KARYOTYPIC ANALYSIS OF THE GOBIID FISH GENUS *QUIETULA* JORDAN AND EVERMANN

by

Peter Calvert Cook

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SIGNED: Peter C. Cook

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

D A Thomson

Donald A. Thomson
Associate Professor of
Ecology and Evolutionary Biology

Sept. 16, 1976
Date

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ABSTRACT

The karyotypes of the two species of the gobiid fish genus Quietula, Q. y-cauda (Jenkins and Evermann) and Q. guaymasiae (Jenkins and Evermann), are reported for the first time.

The fishes were collected from estuaries along the Sonoran coast of the Gulf of California. The karyotypes were prepared from gill epithelium.

Analysis of the karyotypes revealed the diploid complements of the two species to contain the same number of chromosomes, but to differ in the number of biarmed chromosomes. Quietula y-cauda has 42 acrocentric chromosomes (N.F.=42). Quietula guaymasiae has 42 chromosomes consisting of six metacentrics, four submetacentrics, and 32 acrocentrics (N.F.=52).

It is hypothesized that the karyotype of Q. guaymasiae might be derived from that of Q. y-cauda. In addition, it is hypothesized, from comparison of the karyotypes of the genera Quietula and Gillichthys, that the karyotype of Q. y-cauda might be derived from that of the closely related Gillichthys mirabilis, or a mirabilis-like ancestor.

INTRODUCTION

The karyotypes of the two species of the gobiid fish genus Quietula, Q. y-cauda (Jenkins and Evermann, 1888) and Q. guaymasiae (Jenkins and Evermann, 1888), are presented here for the first time. The data are used in discussing the separate species status of the two species, the evolutionary relationship of the two species, and the evolutionary relationship of the two genera, Quietula and Gillichthys.

The karyotypes of Gillichthys mirabilis Cooper and G. seta (Ginsburg), the only two species in that genus, were previously reported by Chen and Ebeling (1971). They were able to hypothesize, from analysis of the evolution of the karyotypes, the derivation of G. seta from G. mirabilis. Earlier, Barlow (1961) had hypothesized this derivation based on analysis of the morphology and ecology of the two species. The karyotypes and the mode of evolution of the karyotypes of the Quietula species may be similar to those of the species of Gillichthys because of the great amount of similarity between these genera.

Similarities exist between the genera in number of species, distribution, ecology, and morphology. Each genus has two species, one of which is endemic to the upper Gulf of California, the other occurring on the Pacific coast

of California and Baja California as well as in the upper Gulf. All of the species are estuarine inhabitants except G. seta which inhabits rocky intertidal pools located near the mouths of estuaries (Barlow, 1961). Morphological similarities have been recognized and reported by several workers, including Jenkins and Evermann (1888), Gilbert (1889), Jordan and Evermann (1896), and Ginsburg (1945).

Gillichthys mirabilis and Quietula y-cauda occur sympatrically in estuaries along the Pacific coast of California and Baja California, and in the upper Gulf. Quietula guaymasiae is endemic to the upper Gulf inhabiting estuaries sympatrically with G. mirabilis and Q. y-cauda. Gillichthys seta is endemic to the upper Gulf and may also be found in the Gulf, sympatric with any, or all, of the three gobies.

This study presents the heretofore unknown karyotypes of Quietula y-cauda and Q. guaymasiae. The evolutionary relationship of these species is analyzed, as is the evolutionary relationship of the genus Quietula to the genus Gillichthys on the basis of the karyotypes and the probable mode of karyotype evolution.

MATERIALS AND METHODS

Collection data for the fishes used in this study are given in Table 1. The collections were made with a beach seine in three estuarine habitats along the Sonoran coast of the Gulf of California. The fishes were transported to Tucson, Arizona, in plastic bags containing a small amount of sea water saturated with oxygen. Despite frequent collecting efforts in suitable habitats no Quietula y-cauda were caught in the Gulf of California by anyone associated with The University of Arizona between April 1973 and February 1976.

The karyotyping procedure used is basically that of Fisher and Rachlin (1972). The source tissue for the karyotypes was gill epithelium. The procedure involves the following steps: pretreatment with colchicine, hypotonic treatment, tissue fixation, staining and mounting, and microinspection.

Pretreatment with Colchicine

Colchicine is a mitotic inhibitor which arrests cell division at the metaphase stage. Two methods of administration were used: intramuscular injection and total immersion bath. The bath was used according to the method of Chen (1967), for individuals too small to be injected easily.

Table 1. Collection data of specimens of *Quietula guaymasiae* and *Quietula y-cauda* used in the preparation of karyotypes for analysis. — All collections are from the Sonoran coast of the Gulf of California. See Appendix A for voucher specimen reference.

Species	Collection Date	Location	N	Size Range (S.L., mm)	Sex Ratio (M:F)
Quietula guaymasiae	June 1973	Bahia Kino; ca. 1 mi N of Pto. Ignacio (28° 53' N; 112° 2' W)	3	56.0–65.0	3:0
	Summer 1973	Pto. Penasco; Bahia Cholla, tide channels (31° 22' N; 113° 40' W)	2	42.0–46.0	0:2
	Sept. 1973	Pto. Penasco; ca. 1 mi N of Bahia Cholla (31° 22' N; 113° 40' W)	4	46.0–59.0	2:2
	Feb. 1976	Guaymas; Estero Soldado, main lagoon (27° 57' N; 111° W)	2	34.0–35.5	2:0
Quietula y-cauda	Feb. 1976	Guaymas; Estero Soldado, main lagoon (27° 57' N; 111° W)	8	30.0–39.0	0:8

The dosage and concentration of the colchicine dissolved in distilled H_2O was varied (Table 2) as seemed appropriate, but within the range of amounts reported as successful by Denton (1973). The length of the pretreatment period was varied (Table 2) to test the relative effects of short and long periods of exposure to colchicine on the quantity and quality of chromosome spreads produced. The results of this test are indicated as positive or negative in Table 2.

Hypotonic Treatment

This treatment was begun with the live animal and was completed with the dissected rightside gill arches after the fish had been sacrificed with the anesthetic Quinaldine (Eastman Kodak Co.). The live animal was subjected to consecutive one-hour treatments with solutions of 50% seawater/50% freshwater and 100% freshwater. The dissected gill arches were subjected to a 90 minute treatment in a hypotonic salt solution consisting of equimolar (0.055 M) solutions of KCl, $NaNO_3$, and CH_3COONa in a ratio of 4:2:0.8 by volume.

Tissue Fixation

The fixative solution used differed from that of Fisher and Rachlin (1972) by the use of methanol instead of ethanol. Denton (1973) states that methanol is preferred by most workers. Freshly made fixative was administered in three washes of 30, 15 and 15 minutes.

Table 2. Procedural variations in colchicine pretreatment*. — Preparations producing satisfactory results are indicated by + under "Usable Material".

Species	Method	Dosage (ml)	Concentration Colchicine Sol.	Pretreatment Length (hr)	Usable Material
Quietula guaymasiae	Injection	0.10	0.05%	24	+
	"	"	"	6	−
	"	"	"	24	−
	"	"	"	2	−
	"	"	"	4	−
	"	0.02	0.50%	3	−
	"	"	"	15	−
	"	0.01	"	24	+
	"	0.02	"	24	+
Quietula y-cauda	Bath	250.00	0.02%	4.5	+
	"	"	"	4.5	+
	"	"	"	15	+
	"	"	"	15	+
	"	"	"	11	+
	"	"	"	11	+
	"	"	0.10%	5	+
	"	"	"	5	+
Quietula guaymasiae	"	"	"	5	+
	"	"	"	5	+

* Per individual fish; listed in chronological order.

Staining and Mounting

Following the final fixative wash the epithelial cells were sloughed off onto microscope slides. After the slides had dried in air, they were stained in either Giemsa or aceto-orcein (2%) stain. Giemsa stain was used for all but the last two Quietula guaymasiae. Material from the last two Q. guaymasiae and all of the Q. y-cauda was stained with aceto-orcein. Although most of the material stained with Giemsa was acceptable, some material was obviously poorly stained. I learned, later, that for successful use of Giemsa it should be maintained in a phosphate buffer at a pH of 6.4 (Denton, 1973). Aceto-orcein is not as sensitive to pH and, therefore, does not require special technical consideration. This might explain the staining inconsistencies noticed in the present study when Giemsa was used. All preparations were stained for about 20 minutes (Chen, 1967). They were then cleared in a series of five washes (two acetone, one 1:1 acetone-xylene, and two xylene) and mounted with Permount (Fisher Scientific Co.).

Microinspection

Slides were observed with a light microscope with a green filter at oil immersion magnification (950x). Photographs were made with a 4x5 inch camera using Kodalith Ortho Type III film and Kodabromide F-5 paper (Eastman Kodak Co.).

Chromosome counts were made by direct observation through the microscope for most of the material. In cases in which the chromosomes were excessively crowded or overlapped, photographs were helpful in identifying individual chromosomes.

Karyograms were made from the best photographs. The chromosomes were first classified according to morphotype using the classification of Chen (1967). This classification recognizes three morphotypes based on the ratio of the short arm of the chromosome to the long arm. The three morphotypes are: metacentric (ratio greater than or equal to 0.9), submetacentric (ratio greater than or equal to 0.3, but less than 0.9), acrocentric (ratio less than 0.3). The ratios were determined from measurements made with dial calipers on enlarged photographs. The chromosomes were then cut from the photographs and arranged on paper in horizontal rows with the largest chromosome at the left end of the row. The chromosome morphotypes were grouped from top to bottom in the order: metacentric, submetacentric, acrocentric. If a morphotype was not represented in the complement the succeeding group was moved up in the karyogram to fill in.

RESULTS

Of the 19 individuals used in this study, usable material was obtained from 14. Lack of usable material from the other five fish might be explained by the variations employed in the colchicine pretreatment period or by the poor staining that was noticed. The 14 individuals providing the karyotypes were eight females of Quietula y-cauda and two females and four males of Quietula guaymasiae. For Quietula y-cauda, 109 chromosome spreads were analyzed, and for Q. guaymasiae, 188 chromosome spreads were analyzed.

The diploid number of both species of Quietula is 42. The karyotypes are distinguishable on the basis of chromosome morphology. The karyotype of Quietula y-cauda has 42 acrocentric chromosomes (Fig. 1). The karyotype of Quietula guaymasiae has 32 acrocentric, 4 submetacentric, and 6 metacentric chromosomes (Fig. 2). The chromosome arm numbers (N.F.) are 42 and 52, respectively. There are no obvious sex chromosomes in Q. guaymasiae, and I assume that there are none in Q. y-cauda (no male Q. y-cauda were caught).

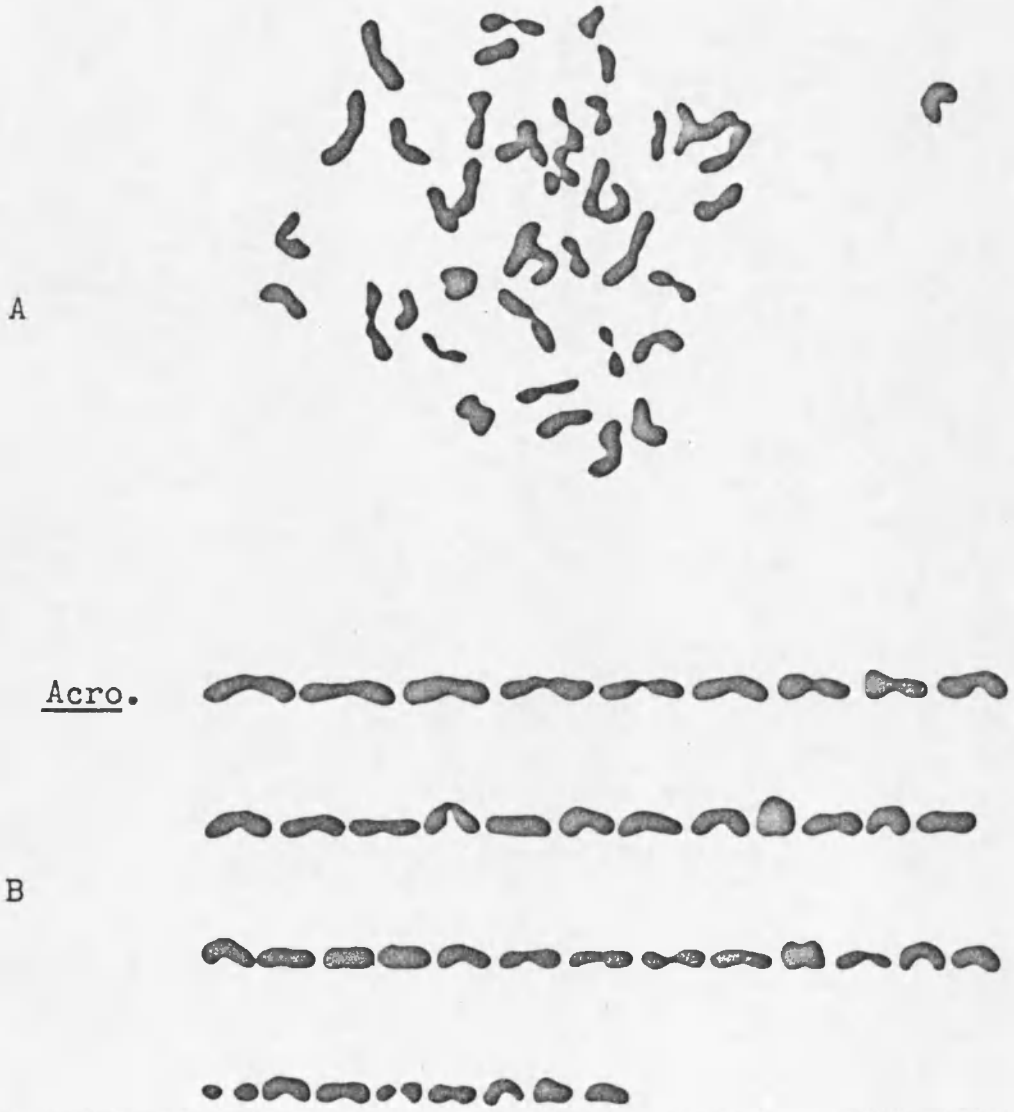
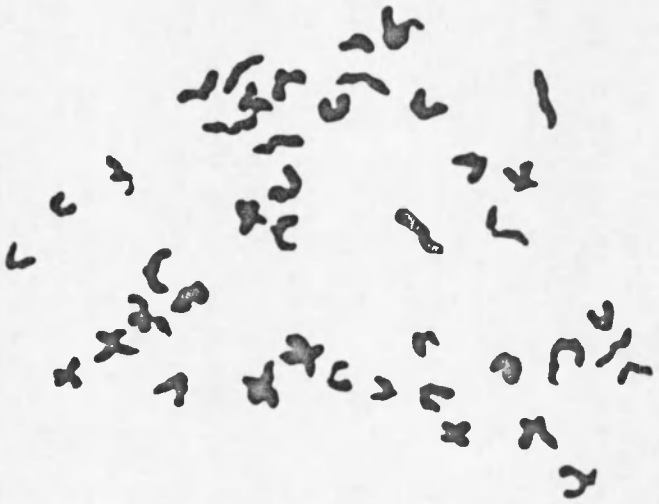


Figure 1. Karyotype (A) and karyogram (B) of Quietula y-cauda.

Acro. = acrocentric.

A



B Meta.

Smet.

Acro.

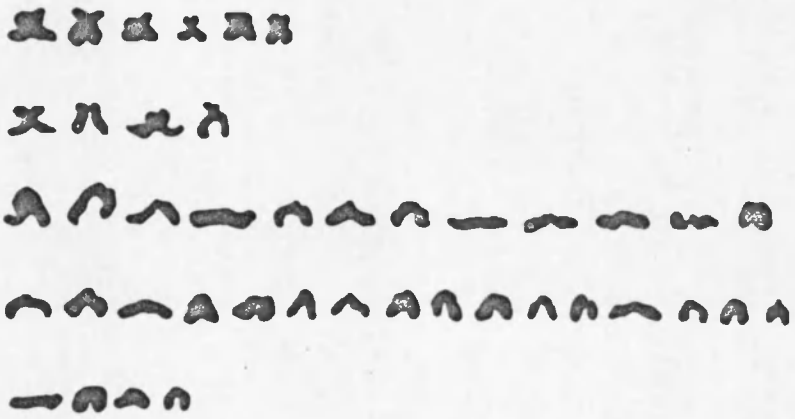


Figure 2. Karyotype (A) and karyogram (B) of Quietula guaymasiae.

Met. = metacentric, Smet. = submetacentric, Acro. = acrocentric.

DISCUSSION

With few exceptions it is generally recognized that every species has a unique karyotype (White, 1973). The karyotypes of Quietula y-cauda and Q. guaymasiae are distinctly different. Therefore, even though Gilbert (1889) and Jordan and Evermann (1896) were doubtful of the distinctness of the two species, the karyotype data support the presently recognized separate species status.

The evolutionary relationship of the two species of Quietula may be hypothesized from analysis of the karyotypes. The evolution of fish karyotypes, and of gobiid fishes in particular, seems to follow a trend toward a smaller complement with a greater proportion of biarmed chromosomes (Chen, 1967; Chen and Ebeling, 1971). Both species have 42 chromosomes but Q. guaymasiae has a greater proportion of biarmed chromosomes. This suggests that the karyotype of Q. guaymasiae is more recently derived than that of Q. y-cauda. I conclude, therefore, that Q. guaymasiae might have derived from Q. y-cauda or a y-cauda like ancestor. The derivation of the karyotype of Q. guaymasiae from the karyotype of Q. y-cauda must have involved, at least, 10 pericentric inversions.

Comparison of the karyotypes of Quietula and Gillichthys (Table 3), using the same criteria, suggests that,

Table 3. Chromosome morphotype classification of the karyotypes of *Quietula y-cauda*, *Q. guaymasiae*, *Gillichthys mirabilis*, and *G. seta*.

Species	2N	Met.	Smet.	Acro.	N.F.
Q. y-cauda	42	0	0	42	42
Q. guaymasiae	42	6	4	32	52
*G. mirabilis**	44	0	12	32	56
*G. seta**	44	6	14	24	64

* Chen and Ebeling, 1971.

since they are closely related genera, the karyotype of Quietula y-cauda might have been derived from the karyotype of Gillichthys mirabilis or a mirabilis-like ancestor. This derivation of karyotype must have involved a reduction in chromosome number from 44 to 42. This might have occurred, with highest probability, by centric fusion of, at least, four acrocentric chromosomes forming two biarmed chromosomes. Since the karyotype of Q. y-cauda does not contain biarmed chromosomes, those formed as a result of centric fusions, and any that might have been present in the ancestral karyotype, must have been transformed into uniarmed chromosomes by pericentric inversions (at least 14). The reduction in chromosome number might also have occurred by tandem fusions of two pairs of acrocentric chromosomes, thereby not forming the two biarmed chromosomes, but requiring, none the less, at least, 12 pericentric inversions to convert the existing biarmed chromosomes of the ancestral karyotype to acrocentrics. The karyotype of Quietula y-cauda could have been derived, by either of these mechanisms, from the karyotype of Gillichthys mirabilis.

On the basis of the karyotype information presented here, and the speciation model described for Gillichthys seta by Barlow (1961), I hypothesize that Quietula y-cauda was derived from Gillichthys mirabilis in the estuaries on the Pacific coast of California and Baja California. These species entered the Gulf of California together at a time

when their ranges were displaced southward by lowering temperatures. When the waters warmed again their ranges were displaced northward, separating the Gulf populations from the outer coast populations, until they reached their present locations in the upper Gulf. There, Quietula guaymasiae and Gillichthys seta were speciated.

Analysis of the evolution of the karyotypes of these two genera has allowed me to hypothesize a clear-cut and credible sequence of speciational events resulting in the derivation of another species of goby endemic to the Gulf of California. This same evolutionary model may be found again in the genus Ilypnus. One species, Ilypnus gilberti (Eigenmann and Eigenmann) occurs sympatrically with Quietula y-cauda and Gillichthys mirabilis on the Pacific coast of California and in the Gulf of California. Two other species, Ilypnus luculentus (Ginsburg) and an undescribed species, are endemic to the Gulf of California. Analysis of the karyotypes of these species may show an extremely close relationship of these three genera.

APPENDIX A

VOUCHER SPECIMENS

The fishes used in this study are deposited in the Fish Collection of The University of Arizona under the following catalog numbers: *Quietula guaymasiae*-- UA73-51, UA73-102, UA76-8; *Quietula y-cauda*-- UA76-8.

LITERATURE CITED

Barlow, G. W. 1961. Gobies of the genus Gillichthys, with comments on the sensory canals as a taxonomic tool. Copeia 1961:423-437.

Chen, T. R. 1967. Comparative karyology of selected deep-sea and shallow-water teleost fishes. PhD. Dissertation, Yale University. 212pp.

_____ and A. W. Ebeling. 1971. Chromosomes of the goby fishes in the genus Gillichthys. Copeia 1971:171-174.

Denton, T. E. 1973. Fish Chromosome Methodology. Charles C. Thomas-Publisher, Springfield, Illinois. 166pp.

Fisher, P. B. and J. W. Rachlin. 1972. Karyotypic analysis of two sympatric species of fish, Fundulus heteroclitus L. and F. majalis. J. Fish Biol. 4:67-71.

Gilbert, C. H. 1889. Notes on the occurrence of Gillichthys y-cauda at San Diego, California. Proc. U. S. Nat'l. Mus. 12:363.

Ginsburg, I. 1945. Contribution to a methodology in the caudal fin ray count of fishes and its use in classification. Copeia 1945:133-142.

Jenkins, O. P. and B. W. Evermann. 1888. Description of eighteen new species of fishes from the Gulf of California. Proc. U. S. Nat'l. Mus. 11:137-158.

Jordan, D. S. and B. W. Evermann. 1896. The fishes of North and Middle America: a descriptive catalogue of the species of fish-like vertebrates found in the waters of North America north of the isthmus of Panama. III. Bull. U. S. Nat'l. Mus. 47:2183-3136.

White, M. J. D. 1973. Animal Cytology and Evolution. Cambridge University Press. 961pp.