



# From the mountains to the sea: phylogeography and cryptic diversity within the mountain mullet, *Agonostomus monticola* (Teleostei: Mugilidae)

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## ABSTRACT

**Aim** The mountain mullet, *Agonostomus monticola*, is a diadromous fish widespread throughout Middle America, occurring in rivers along the Atlantic and Pacific slopes, as well as in the West Indies. Based on the disjunct distribution of this species, it has been hypothesized that this fish may represent more than one taxon. The purpose of this study was to conduct a robust phylogeographical analysis of *A. monticola* across its range, using mitochondrial and nuclear markers. We aimed to investigate the potential for cryptic diversity and the time-scale of divergence in an effort to elucidate biogeographical episodes within Middle America in relation to the evolutionary history of *Agonostomus monticola*.

**Location** North and Middle America, including the West Indies.

**Methods** Mitochondrial and nuclear sequence data (cyt *b*, S7-1) from individuals throughout the range of the species were analysed phylogenetically using maximum-likelihood methodology. The oldest known fossil mullet was used as a calibration to investigate divergence times for clades within *A. monticola*.

**Results** Results indicate four distinct lineages within the known range of *A. monticola* that largely correspond to oceanic basins (Caribbean, Gulf of Mexico, Pacific-A, Pacific-B). Divergence time estimates indicate early to mid-Miocene divergences for all four *A. monticola* clades, with Oligocene to Miocene divergences of internal nodes.

**Main conclusions** These findings are congruent with geological hypotheses regarding movement of the Chortís block, as well as recent studies on the age of beginning emergence of the Panama Arc.

## Keywords

*Agonostomus monticola*, diadromous, divergence times, fish, Middle America, mullet, North America, phylogeography.

## INTRODUCTION

Middle America – the region comprising Mexico, Central America and the Greater Antilles – hosts a diverse array of freshwater fishes that have been the focus of evolutionary and biogeographical studies (e.g. catfishes, Perdices *et al.*, 2002; swamp eels, Perdices *et al.*, 2005; tetras, Bermingham & Martin, 1998; Ornelas-García *et al.*, 2008; cichlids, Martin & Bermingham, 1998; poeciliids, Rauchenberger, 1989). Diadromous fishes, which utilize both freshwater and marine environments during their life cycle, occur throughout

Middle America (e.g. gobies, sleepers and mullets). However, currently there are few studies that have investigated phylogeographical or population genetic patterns of a diadromous fish in this region (Cook *et al.*, 2009). Given the nature of their life history, diadromous species have the potential to offer a different biogeographical perspective from that of primary or secondary freshwater fishes. Processes that may have shaped the evolution of strictly freshwater or marine taxa may have had different or no effects on diadromous taxa.

The mullet genus *Agonostomus* (Teleostei: Mugilidae) includes three species and is hypothesized to be sister to

most other mugilids (Harrison & Howes, 1991; Thomson, 1997); recent work, however, suggests that *Agonostomus* is sister to the genus *Mugil* (Durand *et al.*, 2012). The mountain mullet, *Agonostomus monticola* (Bancroft, 1834), was originally described from Jamaica and occurs in North, Central and South America along the Atlantic slope from North Carolina south to Venezuela (Berra, 2001) and along the Pacific slope from Baja California to Colombia (Miller *et al.*, 2005). The species also occurs throughout the West Indies (Greenfield & Thomerson, 1997). Most mainland records are from Mexico and further south, although some records from the United States exist (summarized in Matamoros *et al.*, 2009). The remaining two species, *Agonostomus telfairii* Bennett, 1832 and *Agonostomus catalai* Pellegrin, 1932, occur sympatrically along the coast of Madagascar and Mauritius, with reports of each from surrounding areas (Thomson, 1997). *Agonostomus* has largely been neglected in systematic and biogeographical studies.

*Agonostomus monticola* is typically found in tropical rivers with high flow (Matamoros *et al.*, 2009), and the common name mountain mullet comes from the propensity of this species to travel far upstream to elevations as high as 1500 m (Miller *et al.*, 2005), although Loftus *et al.* (1984) reported collections from lentic bodies of water. In general, there is much to learn regarding the life history and ecology of this species. While there is consensus that *A. monticola* is a diadromous species, there is substantial debate as to whether the species is catadromous (living in fresh water and travelling to the ocean to spawn; Anderson, 1957; Cruz, 1987; Phillip, 1993) or amphidromous (with spawning events occurring in fresh water and juveniles subsequently carried out to sea; Erdman, 1972; Pezold & Edwards, 1983).

Past morphological studies of *A. monticola* have led to contradictory findings that have resulted in 20 synonyms for the species (see Thomson, 1997, for a detailed taxonomic history of this species). This is in part because many of the characters previously used to diagnose populations of *A. monticola* were found to be variable or associated with growth and development (e.g. thickness of the lip; Thomson, 1997). The family Mugilidae has been the focus of very few systematic studies (Schultz, 1946; Harrison & Howes, 1991; Thomson, 1997; Durand *et al.*, 2012), and there are no previous works that specifically investigated the intraspecific geographical variation of *A. monticola*. Given the allopatric distribution of this species (Pacific/Atlantic), some authors have suggested that *A. monticola* may include cryptic species diversity (Harrison, 2002; Miller *et al.*, 2005). Studies of similarly distributed aquatic species (shrimp, Hurt *et al.*, 2009; fish, Grant & Leslie, 2001; Tringali *et al.*, 1999; for a summary, see Lessios, 2008) indicate that cryptic diversity could be present within *A. monticola*.

The distribution of this species fits a general model of transisthmian geminate species pairs, separated by the Isthmus of Panama (e.g. Tringali *et al.*, 1999; Grant & Leslie, 2001; Concheiro Pérez *et al.*, 2007; Lessios, 2008). Most studies have concluded that divergences between Caribbean

and Pacific lineages took place around 3–4 Ma (Lessios, 2008), although some taxa show divergences prior to 4 Ma (Murphy & Collier, 1996; Martin & Bermingham, 1998; Hurt *et al.*, 2009). In addition, recent geological evidence (1) shows that uplift of the Isthmus of Panama began in the late Oligocene (Farris *et al.*, 2011), (2) suggests a narrower seaway between Central and South America, and (3) reconstructs the isthmus as an uninterrupted chain above sea level from the late Eocene to late Miocene (Montes *et al.*, 2012). Depending on conditions, this could have led to earlier divergences than previously hypothesized. Given the broad distribution of *A. monticola*, it is possible that other geological features of Middle America may have played a role in shaping the phylogeographical patterns of this species. The Chortís block (Nuclear Middle America; encompassing El Salvador, Honduras, southern Guatemala and part of Nicaragua) is one of the main geological structures in Middle America. Most models show the origin of the Chortís block in south-western Mexico, with a mid-Eocene eastward movement along the Motagua–Polochic fault, the current northern edge of the Chortís block (Rogers *et al.*, 2007; Hulsey & López-Fernández, 2011). This structure has been shown to have played an important role in the present-day distribution of Middle American fishes (Matamoros *et al.*, 2012).

The objectives of this study are twofold: first, to conduct a robust phylogeographical investigation of *A. monticola* across the range of this widespread species using both mitochondrial and nuclear markers in an effort to identify cryptic diversity and biogeographical patterns; and second, to use this hypothesis of evolutionary relationships to investigate the time-scale of divergence for lineages within *A. monticola*, to elucidate the timing of biogeographical events within Middle America in relation to the evolutionary history of the species. Given the wide distribution of this species, the complex geological history of the region and the diversity of similarly distributed taxa, we hypothesize that multiple evolutionary lineages will be recovered within this species.

## MATERIALS AND METHODS

### Specimen collection and taxon sampling

Samples of *A. monticola* were acquired from throughout the range of the species (see Appendix S1 in Supporting Information). Most specimens were collected with seines and backpack electrofishers from freshwater rivers across Mexico, Cuba, Hispaniola and Jamaica. Various researchers provided the remaining samples from across the range of the species. Tissue samples (fin clips and/or muscle) were preserved in 95% ethanol and voucher specimens were preserved when possible and deposited in the Southeastern Louisiana University Vertebrate Museum, Museo Nacional de Ciencias Naturales in Spain, or other research collections. See Appendix S1 for a detailed listing of samples and localities.

## Molecular methods

Whole genomic DNA was extracted from ethanol-preserved tissue samples using the Qiagen DNeasy Tissue Kit (Qiagen, Inc., Valencia, CA). The mitochondrial gene cytochrome *b* (cyt *b*, 1097 base pairs) was amplified using the primers GluDG.L and H16460 (Perdices *et al.*, 2002); the PCR protocol followed Perdices *et al.* (2002). The first intron of the nuclear ribosomal *S7* gene (*S7*-1; 594 base pairs) was amplified with the primers RP1 and Rpx (Chow & Hazama, 1998); amplification followed Chow & Hazama (1998). PCR products were electrophoresed on a 0.8% agarose gel and compared to a standard to assess the presence, size and intensity of amplified fragments. Products were purified with ExoSAP (USB Corporation, Cleveland, OH) and subsequently used in sequencing reactions according to the manufacturer's recommendations (Applied Biosystems, San Francisco, CA). Prior to sequencing, gel filtration (Edge Biosystems, Gaithersburg, MD) or X-terminator (Applied Biosystems) was used to remove excess dye terminators, primers and nucleotides. Chromatographs were checked by eye for ambiguities and sequences were aligned using SEQUENCHER 4.8 (Gene Codes, Ann Arbor, MI) and CLUSTALW (EMBL-EBI, Cambridgeshire, UK). GenBank accession numbers for sequences can be found in Appendix S1.

## Phylogenetic methods

Phylogenetic hypotheses were inferred with maximum-likelihood methodology for the individual markers both independently and combined. For the combined analysis, the matrix included only those terminals with data for both cyt *b* and *S7*. The likelihood program GARLI 2.0 (Zwickl, 2006) was used for topology reconstruction, with two partitions in the concatenated analysis, corresponding to the two gene fragments. The model of evolution for each partition was selected in jMODELTEST (Posada, 2008) using the Akaike information criterion (AIC; Posada & Buckley, 2004), and the HKY+I model was selected for both genes. Ten independent likelihood searches were conducted for each analysis (cyt *b*, *S7*, combined). Additionally, a nonparametric bootstrap with 100 pseudoreplicates was also conducted for each analysis. *Joturus pichardi* has been hypothesized to be a close relative of the genus *Agonostomus* and was used as an additional ingroup taxon (Harrison & Howes, 1991; Thomson, 1997). For the cyt *b* data set, outgroups included *Mugil cephalus* ( $n = 3$ ), *Mugil curema* ( $n = 1$ ) and *Crenimugil crenilabis* ( $n = 1$ ). *Mugil curema* was the outgroup taxon for the *S7*-1 data set. The *S7*-1 data set is composed of a subset of individuals from the cyt *b* data set. In addition, a *S7*-1 sequence was available for *A. telfairii*, and this was included in *S7*-1 and combined analyses.

## Haplotype analysis

We used rcs 1.21 (Clement *et al.*, 2000) to examine the relationships among *A. monticola* cyt *b* haplotypes using

statistical parsimony (Templeton *et al.*, 1992). An unrooted haplotype network was constructed using the default settings, including a 95% connection limit, in rcs.

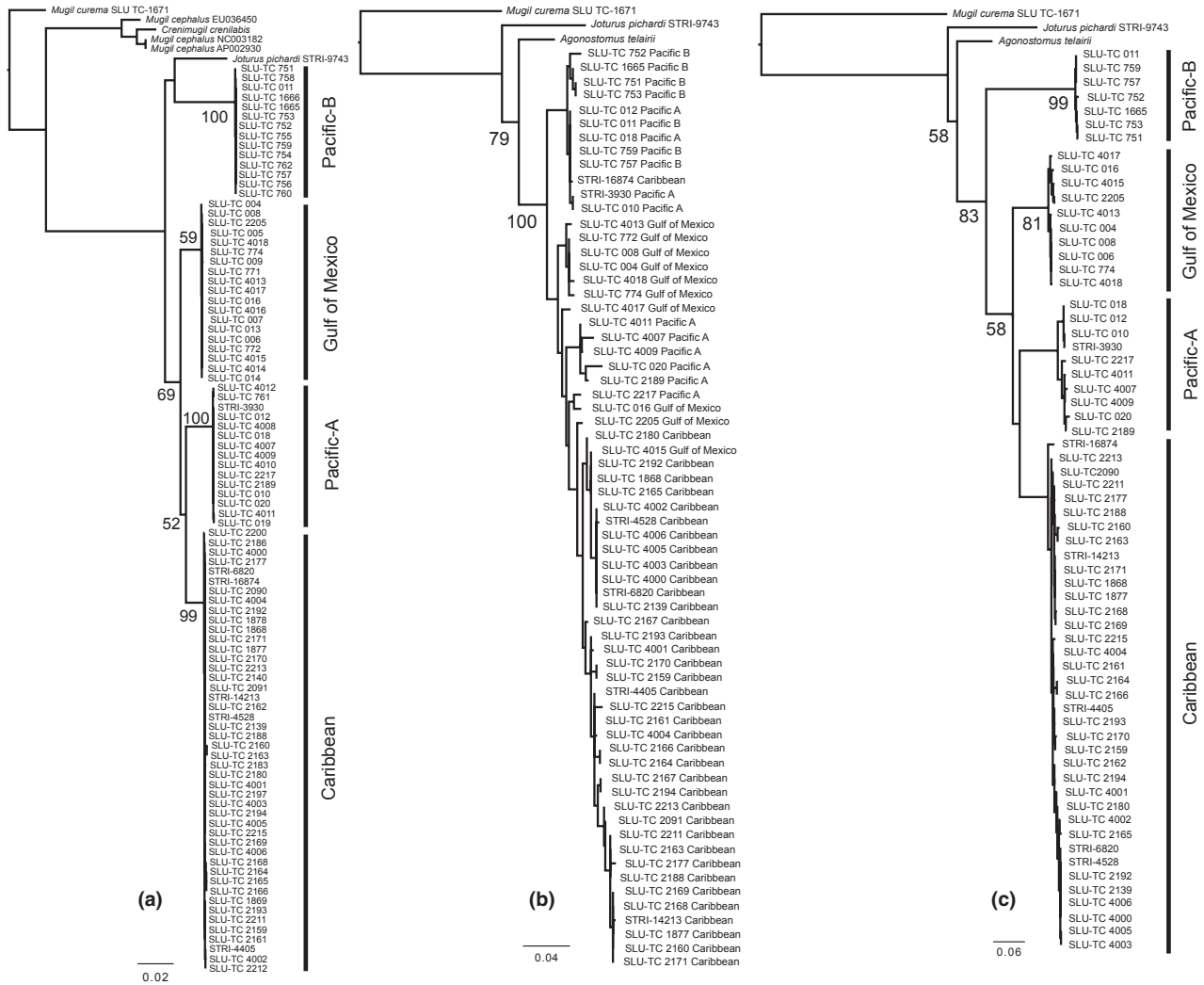
## Divergence time estimation

A combined cyt *b* + *S7*-1 data set, partitioned by gene and with the same models as the maximum-likelihood analysis, was used to estimate divergence times under a relaxed lognormal clock (Drummond *et al.*, 2006) in BEAST 1.5.4 (Drummond & Rambaut, 2007). The concatenated data set included the same individuals as the *S7*-1 data set, such that cyt *b* and *S7*-1 sequences were available for all individuals. Mean substitution rates were not fixed, and a Yule birth–death prior was used for speciation. The oldest fossil mullet species is †*Mugil princeps* (Agassiz, 1818), which dates to the early Oligocene–late Eocene (30–40 Ma; Patterson, 1993). We conservatively calibrated the node that represents the most recent common ancestor of *Mugil* + (*Joturus* + *Agonostomus*) following the calibration recommendations of Parham *et al.* (2012) and Sauquet *et al.* (2012). This calibration was given a lognormal prior, and we calibrated the root of the tree with a hard minimum age of 30 Ma. A soft maximum was set at 95 Ma (mean = 2.15, SD = 1.0), the approximate age of the oldest acanthomorph fossils representing the stem acanthomorph lineages *Polymixia* and *Beryciformes* (Patterson, 1993). Four independent analyses were performed with 50 million generations each, and trees were sampled every 1000 generations (burn-in 5 million generations per analysis). TRACER 1.5 (Rambaut & Drummond, 2007) was used to check the effective sample sizes (ESS) of parameters and to verify stationarity. All parameters had an ESS > 200, suggesting that all analyses adequately sampled the posterior distributions of each parameter and that stationarity was achieved. A 50% maximum clade compatibility tree was then generated from the posterior tree distribution following burn-in.

## RESULTS

### Cytochrome *b* and *S7*-1 data sets

The 10 independent likelihood analyses of the cyt *b* data set resulted in identical topologies (Fig. 1a), with four distinct clades of *A. monticola* that largely correspond to oceanic basins, but with two distinct lineages of *A. monticola* in the Pacific (here called 'Pacific-A' and 'Pacific-B'). Sequence divergences between the clades are shown in Table 1a. Within each of these four clades, average uncorrected sequence divergence ( $p$ ) was low (0.0–0.2%), but there was wide divergence between the clades of *A. monticola* ( $c.$  8–9%; see Table 1a). A sister relationship between Caribbean and Pacific-A clades was recovered (Fig. 1a), with the Gulf of Mexico lineage sister to this clade. The low bootstrap values are probably due to the inability of this marker to sort out relationships among the well-supported Pacific-A, Caribbean and Gulf of Mexico



**Figure 1** Trees resulting from maximum-likelihood analyses for (a) *cyt b*, (b) *S7-1* and (c) concatenated *cyt b* and *S7-1* data sets for *Agonostomus monticola*. Bootstrap values provided for clades with values > 50% are shown. Scale bars show substitutions per site. Numbers beside operational taxonomic units (OTUs) correspond to the locality information in Appendix S1.

clades, as these clades have comparable levels of genetic divergence. *Joturus pichardi* was recovered as sister to the Pacific-B lineage, with 14.9–15.2% sequence divergence from the other three lineages. The Pacific-B lineage consists of individuals that are sympatric with individuals from the Pacific-A clade (in Río Mezquital and Río Ayuquila). It has an average sequence divergence of 15.4–15.7% from the Gulf, Caribbean and Pacific-A lineages, and a 17.1% average divergence from *J. pichardi*. The sequence divergence between ingroup and outgroup taxa was 27.6–29.0%.

Unsurprisingly, the nuclear gene *S7-1* was more conserved across taxa, and the likelihood analyses of the *S7-1* data set showed little clade-specific genetic differentiation (Fig. 1b). Interrelationships within *A. monticola* were largely unresolved, and on average sequence divergence was 0.8% among all individuals. This lack of resolution is probably due to incomplete lineage sorting and the inherent level of variability and mutation rate of this marker for this species. As noted by Zink & Barrowclough (2008), the recovery of

geographically structured mitochondrial trees without nuclear corroboration is a common occurrence in phylogeographical studies utilizing both mitochondrial and nuclear data.

Analysis of the concatenated data set resulted in an overall topology that was largely congruent with that of *cyt b* alone. The same four clades of *A. monticola* were recovered, and sequence divergences are shown in Table 1b. *Agonostomus telfairii* was recovered as sister to *A. monticola*, and *J. pichardi* as sister to *Agonostomus*.

### Haplotype network

Four distinct haplotype networks were recovered, corresponding to the clades recovered in the likelihood analyses (Fig. 2). A total of 31 haplotypes were recovered, with the Caribbean network being the most diverse, consisting of 17 haplotypes. The other three networks were less diverse, containing 3–6 haplotypes. No haplotypes were shared among clades, indicating a lack of gene flow among clades.



**Table 1** Uncorrected (a) *cyt b* and (b) combined *cyt b* + *S7-1* sequence divergences (percentages) for clades of *Agonostomus monticola* ( $n = 94$ , in a;  $n = 64$ , in b), *A. telfairii* ( $n = 1$ ), *Joturus pichardi* ( $n = 1$ ) and outgroups ( $n = 5$ , in a;  $n = 1$ , in b).

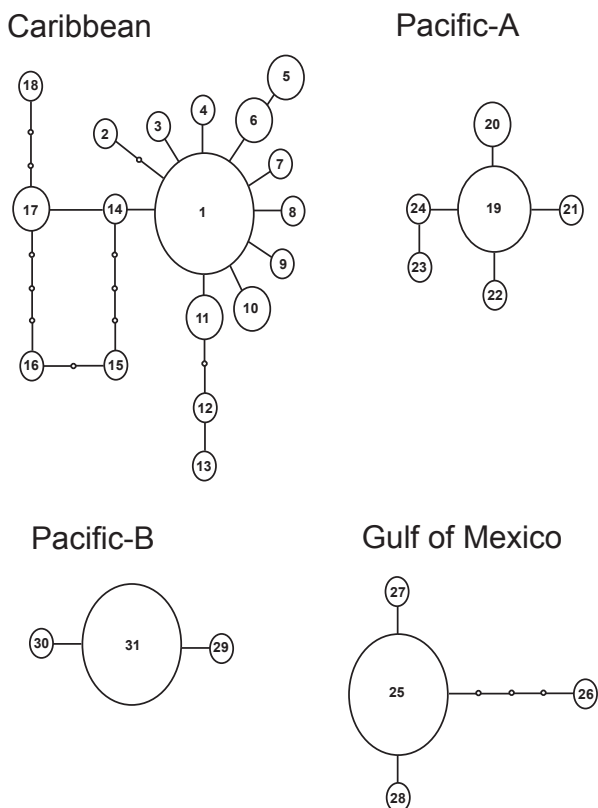
(a)	Clade	1	2	3	4	5
1	Caribbean					
2	Pacific-A	8.3				
3	Gulf of Mexico	8.2	9.2			
4	Pacific-B	15.4	15.4	15.7		
5	<i>J. pichardi</i>	14.9	15.6	15.2	17.1	
6	Outgroups	28.4	29.0	27.6	27.8	28.1

(b)	Clade	1	2	3	4	5	6
1	Caribbean						
2	Pacific-A	6.3					
3	Gulf of Mexico	5.9	6.4				
4	Pacific-B	11.4	11.5	11.6			
5	<i>A. telfairii</i>	6.2	6.3	6.1	8.8		
6	<i>J. pichardi</i>	5.3	5.7	5.4	6.1	5.9	
7	Outgroup	16.8	17.1	16.9	17.0	16.5	17.6

### Molecular dating

Results from the divergence time analysis (Fig. 3, Table 2) indicate that all four lineages of *A. monticola* date to at least the late to mid-Miocene (14.7–7.0 Ma). The Caribbean and



**Figure 2** Four distinct haplotype networks recovered from analysis of the *cyt b* data set for *Agonostomus monticola*.

Pacific-A clades were estimated to have diverged in the early Miocene, 20.7 Ma (95% highest posterior density, HPD: 31.8–11.8 Ma). The slightly older Gulf of Mexico lineage is likely to have diverged in the early to mid-Miocene, *c.* 23.8 Ma (95% HPD: 35.4–13.8 Ma). The Pacific-B lineage was estimated to have diverged in the mid-Oligocene, 29.6 Ma (95% HPD: 42.8–17.5 Ma). Congruent with the fossil calibration, the divergence of *Mugil curema* and the ingroup occurred 41.5 Ma (95% HPD: 55.0–34.3 Ma), in the mid-Eocene.

### DISCUSSION

Investigations of genetic structure and variation can be used to identify cryptic lineages within widespread taxa (e.g. spiders, Duncan *et al.*, 2010; bivalves, Marko & Moran, 2009; geckos, Georges & Rodrigues, 2010; fishes, Haney *et al.*, 2009; insects, Haruyama *et al.*, 2008), and identification of these distinct lineages can be used to improve understanding of biogeographical history and the potential timing of diversification of lineages. Because of its diadromous life history and widespread distribution throughout three oceanic basins around Middle America, *A. monticola* represents an interesting taxon for investigating cryptic species diversity and the biogeographical history of Middle America. For example, geological processes that may have been important in shaping the evolution of a freshwater species may have had no effect on a diadromous species utilizing freshwater and marine environments.

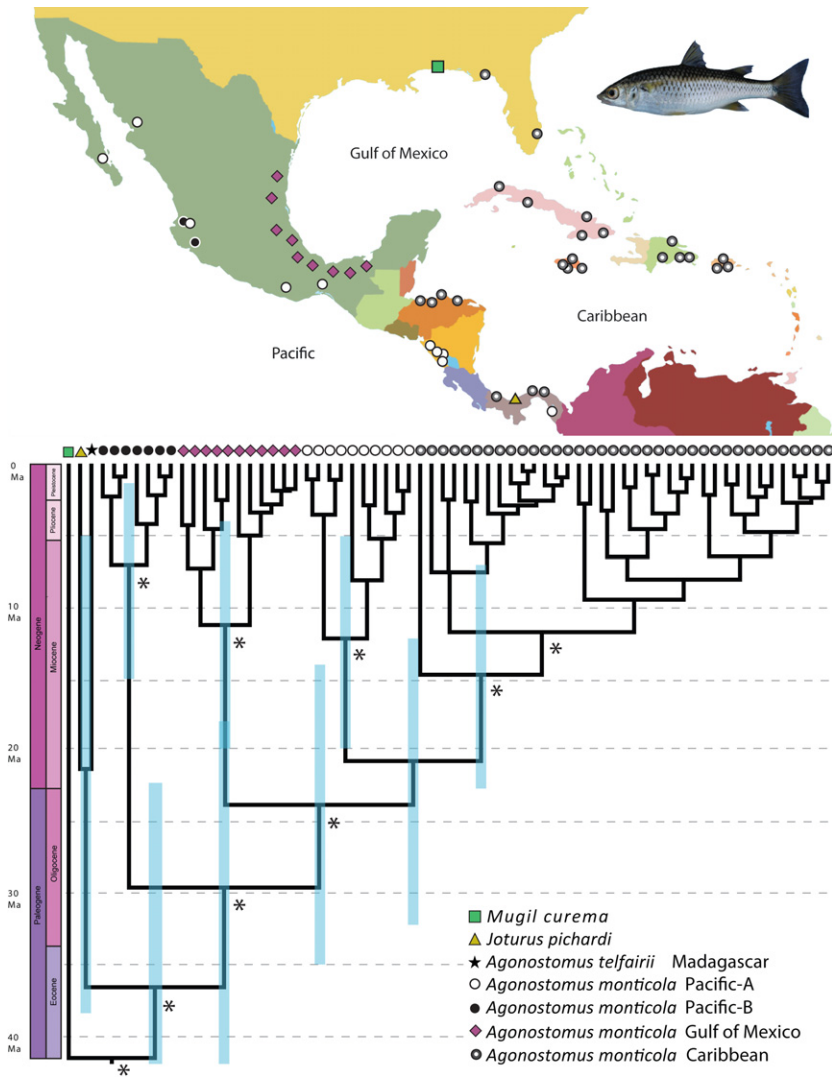
### Divergence time estimates

The calibration used for divergence time estimation was conservative, with a minimum age of 30 Ma based on the oldest mullet fossil ( $\dagger$ *Mugil princeps*; Patterson, 1993). This study was not calibrated with geological information in order to avoid biasing calibrations *a priori* with a hypothesized geological vicariance event. Our results indicate that there are four distinct and highly divergent lineages within the range of *A. monticola*, and that these lineages largely correlate with oceanic basins (Gulf of Mexico, Caribbean, Pacific-A, Pacific-B), as seen in Fig. 3. Each of the distinct geographical lineages was estimated to have diverged during the Miocene, *c.* 14.7–7.0 Ma (Fig. 3).

Little is known regarding the interrelationships among *Agonostomus* species or the relationships of these species to *J. pichardi*. Further work is needed to investigate these relationships in a broader phylogenetic context with greater taxonomic sampling. Our results indicate, however, that the four lineages of *A. monticola* are certainly distinct from *A. telfairii* and *J. pichardi* (Figs 1c & 3) and that cryptic species diversity is likely to exist within the known range of *A. monticola*.

### Phylogeographical patterns

As the life history of a diadromous species such as *A. monticola* includes multiple environments, the phylogeographical patterns of this species may differ from results and conclusions



**Figure 3** Time-calibrated phylogeny for *Agonostomus monticola* constructed in BEAST. Asterisks indicate posterior probabilities  $\geq 0.95$ .

**Table 2** Estimated divergence times of *Agonostomus* and other mugilid clades. HPD, highest posterior density.

Clade	Mean age (Ma)	95% HPD age (Ma)
Caribbean ( <i>A. monticola</i> )	14.7	23.4–6.9
Pacific-A	12.2	20.3–5.0
Gulf of Mexico	11.2	19.7–4.2
Pacific-B	7.0	14.5–1.5
Caribbean + Pacific	20.7	31.8–11.8
Caribbean/Pacific + Gulf of Mexico	23.8	35.4–13.8
<i>Agonostomus</i>	29.6	42.8–17.5
Mugilidae	41.5	55.0–34.3

based on marine or freshwater fishes (Tringali *et al.*, 1999; Perdices *et al.*, 2002; Craig *et al.*, 2004; Ornelas-García *et al.*, 2008). There appears to be little to no gene flow among the four distinct geographical lineages, which is indicated by the genetic uniformity within each of these clades (Fig. 1), the high level of sequence divergence (*cyt b*) among the four lineages (Fig. 1, Table 1a), and the lack of shared haplotypes

(Fig. 2). Based on the high degree of sequence divergence and geological history of Middle America (Pindell *et al.*, 2006; Rogers *et al.*, 2007), it seems likely that these lineages have remained out of contact with each other since their separation. The two Pacific lineages are sympatric in the Río Ayuquíla drainage in Mexico, but based on our sampling thus far we have not found individuals from the Pacific-B clade south of the Río Ayuquíla. Several studies indicate a colonization of Middle America by some fishes 4–7 Ma, before the final closure of the Panama Strait (e.g. Perdices *et al.*, 2002, 2005; Ornelas-García *et al.*, 2008). However, these studies are based on primary or secondary freshwater fishes (*sensu* Myers, 1949), and this colonization has been found only in taxa that are hypothesized to have dispersed from South America to Central America. Our results indicate that dispersal from South America to Central America does not readily explain the present-day distribution of *Agonostomus* throughout Middle America, and that, because of the diadromous life history of this lineage, the current distribution is more directly tied to the geological history of the oceanic basins in the region, as discussed below.

*Gulf of Mexico, Caribbean and Pacific-A lineages*

Surprisingly, the Gulf of Mexico and Caribbean lineages were not recovered as each other's closest relatives (Fig. 3), and it seems unlikely that there is significant gene flow between the two oceanic basins, despite their connectivity and the diadromous behaviour of this fish. One explanation for this pattern is the lack of suitable habitat on the Yucatán Peninsula. As noted earlier, *A. monticola* typically occurs in high-flow rivers (Miller *et al.*, 2005; Matamoros *et al.*, 2009), which are scarce on the Yucatán Peninsula. The lack of suitable freshwater habitat has previously been hypothesized as a historical factor responsible for the impoverished fish fauna of the Yucatán as a whole (Miller *et al.*, 2005).

The Gulf of Mexico lineage was recovered as the sister group to a Caribbean + Pacific-A clade (Fig. 3), with this clade most likely diverging in the early Miocene (23.8 Ma, Table 2). Palaeogeographical reconstructions and geological evidence indicate that the Chortís block was no longer in its early Eocene position in southern Mexico around this time (20 Ma), but was moving eastwards towards its present-day position (Rogers *et al.*, 2007). In addition, the Yucatán Peninsula (at least its northern reaches), hypothesized to be the geological barrier leading to the divergence of the Gulf of Mexico lineage, was almost completely submerged under water (Blakey, 2010). The current configuration of the Yucatán was not reached until the Pliocene (López Ramos, 1975; Miller *et al.*, 2005). Therefore, it is likely that the Yucatán Peninsula would not have been a barrier to dispersal for *Agonostomus* at that time; shortly thereafter, however, with the emergence of the peninsula (and its lack of large river systems), *A. monticola* may not have been able to make it around this barrier with a high enough occurrence to maintain routine gene flow between the two basins. Divergence time estimates suggest that the Gulf of Mexico lineage diverged from the common ancestor of the Gulf of Mexico + (Pacific-A + Caribbean) clade *c.* 24 Ma, with the Gulf of Mexico lineage itself dating to *c.* 8 Ma (Table 2).

The Caribbean and Pacific-A lineages probably diverged from their common ancestor during the early Miocene, 20.7 Ma, and each geographical lineage was estimated to have evolved since the mid-Miocene, 14.7–12.2 Ma. The early Miocene divergence of the Caribbean and Pacific-A mullets is largely consistent with previous studies that found a Miocene colonization of Middle America by some taxa before the hypothesized 3–3.5 Ma final closure of the Panama Strait (Perdices *et al.*, 2002, 2005; Ornelas-García *et al.*, 2008), although substantially older ages are recovered for *Agonostomus*. Furthermore, recent work suggests that the collision between South America and the Panama Arc crust began 25–23 Ma (Farris *et al.*, 2011). Additionally, the isthmus may have been an uninterrupted chain above sea level from the late Eocene to late Miocene, restricting the width of the seaway between Central and South America (Montes *et al.*, 2012). Our present study seems largely congruent with these geological findings, suggesting that at least for a diadromous fish such

as *A. monticola*, the Caribbean/Pacific divergence appears to have taken place before the final isthmus closure, with conditions such that a barrier was created for these fishes by the early collision of South America and the Panama Arc.

In our study, we included a few specimens of *A. monticola* from the United States (Fig. 3). It is hypothesized that the presence of *A. monticola* in the United States could be due to recruitment from areas such as Cuba (Anderson, 1957; Loftus *et al.*, 1984), and not necessarily breeding populations, although Loftus *et al.* (1984) discuss the possibility of a breeding population of *A. monticola* in Miami, Florida. Data from the present study shed some light on this notion. Individuals collected from the Atlantic coast of the United States in Miami, as well as from the panhandle of Florida along the Gulf of Mexico, were all recovered as belonging to the Caribbean lineage of *A. monticola*. This supports the notion that individuals of *A. monticola* collected in Miami (Loftus *et al.*, 1984) may indeed be drifters (transients), but this begs explanation for the recovery of individuals from north-western Florida (Gulf of Mexico) in the Caribbean clade.

Ocean currents may have an influence on the occurrence of Caribbean-clade individuals in the Gulf of Mexico (e.g. Eglin, Florida). The Loop Current (Fig. 4) is a warm ocean current that moves between the Yucatán Peninsula and Cuba into the Gulf of Mexico, then loops south-east and travels between Florida and Cuba (Coats, 1992). Studies have shown that the velocity and position of the Loop Current can vary (Coats, 1992). Regardless of the reproductive mode, larval *A. monticola* eventually inhabit marine waters, having either been born there or drifted in. It is probable that young *A. monticola* may be carried in ocean currents such as the Loop Current, and become dispersed to areas such as Eglin, Florida. Oceanic currents along the Gulf coast of Mexico do not appear to be particularly strong. *Agonostomus monticola* from this area may not be exposed to currents that carry it to far-reaching areas (e.g. the panhandle of Florida



**Figure 4** Map illustrating the location of the Loop Current. White arrows indicate higher flow velocities. The Loop Current is the black band travelling through the Yucatán Channel and looping through the Gulf of Mexico before exiting between Florida and Cuba. Map is not drawn to scale.

and elsewhere), which may be an aid in explaining the genetic isolation of the Gulf of Mexico clade. In conjunction with the lack of large river systems on the Yucatán Peninsula, the Loop Current could also help explain the distinctiveness of the Caribbean and Gulf of Mexico clades.

#### *Pacific-B lineage and cryptic diversity within A. monticola*

Interestingly, some individuals in the Pacific-B clade were collected at the same sampling time and locality – even the same seine haul – as individuals in the Pacific-A clade. Individuals of Pacific-B phenotypically resemble *A. monticola* specimens, and because this is the only such mullet species occurring in Pacific rivers of Middle America, these specimens were initially identified as *A. monticola*. The two Pacific lineages are never recovered as each other's closest relative, and there exists a high degree of genetic divergence between the Pacific-B lineage and all other *Agonostomus* clades (Fig. 1, Table 1). Divergence time estimates indicate that the Pacific-B lineage initially diverged 29.6 Ma, with the Pacific-B clade dated to *c.* 7 Ma (Table 2). We find the Chortís block to be the only geological structure in the vicinity of the distribution of this lineage, and nothing seems apparent about this structure at 30 Ma that would lead to divergence of this lineage. It is possible that some geological event separated the Pacific-B lineage, followed by later dispersal of Atlantic individuals into the Pacific. At present, pending further work, we can conclude little about geological events leading to divergence of this lineage. In addition, the fact that we found these individuals from only two points in western Mexico does not mean it is absent from southern drainages.

Nevertheless, we hypothesize that individuals from the Pacific-B, Pacific-A and Gulf of Mexico clades may represent their own lineages distinct from the Caribbean lineage, and preliminary morphological data (C.D.M., unpublished data) indicates that individuals from the Pacific-B clade do possess diagnosable morphological characters that differentiate these specimens from the other lineages of *Agonostomus*. The recovery of potential cryptic species within the known range of *A. monticola* is novel but not necessarily unexpected given the taxonomic confusion surrounding *A. monticola* (Harrison, 2002; Miller *et al.*, 2005), and it is common for diadromous fish lineages to exhibit intraclade genetic uniformity (McDowall, 2008).

#### **Taxonomic comments**

We are currently in the process of investigating morphological differentiation across the distinct geographical lineages of *Agonostomus*, as we believe that the high degree of genetic divergence, in combination with their geographical and genetic isolation, suggests that these distinct lineages may represent distinct species. While available names exist for some clades, specific epithet revisions are currently premature and beyond the scope of the present study, and we wish to complete a taxonomic revision (complete with diagnoses and

descriptions, currently in preparation) of this widespread group before formally assigning names to these clades.

#### **CONCLUSIONS**

This study represents the first phylogeographical study of the mountain mullet, *Agonostomus monticola*, and indicates that cryptic species diversity is likely to exist throughout the range of this widespread species. The recovery of four divergent lineages of *Agonostomus* across oceanic basins is novel, and three of these correspond to those of Durand *et al.* (2012). While our results indicate that these four geographical regions are genetically distinct and isolated from each other, we are currently in the process of conducting a thorough study of morphological variation before making any alpha-level taxonomic revisions of this broadly distributed species.

Combined analysis of mitochondrial and nuclear data clearly shows biogeographical structure among the clades, and it appears this structure is largely congruent with geological events. The movement of the Chortís block and early stages in the formation of the Isthmus of Panama are likely to have led to divergence of Gulf of Mexico, Caribbean and Pacific-A clades. Some attribute of the Chortís block may also explain the divergence of the Pacific-B clade. In addition, the effects of available freshwater habitat and ocean currents may explain the separation of these lineages despite no obvious geological barrier between some lineages. Within each of the four distinct lineages of *A. monticola* in the Americas, there is little phylogeographical structure or genetic difference, indicating that there is a high degree of gene flow within the range of each lineage. However, the use of more variable markers (e.g. microsatellites) could potentially show population structure within these clades.

A thorough understanding of the life history of *A. monticola* will also be important for unravelling its evolutionary history. For other diadromous species with similar distributions, (e.g. Gobiidae, Eleotridae) multiple ( $n > 2$ ) species are currently recognized within individual oceanic basins (Pacific, Caribbean). With near genetic uniformity within clades, this does not appear to typically be the case for *Agonostomus*. In the absence of basic life-history knowledge for this group, it is impossible to address certain questions related to their evolution beyond phylogenetic relationships. The close association between these species and freshwater river systems of Middle America indicates their importance to its evolutionary history. The inclusion of additional information on the timing and appearance of lotic systems in the region could prove to be instrumental in understanding the diversification of this species.

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## REFERENCES

- Anderson, W.W. (1957) Larval forms of the fresh-water mullet (*Agonostomus monticola*) from the open ocean off the Bahamas and South Atlantic coast of the USA. *Fishery Bulletin*, **57**, 415–425.
- Bermingham, E. & Martin, A.P. (1998) Comparative mtDNA phylogeography of neotropical freshwater fishes: testing shared history to infer the evolutionary landscape of lower Central America. *Molecular Ecology*, **7**, 499–517.
- Berra, T.M. (2001) *Freshwater fish distribution*. Academic Press, San Diego, CA.
- Chow, S. & Hazama, K. (1998) Universal PCR primers for S7 ribosomal protein gene introns in fish. *Molecular Ecology*, **7**, 1255–1256.
- Clement, M., Posada, D. & Crandall, K.A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1659.
- Coats, D.A. (1992) The Loop Current. *The physical oceanography of the U.S. Atlantic and Eastern Gulf of Mexico* (ed. by J.D. Milliman and E. Imamura), pp. 1–45. United States Department of the Interior, Mineral Management Service, Atlantic OCS Region, Herndon, VA.
- Concheiro Pérez, G.A., Řičan, O., Ortí, G., Bermingham, E., Doadrio, I. & Zardoya, R. (2007) Phylogeny and biogeography of 91 species of heroine cichlids (Teleostei: Cichlidae) based on sequences of the cytochrome *b* gene. *Molecular Phylogenetics and Evolution*, **43**, 91–110.
- Cook, B.D., Bernays, S., Pringle, C.M. & Hughes, J.M. (2009) Marine dispersal determines the genetic population structure of migratory stream fauna of Puerto Rico: evidence for island-scale population recovery processes. *Journal of the North American Benthological Society*, **28**, 709–718.
- Craig, M.T., Hastings, P.A. & Pondella, D.J., II (2004) Speciation in the Central American Seaway: the importance of taxon sampling in the identification of trans-isthmian geminate pairs. *Journal of Biogeography*, **31**, 1085–1091.
- Cruz, G.A. (1987) Reproductive biology and feeding habits of Cuyamel, *Joturus pichardi*, and Tepemechin, *Agonostomus monticola* (Pisces; Mugilidae) from Rio Platano, Mosquitia, Honduras. *Bulletin of Marine Science*, **40**, 63–72.
- Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.
- Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology*, **4**, e88.
- Duncan, R.P., Rynerson, M.R., Ribera, C. & Binford, G.J. (2010) Diversity of *Loxosceles* spiders in Northwestern Africa and molecular support for cryptic species in the *Loxosceles rufescens* lineage. *Molecular Phylogenetics and Evolution*, **55**, 234–248.
- Durand, J.-D., Shen, K.-N., Chen, W.-J., Jamandre, B.W., Blel, H., Diop, K., Nichio, M., Garcia de León, F.J., Whitfield, A.K., Chang, C.-W. & Borsa, P. (2012) Systematics of the grey mullets (Teleostei: Mugiliformes: Mugilidae): molecular phylogenetic evidence challenges two centuries of morphology-based taxonomy. *Molecular Phylogenetics and Evolution*, **64**, 73–92.
- Erdman, D.S. (1972) *Inland game fishes of Puerto Rico*. Department of Agriculture, Commonwealth of Puerto Rico, San Juan, Puerto Rico.
- Farris, D.W., Jaramillo, C., Bayona, G., Restrepo-Moreno, S.A., Montes, C., Cardona, A., Mora, A., Speakman, R.J., Glascock, M.D. & Valencia, V. (2011) Fracturing of the Panamanian Isthmus during initial collision with South America. *Geology*, **39**, 1007–1010.
- Georges, S.R. & Rodrigues, M.T. (2010) The hidden diversity of *Coelodactylus amazonicus* (Sphaerodactylinae: Gekkota) revealed by molecular data. *Molecular Phylogenetics and Evolution*, **54**, 583–593.
- Grant, W.S. & Leslie, R.W. (2001) Inter-ocean dispersal is an important mechanism in the zoogeography of hakes (Pisces: *Merluccius* spp.). *Journal of Biogeography*, **28**, 699–721.
- Greenfield, D.W. & Thomerson, J.E. (1997) *Fishes of the continental waters of Belize*. University of Florida Press, Gainesville, FL.
- Haney, R.A., Turner, B.J. & Rand, D.M. (2009) A cryptic lineage within the pupfish *Cyprinodon dearborni* suggests multiple colonizations of South America. *Journal of Fish Biology*, **75**, 1108–1114.
- Harrison, I.J. (2002) Order Mugiliformes, Mugilidae. *The living marine resources of the western central Atlantic, Vol. 2: Bony fishes part 1 (Acipenseridae to Grammatidae)* (ed. by K.E. Carpenter), pp. 1071–1085. Food and Agriculture Organization, Rome.
- Harrison, I.J. & Howes, G.J. (1991) The pharyngobranchial organ of mugilid fishes: its structure, variability, ontogeny, possible function and taxonomic utility. *Bulletin of the British Museum of Natural History (Zoology)*, **57**, 111–132.

- Haruyama, N., Hideshi, N., Mochizuki, A. & Nomura, M. (2008) Mitochondrial phylogeny of cryptic species of the lacewing *Chrysoperla nipponensis* (Neuroptera: Chrysopidae) in Japan. *Annals of the Entomological Society of America*, **101**, 971–977.
- Hulsey, C.D. & López-Fernández, H. (2011) Nuclear Central America. *Historical biogeography of neotropical freshwater fishes* (ed. by J.S. Albert and R.E. Reis), pp. 279–291. University of California Press, Berkeley, CA.
- Hurt, C., Anker, A. & Knowlton, N. (2009) A multilocus test of simultaneous divergence across the Isthmus of Panama using snapping shrimp in the genus *Alpheus*. *Evolution*, **63**, 514–530.
- Lessios, H.A. (2008) The Great American Schism: divergence of marine organisms after the rise of the Central American Isthmus. *Annual Review of Ecology, Evolution, and Systematics*, **39**, 63–91.
- Loftus, W.F., Kushland, J.A. & Voorhees, S.A. (1984) Status of the mountain mullet in southern Florida. *Florida Scientist*, **47**, 256–263.
- López Ramos, E. (1975) Geological summary of the Yucatan Peninsula. *The ocean basins and margins*, Vol. 3 (ed. by A.E.M. Narin and F.G. Stehli), pp. 257–282. Plenum Press, London.
- Marko, P.B. & Moran, A.L. (2009) Out of sight, out of mind: high cryptic diversity obscures the identities and histories of geminate species in the marine bivalve genus *Acar*. *Journal of Biogeography*, **36**, 1861–1880.
- Martin, A.P. & Bermingham, E. (1998) Systematics and evolution of lower Central American cichlids inferred from analysis of cytochrome b gene sequences. *Molecular Phylogenetics and Evolution*, **9**, 192–203.
- Matamoros, W.A., Schaefer, J., Mickle, P., Arthurs, W., Ikoma, R.J. & Ragsdale, R. (2009) First record of *Agonostomus monticola* (family: Mugilidae) in Mississippi freshwaters with notes of its distribution in the southern United States. *Southeastern Naturalist*, **8**, 175–178.
- Matamoros, W.A., Kreiser, B.R. & Schaefer, J.F. (2012) A delineation of Nuclear Middle America biogeographical provinces based on river basin faunistic similarities. *Reviews in Fish Biology and Fisheries*, **22**, 351–365.
- McDowall, R.M. (2008) Diadromy, history and ecology: a question of scale. *Hydrobiologia*, **602**, 5–14.
- Miller, R.R., Minckley, W.L. & Norris, S.M. (2005) *Freshwater fishes of México*. University of Chicago Press, Chicago, IL.
- Montes, C., Cardona, A., McFadden, R., Morón, S.E., Silva, C. A., Restrepo-Morena, S., Ramírez, D.A., Hoyos, N., Wilson, J., Farris, D., Bayona, G.A., Jaramillo, C.A., Valencia, V., Bryan, J. & Flores, J.A. (2012) Evidence for middle Eocene and younger land emergence in central Panama: implications for Isthmus closure. *Geological Society of America Bulletin*, **124**, 780–799.
- Murphy, W.J. & Collier, G.E. (1996) Phylogenetic relationships within the aplocheiloid fish genus *Rivulus* (Cyprinodontiformes: Rivulidae): implications for Caribbean and Central American biogeography. *Molecular Biology and Evolution*, **13**, 642–649.
- Myers, G.S. (1949) Salt-tolerance of fresh-water fish groups in relation to zoogeographical problems. *Bijdragen tot de Dierkunde*, **28**, 315–322.
- Ornelas-García, C.P., Domínguez-Domínguez, O. & Doadrio, I. (2008) Evolutionary history of the fish genus *Astyanax* Baird & Girard (1854) (Actinopterygii, Characidae) in Mesoamerica reveals multiple morphological homoplasies. *BMC Evolutionary Biology*, **8**, 340.
- Parham, J.F., Donoghue, P.C.J., Bell, C.J. *et al.* (2012) Best practices for justifying fossil calibrations. *Systematic Biology*, **61**, 346–359.
- Patterson, C. (1993) Osteichthyes: Teleostei. *The fossil record 2* (ed. by M.J. Benton), pp. 621–656. Chapman & Hall, London.
- Perdices, A., Bermingham, E., Montilla, A. & Doadrio, I. (2002) Evolutionary history of the genus *Rhamdia* (Teleostei: Pimelodidae) in Central America. *Molecular Phylogenetics and Evolution*, **25**, 172–189.
- Perdices, A., Doadrio, I. & Bermingham, E. (2005) Evolutionary history of the synbranchid eels (Teleostei: Synbranchidae) in Central America and the Caribbean islands inferred from their molecular phylogeny. *Molecular Phylogenetics and Evolution*, **37**, 460–473.
- Pezold, F.L. & Edwards, R.J. (1983) Additions to the Texas marine ichthyofauna, with notes on the Rio Grande estuary. *The Southwestern Naturalist*, **28**, 102–105.
- Phillip, D.A.T. (1993) Reproduction and feeding of the mountain mullet, *Agonostomus monticola*, in Trinidad, West Indies. *Environmental Biology of Fishes*, **37**, 47–55.
- Pindell, J., Kennan, L., Stanek, K.P., Maresch, W.V. & Draper, G. (2006) Foundations of Gulf of Mexico and Caribbean evolution: eight controversies resolved. *Geologica Acta*, **4**, 303–341.
- Posada, D. (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256.
- Posada, D. & Buckley, T.R. (2004) Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology*, **55**, 793–808.
- Rambaut, A. & Drummond, A.J. (2007) *Tracer v1.4*. Available at: <http://beast.bio.ed.ac.uk/Tracer>.
- Rauchenberger, M. (1989) Systematics and biogeography of the genus *Gambusia* (Cyprinodontiformes, Poeciliidae). *American Museum Novitates*, **2951**, 1–74.
- Rogers, R.D., Mann, P. & Emmet, P.A. (2007) Tectonic terranes of the Chortis block based on integration of regional aeromagnetic and geologic data. *The Geological Society of America Special Papers*, **428**, 65–88.
- Sauquet, H., Ho, S.Y.W., Gandolfo, M.A., Jordan, G.J., Wilf, P., Cantrill, D.J., Bayly, M.J., Bromham, L., Brown, G.K., Carpenter, R.J., Lee, D.M., Murphy, D.J., Sniderman, J.M.K. & Udovicic, F. (2012) Testing the impact of calibration on molecular divergence times using a fossil-rich group: the case of *Nothofagus* (Fagales). *Systematic Biology*, **61**, 289–313.

- Schultz, L.P. (1946) A revision of the genera of mullets, fishes of the family Mugilidae, with descriptions of three new genera. *Proceedings of the United States National Museum*, **96**, 377–395.
- Templeton, A.R., Crandall, K.A. & Sing, C.F. (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics*, **132**, 619–633.
- Thomson, J.M. (1997) The Mugilidae of the world. *Memoirs of the Queensland Museum*, **41**, 457–562.
- Tringali, M.D., Bert, T.M., Seyoum, S., Bermingham, E. & Bartolacci, D. (1999) Molecular phylogenetics and ecological diversification of the transisthmian fish genus *Centropomus* (Perciformes: Centropomidae). *Molecular Phylogenetics and Evolution*, **13**, 193–207.
- Zink, R.M. & Barrowclough, G.F. (2008) Mitochondrial DNA under siege in avian phylogeography. *Molecular Ecology*, **17**, 2107–2121.
- Zwickl, D.J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. PhD Thesis, University of Texas at Austin, Austin, TX.

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## BIOSKETCH

**Caleb McMahan** is a graduate student in ichthyology at the LSU Museum of Natural Science, whose research interests include the systematics, taxonomy and biogeography of Neotropical fishes. This work was completed as part of his Master's thesis at Southeastern Louisiana University. All authors are interested in the systematics and biogeography of Middle American freshwater fishes.

Author contributions: C.D.M. and K.R.P. conceived the ideas; all authors assisting in collecting samples; C.D.M. collected the data; C.D.M., K.R.P. and M.P.D. analysed the data; and C.D.M. led the writing.

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