*Copeia*, 1986(4), p. 1024


A HERMAPHRODITIC ARCTIC FLOUNDER, *LIOPSETTA GLACIALIS* (PLEURONECTIDAE), FROM ALASKA.—We discovered a hermaphroditic *Liopsetta glacialis* while examining tissues from a sample of 30 representatives of this species for a histopathological survey of fishes from the Beaufort Sea, Alaska. *L. glacialis* occurs in the Arctic Ocean from Queen Maude Gulf in arctic Canada, along the North American and Siberian coasts to the White Sea and Barents Sea (Morrow, 1980). The specimen was collected from Beaufort Lagoon (69°50′N, 142°15′W) on July 29, 1984. It weighed 15 g, measured 100 mm (total length) and was between 3 and 4 yr old.

The gonad was preserved in Bouin's fixative and embedded in paraffin. Histological sections were cut at 5 μm and stained with Harris' hematoxylin followed by eosin counterstain (Humason, 1979).

The gonad contained occasional to frequent primary oocytes which were scattered among seminiferous tubules (Fig. 1). The primary oocytes were at a stage of development comparable to the peri-nucleolus stage described by Yamamoto and Yamazaki (1961). The seminiferous tubules contained cysts of cells in various stages of spermatogenesis. Because sperm was present in some of these tubules, this fish could have probably functioned as a male.

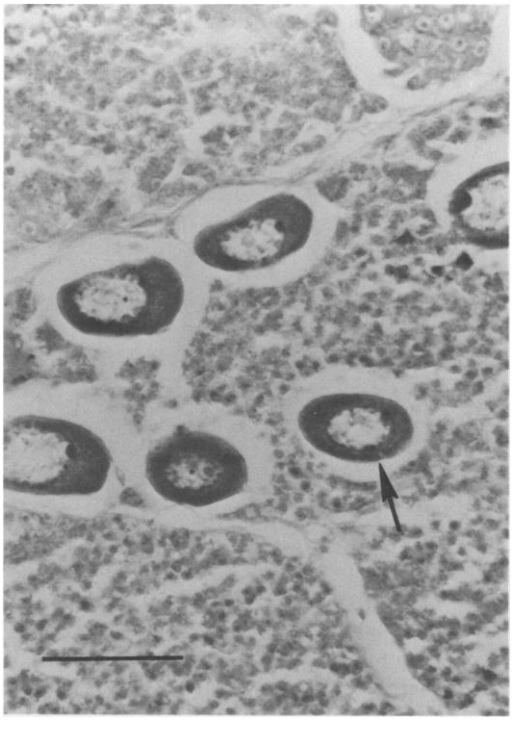

Fig. 1. Gonad of *Liopsetta glacialis* showing primary oocytes (arrow) interspersed among seminiferous tubules. Bar indicates 50 μm.

Atz (1964) reported hermaphroditism to occur significantly less often in flatfishes than in other groups of fishes. To our knowledge, hermaphroditism has not been previously reported in *L. glacialis*.

*Acknowledgments.*—Histological slides were prepared by Douglas W. Eib.

## Literature Cited

Atz, J. W. 1964. Intersexuality in fishes, p. 145–232. *In:* Intersexuality in vertebrates including man. C. N. Armstrong and A. J. Marshall (eds.). Academic Press, New York, New York.

Humason, G. L. 1979. Animal tissue techniques. W. H. Freeman, Co., San Francisco, California.

Morrow, J. E. 1980. The freshwater fishes of Alaska. Alaska Northwest Publishing Co., Anchorage, Alaska.

Yamamoto, K., and F. Yamazaki. 1961. Rhythm of development in the oocyte of the gold-fish, *Carassius auratus*. Bull. Fac. Fish. Hokkaido Univ. 12:93–110.

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*Copeia*, 1986(4), pp. 1024–1028


THE KARYOTYPE OF THE MEXICAN BLINDCAT, *PRIETELLA PHREATOPHILA* CARRANZA (ICTALURIDAE).—Organisms inhabiting troglobitic (cave) environments commonly experience the degeneration of eyes and pigments and the compensatory development of adaptations for cave dwelling (Eigenmann, 1909; Barr, 1968). Of the vertebrates, catfishes

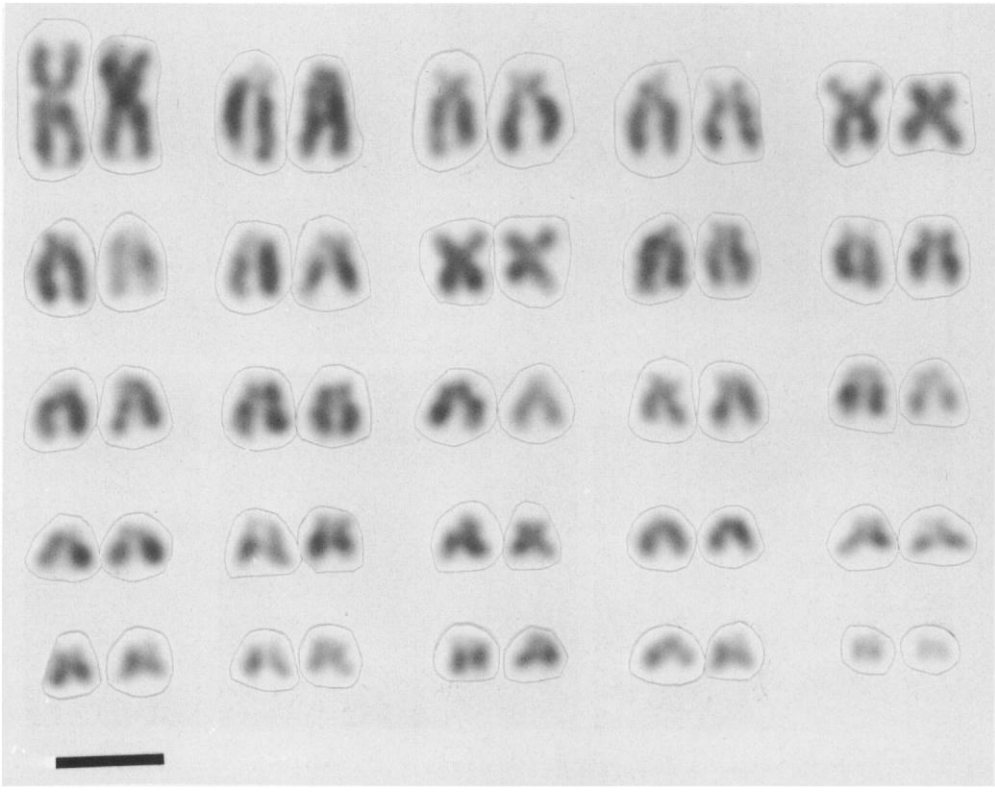

Fig. 1. Standard karyogram of *Prietella phreatophila* (2n = 50, FN = 80±). Scale bar = 5 μm.

are considered preadapted for life in cave environments due to their highly developed tactile sensory structures and to certain other biological attributes (Lundberg, 1982; Miller, 1984). Indeed, some 40% of all the blind fishes inhabiting fresh water are siluroids (Miller, 1984).

Within the Ictaluridae there are three blind species: the toothless blindcat, *Trogloglanis pattersoni* Eigenmann (1919); the widemouth blindcat, *Satan eurystomus* Hubbs and Bailey (1947); and the Mexican blindcat, *Prietella phreatophila* Carranza (1954). The former two are known only from artesian wells near San Antonio, Texas; the latter is known only from phreatic wells in Coahuila, Mexico. All three species are rare and extremely difficult to obtain alive. The three species also apparently arose from different evolutionary lineages and are not considered closely related (Taylor, 1969; Lundberg, 1982). Herein, we document the karyotype of *P. phreatophila.*

*Materials and methods.*—A single specimen of *P. phreatophila* was collected on Nov. 7, 1984, 5.1 km west of the plaza in Melchior Múzquiz, Coahuila, Mexico. The specimen was transported live to College Station, Texas, where it was maintained in a well-aerated aquarium and fed frozen brine shrimp daily for ca. three months.

Approximately 90 min prior to its sacrifice, the specimen, measuring 27.7 mm in standard length and 0.6 g in weight, received a 0.05 cc intraperitoneal injection of 1% colchicine. The specimen was sacrificed by pithing and all of its gill arches were excised and transferred to a small petri dish containing 5.0 ml of 0.4% KCl. The gill arches were then teased apart using fine-tipped forceps and allowed to incubate at room temperature for 35 min, at which time they were fixed in four changes (10 min each) of ice-cold Farmer's solution (3:1 MeOH/HAc). Slides were prepared following Gold (1984). The specimen was deposited in the Texas Cooperative Wildlife Collection at Texas A&M University (TCWC 5144-1).

In addition to conventional Giemsa staining, various chromosome banding methods were

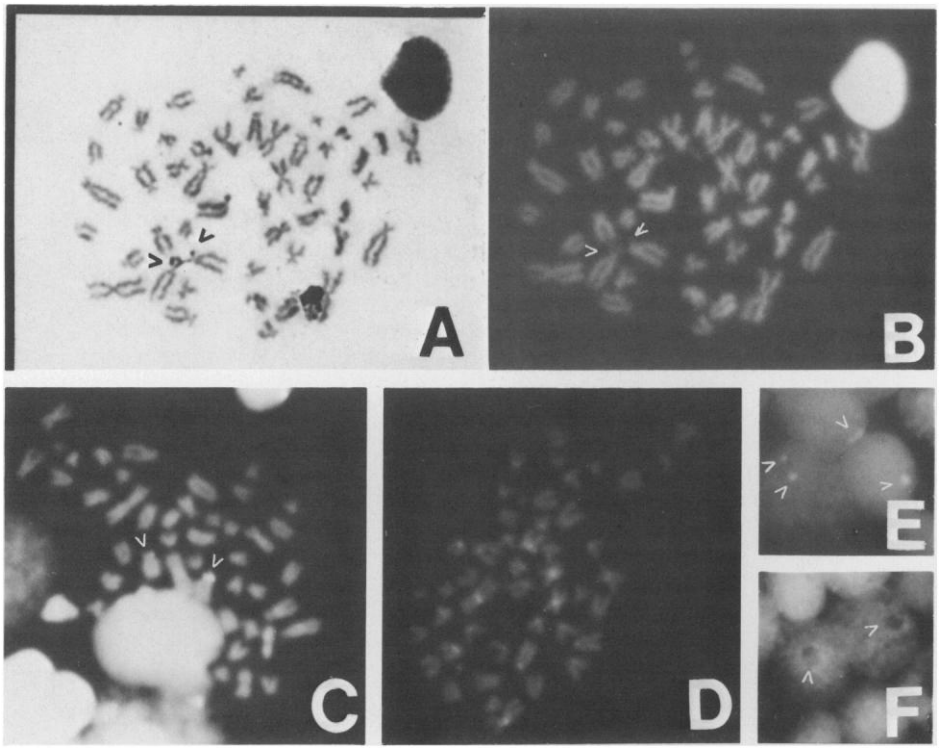


Fig. 2. The effect of various staining treatments on cytological preparations of *Prietella*. (A)–(D) Photos of metaphases after: (A) AgNOR staining; (B) DAPI staining of same preparation in (A) after silver destaining; (C) CMA staining; and (D) C-banding and staining with DAPI (instead of Giemsa). The arrowheads in (A)–(C) indicate the NORs. Note in (B) the quenched fluorescence of NOR sites after DAPI staining. This is theoretically due to a relative paucity of AT base pairs in NOR sites (see text). (E)–(F) Photos of interphase nuclei after staining with: (E) CMA; and (F) DAPI. The arrowheads point to nucleoli. Note the absence of conspicuously fluorescing chromocenters in both (E) and (F) as well as the quenched DAPI fluorescence of the nucleoli in (F).

employed. These were silver staining (Gold and Ellison, 1983), chromomycin staining (Amemiya and Gold, 1986), DAPI staining (Amemiya and Gold, 1986) and C-banding (Gold et al., 1986). Due to the limited number of slides, preparations were usually stained with at least two different staining procedures. This required destaining of stained preparations. Silver destaining was done by treating slides for 1–5 h in Kodak photographic fixer; fluorochrome and Giemsa destaining were done by treating slides in Farmer's solution (overnight for fluorochromes and 10 min for Giemsa).

We used a Zeiss Universal microscope equipped with epifluorescence and fitted with an Olympus OM2 camera. Full details are given in Amemiya and Gold (1986). Photomicrographs were taken on Kodak technical pan 2415 film developed in Diafine (Acufine). Typical exposure times were 2 sec for bright field and 15–90 sec for fluorescence. Chromosome counts were made by direct observation of conventionally (i.e., Giemsa) stained metaphases. A strong mode was established after ca. 20 spreads. Arm number estimates (Levan et al., 1964) were made using dial calipers from projected photographic negatives of five well spread metaphases. The amount of C-band heterochromatin (expressed as a proportion of total chromosome length) was measured using dial calipers from photographic prints of three C-banded metaphases.

*Results and discussion.*—A standard karyogram of *P. phreatophila* is shown in Figure 1. The specimen had a diploid chromosome number of 50 and an estimated diploid arm number of 80. Figure 2 shows the effects of the various staining

procedures. Silver and chromomycin (CMA), which specifically differentiate nucleolus organizer regions (NORs) on fish chromosomes (Amemiya and Gold, 1986), revealed that the NOR in *Prietella* is found terminally on the short arm of a medium-large subtelocentric chromosome pair (Fig. 2A, C). The fluorochrome DAPI, which generally results in a banding pattern complementary to that of CMA (Amemiya and Gold, 1986), did not induce notable differential chromosome banding except for the expected quenching of the NORs (Fig. 2B).

C-banding revealed that the chromosomal heterochromatin in *Prietella* is localized exclusively around the centromeres (Fig. 2D). Quantitative measurements showed that roughly 12–16% of the total chromosome length is comprised of C-band heterochromatin, a range which is approximately the same as that found in other fishes examined to date (Gold et al., 1986). The strictly centromeric distributional pattern, however, is atypical in that most of the fishes thus far examined possess both non-centromerically as well as centromerically located heterochromatin (Gold et al., 1986). Based on the fluorescent patterns of CMA and DAPI (Fig. 2B–C), it is doubtful that the heterochromatin in *Prietella* is particularly enriched in either adenine-thymine (AT) or guanine-cytosine (GC) DNA base pairs. This follows from published observations that CMA and DAPI selectively enhance the fluorescence of chromosomal regions rich in GC and AT base pairs, respectively (Schweizer, 1976; Jorgenson et al., 1978). In addition, chromocenters, interphase nuclear entities consisting of heterochromatic aggregations, are usually strikingly enhanced by CMA or DAPI if their base composition is largely GC- or AT-rich (Schweizer, 1976). The CMA and DAPI stained nuclei in Figures 2E and 2F show essentially an absence of conspicuously fluorescing chromocenters.

A phylogenetic hypothesis of systematic relationships within the Ictaluridae was proposed by Lundberg (1982). The genus *Noturus*, the presumed sister group to *Prietella*, has a diploid karyotype comprised of 40–72 chromosomes and 62–82 chromosome arms (FN). The ancestral condition in *Noturus* is thought to be 2n = 54–56 and FN = 82± (LeGrande, 1981). Qualitatively and quantitatively, the standard karyotype of *Prietella* cannot be distinguished from a *Noturus*-type karyotype (LeGrande, 1981). It does, however, differ from the proposed ancestral *Noturus* karyotype in chromosome number.

The finding that *Prietella* has a single pair of NORs is interesting in view of the pattern of chromosomal NOR variation thus far observed in the Ictaluridae. Preliminary investigations in our laboratory and by W. H. LeGrande (University of Wisconsin at Stevens Point) have revealed that at least three species in *Noturus* have multiple NORs (i.e., more than one pair): *N. gyrinus*, *N. munitus* and *Noturus* sp. (undescribed form referred to as the "broadtail madtom" from the Waccamaw drainage, North Carolina). Conversely, all non-*Noturus* species thus far examined have only a single pair of NORs. These include *Pylodictis olivaris*, *Ictalurus* (*Amiurus*) *melas*, *Ictalurus* (*Amiurus*) *natalis* and *Ictalurus* (*Ictalurus*) *punctatus*. Since single NORs generally appear to be ancestral to multiple NORs (Hsu et al., 1975; Gold, 1984; Bickham and Rogers, 1985), these findings suggest that a single pair of NORs is primitive for the Ictaluridae and that the multiple NORs in *Noturus* represent a derived condition which arose after the divergence of *Noturus* and *Prietella*. Confirmation of this hypothesis awaits the examination of additional taxa.

In summary, we have examined the karyotype of *P. phreatophila* Carranza. The diploid karyotype consists of 50 chromosomes, 80± chromosome arms, a single pair of NOR-bearing chromosomes and C-band heterochromatin comprising ca. 12–16% of the total chromosome length and localized exclusively around the centromeres. Cytosystematically, the standard karyotype of *Prietella* is not distinct enough to separate it from a *Noturus* karyotype. The single NOR in *Prietella*, however, may be the ancestral condition for the Ictaluridae, whereas the multiple NORs in *Noturus* may be derived.

*Acknowledgments.*—We thank the Mexican government for its continued cooperation with our (SWK, FSH) work on the catfishes of Mexico. Funds for the research were provided by the Texas Agricultural Experiment Station on Projects H-6703 (JRG) and H-1678 (FSH), by a Tom Slick Graduate Research Fellowship (SWK), and by the National Science Foundation under Grant BSR-8415428 (JRG). We also thank W. H. LeGrande for providing unpublished chromosome data and J. Crenshaw for maintaining the specimen alive.

## Literature Cited

Amemiya, C. T., and J. R. Gold. 1986. Chromomycin $A_3$ stains nucleolus organizer regions of fish chromosomes. Copeia 1986:226–231.

BARR, T. C. 1968. Cave ecology and the evolution of troglobites, p. 35–102. *In:* Evolutionary biology, Vol. 2. T. Dobzhansky, M. K. Hecht and W. C. Steere (eds.). Appleton-Century-Crofts, New York, New York.

BICKHAM, J. W., AND D. S. ROGERS. 1985. Structure and variation of the nucleolus organizer region in turtles. Genetica 67:171–184.

EIGENMANN, C. H. 1909. Cave vertebrates of America, a study in degenerative evolution. Carnegie Inst. Washington Pub. 104:1–241.

GOLD, J. R. 1984. Silver-staining and heteromorphism of chromosomal nucleolus organizer regions in North American cyprinid fishes. Copeia 1984: 133–139.

———, AND J. R. ELLISON. 1983. Silver staining for nucleolus organizing regions of vertebrate chromosomes. Stain Technol. 58:51–55.

———, C. T. AMEMIYA AND J. R. ELLISON. 1986. Chromosomal heterochromatin differentiation in North American cyprinid fishes. Cytologia. (In press).

HSU, T. C., S. E. SPIRITO AND M. L. PARDUE. 1975. Distribution of 18 + 28S ribosomal genes in mammalian genomes. Chromosoma 53:25–36.

JORGENSON, K. F., J. H. VAN DE SANDE AND C. C. LIN. 1978. The use of base pair specific DNA binding agents as affinity labels for the study of mammalian chromosomes. Chromosoma 68:287–302.

LEGRANDE, W. H. 1981. Chromosomal evolution in North American catfishes (Siluriformes: Ictaluridae) with particular emphasis on the madtoms, *Noturus*. Copeia 1981:33–52.

LEVAN, A., K. FREDGA AND A. SANDBERG. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52:201–220.

LUNDBERG, J. G. 1982. The comparative anatomy of the toothless blindcat, *Trogloglanis pattersoni* Eigenmann, with a phylogenetic analysis of the ictalurid catfishes. Misc. Publ. Mus. Zool. Univ. Mich. 163: 1–85.

MILLER, R. R. 1984. *Rhamdia reddelli*, new species, the first blind pimelodid catfish from Middle America, with a key to the Mexican species. Trans. San Diego Soc. Nat. Hist. 20:135–144.

SCHWEIZER, D. 1976. Reverse fluorescent chromosome banding with chromomycin and DAPI. Chromosa 58:307–324.

TAYLOR, W. R. 1969. A revision of the catfish genus *Noturus* Rafinesque with an analysis of the higher groups in the Ictaluridae. U.S. Nat. Mus. Bull. 282: 1–35.

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*Copeia*, 1986(4), pp. 1028–1030


DEVELOPMENTAL TRANSITION IN FEEDING MORPHOLOGY OF THE MIDAS CICHLID.—In a recent review of the relationship between morphology and feeding in cichlid fishes, Barel (1983) concluded that this speciose group can be divided into two fundamentally different functional morphotypes—"Biters" and "Suckers." Simply stated, biters have stout, reinforced elements in the lower jaws and suspensorium, strengthened neurocranial-suspensorial articulations, limited mouth protrusibility and blunt, deep-bodied heads. In contrast, suckers have relatively longer, more delicately constructed head elements and weaker neurocranial-suspensorial articulations, highly protrusible mouths and slender, pointed head profiles. Biters include algae scrapers, mollusc crushers, insect pickers and scale and sponge eaters; suckers prey on other fishes, aquatic invertebrates and their own larvae as well as those of other species.

Although the division briefly outlined above is an easily understood model of cichlid functional morphology, it does not consider two highly significant aspects of feeding-related morphology—developmental changes within species and opportunistic switching from regular feeding mode. In this regard, I studied an ontogenetic series (120 total) of both alcohol-preserved and cleared-and-stained Midas cichlids (*Cichlasoma citrinellum*). Seven of the specimens were caught in Lake Masaya, Nicaragua; the remainder were raised in the experimental fish ponds at the University of California (Berkeley). These cichlids show great changes during ontogeny in the shape and relative proportions of the skull and lower jaws. The juvenile and adult ends of the developmental transition within this species conform, respectively, to Barel's (1983) suction-feeding and biting morphotypes. However, as discussed below, adults are not strictly constrained by their morphology to one feeding mode.

Two components of the ontogenetic change in *C. citrinellum* will be considered: the shape and robustness of the lower jaw complex and the relative depth of the head in lateral view. According to Barel (1983), these two features are strongly correlated with whether a given cichlid is a suction feeder or a biter.

The lower jaw complex (Fig. 1) in juvenile *C. citrinellum* is long and narrow with relatively