

Chromosomal NOR Karyotypes and Genome Size Variation among Squawfishes of the Genus *Ptychocheilus* (Teleostei: Cyprinidae)

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Nucleolus organizer region (NOR) chromosomes from species of the North American cyprinid genera *Ptychocheilus*, *Mylopharodon*, and *Lavinia* were documented and tested for presumed homology using trypsin G-banding. Four species of *Ptychocheilus* (*P. lucius*, *P. grandis*, *P. umpqua*, and *P. oregonensis*), *M. conocephalus*, and *L. exilicauda* possessed two pair of NOR-bearing chromosomes with the NORs situated terminally on the short arms of medium-sized acrocentric chromosomes (NOR phenotype A). Both pair of NOR chromosomes in all three genera were identical in trypsin G-banding pattern and hence were uninformative for inference of phylogenetic relationships within *Ptychocheilus*. The apparent conservatism of chromosomal NOR evolution in these cyprinid genera from western North America is in striking contrast to that documented among cyprinids from eastern North America. Genome sizes (DNA contents) of the six species also were documented using flow cytometry. Heterogeneity tests revealed three homogeneous groups of samples: (1) *L. exilicauda*, *P. lucius*, *P. grandis*, and *P. umpqua* from the Siuslaw River with low genome sizes; (2) *M. conocephalus* with an intermediate genome size; and (3) *P. oregonensis* and *P. umpqua* from the Umpqua River drainage with high genome sizes. The difference in genome size between *P. umpqua* from the Siuslaw and Umpqua river drainages is greater than that found, on average, between any two North American cyprinid species drawn at random. The difference in genome size, coupled with morphological differences documented previously, suggests that *P. umpqua* from the two drainages may represent distinct species. Using an optimization procedure, genome sizes were partitioned among branches of two contrasting hypotheses of relationships in *Ptychocheilus*. The hypothesis of {*P. lucius* [*P. grandis* (*P. umpqua*, *P. oregonensis*)]} required about 20% less change in genome size (i.e., was more parsimonious) than the hypothesis of {*P. oregonensis* [*P. umpqua* (*P. grandis*, *P. lucius*)]}.

SQUAWFISHES (genus *Ptychocheilus*) include the largest native species of the family Cyprinidae in North America, with the Colorado squawfish, *Ptychocheilus lucius*, reported to obtain a length of not quite 2 m and a weight of up to 45 kg (Minckley, 1973). Four extant species of *Ptychocheilus* are currently recognized: *P. lucius*, *P. grandis*, *P. umpqua*, and *P. oregonensis*. A description of the native ranges of the four species of *Ptychocheilus* may be found in Mayden et al. (1991). Two fossil species of *Ptychocheilus* are also recognized: *P. prelucius* from Miocene or Pliocene deposits in Arizona (Uyeno and Miller, 1965; Smith, 1981), and *P. arciferus* from Miocene-Pliocene deposits in Idaho (Kimmel, 1975; Smith 1975). The former (*P. prelucius*) is considered ancestral to *P. lucius*, whereas *P. arciferus* is considered to be ancestral to *P. oregonensis* (Uyeno and Miller, 1965; Smith, 1975).

Hypotheses of relationships among extant species of *Ptychocheilus* have been proposed by Carney and Page (1990) and Mayden et al. (1991). Carney and Page (1990) proposed a hy-

pothesis of {*P. lucius* [*P. grandis* (*P. umpqua*, *P. oregonensis*)]}; whereas Mayden et al. (1991) proposed a hypothesis of {*P. oregonensis* [*P. umpqua* (*P. grandis*, *P. lucius*)]}. The two hypotheses differ only with regard to the placement of the root, i.e., the unrooted networks are equivalent. Carney and Page (1990) considered *P. lucius* as primitive based on the premise that *P. prelucius* was the older of the two described fossil species of *Ptychocheilus*. Mayden et al. (1991), alternatively, employed two monotypic genera, *Mylopharodon* (*conocephalus*) and *Hesperoleucus* (*symmetricus*), as outgroups to infer relationships among *Ptychocheilus*. Their choice of *Mylopharodon* and *Hesperoleucus* as outgroups was based on earlier allozyme studies by Avise and Ayala (1976) where *Hesperoleucus* and the related monotypic genus *Lavinia* (*exilicauda*) were suggested to be the sister group to a lineage comprised of *Mylopharodon* and *Ptychocheilus*. Carney and Page (1990) considered the allozyme data of Avise and Ayala (1976) but pointed out that the methods used to analyze the allozyme data

phylogenetically had been criticized by Buth (1984).

In a recent study, Jenkin et al. (1992) documented chromosome numbers and chromosomal nucleolar organizer region (NOR) phenotypes of the four extant species of *Ptychocheilus* and several other species belonging to the "western clade" (Coburn and Cavender, 1992) of North American Cyprinidae. All four species of *Ptychocheilus*, *Mylopharodon*, and *Lavinia* possessed $2n = 50$ chromosomes and two pair of NOR-bearing chromosomes of the A NOR phenotype (defined as a NOR situated terminally on the short arm of a medium-sized acrocentric chromosome). Jenkin et al. (1992) suggested that the AA NOR state might represent a derived condition uniting several members of the western clade (including *Ptychocheilus*, *Mylopharodon*, and *Lavinia*) into a monophyletic group but noted that it remained to be shown that the A NOR chromosomes were homologous among species. In this study, the NOR-bearing chromosomes of three of the four species of *Ptychocheilus* and of *Mylopharodon* and *Lavinia* were tested for presumed homology using G-banding to determine whether the chromosomal NORs were informative phylogenetically (1) within *Ptychocheilus* and/or (2) within the western clade of North American Cyprinidae. In addition, genome sizes (DNA contents) of all four species of *Ptychocheilus* (and of *Mylopharodon* and *Lavinia*) were estimated using flow cytometry to determine whether genome size evolution might contribute to the systematics and phylogeny of *Ptychocheilus*.

METHODS

Specimens examined in this study were obtained primarily by seine from natural populations. Collection localities and voucher material are listed in Material Examined. Two samples of *P. umpquae* were examined: one from the Umpqua River drainage [referred to hereafter as *P. umpquae* (Umpqua)] and one from the Siuslaw River [referred to hereafter as *P. umpquae* (Siuslaw)]. Fibroblast cultures were seeded in the field following procedures outlined in Gold et al. (1990a). Metaphase chromosomes were prepared after Amemiya et al. (1984), following modifications specified in Gold et al. (1990a). G-banding (using trypsin) of NOR chromosomes employed methods in Gold et al. (1990a) and Gold and Li (1991). G-banding of NOR chromosomes was carried out on at least two individuals each from all samples of *Ptychocheilus* (except *P. lucius*) and from one individual each of *M. conocephalus* and *L. exilicauda*. Re-

peated attempts to obtain trypsin G-bands on NOR-bearing chromosomes of *P. lucius* were unsuccessful. Bright-field microscopy followed Gold and Amemiya (1986).

Whole blood for genome size determinations was taken in the field and cryopreserved following procedures outlined in Gold et al. (1991b). Genome size determinations of individual fish were made using flow cytometry of erythrocyte nuclei as described in Gold et al. (1991b). Erythrocytes from both chicken and common carp were used as internal standards. Homogeneity of sample mean DNA values was tested using one-way analysis of variance, and mean separation was accomplished using Duncan's multiple range test. All statistical analyses were carried out on the Texas A&M University mainframe computer using Statistical Analysis Systems (SAS, 1985) programs.

RESULTS

All specimens of *Ptychocheilus*, *Mylopharodon*, and *Lavinia* possessed $2n = 50$ chromosomes and two pairs of NOR-bearing chromosomes, as reported by Jenkin et al. (1992). Both pairs of NOR chromosomes in all individuals were of the A NOR phenotype, defined as a NOR situated terminally on the short arm of a medium-sized acrocentric chromosome (Gold and Amemiya, 1986; Amemiya and Gold, 1988). Trypsin G-banding patterns of the NOR chromosomes were obtained from individuals of all samples except for *P. lucius*. Both pair of A NOR chromosomes in all specimens examined possessed four G^+ (dark) bands on the long arm and a single G^+ band on the short arm (Fig. 1). The two pair of A NOR chromosomes, however, differed by the presence of a large G^- (light) band near the middle of the long arm on one of the NOR chromosome pairs, hereafter designated NOR chromosome A₁ (Fig. 1). The large G^- band was observed on two of the four NOR chromosomes identified in all G-banded, mid-metaphase (diploid) preparations examined and is hypothesized to represent a fixed chromosomal feature permitting identification of the two, nonhomologous A NOR chromosomes.

Mean genome size values (Table 1) among the six samples of *Ptychocheilus* ranged from 2.52 picograms (pg) of DNA in *P. lucius* to 2.81 pg in *P. umpquae* (Umpqua). The remaining two species (*L. exilicauda* and *M. conocephalus*) possessed 2.49 pg and 2.66 pg of DNA, respectively (Table 1). Heterogeneity testing of mean genome sizes (Table 1) revealed three more-or-less homogeneous groups of samples: (1) *L. exilicauda*, *P. lucius*, *P. grandis*, and *P. umpquae*

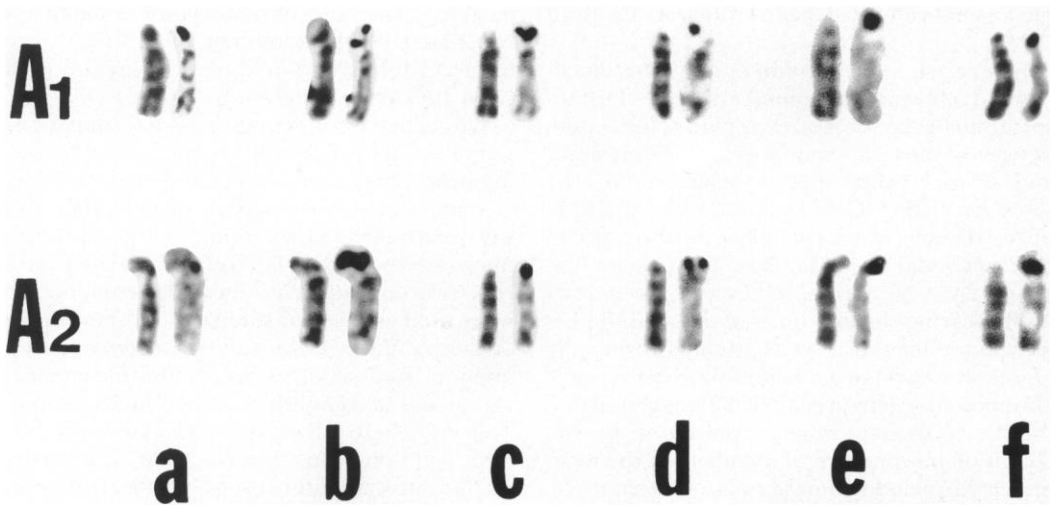


Fig. 1. Trypsin G-banded NOR chromosomes from (a) *Lavinia exilicauda*, (b) *Mylopharodon conocephalus*, (c) *Ptychocheilus oregonensis* (Maries River), (d) *P. grandis*, (e) *P. umpquae* (Siuslaw River), and (f) *P. umpquae* (Umpqua River drainage). For each pair, the chromosome on the left is from a G-banded metaphase; the chromosome on the right is from sequential silver staining (to identify NOR chromosomes) from the same metaphase. Bar denotes large G⁻ (negative) band on the A₁ NOR chromosome.

(Siuslaw) (2.49–2.57 pg of DNA); (2) *M. conocephalus* (2.66 pg of DNA); and (3) *P. oregonensis* from western and eastern Oregon and *P. umpquae* (Umpqua) (2.75–2.81 pg of DNA). The difference in genome size between *P. umpquae* (Siuslaw) (2.54 pg of DNA) and *P. umpquae* (Umpqua) (2.81 pg of DNA) is striking and is greater than the average difference in genome size between any two species of North American Cyprinidae drawn at random (Gold et al., 1990b).

DISCUSSION

The finding of presumably homologous A₁ and A₂ NOR chromosome pairs in the samples of *Ptychocheilus*, *Mylopharodon*, and *Lavinia* is uninformative relative to resolving phylogenetic relationships within *Ptychocheilus*. However, assuming *P. lucius* also possesses A₁ and A₂ NOR chromosome pairs [which seems likely given that *P. lucius* possesses two pairs of A NOR chromosomes of a size similar to those in other *Ptychocheilus* (Jenkin et al., 1992)], the implication is that there have been no detectable changes in NOR chromosome phenotypes since all three genera evolved from a common ancestor. Given that fossils of *Ptychocheilus* and *Mylopharodon* are known from Miocene-Pliocene deposits (Smith, 1981), the apparent conservatism of chromosomal NOR evolution is in striking contrast to that documented among cyprinids endemic to eastern North America where differ-

ences in chromosomal NOR phenotypes among species within genera and among genera are common (Amemiya and Gold, 1990; Amemiya et al., 1992). As discussed by Jenkin et al. (1992), the apparent chromosomal conservatism in western North American cyprinids is reminiscent of the “canalization” hypothesis (Bickham and Baker, 1979), where the karyotype is strongly affected by natural selection, and older lineages presumably evolve an “optimum” karyotype.

Among other North American cyprinids, A NOR chromosomes have been found only in (1) *Camptostoma anomalum*, a member of the “chub” clade (Mayden, 1989); (2) *Notemigonus crysoleucas*, the only endemic North American cyprinid in the Leuciscini (Cavender and Coburn, 1992); and (3) other cyprinids endemic to western North America, including species of *Acrocheilus*, *Gila* (*Temiculina*), *Orthodon*, *Plagopterus*, *Rhinichthys*, and *Richardsonius*, all of which have at least two pairs of A NOR chromosomes (Jenkin et al., 1992). Because the A NOR chromosomes in *C. anomalum* appear to be independently derived within the chub clade (Amemiya and Gold, 1990), Jenkin et al. (1992) suggested that a single pair of A NOR chromosomes (as found in *N. crysoleucas*) might be plesiomorphic for North American Cyprinidae and that the addition of an A NOR chromosome pair might represent a chromosomal synapomorphy uniting several western cyprinid genera into a clade. The G-banding patterns of both pair of A NOR

TABLE 1. SUMMARY GENOME SIZE DATA. $n = 5$ for all samples. Data are in picograms of DNA per nucleus. Mean DNA values of samples with the same letter superscript (Duncan's test grouping) do not differ at $\alpha = 0.05$.

Sample	Mean \pm SE	Range
<i>Ptychocheilus lucius</i>	2.52 \pm 0.01 ^{a,b}	2.48–2.56
<i>Ptychocheilus grandis</i>	2.57 \pm 0.01 ^b	2.54–2.59
<i>Ptychocheilus umpquae</i> (Siuslaw River)	2.54 \pm 0.01 ^{a,b}	2.52–2.56
<i>Ptychocheilus umpquae</i> (Lookingglass Creek)*	2.81 \pm 0.02 ^c	2.75–2.87
<i>Ptychocheilus oregonensis</i> (Maries River)	2.75 \pm 0.03 ^d	2.66–2.85
<i>Ptychocheilus oregonensis</i> (Umatilla River)	2.76 \pm 0.02 ^{c,d}	2.73–2.83
<i>Mylopharodon conocephalus</i>	2.66 \pm 0.03 ^c	2.63–2.77
<i>Lavinia exilicauda</i>	2.49 \pm 0.01 ^a	2.45–2.52

* Umpqua River drainage.

chromosomes in the western cyprinids examined in this study, however, differ markedly from the G-banding pattern of the A NOR chromosome in *N. crysoleucas* (Gold et al., 1991a), indicating that the A NOR chromosome in the latter is not homologous to either of the A NOR chromosomes in *Ptychocheilus*, *Mylopharodon*, or *Lavinia*. This indicates that the A₁A₂ NOR state may represent a synapomorphy uniting *Ptychocheilus*, *Mylopharodon*, and *Lavinia* into a clade which also may include other western cyprinids. G-banding of both pairs of A NOR chromosomes in other western cyprinids is currently in progress to test the latter hypothesis.

The genome size data are of significance to the evolution of *Ptychocheilus* for two reasons. The first is the possibility that *P. umpquae* (Siuslaw) may represent an undescribed species of squawfish. The difference in genome size (0.29 pg of DNA) between *P. umpquae* (Siuslaw) and *P. umpquae* (Umpqua) is greater than the genome size difference, on average, between any two North American cyprinid species drawn at random (Gold et al., 1990b) and considerably greater than the genome size difference between putative pairs of North American cyprinid sister species (Gold et al., 1992; unpubl. data). Mayden et al. (1991) found *P. umpquae* (Siuslaw) to be quite distinctive in multivariate (morphometric) hyperspace from *P. umpquae* (Umpqua), *P. oregonensis*, and *P. grandis* and suggested that the distinctiveness of *P. umpquae* (Siuslaw) may "... alter ... the number of species in the genus." Mayden et al. (1991) also cited a personal communication from C. Bond to D. Both where reservations regarding the relationship between *Ptychocheilus* in the Siuslaw and Umpqua rivers were noted. Whether *P. umpquae* (Siuslaw) merits recognition as a dis-

tinct species awaits further study (as discussed by Mayden et al., 1991), but it is interesting to note that *P. umpquae* (Siuslaw) and *P. umpquae* (Umpqua) are less similar in morphometry and genome size than are *P. umpquae* (Umpqua) and *P. oregonensis* (Mayden et al., 1991; this paper).

The second reason regards the differing hypotheses of phylogenetic relationships among *Ptychocheilus* proposed by Carney and Page (1990) and Mayden et al. (1991). To examine whether genome size evolution might contribute to an understanding of phylogeny in *Ptychocheilus*, changes in genome size were partitioned among branches in both phylogenies using the optimization procedure of Farris (1970) as employed by Larson (1984), Sessions and Larson (1987), and Gold et al. (1992). In analyses of both phylogenies, *Mylopharodon* was placed outside of a presumed monophyletic *Ptychocheilus* [using only *P. umpquae* (Umpqua) to represent *P. umpquae*], and the hypothetical genome size at the basal node of each phylogeny was inferred by optimization using *Lavinia* as the root. For the phylogeny proposed by Carney and Page (1990), the optimization procedure indicated a minimum (absolute) genome size change of 0.46 pg of DNA; whereas for the phylogeny proposed by Mayden et al. (1991), the optimization procedure indicated a minimum (absolute) genome size change of 0.55 pg of DNA. The difference between 0.46 pg and 0.55 pg of DNA is almost 20% and indicates that the phylogeny of Carney and Page (1990) is the more "parsimonious" of the two relative to genome size change. Similar analyses of the remaining 13 possible, fully resolved trees involving the four species of *Ptychocheilus* revealed no other trees with a minimum (absolute) genome size change less than 0.46 pg of DNA.

The above could be taken as evidence in support of the phylogeny for *Ptychocheilus* proposed by Carney and Page (1990), and, indeed, some authors (e.g., Novacek and Norell, 1989) have treated genome size as a phylogenetically informative character, with different genome size values representing different character states. Treating genome size as a single character in phylogenetic inference, however, is problematic for a number of reasons, not the least of which is that genome size may be strongly influenced by natural selection (Szarski, 1983; Cavalier-Smith, 1985), and similarities in genome size among taxa could be a result of convergence. As a consequence, we conclude only that the phylogeny of *Ptychocheilus* proposed by Carney and Page (1990) requires less (minimum) change in genome size than does the phylogeny proposed by Mayden et al. (1991). A major impediment to phylogenetic study of *Ptychocheilus* appears to be a lack of agreement about which taxa could serve as the most appropriate outgroups to the genus (Carney and Page, 1990; Mayden et al., 1991); and in a recent phylogenetic analysis of morphological character variation in western North American cyprinids, Coburn and Cavender (1992) placed *Ptychocheilus* in a large, unresolved polytomy with several other members (including *Mylopharodon*, *Lavinia*, and *Hesperoleucus*) of the western clade.

MATERIAL EXAMINED

Collection localities of material examined are listed below. Catalog numbers of voucher specimens deposited in the Ichthyological Research Collection at the University of California at Los Angeles (UCLA) are given in parentheses. *Lavinia exilicauda*—Coyote Creek, coastal drainage, Santa Clara County, California (W90-11); *Mylopharodon conocephalus* and *Ptychocheilus grandis*—Pit River, Pit River drainage, Modoc County, California (W90-7); *Ptychocheilus oregonensis*—Maries River, Willamette River drainage, Benton County, Oregon (W90-5) and Umatilla River, Columbia River drainage, Umatilla County, Oregon (W90-6); *Ptychocheilus umpquae*—Lookingglass Creek, Umpqua River drainage, Douglas County, Oregon [W90-2(A)] and Siuslaw River, Siuslaw River drainage, Lane County, Oregon (W90-3). Specimens of *Ptychocheilus lucius* were obtained from the Dexter National Hatchery in Dexter, New Mexico, and originated from parental stocks of *P. lucius* taken from the Yampa River. Voucher specimens of *P. lucius* were deposited in the Texas Cooperative Wildlife Collections (catalog #6921.04) at Texas A&M University.

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