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Correlating hydrographic events and divergence times of speckled dace (Rhinichthys: Teleostei: Cyprinidae) in the Colorado River drainage

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ABSTRACT

We estimated the timing of paleodrainage connections in the Colorado River Basin using mitochondrial deoxyribonucleic acid (DNA) sequence divergences among populations of the speckled dace, *Rhinichthys osculus.* Cytochrome *b* and ND4L sequences were analyzed by maximum likelihood methods to estimate phylogenetic branch lengths, which were calibrated to geological time with a fossil age estimate. We assume that heterogeneity in rate of evolution of mitochondrial DNA is caused in part by differences in body size, temperature, and correlated life-history traits; therefore, branch lengths are used directly to calculate rates of nucleotide substitution and ages of nodes on the phylogenetic tree. *Rhinichthys osculus* is estimated (by the corrected age of the oldest fossil) to have diverged from its sister species at 6.3 Ma. We estimate that speckled dace have been in the Colorado drainage for 3.6 m.y., and they have dispersed through the drainage and to former connectives, such as the Los Angeles Basin, in the past 1.9 m.y. Divergence among lineages of the upper and lower Colorado River drainages (above and below Grand Canyon) is estimated to have occurred ca. 1.9–1.3 Ma. Genetic divergence of allopatric lineages in the lower Colorado River drainage was accompanied by morphological adaptations to different stream gradients, but small genetic distances among these forms indicate recent gene flow and lack of reproductive isolation.

Keywords: molecular clock, biogeography, Great Basin, Los Angeles, fish.

INTRODUCTION

Aquatic biologists and geologists interested in geological history of the Great Basin recognized long ago that patterns of population relationships and drainages correspond to each other in ways that suggest reciprocal hypotheses and explanations (e.g., Blackwelder, 1933; Hubbs and Miller, 1948). Evolutionary relationships often correlate with drainage-basin configuration (Hubbs and Miller, 1948; Hubbs et al., 1974; Taylor, 1985; Liu

and Hershler, 2005; Hershler and Liu, this volume). Noncongruence between hydrographic basin configuration and the distribution pattern of an aquatic species or its populations often suggests a former drainage connection (Jordan, 1928; Minckley et al., 1986). Patterns of morphological similarity of organisms have traditionally been used to infer biological relationships and evolutionary timing, but variable divergence rates have caused erroneous estimates (Smith et al., 2002). Mitochondrial deoxyribonucleic acid (mtDNA) sequences, however, may change at

stochastically constant rates within a taxon and a gene (Kimura, 1994); differences among these mtDNA sequences are therefore useful for estimating the dates of evolutionary branching events among populations and higher taxa. As causes of variation in substitution rates become better understood, the measurement of differences in DNA sequences as a means to estimate chronology of paleodrainage changes will become increasingly useful. In this paper, we explore mtDNA differences among speckled dace and use these data to estimate the timing of Pliocene and Pleistocene hydrographic events that allowed these small, fluvial fishes to disperse and differentiate in the Colorado River drainage.

As far as is known, fish distributions reflect present and former habitats rather than jump dispersal events. The assumption that birds do not disperse desert fish or fish eggs is critical to this interpretation and is supported by nonhaphazard distribution patterns that have persisted for thousands of years: In the western United States and other arid areas, distinctive fish populations are often restricted to small, connected drainages, even within major waterfowl flyways. Great Basin fishes are usually distributed within drainages or among drainages that were formerly connected, such as within the Bonneville or Lahontan Basins, or between the Virgin River and pluvial White River tributaries (Hubbs and Miller, 1948). Temperature extremes and dry air apparently preclude aerial transport of fish or fish eggs, particularly across deserts or mountains. Transplants of game fish by fisheries managers and bait by fishermen may cause analytical problems, but these can often be identified or ruled out with genetic markers of the sort studied in this paper. In some cases, paleohydrographic connections have been proposed based on geologic evidence, and these can be tested by analysis of the relationships and genetic distances among populations that inhabit the separate areas. The relationships among populations provide information about the direction of gene flow or immigration; the genetic distances provide information about time of isolation.

Three topographic events are recognized here as having the potential to split a population on opposite sides of a drainage divide, initiating divergence in DNA. Vicariance is the separation of populations by emergence of a barrier such as a basalt flow or tectonic change in elevation, development of falls, or desiccation of intervening habitat (Hubbs and Miller, 1948). Stream capture results from headward erosion to a point of intersection with a separate drainage, causing a new hydrographic (and biogeographic) connection and eventual severance of the old path of the captured water body (Jordan, 1928). Lake spillovers release water and possibly fishes over a divide, sometimes ceasing when the barrier function of the divide sill is reestablished (Hubbs and Miller, 1948). A fourth form of geographical separation is isolation by distance (Wright, 1938, 1940).

Fish populations are commonly transferred to neighboring streams by stream piracy (Kuehne and Bailey, 1961; Ross, 1972; Jenkins and Burkhead, 1993), which initiates isolation of the captured population from the parent population. Population isolation by desiccation of intervening habitats was described in great detail by Hubbs and Miller (1948), who explicitly used geologi-

cal evidence of the timing of such climatic events as markers for measuring evolutionary rate. Lineages that disperse widely and become separated by geographic distance in a large drainage are important in the Colorado River drainage. Each mode of isolation has the potential to establish separately evolving lineages, and the resulting relationships can be identified in a genetic analysis. Our goal is to estimate the direction of movement and the timing of isolating events, and to test these hypotheses with geologic data.

The molecular clock hypothesis (Gillespie, 1991; Bromham and Penny, 2003), supported by the neutral theory of evolution (reviewed in Kimura, 1994), forms the basis for the wide application of DNA sequencing technology to test hypotheses about timing of divergence of lineages (e.g., Hedges and Kumar, 2002; Sanderson, 2002). The number of nucleotide substitutions in DNA sequences can be measured with precision and applied to estimations of rates and ages of divergence, although some theoretical and methodological problems surrounding calibration and rate heterogeneity remain to be solved. For example, use of recognizable taxa in the fossil record to calibrate evolutionary rates to geologic time is desirable, but the fossil record is incomplete, and it probably never records the first members of a new lineage in a divergence event. The improbability of discovering fossils of the earliest members of a lineage can be mitigated by statistical methods that add confidence limits or a correction factor based on the density distribution of the fossil record (Strauss and Sadler, 1989; Marshall, 1990). Rate heterogeneity among genes, populations, and taxa also causes erroneous time estimates, but the effects of generation time and correlated life-history traits (Martin and Palumbi, 1993) and the mass-specific metabolic rate theory (Gillooly et al., 2005) suggest that rate heterogeneity caused by generation time, temperature, and body size might be treated as deterministic data rather than error to be averaged out or discarded (Estabrook et al., 2007). In this paper, in an attempt to provide a more accurate application of molecular dating to the reconstruction of the relationship between DNA sequence variations and drainage histories, we apply corrections of fossil ages as well as maximum likelihood branch lengths as proxies for corrected estimates of metabolic effects on molecular rates. Habitats, barriers, and aquatic connections among adjacent drainages have shaped phylogenetic relationships, while temperatures of environments, body size, and life-history traits have influenced the rates of evolution of speckled dace since the late Miocene.

The speckled dace is the most abundant and widespread species of fish in the Colorado River drainage (Lee et al., 1980; Fig. 1). They are small (adults are 5–9 cm long) and usually live in flowing water. Some are capable of maintaining station and swimming vigorously in swift currents; these possess morphological adaptations, including streamlined bodies and large, falcate fins. Speckled dace range from near sea level to 1400 m elevation in the north, and from 200 to 2400 m in the south. Our goal is to examine relationships among populations of Colorado River basin speckled dace and test hypotheses about timing of connections among drainages based on new estimates of rates of molecular change.
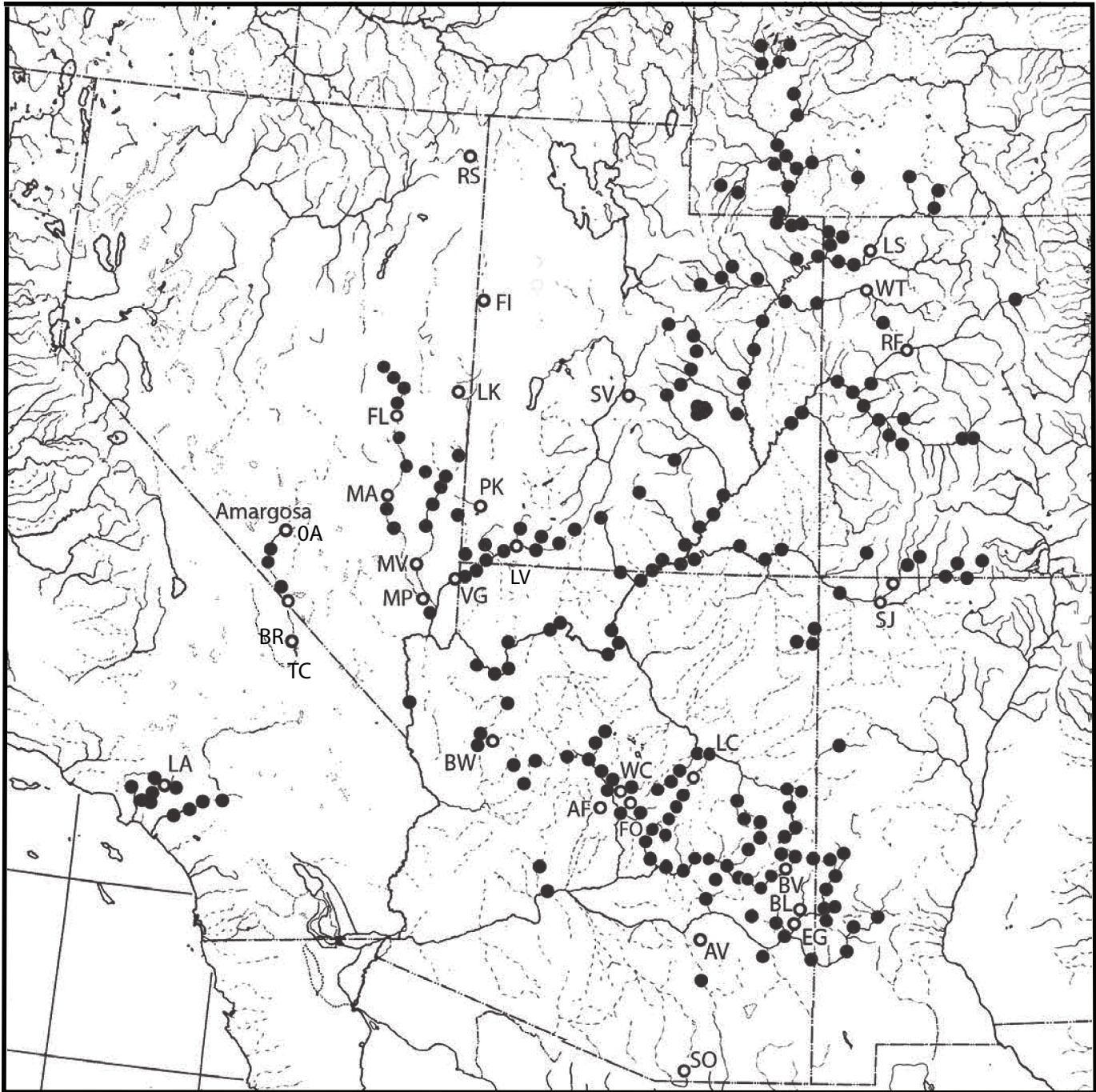
Figure 1. Distribution of *Rhinichthys osculus* (based on museum collections) in the Colorado River Basin and its former connectives (filled circles) and locations of samples used in this study (open circles). Locality abbreviations: Arizona: AF—Agua Fria; AV—Aravaipa Creek; BL—Little Blue River; BV—Beaver Creek; BW—Bill Williams drainage; EG—Eagle Creek; FO—Fossil Creek; LC—Little Colorado River; SO—Sonoita Creek; WC—West Clear Creek. California: LA—Los Angeles Basin; TC—Tecopa (town). Colorado: LS—Little Snake River; RF—Rifle River; WT—White River. Nevada: BR—Bradford Spring; FL—Flag Spring; LK—Lake Creek; MA—Maynard Spring; MV—Meadow Valley Wash; MP—Moapa; OA—Oasis Valley; RS—Rock Spring; VG—Virgin River. New Mexico: SJ—San Juan drainage. Utah: FI—Fish Springs; LV—LaVerkin Creek; PK—Park Valley; SV—Sevier River. (See Methods section for more detailed locality information.)

METHODS

Testing Direction and Timing of Gene Flow and Isolation

Biogeographic histories of populations may be analyzed using the following steps: (1) establish the phylogenetic relationships of the populations of a natural group with genetic (DNA) analysis; (2) establish the points of vicariance or directions of dispersal of the ancestors of lineages of the populations; (3) quantify the genetic distances or phylogenetic branch lengths among populations so that molecular clock methods can provide estimates of time of separation of the lineages; and (4) test the putative timing and direction of the separation of the lineages with geological evidence.

The direction of population transfer is estimated by comparing general versus local patterns of distribution and phylogenetic relationships: the occurrence of an isolated population in a small watershed across a drainage divide from more broadly distributed and diverse relatives usually implies movement of the divide to isolate the former from the latter. Similarly, the occurrence of a locally distinctive population that is phylogenetically embedded within a larger clade that is distributed on the other side of a drainage divide implies capture of the former (and its stream habitat) across the divide, rendering it geographically disjunct from its group of relatives. Putative examples based on fish relationships can usually be tested geologically by looking for barbed tributaries in reversed stream segments (Wheeler and Cook, 1954), abandoned valleys between the captured and former distributary (Smith et al., 1983), and fluvially active young canyons with steep slopes exposing young cosmogenic isotopes (Wolkowinsky and Granger, 2004). Western examples of transfer via stream piracy include the Pleistocene capture of the Zuni mountain sucker from the Rio Grande to Colorado River headwaters in New Mexico (Smith et al., 1983) and Pliocene transfer of three species of minnows (downstream) and one species of sucker (upstream) (Smith et al., 2000) when the upper Snake River was captured by the Columbia drainage through Hells Canyon (Wheeler and Cook, 1954).

Molecular Methods

For this study, samples of *Rhinichthys osculus* were collected from local populations inhabiting Colorado River tributaries; comparative specimens from related populations in the Great Basin, Amargosa River, Los Angeles Basin, and Columbia River drainages (Fig. 1) were drawn from previous work (see following for GenBank numbers). Locality data, including global positioning system (GPS) coordinates and sample sizes are available from Dowling at the Arizona State University Native Fishes Laboratory. Samples collected for this paper are: **Arizona**—Trout Creek, Bill Williams drainage, Mohave County; Sycamore Creek at Forest Service Route 677, Agua Fria River drainage, Yavapai County; West Clear Creek at Bullpen Ranch, Yavapai County; Fossil Creek, Verde River drainage, Gila County; Aravaipa Creek, San Pedro River, Pinal County; Beaver Creek on FR 26, Greenlee County; Eagle Creek, Gila River, Greenlee/Graham County line; Sonoita Creek just off of State Route 82, Santa Cruz County; Little Blue Creek, Greenlee County; Chevelon Creek at Mormon Crossing, Coconino County; **California**—Amargosa River at Tecopa, San Bernardino County; East Fork of San Gabriel River at Los Angeles, Los Angeles County; **Colorado**—Little Snake River at Road (Rd) 26, Moffat County; Rifle Creek, tributary to Colorado River at Rifle, Garfield County; White River at Rd 77, Rio Blanco County; **Nevada**—Flag Springs, Pahranagat Valley, Lincoln County; Maynard Spring, Pahranagat Valley, Lincoln County; Meadow Valley Wash, Clark County; Muddy River, Moapa, Clark County; Virgin River at Mesquite, Clark County; Oasis Valley, Nye County; Bradford Spring, Nye County; Lake Creek, White Pine County; Rock Springs, Elko County; **New Mexico**—Animas River on SR 516 in Aztec, San Juan County; Animas River at Boyd Park Landing in Farmington, San Juan County; **Utah**—La Verkin Creek, tributary to the Virgin River, Washington County; tributary to Fish Springs, Gandy, Snake Valley, Millard County; Park Canyon Creek, Washington County; Salina Canyon Creek, at Salina, Sevier County.

Whole specimens or fin clips were preserved in 95% ethanol or were frozen on dry ice and maintained at –20 °C until use. DNA was obtained from muscle tissue or fin clips as described in Tibbets and Dowling (1996).

DNA fragments for sequencing were obtained by polymerase chain reaction through 20–30 cycles of denaturation at 94 °C for 1 min, annealing at 48 °C for 1 min, and extension at 72 °C for 2 min using pairs of primers LA-HD and LD_{LUX} or LD_{RHINO}-HA for cytochrome *b* (cyt*b*) and primers arg*b*-L and $ND4L_{Agosia}$-H for NADH dehydrogenase 4L (ND4L) in 25 or 50 μL reaction volumes (Gerber et al., 2001). Sequences were obtained with an automated sequencer (either ABI377 or ABI3730) and aligned by eye as described in Gerber et al. (2001). All sequences were deposited in GenBank (DQ990149–DQ990316).

Individuals were drawn at random from various localities and sequenced. Each individual has a single sequence (its haplotype). Variant sequences exist within many populations of *Rhinichthys osculus*, and some of these are sometimes shared among populations. Phylogenetic analysis of the sequence differences among population samples permits us to infer branching of evolutionary lineages and groups, or clades, representing higher-order evolutionary divergence within and between species.

Estimation of Phylogenetic Branching Sequence

An estimation of the rate of evolution depends on accuracy of branch lengths and branching order within a hypothesized phylogenetic tree. We present results of two of the most common methods used to estimate branching pattern and branch lengths, maximum parsimony and maximum likelihood. Parsimony methods use the pattern of shared, derived character states among taxa to estimate branching models that minimize the numbers of steps and ad hoc hypotheses (Farris, 1983; Swofford et al., 1996), but on these trees, branch lengths must be estimated separately by

least squares analysis. Maximum likelihood methods use frequencies of the four nucleotide bases and appropriate gamma distributions to estimate tree topologies, where branch lengths represent the best fit to the pattern of sequence similarities among taxa.

The computer program PAUP (Phylogenetic Analysis Using Parsimony; Swofford, 2002) was used to estimate parsimony trees from mtDNA cyt*b* (1040 base pairs [bp]) + ND4L (297 bp) sequences using full heuristic search and the tree bisection-reconnection (TBR) method with 10 random-addition sequences and uninformative characters excluded. Relative strength of nodes was evaluated by bootstrap analysis (1000 replicates) using full heuristic search and TBR with simple addition. Maximum likelihood trees were generated for the same sequences through heuristic search using the model identified by the Akaike information criterion (GTR + I + G) as implemented by ModelTest 3.06 (Posada and Crandall, 1998); the relative strength of nodes was evaluated by bootstrap analysis (100 replicates).

Estimation of Rates of Molecular Evolution

Amounts of change among taxa were measured by counting the number of base-pair differences in homologous samples of their DNA in the context of a phylogenetic model. Two homologous DNA sequences accumulate differences over time, presumably describable with a Poisson process. The number of actual changes can be used to estimate divergence, *d:*

$$d = -\ln(1 - [n/L]),$$

where *n* is the number of observed changes between a pair of sequences, and *L* is the length of the sequence (Graur and Martin, 2004). Single lineage rate of change over time, *r* (inferred from fossil data, *T* in m.y.), is *r* = (*d*/2*T*).

Measurements of *n* and *L* are no longer significant sources of error because of laboratory and quantitative methods developed by molecular biologists over the past several decades (Gillespie, 1991; Tamura and Nei, 1993). Methods for calculating *d* and *r* are also precise, since there are corrections for many potential biases (Swofford et al., 1996; Nei and Kumar, 2000; Swofford, 2002). Loss of alleles in small populations that were bottlenecked in their history may distort the number of changes (in either direction) and obscure our interpretation of the relation between evolution and time, although in the future, population genetics and coalescent theory may help to recover information about such population histories (Kingman, 1982).

Estimation of Divergence Times from Fossil Ages

The age of first appearance of a lineage is the largest source of error in molecular clock work because it does not necessarily correspond to the age of the earliest known fossil. Divergence time can be more accurately estimated by adding a correction factor based on the frequency distribution of known fossil samples of the taxon being studied, using the method of Strauss and Sadler (1989) and Marshall (1990):

$$\alpha = (1 - C_1)^{-1/(H-1)} - 1,$$

where α is the correction factor expressed as a fraction of the total known stratigraphic range, C_1 is the confidence level, e.g., 50% or 95%, and *H* is the number of horizons in which the taxon occurs (1 m.y. intervals in this study). This development of a statistical method for estimating confidence intervals for fossil ages is an important advancement, but this method assumes a random distribution and equal sampling of stratigraphic horizons—assumptions that are rarely met (Marshall, 1990). Future refinements of fossil age confidence intervals will require methods that also correct for the unevenness of the fossil record (Foote and Raup, 1996; Holland, 1995).

Calibration of divergences with ages of putative geological isolating events, such as inception of falls, desiccation of lakes, or disruption of drainages may lead to underestimation of lineage ages (similar to use of the fossil record) because older geographic variation usually exists in widespread populations (Knowlton and Weigt, 1998). Therefore, any previous genetic isolation by distance will elevate the estimate of genetic distance that is used to calculate the rate. The most serious problem with use of such data is that statistical correction based on sample density is not possible for historically unique geologic events.

Previously, we used calibrations based on earliest known fossils that yielded molecular substitution rates of ~1.1% per m.y. (pair-wise sequence divergence, equivalent to 0.55 per m.y. single-lineage rate) in the mitochondrial cytochrome *b* (cyt*b*) gene of western cyprinids (Smith et al., 2002). This slow (papers in Kocher and Stepien, 1997) rate caused estimation of older events than previously hypothesized, but we erred in two ways: (1) by using the age of the first fossil appearance at face value and (2) by assuming a uniform molecular substitution rate among cyprinid fishes. Although we were concerned about variation correlated with body size and generation time, based on the pioneering work of Martin and Palumbi (1993) (but see Slowinski and Arbogast, 1999), a theoretical basis for interpreting this variation was not available until Gillooly and others applied their mass-specific metabolic rate theory (Gillooly et al., 2001) to rates of molecular substitution (Gillooly et al., 2005). A demonstration of the applicability of the mass-specific metabolic rate hypothesis to molecular rates in mtDNA of cyprinid fishes (Estabrook et al., 2007) suggests that our early work based on fossils of large, northern cyprinid fishes underestimated evolutionary rates in both small-bodied and southern fishes. Small-bodied organisms and those that live in warm temperatures, such as dace (*Rhinichthys*) and pupfish (*Cyprinodon*) have higher metabolic rates, which lead to higher rates of cell division, mutation, and substitution (Gillooly et al., 2005). They also have shorter generation times. Estabrook et al. (2007) demonstrated a threefold difference in substitution rates between large northern and small southern cyprinid fishes. This disparity was concordant with different

climatic histories experienced by different lineages in the Pliocene and Pleistocene. The speckled dace is distributed among all major drainages in the western United States (except the Mojave River) from the Columbia River Basin in southern British Columbia to the southern reaches of the Gila River drainage near Nogales, Sonora. Its single-lineage rate of change in cyt*b* varies from 1.2% per m.y. in the north to 2.5% per m.y. in the southwest.

Several methods have been used to estimate and check rates of substitution in *Rhinichthys*. Cross validation (e.g., Near and Sanderson, 2004) of molecular and fossil estimates of substitution rate in a sample of 54 genera and species of (primarily) western North American cyprinids, 12 of which have fossil records (Smith et al., 2002), showed that *Rhinichthys* has rapidly changing cyt*b* and ND4L, and the single-lineage rate is estimated to be at least 1.3% per m.y. To calibrate our rate estimate, we identified the oldest fossils having *Rhinichthys osculus* characters, which are from the Glenns Ferry Formation on the Snake River Plain (ca. 4–5 Ma). There are only three time horizons containing *Rhinichthys osculus,* so applying Marshall's method at the 50% level to approximate the center of the distribution, an alpha value of 1.8 m.y. yields an estimated time of origin of 6.3 Ma. If we sum the branch lengths from the ancestral node to individual terminals on the *Rhinichthys* phylogenetic tree and divide by 6.3, we calculate a range of rates from 1.2% to 2.5% per m.y. (mean value 1.8% per m.y.). We assume (based on Estabrook et al., 2007; see following) that some of the heterogeneity in rates is attributable to body size and habitat temperature, so individual rates and branch lengths are used directly in the estimation of divergence times (branch lengths divided by rate). That is, by dividing any lineage's branch length, measured from the common ancestor with its sister group, by that lineage's estimated rate, the estimated age of the isolation time of interest can be found. The estimated node age is the average of the two sister lineages, bracketed by their individual ages.

Rate Heterogeneity and Mass-Specific Metabolic Rate

Martin and Palumbi (1993) showed that a suite of correlated traits, including generation time and body size, are correlated with substitution rates in animals. A general model, supported by data from a diversity of organisms (Gillooly et al., 2001, 2005), relates the DNA base substitution rate to metabolic rate:

$$B = bM^{-1/4}\,e^{-E/kT},$$

where the metabolic rate, *B,* is inversely proportional to the negative fourth root of body mass times the Naperian base raised to the power of the quotient (activation energy for biological reactions = 0.65 eV) divided by Boltzmann's constant ($k = 8.62 \times 10^{5}$ eV/K × body temperature [*T*] in K). Larger organisms and those living in colder habitats have slower metabolic rates.

Estabrook et al. (2007) found that estimated metabolic rate significantly ($p = 0.011$) co-occurred with substitution rates among pairs within three clades of 54 genera and species of American cyprinid fishes, which included *Rhinichthys osculus, R. cataractae,* and *R. obtusus* (fishes grow at their environmental temperature). Comparison of predicted versus observed genetic distances demonstrated that among sister pairs, the longer maximum likelihood branch lengths are significantly predicted by smaller body size and warmer temperature. We therefore used the maximum likelihood branch lengths divided by the substitution rate of each lineage pair to estimate ages of hydrographic barriers.

Confidence Estimates

Confidence estimates are often based on standard errors of maximum likelihood branch lengths. However, branch lengths are estimated with so little error that they inappropriately inflate confidence compared to the error associated with the estimated time of first occurrence of a lineage based on fossils. Depending on the temporal distribution of fossils of the taxon of interest, the 95% confidence interval expressed as a fraction of the total stratigraphic range can range from 1900% in the case of a single fossil, to 39% in the case of fossils from 10 different horizons, to 3% in the case of fossils from 100 horizons (Marshall, 1990; Graur and Martin, 2004). In this paper, we provide the standard errors for maximum likelihood branch lengths as well as standard deviations for averages of branch lengths. Neither is entirely suitable since these compound errors have not been investigated statistically.

RESULTS

The maximum likelihood tree with relative branch lengths and bootstrap values is shown in Figure 2, and a consensus of parsimony trees with bootstrap values is shown in Figure 3. (Maximum parsimony analysis yielded 1536 most parsimonious trees of 746 steps, with a consistency index of 0.53, excluding uninformative characters, and a retention index of 0.84.) Figures 2 and 3 show an acceptable level of agreement of major clade composition. The maximum likelihood tree structure (list of taxa and nodes with branch lengths and standard errors) is detailed in Table 1. Table 2 provides estimated lineage rates and ages. Previous work (Oakey et al., 2004) identified a major division within *Rhinichthys osculus* between a Colorado River clade and a sister clade distributed to the north and west (Columbia River drainage, Lahontan and northern Bonneville Basins, northern California, and Owens and Amargosa River drainages). The closest relatives of *Rhinichthys osculus* are *R. cataractae,* which lives to the north and east of the study area, and *R. obtusus,* which is distributed in midwestern North America. These species differ from *R. osculus* by an average of 11% sequence divergence and were estimated to have separated from *R. osculus* at 6.3 Ma, yielding an average divergence rate of 1.8% per m.y. (Table 2). The broader diversity of lineages in the vicinity of the Columbia drainage has contributed to tree hypotheses rooted in that area in both previous analyses (Pfrender et al., 2004; Oakey et al., 2004). The oldest fossil *Rhinichthys* (4.5 Ma) is also found in that region (Snake River Plain).
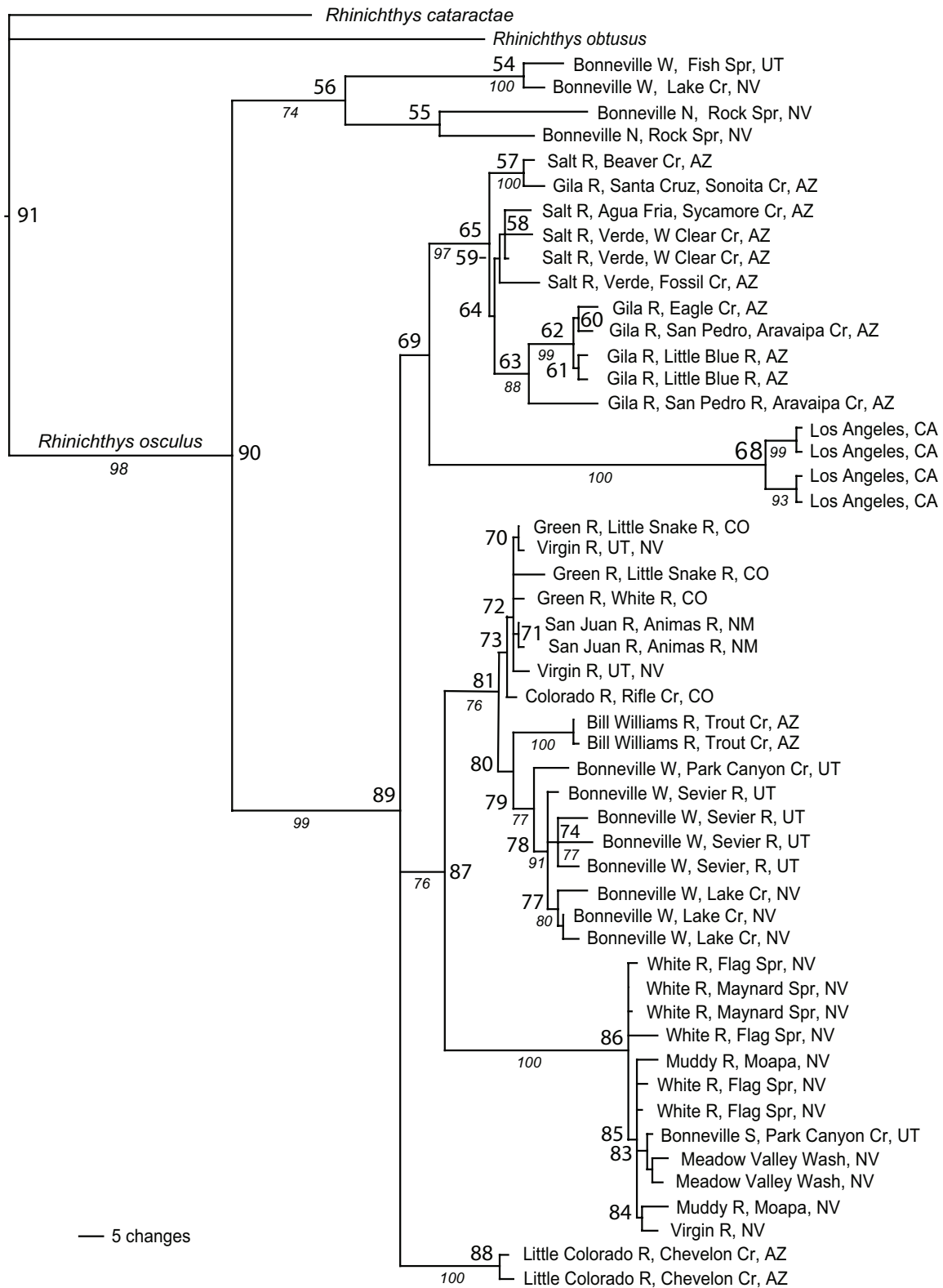
Figure 2. Maximum likelihood tree based on cyt*b* and ND4L sequences (ln likelihood = 5881.45691). Branch lengths are proportional to amount of divergence. Nodes are shown in large Roman type; bootstrap support is shown in small italic type when greater than 70%. See Table 1 for values. Node numbers identify divergence events; estimated node ages are in Table 2.

Figure 3. Bootstrap consensus from maximum parsimony analysis of cyt*b* and ND4L sequences. Bootstrap values at nodes indicate the percentage of 1000 replicates that obtained the exact descendants shown at that node. Only values greater than 50% are shown.

TABLE 1. MAXIMUM LIKELIHOOD TREE FILE FOR *RHINICHTHYS OSCULUS* FROM
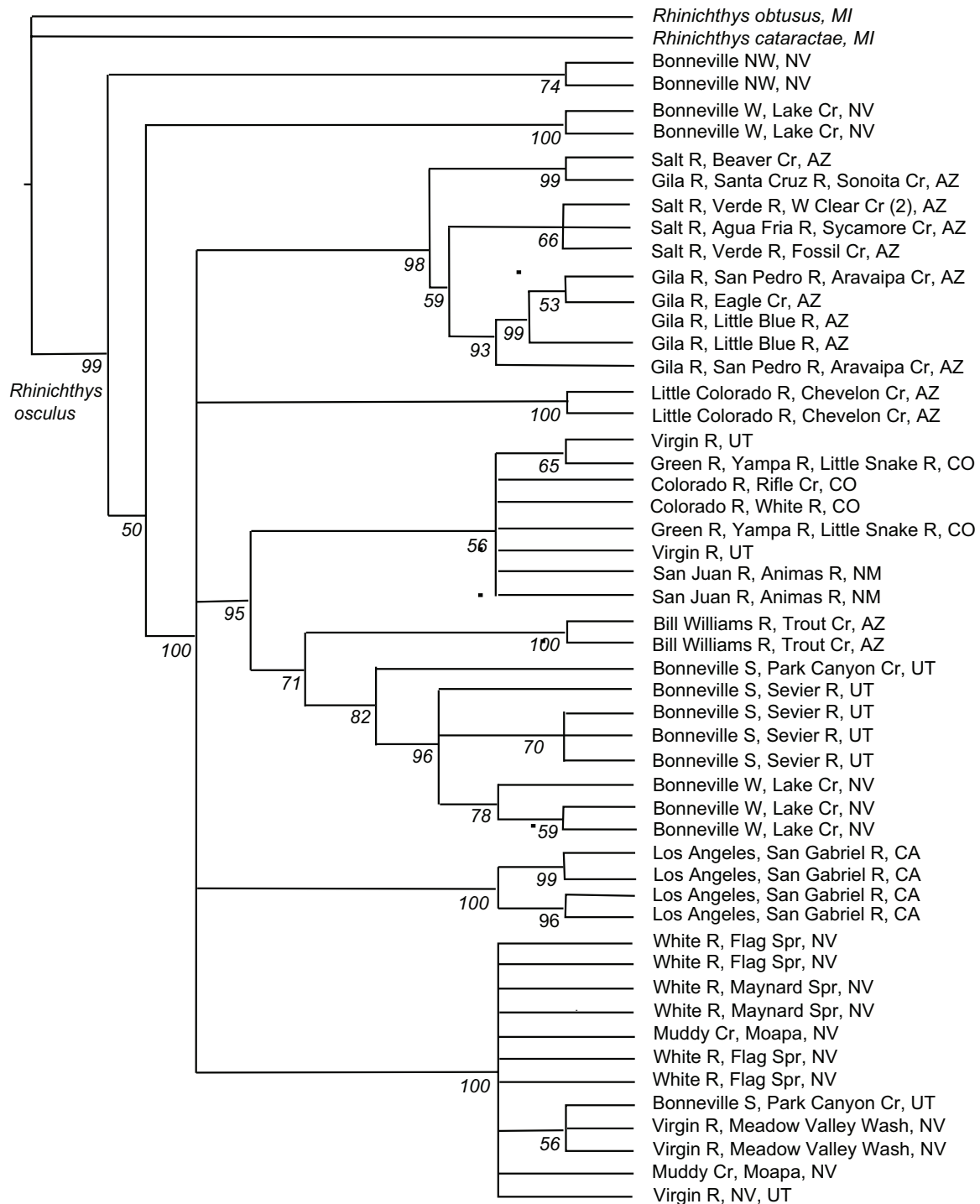THE COLORADO BASIN AND ASSOCIATED DRAINAGES

Sample, state, node	Locality abbreviation	Connect to node	Branch length	Standard error
R. cataractae, MI		91	0.0615	0.011
R. obtusus, MI		91	0.1393	0.019
90		91	0.0380	0.010
56		91	0.0380	0.010
54		56	0.0454	0.008
Bonn, Fish Spr, UT	FI	54	0.0062	0.002
Bonn, Lake Cr, NV	LK	54	0.0027	0.002
55		56	0.0106	0.004
Bonn, Rock Spr, NV	RS	55	0.0235	0.005
Bonn, Rock Spr, NV	RS	55	0.0168	0.004
89		90	0.0446	0.008
69		89	0.0012	0.002
65		69	0.0104	0.003
57		65	0.0043	0.002
Salt R, Beaver Cr, AZ	BV	57	0.0014	0.001
Gila R, Sonoita Cr, AZ	SO	57	0.0029	0.001
64		65	0.0014	0.001
59		64	0.0009	0.001
58		59	0.0007	0.001
Salt R, Agua Fria, AZ	AF	58	0.0036	0.002
Salt R, W Clear Cr, AZ	WC	58	0.0043	0.002
Salt R, W Clea rCr, AZ	WC	58	0.0007	0.002
Salt R, Fossil Cr, AZ	FO	59	0.0058	0.002
63		64	0.0052	0.002
62		63	0.0065	0.002
60		62	0.0007	0.001
Gila R, Eagle Cr, AZ	EG	60	0.0029	0.001
Gila R, Aravaipa CR, AZ	AV	60	0.0022	0.001
61		62	0.0007	0.001
Gila R, L Blue Cr, AZ	LB	61	0.0015	0.001
Gila R, L Blue Cr, AZ	LB	61	0.0014	0.001
Gila R, Aravaipa Cr, AZ	AV	63	0.0109	0.003
68		69	0.0646	0.009
66		68	0.0053	0.002
LA, San Gabriel R, CA	LA	66	0.0008	0.001
LA, San Gabriel R, CA	LA	66	0.0007	0.001
67		68	0.0036	0.002
LA, San Gabriel R, CA	LA	67	0.0007	0.001
LA, San Gabriel R, CA	LA	67	0.0007	0.001
87		89	0.0061	0.003
81		87	0.0061	0.003
73		81	0.0015	0.001
72		73	0.0007	0.001
70		72	0.0007	0.001
Green R, L Snake R, CO	LS	70	0	undefined
Virgin R, NV	VG	70	0.0007	0.001
Green R, L Snake R, CO	LS	72	0.0043	0.002
White R, CO	WT	72	0.0014	0.001
71		72	0.0007	0.001
S Juan R, Animas R, CO	SJ	71	0	undefined
S Juan R, Animas R, CO	SJ	71	0.0007	0.001
Virgin R, NV, UT	VG	72	0.0021	0.001
Colo R, Rifle Cr, CO	RF	73	0	0.002
80		81	0.0022	0.000
74		80	0.0101	0.003
Bill Williams R, AZ	BW	74	0	undefined
Bill Williams R, AZ	BW	74	0.0007	0.001
79		80	0.0022	0.001
Bonn, Park Cn Cr, UT	PK	79	0.0044	0.002
78		79	0.0027	0.001
Bonn, Sevier R, UT	SV	78	0.0014	0.001
75		78	0.0014	0.001
Bonn, Sevier R, UT	SV	75	0.0043	0.002
Bonn, Sevier R, UT	SV	75	0.0050	0.002

(*continued*)

TABLE 1. MAXIMUM LIKELIHOOD TREE FILE FOR *RHINICHTHYS OSCULUS* FROM THE COLORADO BASIN AND ASSOCIATED DRAINAGES (*continued*)

Sample, state, node	Locality abbreviation	Connect to node	Branch length	Standard error
Bonn, Sevier R, UT	SV	75	0.0028	0.001
77		78	0.0014	0.001
Bonn, Lake Cr, NV	LK	77	0.0043	0.002
76		77	0.0007	0.001
Bonn, Lake Cr, NV	LK	76	0	undefined
Bonn, Lake Cr, NV	LK	76	0.0021	0.001
86		87	0.0341	0.005
White R, Flag Spr, NV	FL	86	0.0014	0.001
White R, Maynard, NV	MA	86	0	undefined
White R, Maynard, NV	MA	86	0.0007	0.001
White R, Flag Spr, NV	FL	86	0.0042	0.002
85		86	0.0014	0.001
Muddy R, Moapa, NV	MP	85	0.0028	0.001
White R, Flag Spr, NV	FL	85	0.0014	0.001
White R, Flag Spr, NV	FL	85	0.0007	0.001
83		85	0.0014	0.001
Bonn, Park Cn Cr, UT	PK	83	0.0007	0.001
82		83	0.0007	0.001
Meadow V Wash, NV	MV	82	0.0021	0.001
Meadow V Wash, NV	MV	82	0.0014	0.001
84		85	0.0007	0.001
Muddy R, Moapa, NV	MP	84	0.0035	0.002
Virgin R, NV	VG	84	0.0021	0.001
88		89	0.0180	0.004
Little Colorado R, AZ	LC	88	0.0013	0.001
Little Colorado R, AZ	LC	88	0.0022	0.001

Notes: Sum of branch lengths = 0.6922. –Ln likelihood = 5881.4569. *Rhinichthys cataractae* and *Rhinichthys obtusus* are out-groups. Locality names and node numbers are from Figure 2.

Key: Cn—Canyon; Cr—Creek; L—Little; R—River; Spr—Spring; V—Valley.

The major groups of fish (referred to by drainage) within the Colorado River drainage (nodes 90–89; Fig. 2; Table 2) are (1) Little Colorado River (nodes 89–88); (2) Gila and Salt Rivers (nodes 69–65); (3) Los Angeles Basin (nodes 69–68); (4) upper Colorado River drainage (nodes 87–81); (5) Bill Williams drainage (node 80) and its sister clade in the Bonneville Basin (node 79); and (6) a group of related lineages in the Virgin River, Meadow Valley Wash, White River, and Muddy or Moapa River drainages ("Virgin-White drainages," nodes 87–86; Fig. 2; Table 2).

Examination of Figure 2 reveals considerable heterogeneity of rates, recognizable, for example, as the large differences between the branch lengths from nodes 69 and 89. It is clear that when large rate differences are seen, they tend to be associated with differences in environmental temperature, body size, latitude, and elevation: i.e., the small (body size), lowland Los Angeles Basin lineage evolved much faster from the common ancestor at node 69 than high-elevation Gila and Salt River lineages or Virgin-White River drainage lineages. Furthermore, the Virgin-White River drainage lineages, from southern Nevada and Utah, have accumulated substitutions at a much faster rate than the more northern, higher-elevation lineages from the upper Colorado River drainage and the Bonneville Basin. These differences in rate are consistent with late Cenozoic and present-day climatic differences. The resulting differences in branch lengths form the basis for our estimation of ages of divergence events.

Several relationships are unexpected and require special biogeographic and hydrographic explanations: the close relationship between lineages from the Los Angeles and lower Colorado River drainages (node 69–65 and 68), the derivation of the Bill Williams River lineage plus southern and western Bonneville Basin lineages from upper Colorado River lineages (node 81–80 and 79), and the sister relationship between haplotypes of Meadow Valley Wash and Park Canyon Creek, southwestern Bonneville Basin (node 83). The Amargosa River populations might have been expected to be closely related to populations living in the lower Colorado River drainage (unnumbered nodes at bottom of Table 2) based on the pattern seen in pupfishes (Echelle, this volume), but Amargosa *Rhinichthys* are instead most closely related to Owens and Lahontan drainage lineages.

Early Pliocene *Rhinichthys osculus*

The early split of *Rhinichthys osculus* into a northwest (Columbia–northern Bonneville–northern California) clade and a southeast (Colorado drainage—this study, node 90) clade is estimated to have occurred at 3.6 Ma (Table 2). At that time the western Snake River Plain was occupied by the Pliocene Glenns Ferry lake, which in the late Miocene had emptied westward across Oregon to the Klamath and Sacramento drainages and at other times was connected, perhaps indirectly, to the eastern Great

TABLE 2. ESTIMATED MEAN AGES OF NODES, BRANCH LENGTHS, AVERAGE
RATES, AND STANDARD DEVIATIONS OF AGE ESTIMATES

Node	Population lineages to node	Branch lengths to node	Mean rate per m.y.	Mean age (Ma)	SD
91	Ancestors of *Rhinichthys osculus*	0.1137	0.018	6.3	NA
90	(Colorado) * (Great Basin)	0.0560	0.015	3.6	0.310
89	(Gila, LA) * (Upper Colo)* (LCR)	0.0307	0.018	1.7	0.949
88	LCR Chevelon Creek 1, 2	0.0018	0.014	0.128	0.001
87	(Upper Colo + Bonn) * (middle Colo)	0.0234	0.018	1.3	0.678
86	Muddy Creek + Park, Virgin, polytomy	0.0034	0.020	0.170	0.112
85	Muddy Creek + Park, Virgin, polytomy	0.0029	0.020	0.144	0.072
84	(Moapa) * (Virgin River)	0.0029	0.020	0.143	0.051
83	(Meadow Vally Wash) * (Park)	0.0019	0.020	0.095	0.001
82	Meadow Valley Wash 1, 2	0.0018	0.020	0.087	0.025
81	(Upper Colorado) * (S Bonn)	0.0079	0.016	0.487	0.268
80	(Bill Williams) * (Bonneville)	0.0092	0.017	0.546	0.110
79	(Park) * (Bonneville S, Bonn W)	0.0067	0.017	0.398	0.120
78	(Bonn S) * (Bonn W) polytomy	0.0043	0.017	0.253	0.112
77	(Bonn W 5) * (Bonn W 3, 4)	0.0043	0.017	0.255	0.000
76	Bonneville W 3, 4	0.0004	0.017	0.021	0.001
75	Bonn Sevier River 2, 3, 4	0.0040	0.016	0.253	0.001
74	Bill Williams Trout Creek 1, 2	0.0004	0.017	0.021	0.000
73	(Upper Colo.) * (Rifle River, CO)	0.0024	0.016	0.155	0.070
72	Upper Colo. River polytomy	0.0016	0.016	0.105	0.080
71	(Aztec Creek) * (Animas River) NM	0.0007	0.016	0.045	0.030
70	(Little Snake, CO) * (Virgin, NV)	0.0007	0.016	0.045	0.030
69	(Gila drainage) * (Los Angeles drainage)	0.0355	0.019	1.9	1.180
68	LA, San Gabriel (1, 2) * (3,4)	0.006	0.024	0.229	0.024
67	LA, San Gabriel 3, 4	0.0010	0.024	0.042	0.000
66	LA, San Gabriel 1, 2	0.0010	0.025	0.041	0.000
65	(Beaver, Sonoita) * (Gila, Salt)	0.0126	0.017	0.742	0.440
64	(Salt drainage)* (Gila drainage)	0.0125	0.017	0.728	0.450
63	(Aravaipa 2)* (other Gila)	0.0132	0.018	0.727	0.230
62	(Gila) * (Eagle, Aravaipa 1)	0.0072	0.018	0.398	0.250
61	Gila Little Blue 1, 2	0.0104	0.019	0.548	0.000
60	Gila Eagle Creek, Aravaipa	0.0025	0.018	0.141	0.001
59	(Fossil Creek) * (other Salt drainage)	0.0042	0.016	0.261	0.119
58	Sycamore Creek, W Clear Creek 1, 2	0.0030	0.016	0.188	0.002
57	Gila Beaver Creek, Sonoita Creek	0.0020	0.016	0.125	0.001
	(Lahontan) * (Amargosa)	0.0360	0.016	1.7	0.062
	Amargosa: Jackrabbit, Tecopa	0.0090	0.016	0.540	0.070
	Amargosa: Oasis; Owens River	0.0070	0.016	0.441	0.080

Note: Node numbers are from Figure 2. An asterisk (*) is used to identify the two populations that evolved from the node in column 1.

Key: Bonn—Bonneville Basin; Colo—Colorado River; LA—Los Angeles Basin; LCR—Little Colorado River; S—south; W—west.

Basin (Spencer et al., this volume). The Glenns Ferry lake was captured by a headwater stream at the Oxbow in what is now the head of Hells Canyon of the Snake River (Wheeler and Cook, 1954), probably ca. 2.8–3.0 Ma (Smith et al., 2000). This drainage contained *Rhinichthys* in the Pliocene and had occasional headwater connections to the Lahontan area in the Pliocene and Pleistocene (Reheis et al., 2002; Smith et al., 2002) as well as late Miocene connections to the Cache Valley area, as represented in the Salt Lake Group (McClellan, 1977; Taylor, 1985). In the late Pleistocene, the Bonneville Basin drained to the Snake and Columbia River basin (Gilbert, 1890; Oviatt et al., 1992), providing opportunities for intermingling between the northern Bonneville and upper Snake River fish faunas (Hubbs and Miller, 1948); however, the relationship between the northern Bonneville Basin, Columbia drainage, and northern California speckled dace dates from a much earlier connection (ca. 2.8 Ma, about the time of the Snake River capture; Smith et al., 2000).

Late Pliocene Divergence of Lineages in the Colorado River Drainage

A basal three-way split in the phylogenetic tree (Fig. 2, node 89) suggests divergence among lineages in the upper Colorado River, Little Colorado River, and lower Colorado River drainages ca. 1.9–1.7 Ma (splits at nodes 88, 87, and 69; Fig. 2; Table 2). This implies that populations were mixing in the drainage at times during or prior to this stage of Colorado River history. Discovery of older haplotypes in the upper Colorado drainage in the future would require reevaluation of this hypothesis, but our data imply that the original Colorado drainage speckled dace separated from

the Northwest clade ca. 3.6 Ma (node 90), and divergence of populations in the Colorado drainage began at 1.9–1.7 Ma (node 89). The consensus of the maximum parsimony trees (Fig. 3) conservatively suggests that five major branches of *Rhinichthys*—those from the Gila-Salt, Little Colorado, upper Colorado (and Bill Williams plus southern Bonneville) drainages, plus the Los Angeles Basin, and Virgin-White drainages—date from the period between 1.3 and 1.9 Ma, based on the branch lengths between node 89 and nodes 69, 87, and 88 in Figure 2 and the polytomy (nondichotomous branching) of those clades in Figure 3.

Speckled Dace of the Colorado and Los Angeles River Basins

The biologically and geographically puzzling connection between the Los Angeles Basin speckled dace and its relatives in the lower Colorado drainage (Gila-Salt, Fig. 2) or Virgin-White drainages (Fig. 3) occurred coeval with the divergence of other Colorado River drainage speckled dace (Fig. 1). The timing of colonization of the Los Angeles Basin by speckled dace from the lower Colorado River drainage (Cornelius, 1969) is bracketed here by two very different branch lengths—the long branch to the Los Angeles Basin populations (branch length 7.1%, rate 2.4% per m.y., age estimate 2.96 Ma) and short average branch length from the Gila and Salt lineages (branch length 1.3%, rate 1.9% per m.y., age estimate 0.66 Ma). These suggest an average age estimate of 1.9 Ma, with a standard deviation of 1.18 Ma (Table 2; Fig. 2). The lack of a constrained time estimate and ambiguous relationships leave this problem unresolved (Spencer et al., this volume).

Divergence of lineages scattered among separate drainages within the Gila and Salt River Basins suggests periodic isolation by distance and aridity between ca. 742 and 125 ka (nodes 65–57). The Gila and Salt River lineages have been diverging since ca. 742 ka, as indicated by haplotypes found in Sonoita Creek of the southern Gila drainage and Beaver Creek of the Salt drainage (node 65). The divergence of most Gila and Salt River lineages from each other occurred from a second event dating ca. 728 ka (node 64), possibly as a result of isolation by occasional aridity as well as by distance downstream to the confluence of the Gila and Salt Rivers.

Upper Colorado River and Bonneville Basins

Lineages of the upper Colorado River drainage above Grand Canyon diverged from those of the lower basins (node 87) ca. 1.34 Ma (S.D. = 0.68), much later than the 5.5–5.2 Ma integration of the upper and lower parts of the Colorado River (Spencer et al., this volume). Haplotypes from the upper drainage spread south as far as the Virgin River (nodes 72 and 70) at ca. 105 ka and 45 ka, respectively. Our sampling failed to discover lower or middle basin haplotypes in the upper Colorado drainage.

This portion of the phylogeny depicts an unexpected but significant sister relationship between the southern and western Bonneville Basin lineages and fish from the Colorado River Basin, including the Bill Williams drainage, west-central Arizona

(nodes 81, 80, 79), dating from ca. 487 Ka (S.D. = 287 ka). These exchanges could have been facilitated through the Virgin River–Bonneville Basin pass south of Cedar City, Utah, and the low divide between the Escalante arm of the Bonneville Basin and the headwaters of Meadow Valley Wash near Crestline, Nevada.

The most plausible conduit for colonization of the southern pre–Bonneville Basin from the upper Colorado River drainage was near the Virgin River headwaters at an alluvial-fan dam (current elevation 1670 m, 5480 ft) west of the Hurricane fault, south of Cedar City, Utah. Three kilometers north of Kanarraville, Utah, the canyon axis is directed southwest toward the Virgin River headwaters, but upon emerging from the mouth, the creek bed turns northwest and passes on the north side of its alluvial fan (at 37°33′47″N; 113°10′36″W) toward the Bonneville Basin before turning south again to the Virgin River drainage. The surface of the fan shows evidence of ancient distributary channels directed both northwest and southwest (Hubbs and Miller, 1948, p. 30). This fluctuating drainage pattern might have been responsible for some of the presumed ancient exchanges between the Colorado drainage and Bonneville Basin that are required to explain sister relationships of DNA haplotypes across the Virgin River–Bonneville Basin divide (Hubbs and Miller, 1948; Smith, 1966). The large standard deviation for this divergence estimate spans the time of a highstand of an early lake in the Bonneville Basin (Oviatt, 2002) marked by the 650 ka Lava Creek B ash. Widespread gene exchange is implied by an approximately contemporaneous divergence event (node 80, 546 ka; S.D. = 110 ka) that separated the southern Bonneville lineages from those of the Bill Williams drainage in west-central Arizona.

A variant of a Meadow Valley Wash–Virgin River haplotype is found across the divide in Park Canyon Creek, a tributary to the Escalante arm of the southern Lake Bonneville drainage, downstream from possible capture site near Crestline, Nevada (37°39′48″N, 114°07′33″W, elevation 1826 m [5992 ft]). Inspection of the site on the ground and from the air in 2004 suggests that Sheep Springs Draw, an 8 km tributary, once flowed south to Clover Creek (tributary to Meadow Valley Wash) at the capture site, now the summit of the Union Pacific Railroad grade. Sheep Creek Draw takes an abrupt turn to the northeast and drains to the Escalante Desert of the southern Bonneville Basin. Local topography suggests that it formerly drained from Crestline southeast to Shoal Creek (which receives Park Canyon Creek west of Enterprise, Utah), which is also a tributary to the Escalante Desert.

Speckled dace of the southern and northern portions of Bonneville Basin are genetically distinct from each other and were derived from separate immigration events from southern and northern lineages that diverged ca. 3.6 Ma (Tables 1 and 2). Haplotypes of speckled dace in the Sevier River drainage, Utah (nodes 77, 75), and Lake Creek, Nevada, in the southern Bonneville Basin (nodes 77, 76) diverged from each other ca. 253 ka. A Park Canyon Creek haplotype in the southwest corner of the basin (node 79) diverged from the ancestor of these two ca. 398 ka (S.D. = 120 ka), possibly coincident with regression of one of the several pre-Bonneville lakes (see Oviatt, 2002). Northern

Bonneville Basin haplotypes are distinct, diverse, and not closely related to Colorado River drainage or southern Bonneville Basin haplotypes, although a northern haplotype occurs as far south as Lake Creek, in the same population as the southern haplotype.

Virgin-White River Drainages

Divergence among fishes of the lower Colorado River tributaries in southern Nevada and Utah (White River, Muddy River, Meadow Valley Wash, and Virgin River lineages) began after the basal divergence at 1.3 Ma (node 87). Estimated divergence times among these lineages range from 170 (node 86) to 87 ka (nodes 85, 84, 83, 82; Table 2; Fig. 2). These short divergence times are inconsistent with the considerable morphological divergence (e.g., Fig. 4) among populations of the lower Colorado River drainages, which have been treated as distinct subspecies and species (Hubbs and Miller, 1948; Williams, 1978; Miller, 1984).

DISCUSSION

Dispersal and evolution of diverse lineages of *R. osculus* in the Colorado River drainage are estimated to have occurred within the Pliocene and Pleistocene. Recalibration with a corrected (older) age estimate of 6.3 Ma for the ancestral *R. osculus* split yields rates between 1.2% per m.y. and 2.5% per m.y., resulting in age estimates that range from slightly younger to half as old as previously suggested for small minnows in this area (Dowling et al., 2002; Smith et al., 2002). Recognition that rates of molecular evolution are influenced by habitat temperatures, body size, and generation time permits cross-checking of rates and ages estimated from the metabolic rate equation of Gillooly et al. (2005) and fossil calibration. These two constraints justify direct use of maximum likelihood branch lengths to estimate heterogeneous rates (Fig. 2; Table 2). The corrected fossil age and the direct use of maximum likelihood branch lengths are assumed to increase accuracy of the estimated ages of hydrological connections and isolation events, although confidence limits of fossil ages and rate heterogeneity are still large compared to the precision of substitution counts and the narrow standard errors on maximum likelihood estimates.

We hypothesize that speckled dace colonized the upper Colorado River Basin and began to diverge from their sister lineages in the northern Bonneville and Snake drainages ca. 3.6 Ma (node 90) (Table 2; Figs. 2 and 5). They spread south throughout the upper basin but were perhaps limited in their dispersal through Grand Canyon by the rapids, falls, and lava dams, which were present periodically (Hamblin, 1994; Billingsley, 2001). Access to the Little Colorado River, Virgin River, White River, Meadow Valley Wash, and Gila-Salt drainages (nodes 89, 87) allowed divergence among lineages in these basins to begin at ca. 1.7–1.9 Ma. Divergence among the lineages in the Virgin-White drainage group (node 87) began in the next few hundred thousand years.

Several puzzles still exist. The geographic distribution of the Virgin River–White River group (including pluvial White River, Muddy River, Meadow Valley Wash, and Beaver Dam Wash) of lineages of speckled dace conforms rather closely to the areal extent of the Muddy Creek Formation (Pederson, 2001) (Fig. 1).
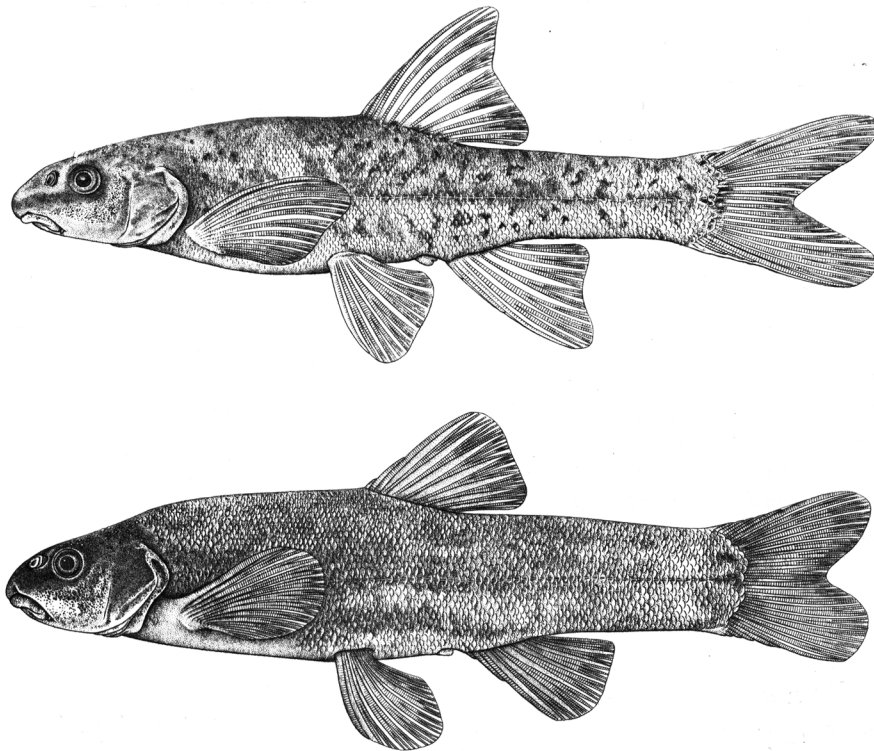

Figure 4. Morphologically divergent forms of speckled dace. Top—streamlined, swift-water morphotype, UMMZ 162761, Salt River, Gila County, Arizona, male, 70 mm standard length with large, falcate fins and slender caudal peduncle. Bottom—robust, slow-water morphotype, UMMZ 162735, Gila drainage, Catron County, New Mexico, male, 66 mm standard length. The two morphotypes occur allopatrically in the lower Colorado River drainage but sympatrically in the Columbia drainage. The robust morphotype also occurs elsewhere.
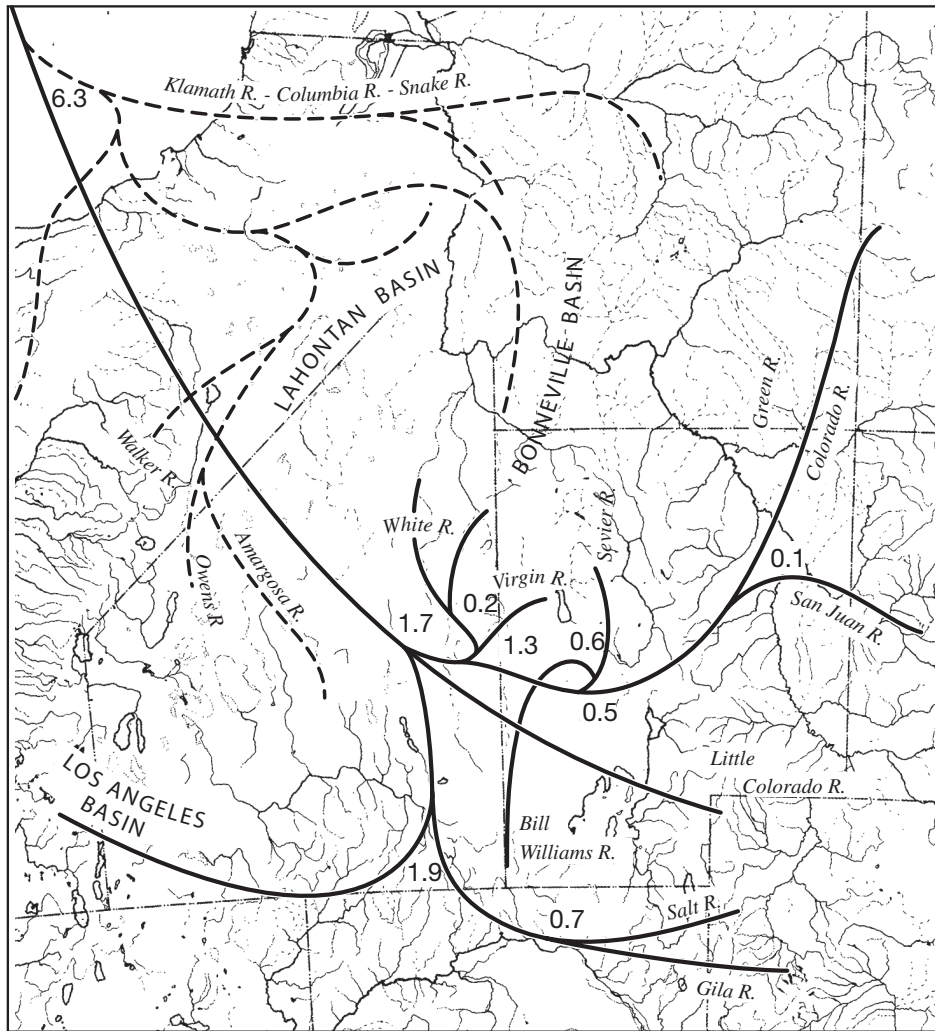
Figure 5. Diagrammatic phylogenetic tree diagram of *Rhinichthys osculus* overlaid on map of the study area showing estimated node ages from Table 2. Dashed lines represent branches in a companion study.

Node ages estimated here (younger than 1.34 Ma) postdate the age of the Muddy Creek Formation (4.5–6 Ma and older; Pederson, 2001). The geographic coincidence of *Rhinichthys* and the drainages that cross the Muddy Creek Formation suggests that fish dispersal was approximately constrained by the same Basin and Range topography that bounded the Muddy Creek Formation upstream and the Grand Canyon downstream. Several species of catostomid and cyprinid fishes are similarly centered on this Basin and Range segment of the Colorado River drainage (Spencer et al., this volume). This shared pattern of distribution suggests that the Grand Canyon has served as an effective barrier to upstream immigration. It is also possible that these fishes (but not their haplotypes) are much older than estimated here, and they coevolved with the Muddy Creek Formation in the Pliocene. Evidence of movement through the Grand Canyon is limited to two minor Pleistocene colonizations from the upper basin to the Virgin River (Fig. 2; Table 2; nodes 70, 72).

Speckled dace in the Los Angeles Basin have been isolated in a uniquely warm, lowland environment throughout the Pleistocene and might be expected to exhibit species-level morphological characters based on their genetic distinctiveness (more than 7% sequence divergence), yet no diagnostic morphological features have been discovered. Plausible dispersal routes from the lower Colorado River drainage to the Los Angeles Basin are obscure (Spencer et al., this volume). Colonization of the Los Angeles Basin from the lower Colorado River drainage might have involved sections of what is now the Mojave River (but not the Amargosa River, see following), although the Mohave River drainage is one of the few western drainages that lack *Rhinichthys*. Surprisingly, speckled dace of the Los Angeles Basin are not closely related to Owens River, Amargosa River, or northern California lineages, which are divergent members of Columbia Basin and Lahontan Basin clades (Table 1). There is considerable haplotype diversity in the sample from the Los Angeles River Basin (San Gabriel River), and estimated divergence times range from 229 ka to 41 ka (nodes 68, 67, 66; Table 2). The long branch leading to the Los Angeles population is apparently the consequence of rapid molecular evolution of these fishes, which are small, have

one-year generations, and have lived in frequent low-elevation warm conditions during the past 1.9 m.y. Their relatives in the Colorado River drainage live in colder waters at higher elevations and attain larger body sizes over 2–4 yr generations.

Morphology of Virgin, White, Gila, and Salt River Speckled Dace

The morphological divergence among Gila River, Salt River, pluvial White River, White River, Muddy River, Meadow Valley Wash, and Virgin River lineages is greater (Fig. 4) than that observed among the rest of the Colorado River drainage clades, including fishes of the Los Angeles Basin. Morphotypes correspond to habitat, not drainage: swift-water forms are streamlined, with large falcate fins and variegated color patterns; slow-water forms are robust, with small, rounded fins and more uniform colors (Fig. 4). These lineages date from the time of basal divergence at 1.3 Ma (node 87), but the observed molecular evolution does not preclude polymorphism present in their ancestors because the same morphotypes are found in the Columbia River drainage (Peden and Hughes, 1988). Estimates of divergence times range from 170 to 87 ka in the Virgin-White River drainage (nodes 86, 85, 84, 83, 82; Table 2; Fig. 2) and 742–261 ka in the Gila-Salt River drainage (nodes 59–65; Table 2). Since morphotypes adapted to swift versus slow currents are found in the Colorado River drainage, Virgin-White River basin streams, and the Columbia River drainage, they might represent polymorphisms that are as old as 3.6 Ma but that have been locally maintained. In the Columbia Basin, these morphotypes have achieved reproductive isolation (Peden and Hughes, 1988) either because the lineages are older or because northwest fluvial systems have been less arid and more hydrologically stable through the Pliocene and Pleistocene. Fluvial fish are distributed at lower elevations in cooler climates of northwestern North America because of temperature, and northern populations of *Rhinichthys* thus tend to be more readily isolated by topographic barriers, while southwestern North American populations are more often isolated by aridity and hydrologic instability (also see Ghalambor et al., 2006).

CONCLUSIONS

Rhinichthys osculus is estimated to have begun to diverge from its sister species ca. 6.3 Ma (Figs. 2 and 5; Table 2). Using this date to calibrate genetic distances among populations and infer ages of barriers in the history of the species, we estimate the time of appearance of these fishes in the Colorado River drainage at ca. 3.6 Ma (Fig. 5). If this calibration is correct, the spread of *R. osculus* from the upper Colorado Basin to the lower basin and then to the Los Angeles Basin probably occurred ca. 1.9–1.7 Ma (Fig. 5). Speckled dace phylogeography suggests a 487 ka connection between the upper Colorado River drainage and the southern Bonneville Basin and a 546 ka connection between the southern Bonneville Basin and the lower Colorado River Basin (Fig. 5). The standard deviations of these estimated ages are broad enough that both are consistent with the 650 ka high lake highstand in the pre–Bonneville Basin (Oviatt, 2002). They are indicative of a wetter climate in which stream captures or overflows might have occurred at saddles on the southern rim of the Bonneville Basin.

Divergence of fast- and slow-water morphotypes (Fig. 4) is substantial among speckled dace of corresponding fluvial habitats in Gila-Salt, White River, Meadow Valley Wash, and Virgin River localities. Although evidence of reproductive isolation among these morphologically specialized dace of the Colorado drainage has not yet been documented, similar morphotypes have been shown to be reproductively isolated from each other in the Pacific Northwest (Peden and Hughes, 1988).

As a result of transfers across drainage divides, the speckled dace of the Colorado River drainage are not monophyletic, that is, they do not form a group of mutually closest relatives restricted to the basin. The most significant barrier dividing genetic lineages within the Colorado River Basin is the Grand Canyon.

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REFERENCES CITED

Billingsley, G.H., 2001, Volcanic rocks of the Grand Canyon area, *in* Young, R.A., and Spamer, E.E., eds., The Colorado River: Origin and Evolution: Grand Canyon, Arizona, Grand Canyon Association Monograph 12, p. 223–229.

Blackwelder, E., 1933, Lake Manly: An extinct lake of Death Valley: Geography Reviews, v. 23, p. 464–471, doi: 10.2307/209632.

Bromham, L., and Penny, D., 2003, The modern molecular clock: Nature Reviews: Genetics, v. 4, p. 216–224, doi: 10.1038/nrg1020.

Cornelius, R.H., 1969, The Systematics and Zoogeography of *Rhinichthys osculus* (Girard) in Southern California [Master's thesis]: Fullerton, California State University, 195 p.

Dowling, T.E., Tibbets, C.A., Minckley, W.L., and Smith, G.R., 2002, Evolutionary relationships of the minnow tribe Plagopterini (Teleostei: Cyprinidae) from cytochrome *b* sequences: Copeia, v. 2002, p. 665–678, doi: 10.1643/0045-8511(2002)002[0665:EROTPT]2.0.CO;2.

Echelle, A.E., 2008, this volume, The western North American pupfish clade (Cyprinodontidae: *Cyprinodon*): Mitochondrial DNA divergence and drainage history, *in* Reheis, M.C., Hershler, R., and Miller, D.M., eds., Late Cenozoic Drainage History of the Southwestern Great Basin and Lower Colorado River Region: Geologic and Biotic Perspectives: Geological Society of America Special Paper 439, doi: 10.1130/2008.2439(02).

Estabrook, G.F., Smith, G.R., and Dowling, T.E., 2007, Body mass and temperature influence rates of mitochondrial DNA evolution in western North American cyprinid fish: Evolution, v. 61, p. 1176–1187.

Farris, J.S., 1983, The logical basis of phylogenetic analysis, *in* Platnick, N.I., and Funk, V.A., eds., Advances in Cladistics, Volume 2: New York, Columbia University Press, p. 7–36.

Foote, M., and Raup, D.M., 1996, Fossil preservation and the stratigraphic ranges of taxa: Paleobiology, v. 22, p. 121–140.

Gerber, A.S., Tibbets, C.A., and Dowling, T.E., 2001, The role of introgressive hybridization in the evolution of the *Gila robusta* complex (Teleostei: Cyprinidae): Evolution; International Journal of Organic Evolution, v. 55, p. 2028–2039.

Ghalambor, C.K., Huey, R.B., Martin, P.R., Tewksbury, J.J., and Wang, G., 2006, Are mountain passes higher in the tropics? Janzen's hypothesis revisited: Integrative and Comparative Biology, v. 46, p. 5–17, doi: 10.1093/icb/icj003.

Gilbert, G.K., 1890, Lake Bonneville: U.S. Geological Survey Monograph 1, 438 p.

Gillespie, J.H., 1991, The Causes of Molecular Evolution: New York, Oxford University Press, 336 p.

Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M., and Charnov, E.L., 2001, Effect of size and temperature on metabolic rate: Science, v. 293, p. 2248–2251, doi: 10.1126/science.1061967.

Gillooly, J.F., Allen, A.P., West, G.B., and Brown, J.H., 2005, Metabolic rate calibrates the molecular clock: Reconciling molecular and fossil estimates of evolutionary divergence: Proceedings of the National Academy of Sciences of the United States of America, v. 102, p. 140–145, doi: 10.1073/pnas.0407735101.

Graur, D., and Martin, W., 2004, Reading the entrails of chickens: Molecular timescales of evolution and the illusion of precision: Trends in Genetics, v. 20, p. 80–86, doi: 10.1016/j.tig.2003.12.003.

Hamblin, W.K., 1994, Late Cenozoic Lava Dams in the Western Grand Canyon: Geological Society of America Memoir 183, p. 331–376.

Hedges, S.B., and Kumar, S., 2002, Vertebrate genomes compared: Science, v. 297, p. 1283–1285, doi: 10.1126/science.1076231.

Hershler, R., and Liu, H.-P., 2008, this volume, Ancient vicariance and recent dispersal of springsnails (Hydrobiidae: *Pyrgulopsis*) in the Death Valley system, California-Nevada, *in* Reheis, M.C., Hershler, R., and Miller, D.M., eds., Late Cenozoic Drainage History of the Southwestern Great Basin and Lower Colorado River Region: Geologic and Biotic Perspectives: Geological Society of America Special Paper 439, doi: 10.1130/2008.2439(04).

Holland, S.M., 1995, The stratigraphic distribution of fossils: Paleobiology, v. 21, p. 92–109.

Hubbs, C.L., and Miller, R.R., 1948, Correlation between fish distribution and hydrographic history in the desert basins of western United States, *in* The Great Basin, with Emphasis on Glacial and Postglacial Times: Bulletin of the University of Utah 38, Biological Series, v. 10, p. 17–144.

Hubbs, C.L., Miller, R.R., and Hubbs, L.C., 1974, Hydrographic History and Relict Fishes of the North-Central Great Basin: Memoirs of the California Academy of Sciences 7, 254 p.

Jenkins, R., and Burkhead, N., 1993, Freshwater Fishes of Virginia: Bethesda, Maryland, American Fisheries Society, 1080 p.

Jordan, D.S., 1928, The distribution of freshwater fishes: Smithsonian Institution Annual Report, v. 1927, p. 355–385.

Kimura, M., 1994, Population Genetics, Molecular Evolution, and the Neutral Theory: Selected Papers (N. Takahata, ed.): Chicago, University of Chicago Press, 704 p.

Kingman, J.F.C., 1982, The coalescent: Stochastic Processes and Their Applications, v. 13, p. 235–248, doi: 10.1016/0304-4149(82)90011-4.

Knowlton, N., and Weigt, L.A., 1998, New dates and new rates for divergence across the Isthmus of Panama: Proceedings of the Royal Society of London, series B, v. 265, p. 2257–2263.

Kocher, T.D., and Stepien, C.A., 1997, Molecular Systematics of Fishes: San Diego, Academic Press, 314 p.

Kuehne, R.A., and Bailey, R.M., 1961, Stream capture and the distribution of the percid fish *Etheostoma sagitta,* with geologic and taxonomic considerations: Copeia, v. 1961, p. 1–8, doi: 10.2307/1440163.

Lee, D.S., Gilbert, C.R., Hocutt, C.H., Jenkins, R.E., McAllister, D.E., and Stauffer, J.R., Jr., 1980, Atlas of North American Freshwater Fishes: Raleigh, North Carolina State Museum of Natural History, 834 p.

Liu, H.-P., and Hershler, R., 2005, Molecular systematics and radiation of western North American nymphophiline gastropods: Molecular Phylogenetics and Evolution, v. 34, p. 284–298, doi: 10.1016/j.ympev.2004.09.013.

Marshall, C.R., 1990, Confidence intervals on stratigraphic ranges: Paleobiology, v. 16, p. 1–10.

Martin, A.P., and Palumbi, S.R., 1993, Body size, metabolic rate, generation time, and the molecular clock: Proceedings of the National Academy of Sciences of the United States of America, v. 90, p. 4087–4091, doi: 10.1073/pnas.90.9.4087.

McClellan, P.H., 1977, Paleontology and Paleoecology of Neogene Freshwater Fishes from the Salt Lake Beds, Northern Utah [Master's thesis]: Berkeley, California, University of California, 243 p.

Miller, R.R., 1984, *Rhinichthys deaconi,* a new species of dace (Pisces: Cyprinidae) from southern Nevada: University of Michigan Museum of Zoology Occasional Papers 707, p. 1–21.

Minckley, W.L., Hendrickson, D.A., and Bond, C.E., 1986, Geography of western North American freshwater fishes: Description and relationships to intracontinental tectonism, *in* Hocutt, C.H., and Wiley, E.O., eds., The Zoogeography of North American Freshwater Fishes: New York, John Wiley and Sons, p. 519–613.

Near, T.J., and Sanderson, M.J., 2004, Assessing the quality of molecular divergence time estimates by fossil calibrations and fossil-based model selection: Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences, v. 359, p. 1477–1483, doi: 10.1098/rstb.2004.1523.

Nei, M., and Kumar, S., 2000, Molecular Evolution and Phylogenetics: New York, Oxford University Press, 333 p.

Oakey, D.D., Douglas, M.E., and Douglas, M.R., 2004, Small fish in a large landscape: Diversification of *Rhinichthys osculus* (Cyprinidae) in western North America: Copeia, v. 2004, p. 207–221, doi: 10.1643/CG-02-264R1.

Oviatt, C.G., 2002, Bonneville Basin lacustrine history: The contributions of G.K. Gilbert and Ernst Antevs, *in* Hershler, R., Madsen, D.B., and Currey, D.R., eds., Great Basin Aquatic Systems History: Smithsonian Contributions to the Earth Sciences, v. 33, p. 121–128.

Oviatt, C.G., Curry, D.R., and Sack, D., 1992, Radiocarbon chronology of Lake Bonneville, eastern Great Basin, USA: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 99, p. 225–241, doi: 10.1016/0031-0182(92)90017-Y.

Peden, A.E., and Hughes, G.W., 1988, Sympatry in four species of *Rhinichthys* (Pisces), including the first documented occurrences of *R. umatilla* in the Canadian drainages of the Columbia River: Canadian Journal of Zoology, v. 66, p. 1846–1856.

Pederson, J.L., 2001, Searching for the pre–Grand Canyon Colorado River: The Muddy Creek Formation north of Lake Mead, *in* Young, R.A., and Spamer, E.E., eds., The Colorado River: Origin and Evolution: Grand Canyon, Arizona, Grand Canyon Association Monograph 12, p. 71–76.

Pfrender, M.E., Hicks, J., and Lynch, M., 2004, Biogeographic patterns and current distribution of molecular-genetic variation among populations of speckled dace, *Rhinichthys osculus* (Girard): Molecular Phylogenetics and Evolution, v. 30, p. 490–502, doi: 10.1016/S1055-7903(03)00242-2.

Posada, D., and Crandall, K.A., 1998, Modeltest: Testing the model of DNA substitution: Bioinformatics (Oxford, England), v. 14, p. 817–818, doi: 10.1093/bioinformatics/14.9.817.

Reheis, M.C., Sarna-Wodjcicki, A.M., Reynolds, R.L., Repenning, C.A., and Mifflin, M.D., 2002, Pliocene to middle Pleistocene lakes in the western Great Basin: Ages and connections, *in* Hershler, R., Madsen, D.B., and Curry, D.R., eds., Great Basin Aquatic Systems History: Smithsonian Contributions to the Earth Sciences, v. 33, p. 53–108.

Ross, R.D., 1972, The Drainage History of the Tennessee River: Blacksburg, Virginia Polytechnic Institute and State University Research Division Monograph 4, p. 11–42.

Sanderson, M.J., 2002, Estimating absolute rates of molecular evolution and divergence times: A penalized likelihood approach: Molecular Biology and Evolution, v. 19, p. 101–109.

Slowinski, J.B., and Arbogast, B.S., 1999, Is there an inverse relationship between body size and the rate of molecular evolution?: Systematic Biology, v. 48, p. 396–399, doi: 10.1080/106351599260364.

Smith, G.R., 1966, Distribution and evolution of the North American catostomid fishes of the subgenus *Pantosteus,* genus *Catostomus:* University of Michigan Museum of Zoology Miscellaneous Publications 129, p. 1–132.

Smith, G.R., Hall, J.G., Koehn, R.K., and Innes, D.J., 1983, Taxonomic relationships of the Zuni mountain sucker, *Catostomus discobolus yarrowi:* Copeia, v. 1983, p. 37–48, doi: 10.2307/1444696.

Smith, G.R., Morgan, N., and Gustafson, E., 2000, Fishes of the Mio-Pliocene Ringold Formation, Washington: Pliocene Capture of the Snake River by the Columbia River: University of Michigan Museum of Paleontology Papers 32, p. 1–47.

Smith, G.R., Dowling, T.E., Gobalet, K., Lugaski, T., Shiozawa, D., and Evans, P., 2002, Biogeography and rates of evolution of Great Basin fishes, *in* Hershler, R., Madsen, D.B., and Curry, D.R., eds., Great Basin Aquatic Systems History: Smithsonian Contributions to the Earth Sciences, v. 33, p. 175–234.

Spencer, J.E., Smith, G.R., and Dowling, T.E., 2008, this volume, Middle to late Cenozoic geology, hydrography, and fish evolution in the American Southwest, *in* Reheis, M.C., Hershler, R., and Miller, D.M., eds., Late Cenozoic Drainage History of the Southwestern Great Basin and Lower Colorado River Region: Geologic and Biotic Perspectives: Geological Society of America Special Paper 439, doi: 10.1130/2008.2439(12).

Strauss, D., and Sadler, P., 1989, Classical confidence intervals and Bayesian probability estimates for ends of local taxon ranges: Mathematical Geology, v. 21, p. 411, doi: 10.1007/BF00897326.

Swofford, D.L., 2002, PAUP*: Phylogenetic Analysis Using Parsimony, Version 4.0b10: Sunderland, Massachusetts, Sinauer Associates.

Swofford, D.L., Olson, G., Waddell, P., and Hillis, D., 1996, Phylogenetic inference, *in* Hillis, D., Moritz, C., and Mable, B., eds., Molecular Systematics (2nd edition): Sunderland, Massachusetts, Sinauer Associates, p. 407–514.

Tamura, K., and Nei, M., 1993, Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees: Molecular Biology and Evolution, v. 10, p. 512–526.

Taylor, D.W., 1985, Evolution of freshwater drainages and mollusks in western North America, *in* Smiley, C.J., ed., Late Cenozoic History of the Pacific Northwest: San Francisco, American Association for the Advancement of Science (Pacific Division), p. 265–321.

Tibbets, C.A., and Dowling, T.E., 1996, Effects of intrinsic and extrinsic factors on population fragmentation in three species of North American minnows (Teleostei: Cyprinidae): Evolution; International Journal of Organic Evolution, v. 50, p. 1280–1292.

Wheeler, H.E., and Cook, E.F., 1954, Structural and stratigraphic significance of the Snake River capture, Idaho and Oregon: The Journal of Geology, v. 62, p. 525–536.

Williams, J.E., 1978, Taxonomic status of *Rhinichthys osculus* (Cyprinidae) in the Muddy River, Nevada: The Southwestern Naturalist, v. 23, p. 511–518, doi: 10.2307/3670257.

Wolkowinsky, A.J., and Granger, D.E., 2004, Early Pleistocene incision of the San Juan River, Utah, dated with ^{26}Al and ^{10}Be: Geology, v. 32, p. 749–752, doi: 10.1130/G20541.1.

Wright, S., 1938, Size of population and breeding structure in relation to evolution: Science, v. 87, p. 430–431.

Wright, S., 1940, Size of populations in relation to speciation: American Naturalist, v. 74, p. 232–248, doi: 10.1086/280891.