ORIGINAL ARTICLE

Inter-basin exchange and repeated headwater capture across the Sierra Madre Occidental inferred from the phylogeography of Mexican stonerollers

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ABSTRACT

Aim Geomorphic evolution of river basins can shape the structure and diversity of aquatic communities, but understanding the biological significance of basin evolution can be challenging in semi-arid regions with ephemeral or endorheic conditions and complex drainage configurations such as the Sierra Madre Occidental (SMO) in North America. In this study, we characterized range-wide patterns of genetic variation in the Mexican stoneroller (*Campostoma ornatum*) to infer how orogenic and erosional influences on river basin connectivity have given rise to the diverse and largely endemic freshwater communities across the SMO region.

Location Twelve drainage basins across northern Mexico and the south-western United States, centred on the SMO.

Methods We collected 202 specimens from 98 localities across the range of *C. ornatum*. We performed phylogenetic analyses of DNA sequences from one mitochondrial (cytochrome *b*) and one nuclear (intron S7) gene. Phylogenetic trees were estimated for each data set using maximum likelihood and Bayesian inference.

Results Phylogenetic analyses consistently resolved a monophyletic *C. ornatum* composed of multiple evolutionary lineages within two markedly divergent clades that differentiate northern drainages from southern drainages in the SMO region. Within-clade patterns of divergence corresponded to fine-scale geographic structure within and among SMO drainage basins. However, the geographic distribution of evolutionary lineages within the northern and southern clades did not always correspond to the geographic configuration of drainage basins. Some subclades encompassed multiple drainages, and individuals from a single drainage were sometimes recovered in multiple subclades.

Main conclusions Our findings indicate that a common ancestor of Mexican *Campostoma* is likely to have entered north-west Mexico through an ancient Rio Grande system that extended as far south as the Rio Nazas and Rio Aguanaval. The geographic orientation of the two strongly divergent clades recovered within *C. ornatum* provides evidence of long-standing isolation of southern basins from northern basins within the ancestral Rio Grande system, possibly due to the combined influence of tectonic events and increasing regional aridity. Geographic patterns of genetic variation also provide evidence of range expansion from Atlantic to Pacific drainages due to drainage evolution and river capture events, as well as further inter-basin exchange via more recent headwater capture events, hydrological connections and possible anthropogenic introductions.

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INTRODUCTION

Geomorphic evolution of river basins can shape the structure and distribution of freshwater biological diversity, but understanding the biological significance of basin evolution can be challenging in semi-arid regions with ephemeral or endorheic conditions. It is unclear, for example, whether ichthyofaunal distributions across western North America reflect persistent isolation of river basins rather than structural integration of sub-basins or hydrological connections and transfers via stream piracy (Minckley *et al.*, 1986). The relative influence of inter-basin isolation and exchange is particularly unclear in semi-arid regions with geographically proximate headwaters such as highlands that function as continental divides (Hubbs & Miller, 1948; Smith *et al.*, 1983; Smith & Miller, 1986; Minckley *et al.*, 1986).

Phylogenetic relationships among closely related taxa and geographic patterns of intra-specific genetic variation can serve as biological records of geomorphic evolution in semi-arid regions (Smith *et al.*, 2002; Echelle, 2008; Schönhuth *et al.*, 2008). By promoting isolation or connectivity, for example, drainage evolution has the potential to establish or mix independently evolving lineages. The timing and sequence of geomorphic events can also be inferred from estimates of genetic divergence within and among lineages (Smith & Dowling, 2008). Increasing use of phylogenetic and phylogeographic analysis of freshwater fishes is beginning to resolve relationships between drainage evolution and the structure of freshwater diversity in semi-arid regions of the world (Smith *et al.*, 2002; Schönhuth *et al.*, 2006; Echelle, 2008). Nonetheless, many areas with diverse and largely endemic communities have yet to receive much attention, including the geologically complex north-northwest trending Sierra Madre Occidental (SMO) volcanic arc in Mexico (Schönhuth & Doadrio, 2003; Schönhuth *et al.*, 2006).

The SMO is a 289,000 km^2 region (comprising one-sixth of Mexico) that is thought to be an important corridor for historical colonization and vicariance of freshwater fishes across North America (Miller & Smith, 1986). The region is noted for supporting diverse and endemic freshwater communities, yet little is known about the conditions that have contributed to community formation or species distributions. Intra-continental tectonism has been singled out as a key driver of freshwater fish biogeography across the SMO region (Miller & Smith, 1986), but it is also thought that other geomorphic events and climatic trends of increasing aridity have influenced species distributions over time (Miller & Smith, 1986; Minckley *et al.*, 1986; Smith & Miller, 1986). Both

phylogenetic and phylogeographic approaches have been taken to examine the relative importance of geomorphic events and climate on freshwater fishes in western North America (Schönhuth *et al.*, 2006, 2008; Echelle, 2008), but none have focused on wide-ranging species with distributions that span the SMO. Indeed, few species have a sufficiently broad range across the highlands of west and central Mexico to permit comprehensive examination of biogeographic patterns and to identify events or conditions responsible for the origins and maintenance of the region's freshwater diversity (Schönhuth *et al.*, 2001, 2008; Domínguez-Domínguez *et al.*, 2006; Echelle, 2008; Pérez-Rodríguez *et al.*, 2009).

In this study, we examined geographic patterns of genetic variation within the Mexican stoneroller, *Campostoma ornatum* Girard, 1856, to infer the biological significance of drainage evolution across the SMO region. The Mexican stoneroller is among the most widely distributed species in western and central Mexico, inhabiting drainages on both the Atlantic and Pacific sides of the continental divide (Miller *et al.*, 2005). Hence, the species represents a useful model for assessing how geomorphic events have shaped the biogeographic history of freshwater biota across the SMO. No studies have yet been carried out that detail the evolutionary history and biogeography of *C. ornatum*. Herein, we examine range-wide patterns of mitochondrial and nuclear DNA variation to assess relationships among populations of Mexican stoneroller inhabiting drainages throughout the SMO and adjacent regions. We assessed both phylogenetic and geographic patterns of evolutionary diversity to infer potential historical conditions and geological processes that: (1) have influenced the species' current distribution; (2) are responsible for evolutionary diversity in the taxon; and (3), by extension, have shaped patterns of freshwater diversification in the highlands of central and western Mexico.

MATERIALS AND METHODS

Study area

The SMO formed as a result of Cretaceous–Cenozoic magmatic and tectonic episodes generated from the subduction of the Farallon Plate beneath North America and the opening of the Gulf of California (Ferrari *et al.*, 1999, 2005). The SMO is an arc of ancient volcanic mountains 125–300 km wide with peaks as high as 3000 m. The mountain range stretches 1500 km along the Gulf of California from Arizona in the United States (USA) to Jalisco in central Mexico (Fig. 1). The region has annual fluctuations in temperature and rainfall that
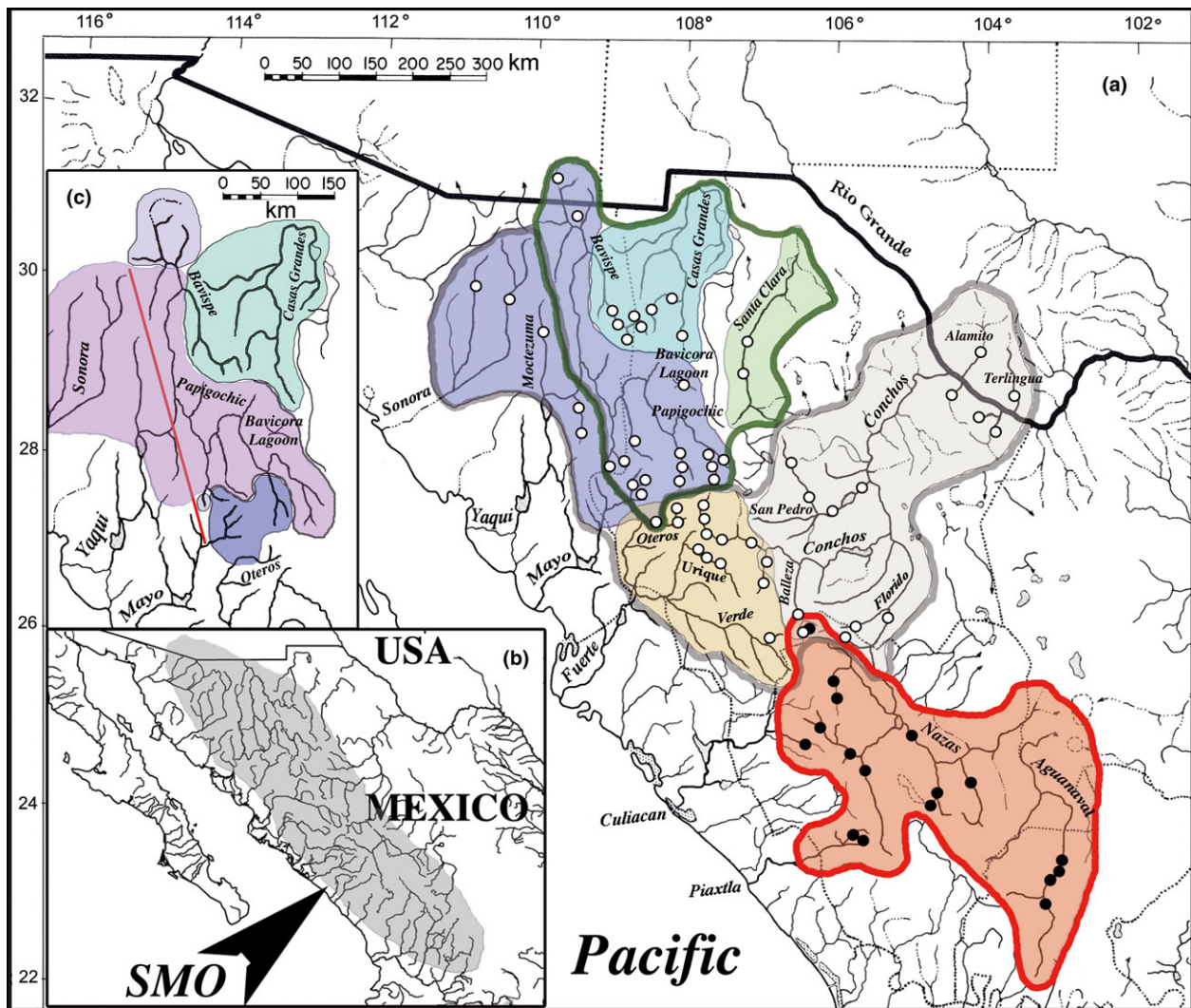
Figure 1 Maps of the study area in western North America. (a) Distribution of Mexican stonerollers with sample localities. White dots indicate localities with the newly defined *Campostoma ornatum* and black dots indicate localities with the newly proposed sister species. Differently shaded areas correspond to different mitochondrial cytochrome *b* clades, and heavy lines correspond to different nuclear S7 clades. (b) Location of the Sierra Madre Occidental (SMO) in Mexico. (c) Rio Yaqui drainage subclade distributions recovered in cytochrome *b* genealogy. The straight line corresponds to the geographic separation of clades recovered in S7 analyses.

differ between northern and southern sections of the range due to regional variation in elevation. The western side of the SMO generally receives more rainfall and has consistently milder winters (Brown, 1994).

River drainages within the SMO exhibit complex hydrological and hydrographical patterns. The predominant drainage direction is towards the Gulf of California and Pacific Ocean (hereafter referred to as the Pacific coast). Rivers emptying towards the Pacific coast extend eastwards from arid lowlands into the highlands of the SMO. Both of the primary Pacific drainages – the Rio Yaqui and Rio Fuerte basins – encompass a complex of sub-basins. The Rio Yaqui, which drains *c.* 73,000 km^2 and is one of the largest rivers in western Mexico, contains the Rio Papigochic sub-basin to the south (Chihuahua) and the Rio Bavispe sub-basin to the north (Sonora) (Hendrickson *et al.*, 1981). The Rio Fuerte drainage

has three main tributaries: the Rio Oteros to the north, the Rio Verde in the south, and the Rio Urique between the Rio Oteros and the Rio Verde. The Rio Conchos, draining *c.* 68,000 km^2, is the largest river that feeds into the Rio Grande from Mexico. It courses through the Chihuahuan Desert from a series of tributaries (Rio Florido, Rio Conchos, Rio San Pedro) originating in the SMO. Like other rivers in well-irrigated semi-desert regions of North America, the Rio Conchos has been heavily impacted by agricultural practices and groundwater removal – droughts from 1994 to 2006 depressed the amount of water delivered to the Rio Grande and in 1999 the Rio Grande failed to reach the sea for the first time in recorded history (Trueba *et al.*, 2008). The Rio Nazas and Rio Aguanaval drainages are two endorheic basins that occur within the hydrological system of the southern SMO. In northern and eastern areas of the SMO, the Guzman Basin includes four

major endorheic sub-basins: the Rio Casas Grandes, Rio Santa María, Rio Santa Clara and Laguna Bustillos. This vast endorheic region (the pluvial Lago de Guzman system of Smith & Miller, 1986) once supported a complex of lakes, streams and springs, including part of the ancient pluvial Lake Palomas, which encompassed more than 7000 km^2 during the late Pleistocene (Smith & Miller, 1986; Castiglia & Fawcett, 2006). Tributaries of the Guzman Basin now form a disrupted array of desiccated inter-montane basins that can be identified by distributions of at least 11 native fishes found in remnant aquatic habitats (Smith & Miller, 1986; Minckley *et al.*, 2002). The 2100 km^2 Bavicora Lagoon lies between the Guzman Basin and the upper Yaqui drainage at elevations from 2100 m to 3000 m. The lagoon was a large pluvial lake in the Pleistocene (Smith & Miller, 1986; Metcalfe *et al.*, 2002) and is now a small flat seasonally flooded lake bed (referred to as a 'playa') with several tributaries that are diverted for irrigation (Stefferud & Propst, 1996).

Study organism

Members of the genus *Campostoma*, commonly referred to as 'stonerollers', are among the most abundant and widespread fishes in eastern and south-western North America, extending northwards into southern Canada and southwards into central Mexico (Lee *et al.*, 1980; Burr & Cashner, 1983; Blum *et al.*, 2008). The Mexican stoneroller, *C. ornatum*, or 'rodapiedras mexicano', is the southernmost distributed species of the genus. This species inhabits 12 drainages across south-western North America and does not occur in sympatry with any other species of the genus. *Campostoma ornatum* is a prominent member of the freshwater fish fauna endemic to the highlands of west and central Mexico, and is among the most widely distributed species within the SMO. The species ranges as far north as the Big Bend region of the Rio Grande in Texas, westward through the Rio Conchos basin and endorheic systems of Chihuahua, into the Rio Yaqui and Rio Sonora of Chihuahua and Sonora. South of Rio Yaqui it occurs on the west slope of the SMO in the upper parts of the Mayo, Fuerte and Piaxtla drainages. It is has been recorded eastward into the endorheic Rio Nazas and Rio Aguanaval basins of Durango and Zacatecas, and was first recorded here in the upper Rio Culiacan (Fig. 1).

Campostoma ornatum is a meristically and morphologically variable species that displays features once considered to be primitive compared with other members of the genus (Burr, 1976, 1980). Buth & Burr (1978) assigned *C. ornatum* as the basal member of the genus due to strong divergence across multiple isozyme loci, and suggested that several diagnostic morphological traits (e.g. small scale size, reduced cephalic tuberculation, gut rarely coiled around the gas bladder) exhibited by the species represent ancestral character states. More recent molecular analyses recovered *C. ornatum* as a derived lineage, indicating that diagnostic traits exhibited by the species are apomorphies rather than ancestral character states (Blum *et al.*, 2008). Burr (1976) observed extensive

variation in both meristic and other morphological characters, and proposed that the species was polytypic according to what appeared to be a mosaic distribution of character variation across its range. Later surveys, noted discordant patterns of morphological variation within *C. ornatum* (R.L.M. and others, pers. obs.). Little is known, however, about genetic variation within *C. ornatum*. Genetic evidence of extensive cryptic variation across the genus (Blum *et al.*, 2008) suggests that the species may be composed of more than one evolutionary lineage.

Field collections and laboratory analysis

We collected 202 specimens of *C. ornatum* by electrofishing and seining 98 locations, from the Rio Aguanaval drainage at latitude 23°32′ N in Zacatecas, Mexico, to the Rio Yaqui drainage at latitude 31°46′ N in Arizona, USA. The specimens represent populations from the 12 drainages the species is known to inhabit (Rio Grande–Rio Conchos, Rio Sonora, Rio Yaqui, Rio Mayo, Rio Fuerte, Rio Culiacan, Rio Piaxtla, Rio Nazas, Rio Aguanaval, Rio Casas Grandes, Rio Santa Clara and Bavicora Basin) (Fig. 1). Specimens from the two closely related species *Campostoma pullum* and *Campostoma plumbeum* were used as outgroup taxa, reflecting the most recent molecular phylogeny of the genus (Blum *et al.*, 2008). A list of specimens examined is provided in Appendix S1 in the Supporting Information; institutional abbreviations follow those of Leviton *et al.* (1985) and Leviton & Gibbs (1988). Other voucher materials are deposited in ichthyological collections at Saint Louis University (STL), St. Louis, MO, USA.

Two genomic regions were selected for study: the complete mitochondrial cytochrome *b* gene (cyt *b*, 1140 bp) and the nuclear locus S7 (from 869 to 875 bp). Nuclear DNA and mitochondrial DNA (mtDNA) sequences were obtained from the same individuals. DNA extraction from tissue samples was performed using a DNeasy Tissue extraction Kit (Qiagen, Valencia, CA, USA). Amplification and primers for the cyt *b* gene are detailed in Schönhuth & Doadrio (2003) and Blum *et al.* (2008). Primers used for the nuclear S7 gene are detailed in Chow & Hazama (1998). All polymerase chain reaction (PCR) amplifications were conducted in 50 μL reactions. When necessary, nested PCR was performed for the S7 region with two newly designed internal primers: S72-F (5′-GAT ATC AAG TTA AAA TGT-3′) and S72-R (5′-TCG CAC TGG TAC TGA ACA ATG-3′). Nested PCR amplifications were carried out in a 50 μL solution containing 2 μL of template from the initial PCR, 5 μL 10× PCR Ex Taq Buffer [Mg^+ free, 20 nM TRIS-HCl, pH 8, 100 mM Kcl, 0.1 mM ethylenediaminetetra-acetic acid (EDTA)], 0.2 μM of each primer, 0.6 mM deoxy-nucleotide triphosphate (dNTP), 2 mM of $MgCl_2$, and 1.25 units of TaKaRa Ex Taq (TaKaRa Bio, Madison, WI, USA). After an initial denaturation step at 95 °C for 15 min, 35 cycles were performed as follows: denaturation at 94 °C (30 s), annealing at 48 °C (60 s) and extension at 72 °C (90 s), with a final extension of 5 min at 72 °C. PCR was carried out in a Peltier Thermal Cycler-200 (MJ Research, Waltham, MA,

USA). When more than one product resulted from PCR amplification of the S7 region, the target product was gel extracted and purified using a DNA Gel Extraction Kit (Qiagen). Primers for direct sequencing of the purified PCR were the same as those used for the PCR amplification. Purified PCR products were sent to FinchLab (University of Washington High-Throughput Genomics Unit) for sequencing. Sequences specifically obtained for this study have been deposited in GenBank (accession numbers HQ608523 to HQ608820).

A representative subset of specimens from each mitochondrial clade were selected for nuclear S7 analysis. This resulted in a set of S7 sequences from 100 specimens from 59 localities out of the larger set examined for cyt *b* variation (Appendix S1). All sequences were aligned manually and with CLUSTALX v.1.85 (Thompson *et al.*, 1997) alongside sequences obtained from the outgroup species *C. plumbeum* and *C. pullum*. No ambiguous alignments or gaps were found in the cyt *b* alignment, and no characters were excluded from analyses. Multiple indels were detected in the S7 alignment. The length of S7 sequences for all specimens of *C. ornatum* examined varied from 869 bp to 875 bp. The cyt *b* and S7 alignments were analysed as two separate data sets. Observed genetic divergences noted herein are based on cyt *b* uncorrected pairwise (*p*)-distances.

Phylogenetic trees were estimated for each data set using maximum likelihood (ML) and Bayesian inference (BI) as implemented in RAxML v.7.0.4 (Stamatakis, 2006) and MrBayes v.3.03 (Huelsenbeck & Ronquist, 2001), respectively. The search for the optimal ML trees was conducted on a high-performance iDiscover cluster computing facility (32 nodes) located at Saint Louis University. Inferences included mixed-model analyses; partitions were assigned with respect to codon positions of the cyt *b* protein-coding gene. For the ML search with the mixed model of nucleotide substitution, we used the GTR+I+G model (with four discrete rate categories). The ML tree search was conducted by performing 100 distinct runs using the default algorithm of the program for random trees (−d option) as a starting tree for each run. The final tree was determined by a comparison of likelihood scores under the GTR+I+G model among suboptimal trees obtained per run. Robustness of the inferred ML tree was evaluated using bootstrap analysis (Felsenstein, 1985) according to 1000 pseudoreplications. ML bootstrap results via analyses using RAxML web-servers (Stamatakis *et al.*, 2008) were obtained from the CIPRES cluster (CIPRES portal v.1.13) at the San Diego Supercomputer Center at http://www.phylo.org/sub_sections/portal/. BI analyses were conducted for each gene data set. The hierarchical likelihood ratio test (hLRT) implemented in MODELTEST v.3.4 (Posada & Crandall, 1998) was used to find the best-fit evolutionary model for each sequence data set. For BI, 1,000,000 generations were implemented, sampling the Markov chain at intervals of 100 generations. A total of 1000 out of 10,000 resulting trees were discarded as 'burn-in'. Bayesian inferences were performed twice beginning with different starting trees. Support for tree nodes was determined based on values of Bayesian posterior probabilities obtained from a majority-rule consensus tree conducted with PAUP* v.4.0b10 (Swofford, 2001).

RESULTS

Sequence analyses

Plots of transitions and transversions against uncorrected genetic distance indicated an absence of nucleotide saturation in the cyt *b* and S7 data sets (data not shown). Of the 1140 bp aligned for cyt *b*, 274 sites were variable and 240 (8.3% first position; 2.5% second; 87.5% third) were parsimony informative. A low frequency of guanine in third-codon positions (G: 11.06%) was found in the cyt *b* gene region, as also occurs in *Notropis* (Bielawski & Gold, 1996) and *Dionda* (Schönhuth *et al.*, 2008). Of the 888 bp aligned for S7, 98 sites were variable and 72 were parsimony informative. This increases to 121 variable and 98 parsimony informative characters if gaps are treated as a fifth character state.

The amount of cyt *b* sequence divergence – calculated as uncorrected *p*-distances – among specimens from different populations ranged from 0% to 5.59% (Table 1). Uncorrected *p*-distances for S7 ranged from 0.0% to 2.3% (*c.* 2.4 times lower than those observed in cyt *b*) among specimens from different populations. Maximum divergence values in both genes (4.49–5.59% for cyt *b*, and 0.9–2.3% for S7) occurred between specimens from southern and northern drainages. Minimum genetic divergence at both mitochondrial and nuclear genes occurred between several geographically proximate (yet separate) drainages.

Phylogenetic analyses

Mexican stonerollers were recovered as a well-supported monophyletic group in all analyses. However, two strongly divergent clades with north–south orientation (hereafter referred to as the 'northern clade' and the 'southern clade') were recovered within the group, supporting previous hypotheses of polytypy in the species. The widely distributed northern clade includes populations from the following eight drainages: the Rio Grande–Conchos, Sonora, Yaqui, Mayo, Fuerte, Casas Grandes, Santa Clara and Bavicora Basin. The southern clade is composed of populations from the Nazas, Aguanaval, Culiacan and Piaxtla drainages. These two well-supported clades were recovered as deeply divergent sister groups in all analyses of mitochondrial (cyt *b*) and nuclear (S7) sequence variation. Depending upon the gene examined, however, analyses recovered different groupings within each clade. Analyses of cyt *b* sequences always recovered five well-differentiated subclades within the northern clade (subclades A to E) and four subclades within the *Southern Clade* (Fig. 2). While analyses of the S7 intron region always recovered two subclades within the northern clade, little structure was found within the southern clade, probably due to the smaller amount of divergence present among individuals within the clade

Table 1 Chronosequence of biogeographic events suggested by the mitochondrial DNA (mtDNA) genealogy of *Campostoma* across the Sierra Madre Occidental (SMO).

Lineages	mtDNA genetic divergences (%)	Geographic distribution	Biogeographic perspective	Phylogenetic perspective
Common ancestor for Mexican stonerollers	0–5.59	Ancient Rio Grande system, from Rio Grande to Nazas and Aguanaval	Wide distribution, closely related to congeneric species in the Great Plains	Monophyletic clade encompassing all of these drainages, with a wide range of mtDNA sequence divergence values
Separation between the Northern Clade and Southern Clade	4.49–5.59	Northern clade: from Rio Grande to southern Conchos. Southern clade: Nazas and Aguanaval	Separation of southern drainages from ancestral Rio Grande system due to SMO formation and increasing regional aridity	Deep split between the southern and northern lineages in the phylogeny
Northern Clade diversification into five subclades	2.07–3.42	From Rio Grande to southern Rio Conchos but also extending to Pacific drainages (the Yaqui and Sonora)	Range expansion to Pacific drainages from northern ancestral Rio Grande through river captures from Rio Conchos and Guzman Basin to the Yaqui	Individuals in Pacific drainages are closely related and fall within the Northern Clade, high genetic divergence values among Yaqui specimens
Southern Clade diversification into subclades	1.4–1.8	Endorheic Nazas and Aguanaval drainages	Increasing aridity may have separated the drainages and limited gene flow within the Nazas	Separate lineages in each endorheic basin and also within the Nazas
Subclade-E diversification	0–2.89	Upper and Lower Yaqui drainage, Sonora drainage and Bavicora Basin	Limited gene flow within the Yaqui, but possible connections among the three drainages	Well-supported group with unresolved relationships and marked genetic divergences within the subclade
Upper Conchos lineage	0–1.67	Upper Rio Conchos and Fuerte drainages (Rios Oteros, Urique and Verde)	Recent headwater connections or river captures among both drainages, range expansions to Fuerte from Conchos	Low divergence values among individuals from the two drainages
Casas Grandes lineage	0–0.35	Casas Grandes and South Bavispe (upper Yaqui)	Recent headwater connections among both drainages; South Bavispe likely captured part of the Casas Grandes, bringing the lineage into upper Yaqui	Low divergence values among individuals from South Bavispe and Casas Grandes; distant relations with other Yaqui populations
Upper Yaqui lineage	0–0.1	Rivers in the upper Yaqui, Mayo, and upper Oteros in the Fuerte drainage	Recent hydrological connections of three Pacific drainages	Negligible genetic divergence values between upper waters of the three drainages
West Nazas lineage	0	Western rivers of the Nazas, upper Piaxtla and upper Culiacan drainages	Recent headwater capture of western sections of the endorheic Nazas by two Pacific drainages (Piaxtla and Culiacan)	Negligible genetic divergence values between the western Nazas and two Pacific drainages

(Fig. 3). ML and BI topologies were concordant, and for brevity the following sections only detail findings from the ML analysis of the cyt *b* and S7 gene regions.

Phylogenetic and biogeographic analysis of the cytochrome *b* mtDNA gene region

Two major well-supported sister clades were recovered in all analyses. The northern clade is composed of specimens from eight northern drainages distributed across five well-supported subclades. The highly differentiated southern clade encompasses specimens from four southern drainages in the SMO, including the Rio Nazas, Rio Aguanaval, Rio Culiacan and Rio Piaxtla, plus two specimens from the southernmost part of the Rio Conchos drainage in the upper Rio Balleza. Both clades exhibit topological structure that does not always align with drainage configurations. Within the northern clade, subclade A includes specimens from the upper Rio Conchos and Rio
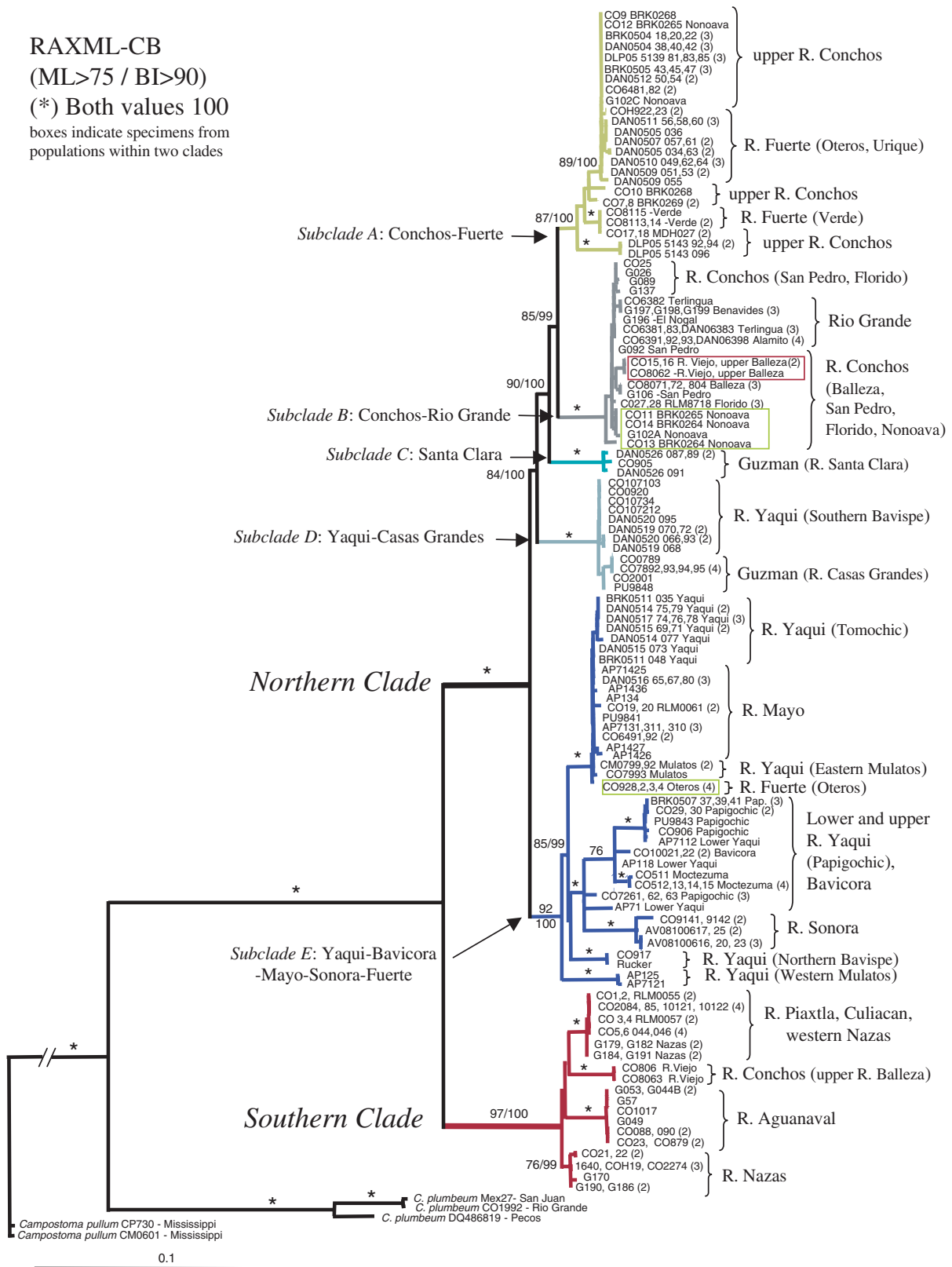
Figure 2 Phylogenetic relationships of all specimens of the Mexican stoneroller (*Campostoma ornatum*), the sister new species and outgroup taxa according to sequence variation across the cytochrome *b* mitochondrial gene; maximum likelihood (ML) tree using the GTR+I+G model with ML bootstrap values (based on 1000 replicates) and Bayesian posterior probabilities, with support values of > 75 and > 90 reported, respectively. Asterisks are given when both values were 100. The number of identical sequences from the same locality is shown in parentheses.
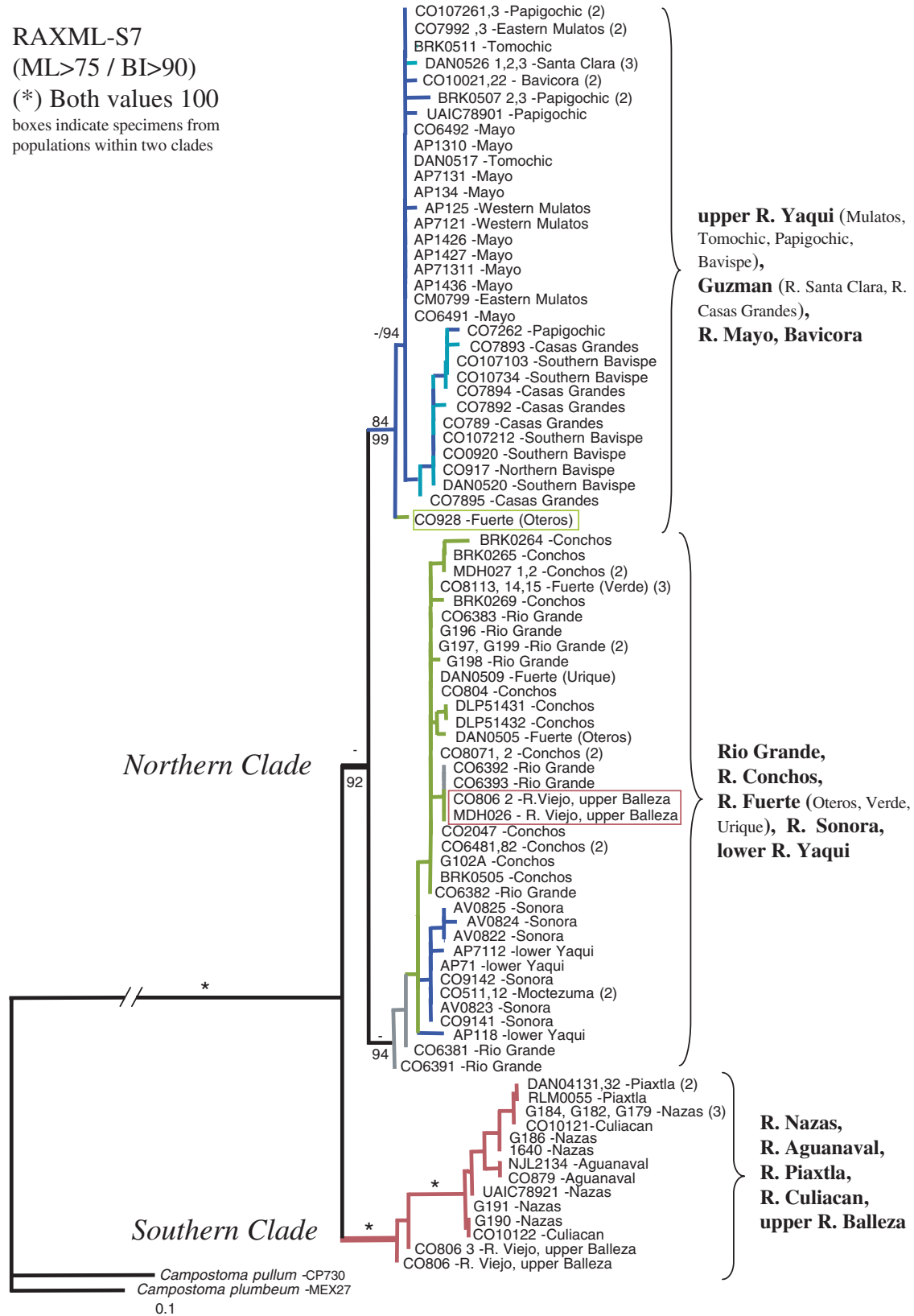
Figure 3 Phylogenetic relationships of selected specimens of the Mexican stoneroller (*Campostoma ornatum*), the sister new species and outgroup taxa according to sequence variation across the nuclear S7 region; maximum likelihood (ML) tree using the GTR+I+G model with ML bootstrap values (based on 1000 replicates) and Bayesian posterior probabilities, with support values of > 75 and > 90 reported, respectively. Asterisks are given when both values were 100. The number of identical sequences from the same locality is shown in parentheses.

Fuerte drainages; subclade B includes specimens from the Rio Conchos and Rio Grande; subclade C is composed of specimens from Rio Santa Clara (part of the Guzman Basin complex); subclade D includes specimens from two independent drainages, Rio Bavispe (upper Yaqui) and Rio Casas Grandes (part of the Guzman Basin); and the large subclade E is composed of specimens from four Pacific drainages (Sonora, Yaqui, Mayo, Fuerte) as well as the interior Bavicora Lagoon (Fig. 1a). Within the southern clade, populations from the endorheic Rio Nazas drainage were recovered within two subclades. Specimens from the eastern part of this drainage form a distinctive clade, while specimens from the western part of the Nazas drainage (Rio Ramos and Rio Tepehuanes) were nested with those from two Pacific drainages (Rio Piaxtla and Rio Culiacan).

Analyses of the cyt *b* gene region recovered additional patterns of divergence that correspond to fine-scale geographic structure within and among SMO drainage basins. Specimens from the Rio Conchos, for example, were recovered in two differentiated subclades. One clade included specimens from the headwaters of the Rio Conchos, whereas the second clade included specimens from tributaries in the middle and lower portions of the Rio Conchos Basin (including locations on the Rio Balleza, Rio Florido, Rio Nonoava and Rio San Pedro). Specimens from the Rio Yaqui basin were also recovered in two strongly divergent subclades; populations from the southern streams of the Rio Bavispe were highly differentiated from remaining populations sampled from the lower Rio Yaqui as well as from the Rio Papigochic and northern Rio Bavispe. Similarly, specimens from the Guzman Basin were recovered in separate clades corresponding to the Rio Casas Grandes and the Rio Santa Clara basins. Specimens from the Rio Oteros within the Rio Fuerte Basin also were recovered in two differentiated clades. Hence, individuals sampled from five basins (the Rio Grande-Conchos, Rio Yaqui, Rio Fuerte, Rio Nazas and Guzman basins) did not form reciprocally monophyletic subclades. Rather, specimens from these basins were distributed as weakly divergent groups nested among different subclades. For example, subclade A contains specimens from the upper Rio Conchos and Rio Fuerte. Subclade B includes specimens from tributaries of the middle and lower Rio Conchos nested with Rio Grande populations. Subclade D contains nested specimens from the Rio Bavisque in the Yaqui drainage with specimens from the Rio Casas Grandes in the Guzman Basin. Within subclade E, specimens from Rio Tomochic in the Rio Papigochic Basin nest with specimens from Rio Mayo and one population from the Rio Oteros in the Rio Fuerte Basin. Two genetically differentiated clades also were recovered within the endorheic Rio Nazas Basin, with specimens inhabiting western regions of the basin (i.e. Rio Ramos and Rio Tepehuanes) being more closely related to those from the Pacific-flowing Rio Piaxtla and Rio Culiacan than to remaining populations from the Rio Nazas drainage. In subclade E, marked genetic divergence was found among some populations from the Yaqui drainage including the northern Rio Bavispe, western Rio Mulatos, and some parts of the Rio Papigochic nested with Rio Mayo and Rio Oteros. A hetero-geneous well-supported group was also recovered within subclade E that includes specimens from Rio Sonora as well as the lower Rio Yaqui, upper Rio Papigochic and Bavicora basins (Figs 1c & 2).

Phylogenetic and biogeographic analysis of the S7 nuclear region

Analysis of sequence variation across the S7 nuclear intron recovered two well-supported clades, corresponding to the north–south geographic structure found in the tree topologies resulting from analysis of mitochondrial sequence variation (Fig. 3). The northern clade is composed of specimens from the following eight northern drainages: Rio Grande-Conchos, Sonora, Yaqui, Fuerte, Mayo, Casas Grandes and Santa Clara, and the endorheic Bavicora Lagoon. The subclades recovered within the northern clade, however, were different in relation to those recovered in tree topologies based on mtDNA sequence variation. One well-supported subclade grouped specimens from the following six drainages: Santa Clara, Casas Grandes, Bavicora, Rio Bavispe and Rio Papigochic in the upper Yaqui, upper Oteros in the Rio Fuerte and Mayo basins. All remaining specimens were recovered in a poorly supported yet separate clade encompassing the Sonora, Fuerte (including Rio Verde, Rio Oteros and Rio Urique), Conchos, Rio Grande and lower Yaqui drainages. Specimens from the Rio Yaqui and Rio Oteros in the Rio Fuerte drainage were also recovered in two subclades within the northern clade. The strongly differentiated southern clade is composed of specimens from four southern drainages, two of which are endorheic basins (the Rio Nazas and Rio Aguanaval), with the other two being Pacific drainages (the upper Rio Culiacan and Rio Piaxtla). Two specimens from the upper Rio Balleza in the southern Rio Conchos drainage were also recovered in this clade (Fig. 1a). The southern clade recovered individuals from the Rio Piaxtla and Rio Aguanaval in reciprocally monophyletic clades, but no substantial structure was found among individuals sampled from the Rio Nazas, Rio Culiacan and upper Rio Balleza basins.

DISCUSSION

Taxonomic implications

Patterns of genetic variation confirm that *C. ornatum* encompasses multiple evolutionary lineages, some of which are strongly divergent from one another (*p*-distances, 5.59% for cyt *b* and 2.3% for S7). Maximum likelihood and Bayesian analyses of both genes supported a monophyletic *C. ornatum* with considerable phylogenetic and geographic structure. Two well-differentiated major clades were recovered in both mitochondrial and nuclear gene tree topologies. Southern populations from four different drainages (Rio Nazas, Rio Aguanaval, Rio Culiacan and Rio Piaxtla) and a population from the upper Rio Balleza (southern headwaters of Rio Conchos) were recovered in a strongly supported clade. The other well-supported clade was composed of individuals from

northern drainages including the Rio Grande–Conchos, Sonora, Yaqui, Mayo, Fuerte, Guzman complex and the Bavicora Basin. The two evolutionary lineages only co-occur in the headwaters of the Rio Balleza, which is a southern Rio Conchos tributary.

Our findings support prior morphological studies indicating that *C. ornatum* is a polytypic taxon (Burr, 1976). We found that *C. ornatum* exhibits patterns of genetic variation comparable to the high levels of diversity and endemicity within other cyprinid taxa inhabiting the SMO and surrounding areas (Schönhuth & Doadrio, 2003; Schönhuth *et al.*, 2006, 2008), including the presence of a distinct evolutionary lineage in the southern SMO that warrants species recognition. The uncorrected *p*-distances of 4.49–5.59% for cyt *b* between the northern clade and southern clade lineages are greater than cyt *b* distances between recognized species of *Campostoma* (Blum *et al.*, 2008), including *Campostoma anomalum michauxi* and *Campostoma pauciradii* (2.89–3.85%), and *Campostoma oligolepis* and *Campostoma anomalum anomalum* (2.22–2.80%).

We propose that the populations constituting the southern clade be considered a new species sister to *C. ornatum*. '*Campostoma ornatum*' should be re-described to include only the northern drainages in the northern clade (the Rio Grande and the Rio Conchos on the Atlantic slope, the Rio Casas Grandes and Rio Santa Clara in the Guzman Basin, the Bavicora Lagoon, and the Rio Sonora, Rio Yaqui, Rio Mayo and Rio Fuerte on the Pacific slope). Based upon our findings, the new southerly species is distributed in the endorheic Rio Nazas and Rio Aguanaval, and also in upper Rio Piaxtla and upper Rio Culiacan. Its presence in the headwaters of the Rio Balleza (Rio Conchos) is likely to be a result of stream capture (Fig. 1). Available morphological data also support recognition of the southern clade as a new species. Although the observed morphological differences were not originally interpreted as having any geographic or taxonomic implications, Burr (1976) found that southern and northern populations of *C. ornatum* exhibited substantially different meristic, morphometric and pigmentation characters.

Finer-scale patterns of genetic variation within the northern and southern clades differ between the mtDNA and nuclear gene genealogies. Mitochondrial divergences among different populations were always greater (*c.* 2.4 times higher) than nuclear values, suggesting that substitution rates in the cyt *b* region are significantly higher in these populations than in the S7 region. Consequently, some populations, such as those in the Rio Yaqui Basin (e.g. specimens from northern versus southern Bavispe), are highly differentiated according to mitochondrial sequence variation but constitute an unresolved subclade according to nuclear sequence variation. Whereas our mtDNA gene genealogy supports early studies characterizing *Campostoma ornatum pricei* Jordan and Thoburn, 1896 (Jordan & Evermann, 1896) as a distinct subspecies in the Chiricahua Mountains (Arizona, USA), our nuclear gene genealogy agrees with later morphological analyses that do not recognize *C. ornatum pricei* as a distinct form within the taxon

(Burr, 1976). As a consequence, no consistent support was found for recognition of additional evolutionary lineages within the northern and southern clades, but further study of fine-scale variation is nonetheless warranted.

Biogeographic implications

The pair of proposed sister taxa – the newly circumscribed Mexican stoneroller and the newly identified southern species – are the southernmost members of the genus *Campostoma*, being allopatric in respect of all other congeners. These sister taxa occur in south-western North America, primarily in north-west Mexico, while remaining members of this ubiquitous genus inhabit most of eastern North America (Lee *et al.*, 1980; Blum *et al.*, 2008). Prior phylogenetic analyses of the genus recovered *C. ornatum* as a derived lineage most closely related to the Plains clade of *C. pullum*, tentatively referred to as *C. plumbeum* by Blum *et al.* (2008). Fossil *Campostoma* remains are known from Pleistocene deposits in Kansas (*c.* 1 Ma) and from localities in Kansas and Texas that are *c.* 100,000 years old (Smith, 1963, 1981; Lundberg, 1967; Cross *et al.*, 1986), raising the possibility that the Plains clade has existed since at least 1 Ma. Blum *et al.* (2008) suggest that the evolutionary origins and biogeography of the Plains clade is consistent with the Plains stream hypothesis (Metcalf, 1966; Cross *et al.*, 1986) in which a pre-glacial drainage encompassed much of the western Great Plains of North America flowing from North Dakota south to Texas and emptying into the Gulf of Mexico. The degree of divergence between *C. ornatum* and the newly identified southern lineage relative to *C. plumbeum*, however, suggests that an ancestral species occurred across the Great Plains and northern Mexico area well before the existence of the hypothesized pre-glacial drainage, and that earlier events, such as the orogeny of the SMO, probably contributed to the diversification of *Campostoma* in south-western North America. Uplift of the SMO near the continental margin of north-western Mexico formed the continental divide and drainage configurations in the region during the Oligocene and Miocene, although local uplift continued through the Pleistocene (Miller & Smith, 1986; Ferrari *et al.*, 2005).

Prior biogeographic studies indicate that climate and drainage evolution have also shaped freshwater species distributions in the southern Great Plains and central Mexico (Minckley *et al.*, 1986; Miller & Smith, 1986). Smith & Miller (1986) proposed, for example, that the current Rio Grande was part of a more highly integrated hydrographical system, including several interior basins of north and central Mexico, and that increasing regional aridity fragmented the system, effectively isolating southern populations. Smith & Miller (1986) further suggested that rivers in western Mexico draining to the Pacific (upper Yaqui and upper Mezquital) through desert regions originated through headwater capture from the ancestral and the extant Rio Grande system, and current trans-divide distributions reflect exchange due to stream capture events, drainage reversals and structural integration of sub-basins.

Our findings on range-wide patterns of genetic variation within Mexican stonerollers permit more detailed reconstruction of colonization pathways and connectivity among drainage basins across the SMO and adjacent areas. A common ancestor of both lineages of Mexican *Campostoma* probably entered north-west Mexico through an ancient Rio Grande system that encompassed the Guzman and Bavicora basins and extended as far south as the Rio Nazas and Rio Aguanaval (Smith & Miller, 1986; Mayden *et al.*, 1992). This would have resulted in occupancy of the Rio Grande, Rio Conchos, the contemporary Guzman complex and the Bavicora Basin as well as the Rio Nazas and Rio Aguanaval. It is unclear, however, how colonization may have progressed among these drainages because extensive palaeolakes may have influenced connectivity during the late Pleistocene (Metcalfe, 2006) when northern Mexico was much wetter than it is today (Metcalfe *et al.*, 2000, 2002).

Consistent recovery of a sister relationship between a widely distributed northern lineage (including Rio Grande–Conchos populations) and a group of southern populations (including populations from the Rio Nazas and Rio Aguanaval drainages) supports Meek's (1904) hypothesis that the Rio Nazas drainage formerly connected to the Rio Grande drainage. The route suggested by Arellano (1951), who posited a connection between the old Rio Nazas and what is now the Rio San Juan, is difficult to reconcile with the present distribution of *C. plumbeum* in this river. Meek (1904) suggested that the large pluvial palaeolakes Lago Mayran and Lago Viesca – located at the mouth of the Rio Nazas and Rio Aguanaval, respectively – were connected at some former time and may have flowed northward toward the Rio Conchos, affording a dispersal route into these drainages (Burr, 1976). Conant (1963) also suggested that a succession of pluvial lakes during glacial maxima may have connected the Rio Grande, Rio Nazas and Rio Aguanaval. Burr (1976) raised the possibility of a more recent connection, noting the lack of strong differentiation between 'C. ornatum' from the Rio Nazas, Rio Aguanaval and the Rio Conchos (despite noting that southern populations of 'C. ornatum' have deeper bodies, low scale numbers and longer snouts). Substantive genetic differentiation between the northern and southern lineages, however, provides evidence of long-standing isolation of southern basins (Rio Nazas and Rio Aguanaval) from northern basins within the ancestral Rio Grande system (Fig. 4, Table 1).

Evolutionary relationships often correlate with the geographic configuration of drainage basins (Hubbs & Miller, 1948; Liu & Hershler, 2005; Smith & Dowling, 2008), but we found that relationships did not always reflect drainage proximity. The two evolutionary lineages that we recovered are currently distributed across 12 proximate drainages across the SMO, although disjunctive populations occur in the Big Bend region of Texas and northern Chihuahua. Further differentiation within each of these lineages might be expected to reflect hydrological proximity or configuration of drainage basins across the region. We found, however, that patterns of genetic differentiation among subclades did not always correspond to current drainage basins. Unexpected relationships

were found, where individuals from one drainage were often recovered within several subclades.

The lack of congruence between basin configurations (present-day and palaeobasins) and relationships among sampled locations has been interpreted as evidence of historical drainage connections or river capture events (Jordan, 1928; Miller & Smith, 1986; Minckley *et al.*, 1986; Schönhuth *et al.*, 2001). As most of the disparate drainages recovered within each subclade occur on opposite slopes of the SMO, the prevailing pattern of transfers probably reflects headwater capture events. This finding is consistent with patterns of geographical variation in elevational distributions of *Campostoma* across the region. For example, *Campostoma* inhabits a broad elevational range in the Rio Grande–Conchos, Yaqui and Sonora drainages, but is restricted to higher elevations in the Rio Mayo, Rio Culiacan, Rio Piaxtla and Rio Fuerte. Evidence of cross-divide exchanges has also been inferred for *Catostomus* and *Oncorhynchus* species in the area and in other fishes inhabiting semi-desert regions of western North America (Minckley *et al.*, 1986; R.L.M., unpublished data).

The recovered gene genealogies suggest that two evolutionary lineages emerged following the ancient isolation of southern populations in the Rio Nazas and Rio Aguanaval drainages from the ancestral Rio Grande system. As both evolutionary lineages were originally restricted to Atlantic drainages (the ancestral Rio Grande system), subsequent range expansions extended their distribution to Pacific drainages. The composition of mtDNA subclades indicates that the expansion to Pacific drainages probably progressed via multiple independent river capture and headwater connection events. The geographic distribution of both evolutionary lineages in several tributaries of Pacific drainages, but only in upper headwaters of the Rio Piaxtla, Rio Culiacan and Rio Mayo drainages, also raises the possibility that two periods of active river capture occurred. An ancient expansion to Pacific drainages involving transfers of major portions of drainages is indicated by the high levels of genetic divergence among individuals sampled from the Rio Sonora and Rio Yaqui basins, whereas the absence of genetic divergence among other geographically disparate collections suggests more recent episodes of admixture by secondary contact through headwater capture across several regions of the SMO, including the upper Conchos–upper Fuerte drainages, upper Bavispe–Casas Grandes drainages, western Nazas–upper Piaxtla–upper Culiacan drainages and the upper Papigochic–upper Mayo drainages (Fig. 4).

Additional colonization events are likely to have involved repeated or sequential transfers or migration among basins, possibly during periodic overflows in floodplains (Meek, 1904; Miller, 1958; Burr, 1976) or through recent anthropogenic introductions. Historical inter-basin exchange is a possible explanation for observations of multiple evolutionary lineages or subclades within a single basin. For example, individuals from the Rio Oteros were recovered in the Mayo-Yaqui clade that encompasses drainages immediately to the north, and in the clade immediately to the east (Rio Fuerte–upper Rio Conchos drainages). The upper Rio Balleza (Rio Conchos
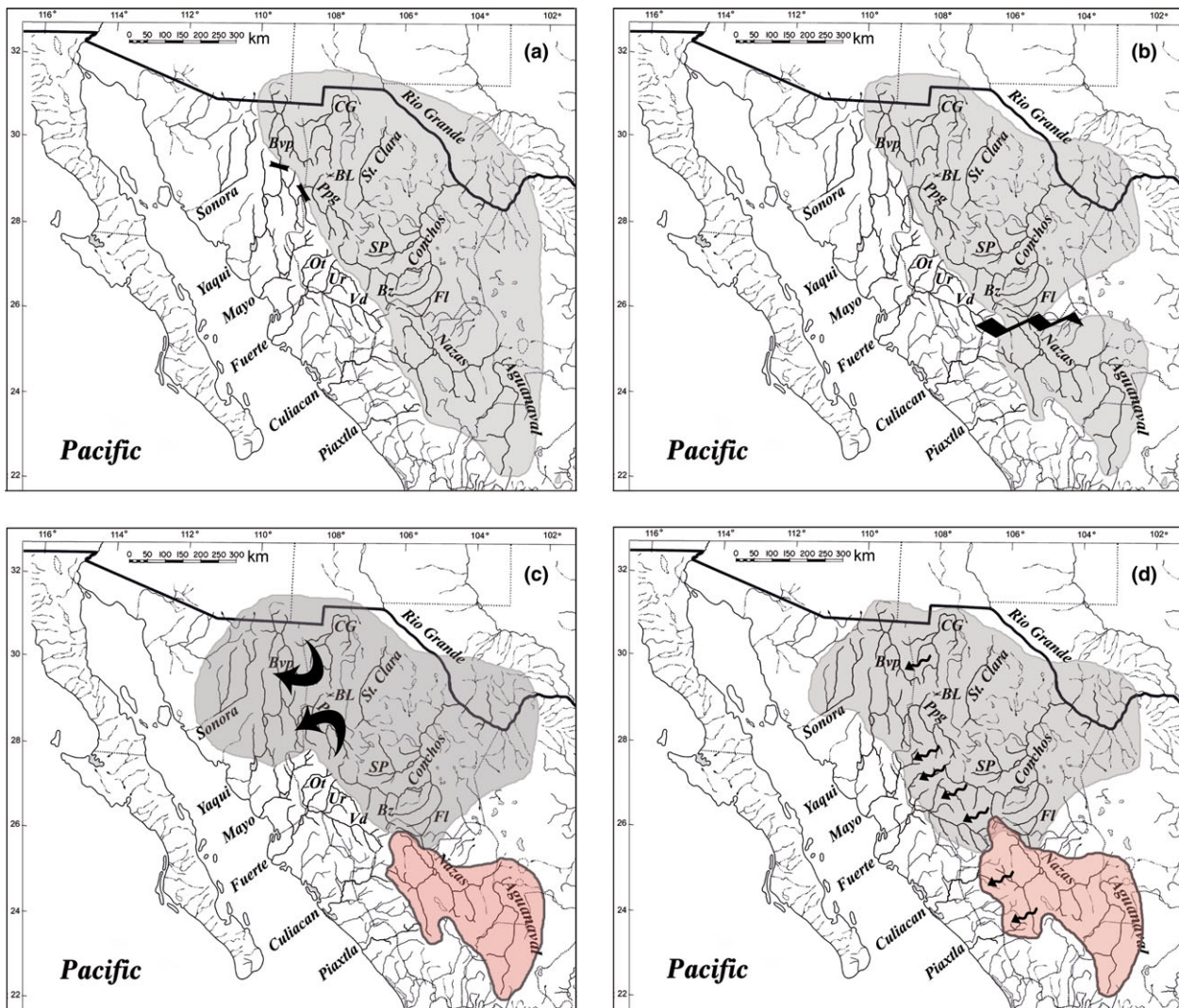
Figure 4 Maps showing suggested distributions of the common ancestor of both Mexican *Campostoma* lineages and correlation with a chronosequence of drainage configurations. (a) Ancient 'Rio Grande system' and ancient continental divide. (b) Separation between northern and southern drainages. (c) Pacific range expansion through river capture of ancestral Rio Grande drainages. (d) Recent headwater capture and hydrological connectivity between areas across the SMO (Rios Casas Grandes–upper Yaqui; Yaqui-Mayo–Fuerte; Conchos–Fuerte; Nazas–Culiacan; Nazas–Piaxtla). CG, Casas Grandes; Bvp, Bavispe; BL, Bavicora Lagoon; Ppg, Papigochic; Bz, Balleza; Fl, Florido; SP, San Pedro; Ot, Oteros; Ur, Urique; Vd, Verde.

drainage) also harbours individuals that are more closely related to the southern lineage than to individuals from the same drainage. Sequential inter-basin dispersal is also a potential mechanism for the occurrence of *Campostoma* in the Rio Sonora, which represents the farthest penetration of a Rio Grande faunal element into western Mexico (Burr, 1976). The presence of multiple evolutionary lineages within a single drainage might also reflect ongoing gene flow among basins. Negligible genetic divergence was found, for example, between disjunctive sites in the middle and lower Rio Conchos and sites on tributaries in the middle Rio Grande, yet restricted gene flow is limiting exchange among populations in the upper and in the middle-to-lower Rio Conchos. Longitudinal gene flow appears to be low enough for the establishment and persistence of independent mtDNA clades within the Rio Conchos Basin,

although mixing does occur at some locations, such as in the Rio Nonoava. A similar pattern was detected within the Rio Nazas drainage, where populations inhabiting the western part of the drainage are more similar to those from the Rio Piaxtla and Rio Culiacan basins than other Rio Nazas locations.

Several genetically distinct populations inhabiting the endorheic Guzman complex and Bavicora Basin appear to be derived from an ancestral lineage that occupied the ancestral Rio Grande system. Individuals from these interior basins were recovered in three different mitochondrial subclades, although analysis of nuclear sequence variation suggests that all specimens from the Guzman complex, the Bavicora Basin and several proximate basins – including the Rio Bavispe and Rio Papigochic in the upper Yaqui drainage, the Mayo Basin, as well as the Rio Oteros within the Fuerte Basin – are a single

well-supported group relative to individuals from the lower Yaqui, Sonora, Conchos, and Fuerte drainages (Figs 1 & 3). Nonetheless, patterns of mtDNA sequence variation among individuals sampled from the Rio Casas Grandes and Rio Santa Clara drainages suggest that occupants of the ancient Rio Grande system diverged into multiple lineages that now occupy different drainages of the Guzman complex. Notably, *C. ornatum* does not occur in the intervening Rio Santa Maria and Laguna Bustillos drainages, possibly because barriers have prevented recolonization from other areas in the Guzman complex. The distribution of mtDNA haplotypes also suggests that colonization of the Guzman complex proceeded from the Rio Conchos and Rio Yaqui basins. We recovered individuals from the highly distinctive Rio Santa Clara Basin in the Guzman complex as close relatives of individuals from the Rio Conchos, which is immediately to the south of the drainage divide. Yet individuals from the Rio Casas Grandes in the Guzman complex were nested among individuals from the southern Rio Bavispe in the upper Rio Yaqui Basin, which is immediately across the drainage divide to the west. On the other hand, individuals from the interior Bavicora Basin were recovered in mtDNA topologies as more closely related to those from the upper and lower Rio Yaqui Basin than to the Guzman complex.

Burr's (1976) morphological review of '*C. ornatum*' found evidence of similarities among specimens from the Guzman complex and proximate drainages suggestive of inter-basin exchange. Burr (1976) also found similarities between individuals from Rio Piaxtla, Rio Aguanaval and Rio Nazas drainages, as well as between the Rio Fuerte and Rio Conchos basins. Except for the Rio Yaqui and Rio Casas Grandes drainages, Burr (1976) did not indicate whether observed similarities among drainages might be due to historical or ongoing exchange. Our data are generally consistent with Burr's (1976) inferences, but what was previously interpreted as mosaic variation may be more parsimoniously interpreted as evidence for multiple stream capture and translocations of divergent lineages formed in allopatry. A similar pattern was observed in the *Cyprinella formosa* species group, where allozyme analyses suggested the Rio Papigochic population resulted from a pre-Pleistocene headwater transfer prior to the break-up of Lake Palomas in the Guzman complex during a period of increasing aridity (Wood & Mayden, 2002).

Molecular data indicate that colonization of the Rio Yaqui drainage proceeded from the northern and southern sections of the ancestral Rio Grande system. Colonization from multiple sources is consistent with the observed pattern of mtDNA and nuclear sequence divergence among individuals from the Rio Yaqui drainage. The observed patterns of divergence, including recovery of individuals in different subclades in both mtDNA and nuclear gene genealogies, also suggests that little exchange has occurred among populations within the drainage following initial colonization from sources in the ancestral Rio Grande system. Patterns of relationships across mtDNA gene genealogies, however, do indicate that recent exchanges have occurred between the Rio Bavispe and

Rio Casas Grandes as well as between the Bavicora Basin and Rio Papigochic (but not between Rio Papigochic and Rio Santa Clara) via headwater capture.

SUMMARY

We examined range-wide patterns of genetic variation among *Campostoma* populations to infer the progression of events that may have shaped species distributions and freshwater diversity across the SMO and adjacent areas. The recovered gene genealogies indicate that: (1) a widespread ancestral form inhabiting an ancient Rio Grande system diverged into two evolutionary lineages following isolation of southern drainages, possibly due to the combined influence of tectonic events and increasing regional aridity (Hubbs & Miller, 1948; Smith, 1981; Miller & Smith, 1986; Metcalfe *et al.*, 2002); (2) divergence into two evolutionary lineages was followed by range expansion from Atlantic to Pacific drainages due to drainage evolution and river capture events; and (3) further inter-basin exchange has occurred via more recent headwater capture events, hydrological connections, and possible anthropogenic introductions. As a consequence, the geographic distribution of genetic variation does not necessarily correspond to drainage boundaries, where geographically close but hydrologically disjunctive populations from independent drainages may be more closely related than geographically distant populations from the same river drainage.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Appendix S1 Sampling localities for the specimens of *Campostoma ornatum* and outgroups analysed in this study.

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BIOSKETCH

Susana Schönhuth's research focuses on investigating the phylogenetic relationships and biogeography of southern North American cyprinids, primarily in arid and semi-arid regions. The research team focuses on fish biodiversity, working at different institutions, using molecular and morphological data to understand different aspects of the evolution, systematics and ecology of freshwater fishes.

Author contributions: R.L.M. and S.S. conceived the ideas; all co-authors worked in the field and collected specimens; D.A.N., M.J.B., A.P. and S.S. collected genetic data; S.S. analysed the data; R.L.M., S.S. and M.J.B. led the writing.

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