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Evolution and Biogeography of the Clinidae (Teleostei: Blennioidei)

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The systematic and biogeographic relationships of the teleost family Clinidae (commonly known as kelpfishes or klipfishes), an antitropically distributed group of nearshore fishes characterized by widespread ecological variation, were analyzed using allozyme and morphological characters. The Clinidae comprises three tribes; the matritrophic (ovoviviparous) Clinini and Ophiclinini, and the oviparous tribe Myxodini. Emphasis of the present study was in resolving the relationships among the eastern Pacific Myxodini, which include the South American genus *Myxodes* and the North American genera *Heterostichus* and *Gibbonsia*. Allozyme data were analyzed from 40 presumptive gene loci for all four North American myxodin species, one species of the Australian clinid tribe Clinini (*Heteroclinus whiteleggei*), 10 species (representing all tribes) of the hypothesized sister blennioid family, the Labrisomidae, and two species of the blennioid family Chaenopsidae (also believed to be closely related; the latter two families were used as outgroups).

Several different phylogenetic approaches for analyzing allozyme data were used for purposes of comparison, including two frequency character methods, a genetic distance method, a discrete character parsimony method (using only fixed alleles), and a multistate character parsimony analysis (the latter two used presumptive gene loci as characters and selected alleles as character states). Discrete morphological characters were also analyzed both separately and included with allozyme data in parsimony analyses.

Results from morphological and allozyme data, as well as the different methods of phylogenetic analysis, were congruent; yielding identical parsimonious relationships among taxa, except within the genus *Gibbonsia*. Two most parsimonious trees were consistently obtained, which differ in whether *G. metzi* or *G. elegans* is the basal clade within the genus (and the sister group to the other two species). The Clinidae is distinguished by a suite of allozyme character state synapomorphies identified in the present study as well as three morphological synapomorphies. The present study indicates that the cryptotremin labrisomids are the sister group to the Clinidae. The trophodermic matritrophic (ovoviviparous) clinids (comprising the tribes Clinini and Ophiclinini) and the Myxodini are sister taxa, and the South American myxodin genus *Myxodes* is sister to the northern myxodin genera. The North American genera *Heterostichus* and *Gibbonsia* are sister groups.

THE systematics of the eastern Pacific members of the teleost family Clinidae, suborder Blennioidei, were last examined by C. Hubbs (1952), and the genus *Gibbonsia* was recently revised by Stepien and Rosenblatt (1991). Clinids are relatively small (less than 300 mm SL, except *Heterostichus rostratus*) benthic fishes, the majority of which inhabit intertidal and shallow subtidal waters (Springer, 1982; Nelson, 1984). George and Springer (1980) redefined the Clinidae, excluding the Tripterygiidae, Labrisomidae, and Chaenopsidae, and adding the tribe Ophiclinini. Clinids are distinguished by the morphological synapomorphy of a cordlike ligament extending from the ceratohyal to the dentary symphysis (Springer et al., 1977). The Clinidae contains three tribes (Myxodini, Clinini, and Ophiclinini), 20 genera, and 71 species, most of which are restricted to Australia and Africa (Nelson, 1984). The family has a mostly antitropical distribution, shown in Figure 1A, and all but five (tropical) species are limited to warm temperate waters (Springer, 1982).

The present study primarily examined relationships among the eastern Pacific myxodin

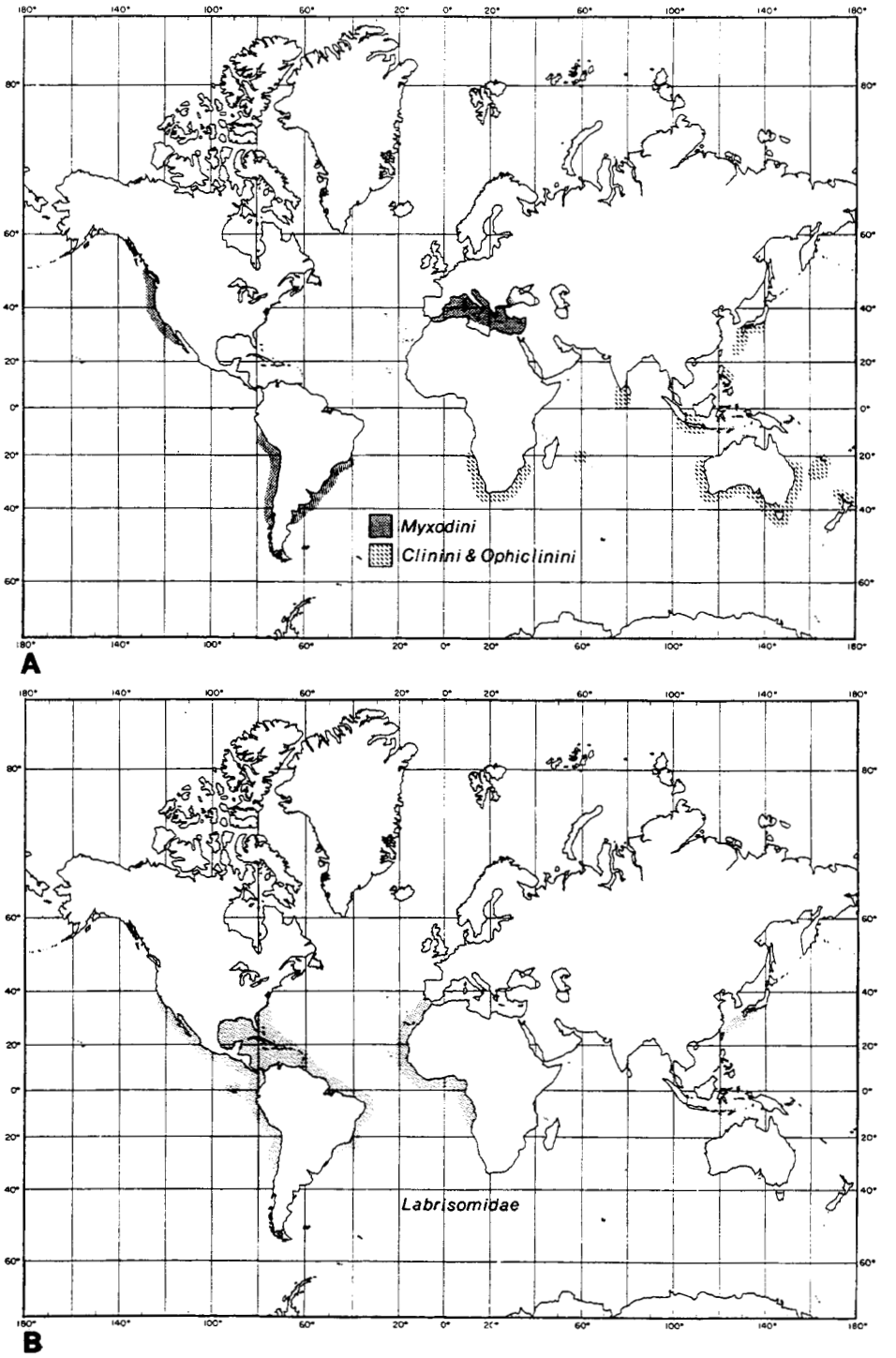

Fig. 1. Distribution maps of the families Clinidae (A) and Labrisomidae (B), based on C. Hubbs (1952), Penrith (1969), Springer (1970), Stephens and Springer (1973), George and Springer (1980).

TABLE 1. PRESENT STATUS OF THE CLINID TRIBE MYXODINI. (After C. Hubbs, 1952; Springer, 1970; Stephens and Springer, 1973; George and Springer, 1980; and Stepien and Rosenblatt, 1991.)

Component taxa	Common distribution
A. *Gibbonsia* (Cooper)	
1. *G. elegans* (Cooper)	Point Conception, California, through central Baja California, Mexico
2. *G. metzi* C. L. Hubbs	North of Point Conception along U.S. Pacific coast and in areas of cold-water upwelling south through central Baja California, Mexico
3. *G. montereyensis* C. Hubbs	North of Point Conception and in subtidal waters and areas of cold-water upwelling south through central Baja California, Mexico, including Guadalupe and San Benito islands
B. *Heterostichus rostratus* Girard	Point Conception through central Baja California, Mexico
C. *Myxodes* Cuvier	
1. *M. viridis* Valenciennes	Southern Peru through central Chile
2. *M. cristatus* Valenciennes	Central through southern Chile
3. *M. ornatus* Stephens and Springer	Chile, range unknown
D. *Ribeiroclinus eigenmanni* (Jordan)	Western Atlantic from southern Brazil through central Argentina
E. *Clinitrachus argentatus* (Risso)	Mediterranean Sea

clinids. All species in the tribe Myxodini are found in shallow temperate waters of the Western Hemisphere, except for one monotypic genus limited to the Mediterranean Sea (Springer, 1970; Stephens and Springer, 1973). The Myxodini is characterized by ovuliparous oviparity and the lack of an intromittent organ in males; both features are regarded as plesiomorphies (C. Hubbs, 1952; Springer, 1970; George and Springer, 1980). The other clinid tribes, Clinini and Ophiclinini (which are primarily found in South Africa, temperate Australia, and New Zealand), share the synapomorphies of matritrophy (Wourms et al., 1988) and a male intromittent organ (George and Springer, 1980).

Five genera are contained in the Myxodini: *Myxodes, Heterostichus, Gibbonsia, Ribeiroclinus,* and *Clinitrachus.* Myxodins are not found in Central America or other tropical waters (Springer, 1970; Stephens and Springer, 1973; Nelson, 1984). *Myxodes* ranges along the west coast of South America, from southern Peru through southern Chile. *Heterostichus* and *Gibbonsia* share a distribution from central Baja California, Mexico, to British Columbia, Canada (C. Hubbs, 1952). *Ribeiroclinus* (monotypic) is found along the southwestern Atlantic coast of South America, and *Clinitrachus* (monotypic) is a Mediterranean endemic (Springer, 1970; Nelson, 1984; see Table 1 for summary of genera, species, and distributions and Figure 1A for a map of their distributions).

The Labrisomidae has been regarded as either the probable sister group to the Clinidae (C. Hubbs, 1952) or a possible sister group (George and Springer, 1980; Springer, pers. comm.), and 10 species of labrisomids (including all of the six tribes) were used as outgroups in the present study. Evolutionary relationships of the Labrisomidae, based on allozyme and DNA sequence data, are further examined in Stepien et al. (Stepien et al., 1992). Labrisomids are distinguished from clinids by the following morphological characters: scales with radii only on anterior margins, and never small and embedded, but sometimes absent (C. Hubbs, 1952; Stephens and Springer, 1973; George and Springer, 1980). The Labrisomidae contains 102 species and is widely distributed throughout the New World tropics, shown in Figure 1B (Nelson, 1984). A fossil labrisomid is known from Miocene deposits in the Mediterranean, where the family is no longer represented. There are no known fossil clinids (Springer, 1970; George and Springer, 1980).

The blennioid family Chaenopsidae (tube

blennies) was also employed as an outgroup. The Chaenopsidae is restricted to the New World tropics and traditionally has been regarded as most closely related to the neoclinin labrisomids (C. Hubbs, 1952; Stephens, 1963).

Allozyme data from 40 presumptive gene loci of all North American myxodin species and subspecies, as defined by C. Hubbs (1952), were analyzed in the present study. Results from the subspecies and population analyses are presented and discussed in Stepien and Rosenblatt (1991). Allozyme data were also collected from the South American myxodin *M. viridis* Valenciennes and the Australian clinin *H. whiteleggei* Ogilby.

There are several approaches for the phylogenetic analysis of allozyme data, including genetic distance, frequency character, discrete character, and multistate character parsimony methods (Buth, 1984, pers. comm.; D. L. Swofford, pers. comm.). In the present study, four different phylogenetic programs (encompassing all four methods) were used to generate and compare trees.

The relationships of the Myxodini and its sister group, the matritrophic tribes Clinini and Ophiclinini, as well as the relationships of the South and North American myxodin genera and the North American myxodin species, were examined in the present study. Phylogenetic trees based on the allozyme data were compared with those based on morphological characters. Allozyme and morphological data were also combined in parsimony analyses. Several allozyme character state synapomorphies were identified which, in addition to morphological characters, distinguish members of the family Clinidae, the eastern Pacific members of the tribe Myxodini, and the North American myxodin clinids.

Materials and Methods

Species and populations analyzed.—Data for the individual clinid subspecies and populations are compared and discussed in Stepien and Rosenblatt (1991), in which *G. erythra* and *G. norae* were placed in the synonymy of *G. montereyensis*. Species, collection locations, and collection numbers of formalin-preserved voucher specimens are given in Materials Examined. The North American Myxodini, as defined by Stepien and Rosenblatt (1991), include *H. rostratus, G. metzi, G. elegans,* and *G. montereyensis* (Table 1). The diversity of sample sites examined in Stepien and Rosenblatt (1991) represented both the centers of the ranges of each species and areas of infrequent occurrence and sympatry with related species. Allozyme data from the South American myxodin *M. viridis*; the Australian clinin *H. whiteleggei*; 10 species of labrisomids, including *Auchenionchus microcirrhis* (Valenciennes) (tribe Cryptotremini), *Alloclinus holderi* (Lauderbach) (tribe Cryptotremini), *Paraclinus integripinnis* (Smith) (tribe Paraclinini), *P. sini* Hubbs (tribe Paraclinini), *Exerpes asper* (Jenkins and Evermann) (tribe Paraclinini), *Starksia spinipenis* (Al-Uthman) (tribe Starksiini), *Labrisomus xanti* Gill (tribe Labrisomini), *Malacoctenus gigas* Springer (tribe Labrisomini), *Neoclinus blanchardi* Girard (tribe Neoclinini), and *Mnierpes macrocephalus* (Günther); and two species of chaenopsids, *Emblemaria hypacanthus* (Jenkins and Evermann) and *Acanthemblemaria macrospilus* Brock were analyzed.

Enzyme electrophoresis.—Specimens were collected by netting intertidally with use of the anesthetic quinaldine or subtidally by scuba diving and were immediately frozen and stored at −40 C. Separate extracts of eye, liver, and muscle were prepared from each specimen. Tissues were homogenized in a 1 : 1 volume : volume mixture of tissue and 0.1 M potassium phosphate buffer (pH 7, Waples and Rosenblatt, 1987) and centrifuged at 20,000 *g* for 10 min. The supernatant fraction was subjected to horizontal starch electrophoresis in 12.5% starch gels (Sigma starch; Sigma Chemical Co., St. Louis, Missouri 63178). Enzymes and tissues surveyed, loci scored, and buffers are given in Stepien and Rosenblatt (1991). Staining methods and recipes were adapted from Selander et al. (1971), Waples (1986), and Buth and Murphy (1990). Enzyme nomenclature follows recommendations of the International Union of Biochemistry (1984).

Relative migration distances were compared with the most common allele (assigned a value of 100) from the population of *G. elegans* at Bird Rock, San Diego, California, from which 63 individuals were surveyed (Stepien and Rosenblatt, 1991). Other alleles were labeled according to the mobility of their products relative to this standard.

Data analysis.—Genetic distance, frequency character, discrete character, and multistate

character parsimony procedures were used to analyze the allozyme data in phylogenetic computer programs to test for method-dependent variation in the generated trees. Different labrisomid tribes and the two species of chaenopsids were designated as the outgroup in separate, independent computer analyses.

Genetic distance clustering procedures are the most widely used approach for analyzing allozyme data but are also the most controversial (Buth, 1984). Genetic distances measure the degree of genetic divergence between each pair of taxa. They lose information by reducing arrays of allozyme frequency data to a single value. BIOSYS-1 vers. 1.7 (Swofford and Selander, 1981, 1989) was used to calculate modified Rogers' (Rogers, 1972; Wright, 1978) genetic distances between all pairwise combinations of taxa. Genetic distances were based on data from one population for each species chosen from the geographic midpoint of their respective ranges. These large population samples included *H. rostratus* and *G. elegans* from La Jolla, California, and *G. metzi* and *G. montereyensis* from San Simeon, California. Data from several other populations for each species, representing other areas of their ranges, were analyzed in Stepien and Rosenblatt (1991). BIOSYS-1 (Swofford and Selander, 1981, 1989) was also used to construct distance Wagner trees (Farris, 1972), which utilized the modified Rogers' genetic distances and the Wagner clustering procedure. Data from 10 species of labrisomids and two species of chaenopsids were included in this analysis.

Frequency character methods compare changes in the frequencies of individual alleles and their gain, loss, and/or fixation among taxa. The phylogenetic trees are based on minimizing the number of genetic changes between taxa. Swofford and Berlocher's (1987, 1988) Frequency parsimony (FREQPARS vers. 2.0) and Felsenstein's (1981, 1984, 1988) Continuous character–Maximum likelihood (CONTML in PHYLIP Phylogeny Inference Package vers. 3.1) methods were used.

FREQPARS generates a tree on which each allele undergoes the least amount of frequency change, while at the same time ensuring that allelic frequencies in the hypothetical ancestors add to 1.00. This program was modified for use on this particular data set by D. Swofford. CONTML models evolutionary change at a locus as a Brownian motion process without requiring the assumption of rate uniformity across lineages. All of the clinid species and the three labrisomid species closest in genetic distance to the Clinidae (*Auchenionchus microcirrhis, Alloclinus holderi,* and *P. integripinnis*) were analyzed. Global branch swapping and 10 separate trials using the random-number jumble option were used in CONTML.

A discrete character parsimony analysis using Phylogenetic Analysis Using Parsimony (PAUP 3.0; Swofford, 1990) was also used, in which the loci served as the characters and fixed (frequencies = 1.00) and predominant alleles (frequencies >0.85, >0.90, and >0.95) were coded as discrete character states (in separate computer analyses; Tables 2–3). Using only fixed alleles is more acceptable to some cladists who do not regard variable characters as providing proper phylogenetic data (Crother, 1990). However, this approach has the disadvantage of discarding the information contained in allelic variation. Allelic frequencies less than the cut-off level were not coded, and, if there was not an allele with a given frequency at a particular locus, the character state data for that species were coded as missing. Exhaustive searches were employed, and trees were rooted with the labrisomid *A. microcirrhis.*

A multicharacter state parsimony analysis using PAUP 3.0 (Swofford, 1990) was also employed, in which, as above, alleles were coded as unordered character states (with multiple character states analyzed as polymorphisms) and loci as the characters (Table 3). This approach has the advantage of including allelic variation but, as in the discrete character analysis, disregards allelic frequency information. Exhaustive searches were used and trees were rooted to *A. microcirrhis.*

Phylogenetic hypotheses generated by the four programs were compared with each other and with those based on morphological data from C. Hubbs (1952), Springer (1970), Stephens and Springer (1973), George and Springer (1980), and Stepien and Rosenblatt (1991) (see characters 41–53, Table 3). Discrete morphological data were analyzed using PAUP (Swofford, 1990), using unordered character states and an exhaustive search (rooted with the cryptotremin labrisomids *Auchenionchus microcirrhis* and *Alloclinus holderi*). These morphological data were also added to the discrete and multistate data sets in separate PAUP analyses, using unordered character states and exhaustive searches (as above) and exhaustive searches

TABLE 2. GENOTYPIC DISTRIBUTIONS OF LOCI FOR CLINIDS AND LABRISOMIDS.

Locus	Number of specimens of each genotype per species (and relative allelic mobilities[1])				
	Myxodes viridis	*Heteroclinus whitelaggei*	*Auchenionchus microcirrhis*	*Alloclinus holderi*	*Paraclinus integripinnis*
sAat-A	bb(090):32 ab(100):01	bb(090):09	aa(100):04	bb(090):10	bb(090):21
sAat-B	aa(100):39	aa(100):10	cc(095):04	cc(095):43	cc(095):42
mAat-A	aa(100):25	aa(100):08 ab(200):01	aa(100):04	aa(100):60	aa(100):21
sAcoh-A	ee(115):40	dd(075):04	cc(105):04	aa(100):38 ad(075):04	ff(125):18
Acp-1	aa(100):22	aa(100):09	cc(140):04	bb(115):10 bc(140):01	bb(115):21
Acp-A	cc(090):36	cc(090):09	cc(090):04	ee(135):10 ef(155):03	hh(120):12 eh(135):04
Adh-A	aa(100):32	aa(100):07	aa(100):04	bb(090):31	aa(100):12
Adh-B	aa(100):30	aa(100):08	bb(050):03 bc(070):01	aa(100):30 ac(070):05	aa(100):12 ac(070):02
Ak-A	bb(200):42	bb(200):16	bb(200):04	bb(200):45	bb(200):20 bc(230):01
Ck-A	bb(115):44	bb(115):09	cc(105):04	cc(105):42	cc(105):21
Ck-B	bb(105):41	cc(095):09	aa(100):04	aa(100):37 ab(105):01	bb(105):13
Ck-C	bb(117):42	bb(117):09	cc(125):04	cc(125):07 cd(140):01	dd(140):13
Est-1	cc(090):11 ac(100):01	bb(110):08 ab(100):01	dd(120):04	aa(100):08	aa(100):07
Est-2	bb(090):11	cc(110):09	cc(110):04	cc(110):09	dd(150):07
Est-3	bb(090):20	bb(090):09	dd(110):04	cc(105):42	dd(085):12
Fumh-A	bb(120):33 ab(100):02	bb(120):09	aa(100):04	aa(100):09 ac(150):03	bb(120):20
G6pdh-1	bb(120):42	bb(120):14	bb(120):04	bb(120):10	bb(120):10 be(130):02
G6pdh-2	cc(090):37 bc(110):02	bb(110):08 bd(080):01	ee(120):04 ef(090):02	ee(120):10	ee(120):10
Gpi-A	dd(070):26	aa(100):10	aa(100):03 ae(115):01	dd(070):40 df(115):03	aa(100):18 ad(070):02
Gpi-B	bb(115):11	aa(100):04	aa(100):04	dd(200):35 df(215):05	ee(065):30
Gtdh-A	aa(100):42 ac(075):01	bb(110):09	bb(110):04	bb(110):24	bb(110):20
Gapdh-A	aa(100):43	aa(100):09	cc(200):03 bc(120):01	cc(200):45	dd(080):21 ad(100):02
Gapdh-C	aa(100):42 ab(120):01	bb(120):10	bb(120):04	bb(120):40	bb(120):20
G3pdh-B	ee(120):27	bb(080):14	ff(060):03 af(100):01	ff(060):42	gg(115):07 gf(060):01
Iddh-A	bb(060):08 ab(100):01	cc(125):04	cc(125):04	dd(200):20 de(105):02	ee(106):06 ce(125):02
sIdh-A	cc(095):39	bb(115):09	bb(115):04	bb(115):42	bb(115):05 bc(095):02
mIdh-A	dd(110):40	cc(120):07	cc(120):04	cc(120):25	cc(120):14
Ldh-A	dd(080):43	aa(100):05 ac(090):01	aa(100):04	aa(100):45	ee(120):10
Ldh-B	cc(105):42	aa(100):07	aa(100):04	aa(100):32	aa(100):12
Ldh-C	aa(100):24	aa(100):08	aa(100):04	aa(100):45	aa(100):12

TABLE 2. CONTINUED.

Locus	Number of specimens of each genotype per species (and relative allelic mobilities[1])				
	Myxodes viridis	*Heteroclinus whiteleggei*	*Auchenionchus microcirrhis*	*Alloclinus holderi*	*Paraclinus integripinnis*
sMdh-A	aa(100):42	cc(110):14	bb(070):02	aa(100):10	ee(095):06
		ac(100):01	ab(100):02		
sMdh-B	dd(110):30	bb(090):07	ff(060):04	cc(080):40	gg(105):10
	bd(090):03	be(070):01			
Mpi-A	aa(100):31	aa(100):04	bb(085):04	cc(120):05	cc(120):05
				ac(100):03	
Pep-A	dd(080):44	aa(100):13	ee(115):03	dd(080):25	dd(080):03
		ad(080):01	de(080):01	ad(100):03	df(085):03
Pep-B	cc(095):30	bb(120):12	bb(120):04	cc(095):40	aa(100):22
				cd(110):05	ad(110):02
Pep-3	cc(050):28	bb(125):12	bb(125):04	bb(125):40	ee(035):07
	cd(080):01	bc(050):02			ef(070):02
Pgm-A	cc(070):41	bb(110):06	bb(110):04	ee(080):39	ee(080):20
		bd(050):01		ef(060):03	
Pgdh-A	cc(120):37	dd(080):10	cc(120):04	cc(120):45	cc(120):21
sSod-A	dd(030):41	ee(120):06	ff(060):04	ee(120):40	hh(110):19
				eg(145):10	fh(050):01
Xdh-A	cc(080):40	bb(110):09	cc(080):04	cc(080):20	bb(110):08
	ac(100):01				bc(080):02

[1] Relative allelic mobilities were measured against the standard of the most common allele in the population of *Gibbonsia elegans* from La Jolla, California (see Materials and Methods).
For heterozygotes, the relative allelic mobility of the rarer allele is given in parentheses.

(Table 3). Strict (Rohlf, 1982), Adams (1972, 1986), and 50% majority rule (Rohlf, 1982) consensus trees were run on the shortest trees from the discrete and multistate PAUP analyses, and 1000 bootstrap replications (Felsenstein, 1985) on the data sets were performed using the branch-and-bound algorithm (Hendy and Penny, 1982). Consistency indices (C.I.; Swofford, 1990), g-1 skewness statistics (Sokal and Rohlf, 1981; Hillis, 1991; Huselsenbeck, 1991), and lengths of most parsimonious trees are reported for all PAUP analyses. The g-1 statistic measures degree of skewness of tree length distributions, providing a test of the relative strength of the phylogenetic signal (Hillis, 1991; Huselsenbeck, 1991).

RESULTS

Allozyme data for 40 presumptive gene loci from the clinids *H. rostratus, G. elegans, G. metzi,* and *G. montereyensis* are given in Stepien and Rosenblatt (1991) and those for *M. viridis, H. whiteleggei,* and the labrisomids *Auchenionchus microcirrhis, Alloclinus holderi,* and *P. integripinnis* are summarized in Table 2. Allozyme data for the chaenopsids and remaining labrisomids are given in Stepien et al. (1992). Zymograms illustrating similarities and differences between taxa are given in Figure 2. Genetic distances for all individual population samples of the North American clinid taxa are presented and analyzed in Stepien and Rosenblatt (1991). Populations sampled in Stepien and Rosenblatt (1991) were in Hardy-Weinberg equilibrium and were thus pooled in the present study.

All methods of analysis of allozyme data, including frequency character, genetic distance, discrete character, and multistate character parsimony approaches yielded one or both of two most parsimonious trees. These two trees were identical except among relationships within the genus *Gibbonsia* (Fig. 3) and were obtained when the trees were rooted with different species of labrisomids and chaenopsids.

The two most parsimonious phylogenetic trees obtained from the various types of data analyses are presented in Figure 3A–B. Branches and nodes are lettered, and allozyme character state synapomorphies and autapomorphies delineating taxa at each of these, for both trees, are given in Tables 4 and 5. Possible homoplasies

TABLE 3. DATA MATRIX FOR PAUP ANALYSES, INCLUDING ALLOZYME (CHARACTERS 1–40) AND MORPHOLOGICAL CHARACTERS (41–53).

Taxa	Presumptive gene locus									
	sAat-A 1	sAat-B 2	mAat-A 3	sAcoh-A 4	Acp-1 5	Acp-A 6	Adh-A 7	Adh-B 8	Ak-A 9	Ck-A 10
H. rostratus	a	a***,b,c	b	b*,a	b	b**,a,c	b*,a	a	b	b
G. elegans	a	a***,b	a	a**,b,c	a	a***,d	a***,b	a	a	a
G. metzi	a	a	b	a**,b,f	b	a***,d	a	a	a	a
G. montereyensis	a	b	b	a*,b	a	a*,d	a	a	a	a
M. viridis	b***,a	a	a	e	a	c	a	a	b	b
H. whiteleggei	b	a	a***,b	d	a	c	a	a	b	b
A. microcirrhis	a	c	a	c	c	c	a	b***,c	b	c

Taxa	Ck-B 11	Ck-C 12	Est-1 13	Est-2 14	Est-3 15	Fumh-A 16	Gadph-A 17	Gapdh-C 18	G3pdh-B 19	G6pdh-1 20
H. rostratus	a	a	a	b	b	a	b	a	a	a
G. elegans	a	a	a	a	a***,b,c	a	a,b	a	a***,b,c,d	a
G. metzi	a	b	a	a	a**,b,c	a	b**,a	a	a	a
G. montereyensis	a	b	a	a	a***,b	a	b**,a	a	a***,b,c,d	a
M. viridis	b	b	c**,a	b	b	a*,b	b**,a	a	e	b
H. whiteleggei	c	b	b**,a	c	b	b	b	a	c	b
A. microcirrhis	a	c	d	c	d	b	a	b*,c	f**,a	b

Taxa	G6pdh-2 21	Gtdh-A 22	Gpi-A 23	Gpi-B 24	sIdh-A 25	mIdh-A 26	Iddh-A 27	Ldh-A 28	Ldh-B 29	Ldh-C 30
H. rostratus	a	a	a**,b	a	b**,a	a	a	b***,a	b*,a	a
G. elegans	a	a	a*,b,c,d	a***,c	a	a	a	a	a	a
G. metzi	a	a	a*,b,c	a***,c	a	b	a	a	a	a
G. montereyensis	a	a	a	a**,c	a	b***,a	a	a	a	a
M. viridis	c**,b	a***,c	d	b	c	d	b**,a	d	c	a
H. whiteleggei	b*,d	b	a	a	b	c	c	a**,c	a	a
A. microcirrhis	e*,f	b	a*,e	a	b	c	c	a	a	a

Taxa	sMdh-A 31	sMdh-B 32	Mpi-A 33	Pep-A 34	Pep-B 35	Pep-3 36	Pgdh-A 37	Pgm-A 38	sSOD-A 39	Xdh-A 40
H. rostratus	a	a*,b	a	c**,a	b	b	b**,a	b	b	a
G. elegans	a	a**,b,c	a	a***,b	a	a	a***,b	a	a**,c,e	a
G. metzi	a	a**,b,d	a	b	c***,a,b	a	b**,a	b***,a	a***,c	a
G. montereyensis	b**,a	a***,b	a	b	c	d**,a	b	a	a**,c	a
M. viridis	a	d*,b	a	d	c	c***,d	c	c	d	c***,a
H. whiteleggei	c*,a	b**,e	a	a**,d	b	b***,c	d	b**,d	e	b
A. microcirrhis	b?,a	f	b	e*,d	b	b	c	b	f	c

TABLE 3. CONTINUED.

Taxa	Morphological characters (see key below)												
	41	42	43	44	45	46	47	48	49	50	51	52	53
H. rostratus	b	b	b	a	a	b	b	a	a	a	a	b	b
G. elegans	b	b	b	a	a	b	b	b	b	a	b	c	b
G. metzi	b	b	b	a	a	b	b	a	a	a	b	c	b
G. montereyensis	b	b	b	a	a	b	b	b	b	a	b	c	b
M. viridis	b	b	b	a	a	b	a	a	a	b	b	b	b
H. whiteleggei	b	b	b	b	b	?	a	a	a	a	a	a	a
A. microcirrhis	a	a	a	a	a	a	a	a	a	a	a	a	a

In discrete character state analyses, only the most common allele (the first listed) was used. ? = frequency of most common allele < 85% (in discrete analyses) or character state unknown (for morphological data).

* = frequency of most common allele <90%.

** = frequency of most common allele <95%.

*** = frequency of most common allele <100%.

In multicharacter state analyses, all alleles were coded as character states.

Key to morphological characters

41. Cordlike ligament connecting ceratohyal and dentary symphysis (George and Springer, 1980). a = absent, b = present
42. Scales absent or with radii on anterior margins only (a), scales with radii on all margins (b) (C. Hubbs, 1952; George and Springer, 1980).
43. Occur in red, green, and brown color morphs which match color of plant backgrounds (C. Hubbs, 1952; Penrith, 1969; Stepien, 1990). a = absent, b = present.
44. Male intromittent organ (C. Hubbs, 1952; Penrith, 1969; George and Springer, 1980). a = absent, b = present.
45. Trophodermic matritrophy (ovoviviparity; C. Hubbs, 1952; Penrith, 1969; George and Springer, 1980). a = absent, b = present.
46. Depth segregation of adult males and adult females; males living deeper than females (Stepien 1987, 1990; Stepien et al., 1988). a = absent, b = present.
47. Size sexual dimorphism; adult females larger in size than adult males at given ages (Stepien 1987, 1990; Stepien et al., 1988). a = absent, b = present.
48. Unequal spacing of posterior soft dorsal fin rays (C. Hubbs, 1952). a = absent, b = present.
49. Two or more dorsal ocelli above lateral line (C. Hubbs, 1952; Stepien and Rosenblatt, 1991). a = absent, b = present.
50. Vomerine teeth (Springer, 1970). a = present, b = absent.
51. Palatine teeth (Springer, 1970). a = present, b = absent.
52. Last dorsal fin ray; a = bound for complete length to caudal peduncle, b = free from caudal peduncle, c = tip only free from caudal peduncle (C. Hubbs, 1952; Springer, 1970).
53. Deep notch between dorsal spines 3 and 4 (C. Hubbs, 1952; Springer, 1970). a = absent, b = present.

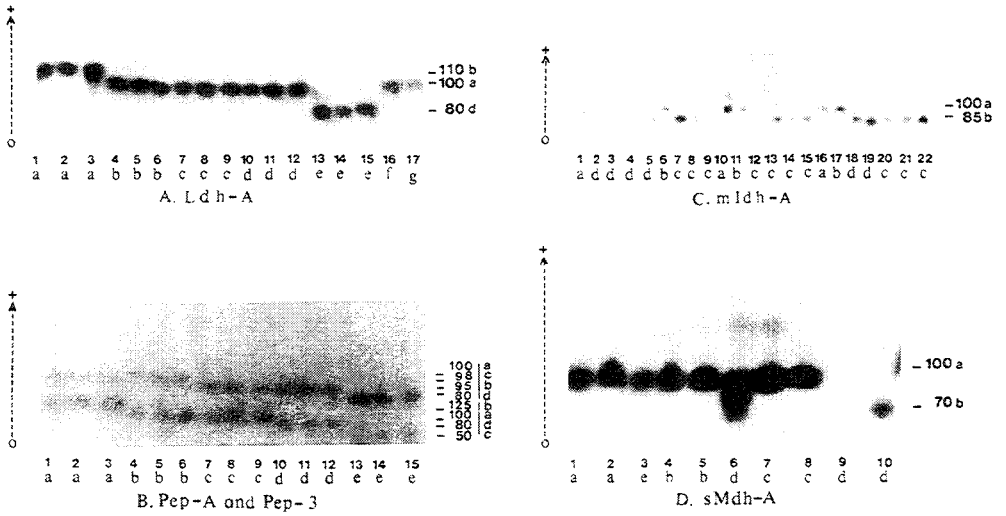

Fig. 2. Examples of zymograms of isozyme expressions from tissue extracts from clinid and labrisomid taxa. o = origin of gel. Taxa are lettered as follows: a = *Heterostichus rostratus*, b = *Gibbonsia elegans*, c = *Gibbonsia metzi*, d = *Gibbonsia montereyensis*, e = *Myxodes viridis*, f = *Heteroclinus whiteleggei*, and g = *Auchenionchus microcirrhis*. Other loci are visible on some of the photographs, e.g., sIdh-A on gel C and sMdh-B on gel D. Heterozygotes include gel A, fish 3-a, alleles a/b; gel B, Pep-A (top bands), fish 12-d, alleles b/d; Pep-3 (lower bands), fish 13-e, alleles d/c; and gel D, fish 6-d, alleles a/b.

of the two trees are compared in Table 5. Morphological synapomorphies at each branch are summarized in Table 3 and the Discussion.

Goodness-of-fit statistics for the Wagner distance tree varied with the number of taxa included (number of labrisomid tribes and inclusion of the Chaenopsidae). Cophenetic correlation (Swofford and Selander, 1981) values ranged from 0.87, when all 10 labrisomid species were included, to 0.93 when rooted to the two most similar labrisomid species (*Auchenionchus microcirrhis* and *Alloclinus holderi*), and 0.99 when only the eastern Pacific myxodin clinid species were analyzed. Cophenetic correlation of the three family data set (Clinidae, Labrisomidae, and Chaenopsidae) was 0.80 (Stepien et al., 1992).

The tree derived from the Wagner genetic distance method (Fig. 3B) is identical to the most parsimonious tree from the frequency character programs (Fig. 3A) in all but the last two branches (the relationships among species in the genus *Gibbonsia*). The North American clinid taxa are separated into two lineages, the genera *Gibbonsia* and *Heterostichus*.

Continuous character–Maximum likelihood (Felsenstein, 1988), a frequency character approach, yielded a single tree (Fig. 3A) in all 10 independent analyses. *Gibbonsia elegans* is depicted as the basal clade within the genus and is the sister group to *G. montereyensis* and *G. elegans*. This tree had Ln likelihood values (Felsenstein, 1988) of 127.26.

Frequency parsimony (Swofford and Berlocher, 1988) yielded two most parsimonious trees (Fig. 3A–B), which differ in whether *G. metzi* (tree A) or *G. elegans* (tree B) is the basal clade in the genus. The lengths of these trees are 134.44 and 134.65, respectively. The next shortest tree places *G. montereyensis* as the basal clade in the genus and has a length of 136.14. The shortest tree (Fig. 3A) obtained with Frequency parsimony was thus identical to the only tree obtained with Continuous character–Maximum likelihood.

Analysis of fixed character state data (allelic frequencies = 100%) using PAUP 3.0 (Swofford, 1990) yielded three most parsimonious trees, which included each of the three species of *Gibbonsia* as sister to the other two (57 steps, C.I.s = 0.93, and g-1 skewness = −1.20 with allozyme data only and 74 steps, C.I.s = 0.91,

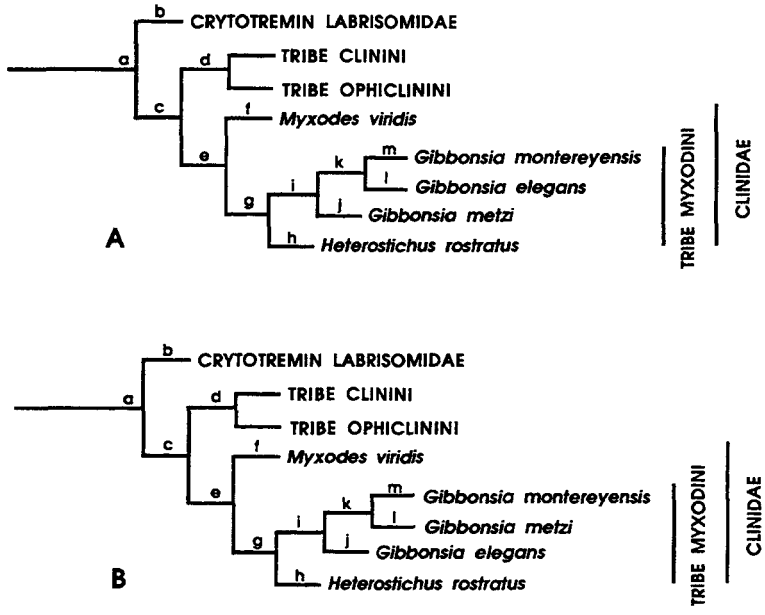

Fig. 3. Most parsimonious phylogenetic trees derived from various approaches for analyzing allozyme data, including two frequency character, one discrete character parsimony, one multicharacter state parsimony, and one genetic distance method. (A) Most parsimonious tree derived from morphological data and frequency character methods for analyzing allozyme data, including Frequency Parsimony and Continuous character–Maximum likelihood, showing relationships of the Clinidae. This tree is also one of the most parsimonious trees from the discrete character and multicharacter state parsimony analyses. (B) Tree derived from the Wagner genetic distance method. This was also the second most parsimonious tree produced by the FREQPARS analysis. This tree is also one of the most parsimonious trees from the discrete and multistate character parsimony analyses. This was the most parsimonious tree from the PAUP analysis of a combination of multicharacter state allozyme and morphological data.

and g-1 skewness = −1.28 with both allozyme and morphological data included). At the 0.95 cut-off level of allelic frequency, trees 3A and 3B were equally parsimonious (65 steps, C.I.s = 0.92, and g-1 skewness = −1.12 with allozyme data only and 82 steps, C.I.s = 0.90, and g-1 skewness = −1.16 with both allozyme and morphological data). At the 0.90 cut-off level, tree 3B was the most parsimonious (78 steps, C.I. = 0.89, and g-1 skewness = −0.92 with allozyme data only and 95 steps, C.I. = 0.91, and g-1 skewness = −0.98 with both allozyme and morphological data). Tree 3B was also the most parsimonious at the 0.85 cut-off level (85 steps, C.I. = 0.93, and g-1 skewness = −0.79 with allozyme data only and 102 steps, C.I. = 0.91, and g-1 skewness = −0.87 with inclusion of morphological data).

Discrete morphological characters for these taxa (characters 41–53 of Table 3) were also analyzed separately using PAUP. Two equally parsimonious trees were obtained (15 steps, C.I.s = 0.93, g-1 skewness = −1.12)—tree 3A and a tree with the positions of *H. rostratus* and *M. viridis* reversed. A bootstrapping consensus tree using branch-and-bound (Hendy and Penny, 1982), 50% majority-rule, and 1000 replications resulted in confidence intervals of 92% for node "e" (of Fig. 3A), 45% for node "g," 68% for node "i," and 87% for node "k."

Multistate character PAUP analysis of allozyme data (see Table 3) resulted in three most parsimonious trees, as in the 100% fixed discrete analysis (63 steps, C.I.s = 0.92, g-1 skewness = −0.95). Bootstrapping with branch-and-bound (Hendy and Penny, 1982), 80% majority-rule, and 1000 replications yielded confidence intervals (Felsenstein, 1985) of 98% for

TABLE 4. ALLOZYME CHARACTER STATES LINKING AND DISTINGUISHING TAXA AT BRANCHES AND NODES A THROUGH F OF TREES SHOWN IN FIGURE 3A–B.

Branch or node					
a	b	c	d	e	f
sAat-A(a)	sAat-B(c)	sAat-A(b)	*sAat-A(a)	Est-2(b)	mAat-A(b)
mAat-A(a)	Acp-1(c)	sAat-B(a)	sAcoh-A(d)	*Est-2(c)	sAcoh-A(e)
Acp-A(c)	Adh-B(b)	mAat-A(b)	*Ck-B(a)	Gadph-C(a)	*Ck-B(a)
Adh-A(a)	Adh-B(c)	Acp-1(a)	Ck-B(c)	Gtdh-A(a)	Ck-B(b)
Ak-A(b)	sAcoh-A(c)	Adh-B(a)	Est-1(b)	*Gtdh-A(b)	Est-1(c)
Ck-B(a)	Ck-A(c)	Ck-A(b)	*Fumh-A(a)	Gpi-A(d)	*G3pdh-B(a)
Est-2(c)	Ck-C(c)	Ck-C(b)	*G3pdh-B(a)	*mIdh-A(c)	G3pdh-B(e)
Fumh-A(a)	Est-1(d)	Est-1(a)	G3pdh-B(c)	Iddh-A(a)	G6pdh-2(c)
Gapdh-C(b)	Est-3(d)	Est-3(b)	G6pdh-2(d)	*Iddh-A(c)	Gtdh-A(c)
G3pdh-B(a)	Gapdh-A(b)	Fumh-A(b)	Ldh-A(c)	Ldh-A(d)	*Gpi-A(a)
G6pdh-1(b)	Gapdh-A(c)	Gapdh-A(a)	sMdh-A(c)	sMdh-B(d)	Gpi-A(d)
Gtdh-A(b)	G3pdh-B(f)	G6pdh-2(b)	sMdh-B(e)	*Pep-A(d)	*Gpi-B(a)
Gpi-A(a)	G6pdh-2(e)	sMdh-B(b)	*Pep-A(a)	Pep-B(c)	Gpi-B(b)
Gpi-B(a)	G6pdh-2(f)	Mpi-A(a)	*Pgdh-A(c)	Xdh-A(a)	*sIdh-A(b)
sIdh-A(b)	Gpi-A(e)	Pep-A(a)	Pgdh-A(d)		sIdh-A(c)
mIdh-A(c)	sMdh-A(b)	Pep-3(c)	Pgm-A(d)		mIdh-A(d)
Iddh-A(c)	sMdh-B(f)		sSod-A(e)		Iddh-A(b)
Ldh-A(a)	Mpi-A(b)		Xdh-A(b)		*Ldh-A(a)
Ldh-B(a)	Pep-A(e)		*Xdh-A(c)		Ldh-A(d)
Ldh-C(a)	sSod-A(f)				*Ldh-B(a)
sMdh-A(a)					Ldh-B(c)
Pep-A(d)					*Pep-A(a)
Pep-B(b)					*Pep-B(b)
Pep-3(b)					*Pep-3(b)
Pgdh-A(c)					Pep-3(d)
Pgm-A(b)					*Pgm-A(b)
Xdh-A(c)					Pgm-A(c)
					sSod-A(d)

* = Character state losses.

node "e," 92% for node "g," and 87% for node "i" (of trees in Fig. 3). Tree 3B was the most parsimonious obtained with a combination of morphological and multistate allozyme data (characters 1–53 of Table 3; 102 steps, C.I. = 0.91, g-1 skewness = −0.87). Bootstrapping with branch-and-bound (Hendy and Penny, 1982), 80% majority-rule, and 1000 replications yielded confidence intervals (Felsenstein, 1985) of 99% for node "e," 99% for node "g," and 92% for node "i" (of trees in Fig. 3).

In all PAUP analyses, strict (Rohlf, 1982), Adams' (1972, 1986), and 50% majority-rule (Rohlf, 1982) consensus analyses of the shortest trees yielded unresolved trichotomies for the genus *Gibbonsia*. Allozyme character state synapomorphies linking taxa at the branches of the two trees are compared in Tables 4 and 5, which show that trees 3A and 3B are supported by equal numbers of total synapomorphies at nodes "g," "i," and "k" (18 vs 16 at node "g," 15 vs. 16 at node "i," and 1 vs 2 at node "k"). Tree 3A has six apparent homoplasies and 67 character state losses, and tree 3B has five apparent homoplasies and 68 character state losses, listed in Table 5.

DISCUSSION

Four different approaches for the phylogenetic analysis of allozyme data yielded parsimonious relationships that were identical, except within the genus *Gibbonsia* (Fig. 3). This set of phylogenetic hypotheses is further supported by the known morphological characters distinguishing these taxa and largely corroborates the relationships proposed by C. Hubbs (1952), Penrith (1969), Springer (1970), and George and Springer (1980). Analysis of allozymes yielded a large data set that provided an

TABLE 5. ALLOZYME CHARACTER STATES LINKING AND DISTINGUISHING TAXA AT BRANCHES AND NODES G THROUGH M OF TREES SHOWN IN FIGURE 3A VERSUS 3B.

g	h	i	j	k	l	m
			Tree A			
*sAat-A(b)	xsAat-B(c)	*Acp-A(c)	*sAat-B(b)	*Acp-1(b)	xmAat-A(a)	*sAat-B(a)
sAat-B(b)	*Acp-1(a)	Acp-A(d)	*Acp-1(a)	xG3pdh-B(c)	*mAat-A(b)	*Adh-A(b)
*mAat-A(a)	Acp-A(b)	Ak-A(a)	sAcoh-A(f)	G3pdh-B(d)	sAcoh-A(c)	*Ck-C(a)
sAcoh-A(a)	*Ck-C(b)	*Ak-A(b)	*Adh-A(b)	*Mdh-B(d)	*Ck-C(b)	*Est-3(c)
sAcoh-A(b)	Fuhm-A(a)	Ck-A(a)	*Ck-C(a)	*Pep-B(b)	*mIdh-A(b)	*Gpi-A(b)
Acp-1(b)	*G3pdh-B(b)	*Ck-A(b)	*G3pdh-B(b)	*Pgm-A(b)	xGpi-A(d)	*Gpi-A(c)
Acp-A(a)	*Gpi-A(d)	Est-2(a)	Gpi-A(b)		sMdh-B(c)	xMdh-A(b)
Acp-A(b)	Ldh-A(b)	*Est-2(b)	*mIdh-A(a)		*Pep-B(c)	*Pep-A(a)
Adh-A(b)	Ldh-B(b)	Est-3(a)	*Pep-A(a)		xsSod-A(e)	*Pep-B(a)
Ck-C(a)	*sMdh-B(d)	Est-3(c)				xPep-3(d)
Gapdh-C(a)	Pep-A(c)	Gpi-A(c)				*Pgdh-A(b)
*Gapdh-C(b)	*Pep-B(c)	Gpi-B(c)				
G6pdh-1(a)	sSod-A(b)	mIdh-A(b)				
*G6pdh-1(b)		*sIdh-A(b)				
G6pdh-2(a)		Pep-A(b)				
*G6pdh-2(b)		Pep-B(a)				
Gpi-A(b)		Pep-3(a)				
sIdh-A(a)		*Pep-3(b)				
mIdh-A(a)		Pgm-A(a)				
sMdh-B(a)		sSod-A(a)				
*Pep-A(d)		sSod-A(c)				
*Pep-3(c)						
Pgdh-A(a)						
Pgdh-A(b)						
Pgdh-A(c)						
*Xdh-A(c)						
			Tree B			
*sAat-A(b)	xsAat-B(c)	*Acp-A(c)	*mAat-A(a)	*mAat-A(a)	*sAat-B(b)	*sAat-B(a)
sAat-B(b)	*mAat-A(a)	Acp-A(d)	sAcoh-A(c)	*Adh-A(b)	sAcoh-A(f)	*sAcoh-A(c)
sAcoh-A(a)	*Acp-1(a)	Ak-A(a)	*Acp-1(b)	*Ck-C(a)	*Acp-1(a)	*Acp-1(b)
sAcoh-A(b)	Acp-A(b)	*Ak-A(b)	*Ck-C(b)	Gpi-A(d)	*G3pdh-B(b)	*Est-3(c)
Acp-1(b)	*Ck-C(b)	Ck-A(a)	sMdh-B(c)	mIdh-A(b)	*G3pdh-B(c)	*Gpi-A(b)
Acp-A(a)	*Fumh-A(a)	*Ck-A(b)	*sMdh-B(d)	*Pep-A(a)	*G3pdh-B(d)	*Gpi-A(c)
Adh-A(b)	*G3pdh-B(b)	Est-2(a)	*Pep-B(b)		*mIdh-A(a)	xsMdh-A(b)
Ck-C(a)	*Gpi-A(d)	*Est-2(b)	*Pep-B(c)			*sMdh-B(d)
Gapdh-C(a)	Ldh-A(b)	Est-3(a)	*Pgm-A(b)			*Pep-B(a)
*Gapdh-C(b)	Ldh-B*b	Est-3(c)	xsSod-A(e)			*Pep-B(b)
G6pdh-1(a)	*Mdh-B(d)	G3pdh-B(b)				xPep-3(d)
*G6pdh-1(b)	*Pep-A(a)	xG3pdh-B(c)				*Pgdh-A(b)
G6pdh-2(a)	Pep-A(c)	G3pdh-B(d)				*Pgm-A(b)
*G6pdh-2(b)	*Pep-B(c)	Gpi-A(c)				
Gpi-A(b)	sSod-A(b)	Gpi-B(c)				
sIdh-A(a)		*sIdh-A(b)				
mIdh-A(a)		Pep-A(b)				
sMdh-B(a)		Pep-B(a)				
*Pep-A(d)		Pep-3(a)				
*Pep-3(c)		*Pep-3(b)				
Pgdh-A(a)		Pgm-A(a)				
Pgdh-A(b)		sSod-A(a)				
*Pgdh-A(c)		sSod-A(c)				
*Xdh-A(c)						

* = Character state losses. x = Possible homoplasy of electromorphs with the same net electric charge.

independent test for the identification of synapomorphies and monophyletic relationships among the Clinidae.

The family Labrisomidae, which was tested as the sister group in the present study, was recently distinguished from the Clinidae by George and Springer (1980). Labrisomids are widely distributed throughout the New World tropics and are also represented by two species in the eastern Atlantic, on the coast of Africa (Nelson, 1984; see Fig. 1B). They are distinguished from the Clinidae by a few morphological characters (see Introduction), and results of the present study support their familial separation.

Results of the present study suggest that the cryptotremid labrisomids, including the South American *Auchenionchus microcirrhis* and the North American *Alloclinus holderi* are sister to the Clinidae (Table 3) and indicate that the Labrisomidae may be paraphyletic (Stepien et al., 1992). In the present study, the cryptotremin labrisomid *Auchenionchus microcirrhis* and clinids share 27 allozyme character state synapomorphies, listed in Table 4. DNA sequence data from nuclear ribosomal and mitochondrial DNA from additional blennioid families and tribes (including the labrisomid tribes) are being used to further elucidate these relationships (Stepien et al., 1992; Stepien, unpubl.).

Allozyme data from the clinid species analyzed in the present study support Springer's hypothesis (Springer, 1970; George and Springer, 1980) that the Clinidae is monophyletic. Ribosomal DNA sequence data from the clinid tribes Clinini and Myxodini and the families Labrisomidae, Chaenopsidae, Tripterygiidae, and Blenniidae also support monophyly of the Clinidae (Stepien et al., 1992). The present study identifies 16 allozyme character state synapomorphies that unite the clinids examined (see branch "c" of Fig. 3A and Table 4). In addition to the morphological synapomorphies previously mentioned (see Introduction and characters 41 and 42 of Table 3), all clinids whose color patterns have been described share similiar variable patterns, unlike most labrisomids (*E. asper,* however, occurs in red and green morphs; C. Hubbs, 1952). These clinids are capable of slow, extensive color changes with background (character 43, Table 3), which involve gain and loss of carotenoid pigments, and often occur in red, green, and brown morphs that match their plant habitats (Stepien 1986a, 1987, 1990; Stepien et al., 1988).

The subfamily Clininae (tribes Clinini and Ophiclinini; C. Hubbs, 1952) is distinguished from the tribe Myxodini by the synapomorphies of matritrophy and a male intromittent organ (characters 44 and 45 in Table 3). Present-day distributions of the matritrophic tribes and the Myxodini do not overlap (Fig. 1A). The present study supports the monophyly of each group and the hypothesis that they have long been separated, proposed by C. Hubbs (1952) and Penrith (1969).

Myxodins examined in the present study share nine allozyme character state synapomorphies (node "e" of Fig. 3 and Table 4). Sister relationships of the North and South American Myxodini suggest that they may have shared a continuous ancestral distribution, extending through the tropics and were later separated by the formation of a barrier to dispersal and gene flow, which led to genetic divergence (a vicariant hypothesis; see Humphries and Parenti, 1987). A vicariant event which may explain this antitropical pattern of relationship and distribution is the Miocene warming of the tropics proposed by White (1986) in his analysis of the silverside fish subfamily Atherinopsinae. Alternatively, such antitropical relationships have traditionally been explained by long-distance dispersal (C. L. Hubbs, 1952).

Briggs' (1974, 1987) competitive exclusion hypothesis offers another explanation for these antitropical biogeographic patterns. He regards antitropically distributed taxa as older, relict groups which were displaced from the tropics by more recently evolved and competitively superior species (Briggs, 1974, 1987; Newman and Foster, 1987).

Myxodes viridis and the North American myxodins share the behavioral synapomorphy of depth segregation of mature males and females, males being found deeper (character 46 of Table 3; Williams, 1954; Stepien, 1987; Stepien et al., 1988). The North American Myxodini are united by 16 allozyme character state synapomorphies and are unequivocally demonstrated to be a monophyletic clade, as all species and subspecies were analyzed in the present study (node "g" of Fig. 3 and Table 5). Unlike *M. viridis* (Stepien, 1990), the North American species share the synapomorphies of sexual differences in size, adult females attaining greater lengths than adult males (character 47 of Table 3; Stepien, 1986b, 1987; Stepien et al., 1988).

Heterostichus rostratus is distinguished from the other northern myxodins by the morphological

apomorphies of a pointed snout and a forked caudal fin, in addition to five allozyme character state apomorphies (Table 5). The genus *Gibbonsia* is a monophyletic clade defined by a suite of 15 allozyme character state synapomorphies (node "i" of Fig. 3 and Table 5). Its reduced dorsal and anal fin and vertebral elements are morphological synapomorphies further distinguishing it from *Heterostichus.*

The phylogeny in which *G. elegans* is the sister species of the other two species in the genus (Fig. 3B) contains one less apparent allozyme character state homoplasy than the tree depicting *G. metzi* as the basal clade (Fig. 3A; see Table 5 and Results). However, morphological characters distinguishing the species of *Gibbonsia* best support tree 3A (Table 3). *Gibbonsia montereyensis* and *G. elegans* share the apparent synapomorphies of reduced fin and vertebral counts and are smaller in total length than *G. metzi* (C. Hubbs, 1952). *Gibbonsia metzi* has relatively equally spaced posterior dorsal soft fin rays and scalation extending onto the caudal fin. This scale pattern is shared with *G. elegans,* whereas *G. montereyensis* exhibits the apomorphic condition of a naked caudal fin (C. Hubbs, 1952; Stepien and Rosenblatt, 1991). The latter two species also share the synapomorphies of unequally spaced posterior dorsal fin rays and the presence of prominent dorsal ocelli on the body (characters 48 and 49 of Table 3; C. Hubbs, 1952; Stepien and Rosenblatt, 1991).

Gibbonsia elegans and *G. montereyensis* share one allozyme character state synapomorphy at branch "k" of the tree shown in Fig. 3A. *Gibbonsia elegans* is distinguished from *G. montereyensis* by two allozyme character state apomorphies (Table 3, branch "l" of Table 5.).

Heterostichus and *Gibbonsia* have planktonic larval stages lasting approximately two months (Stepien, 1986b). The late larval stages, as well as juveniles and adults, are thigmotactic and have been known to raft long distances from shore with pieces of drift algae. They thus appear to be capable of some relatively long-distance dispersal. *Gibbonsia metzi* and *G. montereyensis* share primary distributions north of Point Conception (latitude 34.5°N), California, reappearing in northern Baja California, Mexico, in areas of nearshore cold-water upwelling (C. Hubbs, 1952; Stepien and Rosenblatt, 1991). *Heterostichus rostratus* and *G. elegans* share a common distribution, primarily south of Point Conception through central Baja California. Data from several studies indicated that the temperature boundary at Point Conception is the most important geographic feature affecting the distributions of the California ichthyofauna, separating the warm-temperate Californian biogeographic province to the south from the cold-temperate Oregonian province to the north (C. L. Hubbs, 1952; Horn and Allen, 1978; Davis et al., 1981; Waples and Rosenblatt, 1987), which is also shown in clinid distributions (Stepien and Rosenblatt, 1991; Stepien et al., 1992).

The three species of *Gibbonsia* have been shown to differ significantly in temperature tolerances (Davis, 1974, 1977), which appears to be directly related to their present-day ranges. Although *G. montereyensis* and *G. metzi* appear capable of dispersal and a considerable degree of gene flow between populations north of Point Conception, California, and off Baja California, Mexico, has been shown (Stepien and Rosenblatt, 1991), these species are restricted to cooler waters throughout their ranges. Davis (1974, 1977) found that *G. metzi* has the widest temperature tolerance of the three species but has a lower critical thermal maximum than *G. elegans. Gibbonsia elegans* is more tolerant of the warmer temperatures characteristic of its present-day range and least tolerant of cooler temperatures.

Speciation in the North American Myxodini may be related to shifts in coastal temperatures during the Pliocene and early Pleistocene, which may have served as vicariant events. In the hypothesized relationships shown in the tree in Figure 3A, the first event may have separated the ancestral North American clinid stock into two groups: *Heterostichus rostratus* subtidally in the warmer waters south of Point Conception, California, and *G. metzi* intertidally to the north. A second temperature-related event may have resulted in another north/south separation of ancestral *Gibbonsia, G. elegans* in southern waters and *G. montereyensis* in the northern waters.

Variation in coastal current and temperature patterns may maintain gene flow between populations semi-isolated by temperature barriers (Stepien and Rosenblatt, 1991). There is strong support for dispersal and gene flow between widely separated populations of clinids, which are apparently due to their relatively long larval period, although some clinal variation is also evident. Significant differences in temperature tolerances between the species of *Gibbonsia* (Davis, 1977) and the reappearance of northern California clinids in areas of cold-water upwelling off the coast of Baja California, Mexico

(C. Hubbs, 1952; Stepien and Rosenblatt, 1991; Stepien et al., 1991), indicate that distributions of these species are determined by temperature. Also, El Niño patterns may periodically restrict the ranges of the cooler-water species (*G. metzi* and *G. montereyensis*) and expand those of the warmer-water species (*G. elegans* and *H. rostratus*). It is thus likely that speciation events in the Clinidae are related to coastal temperature patterns.

Materials Examined

Species used for allozyme analyses and voucher numbers.—*Heterostichus rostratus* 35 from Mission Bay, San Diego, California (SIO 90–80), 10 from Santa Catalina Island, California (SIO 90–81), 6 from Punta Clara, Baja California, Mexico (SIO 90–82), 5 from the San Benito Islands, Baja California, Mexico (SIO 90–83), and 6 from Guadalupe Island, Baja California, Mexico (SIO 90–84); *Gibbonsia elegans* 63 from Bird Rock, La Jolla, San Diego, California (SIO 90–85), 10 from Punta Clara, Baja California, Mexico (SIO 90–82), 10 from Santa Barbara, California (SIO 90–87), 3 from San Simeon, California (SIO 90–88), 10 from Middle Coronado Island, northern Baja California, Mexico (SIO 90–89), 4 from Pelican Bay, Santa Cruz Island, California (SIO 90–90), 20 from the San Benito Islands, Baja California, Mexico (SIO 90–83) and 13 from Guadalupe Island, Baja California, Mexico (SIO 90–84); *Gibbonsia metzi* 14 from Soberanes Point, Carmel, California (SIO 90–91), 55 from San Simeon, California; and 21 from Punta Clara, Baja California, Mexico (SIO 90–82); *Gibbonsia montereyensis* 10 from Soberanes Point, Carmel, California (SIO 90–82), 38 from San Simeon, California (SIO 90–88), 2 from Santa Barbara, California (SIO 90–87), 4 from middle Coronado Island, Baja California, mexico (SIO 90–89), 18 from Punta Clara, Baja California, Mexico (SIO 90–82), and 12 from Guadalupe Island, Mexico (SIO 90–84); *Myxodes viridis* 46 from Montemar, Viña del Mar, Chile (SIO 87–132); *Heteroclinus whiteleggei* 17 from Pebble Beach, Sydney, Australia (SIO 90–158). Ten labrisomid species were employed as outgroups, including *Alloclinus holderi* 38 from Santa Catalina Island, California, 6 from the Coronado Islands, Baja California, Mexico, and 2 from La Jolla, San Diego, California (SIO 90–159); *Paraclinus integripinnis* 21 from Bird Rock, La Jolla, San Diego, California (SIO 90–157); *Auchenionchus microcirrhis* 5 from Montemar, Viña del Mar, Chile (SIO 87–139); *Paraclinus sini* 12 from San Carlos Bay, Gulf of California, Mexico (SIO 90–160); *Starksia spinipenis* 7 from the Gulf of California, Mexico; *Exerpes asper* 7 from Kino Bay in the Gulf of California, Mexico (SIO 90–160); *Labrisomus xanti* 10 from Puerto Peñasco, Mexico (SIO 90–162); *Malacoctenus hubbsi* 10 from Puerto Peñasco, Gulf of California, Mexico (SIO 90–162); *Neoclinus blanchardi* 2 from La Jolla, San Diego, California (SIO 90–161) and 3 from Monterey, California, and *Mnierpes macrocephalus* 5 from Azuero Peninsula, Panama. Two species of chaenopsids were also employed as outgroups, including *Acanthemblemaria macrospilus* 4 from central Gulf of California, Mexico and *Emblemaria hypacanthus* 4 from Puerto Peñasco, Gulf of California, Mexico.

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