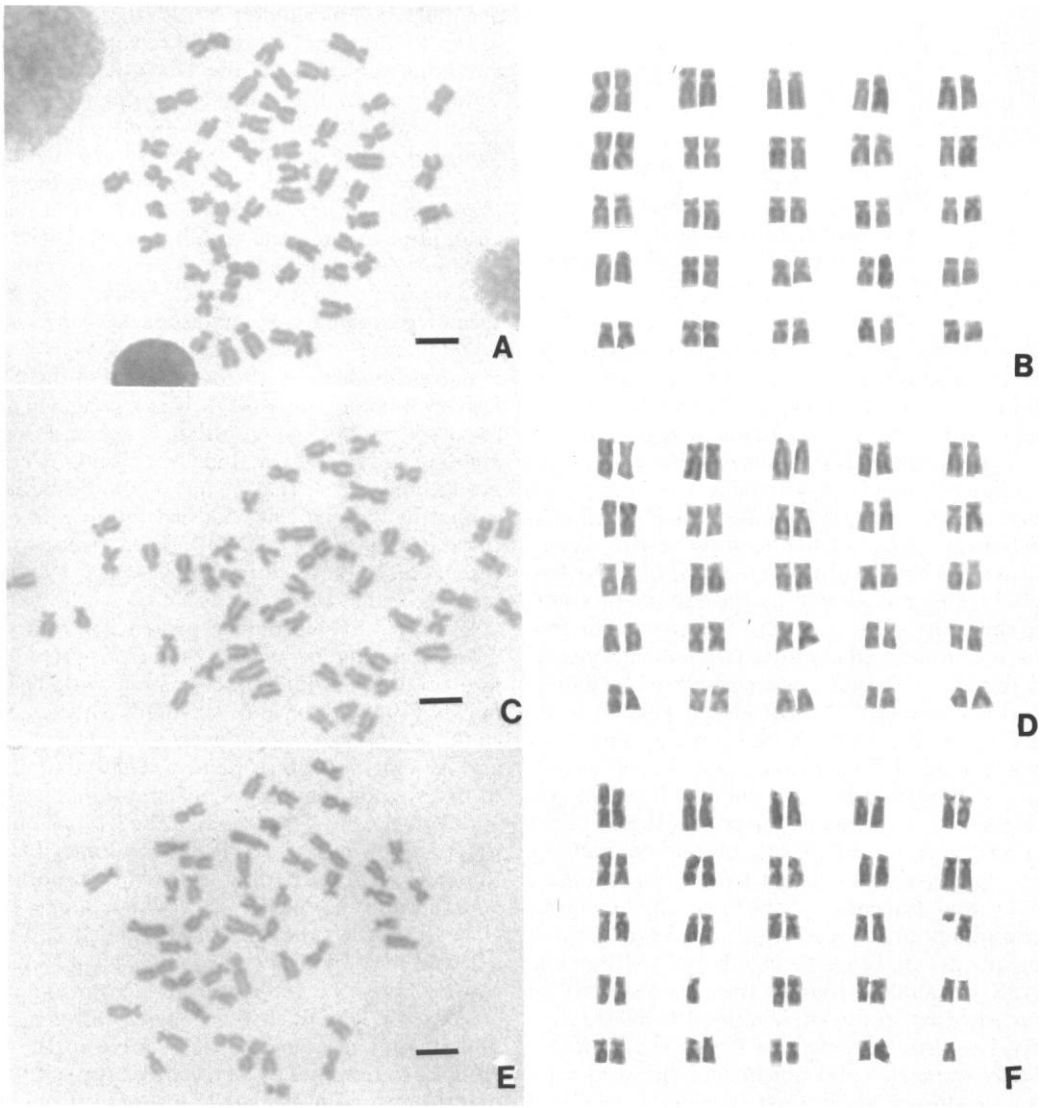

Fig. 4. Standard karyotypes and karyograms from *Pteronotropis hubbsi* (A, B), *P. signipinnis* (C, D), and *P. welaka* (E, F). The karyotype of *P. welaka* is short two chromosomes. Bars are the equivalent of 5 μm.

Gold et al., 1988) are apomorphic conditions. Finally, the inclusion of *H. nuchalis* within the "C′" assemblage adds a complication in that both Mayden (1989) and Coburn and Cavender (pers. comm.) consider the genus *Hybognathus* as allied with other members of the "chub" assemblage (sensu Mayden, 1989) and not with any of the lineages of *Notropis* (*s.l.*). *Hybognathus nuchalis* actually possesses two pair of NOR chromosomes (Amemiya, 1987), one of which—the B NOR phenotype—is found singly in *H. placitus* (Gold and Amemiya, 1986). Initially, we suspected that the C′ NOR chromosome might have arisen in *H. nuchalis* from an interspecific hybridization with one of the species which normally possesses a C′ NOR chromosome. However, we have now examined over 10 specimens of *H. nuchalis* from two localities in Texas and one in Louisiana and all possessed at least one C′ NOR chromosome. The hybridization hypothesis thus appears unlikely, and as a result the phylogenetic affinities of *H. nuchalis* remain problematic.

The genus Pteronotropis.—The genus *Pteronotropis* is generally defined to include three species

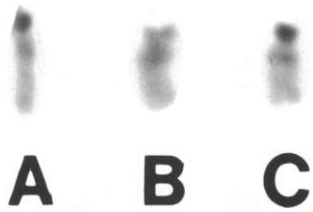

Fig. 5. Comparison of RHG-banded F NOR chromosomes from (A) *Pteronotropis hubbsi*, (B) *P. signipinnis*, and (C) *P. welaka*.

(*P. euryzonus*, *P. hypselopterus*, and *P. signipinnis*) from the southeastern United States (Bailey and Suttkus, 1952; Suttkus, 1955) which share at least three derived morphological features (Mayden, 1989). The genus is of importance to considerations of the systematics of *Notropis* (*s.l.*) because Mayden (1989) considered *Pteronotropis* as a basal clade relative to most of the recognized lineages within *Notropis* (*s.l.*). Mayden (1989) also considered *P. welaka* to be the sister to the above three species of *Pteronotropis* on the basis of a derived condition of the palatine. A fifth species, *P. hubbsi*, was considered by Bailey and Robison (1978) to be closely related to *P. welaka* on the bases of morphology and preferred habitat. They also suggested that *P. hubbsi* and *P. welaka* may be allied to members of the genus *Cyprinella*. Mayden (1989; pers. comm.) examined *P. hubbsi*, but did not include the species in his concept of *Pteronotropis*. Coburn and Cavender (pers. comm.), however, consider both *P. hubbsi* and *P. welaka* to be valid members of *Pteronotropis*. Finally, Dimmick (1987) examined four of these five species in his allozyme study. He identified *P. welaka* as a possible sister to a clade of *P. signipinnis* and *P. hypselopterus*, but did not believe the data supported close relationships between *P. hubbsi* and *P. welaka* or between *P. hubbsi* and *Cyprinella*.

All three species (*P. hubbsi*, *P. signipinnis*, and *P. welaka*) examined possess virtually identical standard karyotypes (Fig. 4), and more importantly, a pair of F NOR chromosomes (Table 1) which by RHG-banding (Fig. 5) appear to be homologous. Two of the species (*P. signipinnis* and *P. welaka*) also possess a D NOR chromosome. By outgroup comparison with *N. stramineus* and *N. longirostris*, and with the assumption that all of the D NOR chromosomes are homologous, the addition of the F NOR chromosome would appear to represent a chromosomal synapomorphy which unites *P. hubbsi*, *P. signipinnis*, and *P. welaka* into a monophyletic assemblage. The single F NOR condition in *P. hubbsi* would then be inferred to represent the autapomorphic loss of the D NOR character state. An alternative explanation which does not assume homology of the D NOR chromosomes would be that a character state transformation from D to F occurred in the branch leading to *P. hubbsi*, *P. signipinnis*, and *P. welaka*, and that an addition of a presumably homologous D NOR chromosome occurred in a subsequent branch leading to *P. signipinnis* and *P. welaka*. This alternative explanation would suggest that *P. signipinnis* and *P. welaka* are sister taxa relative to *P. hubbsi*. Regardless, the occurrence of the putatively homologous F NOR chromosome in all three species does suggest that *P. hubbsi*, *P. signipinnis*, and *P. welaka* comprise a monophyletic group, and hence that *P. hubbsi* should be included in *Pteronotropis*. A third interpretation could be that a single F NOR chromosome represents a symplesiomorphy in *P. hubbsi*, *P. signipinnis*, and *P. welaka*, and that the addition of a D NOR chromosome represents a synapomorphy uniting *P. signipinnis* and *P. welaka*. This interpretation would be consistent with Dimmick's (1987) data and Mayden's views, and could be supported by our previous finding (Gold and Amemiya, 1986; Amemiya, 1987) that one of two chromosome pairs in *Extrarius aestivalis* and *Macrhybopsis storeriana* is of the F NOR phenotype. However, F NOR chromosomes have thus far been found only in these five species, and there are no other data which suggest a close alliance between *E. aestivalis* and *M. storeriana* and the three species of *Pteronotropis*. As a consequence, we interpret the *Extrarius* and *Macrhybopsis* F NOR chromosomes as either nonhomologous or homoplastic relative to the F NOR chromosomes of *Pteronotropis* and suggest that the chromosome data best support Coburn and Cavender's (pers. comm.) hypothesis that *P. hubbsi* probably belongs in the genus *Pteronotropis*.

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Osteology and Interrelationships of the Sand Lances (Teleostei: Ammodytidae)

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***Embolichthys mitsukurii* (Jordan and Evermann), thought to be the least derived member of the teleost family Ammodytidae, is compared anatomically with other ammodytids, and with an assemblage of outgroup taxa (Cheimarrichthyidae, Pinguipedidae, Percophidae, Trichonotidae, Creediidae, Champsodontidae, Chiasmodontidae, Leptoscopidae, Trachinidae, and Uranoscopidae) shown previously by the senior author to represent the core of, but not necessarily to delimit, the perciform suborder Trachinoidei. Evidence is provided to show that ammodytids are trachinoids and that the family represents the sister group of the Trachinidae plus Uranoscopidae. Although there is considerable homoplasy, a large number of unique (among the groups examined) derived features, including osteological, myological, and soft-tissue characters (as well as perhaps physiological and behavioral adaptations), serve to support a hypothesis of monophyly for these three families.**

THE teleost family Ammodytidae, currently the sole member of the perciform suborder Ammodytoidei, includes five or six genera and approx. 19 species (Stevens et al., 1984; H. Ida, pers. comm., 25 July 1978, 30 April 1983). Members of the group are characterized most strikingly by having a narrow, elongate body; a small head, with the lower jaw protrud-