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# Disjunct Sea of Cortez–Pacific Ocean *Gillichthys mirabilis* populations and the evolutionary origin of their Sea of Cortez endemic relative, *Gillichthys seta*

**Abstract** The shortjaw mudsucker, *Gillichthys seta*, an intertidal goby endemic to the Sea of Cortez, has been proposed to be the paedomorphic derivative of the longjaw mudsucker, *Gillichthys mirabilis*. *G. mirabilis* is a disjunct species, with populations found along the Pacific coast of central California to central Baja California, and with isolated populations found in the northern Sea of Cortez. Previous studies have suggested that the endemic paedomorph form speciated in sympatry with the Sea of Cortez population of *G. mirabilis*. Alternatively, this speciation event could have occurred before the separation of *G. mirabilis* populations into two disjunct entities. To test these alternative hypotheses, we collected adult individuals from both species throughout their ranges from December 1997 to November 1998. We amplified and sequenced 142 partial [527 base pairs (bp)] mitochondrial cytochrome *b* regions and 18 nuclear creatine kinase introns (140 bp). We found that Pacific populations of *G. mirabilis* separated into two distinct clades, possibly reflecting a phylogeographic break found in other fish species along the Baja California coast at Punta Eugenia. These two Pacific populations were well separated from Sea of Cortez populations. Furthermore, our results indicate that the split between Sea of Cortez and Pacific populations of *G. mirabilis* occurred well after the speciation event that separated *G. mirabilis* from its paedomorphic counterpart, *G. seta*.

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## Introduction

The origins of endemic species provide a unique opportunity to understand evolutionary processes involved in speciation. These processes, however, are often obscured by several factors, including the extinction of ancestral species and the often long evolutionary time between derived endemic species and non-endemic parental species. The Sea of Cortez, also known as the Gulf of California, is an ideal model system for studying mechanisms of speciation in marine species because of the combination of a relatively large number of endemic fish species (approximately 100 species, Thomson et al. 2000) and a recent geologic history. The fish species from the Sea of Cortez are a mixture of assemblages that comprise representatives from the southern Panamic province and the northern Californian province (Walker 1960; Thomson et al. 2000). Endemic species, which represent 17% of all fish species in the Gulf, have origins relating to one or the other of these faunal provinces (Walker 1960).

Several species of fishes with Californian province affinities are present in the Gulf of California as disjunct populations. The term "disjunct" describes species that are found on the Pacific coast of southern California and Baja California and as isolated populations in the upper and central Gulf of California, yet absent from the lower Gulf regions. The disjunct species represent a highly variable assemblage of taxonomic and ecological groups and encompass approximately 28 families and 12 orders (Present 1987). These populations are thought to have been established either by migration, via extinct waterways connecting the Gulf of California to the Pacific Ocean (Durham and Allison 1960; Upton and Murphy 1997), or by migration around the southern tip of Baja California during periods of oceanic cooling associated with glaciating events (Brusca 1973). Presently, gene flow between Pacific and Gulf populations may be limited by physical and physiological barriers (Hubbs 1948, 1960; Present 1987).

The longjaw mudsucker, *Gillichthys mirabilis* (Gobiidae), is a disjunct species commonly found in the intertidal zone along the Pacific coast in sloughs, lagoons, and estuarine habitats from San Francisco, California, to Bahia Magdalena, Baja California (Miller and Lea 1972). *G. mirabilis* spawns from September to July; eggs are attached to the walls of a brood chamber and guarded by the male (Watson 1996). After hatching, pelagic larvae drift in the water column until they reach approximately 11 mm (Watson 1996). In the Gulf of California, its distribution ranges from Mulege, Baja California, to Bahia Agiabampo, Sonora, Mexico (Fig. 1). Walker (1960) observed that the majority of disjunct species exhibit morphological differences between Gulf and Pacific coast populations. Differences are usually small and often limited to color variations. Some disjunct populations, however, exhibit more pronounced differences (Present 1987), as do Gulf of California and Pacific populations of *G. mirabilis* (Barlow 1961a, b, 1963). Gulf members of this species characteristically have longer jaws, higher fins, shorter fin bases, and a more depressed head than their Pacific counterparts. They also tend to be more completely scaled ventrally and have slightly larger scales.

The genus *Gillichthys* comprises only two species: *G. mirabilis* and its sister species, the shortjaw mudsucker, *G. seta*. The shortjaw mudsucker is endemic to the Gulf of California and is abundant in the high, rocky intertidal zone from Puerto Borrascoso to Guaymas. This habitat represents one of the hottest zones occupied by a fish, as it routinely reaches temperatures around 40 °C (Dietz and Somero 1992). The morphology of adult *G. seta* is similar to that of sub-adult *G. mirabilis* at comparable lengths. *G. seta* differs from Pacific coast *G. mirabilis* by having a shallower head, narrower fleshy interorbital, longer snout, and higher anal fin. Although these differences also exist between *G. seta* and Gulf *G. mirabilis*, their magnitude is less extreme.

Barlow (1961a, b) postulated that *G. seta* originated as a paedomorphic form of the larger *G. mirabilis* or a *mirabilis*-like ancestor by adapting to life in the high intertidal zone of the upper Gulf. The rigorous and marginal habitat presented by these shores may have selected for developmentally retarded or slower growing *Gillichthys*. By selectively altering the developmental rates for various body parts (heterochrony), these fish may have eventually branched off into the species *G. seta* (Barlow 1961a, b). This idea has been further supported by chromosomal analysis (Chen and Ebeling 1971).

Two hypotheses have been proposed to explain the evolutionary origin of the endemic *G. seta*. *G. seta* may have derived from Gulf populations of *G. mirabilis* after the geological formation of the Sea of Cortez. This may have occurred within the Sea of Cortez either in sympatry or in parapatry. Parapatry would have occurred if the two species had diverged in two different regions of the Gulf, or if they had diverged in different habitats (upper vs. lower intertidal zone) within the same region. Alternatively, the evolutionary origin of *G. seta* may have predated the separation of *G. mirabilis* into disjunct populations, before the complete formation of the Gulf.

Our first goal was to determine which of these two hypotheses was supported by molecular data. Although morphological and chromosomal analyses provide measures of genetic differentiation, they do not provide a direct estimate of divergence between the two species. Thus, we decided to use mitochondrial (cytochrome *b*) and nuclear [creatine kinase M (Ck-M) intron 7] DNA markers to recover genetic information unattainable with morphological or chromosomal analyses. The second goal of our study was to determine the levels of gene flow between disjunct populations of *G. mirabilis*, and how the dynamics of these populations may have had an effect on speciation processes in *G. seta*.
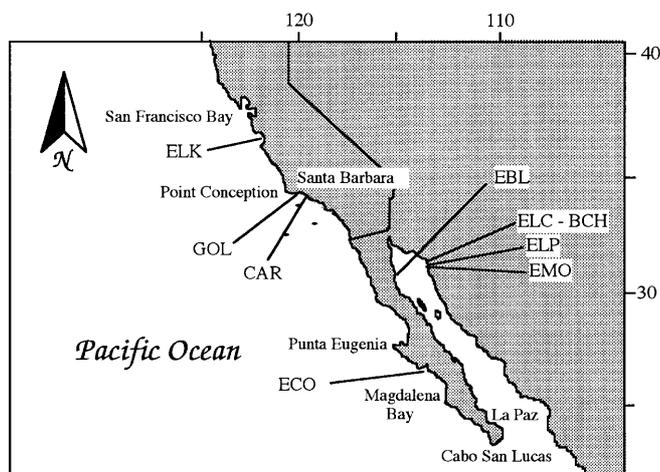


**Fig. 1** Sampling localities of *Gillichthys mirabilis* and *G. seta* on the Pacific coast of California and Baja California and in the Gulf of California (Sea of Cortez). California: Elkhorn Slough (*ELK*), Goleta Slough (*GOL*), Carpenteria Slough (*CAR*). Baja California, Pacific coast : Estero Coyote (*ECO*). Sea of Cortez: Estero La Cholla (*ELC*), Estero Morua (*EMO*), Estero La Pinta (*ELP*), Ensenada Blanca (*EBL*), Bahia Cholla, just outside Estero La Cholla (*BCH*), Estero La Pinta (*ELP*)

## Materials and methods

### DNA samples

Samples used in this study were collected with minnow traps in bays and estuaries. Sampling sites were located as follows: Pacific coast (*G. mirabilis*): Elkhorn Slough (ELK), Goleta Slough (GOL), Carpenteria Slough (CAR), Estero Coyote (ECO); Sea of Cortez (*G. mirabilis*): Estero La Cholla (ELC), Estero Morua (EMO), Estero La Pinta (ELP); Sea of Cortez (*G. seta*): Ensenada Blanca (EBL), Bahia Cholla (just outside Estero La Cholla, BCH), Estero La Pinta (ELP), as described in Fig. 1. Voucher specimens were deposited at the California Academy of Sciences, San Francisco. We used two closely related goby species as out-groups following Nelson's proposed taxonomy of the family (Nelson 1994). *Quietula guaymasae*, which belongs to the same subfamily as *Gillichthys* (Gobionellinae), was collected in Guaymas, Sonora, Mexico, and *Coryphopterus nicholsi,* which belongs to a different goby subfamily,

Gobiinae, was collected in Monterey Bay, California. After collection, samples were placed immediately in 95% ethanol and stored at ambient temperature in the field, and then at 4 °C in the laboratory. Muscle or liver tissue were later dissected from these samples.

Total genomic DNA was prepared from 75–150 mg of muscle or liver tissue by proteinase K digestion in lysis buffer (10 m$M$ Tris, 400 m$M$ NaCl, 2 m$M$ EDTA, 1% SDS) overnight at 55 °C. This procedure was followed by purification using chloroform extractions and alcohol precipitation (Sambrook et al. 1989).

### PCR amplification and sequencing

The initial polymerase chain reaction (PCR) amplification of the mitochondrial cytochrome *b* region was accomplished using the universal primers GLUDG-L and CB3-H (Meyer 1993). Once a few individuals of each species had been sequenced, a specific internal primer (CB1GM-L: 5′-GCCCCMTCSAAYATTWCWGC-TTGATG-3′) was designed to anneal to the light strand for the remaining amplifications. Amplification of Ck M intron 7 followed a nested design. For the primary reaction, the primers CK-F1 and CK-R1 were used, and for the secondary reaction, the primers CK-F2 and CK-R2 (Quattro and Jones 1999).

Each 100 µl reaction contained 10–100 ng DNA, 10 m$M$ Tris HCL (pH 8.3), 50 m$M$ KCl, 1.5 m$M$ MgCl2, 2.5 units of *Taq* DNA polymerase (Perkin-Elmer, Norwalk, Conn.), 150 m$M$ of each dNTP, and 0.3 m$M$ of each primer and was amplified with a cycling profile of 45 s at 94 °C, 45 s at 48 °C, 1 min at 72 °C, for 35 cycles. Secondary amplifications were performed with the same protocol but with annealing temperatures of 54 °C. After purification following the protocol of the manufacturer (ABI, Perkin-Elmer), sequencing was performed in both directions with the primers used in the PCR amplification on an ABI 373 automated sequencer (Applied Biosystems, Foster City, Calif.).

### Phylogenetic analyses and population structure

We used the computer program CLUSTAL V implemented by Sequence Navigator (Applied Biosystems) to align the mitochondrial cytochrome *b* region sequences. Phylogenetic relationships were assessed using the neighbor-joining method (Nei 1987) implemented by the software package PAUP (Phylogenetic Analyses Using Parsimony, version 4.0; Swofford 1998). Maximum parsimony using common algorithms could not be used due to prohibitive computer time. Thus, topological confidence was evaluated with 100 bootstrap replicates (Felsenstein 1985) for neighbor joining and also with 100 replicates using the fast-step method for maximum parsimony (only one tree kept at each replicate). In both neighbor joining and maximum parsimony, bootstrapping analysis was performed with equal weighting of transitions (TR) and transversions (TV), as well as with transversions weighted three times as much as transitions. Average pairwise sequence divergence was calculated using PAUP. Gene flow ($F_{st}$) and haplotype diversity were calculated using the software package DNAsp (Rozas and Rozas 1997) following Hudson et al. (1992). Statistical significance of $F_{st}$ values was computed using the software package Arlequin (version 1.1, Schneider et al. 1997).

## Results

Although *G. mirabilis* and *G. seta* live in close proximity (in some cases, within a few hundred meters), the two species were never caught together in the same trap, supporting the idea that they do not live in the exact same habitats. In general, *G. seta* is found in the uppermost intertidal region, whereas *G. mirabilis* is found in the middle to low intertidal zone. A total of 99 *G. mirabilis* and 43 *G. seta* cytochrome *b* sequences were

obtained (GenBank AY004881-AY005022). Of the 527 bases sequenced, 118 were variable and 82 were phylogenetically informative. As expected, the third codon position contained most of the changes (73%) compared to first (15%) and second (12%) codon positions. The number of transitions was greater than the number of transversions (TR/TV = 3.2). Average pairwise sequence divergences, diversity indices, and haplotype diversity are shown in Table 1. Additionally, nuclear Ck-M introns of 6 *G. seta* and 12 *G. mirabilis* (6 from the Gulf and 6 from the Pacific) were sequenced (GenBank AF288158-AF288159). Out of 140 sequenced nucleotides, 72 corresponded to intronic positions. Out of these 72 bases, two differences were fixed between *G. mirabilis* and *G.seta*. No differences were observed within species.

### Population structure and gene flow

#### Pacific coast G. mirabilis populations

No differences within and between *G. mirabilis* populations were found when using the nuclear Ck-M marker. Thus, the following describes results obtained with the mitochondrial cytochrome *b* marker. *G. mirabilis* samples grouped into three distinct clades (Fig. 2). The first two clades included individuals collected along the Pacific coast (100% bootstrap support). One clade (GM1) contained all the *G. mirabilis* individuals taken at the northern end of the range (i.e. ELK, GOL, CSB, Fig. 1). This clade contained 41 individuals representing 17 haplotypes. The second clade (GM2) included all the individuals taken at Estero Coyote, an estuary approximately 200 km south of Punta Eugenia (ECO, Fig. 1), and two Sea of Cortez *G. mirabilis* (*Gm*11 ELC, *Gm*10 ELP). This clade contained 17 individuals representing 13 haplotypes. These results were reflected by high gene flow levels within regions (north and south, $N_m = 6.92$, $F_{st} = 0.067$), and very low gene flow levels between these regions ($N_m = 0.20$, $F_{st} = 0.718$; see Table 2).

#### Sea of Cortez G. mirabilis populations

The cytochrome *b* marker partially separated individuals collected along the Pacific coast from individuals collected in the Gulf region. Individuals of *G. mirabilis* from the Sea of Cortez formed a clade (GM3, 100% bootstrap support) that included all *G. mirabilis* individuals taken from the Gulf of California, except for two individuals (*Gm*11 ELC and *Gm*10 ELP). This clade contained 43 individuals representing 28 haplotypes. As mentioned above, two Gulf individuals grouped with Pacific individuals, whereas no Pacific individuals grouped with Gulf individuals. These phylogenetic results were therefore accompanied by very low levels of gene flow across Cabo San Lucas between the Gulf of California and the Pacific coast regions ($N_m = 0.31$, $F_{st} = 0.619$). Gene flow across Cabo San Lucas was still very limited when the

**Table 1** *Gillichthys mirabilis* and *G. seta*. Summary of sequence variation within and among populations. Number of haplotypes, number of variable sites, and mean sequence divergence were calculated using DNAsp (Rozas and Rozas 1997). Diversity was calculated according to the equation $h = (1 - \sum x_i^2)n/(n-1)$ where $x_i$ is the frequency of the $i$th mtDNA type (Nei 1987)

| Region name | Locality name | $n$ | Number of haplotypes | Diversity | Number of variable sites | Mean sequence divergence (%) |
|---|---|---|---|---|---|---|
| *G. mirabilis* | | | | | | |
| Total | | 99 | 58 | 0.97 | 60 | 1.4 |
| Pacific Coast | | 56 | 27 | 0.89 | 34 | 0.78 |
| North of Punta Eugenia | | 41 | 17 | 0.81 | 22 | 0.40 |
| | Elkhorn Slough (ELK) | 15 | 6 | 0.78 | 11 | 0.39 |
| | Goleta Slough (GOL) | 15 | 7 | 0.80 | 6 | 0.31 |
| | Carpenteria Slough (CAR) | 11 | 6 | 0.73 | 7 | 0.39 |
| South of Punta Eugenia | Estero Coyote (ECO) | 15 | 10 | 0.91 | 10 | 0.37 |
| Sea of Cortez | | 43 | 29 | 0.97 | 36 | 0.73 |
| | Estero La Cholla (ELC) | 15 | 10 | 0.95 | 18 | 0.64 |
| | Estero Morua (EMO) | 15 | 12 | 0.97 | 10 | 0.57 |
| | Estero La Pinta (ELP) | 13 | 9 | 0.97 | 26 | 0.90 |
| *G. seta* | | | | | | |
| Total | | 43 | 39 | 0.99 | 37 | 1.00 |
| | Ensenada Blanca (EBL) | 14 | 14 | 1.00 | 22 | 1.00 |
| | Bahia Cholla (BCH) | 15 | 13 | 0.98 | 11 | 0.67 |
| | Estero La Pinta (ELP) | 14 | 14 | 1.00 | 20 | 0.96 |
| *G. mirabilis* vs. *G. seta* | | | | | | 11.60 |
| *G. mirabilis* Pacific coast vs. Gulf populations | | | | | | 1.97 |
| *G. mirabilis* north of Punta Eugenia vs. Gulf populations | | | | | | 1.90 |
| *G. mirabilis* south of Punta Eugenia vs. Gulf populations | | | | | | 2.30 |
| *G. mirabilis* north vs. south of Punta Eugenia | | | | | | 1.36 |

regions north of Punta Eugenia and south of Punta Eugenia were considered independently ($N_m = 0.23$, $F_{st} = 0.685$; $N_m = 0.19$, $F_{st} = 0.789$; respectively).

### Gene flow in G. seta populations

*G. seta* populations differ from *G. mirabilis* populations by being included in a single clade (100% bootstrap support). Despite this grouping, *G. seta* individuals showed a remarkably high level of diversity (43 individuals, 39 haplotypes, diversity = 0.99). Furthermore, gene flow between populations was found to be very reduced ($N_m = 2.7$, $F_{st} = 0.19$). For example, gene flow between the east and west coasts of the Gulf, a distance of approximately 100 km, was low ($N_m = 1.80$, $F_{st} = 0.217$). On an even smaller scale, the populations at Bahia Cholla and Estero La Pinta, which are separated by only 35 km, showed relatively low levels of gene flow ($N_m = 4.83$, $F_{st} = 0.094$). This is especially striking when compared with the very high levels of gene flow between *G. mirabilis* from the same locations, Estero La Cholla versus Estero La Pinta, $N_m = 75.27$, $F_{st} = 0.007$(*Gm*10 ELP and *Gm*11 ELC being excluded).

### G. mirabilis and G. seta phylogenetic relationships

Phylogenetic reconstructions based on the cytochrome *b* region separated *G. mirabilis* and *G. seta* into two distinct clades according to their respective species nomenclature (Fig. 2). This separation was strongly supported by bootstrap analysis (100% of the bootstrap replicates). At the nuclear DNA level, 2 (out of 140 aligned base pairs) fixed differences were found between *G. mirabilis* and G. seta. The resulting phylogenetic relationships were therefore concordant with the mitochondrial tree obtained with cytochrome *b* sequences.

### Discussion

#### Phylogeographic barriers and G. mirabilis populations

Concordant phylogeographic patterns between unrelated species provide strong evidence for the influence of vicariant events on population structure (Bermingham and Avise 1986; Avise 1992, 1994; Bermingham et al.

▶

**Fig. 2** Phylogenetic relationships of individuals of *G. mirabilis* and *G. seta* using the neighbor-joining method. Numbers above the major nodes indicate bootstrap support using the neighbor-joining method with equal weights for transitions and transversions (*first figure*), and when transversions are weighted three times as much as transitions (*second figure*). Numbers below the major nodes correspond to bootstrap support using the maximum parsimony method with equal weights and 3:1 weights for transversions and transitions. Only values above 50% are shown. In all cases, 100 bootstrap replicates were used. *Coryphopterus nicholsi* and *Quietula guaymasae* were used as out-groups
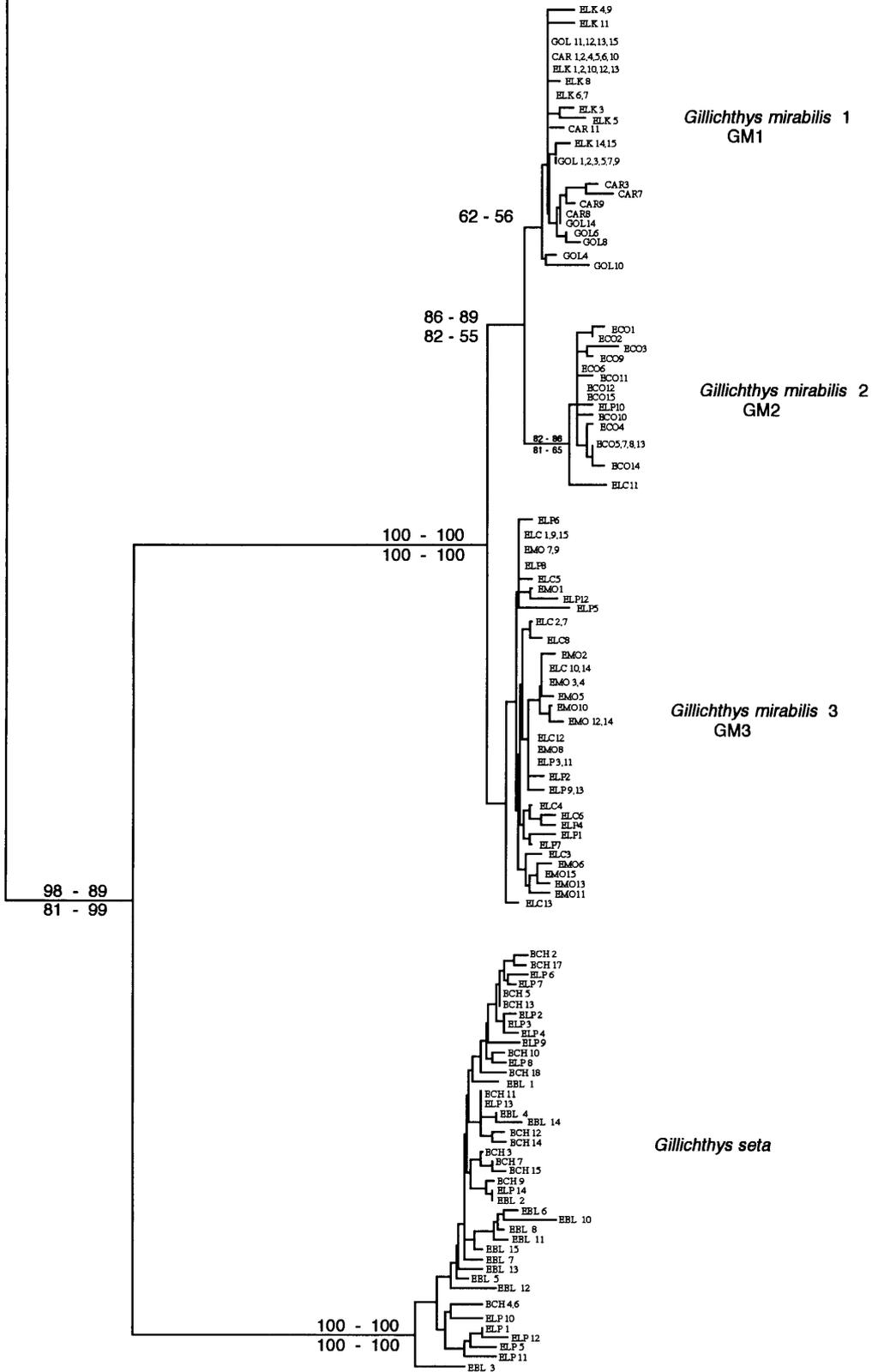
C. nicholsi

Q. guaymasae

ELK 4,9
ELK 11
GOL 11,12,13,15
CAR 1,2,4,5,6,10
ELK 1,2,10,12,13
ELK 8
ELK 6,7
ELK 3
ELK 5
CAR 11
ELK 14,15
GOL 1,2,3,5,7,9
CAR3
CAR7
CAR9
CAR8
GOL14
GOL6
GOL8
GOL4
GOL10

*Gillichthys mirabilis* 1
GM1

62 - 56

86 - 89
82 - 55

ECO1
ECO2
ECO3
ECO9
ECO6
ECO11
ECO12
ECO15
ELP10
ECO10
ECO4
ECO5,7,8,13
ECO14
ELC11

*Gillichthys mirabilis* 2
GM2

82 - 86
81 - 65

100 - 100
100 - 100

ELP6
ELC 1,9,15
EMO 7,9
ELP8
ELC5
EMO1
ELP12
ELP5
ELC 2,7
ELC8
EMO2
ELC 10,14
EMO 3,4
EMO5
EMO10
EMO 12,14
ELC12
EMO8
ELP3,11
ELP2
ELP9,13
ELC4
ELC6
ELP4
ELP1
ELP7
ELC3
EMO6
EMO15
EMO13
EMO11
ELC13

*Gillichthys mirabilis* 3
GM3

98 - 89
81 - 99

BCH 2
BCH 17
ELP 6
ELP 7
BCH 5
BCH 13
ELP2
ELP3
ELP4
ELP9
BCH 10
ELP 8
BCH 18
EBL 1
BCH 11
ELP 13
EBL 4
EBL 14
BCH 12
BCH 14
BCH 3
BCH 7
BCH 15
BCH 9
ELP 14
EBL 2
EBL 6
EBL 10
EBL 8
EBL 11
EBL 15
EBL 7
EBL 13
EBL 5
EBL 12
BCH 4,6
ELP 10
ELP 1
ELP 12
ELP 5
ELP 11
EBL 3

*Gillichthys seta*

100 - 100
100 - 100

**Table 2** Gene flow among *Gillichthys* populations. Pairwise comparisons of geographic regions are represented by values of $F_{st}$ (below diagonal) and $N_m$ (above diagonal). Region labels are described in Fig. 1. The first three rows and columns correspond to *G. seta* populations, all others to *G. mirabilis* populations. Values were calculated using DNAsp (Rozas and Rozas 1997); all values were found to be statistically significant using Arlequin (Schneider et al. 1997)

|      | BCH   | ELP   | EBL   | ELK   | GOL   | CAR   | ECO   | ELC   | EMO   | ELP   |
|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| BCH  | –     | 4.819 | 1.826 | –     | –     | –     | –     | –     | –     | –     |
| ELP  | 0.094 | –     | 1.430 | –     | –     | –     | –     | –     | –     | –     |
| EBL  | 0.215 | 0.259 | –     | –     | –     | –     | –     | –     | –     | –     |
| ELK  | –     | –     | –     | –     | 3.288 | 4.823 | 0.192 | 0.206 | 0.163 | 0.259 |
| GOL  | –     | –     | –     | 0.132 | –     | 2.166 | 0.169 | 0.182 | 0.153 | 0.231 |
| CAR  | –     | –     | –     | 0.094 | 0.187 | –     | 0.186 | 0.212 | 0.167 | 0.273 |
| ECO  | –     | –     | –     | 0.722 | 0.747 | 0.727 | –     | 0.150 | 0.116 | 0.186 |
| ELC  | –     | –     | –     | 0.708 | 0.733 | 0.702 | 0.769 | –     | 4.691 | 26.45 |
| EMO  | –     | –     | –     | 0.754 | 0.766 | 0.749 | 0.811 | 0.096 | –     | 3.984 |
| ELP  | –     | –     | –     | 0.659 | 0.684 | 0.646 | 0.729 | 0.018 | 0.111 | –     |

1992). In the case of the Pacific coast of the Baja California peninsula, a phylogeographic break at Punta Eugenia, which has traditionally been considered a biogeographic boundary (Briggs 1974), has been described for several species of fishes. This genetic break was found in the opaleye, *Girella nigricans* (Terry et al. 2000), the black surfperch, *Embiotoca jacksoni* (Bernardi 2000), and the California killifish, *Fundulus parvipinnis* (Bernardi and Talley 2000). *G. mirabilis* populations show restricted gene flow and significant sequence divergence between northern and southern populations, indicating that Punta Eugenia may also play a role as a phylogeographic barrier in this species. Further sampling around this region will determine if this is the case.

Restricted gene flow across Cabo San Lucas was observed for disjunct populations of *G. mirabilis*. A phylogeographic break at Cabo San Lucas was also found between disjunct populations of mussel blenny, *Hypsoblennius jenkinsi* (Present 1987), and opaleye, *G. nigricans* (Terry et al. 2000). The separation of the Gulf and Pacific coast disjunct populations is thought to have occurred by one of two scenarios. Brusca (1973) proposed that post-glacial warming of waters around the tip of the Baja California peninsula restricted temperate fish to the colder waters found in the northern part of the Gulf. Alternating periods of invasions and separations in the Sea of Cortez may have followed episodes of glacial advances and retreats by allowing fish to round the Cabo San Lucas region when water temperatures were cooler. If this were the case, habitat isolation between populations would have been complete approximately 10,000 years ago when the sea temperatures stabilized to their current conditions. Invading episodes, however, could have occurred during previous glacial episodes during the last 1 million years.

The geological history of the Sea of Cortez is complex. The uppermost region of the Gulf was formed as early as the Miocene, approximately 25 million years ago. The Baja California peninsula was then restricted to a small area in the north, and the southern region of the peninsula was composed of several small and large islands, between which several natural seaways connecting the Pacific Ocean to the Gulf of California may

have existed. This situation lasted until approximately 1 million years ago (Durham and Allison 1960) when the northern peninsula and the southern islands were joined and uplifted to create the present-day Baja California peninsula. The seaways that were once present in the central part of the Baja California peninsula were proposed to be responsible for the genetic differentiation observed between populations of lizards currently found north and south of such presumed seaways (Upton and Murphy 1997). These water channels probably allowed temperate fish to move freely in and out of the area that is now the Gulf of California without having to pass through the warmer waters of the Cape region.

The high degree of sequence divergence observed in cytochrome *b* between the Sea of Cortez and Pacific populations of *G. mirabilis* (1.9–2.3%, Table 1) tends to favor the mid-peninsular seaways scenario but cannot rule out the glacial period hypothesis. Indeed, if we use the generally accepted molecular clock for fish cytochrome b sequences (Meyer 1993; Bermingham et al. 1997; 1%/My to 2.5%/My), the divergence time between Sea of Cortez and Pacific coast fishes can be estimated at between 0.76 and 2.3 million years ago. If we assume that individuals dispersed through a southern route during water-cooling events, this would have happened during the early glaciations, and with little gene flow during the successive glaciating episodes. In contrast, the mid-peninsular seaway scenario allows for dispersal between Pacific Ocean and Sea of Cortez until 1 million years ago, when this dispersion route was definitively stopped. Thus, although our data are compatible with both southern and mid-peninsular seaways routes of dispersal, we think that the latter is more likely. If this were the case, our data would suggest that *G. mirabilis* populations in the Sea of Cortez diverged in allopatry, once the seaways across the Baja California peninsula were closed. Although this conclusion derives from the general results we have described, it is important to note that the two *G. mirabilis* individuals from the Gulf that cluster with Pacific individuals probably correspond to more recent events. These haplotypes may be the result of human transport, as *Gillichthys* is a commonly used bait fish. Alternatively, recent dispersal,

possibly during the more recent glacial times, is not excluded.

### Evolutionary origin of *G. seta*

Our results confirmed and expanded previous work obtained on another nuclear locus, the Lactate Dehydrogenase-A (LDH-A) (Fields and Somero 1997). Lactate dehydrogenase A cDNA sequences obtained from Pacific and Gulf individuals of *G. mirabilis* were identical. In contrast, *G. mirabilis* and *G. seta* individuals exhibited a four-nucleotide difference (all synonymous substitutions). Because only one individual of each *G. mirabilis* population and one *G. seta* individual were sequenced, however, it is not possible to know if LDH-A differences are fixed.

In our case, both mitochondrial and nuclear markers show a high degree of genetic separation between *G. seta* and *G. mirabilis*. The cytochrome *b* divergence (11.6%) is comparable to those found at much higher taxonomic levels (Johns and Avise 1998). It exceeds most other reported values between taxa of marine fish within the same genus and is matched only by the degree found among species of *Chaetodon* in the Pacific Ocean (McMillan and Palumbi 1995).

Using the previously mentioned mitochondrial substitution rates, we estimated the divergence time between *G. mirabilis* and *G. seta* as 4.6–11.6 million years ago. Thus, *G. seta* may have derived from *G. mirabilis* before the complete formation of the Gulf of California.

The upper region of the Gulf of California has been dated as far back as the Miocene, approximately 25 million years ago (Durham and Allison 1960). *G. seta* may have evolved from *G. mirabilis* by invading the ecologically separate habitat presented by the newly formed intertidal region of the upper Gulf. Gene flow may have continued between populations of *G. mirabilis* after the divergence with *G. seta* until the closure of the seaways connecting the Sea of Cortez to the Pacific created disjunct populations. Many of the Sea of Cortez endemic species are shallow-water or warm-water inhabitants (Walker 1960). Our data suggest that at least some of these endemic species could have been established before the Pleistocene when the final formation of the Gulf occurred.

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