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Biogeography of *Cyprinodon* Across the Great Plains-Chihuahuan Desert Region and Adjacent Areas

Christopher W. Hoagstrom* 

Department of Zoology, Weber State University, 1415 Edvalson, Dept. 2505, Ogden, Utah 84408, USA

Megan J. Osborne 

Department of Biology and Museum of Southwestern Biology, University of New Mexico, MSC03 2020, Castetter Hall, Albuquerque, New Mexico, 87131, USA

Abstract

Cyprinodon is renowned for localized endemism across the North American desert. Competing molecular studies have made elucidating timing of diversification across the desert controversial. Debate has focused on Mojave Desert species, with limited evaluation of other evidence. However, the Great Plains and Chihuahuan Desert harbor more taxonomic diversity and are geographically positioned between the Gulf of México (place of origin for the genus) and Mojave Desert, making them central to understanding the evolution of all desert *Cyprinodon*. This study is a detailed assessment of evidence from literature spanning geomorphology, climate, and biogeography vis à vis the mtDNA phylogeny for *Cyprinodon*. Conclusions of Late Miocene-Early Pleistocene diversification are supported across all major clades. Future studies that could improve understanding and address ongoing dilemmas are identified. Importantly, the geography of each clade corresponds to drainage configurations and their evolution through the proposed period of diversification. Eight hypotheses are presented to address major evolutionary events, with emphasis on exploring interpretive challenges within the phylogeny. Broadly, aridity within the Late Miocene apparently facilitated inland invasion of coastal *Cyprinodon* along the ancestral Brazos River and Río Grande. The following Pliocene warm, wet period enabled survival and range expansion through aridland drainages and into adjacent ones. Mio-Pliocene development of the Río Grande Rift and Gila River drainages, causing inter-drainage transfers, was crucial to range expansion. Development of other Gulf of California drainages (Colorado River, Río Yaqui) played peripheral roles. Climatic cooling in the Quaternary Period evidently caused range contractions for populations living at higher latitudes and elevations. Living *Cyprinodon* of the desert represent an incredible legacy of Pliocene range expansion memorialized by subsequent persistence of tenacious endemic populations. Human impacts now threaten this legacy.

Key words: *Cyprinodon*, geomorphology, niche conservatism, reticulate evolution, Pliocene, pluvial lakes, pupfish, speciation, stream capture

The genus *Cyprinodon* (pupfishes) is iconic throughout the North American desert, representing local endemism and adaptation

(Brown 1971; Soltz and Naiman 1978), perhaps most famous for an arsenal of physiological and behavioral abilities, allowing populations to cope

with harsh and variable conditions (Feldmeth et al. 1974; Lema 2008; Tobler and Carson 2010). Despite these qualities, desert *Cyprinodon* are also recognized for vulnerability to extinction through human appropriations of water resources, destruction of aquatic habitats, and introductions of nonnative species (Miller and Pister 1971; Contreras-Balderas and Lozano-Vilano 1996; Echelle et al. 2003). The evolutionary history of the genus has enduring interest because it reveals a legacy of survival writ large across an extensive desert landscape (Miller 1981), providing an evolutionary context for the recent trend of endangerment and extinction. Evidence from *Cyprinodon* is also useful for evaluating geological hypotheses of landscape and river-drainage evolution (Hubbs and Miller 1948; Echelle 2008).

Divergence times based on mtDNA indicate the genus began to diversify in the Late Miocene (Echelle et al. 2005, 2006). However other research contradicts these estimates (Martin et al. 2016), although alternative divergence estimates for the entire genus are unavailable (Martin and Turner 2018). It has been suggested that available geomorphological evidence does not clearly support phylogenetic patterns in the mtDNA gene tree (Echelle 2008; Knott et al. 2008; Martin and Turner 2018), a conclusion apparently based on the difficult question of *Cyprinodon* colonization throughout the Mojave Desert (Echelle 2008; Martin and Turner 2018). To our knowledge, there has been no comprehensive assessment of how well available geomorphological evidence agrees with the mtDNA gene tree outside the Mojave Desert.

Cyprinodon arose in the Gulf of México and adjacent coasts (Echelle and Echelle 2020a). The Great Plains-Chihuahuan Desert region lies immediately west of the Gulf and houses many

Cyprinodon species (Echelle et al. 2003; Hoagstrom et al. 2011). By geographical necessity, this broad region must have held prehistoric portals for colonizations into the Sonoran and Mojave deserts farther inland. Hence, an understanding of *Cyprinodon* evolution across the Great Plains and Chihuahuan Desert is central to understanding diversification of all desert *Cyprinodon*.

Perplexing relations within the *Cyprinodon* mtDNA tree and conflicts with a companion tree based on allozymes seem to produce more questions than answers. Confusing relations exist within the western and Río Nazas clades and conflicts between trees have been hypothesized as instances of reticulate evolution (Echelle et al. 2005; Echelle 2008). We investigate these relations and hypotheses with a synthesis of biogeographical, geomorphic, and climatic evidence. Importantly, this synthesis covers divergence events across the entire *Cyprinodon* phylogeny to ensure interpretations are compatible among clades. Divergence-time estimates in the mtDNA tree are emphasized because although this timing has been questioned for *Cyprinodon* (Martin et al. 2016), phylogeography of other fishes indicates diversification across the region in the Late Miocene-Pliocene (Domínguez-Domínguez et al. 2011; Hoagstrom et al. 2014; Unmack et al. 2014; Schönhuth et al. 2015; Pérez-Rodríguez et al. 2016; Smith et al. 2017), suggesting *Cyprinodon* could have been their contemporaries. Further, while there are several potential causes of mtDNA-allozyme conflicts (e.g., introgression, incomplete lineage sorting, differential selection among genes, sex-biased dispersal), introgression due to secondary contact after a period of allopatry is common among animals (Toews and Brelsford 2012). Indeed, it is prevalent in freshwater fishes

because changing hydrography and climate readily fragment populations and, just as readily, restore contact between former isolates (Wallis et al. 2017). This process has been commonplace across the North American desert (Unmack et al. 2014; Smith et al. 2017).

Molecular analysis is a powerful tool for understanding phylogenetic diversification among taxa. Likewise, geomorphical and ecological evidence can provide critical understanding, and context for molecular data (Craw et al. 2016; Sousa-Santos et al. 2019). Arguably, phylogenetic interpretations that conflict with relevant evidence from geomorphology and ecology are suspect (Renner 2016; Husson et al. 2020) and understanding is strongest when multiple lines of evidence converge, a process called reciprocal illumination (Santos and Capellari 2009).

Much of the *Cyprinodon* phylogeny has not been evaluated in detail against evidence available from fields outside genetics. Hence, we develop a suite of hypotheses intended to clarify present knowledge and focus future studies. Hypotheses are elaborated for a synthetic explanation of prehistoric diversification of *Cyprinodon* across the Chihuahuan Desert, Great Plains, and adjacent areas. By and large, interpretations are fleshed out, updated versions of long-standing hypotheses. However, not since Miller (1981) have available hypotheses been assembled into a comprehensive set. Given much new information, it is important to assess the feasibility of existing hypotheses in light of the much-revised *Cyprinodon* phylogeny and to verify compatibility among hypotheses across the phylogeny.

This study is intended to create a thorough interpretive context to guide future studies and reveal common biogeographical trends within the genus. Importantly, this effort benefits from

enhanced understanding of river-drainage evolution, which provides detailed syntheses of landscape evolution throughout western North America. This includes evolution of major tributary drainages to the Gulf of México (Ewing and Christensen 2016; Snedden and Galloway 2019), development of the Río Grande Rift (Repasch et al. 2017), and integration of the Gila River drainage (Dickinson 2015). We follow the logic of Santos and Capellari (2009) to promote reciprocal illumination as a critical step for assessing strengths and weaknesses of the existing *Cyprinodon* phylogeny.

We do not expect our proposed hypotheses to be the final word on *Cyprinodon* evolution. Although, so far, rigorous fossil evidence is unavailable (Echelle and Echelle 2020a), reevaluating phylogenetic relations across the genus by incorporating nuclear DNA and morphological data (Bagley et al. 2018) and employing genomic approaches (Olave and Meyer 2020) would be excellent next steps. Increased geographical coverage within taxa and sampling more individuals within populations could also provide new insight (Osborne et al. 2016). This would ideally include sampling museum specimens of extinct taxa and populations (Nedoluzhko et al. 2020). The hypotheses presented here are intended to focus such future work.

Methods

Approach

This synthesis is centered on the *Cyprinodon* phylogeny of Echelle et al. (2005, 2006; Echelle 2008). *Cyprinodon* diversification across the desert (Figure 1) appears to have been largely allopatric and peripatric (i.e., with geographical separation during speciation, Echelle et al. 2005).

An *a priori* assumption of geographical separation, used here, allows for development of simple geographical hypotheses in which inter-taxon relations and divergence-time estimates within the *Cyprinodon* phylogeny are treated as hypotheses of where and when ancestral lineages diverged. Geographical relations among taxa are interpreted and assessed with a thorough literature review of evidence available on the evolution of the relevant river drainages and their prehistoric interconnections at the indicated period of time. Information on biogeography of other aquatic or riparian species and evidence of ecological conditions from the same time and place are also considered. Although it is probably impossible to ensure that all relevant information is included, the explicit intent was to conduct a comprehensive literature review.

To develop a realistic sequence of diversification, geological and climatic events of potential relevance to *Cyprinodon* are evaluated for coincident timing of divergence estimates. Echelle et al. (2005) and Echelle (2008) estimated divergence times between *Cyprinodon* species using multidivtime (Kishino et al. 2001). These conservative estimates are used here. *Aphanius*-clock estimates in Echelle et al. (2005) are not used, but are mostly within the ranges of multidivtime estimates. References to geological epochs (e.g., Late Pliocene) follow the Geological Society of America geologic time scale version 5.0 (Walker et al. 2018), except early, middle, and late subdivision of the Pleistocene follow Ehlers et al. (2018).

Reticulate evolution in the form of secondary contact between previously isolated lineages is common within *Cyprinodon* and can be inferred when phylogenies using mtDNA and allozymes are in conflict (Echelle and Echelle 1993; Echelle et al. 2005). These cases provide additional

insight into biogeographical history and drainage evolution. Evidence for secondary contact implies a new connection or re-connection between drainages isolated during a preceding period of divergence.

Study area

River drainages that are the focus of this study are (1) the Red River of the southern Great Plains, major tributary to the Mississippi River of the Gulf of México; (2) the Brazos River, direct tributary to the Gulf of México; (3) the greater Río Grande, direct tributary to the Gulf of México, but with a complex origin including the important and prehistorically disjunct subdrainages: (3a) Pecos River, (3b) Río Salado, (3c) Río Conchos, (3d) Río Grande Rift; (4) Bolsón de los Muertos, base level within an endorheic basin of the southern Río Grande Rift, once connected to the Ancestral Upper Río Grande, presently subdivided into disconnected subdrainages due to low drainage area in an arid climatic region; and (5) Gila River, major tributary to the Colorado River that flows to the Gulf of California.

To reduce confusion, specific terminology is used in two cases. First, the adjective ‘Old’ is used to denote a prehistoric river system that is now essentially defunct (i.e., Old Río Manzano), whereas ‘Ancestral’ is used for a prehistoric version of a modern river system (i.e., Ancestral Pecos River). Second, the term ‘basin’ (‘bolsón’ in Spanish) is used only for structural basins (i.e., Delaware Basin), whereas the term ‘drainage’ is used to represent a river network or watershed.

As a specific example of the second case, terminology used to describe the drainage of the Bolsón de los Muertos is somewhat inconsistent and potentially confusing. Bolsón de los Muertos is a structural basin that also holds the lowest base level for a large part of the endorheic region in the

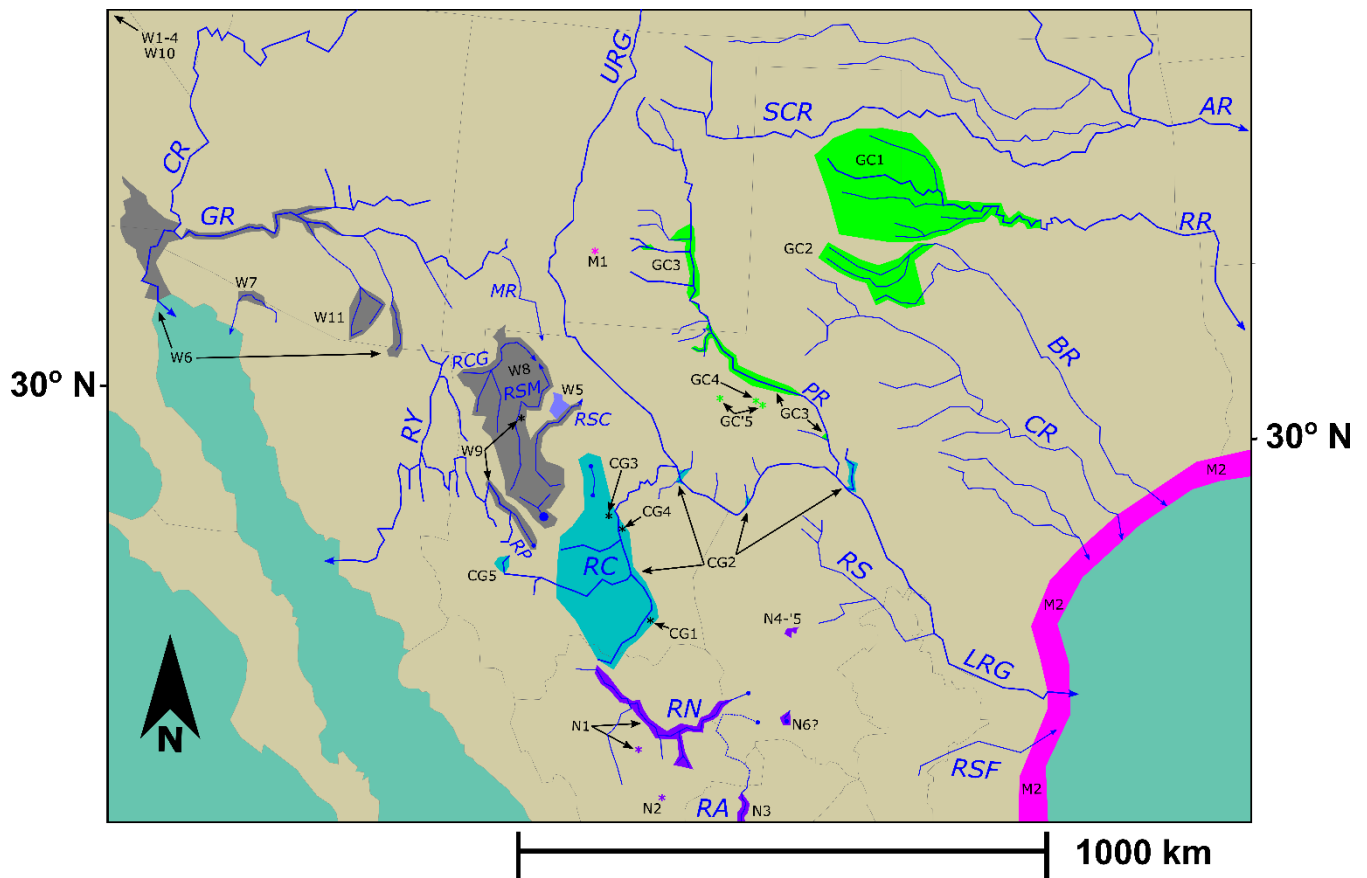


FIGURE 1. Modern hydrography of western North America emphasizing features relevant to *Cyprinodon* inland invasion from the Gulf of México. River drainages (clockwise) are Upper Río Grande (URG), South Canadian River (SCR), Arkansas River (AR), Red River (RR), Brazos River (BR), Colorado River (CR), Pecos River (PR), Río Salado (RS), Lower Río Grande (LRG), Río San Fernando (RSF), Río Aguanaval (RA), Río Nazas (RN), Río Conchos (RC), Río Santa Clara (RSC), Río Santa María, Río Casas Grandes (RCG), Río Papigóchic (RP), Río Yaqui (RY), Mimbres River (MR), Gila River (GR), Colorado River (CR). Shaded areas represent *Cyprinodon* distributions with asterisks for insular populations. Colors indicate clade with individual lineages labeled as follows. **Maritime** (pink): *C. tularosa* (M1), *C. variegatus* (M2); **Great Plains-Chihuahuan Desert** (green): Red River *C. rubrofluvialis* (GC1), Brazos River *C. rubrofluvialis* (GC2), *C. pecosensis* (GC3), *C. bovinus* (GC4), *C. elegans* (GC'5); **Río Nazas** (purple): *C. nazas* (N1), *C. meeki* (N2), *C. sp.* Aguanaval (N3), *C. atrorus* (N4), *C. bifasciatus* (N'5), *C. latifasciatus* (N6?, extinct, presumed in clade based on geography); **Río Conchos-Río Grande** (blue): *C. macrolepis* (CG1), *C. eximius* (CG2), *C. pachycephalus* (CG3), *C. julimes* (CG4), *C. salvadori* (CG5); **western clade subclade** (blue-gray): *C. nevadensis* complex (W1-4, off map); *C. fontinalis* (W5); **western clade subclade** (gray): *C. macularius* (W6), *C. eremus* (W7), *C. pisteri* (W8), *C. albivelis* (W9), *C. radiosus* (W10, off map), *C. arcuatus* (W11, extinct). Labels with apostrophes are for species with different cladal origins but exhibiting mtDNA introgression with their present clades (see text).

southern Río Grande Rift (Hawley 1969; Love and Seager 1996) (Figure 2). Although the drainage of this region is often referred to as the Guzmán Basin, this name strictly applies to Laguna Guzmán, a sub-drainage isolated under arid (non-pluvial) conditions. Under pluvial conditions, Laguna Guzmán either spills into Bolsón de los Muertos or is inundated by its rising lake waters. In driest conditions, the drainage of Laguna Guzmán only receives inflow from Río Casas Grandes (Reeves 1965; Castiglia and Fawcett 2006). In intermediate climates, the tributaries to Laguna el Fresnal (Sabinal Playa, Arroyo Fresnal) and Laguna Santa María (Río Santa María) spill into Laguna Guzmán, but do not flow into El Barreal, the laguna at the center of Bolsón de los Muertos. These distinctions are significant because arid-period subdivisions correspond to divergence among separated populations of endemic fishes (Wood and Mayden 2002; Domínguez-Domínguez et al. 2011; Schönhuth et al. 2011; Lozano-Vilano and De la Maza-Benignos 2017; Corona-Santiago et al. 2018).

In addition, the endorheic drainage of Bolsón de los Muertos is often referred to as the Cabeza de Vaca Basin, which technically applies to a pluvial lake of Pliocene-Early Pleistocene age that was fed by all of the modern tributaries to the Bolsón de los Muertos plus the Ancestral Upper Río Grande (Hawley 1975; Miller 1981; Mack et al. 2006) (Figure 3). Within the time frame relevant to this study, the endorheic drainage of Bolsón de los Muertos was most integrated during the Cabeza de Vaca inundation because the lake reached highest elevations and neighboring Late Pleistocene inundation in the Bolsón de los Muertos without inflows from the Upper Río Grande, which was captured by the Lower Río drainages were most prone to overflow (Axtell 1977). Subsequent pluvial Lake Palomas was a Grande. Thus, pluvial Lake Palomas was less

extensive and lower in elevation than pluvial Lake Cabeza de Vaca. Nevertheless, pluvial Lake Palomas was large enough to integrate El Barreal, Laguna Guzmán, and the Patos Playa fed by Río Santa Clara (Reeves 1965; Hawley 1975; Smith and Miller 1986; Castiglia and Fawcett 2006) (Figure 4).

Synthesis

Hypothesis 1 – Major clades of inland *Cyprinodon* subdivide among Late Miocene river drainages

Inland invasion

The mtDNA phylogeny of Echelle et al. (2005) indicates the genus *Cyprinodon* subdivided into seven clades in the Late Miocene. Five of these have representatives in the North American desert. However, the historical distributions of each clade do not all correspond with modern river drainages. For instance, the Great Plains-Chihuahuan Desert and western clades each inhabit three separate drainages (Figure 1). Meanwhile, the contiguous Río Grande drainage includes representatives from two separate clades. If the greater Río Grande drainage is considered, including formerly connected endorheic drainages (Smith and Miller 1986) (Figures 1-3), then all five clades inhabit this drainage.

The most recent common ancestor (MRCA) of these clades has a presumed coastal origin. Clades invading western North America theoretically used tributaries to the Gulf of México. Ewing and Christensen (2016) and Snedden and Galloway (2019) reconstructed major river drainages of the Late Miocene Gulf of México. As will be described through this synthesis, historical Ancestral Brazos River; (2) Sandia-Potosí clade to Ancestral Río San Fernando; (3) Río Conchos-Río

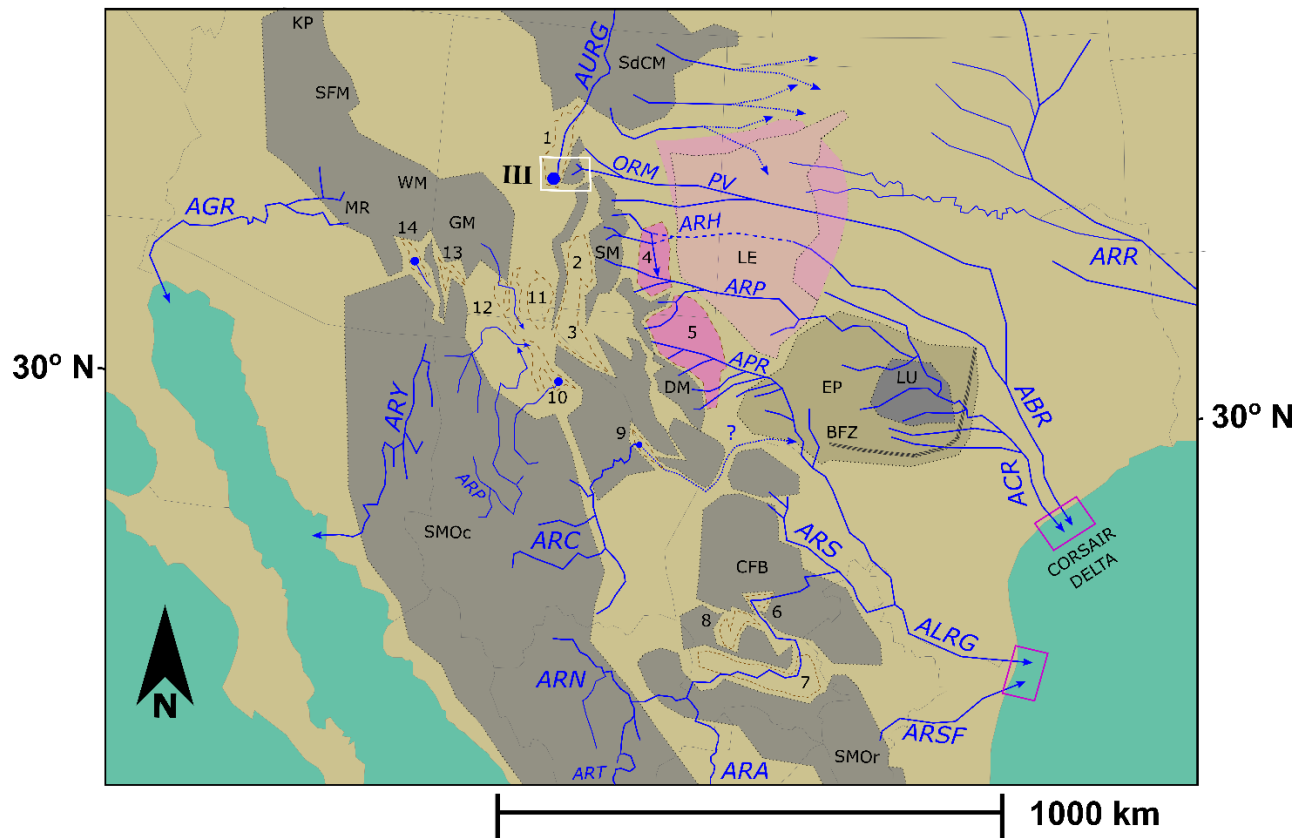


FIGURE 2. Late Miocene hydrography of western North America emphasizing features relevant to *Cyprinodon* inland invasion from the Gulf of México. Primary details are from Snedden and Galloway (2019), Ewing and Christensen (2016), Dickinson (2015), and Repasch et al. (2017) with additional details and references described in accompanying text. River drainages (clockwise) are Ancestral Upper Río Grande (AURG), Old Río Manzano (ORM) including the Portales Valley (PV), Ancestral Río Hondo (ARH), Ancestral Red River (ARR), Ancestral Río Peñasco (ARP), Ancestral Brazos River (ABR), Ancestral Pecos River (APR), Ancestral Colorado River (ACR), Ancestral Río Salado (ARS), Ancestral Lower Río Grande (ALRG), Ancestral Río San Fernando (ARSF), Ancestral Río Aguanaval (ARA), Ancestral Río Tunal (ART), Ancestral Río Nazas (ARN), Ancestral Río Conchos (ARC), Ancestral Río Papigóchic (ARP), Ancestral Río Yaqui (ARY), Ancestral Gila River (AGR). Mountain Ranges and uplifts are Sangre de Cristo (SdCM), Sacramento (SM), Davis (DM), Llano Uplift (LU), Coahuila Fold Belt (CFB), Sierra Madre Oriental (SMOr), Sierra Madre Occidental (SMOc), Gila (GM), White (WM), Mogollon Rim (MR), San Francisco (SFM), Kaibab Plateau (KP). Select structural basins (i.e. bolsóns) relevant to *Cyprinodon* are outlined and numbered: 1 – Albuquerque, 2 – Tularosa, 3 – Hueco, 4 – Roswell, 5 – Delaware, 6 – Cuatro Ciénegas, 7 – Parras, 8 – Sobaco and Hundido (south to north), 9 – Presidio, 10 – de los Muertos, 11 – Mesilla, 12 – Deming and Mimbres (west to east), 13 – Animas-Duncan-Lordsburg, 14 – Safford (with Ancestral San Simon River). Llano Estacado (LE), Edwards Plateau (EP), and the Balcones Fault Zone (BFZ) are also outlined. Pinkish areas indicate a region of salt dissolution with darker areas experiencing most extreme subsidence. Figure depicts diversion of Ancestral Río Hondo into Río Peñasco within the Roswell Basin (dated 11.0–4.5 Ma, Gustavson 1996). White box indicates area featured in Appendix III.

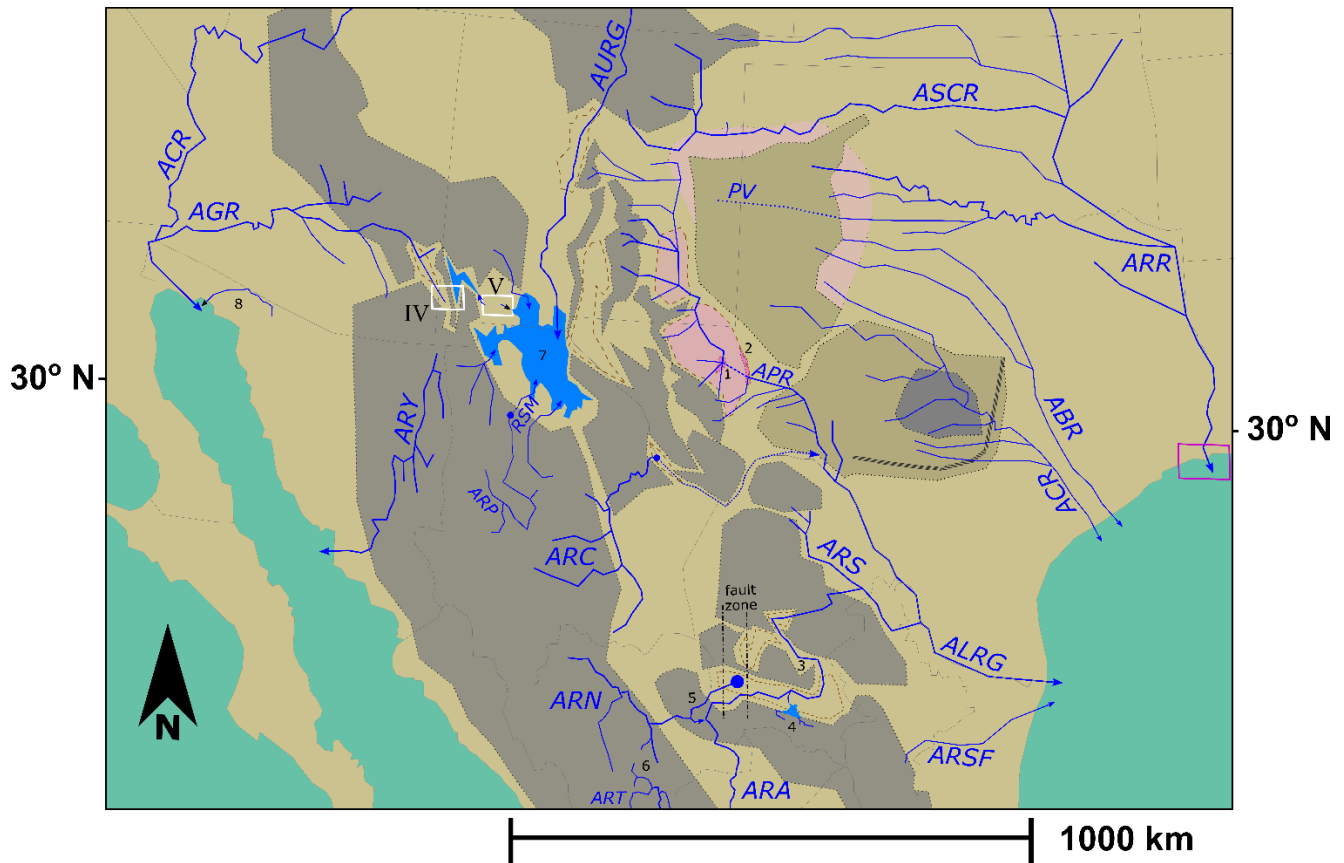


FIGURE 3. Pliocene hydrography of western North America emphasizing features and drainage changes from Late Miocene (Figure 2) most relevant to *Cyprinodon* inland diversification. Primary details follow Figure 2. Only select pluvial lakes are illustrated. Note integration of Pliocene Ancestral Red River (ARR) including emergence of Ancestral South Canadian River (ASCR), development of Ancestral Colorado River (ACR, tributary to Gulf of California), headward extension of the Ancestral Gila River (AGR), and downstream extension of Ancestral Upper Río Grande (AURG). Other important changes are integration of Ancestral Pecos River (APR) and isolation of Ancestral Río Nazas (ARN), possibly attributable to faulting. Llano Estacado became a remnant upland via dissolution subsidence around its margins, causing abandonment of the Portales Valley (PV). New numbered features are: (1) Pecos Trough (Toyah Trough), (2) Monument Draw Trough, (3) San Marcos Valley, (4) Mayrán basin system (ancestral habitat for extinct *C. latifasciatus*), (5) proposed capture of Río Nazas above Cañon de Fernández, (6) Ancestral Río Tunal (ART) transferred to Río Mezquital (Pacific slope), (7) pluvial Lake Cabeza de Vaca, (8) Ancestral Río Sonoyta joining delta of Colorado River at Bahía Adair. White boxes indicate areas featured in Appendixes IV and V.

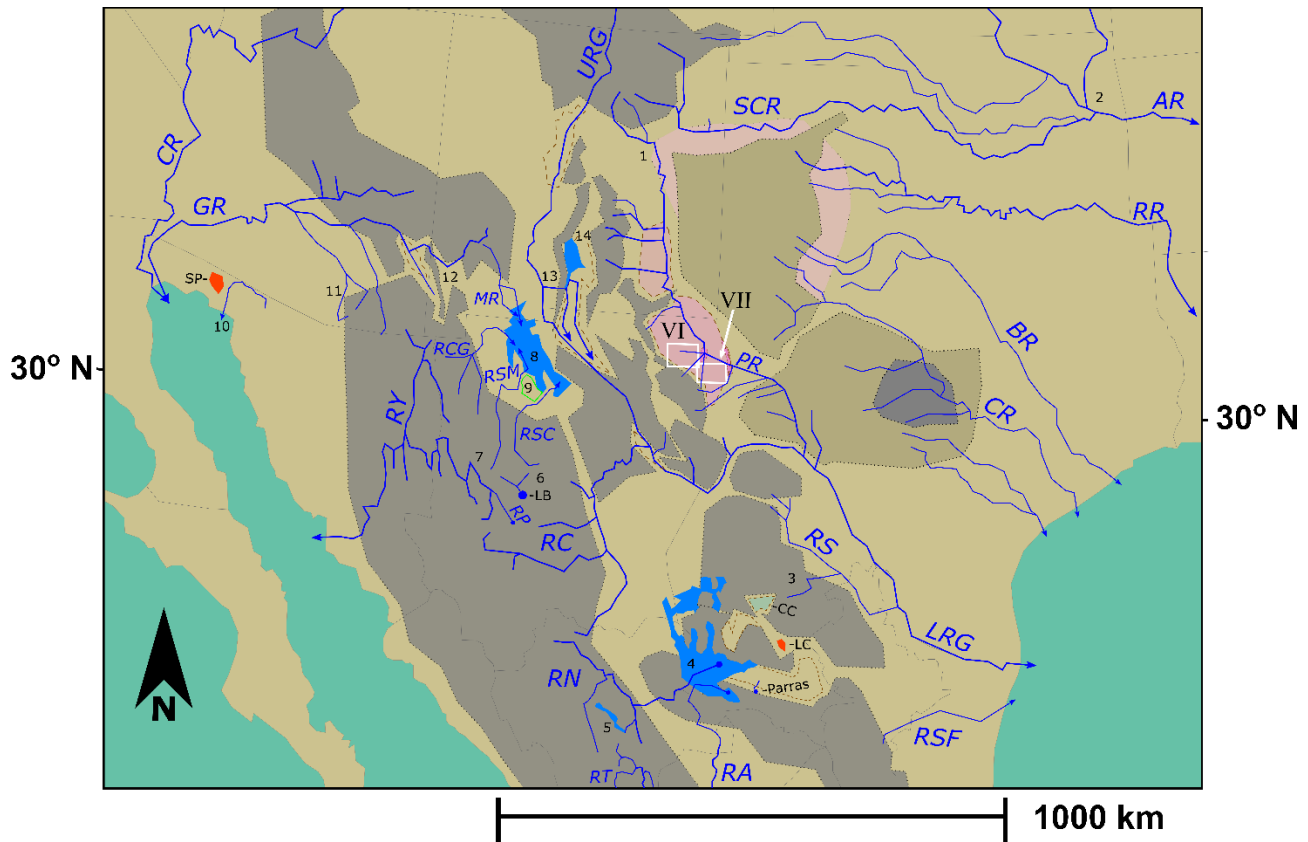


FIGURE 4. Pleistocene hydrography of western North America emphasizing drainage changes from the Pliocene (Figure 3) most relevant to *Cyprinodon* inland diversification. Primary details follow Figures 1 and 2. Note: only select pluvial lakes, not necessarily contemporaries in time, are illustrated. Important events are numbered clockwise: (1) disintegration of Old Río Manzano and integration of upper Pecos River from Sangre de Cristo Mountains, (2) integration of Arkansas River drainage (AR) including capture of South Canadian River (SCR), (3) fragmentation of Río Salado (RS)-Río Aguanaval (RA) drainage due to eruption of Las Coloradas volcanic field (LC) and other regional faulting (Chávez-Cabello et al. 2007) isolating RA, and spring at Parras de la Fuente (Parras); in pluvial conditions, Bolsón de Cuatro Ciénegas (CC) may connect Río Nazas (RN) to Río Salado, (4) pluvial Lake Irritila inundates northwestern Parras Basin and adjacent areas, joins lagunas Mayrán (Nazas) and Viesca (Aguanaul) (Czaja et al. 2014b), possibly spilling into Sobaco Valley, (5) connection of pluvial Lake Santiaguillo with Río Nazas, (6) isolation of Laguna Bustillos (LB) from Río Santa María (RSM), (7) capture of Río Papigóchic (RP) from Río Santa María (RSM) (Figure 3), isolating *C. albivelis* in Río Yaqui (RY) drainage, (8) inundation of pluvial Lake Palomas, (9) isolation of springs inhabited by *C. fontinalis* in Río Bajío de Ojo Caliente drainage (green outline), above shoreline of pluvial Lake Palomas, (10) diversion of Río Sonoyta by Sierra Pinacate (SP) volcanic event (orange polygon) and northwestward shift of Colorado River delta isolate *C. eremus* from *C. macularius*, (11) upper Santa Cruz River, home to extinct *C. arcuatus*, (12) integration of Duncan Valley (formerly part of endorheic basin, Figure 3) into Gila River drainage (GR), (13) flow of Ancestral Upper Río Grande across Fillmore Pass and through Hueco Bolsón provides *C. tularosa* access to Tularosa Basin, (14) pluvial Late Otero, ancestral home for *C. tularosa*, fed by Ancestral Upper Río Grande. In the Holocene, pluvial Lake Palomas desiccated, separating drainages draining to Laguna Guzmán (Mimbres River (MR), ríos Casas Grandes (RCG), RSM) from tributaries draining to the Patos Playa (Río Santa Clara - RSC, Campos-Enriquez et al. 1999). White boxes indicate areas featured in Appendixes VI and VII.

Grande clade to Ancestral Lower Río Grande; and (4) Río Nazas clade to Ancestral Río Salado (Figure 2). The Sandia-Potosí clade is not considered further here because it is not of immediate relevance for *Cyprinodon* penetration into the desert region.

The western clade, which is most remote from the Gulf of México (Figure 1), is enigmatic because it inhabits regions no longer connected to the Gulf of México (Figure 2). If the progenitor of this clade used an aquatic route to reach its present domain, it must have used one of the aforementioned tributaries. Below (Hypothesis 2), a putative scenario is developed for the western clade. Hypothesis 1 focuses on the ancestral Brazos River and Lower Río Grande.

Ancestral Brazos River

The Ancestral Brazos River delivered sediments to the Corsair Delta and extended inland on a northwest trajectory, across the High Plains, to the Manzano Mountains on the eastern flank of the Río Grande Rift (Ewing and Christensen 2016; Snedden and Galloway 2019) (Figure 2). This drainage peaked in area and sediment discharge in the Middle Miocene and declined through the Late Miocene and Pliocene (Ewing and Christensen 2016; Snedden and Galloway 2019).

To explain why *Cyprinodon* invaded western, but not eastern, tributaries to the Gulf of México, Martin (1968, 1972) concluded that competition and predation by freshwater fishes can exclude *Cyprinodon*. Continental populations of *Cyprinodon* typically persist in freshwater systems where they can live sequestered from freshwater fishes, often in evaporative habitats that develop elevated ion concentrations during low-flow periods (Echelle et al. 1972; Hoagstrom and Brooks 1999; Echelle and Echelle 2020a). Nevertheless, representatives are capable of

dispersing among fresher habitats and in some cases, mix with freshwater fishes either in depauperate assemblages such as isolated springs (Minckley 1969) or where alternative habitat conditions allow avoidance of interspecific interactions (Guillory and Johnson 1986).

During the Late Miocene, the Ancestral Brazos River may have provided suitable habitats along its length to allow gradual upstream expansion of *Cyprinodon*. Extreme aridity on the southern Great Plains (Chapin 2008) contributed to the Late Miocene decline of the Ancestral Brazos River (Ewing and Christensen 2016; Snedden and Galloway 2019). Sediment overload likely maintained braided river channels resembling modern rivers. Braided rivers of the plains characteristically support relatively depauperate fish faunas (Matthews 1988), but hold abundant evaporative habitats suitable for *Cyprinodon* (Echelle et al. 1972; Ostrand and Wilde 2004). Upper reaches of the Ancestral Brazos River tap saline groundwater issuing from a broad area of subsurface-salt dissolution (Ewing and Christensen 2016; Figure 2), which boosts ion concentrations downstream, especially in evaporative habitats. This strongly suggests fish faunas in a more arid Late Miocene climate would have been depauperate and evaporative habitats would have been plentiful, ideal for inland invasion by *Cyprinodon*.

Ancestral Colorado River, Gulf of México tributary

A Late Miocene Colorado River extended northwest from the Corsair Delta (shared with the Ancestral Brazos River), across the eastern Edwards Plateau (Hayward and Allen 1987), all the way to the southern Sacramento and northern Guadalupe mountains (Ewing and Christensen 2016). The upper drainage included an Ancestral

Río Peñasco and, by the Early Pliocene, an ancestral Río Hondo (Figure 2). The Ancestral Río Peñasco is now a tributary to the Pecos River, but in the Late Miocene and Early Pliocene continued east across the southern Llano Estacado via the Querecho Plains and Laguna Valley (Nicholson and Clebsch 1961), reaching the North Concho River via Mustang Draw and the, now abandoned, Panther Valley (Frye and Leonard 1964; Byrd 1971; Walker 1978). Although nonnative populations of *Cyprinodon* have been introduced into the upper Colorado River (Ashbaugh et al. 1994), there is no evidence of a prehistoric invasion. Springs were well developed on the Edwards Plateau by the Middle Miocene (Abbott 1975; Veni 2018) and waning sediment delivery to the Gulf of México suggests they were the dominant source of discharge. Spring dominance could explain why Snedden and Galloway (2019) found little evidence for a separate Colorado River in coastal deposits.

Inflow from saline aquifers was presumably present in the headwaters of the Ancestral Colorado River, but discharge from fresher aquifers and an extensive drainage basin on the Edwards Plateau (Woodruff and Abbott 1986) (Figure 2), may have created a barrier to *Cyprinodon* by maintaining more diverse freshwater faunas on the plateau and coastal plain downstream. In addition, *Cyprinodon* appears to avoid canyons and river narrows where evaporative habitats and refuges from flooding are limited (Hubbs and Garrett 1990; Hoagstrom and Brooks 1999). The Colorado River differs from the Brazos River by crossing the Llano Uplift (Figure 2) where resistant bedrock has produced a narrow and incised channel (Heitmüller et al. 2015), suggesting this as another hindrance for *Cyprinodon* invasion. Although the Ancestral Colorado River was a source of fishes to the Pecos

River (Smith and Miller 1986), taxa linking the two drainages (i.e., *Dionda* spp., *Notropis amabilis*-*N. jemezianus*, *Gambusia nobilis* species group, *Etheostoma lepidum* species group) are characteristic of the Edwards Plateau (Hubbs and Springer 1957; Echelle et al. 1984; Schönhuth et al. 2012; Conway and Kim 2016), suggesting this inland uplift as the main source for the fish assemblage in the Late Miocene Colorado River upstream of the Llano Uplift.

Ancestral Lower Río Grande

The length of the Late Miocene Río Grande was truncated compared to the modern version (Snedden and Galloway 2019). Inland from the Río Salado confluence, the Ancestral Lower Río Grande continued northwest only to the Devils and Lower Pecos rivers on the Edwards-Stockton Plateau (Figure 2). The Upper Río Grande (Río Grande Rift) and Middle-Upper Pecos River did not yet exist (Repasch et al. 2017). Possibly, karst conduits connected an Ancestral Río Conchos with the Lower Pecos River (Veni 2009). Like the Brazos River, the Río Grande diminished through the Late Miocene and Early Pliocene (Snedden and Galloway 2019), presumably lowering current velocities, increasing availability of evaporative habitats, and reducing suitability for freshwater fishes, all potentially facilitating inland invasion of *Cyprinodon*.

Hypothesis 2 - Río Grande Rift captures western clade from Ancestral Brazos River

Inland invasion

The pathway inland for the progenitor of the western clade to reach its present distribution (Figure 1) is a conundrum. On the Gulf of México side of the Continental Divide, the clade is historically known from the southern, endorheic

portion of the Río Grande Rift and the adjacent eastern front of the Sierra Madre Occidental. On the Gulf of California side, the clade is known from the Río Yaqui, Gila-Lower Colorado River, Río Sonoyta, and endorheic Owens-Amargosa River drainages (Echelle 2008). Given that (1) the progenitor of all *Cyprinodon* arose 12.6-5.3 Ma, (2) the western clade is estimated at ~6.3 (Echelle et al. 2005) or ~5.4 (Echelle 2008) million years old, and (3) diversification of the western clade was underway by Early Pliocene (Echelle et al. 2005; Echelle 2008), the MRCA of the western clade was theoretically living inland, in isolation from other clades, by the end of the Late Miocene.

The Río Grande Rift is reasonably hypothesized as the initial point of inland isolation because it is the most proximate area to the Gulf of México within the historical range of the western clade (Echelle et al., 2005). However, in the Late Miocene, the Rift was a series of endorheic basins with no aquatic connection to the Gulf of México (Repasch et al. 2017; Snedden and Galloway 2019) (Figure 2). Consistent with this, western clade *Cyprinodon* are distinct from those in the Río Conchos-Río Grande clade, with no evidence for inter-clade secondary contact (Echelle et al. 2005). Thus, although both now inhabit portions of the greater Río Grande drainage, it appears that they have been separated since their original divergence.

Without access via the Ancestral Lower Río Grande, a more northerly route to the rift is most feasible because drainages farther south were confined to eastern slopes of the Sierra Madre Oriental and Mesa del Norte (Snedden and Galloway 2019) (Figure 2), as they are today. This leaves the Ancestral Brazos River as the best candidate for Late Miocene colonization into the Río Grande Rift. Relationships among basal lineages of *Cyprinodon* are unresolved by mtDNA

(Echelle et al., 2005). Additional genetic data is required to critically evaluate alternative hypotheses. Hypotheses considered here are: (1) the Great Plains-Chihuahuan Desert clade of *Cyprinodon* is sister to the western clade and (2) the western clade descends from an earlier invader of the Ancestral Brazos River.

Potential route via Ancestral Brazos River

Without support from molecular data, this hypothesis relies on geomorphic evidence for a Late Miocene connection between the Ancestral Brazos River and the Río Grande Rift. Unlike the Ancestral Lower Río Grande, the Ancestral Brazos River did extend to the Río Grande Rift, reaching the rift-flank Manzano Mountains (Snedden and Galloway 2019). Uplift of the Manzano Mountains 46-20 Ma (Kelley and Chapin 1995; Ricketts et al. 2016) accelerated erosion that contributed to the Ogallala Formation, which blanketed the region east from the mountain front (Kelley 1972; Frye et al. 1982). Sediments traceable to the Manzano Mountains were deposited as far east as the Cross Timbers ecoregion and Lampasas Limestone Cut Plain (present Dallas County, Texas; Byrd 1971; Menzer and Slaughter 1971; Walker 1978). The ancient drainage delivering these deposits is here named Old Río Manzano (after modern Arroyo de Manzano, Appendix III). It flowed east from the Manzano Mountains to the (now abandoned) Portales Valley and thence into the Ancestral Brazos River (Figure 2). If *Cyprinodon* colonized upstream to the foothills of the Manzano Mountains, the population would have lived at the edge of the Albuquerque Basin section of the Río Grande Rift.

Barbed drainages on southeast slopes of the Manzano Mountains are consistent with a hypothesis that a tributary of the Old Río Manzano

once extended along the south side of the Manzano Mountains, draining the eastern fronts of the Manzano and Los Pinos mountains and northern front of Chupadera Mesa (Appendix III). This vicinity shares a relatively low (~2000 m asl) divide with the Albuquerque Basin. Note, the area east of the Manzano Mountains presently holds the endorheic Estancia Valley (Allen 2005), but this did not form until Late Pliocene or Early Pleistocene (Kelley 1972; Allen and Anderson 2000).

Interdrainage transfers of *Cyprinodon* at high elevation (>1500 m asl) most likely occurred between basins rather than over steep divides as *Cyprinodon* rarely occupy steep headwater streams (Echelle and Echelle 1993). High elevation populations within perched valleys and basins are documented for numerous species (Appendix I), in addition to well documented highland populations of *Cyprinodon albivelis* and *C. salvadori* (Minckley and Marsh 2009; Lozano-Vilano and de la Maza-Benignos 2017). High elevation collections from representatives of all inland clades (Appendix I) suggest the MRCA of desert *Cyprinodon* could live at the elevation of the proposed Old Río Manzano capture, especially in a warmer Late Miocene climate. Thus, transfer into the Albuquerque Basin from Old Río Manzano appears to be a good candidate for entry of the MRCA of the western clade into the Río Grande Rift. As discussed below, subsequent climatic cooling could explain the historical absence of *Cyprinodon* from higher elevations at the northern edge of its distribution.

Significantly, faulting that accelerated stream incision in the Albuquerque Basin extended southward to the vicinity of this divide in the Late Miocene (Connell 2004). Subsidence due to faulting should have conferred an erosional advantage to Ancestral Abo Arroyo, allowing it to

advance headward from the Albuquerque Basin into the gap between the Manzano and Los Pinos mountains. Estimated timing of divergence of western clade *Cyprinodon* (~6.3 Ma, Echelle et al. 2005; or ~5.4 Ma, Echelle 2008) is consistent with a hypothesis that Late Miocene stream incision within the Albuquerque Basin led to this capture, transferring some *Cyprinodon* into the Río Grande Rift (Appendix III). Fortuitously, playa lake and fluvial-deltaic habitats, seemingly suitable for *Cyprinodon*, were present in the Late Miocene Albuquerque Basin near the proposed capture zone (Connell 2004). Finally, *Notropis simus simus* is a possible companion taxon in this hypothetical capture. The species is only known from the Middle Pecos River (initially part of the ancestral Brazos River, see below) and Río Grande Rift. Morphological differentiation between Pecos and Río Grande subspecies of *Notropis simus* suggests long isolation (Chernoff et al. 1982), but their date of divergence is unestimated.

Biogeography in Upper Río Grande

A challenge to the above stream-capture hypothesis is the lack of fossil or recent evidence of western-clade *Cyprinodon* in the Upper Río Grande. Although this is problematic, it is possible to envision extinction of the western pupfish clade from the Upper Río Grande after range expansion. In the Early Pliocene, the terminus of the Ancestral Upper Río Grande prograded southward from the Albuquerque Basin and reached the Bolsón de los Muertos, feeding pluvial Lake Cabeza de Vaca (Mack et al. 2006; Repasch et al., 2017) (Figure 3). The incremental process of integration would have allowed gradual southward advance by western clade *Cyprinodon* into the southern Rift, where they persist. Under the relatively warm and less seasonal climate of the Early Pliocene (Salzmann et al. 2011; Ibarra et

al. 2018), conditions throughout the Ancestral Upper Río Grande—between the Albuquerque Basin and pluvial Lake Cabeza de Vaca—were presumably favorable for *Cyprinodon*.

For instance, western-clade *C. albivelis* living in a warmer climate to the south occur in the Río Papigóchic, which was captured by the Río Yaqui (Gulf of California drainage) in the Pleistocene (Figure 4). This was a high elevation transfer and the species persists within an elevated valley (Minckley and Marsh 2009; Lozano-Vilano and de la Maza-Benignos 2017). *Cyprinella formosa* (Wood and Mayden 2002) and *Gila nigrescens* (Osborne et al. 2012; Schönhuth et al. 2014) likely shared this transfer. A population within the Río Santa María drainage (Ojos de Arrey, near Galeana, Gulf of México drainage) is long suspected introduced (Minckley et al. 2002), consistent with slight divergence from the Río Papigóchic (Echelle and Dowling 1992). Similarity of mtDNA haplotypes between *C. albivelis* and *C. pisteri* across the drainage of Bolsón de los Muertos suggests the two species remained allopatric on either side of the Continental Divide until reintroduction of *C. albivelis* (likely by humans) facilitated recent secondary contact.

Isolation in endorheic remnant of Ancestral Upper Río Grande

The Ancestral Upper Río Grande remained separate from the Río Conchos-Lower Río Grande until 1.0-0.8 Ma, when the Upper Río Grande abandoned Bolsón de los Muertos (Mack et al. 2006; Repasch et al., 2017) (Figure 4). At this time, western clade *Cyprinodon* in the Bolsón de los Muertos became isolated from any remaining in the Río Grande. Lack of evidence for secondary contact between the western and Río Conchos-Río Grande clades suggests if any western-clade

populations persisted within the Upper Río Grande, they did not disperse downstream and, similarly, those from the Río Conchos-Río Grande clade did not disperse upstream. This could be because valley incision (Pazzaglia and Hawley 2004; Pazzaglia 2005) caused by a climatic shift to more sustained glaciations 0.1 Ma duration (Ehlers et al. 2018), reduced habitat suitability. Within the Upper Río Grande, periods of cooling, with pluvial environments, would have also lowered water temperatures and diluted river water. Notably, proglacial flooding by spillover of pluvial Lake Alamosa and lakes of the Valles Caldera 0.6-0.4 Ma inundated the valley with cold waters and accelerated valley incision (Pazzaglia and Hawley 2004; Repasch et al. 2017), perhaps causing final elimination of *Cyprinodon*. Failure of Río Conchos-Río Grande clade (Hypothesis 5) and maritime-clade (Hypothesis 8) *Cyprinodon* to colonize the Upper Río Grande from downstream, despite many millennia to do so, supports the inference of unsuitable conditions for *Cyprinodon* within the Quaternary Upper Río Grande. One more challenge to this hypothesis is to explain a lack of Early Pleistocene contact between the western and maritime clades of *Cyprinodon* in the Río Grande Rift. This is considered within Hypothesis 8.

Hypothesis 3 – Gila River drainage captures western-clade *Cyprinodon*

Entry of western-clade *Cyprinodon* into the Gila River drainage is poorly understood. Anthony A. Echelle and colleagues (provisionally) favor a hypothesis of sequential colonization to the Death Valley region by divergent lineages of western-clade *Cyprinodon* (Echelle and Dowling 1992; Echelle and Echelle 1993; Echelle 2008). This most likely occurred from an ancestor that included diverse populations within the Río

Grande Rift, which were weakly divergent for three mtDNA lineages by the time there was access into the Gila River drainage (Echelle and Echelle 1993). Subsequent lineage sorting produced the Amargosa clade, *C. eremus-C. macularius*, and *C. radiosus* as three distinct clades west of the Continental Divide. Similarly, *C. albivelis-C. pisteri* (most closely related to *C. eremus-C. macularius*) and *C. fontinalis* (most closely related to the Amargosa clade) persisted east of the Continental Divide (Figure 5).

Divergence between Gila River and Río Grande Rift lineages is estimated as 4.2-1.5 Ma (Echelle et al. 2005) or ~2.0-1.9 Ma (Echelle 2008). Through the Pliocene, the Ancestral Gila River was truncated compared to the modern drainage, but was head cutting eastward from the Gulf of California, incorporating endorheic basins as it went (Dickinson 2015). Notably, incision of the Gila River into the Safford Basin 3.6-2.2 Ma (Dickinson 2015) corresponds with timing of trans-divide divergence within the western clade (Figure 5), suggesting this facilitated colonization of the Gulf of California drainage. A specific aquatic connection between the Safford Basin and the southern Río Grand Rift is so-far unproposed, but Axtell (1977) suggested highland amphibians and reptiles used low passes through the Peloncillo Mountains. Antelope Pass (traversed by NM Highway 9) is a possible aquatic route. An ephemeral tributary of the San Simon River (Safford Basin) now reaches through the pass into the Animas Valley, with headwaters stretching north and south along the eastern slopes of the Peloncillo Mountains (Appendix IV). Barbed courses of feeder streams suggest this tributary to the San Simon River includes reaches captured from an Animas Valley drainage. Evidence that Safford Basin was rifting and subsiding from the Middle Miocene into the Pliocene (Kruger et al.

1995) opens the possibility that this capture occurred within the time frame necessary to account for entry of *Cyprinodon* into the Gila River drainage. Accordingly, elevated erosion is documented in this period for streams in the adjacent Pinaleño Mountains (Jungers and Heimsath 2016), suggesting erosion was also occurring in Antelope Pass.

Climatic evidence suggests the Late Pliocene environment could have been favorable for aquatic exchange across Antelope Pass. Regional precipitation peaked 3.3-2.9 Ma (Ibarra et al. 2018) and a lake potentially harboring western-clade *Cyprinodon* (Miller 1981) was present in the Animas Valley (Morrison 1965; Axtell 1977). The capture occurred at roughly 1400 m asl (modern elevation), well within the elevational range of western clade *Cyprinodon* (Appendix I). Indeed, Late Pliocene-Early Pleistocene conditions in Safford Basin may have been ideal for *Cyprinodon* because lacustrine habitats deposited salts around the basin (Morrison 1991; Harris 2000), indicating the presence of brackish wetlands. Note, the Middle Pleistocene Animas basalt flow (0.5 Ma, Deal et al. 1978) now occurs across the mouth of Antelope Pass, obscuring Pliocene drainage patterns east of the capture zone (Appendix IV).

Multiple weakly divergent lineages from the western clade theoretically used this route into the Gila River drainage. Whereas *C. radiosus* and the Amargosa clade only survived peripheral to the Colorado River drainage (Echelle 2008), *C. eremus-C. macularius* lineage apparently remained widespread within the Gila-Lower Colorado River drainage. Presence of *C. eremus* in Río Sonoyta, a drainage separate from the Gila-Colorado River, is generally attributed to diversion of Ancestral Río Sonoyta by eruption of the Pinacate Volcanic Field (Hubbs and Miller

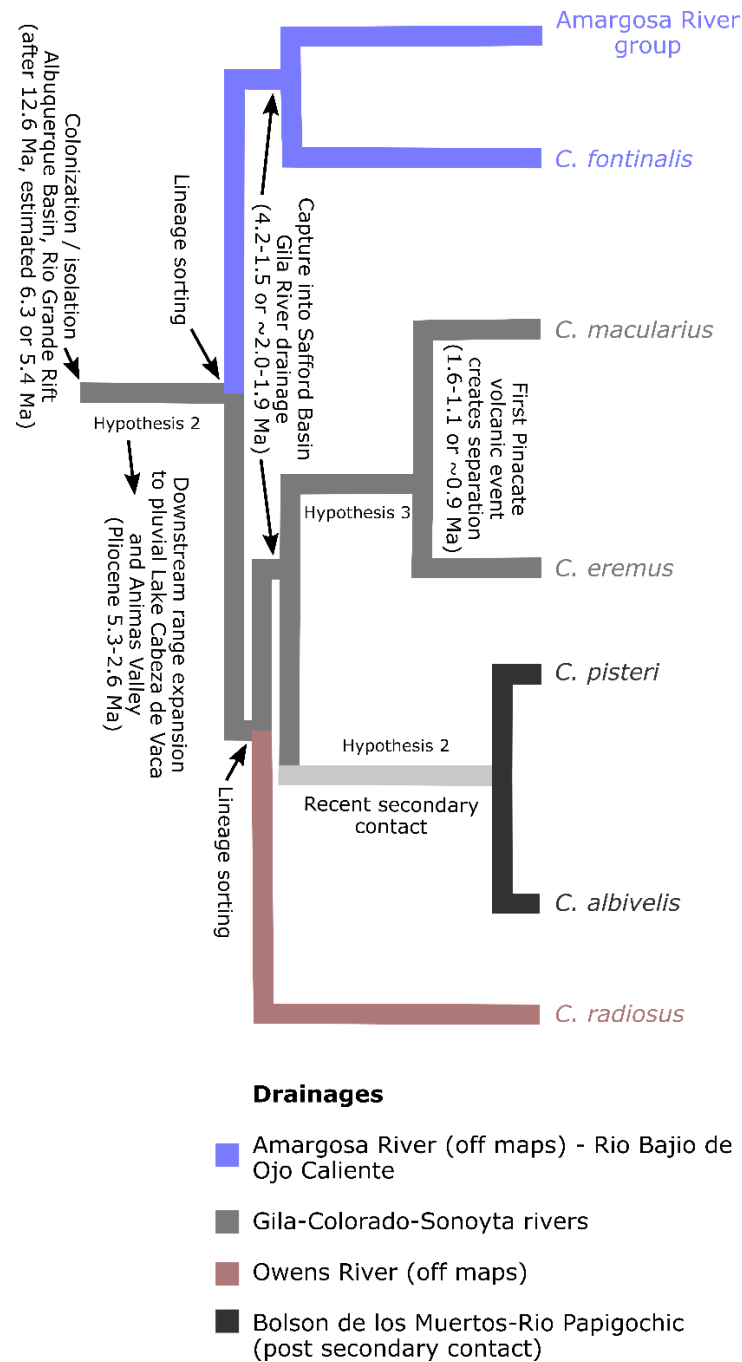


FIGURE 5. Reconstructed mtDNA tree for western clade *Cyprinodon* from chronogram of Echelle et al. (2005), annotated with biogeographical interpretation. Hypotheses relevant to phylogenetic branches are noted, with additional details provided in text. Branch and taxon colors correspond to major river drainages. Divergence dates are from Echelle et al. (2005) and Echelle (2008). Pliocene dates are from the Geological Society of America time scale version 5.0. Amargosa River group includes *C. diabolis*, *C. nevadensis* species complex, and *C. salinus*.

1948; Minckley and Marsh 2009). Before the Pinacate volcanic event, the Ancestral Río Sonoyta continued southwest to Bahía Adair in the Altar basin (Ives 1936) (Figure 3), which also received the Ancestral Colorado River 4.3–3.3 Ma (Howard et al. 2015). These river deltas were proximate and may have connected, but this preceded estimated entry of the *C. eremus*–*C. macularius* into the Gila River drainage. By 3.3 Ma, the Colorado River delta began to retreat inland, so it is likely the MRCA of *C. eremus*–*C. macularius* dispersed some distance along the coast to reach the Ancestral Río Sonoyta. Divergence estimates for *C. eremus* from *C. macularius*, 1.6–1.1 Ma (Echelle et al. 2005) or ~0.9 Ma (Echelle 2008), indicate Early Pleistocene divergence (Figure 5). This corresponds with the 1.7–1.1 Ma first phase of the Pinacate volcanic event (Rodríguez-Trejo et al. 2019), which blocked the Ancestral Río Sonoyta. The distance between the two deltas also increased by gradual northward retreat of the Colorado River delta, which is now ~150 km up the coast from the Río Sonoyta delta.

Cyprinodon in the Animas Valley

A weakness in this hypothesis is that no *Cyprinodon* are known from the drainage of the Pliocene Animas Valley, which was outside the Ancestral Upper Río Grande drainage (Miller 1981; Dickinson 2015). Thus, before transfer into the Gila River drainage, *Cyprinodon* first needed access into the Animas Valley, which included the Animas, Duncan, Virden, and Lordsburg basins (Axtell 1977; Dickinson 2015). The Ancestral Upper Río Grande terminated into pluvial Lake Cabeza de Vaca (Mack et al. 2006), which incorporated basins immediately east of the Animas Valley drainage (Figure 3). At its maximum (Figure 3), the drainage of pluvial Lake

Cabeza de Vaca completely flooded the Bolsón de los Muertos and extended into the Columbus, Deming, Hachita, Mesilla, Mimbres, and Playas basins (Axtell 1977). A Cabeza de Vaca–Animas connection is most feasible at the Separ Trough on the divide between the Deming and Lordsburg basins (Appendix V). Axtell (1977) proposed Separ Trough as a corridor for semiaquatic amphibians and reptiles and Miller (1981) mapped potential inter-basin exchange of *Cyprinodon* there (without elaboration). Fleshing out these preliminary observations (Appendix V), the divide is flat, with a string of shallow depressions across it linking Seventy-six Draw (Lake Cabeza de Vaca) and Burro Ciénega (Animas Valley). Periods of flooding across the divide (presumably more common during the aforementioned Pliocene wet period) or east-west avulsions by radial drainages discharging to the trough possibly could have allowed *Cyprinodon* into Animas Valley via Burro Ciénega. After transfer into the Gila River drainage, populations in the Animas Valley could have succumbed to Quaternary climatic cooling as winter precipitation came to dominate local hydrology, contrasting with dominance of summer monsoons in pluvial Lake Palomas (Scuderi et al. 2010), where the clade persists. Presence of *C. albivelis* and *C. salvadori* (Lozano-Vilano 2002; Minckley and Marsh 2009; Lozano-Vilano and De la Maza-Benignos 2017) and other species (Appendix I) at high elevations in the Sierra Madre Occidental, where the climate remains comparatively warm (Peel et al. 2007), supports the hypothesis of climate mediating elevational and latitudinal distribution in the genus. This is an area of potential future study, but further support comes from the montane species *Pantosteus plebeius*, which displays the opposite pattern, having invaded northward from pluvial Lake Cabeza de Vaca, during the long-term trend of

climatic cooling (McPhee et al. 2008; Corona-Santiago et al. 2018). This advance included Holocene invasion into and across high elevation portions of the Río Grande Rift-Gila River drainage divide (Turner et al. 2019), where *Cyprinodon* is absent.

Río Grande Rift

Avulsion of the Upper Río Grande into the Hueco Bolsón, away from Bolsón de los Muertos (Figure 4), is estimated 2.5 Ma (Gustavson 1991; Repasch et al. 2017). This diverted the largest inflow to pluvial Lake Cabeza de Vaca, fragmenting the formerly integrated drainage into subdrainages (i.e., lagunas). Population fragmentation within resident *Pantosteus plebeius* occurred 3.6-0.6 Ma (Corona-Santiago et al. 2018), consistent with estimated timing of Río Grande diversion. More recent divergence estimates between populations of the Upper Río Grande and Laguna Guzmán—1.8-0.3 Ma (Corona-Santiago et al. 2018)—suggests contact persisted there. Gradual withdrawal of the Upper Río Grande is reasonable because full integration with the Lower Río Grande was only achieved 1.0-0.8 Ma (Mack et al. 2006; Repasch et al. 2017). At that time, the MRCA of the *Cyprinella bocagrande*-*C. formosa* species complex also fragmented among remnants of pluvial Lake Cabeza de Vaca (Wood and Mayden 2002). Population fragmentation in the post pluvial drainage formerly inundated by Pliocene Lake Cabeza de Vaca is likely the case for *C. pisteri* and *C. fontinalis*. The two subclades appear to be peripatric in distribution, with *C. fontinalis* confined to springs of the Río Bajío de Ojo Caliente drainage, which drains to the southwestern El Barreal. This small drainage—nestled between the Río Santa María on the west and Río Santa Clara on the east—incorporates

several small lagunas and springs (Henrickson 1977; Smith and Miller 1980; Hershler et al. 2011; Carson et al. 2015; Lozano-Vilano and De la Maza-Benignos 2017) and has been referred to as springs at Ojo de Carbonera (Smith and Miller 1986).

Adjacent to Río Bajío de Ojo Caliente, *C. pisteri* ranges widely within the Laguna Guzmán drainage (ríos Casas Grandes and Santa María), into the adjacent, endorheic Laguna Bustillos, and within the drainage of Patos Playa (Río Santa Clara) (Figure 1). Later Pluvial Lake Palomas inundated a portion of former pluvial Lake Cabeza de Vaca to 1202 m asl in the early Holocene. This was high enough to merge El Barreal, Laguna Guzmán, and Patos Playa as recently as 8.3-8.2 Ka (Castiglia and Fawcett 2006) (Figure 4) and is congruent with the broad distribution of *C. pisteri*. In contrast, even a higher Late Pleistocene level in pluvial Lake Palomas (1210 m asl) was insufficient to inundate the springs of Río Bajío de Ojo Caliente (Castiglia and Fawcett 2006), which are reported at ~1230 m asl (Smith and Miller 1980). Hence, ancestral *C. fontinalis* may have been isolated from *C. pisteri* since desiccation of Lake Cabeza de Vaca. Detailed phylogeographic study is needed to better document genetic (mtDNA and nuclear) diversity and distribution between species and within widespread *C. pisteri*.

Hypothesis 4 – Pecos River captures ancestral *C. bovinus*-*C. pecosensis*

Salt dissolution and the Great Plains-Chihuahuan Desert clade

Fragmentation of the Ancestral Brazos River created distinct lineages within the Great Plains-Chihuahuan Desert clade (Figure 6). The distributions of these lineages (Echelle and Miller 1974; Echelle and Echelle 1978; Ashbaugh et al.

1994; Page and Burr 2011, plate 34) are affiliated with areas of salt dissolution, which occur on the northeast and southwest fringes of the Llano Estacado (Ewing and Christensen 2016) (Figure 2). As an example, within the Pecos River drainage, Great Plains-Chihuahuan Desert *Cyprinodon* inhabit the Roswell Basin (*C. pecosensis*) and adjacent Delaware Basin (*C. elegans* and *C. bovinus*). In both basins, deep artesian aquifers in Permian limestones leak upward into overlying salt deposits, creating dissolution depressions infilled with alluvial sediments delivered by the Ancestral Pecos River and its tributaries (Hill 1996; Hawley 2005; Land and Newton 2008). The prevalence of salt dissolution in these basins was central to formation of the Pecos River valley (Kelley 1972; Gustavson and Finley 1985). The valley still maintains brackish habitats created by dissolution and ideal for *Cyprinodon*, including cenotes (sinkholes), playas, ciénegas, and brackish springs (e.g., Echelle and Miller 1974; Hoagstrom and Brooks 1999). Also, evaporative habitats that concentrate salts occurred along the Pecos River, where they intermingled with springs and river channels, producing a mosaic of fresh, brackish, and saline habitats (Hoagstrom and Brooks 1999).

Roswell Basin captures Old Río Manzano

A general hypothesis for formation of the Pecos River is long standing (e.g., Kelley 1972; Bachman 1976; Gustavson and Finley 1985; Hawley 1993) and is a common textbook example of drainage evolution by stream piracy (Thornbury 1969; Hunt 1974; DiPietro 2013). Nevertheless, details have been debated (Hill 1996; Hawley 2005) and can be fleshed out using divergence dates available for *Cyprinodon*. Prior to abandonment, the Portales Valley formed a corridor across the Llano Estacado, which was a zone of salt dissolution (Gustavson and Finley

1985). This corridor presumably included shallow, evaporative habitats favored by *Cyprinodon*. Pliocene subsidence in the Roswell Basin captured the Old Río Manzano (Kelley 1972; Gustavson and Finley 1985) in two stages. First, Ancestral Río Hondo, a tributary, was diverted from the Slaton Paleovalley into the Roswell Basin 11.0-4.5 Ma (Gustavson 1996) (Figure 2). Second, alluvial deposits suggest that diversion of the mainstem Old Río Manzano (Figure 3) occurred within the Pliocene (Hawley 2005) and fossil evidence constrains this diversion (Figure 3) to sometime after 6.0 Ma (Morgan and Lucas 2001). Divergence of *C. bovinus*-*C. pecosensis* from *C. rubrofluvialis* is estimated at 4.5-2.4 Ma (Echelle et al. 2005) (Figure 6), consistent with Late Pliocene capture. Other fishes potentially captured are ancestral *Hybognathus amarus*, *Macrhybopsis aestivalis*, and *Notropis simus pecosensis*. These Pecos River-Río Grande fishes share Tertiary origins and close relatives in the Brazos river drainage (Martin and Bonett 2015; Echelle et al. 2018).

In addition to the effects of dissolution subsidence in the Roswell Basin, severance of Old Río Manzano from the Portales Valley (Figure 3) was facilitated by: (1) backfilling of the Portales Valley segment of the Old Río Manzano due to declining stream power caused by increasing aridity (Gustavson and Winkler 1988); (2) uplift of the Jemez Lineament, which tilted the northern Llano Estacado (Nereson et al. 2013), possibly deflecting the Old Río Manzano south; and (3) dissolution subsidence in the Fort Sumner Valley (Mourant and Shomaker 1970), which is the capture location (Reeves 1972). This beheaded the Ancestral Brazos River, isolating ancestral *C. rubrofluvialis* east of the Llano Estacado in a zone of salt-dissolution subsidence that became the new headwater region for the truncated Brazos

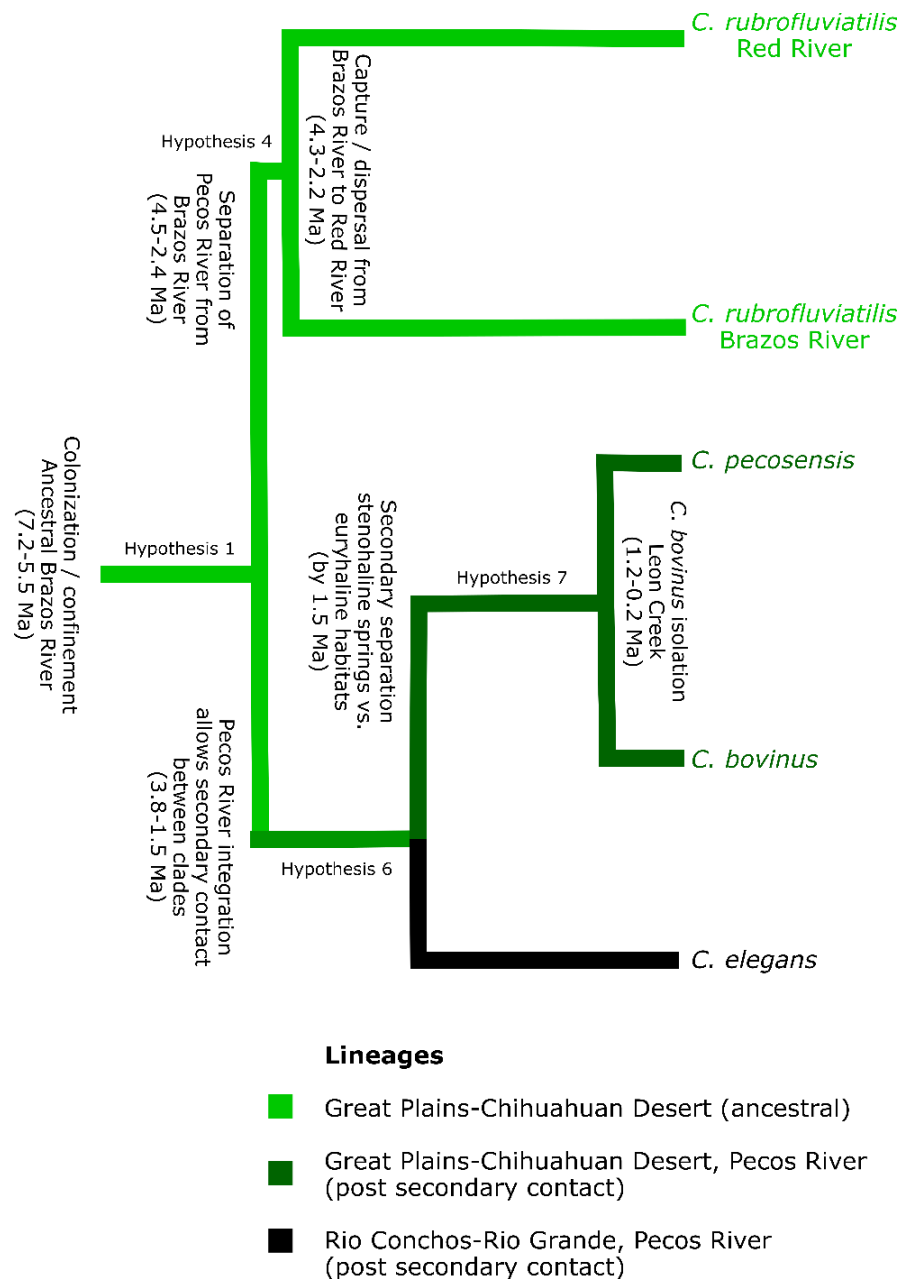


FIGURE 6. Reconstructed mtDNA tree for Great Plains-Chihuahuan Desert clade *Cyprinodon* from chronogram of Echelle et al. (2005), annotated with biogeographical interpretation. Hypotheses relevant to phylogenetic branches are noted, with additional details provided in text. Branch and taxon colors correspond to distinct lineages within the mtDNA clade. Specifically, *C. elegans* descends from the Río Conchos-Río Grande clade, but exhibits mitochondrial introgression with the Great Plains-Chihuahuan Desert clade in the Pecos River drainage, creating an affinity with the *C. bovinus*-*C. pecosensis* sister pair of the Great Plains-Chihuahuan Desert clade. Divergence dates are from Echelle et al. (2005).

River (Figure 3). Ancestral *C. rubrofluvialis* presumably occupied Brazos River tributaries throughout this zone, as they did historically (Figure 1).

Integration of the Middle Pecos River included capture of the Río Peñasco from the Ancestral Colorado River (Figure 3). The 3.8-1.5 Ma mtDNA relation of *C. bovinus*-*C. pecosensis* with *C. elegans* (Echelle et al. 2005) suggests Late Pliocene-Early Pleistocene capture. Southward diversion of the Río Peñasco (with the ríos Hondo and Manzano as tributaries) allowed secondary contact of *C. bovinus*-*C. pecosensis* with *C. elegans*, leading to hybridization and mtDNA capture by the latter. This followed an estimated period of separation likely exceeding two million years (Echelle et al. 2005) (Figure 6).

Interchange between Brazos and Red Rivers

In the Pliocene, expansion of the Ancestral Red River drainage captured northwestern tributaries of the Ancestral Brazos River (Snedden and Galloway 2019) (Figures 1-2). Estimated divergence between *C. rubrofluvialis* of the Red and Brazos river drainages (4.3-2.2 Ma, Echelle et al. 2005) corresponds with these tributary transfers (Figure 6). Multiple exchanges are likely because stream courses varied over time (Seni 1980). Ultimately, *C. rubrofluvialis* persisted in the southern Great Plains and Osage Plains (Figure 2), where salt dissolution remains prevalent (Johnson 1981; Gustavson and Simpkins 1989; Caran and Baumgardner 1990). Through the Quaternary, the modern Brazos and Red rivers entrenched their valleys (Frye and Leonard 1963; Epps 1973; Blum et al. 1992), thereby maintaining separation between populations.

Hypothesis 5 – Río Conchos-Río Grande *Cyprinodon* separate from Río Salado (Río Nazas) lineage; both diversify

Ancestral Río Salado first invasion

The Late Miocene Río Salado was the main branch of the Lower Río Grande (Figure 2). Thus, it stands to reason that a distinct lineage of *Cyprinodon* arose there (Echelle et al. 2005) (Figure 7). Divergence of Río Nazas clade *Cyprinodon* supports a Late Miocene-Early Pliocene connection (6.7-2.8 Ma, Echelle et al. 2005) (Figure 2), further supported by *Cyprinella xanthicara* (Martin and Bonett 2015), the southern Chihuahuan Desert lineage of the *Perognathus flavus* group (Neiswenter and Riddle 2010), and possibly a relict lineage of *Chaetodipus hispidus* (Andersen and Light 2012). Ancestral *C. bifasciatus* potentially pioneered this route as far inland as Bolsón de Cuatro Ciénegas in the Late Miocene because it represents an early diverging lineage, unaffiliated with any major *Cyprinodon* clade (Miller 1968; Echelle and Echelle 1998). Upon secondary contact with the Río Nazas clade, *C. bifasciatus* experienced mtDNA introgression with ancestral *C. atrorus* (Echelle et al. 2005; Carson and Dowling 2006) (Figure 7).

Ancestral Río Salado second invasion

The Río Nazas clade (*sensu stricto*, sans *C. bifasciatus*) represents a second invasion up the Ancestral Río Salado, into Bolsón de Cuatro Ciénegas that also extended into ríos Aguanaval, outlet at 1400 m asl, 100 m below the northwest outlet, making this route more probable (Figure 2). In addition, the upper Río Nazas may have joined the Río Aguanaval upstream of the Parras Basin (Figure 2). The modern Río Nazas makes a sharp bend to the northwest at the gap between El Mulato (present site of Presa Francisco Zarco) and the

head of Cañon de Fernández, but appears to have once continued southeast through a wide valley (at Margarita Machado, Durango) and then east to Río Aguanaval in the valley of Juan Eugenio, Coahuila. Downstream, the valley of Laguna Viesca was erosional before deflation (Kellum 1932), suggesting it was the route of the ancestral Río Aguanaval, which evidently proceeded northeast through Puerto La Peña (an eroded portal between sierras de Jimulco and Parras, Imlay 1936). From there, Río Aguanaval putatively proceeded east across Parras Basin to the northeast outlet of Arellano (1951). Instead of hooking sharply east near the northeast edge of the basin into the drainage of Río Salinas, tributary to Río San Juan (as it does today), the Ancestral Río Aguanaval is hypothesized here to have continued north, curving northwest into San Marcos Valley and thence northeast into Bolsón de Cuatro Ciénegas (Figure 2).

Ancestral Río Nazas separation

The primary node in the Río Nazas clade is estimated 6.0-2.4 Ma, separating western (*C. meeki*-*C. nazas*) and eastern (*C. atrorubens*-*C. sp.* Aguanaval) subclades (Echelle et al. 2005). This divergence estimate closely follows the third stage of faulting in the San Marcos fault zone 14-5 Ma (Chávez-Cabello et al. 2007) (Figure 3). Tectonism associated with the Almagre and El Caballo faults potentially separated the Río Nazas from the Río Aguanaval- Cuatro Ciénegas drainage. If Ancestral Río Nazas joined Río Aguanaval above Cañon de Fernández in the Late Miocene (Figure 2), Mio-Pliocene faulting potentially promoted diversion into or capture by Cañon de Fernández. In any case, the upper Río Nazas shifted west to the northwest outlet of Arellano (1951), into the Sobaco and Hundido valleys (Figure 3). The independent Ancestral Río

Nazas was relatively small once disconnected from Río Aguanaval and, with the end of the 3.3-2.9 Ma Pliocene wet period, may have sustained minimal surface flow to Bolsón de Cuatro Ciénegas. Present faults through the Sobaco and Hundido valleys constitute zones of infiltration and cross-formation flow, which reduce surface-water connectivity by directing subsurface flow from the Río Nazas drainage to springs in Bolsón de Cuatro Ciénegas (Wolaver et al. 2008).

In addition, the Río Mezquital of the Pacific slope captured Río Tunal, which beheaded the ancestral Río Nazas, further reducing its drainage area (Figure 3). Divergence of *C. meeki* from *C. nazas* 4.7-1.6 Ma (Echelle et al. 2005) (Figure 7) dates this capture, consistent with at least three other fish lineages believed to have shared the capture: *Codoma* (~4.2 or 3.1 Ma, Schönhuth et al. 2015), *Moxostoma* (~5.1 or 4.4 Ma, Pérez-Rodríguez et al. 2016), *Pantosteus* (~2.6 Ma, Corona-Santiago et al. 2018). This was a high elevation capture, exceeding 1800 m asl based on modern elevations. *Gila conspersa*-*G. sp.* Mezquital (Schönhuth et al. 2014) and *Etheostoma pottsii* (Smith et al. 1984) also share this biogeography, but their timings of divergence are unestimated.

Flow of Río Nazas to Bolsón de Cuatro Ciénegas must have been re-established at times as indicated by the Quaternary biogeography of gastropods (Hershler 1985; Czaja et al. 2015), turtles (Legler and Vogt 2013; Parham et al. 2015), snakes (Conant 1977); lizards (Montanucci 1974) and fishes (Osborne et al. 2016). The most recent connection may have been Late Pleistocene, when Ancestral Río Nazas formed pluvial Lake Irritila, inundating the western Parras Basin (Laguna Mayrán) and lagunas north and west (Figure 4).

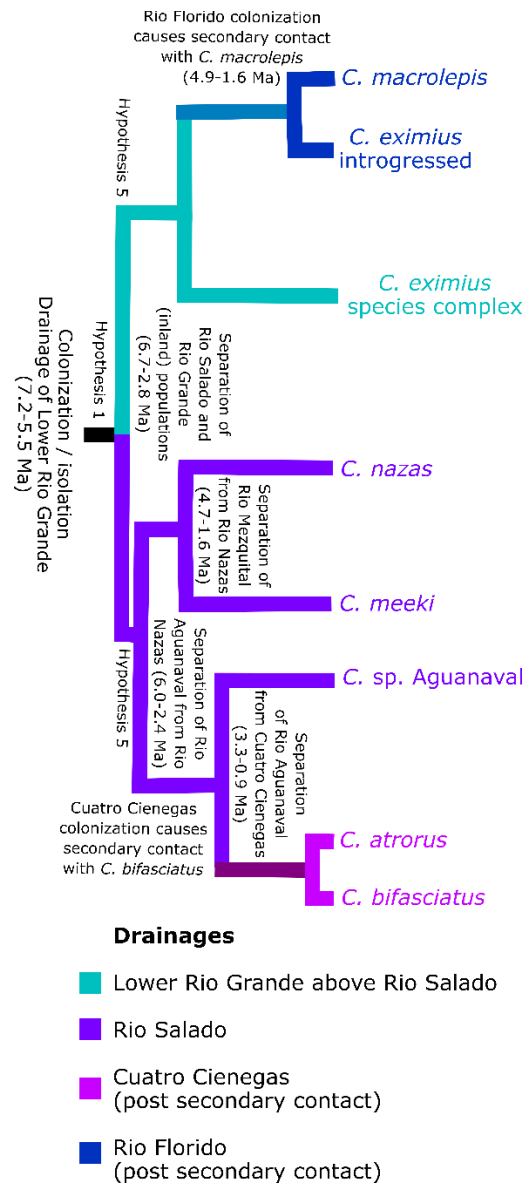


FIGURE 7. Reconstructed mtDNA tree for Río Conchos-Río Grande and Río Nazas sister clades of *Cyprinodon* from chronogram of Echelle et al. (2005), annotated with biogeographical interpretation. Hypotheses relevant to phylogenetic branches are noted, with additional details provided in text. Branch and taxon colors correspond to distinct drainages. The *C. eximius* species complex includes *C. julimes*, *C. pachycephalus*, and *C. salvadori*. Note, *C. elegans* also descends from the Río Conchos-Río Grande clade (blue-green branch), but is missing from this tree because it exhibits mitochondrial introgression with the Great Plains-Chihuahuan Desert clade in the Pecos River drainage (Figure 6). Cases of secondary contact are also present. *Cyprinodon bifasciatus* is not descended from any major clade, but is present here due to mtDNA introgression with *C. atrorus*. *Cyprinodon macrolepis* is descended from the Río Conchos-Río Grande clade and exhibits mtDNA introgression with *C. eximius* within the Río Florido. Divergence dates are from Echelle et al. (2005).

Lake Irritila housed a diverse aquatic fauna typical of a more northern modern lake (Czaja et al. 2014b). Soil geomorphology indicates great moisture in this vicinity during the Last Glacial Maximum (early MIS2, Butzer et al. 2008). Transition to dry climate began ~18 Ka (Butzer et al. 2008) and Holocene gastropod fossils from Sobaco Valley indicate no surface-water connection (Czaja et al. 2014a), consistent with absence of evidence for contact of *C. nazas* with *Cyprinodon* in Cuatro Ciénegas.

Ancestral Río Aguanaval fragmentation

Meanwhile, gene flow continued between Cuatro Ciénegas and Río Aguanaval populations of *Cyprinodon* (Figure 7), presumably via a persistent surface-water connection (Figure 3). The subsequent fourth stage of faulting within the San Marcos fault zone (<5.0-0.1 Ma) included eruption of the Las Coloradas volcanic field (Chávez-Cabello et al. 2007), which blocks the hypothetical northeastern route of the Río Aguanaval through San Marcos Valley (Figure 4) and corresponds in time with the estimated divergence of *C. atrorus* from *C. sp. Aguanaval* (3.3-0.9 Ma, Echelle et al. 2005). This may also correspond with isolation of *Etheostoma pottsii* from congeners in Cuatro Ciénegas and Río Salado (phylogeny in Lang and Mayden 2007). Uplift and volcanic deposits could have blocked the Río Aguanaval, which eventually spilled east into or was captured by Río Salinas (Legler 1990).

It is likely that extinct *C. latifasciatus* either descends from a common ancestor with *C. atrorus*-*C. sp. Aguanaval* or is sister to *C. sp. Aguanaval*. *Cyprinodon latifasciatus* inhabited springs near Parras de la Fuente (Miller et al. 2005; Lozano-Vilano and De la Maza-Benignos 2017), tributary to Ancestral Río Aguanaval (Miller 1981; Figure 3). A pluvial lake in this vicinity (Mayrán

Basin system, distinct from Laguna Mayrán farther west), which supported a diverse aquatic fauna including unidentified fishes, spilled north from the front of the Sierra de Parras, into the eastern Parras Basin (Amezcuca et al. 2012). Resident fishes presumably were ancestors to the now extinct Parras fauna, which included endemic *Gila*, *Stypodon*, and *Characodon* species with *C. latifasciatus* (Miller et al. 2005). A series of Pliocene-Early Pleistocene tufa cascades developed along the lake system as it progressed toward the basin floor (Amezcuca et al. 2012), potentially isolating endemic Parras fishes from the Ancestral Río Aguanaval before their ultimate confinement to springs at Parras de la Fuente.

Lack of C. nazas-C. sp. Aguanaval secondary contact

Cyprinodon sp. Aguanaval is only known from the upper Río Aguanaval near Rancho Grande, Zacatecas (Miller 1976) (Figure 1). This isolation could reflect incision of the Aguanaval River valley and desiccation of the lower river. In >400 river km between the terminal sink at Laguna Viesca and the inhabited valley at Rancho Grande, the river cuts through at least eight narrowed segments that are potential barriers for *Cyprinodon*. There is also a cumulative elevation gain of ~890 m. *Cyprinodon sp. Aguanaval* potentially colonized the upper drainage under wetter conditions and prior to development of river narrows. The course of Río Aguanaval is superimposed (i.e., antecedent; Kellum 1932; Imlay 1936), meaning it developed upon higher sediment layers (now eroded away) and cut down over time. Gradual incision established segments through rock uplifts, which form the present sequence of cañons and boquillas. Isolation of *C. sp. Aguanaval* putatively began as narrow segments, possibly with steep rapids or waterfalls,

developed. Aridity eventually dried the lower river, at least during inter-glacial intervals (Conant 1963), which could explain the restriction of *C. sp.* Aguanaval to wetter valleys perched above the arid desert basins.

However, Pleistocene lakes connected the ríos Aguanaval and Nazas when lagunas Mayrán and Viesca merged (Miller 1981; Czaja et al. 2014b) and it seems likely flow along Río Aguanaval was continuous to Laguna Viesca in a pluvial climate (as already described for Río Nazas). It is notable therefore that *C. sp.* Aguanaval and *C. nazas* show no evidence of secondary contact. In contrast, *Campostoma* cf. *ornatum* (Domínguez-Domínguez et al. 2011; Schönhuth et al. 2011), *Cyprinella garmani* (Schönhuth and Mayden 2010), and *Gila conspersa* (Schönhuth et al. 2014) exhibit little differentiation between the Río Aguanaval and Río Nazas, suggesting Quaternary connectivity. While these more fluvial species dispersed broadly, *C. sp.* Aguanaval evidently remained isolated in the upper drainage, suggesting some combination of river narrows, distance, elevation gain, or other ecological factors (e.g., negative interspecific interactions) were barriers to movement.

Ancestral Lower Río Grande drainage

Karst topography dominated the headwaters of the Late Miocene-Early Pliocene lower Río Grande (Kastning 1983; Smith and Veni 1994; Veni 2009). Karstification initiated in the Middle Miocene (~15 Ma) and the Devils River became a major outlet for the Edwards Aquifer (Veni 2018). The Río Conchos first connected with the Ancestral Lower Río Grande through dissolution corridors that indicate extensive, prolonged karst development (Freeman 1968; Veni 2009). Karst formations across the upper drainage of the Ancestral Lower Río Grande increased surface-

water infiltration at the expense of surface runoff. This positively reinforced karstification, further increasing predominance of subsurface flow. As a result, transport of fluvial sediments declined, consistent with minimal sediment discharge attributable to the Pliocene Río Grande (Snedden and Galloway 2019). Aridity of the Late Miocene and Early Pliocene also reduced surface runoff (Chapin 2008), presumably increasing the ecological significance of springs as climatic refugia (*sensu* Dautreuil et al. 2016; Caldwell et al. 2020).

Karst-spring endemism is common among North American desert fishes (Hubbs 1995), suggesting karst affiliation was important within the Río Conchos-Río Grande clade. The first emigrants into the Río Conchos, for instance, may have been karst-adapted if they occupied the aforementioned dissolution features along the Ancestral Río Conchos. Accordingly, *C. macrolepis*, is confined to springs (Miller 1976; Lozano-Vilano and De la Maza-Benignos 2017) and several populations of *C. eximius* also affiliate with springs (Echelle et al. 2003). Further, *C. julimes* (De la Maza-Benignos and Vela-Valladares 2009) and *C. pachycephalus* (Minckley and Minckley 1986) are spring endemics (see also Lozano-Vilano and De la Maza-Benignos 2017) within the *C. eximius* species complex (Echelle et al. 2005; Carson et al. 2014). *Cyprinodon julimes* and *C. pachycephalus* are hot-spring endemics, geographically close to *C. eximius* (Minckley and Minckley 1986; Miller et al. 2005; De la Maza-Benignos and Vela-Valladares 2009), suggesting peripatric speciation in extreme environments.

Notably, Río Conchos-Río Grande clade *Cyprinodon* failed to colonize the Upper Río Grande (Río Grande Rift), which integrated with the Río Conchos-Lower Río Grande 1.0-0.8 Ma (Mack et al. 2006; Repasch et al. 2017). Lack of

upstream range expansion seems to reflect niche conservatism of species associated with karst geomorphology or a relatively warm climate. Beyond this, the evolutionary history of the Río Conchos-Río Grande clade may prove complex as diversity continues to be described (Lozano-Vilano 2002; De la Maza-Benignos and Vela-Valladares 2009). Secondary contact is documented for *C. eximius* with *C. macrolepis* (Echelle et al. 2005) (Figure 7), *C. julimes* (Carson et al. 2014), and *C. pachycephalus* (Minckley and Minckley 1986). Molecular analyses incorporating more loci and additional specimens from across the range of the clade (including all recognized and proposed taxa), could provide substantial new insight.

Hypothesis 6 – ancestral *C. elegans* colonizes lower Pecos River from Ancestral Lower Río, Grande; secondary contact with *C. bovinus*-*C. pecosensis*

Colonization from Lower Río Grande

The ancestor to *Cyprinodon elegans* descends from the Río Conchos-Río Grande clade (Echelle et al. 2005), implying it diverged after colonizing the Lower Pecos River, a major tributary to the Lower Río Grande (Kastning 1983) (Figure 2). The Lower Pecos River began to entrench its meanders in the Late Miocene-Early Pliocene (Veni 1994). Eventually, it expanded northwest from the Edwards-Stockton Plateau into the southern Delaware Basin (Figure 2) at the southern edge of the Ogallala Formation (Leonard and Frye 1962). Ancestral *C. elegans* presumably expanded up the Pecos River during early valley development. Divergence time of *C. elegans* from the Río Conchos-Río Grande clade has not been estimated due to secondary contact and the capture and fixation of mtDNA from *C. bovinus*-*C.*

pecosensis. Allozyme evidence of divergence order (matched with mtDNA divergence estimates in the Río Conchos-Río Grande clade) indicates it diverged after ~4.6 Ma but before ~3.0 Ma (Echelle et al. 2005). Subsequent incision of the Lower Pecos River canyon (Kastning 1983; Veni 1994), presumably created unfavorable habitat that eventually separated ancestral *C. elegans* from relatives in the Río Grande.

Aforementioned prevalence of karst in the Pliocene Lower Río Grande and Pecos River, along with historical reliance of *C. elegans* on karst habitats (Echelle et al. 2003), suggest the original distribution of ancestral *C. elegans* in the Delaware Basin was among springfed habitats. Springs historically inhabited by *C. elegans* are associated with Cretaceous limestones (Bumgarner et al. 2012) that outcrop on the south rim of the basin (Appendix VI). These housed many early 20th century springs (Brune 1981). Notably, Early Pleistocene Toy Limestone along Salt Draw (Qgt in Stoesser et al. 2006) indicates presence of a large and persistent spring complex. The massiveness of the formation (>9 m thick, ~20 km in near-contiguous length, with several outliers) suggests this could have been a pre-historic habitat for *C. elegans*, a vestige of which persisted as historical springs (Appendix VI).

Disjunct historical distribution

Historical populations of *C. elegans* in Comanche and Balmorhea Springs inhabited disjunct Pecos River tributaries (Appendixes VI and VII). The course of the Pecos River shifted downslope (northward) through the Pleistocene (Leonard and Frye 1962), implying the Late Pliocene course was close to these springs. In addition, the Early Pliocene Lower Pecos River, before integration, may have been relatively small, fed primarily by springs. To the north, Pliocene

dissolution subsidence beginning ~4.0 Ma (Kirkland 2014) created the large Pecos Trough (Anderson 1981), which gradually captured surface streams (Hill 1996). Salt Draw and Toyah Creek—which hold the springs of the Balmorhea area and Toy Limestone, respectively—eventually became tributaries to the trough (Figure 3; Appendix VI). This could have initiated separation of Toyah Creek *C. elegans* from Comanche Creek *C. elegans*.

Secondary inter-clade contact

As already discussed, the Upper Pecos River evidently connected with the Lower Pecos River 3.8–1.5 Ma (Hypothesis 4). During integration, the Upper Pecos River first advanced to the Pecos Trough and this is presumably where *C. bovinus*-*C. pecosensis* contacted *C. elegans* of Toyah Creek (Appendix VI). The estimated timing of contact (3.8–1.5 Ma, Echelle et al. 2005) (Figure 6) supports this scenario. Through time, the subsiding Pecos Trough filled with alluvium >450 m deep (Jones 2001). While this occurred, the trough could have been the terminus of the Upper Pecos River, possibly for much of the Late Pliocene. Even the Early Pleistocene Pecos River had low energy and accumulated sediments that suggest the Pecos Trough was still a terminal sink (Powers and Holt 1993).

This suggests secondary contact may not have occurred with *C. elegans* in Comanche Creek to the east. Comanche Creek joined the Pecos River ~180 river kilometers downstream from the Pecos Trough (Figure 3, Appendix VI) and may not have integrated with the Upper Pecos River until the river spilled east out of the trough. Morphological differences between *C. elegans* populations (Echelle 1975) could reflect a lack of introgression, as morphological change tends to accompany inter-specific introgression in

Cyprinodon (Stevenson and Buchanan 1973; Wilde and Echelle 1997; Tobler and Carson 2010).

Ecological segregation of clades

Despite secondary contact, separate clades of *Cyprinodon* remained distinct in the Delaware Basin after secondary contact (Echelle et al. 2005). Ecological segregation and reproductive isolation may have ultimately limited gene flow between *C. bovinus*-*C. pecosensis* (Great Plains-Chihuahuan Desert clade) and *C. elegans* Río Conchos-Río Grande clade). This is consistent with reproductive isolation of *C. elegans* from invasive, nonnative *C. variegatus* of the maritime clade (Stevenson and Buchanan 1973, Echelle and Echelle 1994; Tech 2006). Further, it is consistent with the hypothesis that *C. elegans* was spring-affiliated because longitudinal segregation of spring-endemic versus generalist congeners has been observed in other *Cyprinodon* (Carson et al. 2008), *Fundulus* (García-Ramírez et al. 2006), *Gambusia* (Hubbs 1971), and *Etheostoma* (Echelle et al. 1976).

Hypothesis 7 – peripatric divergence of *C. bovinus* from *C. pecosensis*

*Biogeography of *C. bovinus**

Upper Leon Creek (including Diamond Y Spring) houses endemic *C. bovinus*, Great Plains-Chihuahuan Desert clade (sister species to *C. pecosensis*, Echelle et al. 2005). Perhaps unexpectedly, Leon Creek lies just 15 airline km west of Comanche springs—former home to *C. elegans* (Appendix VII) and 70 airline km east of the Balmorhea Springs complex—last home to *C. elegans*. Thus, Leon Creek is positioned between the tributaries that housed historical populations of *C. elegans* (Appendixes VI and VII). This interspecific biogeography seems initially difficult

to explain. However, Diamond Y Spring (Leon Creek) has distinct water chemistry because underlying faults allow upward leakage from the Rustler aquifer. This gives Diamond Y Spring 1.9 times greater specific conductance than Comanche Spring and 2.8 times more than San Solomon Spring of Toyah Creek (Bumgarner et al. 2012). Waters of Comanche and San Solomon springs, despite being more distant from each other, have fairly comparable ion concentration because the Edwards-Trinity aquifer is an important source for these springs. As *C. elegans* requires water of relatively low salinity (Echelle and Echelle 1978, 2019), higher specific conductance in springs of Leon Creek may have precluded its colonization or persistence, leaving the creek open to colonization by the euryhaline MRCA of *C. bovinus*-*C. pecosensis*.

Peripatric speciation

Estimated timing of divergence between *C. bovinus* and *C. pecosensis* (1.2-0.2 Ma, Echelle et al. 2005) corresponds with excavation of the modern Pecos River valley (Appendix II). Pecos River incision possibly created barriers along lower Leon Creek (e.g., falls, cascades, zones of infiltration). Upper Leon Creek, where *C. bovinus* resides, remained elevated on a horst (Appendix VII) and faulting on the horst boundary (Bumgarner et al. 2012) must have steepened the descent into the Pecos Valley. Through the period of interspecific divergence, the Pecos River continued to excavate its valley and captured its modern headwaters within the Sangre de Cristo Mountains (Appendix II). Subsequent, strong glaciations 0.16 and 0.02 Ma (MIS 6 and MIS 4-2) contributed glacial runoff down the Pecos River (Pazzaglia and Hawley 2004; Pierce 2004). Today, Diamond Y Spring sits ~120 m above the adjacent Pecos River valley over a direct-line

distance of ~31 km. The former outlet of the dam of Lake Leon (once impounding Leon Springs) lies ~14 km from Diamond Y Spring and sits ~60 m higher. The aforementioned northward shift of the Pecos River valley through the Pleistocene (Leonard and Frye 1962) also gradually increased the distance between the horst boundary and the river.

Divergence between *C. bovinus* and *C. pecosensis* (Figure 6) may reflect spring endemism of the former, but available information suggests *C. bovinus* maintains broad temperature and salinity tolerances comparable to *C. pecosensis* (Kennedy 1977; Echelle and Echelle 1978, 2019; Loiselle 1982), which seems to argue against strict ecological differentiation. Nevertheless, in a somewhat comparable scenario, *Cyprinodon variegatus hubbsi* and *C. v. variegatus* have peripatric distributions on the Florida Peninsula (Gilbert et al. 1992; Robins et al. 2018) and likely diverged in the Pleistocene (Guillory and Johnson 1986). Like *C. bovinus* versus *C. pecosensis*, *C. v. hubbsi* has a similar range of thermal and salinity tolerances to *C. v. variegatus* (Jordan et al. 1993; Jung et al. 2019). More detailed studies however, have shown it is more tolerant of confinement to freshwater (Brix and Grosell 2012; Brix et al. 2015), has lower tolerance of hypoxia (Jung et al. 2019), and exhibits evidence for reproductive isolation via mate choice and reduced rate of hatching (Brix and Grosell 2013). Similar comparisons between *C. bovinus* and *C. pecosensis* could improve understanding of their speciation.

Hypothesis 8 – Early Pleistocene colonization of Tularosa Basin by ancestral *C. tularosa* (maritime clade)

Reaching Tularosa Basin

Tularosa basin is an eastern extension of the Río Grande Rift (Repasch et al. 2017), hydrographically isolated from the modern Río Grande. Timing of *C. tularosa* divergence from *C. variegatus* 3.2-1.0 Ma (Echelle et al. 2005) precedes 1.0-0.8 Ma overflow of the Upper Río Grande (Río Grande Rift) via its modern valley (Repasch et al. 2017), but corresponds with initiation of overflow from the Mesilla Basin, across Fillmore Gap, and into the Tularosa-Hueco basin complex ~2.5 Ma (Gustavson 1991; Repasch et al. 2017) (Figure 4). Discharge from there to the Lower Río Grande via the Hueco Bolsón (Gustavson 1991) could have allowed ingress by ancestral *C. tularosa*. In fact, this timing and geography were fortuitous for reaching the Tularosa Basin because immediately downstream of Fillmore Gap, the Río Grande periodically swung north into the Tularosa Basin en route to the Hueco Bolsón (Mack et al. 2006). This would have allowed ancestral *C. tularosa* into pluvial Lake Otero of the Tularosa Basin (Figure 4). Native populations of *C. tularosa* inhabit remnant habitats of the Lake Otero drainage (Pittenger and Springer 1999). Prehistoric development of the Río Grande (Repasch et al. 2017) implies that no other timing would have allowed this access, in strong congruence with the divergence estimate for this taxon.

Based on mtDNA, *Cyprinodon tularosa*—endemic to the Tularosa Basin—descends directly from the maritime clade (Echelle et al. 2006). Conflicting phylogenetic relations for *C. tularosa* between mtDNA and allozymes suggest ancestral *C. tularosa* hybridized with a member of the Great Plains-Chihuahuan Desert clade prior to divergence from the maritime clade (Echelle et al. 2005), but this requires more molecular study. It is hypothesized here that ancestral *C. pecosensis*

remained isolated upstream of the Edwards Plateau when ancestral *C. tularosa* inhabited the Lower Río Grande, consistent with its historical distribution (Echelle and Echelle 1978) and with absence of evidence for secondary contact with ancestral *C. tularosa* (Echelle et al. 2005). The canyon of the lower Pecos River presumably separated *C. tularosa* from *C. pecosensis* (*sensu* Hubbs and Garrett 1990).

Once the Tularosa basin was colonized, *C. tularosa* may have also had access across Fillmore Gap, into the Upper Río Grande. As discussed for western-clade *Cyprinodon* (Hypothesis 2), populations of maritime-clade *Cyprinodon*, wherever they were in the Late Pliocene-Early Pleistocene Río Grande, may have succumbed to the cooling Quaternary climate, which was associated with theoretically unfavorable glacial/proglacial flooding, valley incision, and canyon formation. As mentioned previously, breaching of pluvial Lake Alamosa and lakes of the Valles Caldera 0.6-0.4 Ma (Pazzaglia and Hawley 2004; Repasch et al. 2017) may have been catastrophes for riverine *Cyprinodon*.

No secondary contact with Río Conchos-Río Grande clade

For ancestral *C. tularosa* to reach the Hueco Bolsón, it necessarily dispersed along the Lower Río Grande, past the Río Conchos. Yet, Río Conchos *Cyprinodon* show no evidence of secondary contact with the maritime clade (Echelle et al. 2005). The lower course of the Río Conchos through the Chihuahuan Fold Belt traverses a series of uplifts through rugged gorges and canyons (Burrows 1910; King and Adkins 1946; Hennings 1994). These are not occupied by *C. eximius*, which occur above and below the canyon region (Miller et al. 2005). It is perplexing, however, that evidence is lacking for introgression

of ancestral *C. tularosa* with the Río Conchos-Río Grande clade of the Río Grande corridor (i.e., *C. eximius*). Following Hypothesis 5, it is proposed that the Río Conchos-Río Grande clade was karst affiliated early in its history, perhaps promoting habitat segregation when ancestral *C. tularosa* colonized the Lower Río Grande. Notably, *C. eximius* is uncommon within the mainstem river, with populations centered in tributaries (Echelle et al. 2003; Heard et al. 2012). Study of reproductive isolation between maritime and Río Conchos-Río Grande *Cyprinodon* could help clarify this dilemma and establish whether inter-specific barriers observed in *C. elegans* (or others) are present in *C. eximius*. An alternative possibility is that *C. eximius* may have lived only in the Río Conchos at the time of *C. tularosa* invasion, being eliminated by earlier Pleistocene floods. If so, then the species may have re-colonized the Río Grande valley after *C. tularosa* extirpation, for instance, after the 0.6-0.4 Ma flood of pluvial Lake Alamosa.

Absence from Bolsón de los Muertos

Because the Río Grande was separating from Bolsón de los Muertos ~2.5 Ma (Gustavson 1991; Repasch et al. 2017), ancestral *C. tularosa* probably did not reach the upper Río Grande until the interconnection was severed. As already described, ancestral *C. tularosa* could not have reached the Tularosa Basin until the Río Grande flowed through Fillmore Gap (away from Bolsón de los Muertos), which occurred episodically 2.6-0.8 Ma (Mack et al. 2006) when the Upper Río Grande first spilled into the Lower Río Grande (Gustavson 1991). This sequence of events is unlikely to have allowed ancestral *C. tularosa* access to the drainage of Bolsón de los Muertos.

Conservation Biogeography

Pioneering ancestors

This synthesis illustrates how the historical and present distributions of *Cyprinodon* could be regarded as legacies of pioneering ancestors with tremendous capacity to traverse the North American desert. Ability to exploit interconnections exceptionally well (compared to other fishes) should be anticipated in light of the presence of *Cyprinodon* in waters where no other (or few other) fishes exist (Miller 1981). Stream captures occur over relatively short time frames (years to decades), thus they epitomize the unique histories of each *Cyprinodon* population whose ancestors capitalized on rare opportunities to expand their ranges and reach persistent aquatic ecosystems. Indeed, the global significance of fish faunal transfers via interdrainage rearrangements and dispersal corridors is increasingly recognized (Waters et al. 2015, 2019; Albert et al. 2018).

Fossil evidence indicates that fish distributions respond to climate change (Cross 1970; Cross et al. 1986; Newbrey and Ashworth 2004), consistent with documentations of fish distributional adjustments in response to anthropogenic climate change (Alofs et al. 2014) and with predictive models of future range expansions (Sharma and Jackson 2008; Campana et al. 2020). Although there is no significant fossil record for desert *Cyprinodon* (Echelle and Echelle 2020a), distributional evidence, combined with mtDNA divergence dates (Echelle et al. 2005; Echelle 2008), implies the Pliocene Epoch (5.3-2.6 Ma) was especially favorable for *Cyprinodon* expansion across the desert region. This makes sense because the climate was warmer than present (Salzmann et al. 2011), which could facilitate survival at higher latitudes and elevations—as seen in various *Cyprinodon* distributed across the

relatively warm Sierra Madre Occidental. Further, higher precipitation increased the abundance of lakes 3.3–2.9 Ma (Ibarra et al. 2018), presumably also raising the abundance of streams and wetlands while boosting surface-water connectivity. Higher precipitation also intensified drainage incision (Jungers and Heimsath 2016), which promoted stream captures by drainages that were expanding in response to rifting, as in the Upper Río Grande (Appendix III) and Gila River (Appendix IV).

During Quaternary glacial cycles, *Cyprinodon* evidently disappeared from some areas occupied in the Pliocene. Climatic cooling—intensified during glaciations (especially in the Late Pleistocene)—would have most affected populations in northern locales (*sensu* Haney et al. 2009). Periods of aridity also eliminated populations where refugia were insufficient for persistence and isolation precluded recolonization (Smith and Miller 1986; Smith et al. 2002). Hence, aquatic ecosystems of larger volume and in wetter environments were most likely to sustain populations (Smith 1981; Minckley 1984). *Cyprinodon* persistence appears to be exceptionally high among desert fishes, given their survival in remote localities across the desert region (Figure 1) and ability to tolerate extreme environmental conditions (Miller 1981; Smith and Miller 1986; Echelle and Echelle 2020a). In summary, Late Miocene–Early Pleistocene range expansion allowed ancestral *Cyprinodon* to found widespread populations, a subset of which inhabited ecosystems where long-term survival was possible. As an interesting contrast, fishes of northern origin that occupy the desert region display opposite geographical trends of range expansion and persistence (Smith 1978). For example, the *Rhinichthys osculus* species complex had great ability to traverse the desert region in the Pliocene (Smith et al. 2017), like *Cyprinodon*.

However, pluvial lakes and high-elevation inter-drainage corridors aided expansion of *R. osculus*, even in the Pleistocene (Smith et al. 2017), when *Cyprinodon* appears to have retreated to lower elevations and latitudes.

Geomorphological support for mtDNA tree and reciprocal illumination

Although some authors have voiced concern for a lack of geomorphological support for the mtDNA tree of Echelle et al. (2005) (Echelle 2008; Knott et al. 2008; Martin and Turner 2018), we contend this general conclusion has resulted from a focus primarily on the Mojave Desert portion of the tree. Our detailed review for the rest of the genus, outside the Mojave Desert, considers geomorphological evidence along with results from studies of climate and of other native animal taxa, finding reasonable support across the remainder of the tree. Timing and geography indicated in the mtDNA *Cyprinodon* chronogram (Echelle et al. 2005; Echelle 2008) is consistent with Late Miocene–Late Pliocene drainage integration within the Río Grande Rift (Repasch et al. 2017) and Gila–Colorado River (Dickinson 2015; Howard et al. 2015). In several cases, there appears to be correspondence of timing for *Cyprinodon* divergence or secondary contact with hydrographic or climatic conditions (Table 1). In addition, the pattern of inter-relations within the phylogeny universally indicates geographical relations between drainages either once integrated or sharing a boundary and potentially exchanging faunas. If range expansion did not rely on such connections, then this arrangement of species in the phylogeny would be unlikely.

Although beyond the scope of this study, hydrographic reconstructions of Quaternary drainages (Ewing and Christensen 2016; Snedden and Galloway 2019) (Figures 1 and 4) suggest that

many of the opportunities for range expansion available in the Late Miocene-Pliocene were unavailable in the Quaternary. Further, as highlighted throughout, the Pliocene wet period was warm in contrast to the cold (i.e., glacial) Pleistocene wet periods (Ibarra et al. 2018). It should not be surprising therefore that, especially at higher latitudes, this earlier wet period was favorable for range expansion of cold-sensitive *Cyprinodon*, whereas the later wet periods were better for range expansion of cold-tolerant fishes like *Rhinichthys* (Kim and Conway 2014; Smith et al. 2017), *Pantosteus* (McPhee et al. 2008; Corona-Santiago et al. 2018; Turner et al. 2019), and *Oncorhynchus* (Shiozawa et al. 2018).

As illustrated here, abundant geomorphological evidence, complemented by climatic evidence, is available to help interpret the *Cyprinodon* phylogeny. In a similar fashion, the *Cyprinodon* phylogeny provides potential time constraints for otherwise undated geomorphic events, such as integration of the Pecos River and transfer of the Río Papigóchic. In many other cases, it provides additional, independent support to established hypotheses (Table 1). In other words, the variety of evidence provides reciprocal illumination. Thus, even though geomorphic evidence has not revealed definitive dispersal corridors of the proper vintage between the Owens, Amargosa, and Colorado Rivers (Knott et al. 2018), this is not necessarily justification for discarding evidence from *Cyprinodon* that such corridors existed. No form of evidence is unfailing and each has limitations. Geomorphic evidence often requires relatively prolonged processes (i.e., millennia) to create lasting evidence that then must be preserved for later discovery. It is possible for geomorphic evidence to be eroded away or for aquatic connections to exist, but be so short lived or

lacking in sedimentation as to not leave a clear geomorphic record. In comparison, biological processes, like range expansion, can occur on relatively short time frames (decades to centuries; witness historical range expansions by invasive fishes). This difference in the temporal scale of responsiveness potentially makes *Cyprinodon* a more sensitive indicator of past drainage connections than geomorphic evidence, especially given their incredible capacity for persistence upon the desert landscape.

Mosaic of diversification

The potential of *Cyprinodon* for comprehensive dispersal and ample perseverance (given a modicum of habitat suitability) appears to have combined with a multi-million year history in the desert region to produce a complex phylogeny (Echelle et al. 2005). Processes putatively described in this study include: (1) simple allopatry (e.g., *C. eremus* from *C. macularius*, within Río Nazas clade (sensu stricto), *C. bovinus*-*C. pecosensis* from *C. rubrofluvialis*, *C. tularosa* from *C. variegatus*), (2) allopatry following lineage sorting (e.g., western clade), (3) peripatry (e.g., *C. bovinus*, *C. pachycephalus*, *C. julimes*), and (4) reticulate evolution (e.g., *C. bifasciatus*-*C. atlorus*, Balmorhea *C. elegans*-*C. pecosensis*-*C. bovinus*, Río Florido *C. eximius*-*C. macrolepis*, *C. pisteri*-*C. albivelis*). Remote survival of relict lineages from the western clade (e.g., *C. fontinalis*, *C. radiosus*) is consistent with persistence of relict cyprinodontid genera *Cualac* and *Megupsilon* (Echelle and Echelle 2020a). These diverse phenomena illustrate that *Cyprinodon* remains a rich source of cases for study of evolution and speciation.

TABLE 1. Highlights from the synthesis of congruent timing and geography between the mtDNA tree of Echelle et al. (2005; Echelle 2008) and geomorphic evidence. For additional details and further examples see main text.

Hypothesis 1:

Historical distributions of *Cyprinodon* clades do not match with Quaternary drainage boundaries, but are compatible with Late Miocene-Pliocene drainages

Arid Late Miocene climate in Great Plains and Chihuahuan Desert ideal for inland dispersal of *Cyprinodon*

Hypothesis 2:

Potential Late Miocene route to Río Grande Rift bypasses Lower Río Grande, maintaining clade integrity (impossible in Quaternary)

Late Miocene faulting in the Albuquerque Basin occurred at the vicinity of proposed capture from Old Río Manzano into the Río Grande Rift and presumably suitable lacustrine habitats were present

Early Pliocene drainage integration in the Río Grande Rift permits colonization of the southern Rift at the time of Gila River drainage expansion

Hypothesis 3:

Pliocene warm and wet period corresponds to period of range expansion

Safford Basin rifting Late Miocene-Pliocene and regional tributary incision potentially account for proposed capture from Animas Valley (Río Grande Rift) into the Gila River drainage

Presumably suitable lacustrine habitats present in Safford Basin during Late Pliocene-Early Pleistocene

C. eremus-*C. macularius* divergence estimate concurrent with first phase of Pinacate volcanic event

C. pisteri distribution conforms with drainages connected during inundations of pluvial Lake Palomas (Quaternary)

C. fontinalis is sequestered in habitats perched above the level of inundation by pluvial Lake Palomas

Hypothesis 4:

Divergence of *C. bovinus*-*C. pecosensis* from *C. rubrofluvialtilis* validates proposed Pliocene abandonment of Portales Valley

Estimate of secondary contact between *C. bovinus*-*C. pecosensis* and *C. elegans* corresponds to timing of Pecos River integration

Separation of Brazos River and Red River *C. rubrofluvialtilis* corresponds to Pliocene expansion of Red River drainage

Hypothesis 5:

Separation between *C. atrorus* and *C. sp. Aguanaval* corresponds to eruption of intervening Las Coloradas volcanic field.

Three fish clades sympatric with *Cyprinodon* in the ríos Tunal and Nazas have comparable Pliocene timing of Nazas-Mezquital divergence

Hypothesis 6:

Potential timing of *C. elegans* divergence overlaps with period of canyon formation on Lower Pecos River

Estimate of secondary contact between *C. bovinus*-*C. pecosensis* and *C. elegans* corresponds to timing of Pecos River integration and subsidence within the Pecos Trough

Hypothesis 7:

Divergence of *C. bovinus* from *C. pecosensis* corresponds to prolonged incision of Pecos River valley, which increased vertical, horizontal, and possibly ecological separation from upper Leon Creek

Hypothesis 8:

Timing of *C. tularosa* divergence closely corresponds with potential for aquatic access into the Tularosa Basin

Niche conservatism

Niche conservatism is common among North American freshwater fishes (McNyset 2009; Knouft and Page 2011). Predominance of speciation by allopatry or peripatry, observed in *Cyprinodon* (Echelle et al. 2005; Echelle and Echelle 2020a), is attributable to niche conservatism (Wiens 2004; Wiens and Graham 2005). Niche conservatism is common in marine fishes invading freshwaters and may reflect preferential invasion of inland niches with ecological opportunity (Buser et al. 2019). The environmental niche of *Cyprinodon* is generally predictable as harsh habitats lacking diverse fish faunas (Echelle et al. 1972; Soltz and Naiman 1978; Hoagstrom and Brooks 1999; Tobler and Carson 2010; Pittenger et al. 2019; Echelle and Echelle 2020a). Exceptional tolerance for elevated water temperatures and high concentrations of dissolved ions in *Cyprinodon* is a legacy of estuarine ancestors. An omnivorous diet high in detritus and algae (Echelle and Echelle 2020a) and an ability to extract more nutrition from these sources compared to other fishes (D'Avanzo and Valiela 1990) may allow typical *Cyprinodon* (those not divergent in feeding biology) to not only survive in habitats unsuitable for strict predators, but maintain relatively dense populations (Naiman 1976). Conservatism in thermal sensitivity strongly influences North American freshwater fishes (Hasnain et al. 2013) and cold sensitivity appears to limit the latitude reached by neotropical fishes invading North America (Smith et al. 2012). Hence, sensitivity to climatic cooling observed in *Cyprinodon variegatus* (Haney et al. 2007, 2009), consistent with anecdotal observations of cold-shock die-offs (Echelle and Echelle 2020a), is hypothesized here to have also restricted the Quaternary distribution of the genus within the desert.

Niche conservatism does not necessarily imply a narrow niche, but that the niches of modern taxa remain similar to that of the common ancestor (Buser et al. 2019). *Cyprinodon* inhabiting the desert typically exploit habitat diversity when present. *Cyprinodon pecosensis* is a good example, using a wide range of habitats including springs, wetlands, waterfowl impoundments, streams, and the Pecos River (Hoagstrom and Brooks 1999). *Cyprinodon albivelis*, *C. macularius*, and *C. radiosus* exhibit similarly broad habitat use (Moyle 2002; Minckley and Marsh 2009). Cases of narrower environmental niches typically reflect a more limited range of habitats available in a given ecosystem. For example, *C. salinus salinus* retreats to springs in the dry season, but disperses downstream into rewetted habitats when available (Soltz and Naiman 1978; Moyle 2002; Colvin et al. 2019). *Cyprinodon* populations confined to a single habitat are commonly those without access to other habitats (e.g., Miller and Walters 1972). However, atypical *C. bifasciatus* remains within springs even when other habitats are available, due to intolerance of environmental variations (Carson et al. 2008). A similar case may exist for *C. pachycephalus* (Minckley and Minckley 1986). As populations of the same species isolated in distinctly different habitats may exhibit molecular (Hirshfield et al. 1980), morphological (Collyer et al. 2005), or behavioral (Lema 2006) divergence, it is possible these examples of niche contraction represent legacies of long-term habitat confinement.

Future research

This is the first detailed attempt to propose specific biogeographical scenarios of diversification and range spread for *Cyprinodon*. The hypotheses presented here should be

evaluated further as more focused research on speciation, and ecology is completed (Table 2). Future studies in climate, geomorphology, and biogeography of other animal groups are also certain to shed new light on scenarios presented here. Nevertheless, the broad range of evidence now available indicates the mtDNA phylogeny is compatible with geomorphic and other evidence. Agreement in many portions of the tree (Table 1) reinforces more tentative proposals elsewhere. The western clade, which until now seems to have been the focus for evaluation of the mtDNA tree, appears to present the most tentative case. But now, for the first time, detailed hypotheses with explicit inferences are available to explain the biogeography of the western clade outside of the Mojave Desert. Perhaps these will provide a helpful context for ongoing consideration of evidence for a Pliocene-Early Pleistocene origin of *Cyprinodon* within the Owens-Amargosa River drainage.

The allozyme tree played a valuable role in interpreting the history of *Cyprinodon* diversification by identifying possible cases of secondary contact (Echelle et al. 2005). Similarly, new phylogenetic analyses are needed to better examine relations within *Cyprinodon*. Incorporating new evidence into the phylogeny, like more comprehensive molecular data, morphological traits, and sampling from more specimens would help better characterize diversity within widespread taxa (*C. eximius*, *C. nazas*, *C. pisteri*) and determine phylogenetic positions of extinct taxa (*C. arcuatus*, Comanche Creek *C. elegans*, *C. latifasciatus*). Hypotheses presented here provide detailed frameworks to direct such studies.

Anthropogenic threat

Given the number of *Cyprinodon* taxa suffering extinction or extirpation (Echelle and Echelle 2020a; Lozano-Vilano et al. 2020; Williams and Sada 2020), with most of the remainder afflicted with range declines and, in many cases, recognized as deserving formal protection (Echelle et al. 2003; Hoagstrom et al. 2011; Lozano-Vilano and De la Maza-Benignos 2017), there is reason to be concerned for all remaining populations. Aquatic habitats of the desert have suffered dramatic impacts and widespread disappearance (Hendrickson and Minckley 1985; Pister and Unkel 1989; Unmack and Minckley 2008). The risk of further loss is high in light of ongoing growth of the human population and ongoing water development (Deacon et al. 2007; Udall 2020; Garrett et al. 2020), climate change (Hausner et al. 2013; Jaeger et al. 2014; Overpeck and Bonar 2020), and spread of invasive, non-native species (Moyle 2020). Desert clades of *Cyprinodon* have come full circle in their evolutionary history. First, Late Miocene aridity in the northwestern Gulf of México drainage facilitated inland invasion. Second, expansion across the desert region (Figure 1), apparently exceeded that of any other fishes originating in the Gulf of México drainage. Third, the Quaternary ice ages caused range retractions away from higher latitudes and elevations, into enduring refugia. Fourth, in the Anthropocene, humans are appropriating waters of aquatic ecosystems for agricultural, domestic, and industrial uses, making the once-welcoming and nurturing desert region increasingly inhospitable.

Where suitable aquatic habitats remain, habitat fragmentation is an insidious threat because smaller populations face higher risk of extirpation (Fagan and Holmes 2006).

Table 2. Suggested studies with *Cyprinodon* to improve understanding of speciation and evaluate hypotheses proposed in this synthesis. For additional details see text.

Hypotheses 1-4:

Resolve phylogenetic relations among clades using additional molecular markers

Hypothesis 2:

Estimate age of separation between *Notropis simus pecosensis* and *N. s. simus* to compare with origin of western clade *Cyprinodon*

Determine phylogeography of *C. albivelis*-*C. pisteri* in detail, with inclusion of other taxa from the western clade

Hypothesis 3:

Make behavioral, ecological, and physiological comparisons across western clade to explore persistence of separate lineages

Study *Cyprinodon* distribution to assess if temperature constrains upper elevational limit

Hypothesis 4:

Estimate ages of separation among populations of the *Gila conspersa* complex and *Etheostoma pottsi* to compare with Río Nazas clade *Cyprinodon*

Hypothesis 5:

Resolve phylogenetic placement with divergence estimate for *C. bifasciatus* and extinct *C. latifasciatus*

Determine phylogeography of Río Conchos-Río Grande clade, including multiple specimens of all recognized species and comprehensive geographical sampling of *C. eximius*

Hypothesis 6:

DNA sampling of extinct *C. elegans* from Comanche Creek to place population in phylogeny and estimate divergence time from Balmorhea *C. elegans*

Studies of reproductive isolation mechanisms between *C. elegans* and *C. pecosensis*

Hypothesis 7:

Comparative studies of environmental tolerances (e.g., freshwater tolerance, hypoxia tolerance) between *C. bovinus* and *C. pecosensis*

Studies of reproductive isolation mechanisms between *C. bovinus* and *C. pecosensis*

Hypothesis 8:

Studies of reproductive isolation between *C. tularosa* and representatives of the Río Conchos-Río Grande clade

In *Cyprinodon*, habitat fragmentation can also result in anthropogenic divergence among populations in isolated habitats, diminishing species integrity (Dunham and Minckley 1998; Watters et al. 2003; Collyer et al. 2015). Once subdivided and degraded, remnant populations become increasingly difficult to conserve (Osborne et al. 2013; Lema et al. 2020) and, not

surprisingly, range contraction is a precursor to extinction (e.g., *C. arcuatus*, Minckley et al. 2002).

Aquatic ecosystems of the desert represent a prehistoric legacy of modern significance. They are reservoirs of biodiversity and exemplars of sustainable water use. Those in the North American deserts have preserved a living record of

the incredible feats of colonization accomplished by ancestral *Cyprinodon*. Now, conservation efforts are paramount if desert *Cyprinodon* are to prevail through the Anthropocene (Pister 1999). Habitat conservation and restoration are critical to maintain ecological processes that supported populations of *Cyprinodon* for millennia (Echelle and Echelle 2020b; Sada and Stevens 2020). For as long as populations persist within natural habitats, more detailed studies will reveal new insights into the biology and ecology of range expansion, speciation, and survival, furthering our efforts for conservation and, perhaps, teaching us something metaphorical about perseverance and sustainability in the desert.

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ORCID

Christopher W. Hoagstrom  <https://orcid.org/0000-0002-8416-1308>

Megan J. Osborne  <https://orcid.org/0000-0002-0978-2905>

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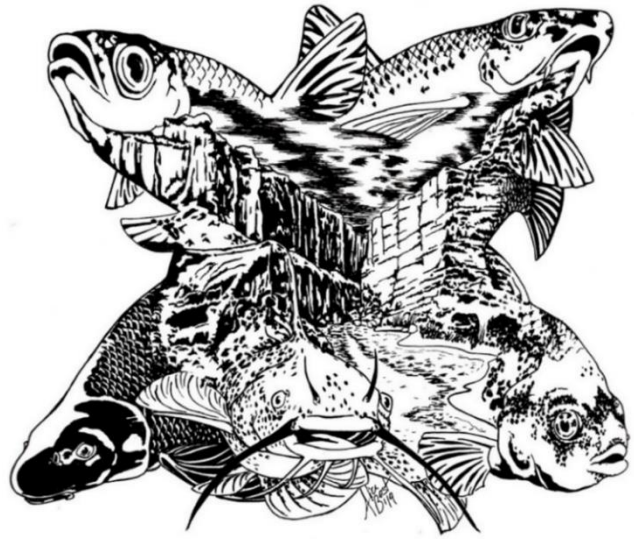
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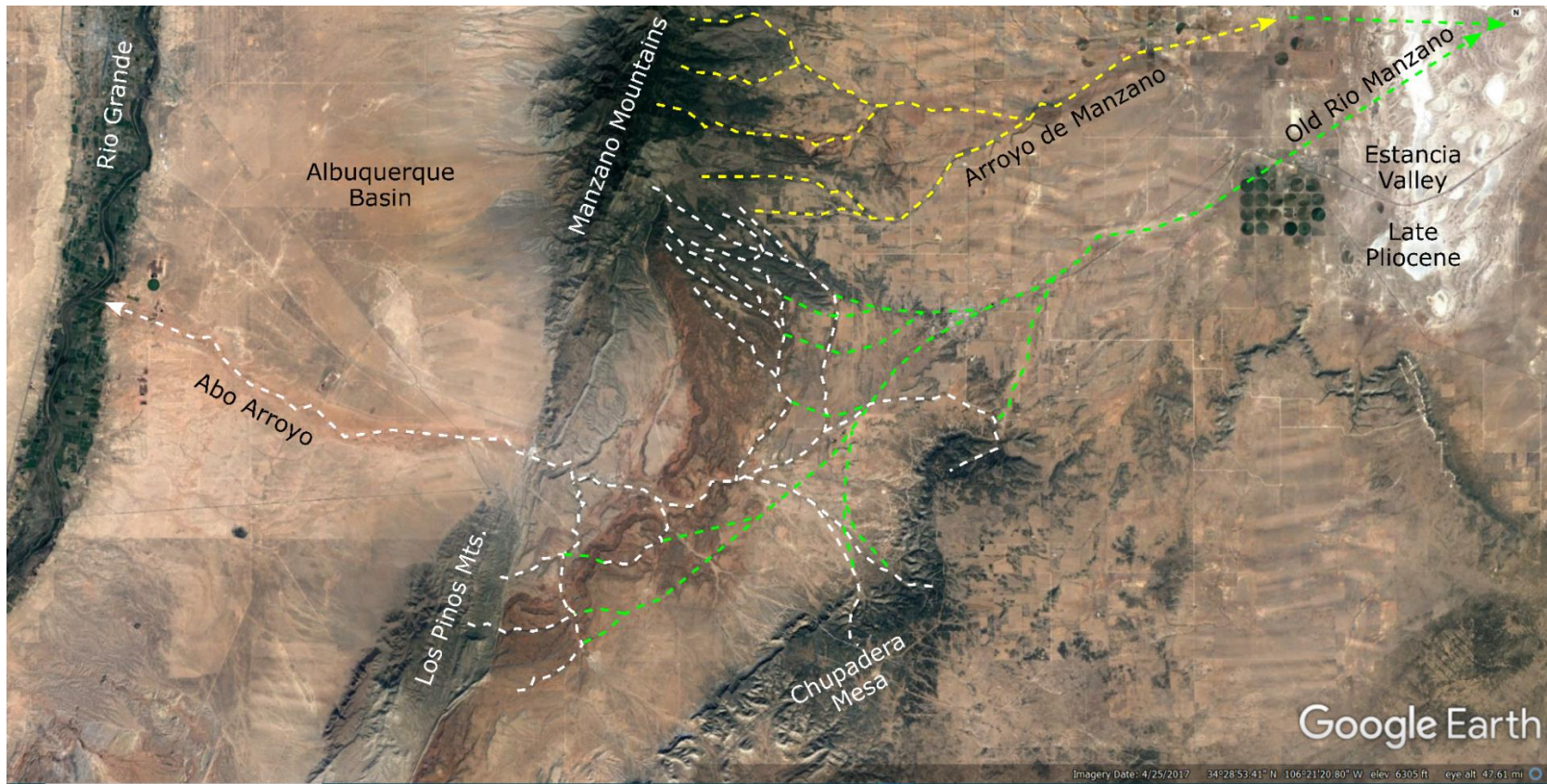
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APPENDIX I. Records of *Cyprinodon* in upland drainages at relatively high elevation, in addition to well documented high-elevation populations of *Cyprinodon albivelis* and *C. salvadori* (Minckley and Marsh 2009; Lozano-Vilano and de la Maza-Benignos 2017). Elevations are estimates from Google Earth Pro.

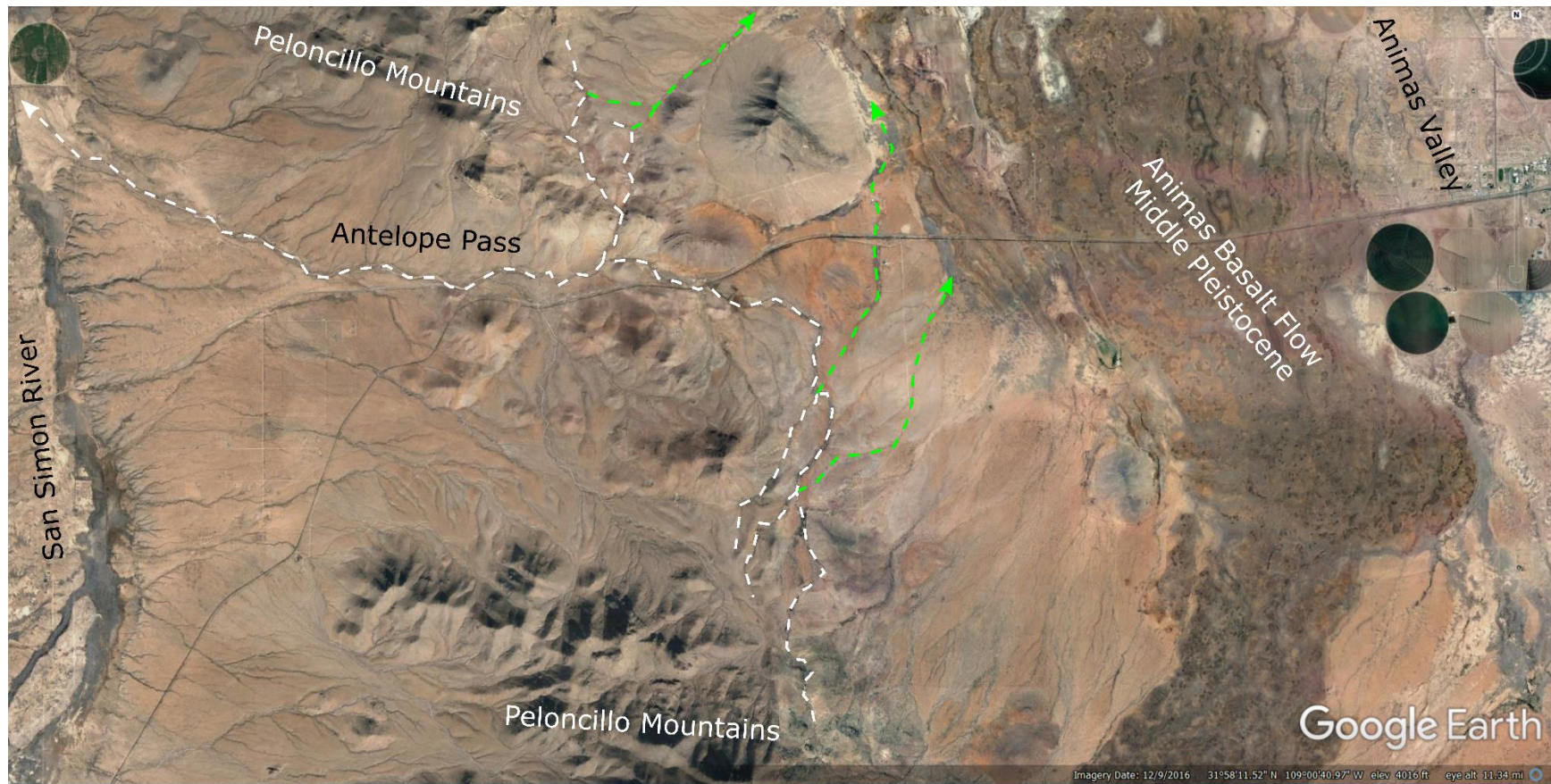
Clade and taxon	Location	Elevation (m)	References
Great Plains-Chihuahuan Desert			
<i>C. pecosensis</i>	Forks of Río Hondo, Río Bonito-Río Ruidoso confluence	~1590	Sublette et al. 1990
Río Conchos-Río Grande			
<i>C. eximius</i>	Río San Pedro, San Francisco de Borja	~1638	De la Maza-Benignos 2009
“	Río Noavaco, Santa Rosalía de Cuevas	~1673	Meek 1904
“	Río Florido, Canutillo	~1676	Miller et al. 2005
“	Río Santa Cruz/Isabel, San Andres/Riva Palacio-Gral. Trías	~1758-1598	Edwards et al. 2002; De la Maza-Benignos 2009
Río Nazas			
<i>C. sp. Aguanaval</i>	Río Aguanaval, Rancho Grande	~2007	Miller 1976, Miller et al. 2005
<i>C. meeki</i>	Known distribution	~1920-1872	“
<i>C. nazas</i>	Río Sextín, San José de Sextín	~1657	“
“	Río Peñon del Covadonga, La Concha to Peñon Blanco	~1746-1730	“
“	Laguna Santiaguillo	~1960	“
Western			
<i>C. pisteri</i>	Upper Río Casas Grandes	~1650	Minckley et al. 2002, Miller et al. 2005
“	Laguna Bustillos	~1955	“
“	Upper Río Santa María at Bachiniva	~2016	“

APPENDIX II. Proposed Quaternary evolution of the Pecos River drainage. Rows in normal font refer to formations within the Pecos River valley believed to be of comparable vintage (see references). Formation abbreviations shown in geological maps for New Mexico (NMBGMR 2003) and Texas (Stoeser et al. 2006) are provided. Rows in italics and small-capital letters include climatic events and drainage rearrangements within the confluent Río Grande drainage, both of which may have contributed to the long-term trend of valley incision along the Pecos River.

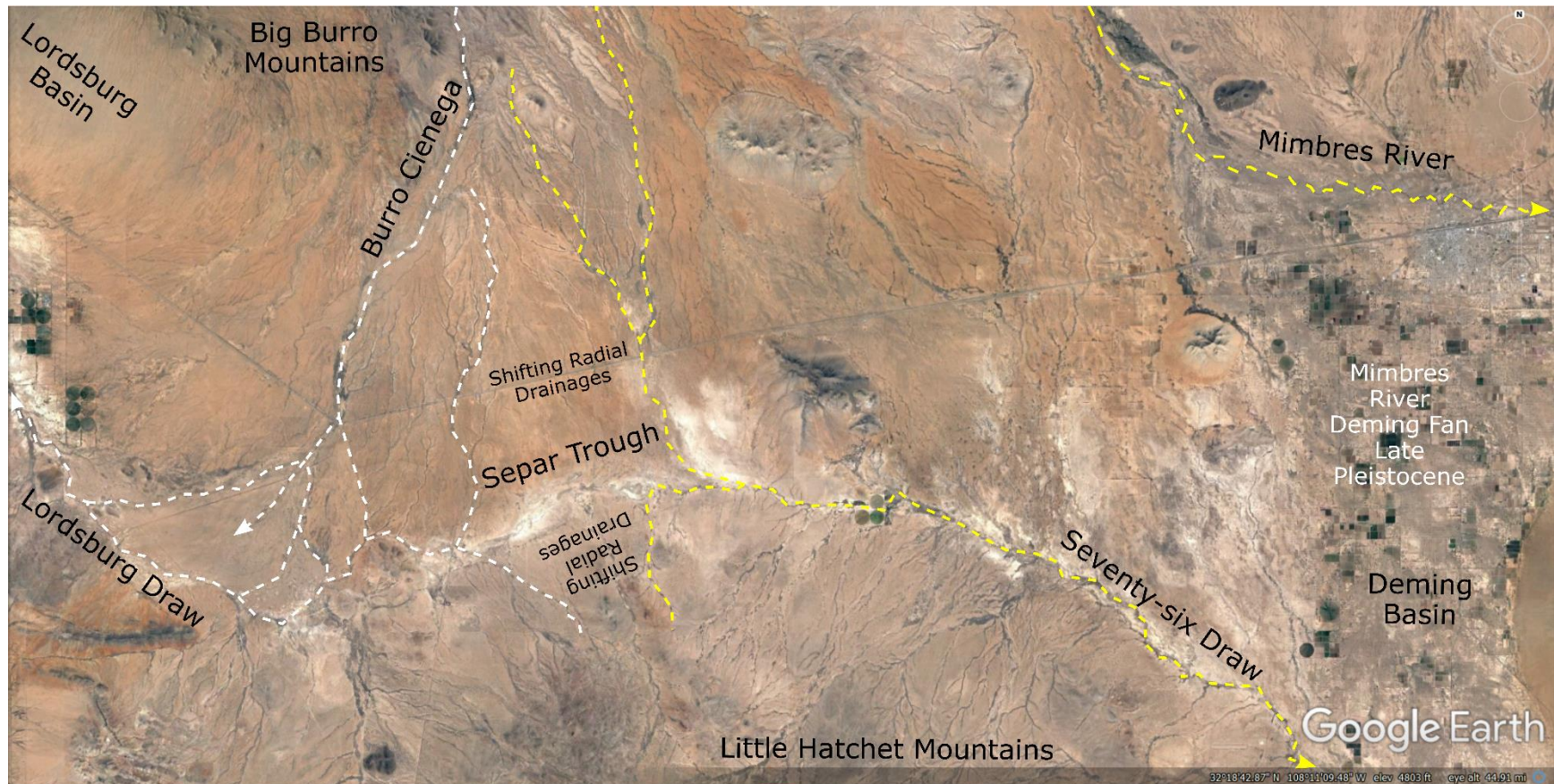
Deposit / formation or <i>EVENT</i>	Date Range Mya (mean)	Stoeser et al. 2006	NMBGMR 2003	References
<i>EARLY-MIDDLE PLEISTOCENE TRANSITION ONSET OF 0.10 MY GLACIAL CYCLES (PLUVIAL PERIODICITY)</i>	1.20-0.80	-----	-----	<i>EHLERS ET AL. 2018</i> -----
<i>UPPER RÍO GRANDE OVERFLOW INTO RÍO CONCHOS-LOWER RÍO GRANDE (BASE-LEVEL LOWERING)</i>	1.00-0.80	-----	-----	<i>REPASCH ET AL. 2017</i> -----
End Gatuña and ‘Surface I’ deposition, Pecos River incision into Capitan Aquifer	1.00-0.56	QTg, Qg, Qsgc	Qoa	Leonard and Frye 1962; Pazzaglia and Hawley 2004; Hawley 2005 Railsback et al. 2015
<i>MIS 16 STRONG GLACIATION (VALLEY DEPOSITION)</i>	0.67-0.62	-----	-----	<i>HEAD AND GIBBARD 2015</i> -----
<i>MIS 15-13 PROLONGED INTERGLACIAL, WEAK GLACIAL (LIMITED VALLEY INCISION)</i>	0.62-0.48	-----	-----	<i>HAO ET AL. 2015</i> -----
<i>LAKE ALAMOSA OVERFLOW IN RÍO GRANDE (BASE-LEVEL LOWERING)</i>	0.60-0.40	-----	-----	<i>REPASCH ET AL. 2017</i> -----
<i>MIS 12 STRONG GLACIATION (VALLEY DEPOSITION)</i>	0.48-0.42	-----	-----	<i>HEAD AND GIBBARD 2015</i> -----
Blackdom alluvial deposits, Neville Formation, ‘Surface II’ (may represent MIS16-12)	?	Qao	Qp	Albritton and Bryan 1939; Leonard and Frye 1962; Hawley 2005
<i>MIS 11-7 PROLONGED INTERGLACIAL, WEAK GLACIAL (VALLEY INCISION)</i>	0.42-0.19	-----	-----	<i>HUGHES AND GIBBARD 2018</i> -----
<i>UPPER PECOS RIVER CAPTURE (INCREASED RUNOFF & GLACIAL INFLUENCE)</i>	0.47-0.09	-----	-----	<i>KIM AND CONWAY 2014</i> -----
<i>MIS 6 STRONG GLACIATION (VALLEY DEPOSITION)</i>	0.19-0.13	-----	-----	<i>EHLERS ET AL. 2018</i> -----
Orchard Park alluvial deposits (Rancholabrean), Calamity Formation, ‘Surface III’ of Delaware Basin, ‘Surface IV’ of Edwards Plateau	0.20-0.14	Qal	Qp	Albritton and Bryan 1939; Leonard and Frye 1962; Hawley 2005; Morgan and Lucas 2006
<i>MIS 5 INTERGLACIAL (VALLEY INCISION)</i>	0.13-0.12	-----	-----	<i>HOFFMAN ET AL. 2017</i> -----
<i>MIS 4-2 PROLONGED STRONG GLACIATION (VALLEY DEPOSITION)</i>	0.12-0.01	-----	-----	<i>HUGHES AND GIBBARD 2018</i> -----
Lakewood Terrace, Kokernot Formation, ‘Surface IV’ of Delaware Basin (may represent MIS5-2)	?	Qt	Qa	Albritton and Bryan 1939; Leonard and Frye 1962; Hawley 2005
<i>MIS 1 INTERGLACIAL (VALLEY INCISION)</i>	0.01- PRESENT	-----	-----	-----



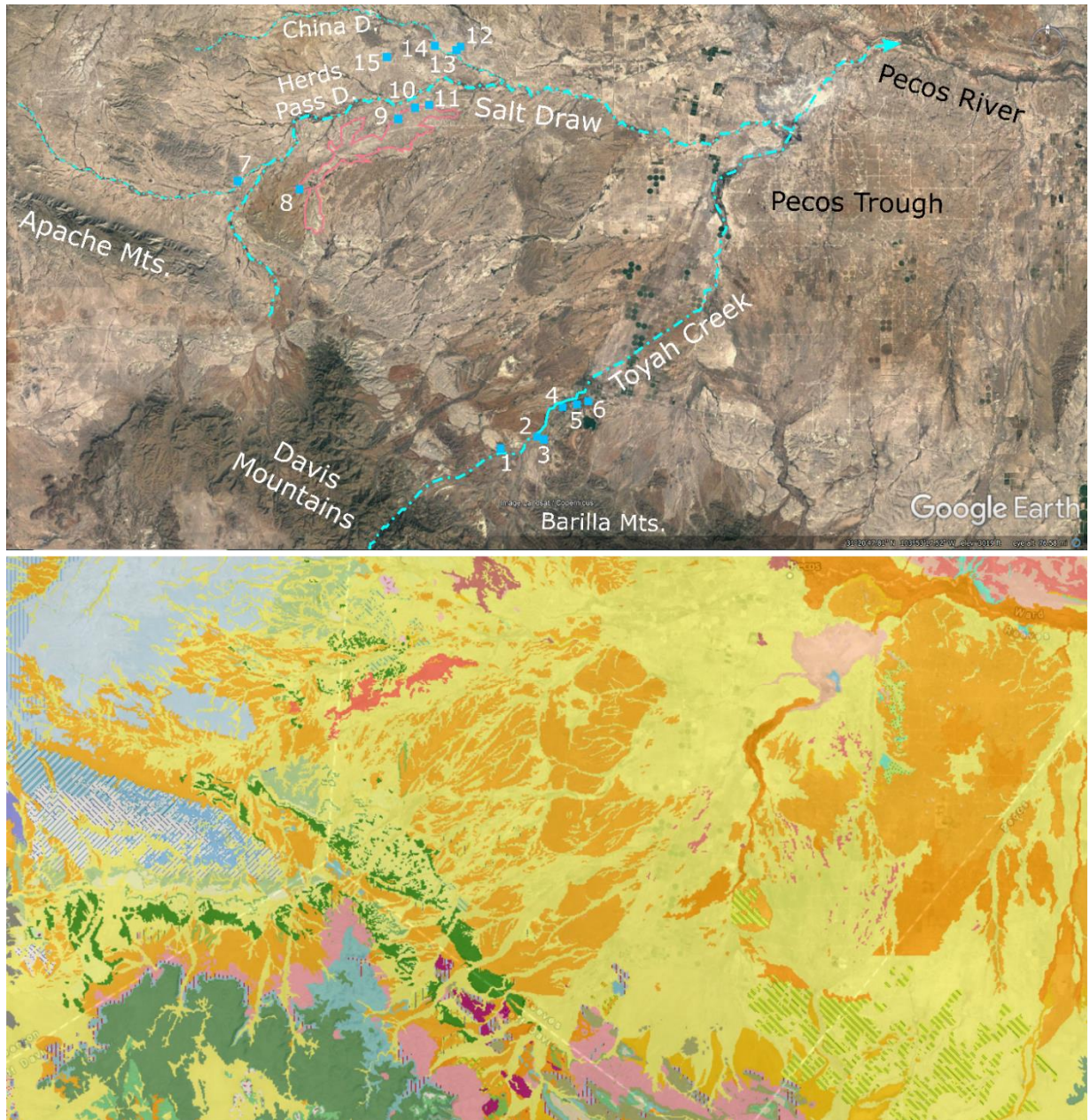
APPENDIX III. Google Earth image for the vicinity of Mountainair, New Mexico (southwest Torrance County), showing proposed Late Miocene beheading of the Old Río Manzano by Abo Arroyo. It is hypothesized that Late Miocene faulting within the Albuquerque Basin (Río Grande Rift, Connell 2004) lowered the basin and initiated tributary incision. Timing of western clade *Cyprinodon* divergence (6.3 Ma, Echelle et al. 2005; 5.4 Ma, Echelle 2008) and Late Miocene hydrography (Figure 2) suggest this as the most feasible route into the incipient Río Grande Rift, from which the clade is hypothesized to have eventually reached its historical distribution. Modern channels are highlighted in white (Abo Arroyo) and yellow (Arroyo de Manzano). Proposed pre-capture channels (generalized), potentially present prior to incision of Abo Arroyo, are highlighted in green. Old Río Manzano is a heretofore unnamed, prehistoric river (now defunct) that deposited a western extension of the Ogallala Formation (Kelley 1972; Frye and Leonard 1982) and transported gravels linked to the Manzano Mountains into the Ancestral Brazos River drainage as far east as the Cross Timbers ecoregion (Dallas County, Texas; Menzer and Slaughter 1971; Walker 1978). Note, although the Estancia Valley playa now lies across the former path of the Old Río Manzano, this is a more recent feature (Late Pliocene or Early Pleistocene, Kelley 1972; Allen and Anderson 2000). An unaltered view can be observed via Google Maps or Google Earth online applications.



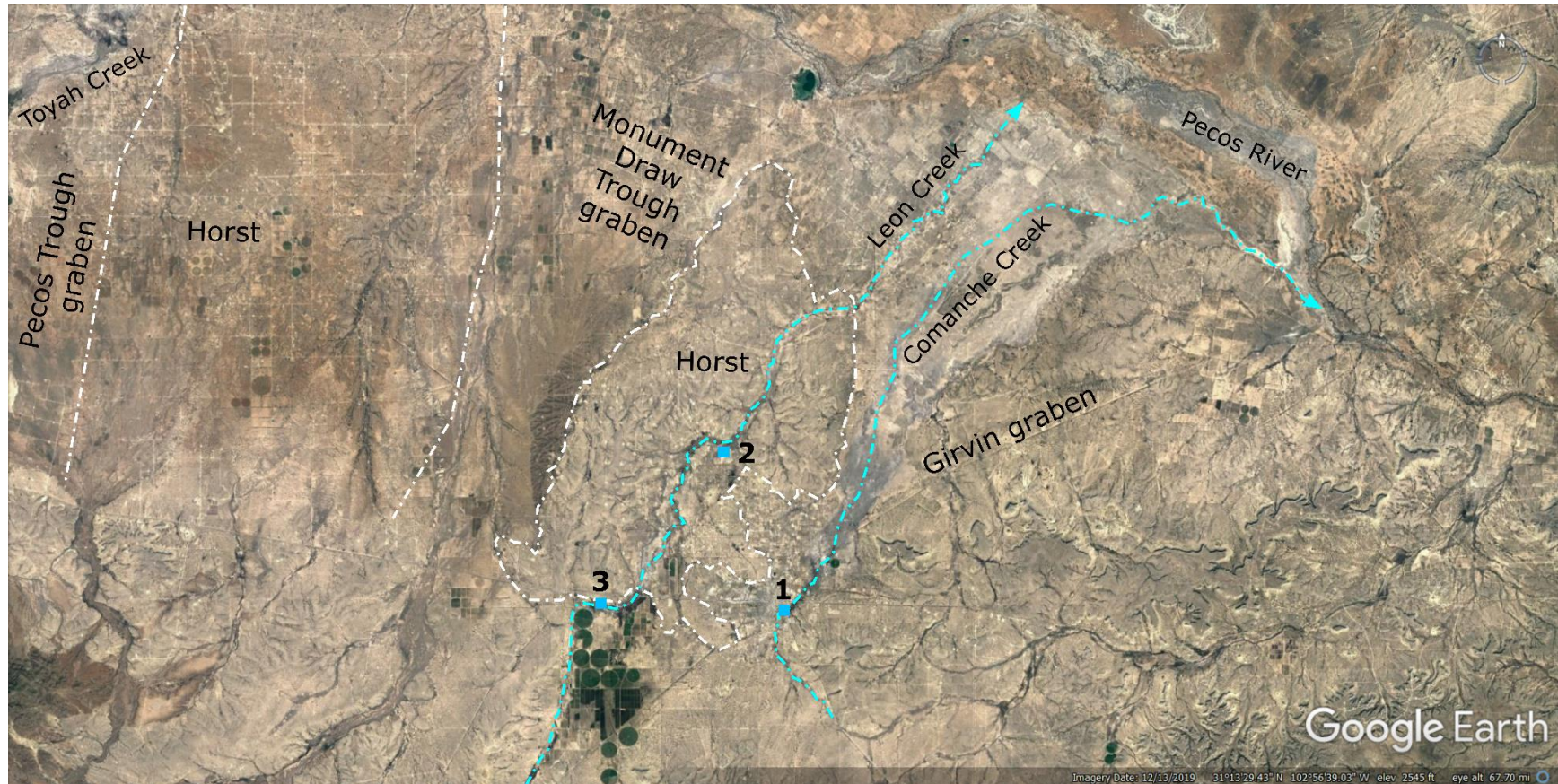
APPENDIX IV. Google Earth image for the vicinity of Animas, New Mexico (central Hidalgo County), showing proposed Early Pliocene beheading of a tributary to the Animas Valley by an unnamed tributary of the San Simon River (Safford Basin) through Antelope Pass. It is hypothesized that Late Miocene faulting within the Safford Basin (Kruger et al. 1995) led to drainage development along the San Simon River, ultimately causing tributary incision through Antelope Pass in the Early Pliocene. Integration with the Gila River basin by 3.6 Ma (Harris 2000) further accelerated river incision and drainage expansion (Dickinson 2015). Timing of divergences between Pacific and Atlantic slope lineages (4.2-1.5 Ma, Echelle et al. 2005; ~2.0-1.9 Ma Echelle 2008) and Pliocene hydrography (Figure 3) suggest this as the most feasible route across the Continental Divide. Modern channels are highlighted in white. Proposed pre-capture channels (generalized), potentially present prior to capture, are highlighted in green. Note, although the Animas basalt flow now lies across the path of streams into Animas Valley, this is a more recent feature (Middle Pleistocene, 0.5 Ma, Deal et al. 1978). An unaltered view can be observed via Google Maps or Google Earth online applications.



APPENDIX V. Google Earth image for the vicinity of Deming, New Mexico (central Luna County), showing potential Pliocene dispersal corridor between the drainages of pluvial Lake Cabeza de Vaca and Animas Valley. It is hypothesized that aquatic habitats were present across this divide in the Pliocene such that either periods of flooding or shifting of radial tributaries flowing off the Big Burro and Little Hatchet mountains provided connectivity or episodic transfer from east (Seventy-six Draw) to west (Burro Ciénega). Based on phylogenetic evidence suggesting multiple *Cyprinodon* lineages colonized the Gila River basin from the drainage of pluvial Lake Cabeza de Vaca (see text), it is possible this route was used multiple times. Modern channels for the drainage of Lordsburg Draw (Animas Valley drainage) are highlighted in white, whereas those of the Mimbres River drainage (pluvial Lake Cabeza de Vaca) are highlighted in yellow. Note, stream courses remain transient and dynamic. Examples include multiple courses for Burro Ciénega where it turns west (highlighted on figure) and Late Pleistocene paths of the Mimbres River through Deming Basin (Deming Fan) (Love and Seager 1996). An unaltered view can be observed via Google Maps or Google Earth online applications.



APPENDIX VI. Google Earth image (top) for the vicinity of Balmorhea, Texas (southern Reeves County), Toyah Creek drainage, with historic springs. Springs along Toyah Creek are associated with historic populations of *Cyprinodon elegans*: (1) Phantom, (2) Giffin, (3) San Solomon, (4) Saragosa, (5) West Sandia, (6) East Sandia. Adjacent Toyah Creek (solid line segment) collected spring overflow and held additional springs (White et al. 1941; Brune 1981). Historic springs (generally defunct today) are also shown within the Salt Draw tributary drainage—(7) Hurds (Herds), (8) seep-fed lake, (9) Bone (Liege), (10) Coyote (Torez), (11) Petican (Pelican). Deposits of Toy Travertine (salmon-color outline) >9 m thick extend upstream along Herd's Pass Draw, indicating Pleistocene springs—presumed precursors to historical springs—discharged over a broad area. Other springs along China Draw—(12) Johnson (with lake), (13) Turin (Twin), (14) Canyon, (15) Burnt—have local travertine deposits (Brune 1981). An unaltered view can be observed via Google Maps or Google Earth online applications. A paired geological map (bottom, Stoesser et al. 2006) shows Toy Limestone travertine along Salt Draw (salmon color). Cretaceous limestones associated with the regional artesian aquifer (shades of green) are also shown.



APPENDIX VII. Google Earth image for the vicinity of Fort Stockton, Texas (central Pecos County), showing position of upper Leon Creek drainage above and across a horst versus surrounding areas in grabens such as the Monument Draw Trough, where dissolution subsidence has lowered the land surface and caused faulting (Bumgarner et al. 2012). The upland portion of the horst is lightly outlined in white and the approximate boundaries of adjacent horsts and grabens are shown. Locations of Comanche Springs (1), Diamond Y Spring (2), and historic location of Leon Spring (3) are shown. *Cyprinodon elegans* (Río Conchos-Río Grande clade) historically occupied Comanche Springs. A section of lower Toyah Creek (Appendix VI) is visible in the upper left. *Cyprinodon bovinus* (Great Plains-Chihuahua Desert clade) historically occupied Leon Spring and persists in Diamond Y Spring. The sister species of *C. bovinus*, *C. pecosensis*, historically occupied the adjacent Pecos River. An unaltered view can be observed via Google Maps or Google Earth online applications.