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Geographical variation in the cephalic lateral line canals of *Eucyclogobius newberryi* (Teleostei, Gobiidae) and its comparison with molecular phylogeography

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Abstract. The tidewater goby, *Eucyclogobius newberryi*, is a small gobiid fish with low dispersal ability that inhabits coastal lagoons and estuaries in California. Analysis of mitochondrial DNA sequence data previously identified six phylogeographic groups within *E. newberryi*, distributed among three biogeographic areas. Here we describe variation in the cephalic canal system of *E. newberryi* and investigate whether this variation is distributed geographically in a manner consistent with the previously reported molecular and biogeographic hypotheses. The northernmost populations, around Cape Mendocino, have only fully developed canals. Populations between Point Arena and the Monterey peninsula mostly have complete supraorbital canals. Populations from south of the Big Sur coastline to the Los Angeles region (LAR) show high variability in canal structure. The southernmost populations have only distinctly reduced canals. Thus, in general, morphological data match closely the molecular and biogeographic hypothesis, distinguishing at least four of the same phylogroups in the same three biogeographic areas, with similar evolutionary implications. Reduction of diversity in the north is consistent with post-glacial expansion from central California. Abrupt reduction of diversity in the south is consistent with a more ancient stronger barrier to gene flow in the vicinity of Los Angeles and founder-effects or bottlenecks in the southernmost populations. By examining specimens from populations collected between 1879 and 1955 that subsequently were extirpated, we have refined the molecularly derived phylogeographic hypothesis and show that the break at LAR occurs in close proximity to genetic discontinuities in other species studied in detail.

Key words: biogeography, California, lateral line cephalic canals, *Eucyclogobius newberryi*

Introduction

The tidewater goby, *Eucyclogobius newberryi* (Girard), has no explicit marine phase and inhabits brackish portions of estuaries, lagoons, marshes and creeks along the California coastline. Its range encompasses a number of phylogeographic boundaries of differing severity (Burton 1998, Bernardi 2000, Dawson et al. 2001, 2002). Mitochondrial DNA (mtDNA) analyses demonstrate that *E. newberryi* is separated into six phylogeographic groups among which the deepest divergence occurs in the Los Angeles region (LAR). This deep phylogenetic gap separates all southern populations from all northern populations and is dated to approximately 2–4 million years BP (Dawson et al. 2001). Despite this ancient divergence and indications of allozyme differences between northern and southern populations (Crabtree 1985), it is currently unknown whether these phylogroups differ significantly phenotypically (for example Love & Larson 1978) – a matter that has evolutionary, taxonomic, and conservation implications.

The tidewater goby should be well suited to analysis of geographic patterns in phenotypic (morphological) variation. It is a small fish, up to ca. 55 mm total length, with reduced squamation (Swift et al. 1989). In gobiid fishes small body size is considered to be specialized (Miller 1996) and is often accompanied by the loss or reduction of the cephalic lateral line canals (Miller 1987, Ahnelt & Bohacek 2004), a paedomorphic feature with head canals absent or only partly developed during ontogeny (Takagi 1988, Iwata et al. 2001, Larson 2001). The sensory canals of gobiid fishes are widely used as a taxonomic tool (for example Akihito 1986, Takagi 1988, Pezold 1993, Larson 2001, Ahnelt & Scattolin 2003, but see Barlow 1961). Preliminary analyses of eastern Pacific bay gobies (Göschl 2002, Ahnelt & Göschl 2003) indicated that the cephalic canals of the lateral line system of *E. newberryi* were unusually variable and generally reduced to the supraorbital canals; postorbital and preopercular canals were absent. Further reductions up to the complete loss of the head canals were observed. Variation in this morphological feature of the tidewater goby therefore offered a rare opportunity to investigate the relationship between patterns of geographic variation in morphological and molecular characters among populations of a single species.

Materials and Methods

Materials

Specimens of *Eucyclogobius newberryi* from 26 collections between Lake Earl, Del Norte County and Agua Hedionda Creek, San Diego County, California (USA) were examined (Fig. 1, Table 1A). The collections were loaned from California Academy of Sciences, San Francisco (CAS, SU), Los Angeles County Museum, Los Angeles (LACM), Naturhistorisches Museum Wien, Vienna (NMW), Scripps Institution of Oceanography, La Jolla (SIO), and University of Michigan Museum of Zoology, Ann Arbor (UMMZ). Full details are given in the Appendix.

In gobiid fishes, the formation of the cephalic lateral line canals is generally completed during juvenile stages (Takagi 1988). As *E. newberryi* settle at a size of 16–18 mm SL (Swift et al. 1989), we considered only specimens ≥ 24 mm SL (n = 546) to avoid ontogenetic variation. Excluding the 52 smallest fish (= 24 mm SL) decreased the number of canal types in a population in only one case—the fully developed canal types 1 and 2 were no longer recorded at Malibu—due to their low initial frequency, indicating that the canal systems of all specimens in the complete dataset (n = 546) were fully developed. Additional smaller fishes were studied to investigate the development of the cephalic lateral line canals.

The specimens were assigned to six phylogeographic regions within three biogeographic areas following the analyses of Dawson et al. (2001) and Dawson (2001) and considering additional information from Burton (1998) and Bernardi (2000) regarding the most likely affiliation of four previously unstudied populations in LAR (Table 1). *Northern area* (north of the Monterey peninsula). Region I (Cape): populations 1, 2, 3, 4, 5. Region II (San Francisco): populations 6, 7, 8, 9 10, 11, 12. These populations are geographically separated from the central populations by approximately 150 km of rocky

Fig. 1. Map of California (USA) showing localities for material examined for *Eucyclogobius newberryi* from six phylogeographic regions. Correlation of regions with phylogeographic groups as defined by Dawson et al. (2001) with additional reference to Burton (1998) and Bernardi (2000): Region I, Cape group; Region II, San Francisco group; Region III, Estero Bay group; Region IV, Point Conception group; Region V, Ventura group; Region VI, San Diego group. Major breaks are indicated by large arrows, and divide the range of the tidewater goby in three principal areas, north of Big Sur coast, between Big Sur coast and LAR and, south of LAR. Less severe breaks are indicated by small arrows. Major cities and landmarks are indicated. Region I: 1, Lake Earl; 2, freshwater lagoon near Highway 101; 3, Stone Lagoon; 4, Big Lagoon; 5, Ten Mile River. Region II: 6, Estero Americano Bay; 7, San Francisco (Cliff House); 8, San Gregorio Creek & Pescadero Creek; 9, Waddell Creek; 10, Scott Creek & Natural Brideges State Park; 11, Santa Cruz; 12, Salinas River. Region III: 13, Little Pico Creek; 14, San Simeon Creek. Region IV: 15, San Luis Obispo & San Luis Obispo Creek; 16, Santa Maria River; 17, Santa Ynez River; 18, Carpinteria Creek. Region V: 19, Calleguas Creek; 20, Malibu Creek; 21, Santa Monica (artesian well). Region VI: 22, Aliso Creek; 23, San Mateo Creek; 24, San Onofre Creek; 25, bird sanctuary near Ocenside; 26, Agua Hedionda Creek.

coastline between about Monterey and San Simeon. In the following we refer to this area as the Big Sur coastline. *Central area* (between the Big Sur coastline and LAR). Region III (Estero Bay): populations 13, 14. Region IV (Point Conception): populations 15, 16, 17, 18. Region V (Ventura): populations 19, 20, 21. *Southern area* (south of LAR). Region VI (San Diego): populations 22, 23, 24, 25, 26. Eight of the 26 populations studied are extirpated and unavailable for molecular analyses: Cliff House (7), Waddell Creek (9), Salinas River (12), Calleguas (19), Malibu (20), Santa Monica (21), Aliso Creek (22), and Agua Hedionda (26). The four extirpated populations spanning the LAR (19, 20, 21, 22) present a novel opportunity to locate more precisely the position of the major evolutionary discontinuity in LAR.

Nomenclature of the lateral line system

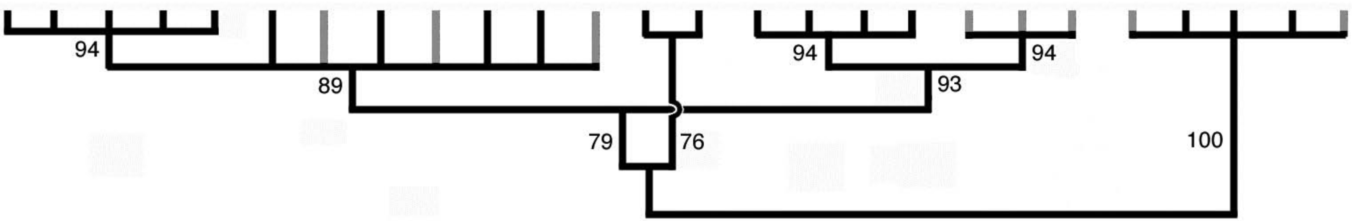
Eucyclogobius newberryi displays high variability in the development of the lateral line system of the supraorbital region. The cephalic canals of the tidewater goby are generally reduced to the supraorbital canals. The nomenclature of the free neuromasts of the lateral line

Table 1. A: The geographic distribution of head canal types in *Eucyclogobius newberryi* by population, arranged from North to South. Collections: Region I (Cape): 1, Lake Earl (G); 2, Freshwater Lagoon; 3, Stone Lagoon (G); 4, Big Lagoon; 5, Ten Mile River (G). Region II (San Francisco): 6, Estero Americano (G); 7, Cliff House, SF † (G – Rodeo Lagoon); 8, San Gregorio & Pescadero Creek (G– San Gregorio); 9, Waddell Creek †; 10, Natural Bridges State Park (G – Moore Creek) & Scott Creek; 11, Santa Cruz; 12, Salinas River † (G – Bennett Slough). Region III (Estero Bay): 13, Little Pico Creek (G); 14, San Simeon Creek (G). Region IV (Point Conception): 15, San Luis Obispo Creek; (G); 16, Santa Maria River (G – Pismo Creek); 17, Santa Ynez River; 18, Carpinteria Creek (G). Region V (Ventura): 19, Calleguas Creek †; 20, Malibu Creek †; 21, Santa Monica (artesian well) †. Region VI (San Diego): 22, Aliso Creek †; 23, San Mateo Creek; 24, San Onofre Creek; 25, near Oceanside (G – Santa Margarita River); 26, Agua Hedionda Creek †. G – indicates genetic sample used in study. † – indicates natural populations that have suffered long term extirpation. **B:** Hypothesis of regional relationships among populations based on the maximum likelihood analyses of Dawson et al. (2001; numbers by branches are bootstrap values) and on Burton (1998) and Bernardi (2000). Grey branches represent populations not analysed in Dawson et al. (2001). **C:** The geographic distribution of head canal types across the six regions indicated by the prior molecular analyses (Fig. 1). Cape, region I; SF, region II; EB, region III; Pt.C, region IV; V, region V; SD, region VI. **D:** The distribution of head canal types across the three principal biogeographic areas (northern, central and southern area) in California (Dawson 2001).

A

Populations		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
n (total 546)		16	1	36	3	7	3	16	122	51	18	4	14	13	15	12	45	4	50	2	38	4	50	7	11	1	3
	1	100	100	100	100	100	67	75	98	94	94	100	93	38	7	58	56	100	64	50	3	50	.	.	.	.	.
T	2	.	.	.	.	.	33	.	2	4	6	.	.	23	27	8	9	.	2	.	5	.	.	.	.	.	.
	3	.	.	.	.	.		25	1	2	.	.	7	.	.	.	2	.	10	50	5	50	.	.	.	.	.
Y	4	.	.	.	.	.	.	.	.	.	.	.	.	15	7	8	22	.	.	.	8	.	.	.	.	.	.
	5	.	.	.	.	.	.	.	.	.	.	.	.	15	7	17	7	.	.	.	29	.	.	.	.	.	.
P	6	.	.	.	.	.	.	.	.	.	.	.	.	8	40	8	4	.	24	.	50	.	86	100	27	.	33
	7	.	.	.	.	.	.	.	.	.	.	.	.	.	13	.	.	.	.	.	.	.	14	.	64	100	33
E	8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	33
	9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	9	.	.

B



system follows Sanzo (1911), that for the pores of the cephalic canals follows Akihito (1971). Neuromasts in the course of the reduced supraorbital canal and their numbers mentioned in text and figures refer to primary neuromasts (canal neuromasts) not enclosed by canal formation. We do not refer to secondary neuromasts, which additionally may occur in the interorbital region of *E. newberryi* (our unpublished data).

Statistic analysis

The frequencies of canal types in each population, region, and area were calculated. Morphological diversity within regions was calculated as the probability that two randomly chosen fish from within a region would be different (i.e. using the equation traditionally used for calculating gene diversity [Nei 1987, cited by Schneider et al. 2000]) and plotted against genetic diversity in *E. newberryi* (Dawson et al. 2001). Heterogenous variances, non-normally distributed data, and unequal sample sizes and numbers of samples at all geographic scales prevented traditional parametric and non-parametric comparative statistical analyses of the frequency data. Instead, canal types were coded as absent (1) or present (2) in each population and used in categorical principal components analysis (CATPCA) and hierarchical cluster analysis. Although all populations were used in CATPCA, only populations with n ≥ 10 were included in hierarchical cluster analyses, using squared-Euclidean distance and centroid clustering, to reduce any potential bias due to small sample sizes. Mean morphological dissimilarity (squared-Euclidean distance) within and between regions was plotted against genetic divergence (Dawson et al. 2001).

Results

Development and variations of the cephalic lateral line canals

The head canals of the lateral line system of *E. newberryi* are reduced to the supraorbital canals of the anterior oculoscapular canal. The postorbital sections of the anterior oculoscapular canals are not developed. The posterior oculoscapular and the preopercular canals are absent. The supraorbital canals are paired, not fused to each other and not connected by a transversal tubule (independent canal

C

Region		Cape	SF	EB	Pt.C	V	SD
Populations		1-5	6-12	13-14	15-18	19-21	22-26
n		63	228	28	111	44	72
	1	100	94	21	61	9	.
T	2	.	3	25	5	5	.
	3	.	3	.	5	11	.
Y	4	.	.	11	10	7	.
	5	.	.	11	5	25	.
P	6	.	.	25	14	43	75
	7	.	.	7	.	.	22
E	8	.	.	.	.	.	1
	9	.	.	.	.	.	1

D

Area		N	C	S
Regions		C-SF	EB-V	SD
n		291	183	72
	1	96	43	.
T	2	2	8	.
	3	2	6	.
Y	4	.	9	.
	5	.	10	.
P	6	.	22	75
	7	.	1	22
E	8	.	.	1
	9	.	.	1

type of Takagi 1988) (Fig. 2). These canals may be completely developed (each with pores B, D and F present), or their interorbital sections may be reduced to different extents. Rarely, these canals may be completely absent.

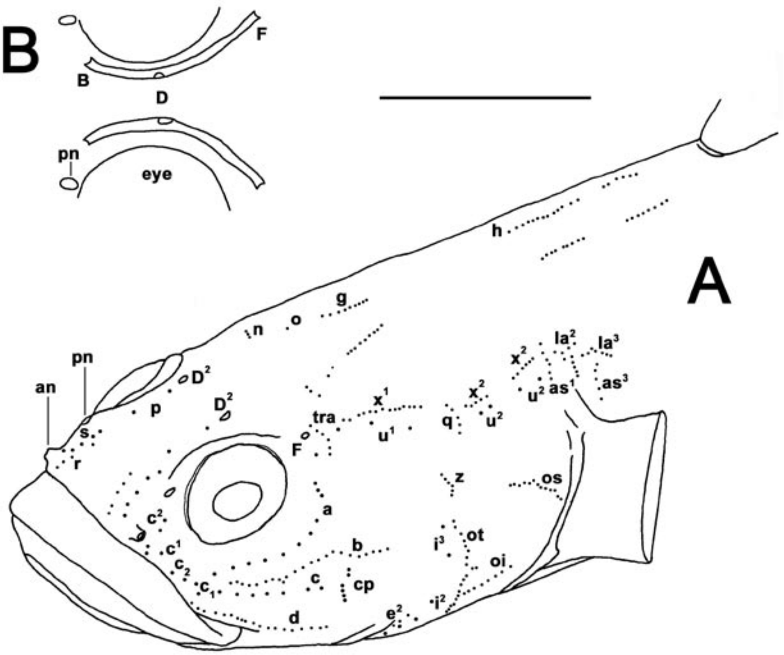


Fig. 2. A: Head lateral line system of *Eucyclogobius newberryi*, CAS 31769, male, 26.6 mm SL, Malibu Creek, in dorso-lateral view. Posterior sections (subcutaneous) of the head canals between pores D^2 and F present, anterior sections absent, representing type 6 (see text, Fig. 3); canal neuromasts (**p**) in the course of the absent anterior sections visible. Labelling of the free neuromasts follows Sanzo (1911). Scale bar = 3 mm. **B:** Interorbital region of *E. newberryi* in dorsal view: SIO 72–87, female, 41.2 mm SL, San Simeon Creek. Head canals completely developed, each with pores B, D and F, representing type 1 (see text). Note different width and irregular course of canals; pores differ in size (B, D) and position (D). The subcutaneous head canals were made visible by blowing air into them. Scale bar = 1 mm. an, pn, anterior and posterior nostrils.

During the ontogeny of the tidewater goby the supraorbital canals first form along the posterior edge of the orbits, followed by the interorbital sections. These parts then fuse and, where they meet, a pore (D) is left medially. Reductions occur in the anterior (interorbital) section of the supraorbital canal. This canal section is always reduced first. No specimen has been found with the interorbital section present (between pores B and D) and the posterior section lacking (between pores D and F).

Supraorbital canals occurred in all specimens examined and were classified as belonging to nine types ranging from complete (type 1) through short rests (type 6) to absent (type 9) (Fig. 3). Completely developed canals extend from the rear of the eye across the interorbital region just posterior to the posterior nostril (Fig. 2B). In the most reduced canal types (types 6, 7) it extends from the rear of the eye to the interorbital region but does not pass anterior of the midline between the eyes (Fig. 2A). The differences in the development of the head canals as shown in types 2, 3 and 5 (Fig. 3) may occur on the left or on the right side of the head. No other types of canal structure, of which numerous intermediates are conceivable, were observed.

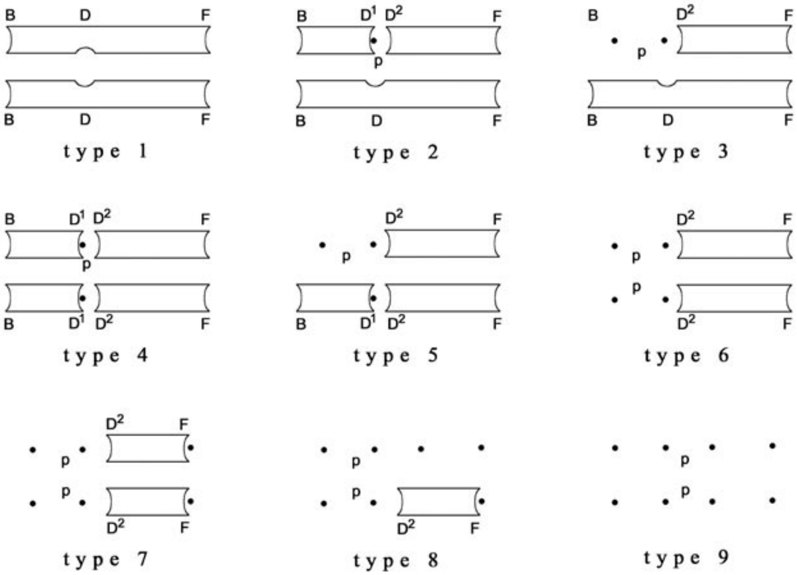


Fig. 3. The types of head canals found in *Eucyclogobius newberryi*, from completely developed (type 1), to head canals lacking (type 9). B, D, anterior and posterior interorbital pores; F, postorbital pore; **p**, canal neuromasts not enclosed during canal formation.

Population subdivision

Variation in the cephalic canal system is clearly not distributed evenly across the range of *E. newberryi,* as particular morphotypes are notably absent from northern and southern extremes. Variation appears to follow a latitudinal gradient. Northernmost populations have only type 1 (complete) canals, while the southernmost populations have only greatly reduced canals (types 6–9). Type 6 and many intermediates between type 6 and type 1 are present in all more central populations (Table 1). Notably, the gradient is not smooth. While there is an intergradation of central and northernmost forms, in which populations in the San Francisco area have a limited subset of canal morphologies, the transition from central to southern morphotypes in the vicinity of LAR is abrupt.

CATPCA indicates greater similarity among the populations in the Cape, San Francisco, Estero Bay, Point Conception, and Ventura regions than with any of the populations in the San Diego clade (Fig. 4). Hierarchical cluster analysis emphasises the depth of this north-south divide at LAR, and indicates additional differences between all central and northern regions. Using evolutionary terminology, the San Diego clade is reciprocally monophyletic with respect to all more northerly populations, the San Francisco group consists of a diverse array of populations and is paraphyletic with respect to the monophyletic Cape clade, Estero Bay populations are paraphyletic with respect to Point Conception populations which, in turn, are paraphyletic with respect to Ventura populations and show some resemblance to some San Francisco populations (Fig. 4).

The previously unstudied extirpated populations in LAR show a number of interesting features. The Malibu Creek population, which is distant from other Ventura populations nevertheless is also highly variable in reduction types and clearly shows an affinity with all populations north of LAR (Table 1, Fig. 4). The Santa Monica population (n = 4), collected

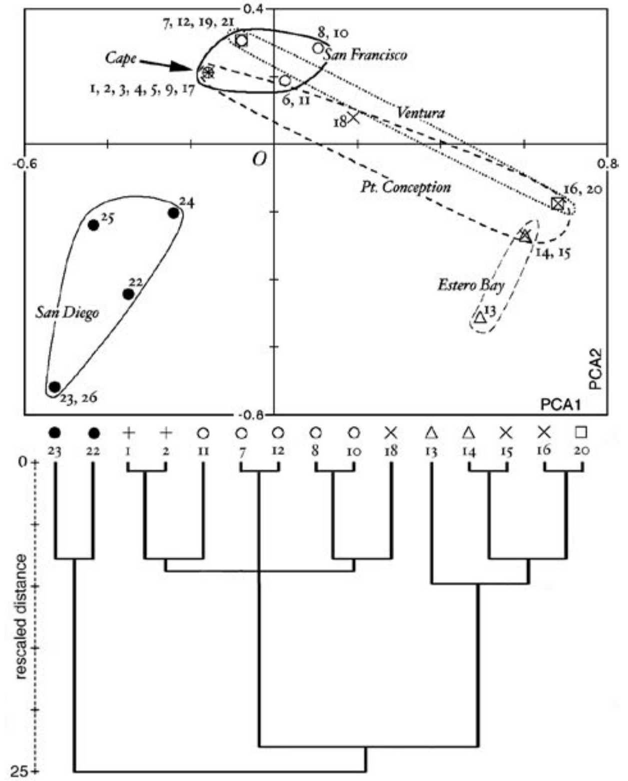


Fig. 4. Categorical Principal Components Analysis of all 26 populations studied (upper) and hierarchical cluster analysis, using squared-Euclidean distance and centroid linkage, of all populations with sample sizes ≥ 10 (lower).

from an artesian well at a depth of about 45 m (Steindachner 1879), also shows the fully developed supraorbital canals of type 1 (n = 2) and slightly reduced type 3 (n = 2) characteristic of central California populations. Given the low sample size it is impossible to determine if this population from the artesian well is distinct from other populations in the same region (Ventura). However, the Santa Monica fish are quite clearly distinct from the Aliso Creek population which is characterized, like other southern populations, by supraorbital canals reduced to their posterior sections (type 6) and occasionally further (types 7– 9).

There was no latitudinal trend in morphological diversity (Fig. 5). Considering all regions, morphological diversity also was not correlated with either haplotype diversity (r = -0.01, p = 0.98) or nucleotide diversity (r = -0.40, p = 0.44). However, data fell into two groups, one showing low morphological diversity (Cape and San Francisco regions) and the other higher morphological diversity (all other regions) and, when only regions III–VI were considered, morphological diversity was significantly correlated with nucleotide diversity (r = 0.99, p = 0.013). The relationship between morphological and haplotype diversity remained marginally non-significant (r = 0.92, p = 0.08).

Considering all pairwise comparisons between all regions, morphological dissimilarity was highly correlated with molecular dissimilarity (r = 0.762, p < 0.001; Fig. 6). Considering only regions I–V, morphological and molecular dissimilarity were also correlated between

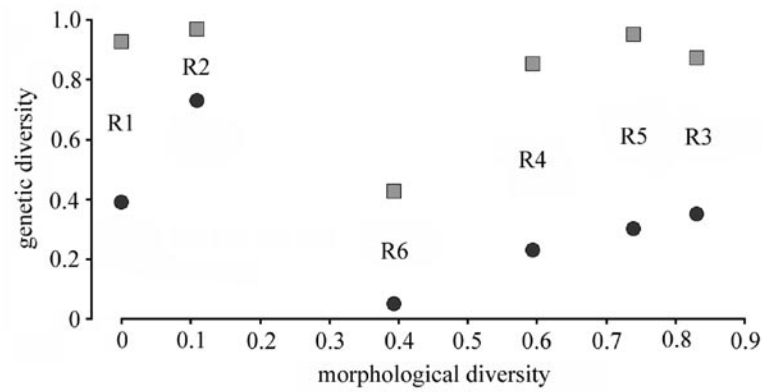


Fig. 5. Morphological and genetic diversity, calculated as the probability that two randomly chosen fish from within a region are different, in six regional groups of *Eucyclogobius newberryi* (Fig. 1). R1, Cape clade. R2, San Francisco group. R3, Estero Bay group. R4, Point Conception clade. R5, Ventura clade. R6, San Diego clade. Light squares, haplotype diversity; black circles, nucleotide diversity (from Dawson et al. 2001).

regions (r = 0.83, p = 0.003). However, morphological and molecular dissimilarity were not correlated within regions (r = 0.13, p = 0.81) or when comparing the San Diego region versus all more northerly regions (r = -0.52, p = 0.37).

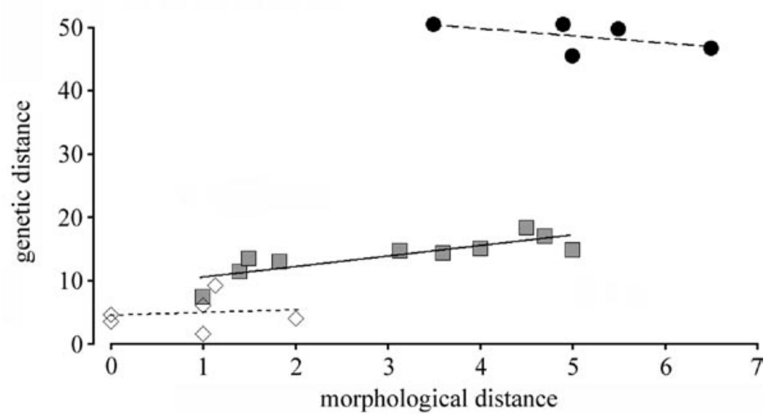


Fig. 6. Mean morphological (squared Euclidean) and genetic (pairwise sequence difference) distances within (open diamonds, y = 0.44x + 4.45) and among (black circles and shaded squares) regional groups of *Eucyclogobius newberryi* (Fig. 1). Black circles indicate comparisons between region 6 and regions 1-5 (y = -1.13x + 54.25). Shaded squares indicated all possible pairwise comparisons among regions 1–5 (y = 1.65x + 8.88). Genetic distances from Dawson et al. (2001).

Discussion

Eucyclogobius newberryi is related to a group of gobiid fishes (Gobionellinae) (Ginsburg 1945, Birdsong et al. 1988) distributed along the California Coast (excluding *Ctenogobius*, *Lethops* and *Typhlogobius*). It is the only species within this group, known as California bay gobies, that displays high variability in the development of the cephalic canals of the lateral line system. Like most other species of California bay gobies (excluding *Gillichthys*), only the supraorbital canals of the anterior oculoscapular canal are developed (our unpublished data). In contrast to the other species of bay gobies, however, the canals on the left and the right side of the head are always completely separated from each other and never connected by a transverse tubule (with the exception of *Ilypnus luculentus* (Ginsburg) from the Gulf of California). Additionally the head canals of the tidewater goby are variously reduced and may be absent (Figs 2A, 3).

A latitudinal gradient in the development of the supraorbital canals of the tidewater goby is obvious. While the northern populations have predominantly complete canals, the southernmost populations have only greatly reduced canals (Fig. 3, Table 1). Populations in central California show both extremes and a high variability of intermediate canal types. Transitions between populations showing these different characteristics occur in the vicinity of the Big Sur coastline and LAR, of which the latter is clearly the more significant, and are therefore in close agreement with biogeographic patterns posited by Dawson (2001). Further subdivision of the northern populations is evident in the presence of type 2 and type 3 fishes in the San Francisco group but their absence from the Cape clade, which is in close agreement with molecular data that indicate a monophyletic clade of *E. newberryi* north of Point Arena (Dawson et al. 2001). Morphological data therefore clearly distinguish at least four of six phylogroups and all three biogeographic areas posited by prior analyses. The morphological data did not indicate so clearly divisions between Estero Bay (region III), Point Conception (region IV), and Ventura (region V) populations in the central biogeographic area, although such divisions may exist on the basis of frequency differences (Table 1, Fig. 4).

Of particular interest is the precise location, if one exists, of the strong phylogeographic boundary which molecular analyses placed somewhere within the approximately 215 km wide LAR (*sensu* Dawson et al. 2001). Morphological analyses of historical collections made before populations in LAR were extirpated allowed us to address this issue. Populations from Calleguas Creek, Malibu, and Santa Monica showed morphotypes (types 1–6) with clear affinities to others in the central California area. In contrast, fish from Aliso Creek were clearly more similar to fishes from San Diego populations to the south (types 6 and 7). Thus, the LAR break is now indicated to occur somewhere between Santa Monica and Aliso Creek, a distance of approximately 100 km, assuming the artesian well population was natural (the distance from Malibu to Aliso Creek is approximately 125 km). Notably, the deepest phylogeographic breaks in *Embiotoca jacksoni* Agassiz and *Tigriopus californicus* Baker, the other two species studied in detail across southern California, also occur within this section of coastline (Burton 1998, Bernardi 2000).

Based on mtDNA sequence analyses and patterns of climate change Dawson et al. (2001) suggested that the Estero Bay region (region III) represented the most likely ancestral range of central and northern *E. newberryi*, being basal to the San Francisco group and Cape clade to the north, and to the Point Conception and Ventura clades to the south. They concluded that there was probably a progressive, possibly repeated, expansion from the ancestral Estero Bay group northward into the San Francisco region, and then further expansion from the San Francisco group northward to form the Cape clade. Assuming morphological similarity is, at least in this case, a reasonable indicator of genetic similarity (Fig. 6) and therefore of phylogeny, canal structure indicates a very similar history of range expansion and colonization (Table 1, Fig. 4). Morphological data show progressively reduced diversity in canal structure in the San Francisco and Cape clades, relative to the Estero Bay group (Table 1), and support the suggestion of Dawson et al. (2001) of a northward expansion and successive founder events. The morphological data, particularly the hierarchical cluster analyses and measures of morphological diversity, presented here are also consistent with expansion, possibly repeated, from Estero Bay group into the Point Conception and Ventura clades. However, this case is perhaps not as clear as northerly expansion because, with six different reduction types (types 1–6), morphological diversity is still high in the Point Conception and Ventura regions, therefore necessitating multiple founder events. The morphological data are also consistent with an alternative scenario: a larger ancestral range of *E. newberryi* that includes the Point Conception and Ventura regions indicating possible glacial refugia in the Santa Barbara Channel and northern Southern California Bight. In contrast to these hypotheses of relatively recent divergences, the abrupt decrease in morphological and genetic diversity and existence of unique morphotypes south of LAR (i.e. in region six) is consistent with a substantial long-term barrier to gene flow and either founder effects at the time of establishment, or greater initial diversity followed by subsequent bottlenecks.

In addition to largely supporting the phylogeographic groups and evolutionary history of *E. newberryi* deduced from molecular analyses and placing the deep LAR break more precisely, the morphological data also raise two more points of interest not addressed previously. First, assuming the differences in cephalic canal system are at least partly hereditary, as they seem to be, then there must be important differences in allele frequencies between the nuclear genomes of southern and northern *E. newberryi* that were not indicated in preliminary analyses of Creatine Kinase intron 6 (Dawson et al. 2001; but see Mendonca et al. 2001). Second, the latitudinal pattern of morphological variation could be consistent with differences due to natural selection and local adaptation, which might be related to habitat variation and the rate or extent of development of the cephalic canal system and the sensory capabilities of different life-stages of *E. newberryi*. The effect might be mediated specifically by the link between canal formation and a niche shift, at 16–18 mm SL (Swift et al. 1989), from larvae inhabiting the water-column to juveniles, and subsequently adults, that are benthic.

As known from other small gobiids, completion of the life-cycle mostly in isolated habitats with stagnant waters may lead to reduction or loss of the cephalic canals (Ahnelt 1995, Ahnelt et al. 1995). With the loss of canals the sensitivity of the lateral line system changes. Thus, more developed canal systems, or earlier development of canal systems, may have advantages in more turbulent waters. More northerly populations of *E. newberryi* occur in generally wetter climes that result in larger habitats influenced more strongly by running water for longer periods of each year; the populations south of the Big Sur coastline, and particularly south of LAR, occur in generally dryer climes and estuaries that are smaller with lower water flow (although all, to our knowledge, are isolated for a portion of each year). Thus capabilities afforded by better developed cephalic canals would have greater benefits in more northerly populations in which fish may more frequently have to retain or recover their positions in estuaries. In a worst-case scenario, if washed into coastal waters, their return to an estuary is critical to their survival and reproduction. If this hypothesis is true, we would expect fish with the most developed canal system to be those that extended their range furthest north most rapidly, which is what we find – only type 1 fish in the northernmost populations. Fish with less developed canals might expand their ranges more slowly (for example from Estero Bay to San Francisco, but not further north at this time). Currently, however, the cline in variation from type 1 to type 9 canals with decreasing latitude, while not favoured simply by chance, cannot be attributed categorically to effects other than random sampling related to founder effects or bottlenecks.

The morphological analyses presented here reinforce prior indications that *E. newberryi* populations across the species' range possess unique attributes, possibly justifying recognition of some populations, most obviously those from the San Diego clade, as a distinct taxonomic entity, perhaps sub-species. The implication of functional benefits to some of this variation emphasizes the value of conserving *E. newberryi* populations throughout the range; adaptive features are given special consideration under the U. S. endangered species act. Moreover, the short distances over which morphological and molecular variation occur in *E. newberryi* have implications for other fishes, such as *Knipowitschia caucasica* (Kawrajsky) (Ahnelt et al. 1995), with similar habitat, life-history and morphological characteristics (e.g. reduced cephalic canals, reduced squamation, small body size) that indicate low dispersal. It seems likely that such a suite of characteristics may be a general indicator of geographic structure and estuarine species of particular evolutionary and conservation interest.

Appendix. Collections, from north to south, were comprised as follows (collection number, number of specimens, SL in mm, sampling site, date).

Region I (Cape): CAS 205698, 16 of 84 spec., 24.1–31.8, Del Norte Co., Lake Earl, 17 Oct. 1964. CAS 31765, 1 spec., 29.5, Humboldt Co., freshwater lagoon near Highway 101, 22 Aug. 1951. LACM 37552-1, 36 of 46 spec., 32.6–41.3, Humboldt Co., Stone Lagoon, 07 July 1977. CAS 13640, 3 of 9 spec., 24.0–30.2, Humboldt Co., Big Lagoon, 11. Sept. 1951. CAS 20138, 7 of 11 spec., 31.9–37.4, Mendocino Co., Ten Mile River, 25 June 1897.

Region II (San Francisco): SIO 64-912-59A, 3 of 4 spec., 30.6–39.0, Sonoma Co., Bodega Bay, Estero Americano Bay, 26 June 1945. NMW 31182, 5 of 6 spec., 25.5–35.2, San Francisco Co., Cliff House near San Francisco, acqu. 1874. NMW 31183, 3 of 7 spec., 24.6–26.6, otherwise same data. NMW 31184, 4 of 7 spec., 25.2–27.4, otherwise same data. NMW 31185, 3 of 4 spec., 28.5–37.9, otherwise same data. CAS 86120, 17 of 19 spec., 24.8–34.3, San Mateo Co., San Gregorio Creek, 26 July 1990. CAS 86281, 53 of 57 spec., 25.0–42.0, San Mateo Co., Pescadero Creek, 12 Sept. 1993. CAS 86119, 6 of 12 spec., 29.0–35.2, San Mateo Co., Pescadero Creek, 14 April 1990. CAS 86184, 46 of 49 spec., 24.1–43.4, San Mateo Co., Pescadero Creek, 29 Aug. 1992. CAS (SU) 654, 13 of 14 spec., 24.8–36.2, Santa Cruz Co., Waddell Creek, 13 April 1892. CAS 28670, 38 of 42 spec., Santa Cruz. Co., Waddell Lagoon, 02 Nov. 1934. CAS 86306, 1 of 2 spec., 31.2, Santa Cruz Co., Scott Creek, 02 Aug. 1990. CAS 86127, 1 spec. 37.9, Santa Cruz Co., Natural Bridges State Park, ? July 1990. CAS 86131, 16 spec., 24.5–36.3, Santa Cruz Co., Natural Bridges State Park, July 1990. NMW 33916, 4 spec., 39.1–40.5, Santa Cruz Co., Santa Cruz, 1880. CAS uncatal., 3 spec., 28.8-32.5, Monterey Co., Salinas River, 11 Aug. 1946. CAS 66857, 1 spec., 29.2, Monterey Co., Salinas River, 11 Aug. 1946. CAS 148290, 4 of 7 spec., 24.4–27.9, Monterey Co., Salinas River, 23 Aug. 1951. CAS 205691, 6 of 19 spec., 26.2–38.9, Monterey Co., Salinas River, 26 May 1927.

Region III (Estero Bay): CAS 82445, 13 of 15 spec., 24.3–33.1, San Luis Obispo Co., Little Pico Creek, 10 Dec. 1950. SIO 72-87, 5 of 13 spec., 32.1–41.5, San Luis Obispo Co., San Simeon Creek, 01 Dec. 1972. CAS 204782, 8 of 25 spec., 24.1–27.6, San Luis Obispo Co., San Simeon Creek, 22. Nov. 1953. CAS 115184, 2 spec., 28.5–28.8, San Luis Obispo Co., San Simeon Creek, 02 Sept. 1948.

Region IV (Point Conception): NMW 31089, 1 spec., 40.2, San Luis Obispo Co., San Luis Obispo, 1900. CAS 86307, 1 spec., 37.9, San Luis Obispo Co., San Luis Obispo Creek, 05 July 1995. CAS 100653, 10 of 11 spec., 24.2–37.4, San Luis Obispo Co., San Luis Obispo Creek, 05 Oct. 1893. CAS 40326, 1 spec., 37.2, San Luis Obispo Co., pond at Santa Maria River mouth, 03 May 1941. CAS 66438, 1 spec., 37.3, Santa Barbara Co., pond E of Santa Maria River, 03 May 1941. CAS 66439, 1 spec., 40.0, Santa Barbara Co., tributary of Santa Maria River, 21 May 1945. CAS 40325, 1 spec., Santa Barbara Co., tributary south side Santa Maria River, 03 May 1941. UMMZ 133770, 41 spec., 31.6–42.2, Santa Barbara Co., tributary of

Santa Maria River, 03 May 1941. CAS 20907, 3 of 4 spec., 28.6- 32.0, Santa Barbara Co., Santa Ynez River Lagoon, 24. Sept. 1940. CAS 40327, 1 spec., 36.9, Santa Barbara Co., Santa Ynez River, 04 May 1941. UMMZ 133067, 50 of 289 spec., 24.0–32.4, Santa Barabara Co., Carpinteria Creek, 08 June 1940.

Region V (Ventura): UMMZ 133072, 2 of 4 spec., 30.7–31.8, Ventura Co., Calleguas Creek, 08 June 1940. CAS 31768, 17 of 95 spec., 24.5–31.4, Los Angeles Co., Malibu Creek at mouth, 12. Oct. 1955. UMMZ 132893, 21 of 27 spec., 24.2–30.0, Los Angeles Co., Malibu Creek, 15 June 1940. NMW 33915, 4 spec., 28.6–33.9, Los Angeles Co., Santa Monica, artesian well, 1879.

Region VI (San Diego): SIO 62-278-59A, 1 of 44 spec., 31.2, Orange Co., Aliso Creek, 13 June 1962. SIO 52-108A&B, 49 of 112 spec., 24.5–35.2, Orange Co., Aliso Creek, 19 July 1952. LACM 36189-2, 7 of 22 spec., 24.2–29.5 San Diego Co., San Mateo Creek on N. edge Camp Pendleton, 26 Dec. 1974. LACM 36188-1, 1 of 7 spec., 30.6, San Diego Co., San Onofre Creek at San Onofre on Camp Pendleton, 26 Dec. 1974. UMMZ 132897, 10 of 19 spec., 24.1–31.8, San Diego Co., San Onofre Creek, 04 July 1940. SIO 55-39, 1 spec., 33.4, San Diego Co., bird sanctuary near Oceanside, 26 April 1955. UMMZ 131809, 3 of 10 spec., 25.6–29.7, San Diego Co., Agua Hedionda Creek near Carlsbad, 29 June 1940.

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