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Rapid range expansion in the Great Plains narrow-mouthed toad (*Gastrophryne olivacea*) and a revised taxonomy for North American microhylids

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ABSTRACT

We investigated genetic variation within the Great Plains narrow-mouthed toad, *Gastrophryne olivacea*, across its geographic range in the United States and Mexico. An analysis of mitochondrial DNA (mtDNA) from 105 frogs revealed remarkably low levels of genetic diversity in individuals inhabiting the central United States and northern Mexico. We found that this widespread matrilineal lineage is divergent (ca. 2% in mtDNA) from haplotypes that originate from the western United States and western coast of Mexico. Using a dataset that included all five species of *Gastrophryne* and both species of the closely related genus *Hypopachus*, we investigated the phylogenetic placement of *G. olivacea*. This analysis recovered strong support that *G. olivacea*, the tropically distributed *G. elegans*, and the temperately distributed *G. carolinensis* constitute a monophyletic assemblage. However, the placement of *G. pictiventris* and *G. usta* render *Gastrophryne* paraphyletic with respect to *Hypopachus*. To complement our mitochondrial analysis, we examined a small fragment of nuclear DNA and recovered consistent patterns. In light of our findings we recommend (1) the resurrection of the nomen *G. mazatlanensis* Taylor (1943) for the disjunct western clade of *G. olivacea* and (2) the tentative placement of *G. pictiventris* and *G. usta* in *Hypopachus*. To explore possible scenarios leading to low levels of genetic diversity in *G. olivacea*, we used mismatch distributions and Bayesian Skyline plots to examine historic population expansion and demography. Collectively these analyses suggest that *G. olivacea* rapidly expanded in effective population size and geographic range during the late Pleistocene or early Holocene. This hypothesis is consistent with fossil data from northern localities and contemporary observations that suggest ongoing northern expansion. Given our findings, we suspect that the rapid range expansion of *G. olivacea* may have been facilitated by ecological associations with open habitats and seasonal water bodies.

1. Introduction

Range dynamics of temperate faunas during the last glacial cycle are becoming better understood. This research has been facilitated by a massive and ever increasing number of intraspecific studies that use molecules to infer recent distributional and demographic shifts. Particularly well represented among these studies are frog and toad species from North America. The available literature includes well-sampled phylogeographic investigations for almost every major Nearctic anuran lineage: Bufonidae, *Anaxyrus americanus* (Fontenot et al., 2011); Hylidae, *Acris crepitans* (Gamble et al., 2008); *Pseudacris regilla* (Recureo et al., 2006), *Pseudacris* spp. (Moriarty Lemmon et al., 2007); Microhylidae, *Gastrophryne carolinensis* (Makowsky et al., 2009); Ranidae, *Lithobates sylvaticus* (Lee-Yaw et al., 2009), *Lithobates clamitans*, *Lithobates catesbeiana* (Austin and Zamudio, 2008). The rate at which these Nearctic frog species are thought to have expanded their post-glacial ranges varies widely. In general there have been three categories of narrative proposed to explain range expansion in North American frogs and toads: (1) species that experienced little expansion and either maintain a regionally restricted distribution or display phylogeographic structure that directly corresponds to patterns of refugial isolation (e.g. Moriarty Lemmon et al., 2007), (2) species that persisted in multiple refugia but subsequently expanded geographically to overlap and hybridize with neighboring populations, thereby obscuring patterns of refugial isolation (e.g., Austin and Zamudio, 2008; Fontenot et al., 2011), and (3) species that appear to have expanded rapidly from a single genetically bottlenecked population (e.g., Makowsky et al., 2009). These narratives have been mostly generated from taxa that inhabit the eastern, western, and northern portions of North America (and mostly in the United States). Notably absent from the literature are studies investigating frogs that are endemic to the expansive Great Plains region, which covers the central portions of the United States and northern Mexico.

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The Great Plains narrow-mouthed toad, *Gastrophryne olivacea* (Anura: Microhylidae), occurs in the Great Plains region of the United States from Missouri, Nebraska, and Kansas southward to the State of Tamaulipas in Mexico. A disjunct series of populations is also known to occur in southern Arizona in the United States and on the west coast of Mexico as far south as the state of Nayarit (Fig. 1; Stebbins, 2003). This western group was previously referred to a separate species, *G. mazatlanensis* (Taylor, 1943), but this taxon was later synonymized with *G. olivacea* (Hecht and Matalas, 1946) although some authors (Chrapliwy et al., 1961) have retained the original name of the western group as a subspecies, *G. o. mazatlanensis* (with members of the eastern populations referred to as *G. o. olivacea*). Great Plains narrow-mouthed toads are small fossorial frogs (19–42 mm snout–vent length [Wright and Wright, 1949; Fitch, 1956a]) that inhabit grasslands, marshy sloughs, and rocky, open wooded slopes and feed almost exclusively on ants (Conant and Collins, 1998). It is presumed that these frogs are capable of rapid range expansion since this phenomenon has been observed in a matter of decades on the northern edge of their range (Blair, 1955). Additionally, *G. olivacea* is the only extant local frog species absent from Pleistocene fossil records in the state of Kansas (Holman, 2003), suggesting that it may have only recently colonized the region.

Phylogenetic relationships of *G. olivacea* have been explored by several authors (Frost et al., 2006; van der Meijden et al., 2007; Makowsky et al., 2009; Greenbaum et al., 2011). Recently, the closely related genus *Hypopachus* was thoroughly reviewed by Greenbaum et al. (2011). These authors included several species of *Gastrophryne* in their analysis and used molecular data to reveal that *Gastrophryne* is probably paraphyletic based on the placement of the Mexican taxon *G. usta*. While previous studies have greatly advanced our understanding of diversity within *Gastrophryne*, a comprehensive molecular analysis has yet to be performed on all members of this likely paraphyletic genus.

In this study we used a fragment of the mitochondrial 16S ribosomal large subunit gene (16S) to characterize genetic variation in populations of *G. olivacea* from throughout their range in the United States and Mexico (Fig. 1). We also examined this mitochondrial variation in respect to all of the currently recognized species of the genera *Gastrophryne* and *Hypopachus*. To complement this examination, we used a gene fragment of nuclear DNA (rhodopsin exon 1) to investigate the higher-level relationships of these taxa. Based on these molecular data, we present descriptions of (1) the matrilineal lineages of *G. olivacea*, (2) the phylogenetic placement of *G. olivacea* and related taxa within the sister genera *Gastrophryne* and *Hypopachus*, and (3) historic patterns of range expansion and population demography in *G. olivacea*.

2. Materials and methods

2.1. Geographic sampling

We collected *G. olivacea* from the United States and Mexico during biological surveys conducted from 2007 to 2011. These specimens were deposited in the herpetological collection of the University of Texas at Arlington (UTA). We augmented this sampling with tissues collected by other researchers at the Texas Natural History Collection (University of Texas at Austin) and the Museum of Vertebrate Zoology (University of California at Berkeley). In total, our sampling included 105 individuals from Arizona, Kansas, Oklahoma, and Texas in the United States, and from Coahuila, Chihuahua, Nayarit, and Tamaulipas in Mexico (Fig. 1). We included geographically redundant sampling from several localities in the United States in order to investigate population level variation. To clarify the phylogenetic placement of *G. olivacea*, we also included several related taxa in our study including: *G. carolinensis*, *G. elegans*, *G. pictiventris*, *G. usta*, *Hypopachus barberi*, *H. variolosus*, *Elachistocleis bicolor*, *Chiasmocleis albopunctata*, and *C. ventrimaculata*. A summary of the taxonomic sampling, specimen vouchers, and their respective locality data is presented in Appendix A.

2.2. Laboratory techniques and genetic sampling

Tissue samples (muscle, liver, or whole limbs) were taken and preserved in either 95% ethanol or tissue lysis buffer (1% SDS; 50 mM Tris, pH 8.0; 100 mM EDTA; 100 mM NaCl). Genomic

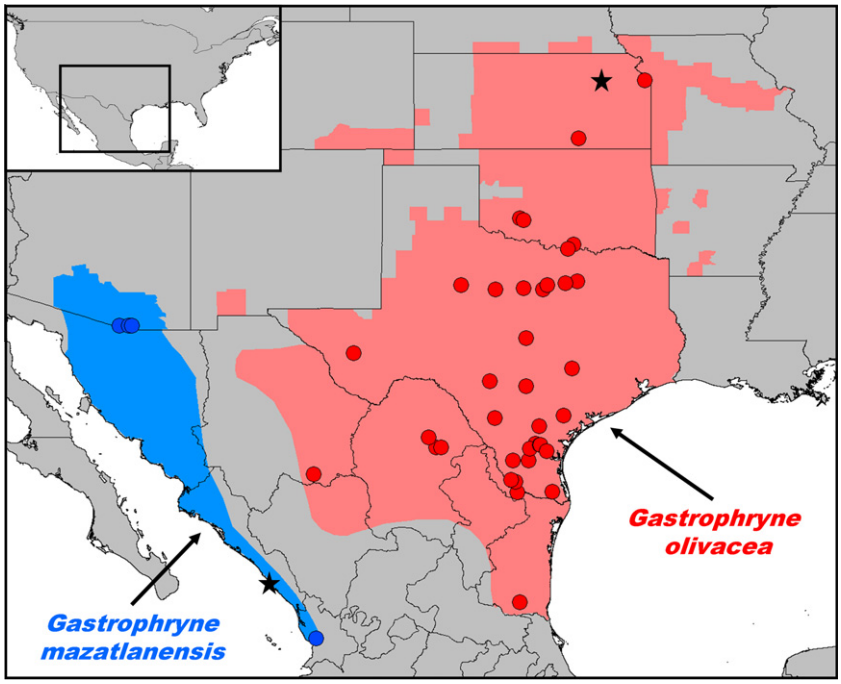

Fig. 1. Geographic sampling and distribution of *Gastrophryne olivacea* and *Gastrophryne mazatlanensis*. Black stars indicate type localities of *G. olivacea* (Geary Co., Kansas, USA) and *G. mazatlanensis* (Mazatlán, Sinaloa, MX). Ranges are modified versions of previous estimates from IUCN et al. (2006).

DNA was extracted using the Qiagen® DNeasy kit (Qiagen Inc. – USA, Valencia, CA). To investigate phylogeographic relationships in *G. olivacea*, we used a fragment of 16S that is frequently employed in studies of anuran phylogeography (Vences et al., 2005; Brown et al., 2010). Two primers modified from other studies (Vences et al., 2005; Brown et al., 2010) were used to amplify a 587 base pair (bp) fragment of 16S (listed 5′ to 3′): forward primer 16SAR, CGC CTG TTT AYC AAA AAC AT and reverse primer 16SBR, CCG GTC TGA ACT CAG ATC ACG T. We used similar PCR conditions and thermal cycling profiles to those listed in Streicher et al. (2009) for 16S. We cleaned PCR products using USB ExoSap-IT (US78201, Ambersham Biosciences, Piscataway, NJ). Cycle sequencing reactions and direct sequencing of DNA fragments were performed by the UTA Genomics Core Facility (Arlington, Texas; http://gcf.uta.edu). All 16S sequences generated for this study were submitted to GenBank (Appendix A).

To investigate phylogenetic relationships in North American microhylids, we also examined a small fragment of the nuclear rhodopsin gene (293 bp; exon 1). These rhodopsin sequence data were obtained from previous studies (Greenbaum et al., 2011; Frost et al., 2006) and included two *G. olivacea* (UTEP 19815, ATH 476), two *G. carolinensis* (UTEP 19907, KU 289624), one *G. usta* (UTA A-60366), two *H. variolosus* (TCWC 83293, JF837002), and two *H. barberi* (UTA A-55222, MVZ 160939).

2.3. Data analysis

DNA sequences were edited and aligned using Sequencher 4.1 (GeneCodes Corporation, Ann Arbor, Michigan, USA). These alignments were further adjusted by eye and examined against vertebrate 16S secondary structure models to identify those regions corresponding to variable loop structures (*sensu* Kjer, 1997). We selected models of molecular evolution, constructed phylogenetic trees and generated genetic distance (uncorrected "*p*") values using the programs MEGA 5 (Tamura et al., 2011) and MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). We used several phylogenetic criteria to generate trees including Bayesian Markov-chain Monte Carlo (BAYES), Maximum Likelihood (ML), Maximum Parsimony (MP), and Minimum Evolution (ME). In ML analyses we employed the GTR + I + G model of nucleotide evolution. In ML, MP, and ME phylogenetic analyses we estimated branch support from 2000 bootstrap pseudoreplicates (Hedges, 1992). In Bayesian phylogenetic reconstructions, we used the GTR + I + G model of nucleotide evolution in paired Markov-chain Monte Carlo searches across 10 million generations sampling every 1000 generations with four chains (three heated, one cold). We examined output parameters in MrBayes 3.1.2 and the program AWTY (Wilgenbusch et al., 2004) to ascertain the convergence of our paired searches. Specifically, we used AWTY to assess topological differences within and among our BAYES runs with a burn-in of 10% and 10 sliding windows. We considered clades to be supported with bootstrap proportions of greater than 70 and posterior probability of greater than 0.95 (Hillis and Bull, 1993). To examine sequence alignments that contained few nucleotide polymorphisms, we used the program TCS 1.18 (Clement et al., 2000) to link haplotypes in statistical parsimony networks. In our examination of *G. olivacea*, we generated measures of haplotype diversity (h) and average per-site nucleotide diversity (π) using the program DNAsp (Rozas et al., 2003).

We examined 16S sequences in the sampled populations of *G. olivacea* for evidence of recent change in effective population size by calculating a mismatch distribution in the program Arlequin 3.1 (Exoffier et al., 2005). To assess putative population expansion and selective neutrality we generated Tajima's *D* statistic in MEGA 5 and Fu's F statistic in DNAsp. To conduct these analyses, our alignment was trimmed and modified to exclude several gapped and missing sites in the loop regions and ends of the 16S fragment. This modification produced a 16S alignment of 494 bp. As an alternative to the traditional methods listed above, we also investigated potential changes in demography by applying a Bayesian Skyline analysis (Drummond et al., 2005) using BEAST (Drummond and Rambaut, 2007). To identify the most appropriate model of nucleotide evolution for the *G. olivacea*-only dataset, we used the program MODELTEST (Posada and Crandall, 1998) implemented in PAUP (Swofford, 2002). We ran this analysis for 10 million steps, using default parameters with sampling every 1000 steps. Prior to convergence diagnostic analysis, the first 10% of samples were discarded as burn-in. We ran two analyses and combined them for the examination of convergence diagnostics in the program TRACER 1.4 (Rambaut and Drummond, 2007).

3. Results

3.1. Mitochondrial DNA

We found that 143 of the 587 bp were variable across our 16S alignment and 87 of these variable sites were parsimony informative. All of the phylogenetic criteria that we used (BAYES, ML, ME, and MP) to analyze 16S recovered highly concordant topologies with similar patterns of nodal support (Fig. 2). The most optimal tree in our ME analysis had a sum branch length of 0.296. In the MP analysis, we recovered 1181 equally parsimonious trees with a length of 186 (consistency index = 0.627; retention index = 0.922). The tree with the highest log likelihood in our ML analysis had a score of −1797.5762. In our BAYES analysis the final deviation of split frequencies (after 10 million generations) had a value of 0.008. Our analysis in AWTY found that while our paired runs never converged topologically, each reached stability after ca. 6 million generations, and the topological differences between runs were small (i.e., <1 symmetric-difference; Penny and Hendy, 1985). Thus, we removed the first 6002 trees (of 10,002) from our BAYES dataset prior to generating posterior probabilities. Collectively, our phylogenetic analyses recovered two major clades of *G. olivacea* and relatively low levels of diversity in both. The first and most geographically wide-spread clade is distributed in areas associated with the Great Plains region of the United States (including the states of Kansas, Oklahoma, Texas; and presumably Colorado, Nebraska, New Mexico, Missouri, and Arkansas) and northern Mexico (including the states of Chihuahua, Coahuila, Tamaulipas; and presumably Durango and Nuevo Leon). The second clade is restricted to west central Arizona in the United States and the west coast of Mexico (including the state of Nayarit; and presumably Sonora and Sinaloa). Our phylogenetic analyses of 16S recovered strong branch support for a sister-group relationship between *G. olivacea* and *G. elegans*. Together these taxa are supported as the sister group to *G. carolinensis* in most of our analyses. The placement of *G. pictiventris* and *G. usta* renders the genus *Gastrophryne* paraphyletic because both of these species were placed in a clade with members of the genus *Hypopachus*. Mean sequence divergence levels also support most topological reconstructions for relationships in *Gastrophryne* and *Hypopachus* (Table 1).

3.2. Nuclear DNA

We found that 33 of the 293 bp in the rhodopsin alignment were variable and 12 of these sites were parsimony informative. The amount of sequence variation within *Gastrophryne* and *Hypopachus* at the rhodopsin locus was quite low (1.6%, within group uncorrected "*p*"). The optimal tree in ME analysis had a sum of branch length of 0.128. Our MP analysis resulted in 33 equally parsimonious trees with a length of 39 (consistency index = 0.949;

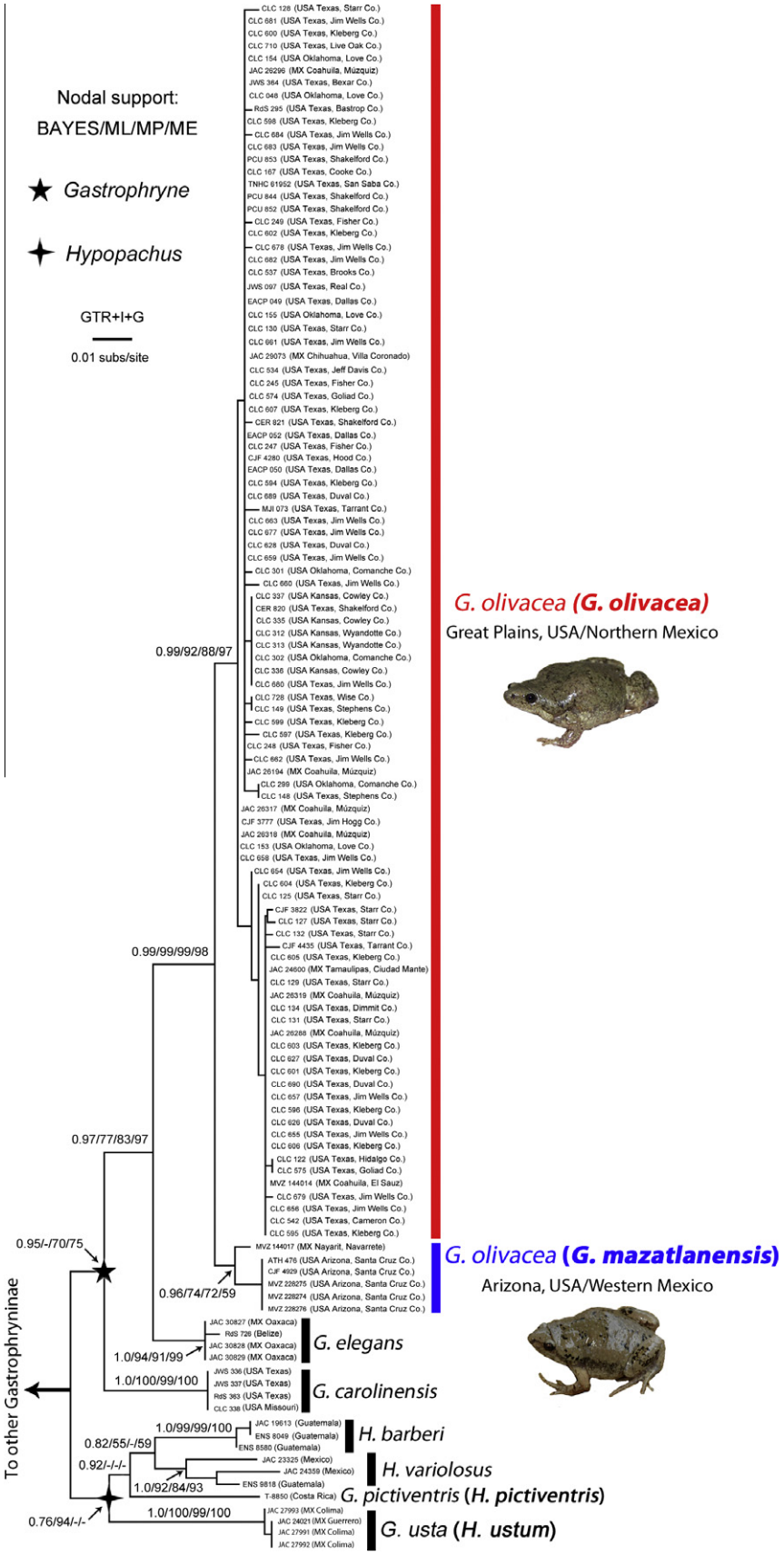

Fig. 2. Maximum likelihood tree for mitochondrial DNA (16S) sampling of *Gastrophryne* and *Hypopachus*. Support values from Bayesian Markov-chain Monte Carlo (BAYES), maximum likelihood (ML), maximum parsimony (MP), and minimum evolution (ME) analyses appear above branches unless otherwise indicated. Photographs are of frogs from Coahuila, Mexico near Múzquiz (*G. olivacea*), and from Sonora, Mexico near Alamos (*G. mazatlanensis*, photographed by Thomas J. Devitt).

Table 1
Mitochondrial 16S mean sequence divergence (uncorrected "*p*") between (below the diagonal) and within (on the diagonal) species of the genera *Gastrophryne* and *Hypopachus*. Outgroup taxa are *Elachistocleis bicolor* and *Chiasmocleis albopunctata*.

	Gastrophryne				*Hypopachus*				Outgroups	
	olivacea	*mazatlanensis*	*elegans*	*carolinensis*	*barberi*	*pictiventris*	*ustum*	*variolosus*	*Elachistocleis*	*Chiasmocleis*
olivacea	0.004									
mazatlanensis	0.022	0.004								
elegans	0.036	0.035	0.001							
carolinensis	0.058	0.055	0.047	0.000						
barberi	0.060	0.053	0.074	0.055	0.001					
pictiventris	0.076	0.073	0.063	0.065	0.057	N/A				
ustum	0.086	0.079	0.077	0.066	0.061	0.075	0.001			
variolosus	0.079	0.070	0.074	0.069	0.042	0.063	0.076	0.033		
Elachistocleis	0.084	0.079	0.081	0.063	0.059	0.086	0.079	0.077	N/A	
Chiasmocleis	0.098	0.106	0.105	0.110	0.108	0.123	0.132	0.119	0.121	N/A

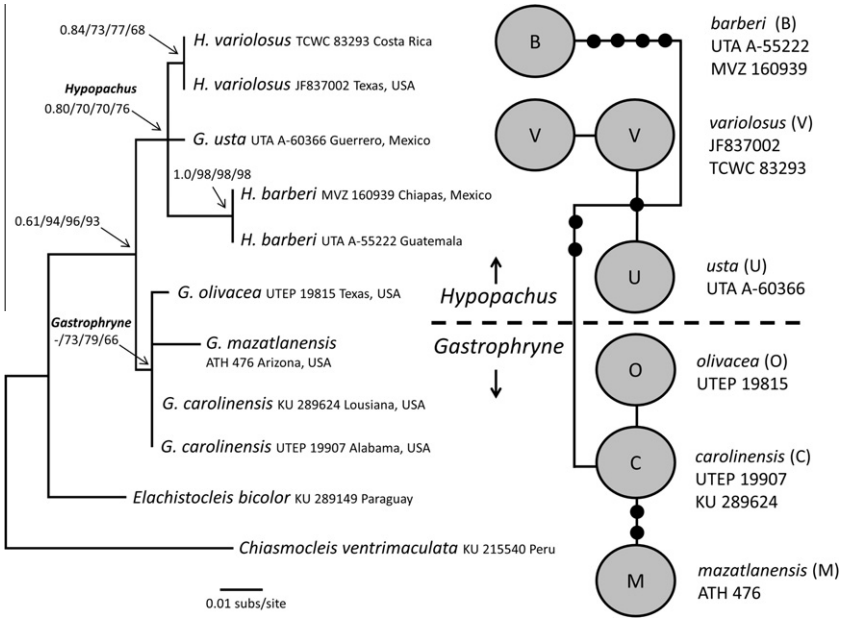

Fig. 3. Maximum likelihood tree (left) and haplotype network (right) of a nuclear DNA fragment (rhodopsin exon 1) for the genera *Gastrophryne* and *Hypopachus*. Branch support is from Bayesian Markov-chain Monte Carlo (BAYES), maximum likelihood (ML), maximum parsimony (MP), and minimum evolution (ME) analyses, respectively. Small black circles in the parsimony network represent mutational steps.

retention index = 0.909). In ML analysis the tree with the highest log likelihood had a score of −610.27. In our BAYES run the final deviation of split frequencies was 0.004 (after 10 million generations). Our analysis in AWTY found that the paired runs converged at 5 million generations, so we excluded the first 5002 trees (of 10,002) as burn-in. Generally, phylogenetic analyses (BAYES, ME, MP, ML) and parsimony networks of the rhodopsin exon fragment support the relationships and major clades found in the mitochondrial dataset. Specifically, these analyses revealed the paraphyly of *Gastrophryne* caused by the placement of *G. usta* within *Hypopachus* (Fig. 3). Additionally, nuclear DNA supported the distinction of the western and eastern clades of *G. olivacea*.

3.3. Matrilineal population demographics

Given the phylogenetic relationships recovered by our analyses of mitochondrial and nuclear DNA, we removed members of the western *G. olivacea* clade ($n = 7$) from our datasets prior to estimating measures of population demography. This modification left a sample containing 98 individuals from the Great Plains in the United States and northern Mexico. This revised alignment contained 21 unique haplotypes and 22 segregating sites (Fig. 4). We found low levels of nucleotide diversity ($\pi = 0.004$) and relatively high levels of haplotype diversity ($h = 0.766 \pm 0.001$). Our *G. olivacea* alignment contained many low-frequency polymorphisms that contributed to a negative Tajima's *D* statistic (−1.57). The Fu's *F* statistic that we recovered is fairly large and negative, which is evidence for an excessive number of alleles as expected during recent population expansion (−11.894). Our observed pairwise difference data generated from *G. olivacea* in the mismatch distribution closely matched the predicted patterns of diversity under a sudden expansion model (Fig. 5). The program MODELTEST selected the K80 model (Kimura, 1980) as the most appropriate model of nucleotide substitution for our dataset. Because we were unable to implement this model in BEAST, we selected the model with the fewest parameters (HKY). The Bayesian Skyline plot also revealed a pattern of constant population growth within the most recent time intervals (Fig. 6).

4. Discussion

4.1. Taxonomic implications

Based on the evolutionary relationships suggested by our analyses, we recommend taxonomic changes within the genera *Gastrophryne* and *Hypopachus*. While our study found *G. olivacea*

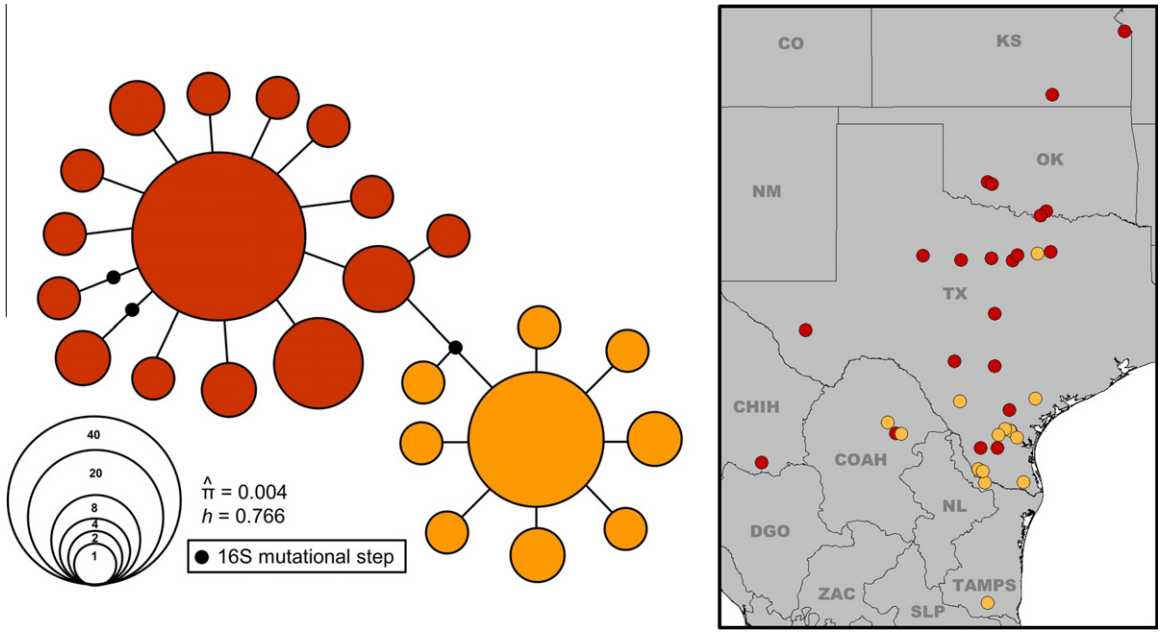

Fig. 4. Evidence for recent range expansion in *Gastrophryne olivacea*: (left) 95% plausible haplotype network generated from mitochondrial 16S data and (right) geographic distribution of two major haplogroups. Note that most orange haplotype localities also contain syntopic red haplotypes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

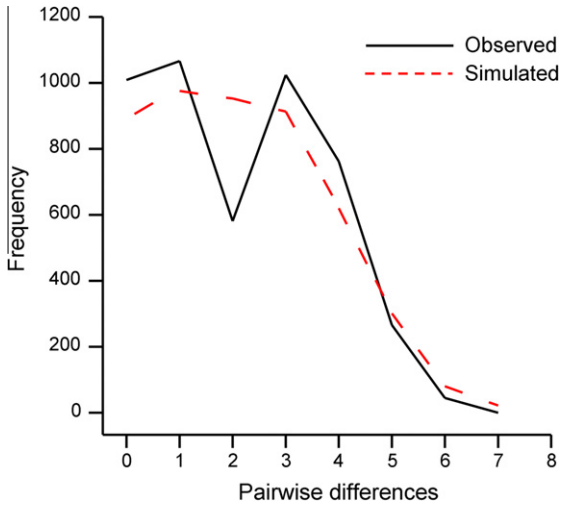

Fig. 5. Mismatch distribution constructed from mitochondrial sequences of 98 *Gastrophryne olivacea* from Mexico and the United States. Dotted line indicates expected distribution under a sudden expansion model, which is closely matched by the observed data (solid line).

to be monophyletic, within this assemblage we observed distinct western and eastern clades in both our mitochondrial and nuclear datasets (Figs. 2 and 3; Table 1). The distributions of these clades are separated by a substantial biogeographic barrier, the Sierra Madre Occidental. Based on our sampling and previously reported data (Stebbins, 2003), the elevational distribution of *G. olivacea* ranges from near sea level to about 1550 m. Between the ranges of the *G. olivacea* clades, the Sierra Madre Occidental consistently exceeds 1700–2500 m, and therefore contemporary gene flow between these groups is most likely minimal to absent. This isolation and differentiation suggests that western populations assignable to *G. o. mazatlanensis* are evolutionarily independent of those in the east. Additionally, one potential contact zone between *G. o. olivacea* and *G. o. mazatlanensis* in lower elevation areas of Arizona was recently surveyed and, while narrow-mouthed toads were abundant in western Arizona, no *Gastrophryne* were observed in the eastern portions of this state (Sullivan et al., 1996), and no records exist for *G. olivacea* in Cochise County, Arizona. The intersection of the Sonoran and Chihuahuan deserts in this area has been proposed as a historic barrier to dispersal for arid-adapted taxa (a.k.a., the Cochise filter barrier), due to the periodic occurrence of woodlands during glaciopluvial periods of the Pleistocene (Van Devender, 1990). This biogeographic pattern of east–west genetic division has been documented in other terrestrial vertebrates with distributions spanning the Cochise filter barrier (e.g., snakes, Castoe et al., 2007; other frogs, Bryson et al., 2010). In a similar manner, the range disjunction between the eastern and western *G. olivacea* clades suggests either small populations or the complete absence of a biotic connection between clades and potentially diminished gene flow.

Given our phylogenetic results and the putatively non-overlapping geographic distributions of the *G. olivacea* clades, herein the nomen *G. mazatlanensis* Taylor, 1943, is resurrected. *Gastrophryne mazatlanensis* was originally described from Sinaloa, Mexico, and applied to the equivalent of the western lineage of *G. olivacea* in our analysis. The species description and diagnosis of *G. mazatlanensis* is found in Taylor (1943) and Nelson (1972). Additional sampling is needed to understand species boundaries of the taxa involved. In particular, future phylogenetic studies should incorporate samples from (1) eastern Arizona and western New Mexico in the United States, (2) along the west coast of Mexico between the localities we sampled for *G. mazatlanensis*, and (3) from localities of intermediate elevation in the Mexican state of Chihuahua.

We found that *G. usta* was more closely related to *Hypopachus* than to other *Gastrophryne* (Table 2, Figs. 2 and 3), similar to the phylogenetic patterns reported by Greenbaum et al. (2011). We feel confident in this placement because our study includes complete taxonomic sampling for both *Gastrophryne* and *Hypopachus* as well as three additional *G. usta* samples that we collected from near the type locality of Tecomán, Colima in western Mexico (Nelson, 1972). We also found that 16S sequence divergence levels observed in relation to *G. pictiventris* suggest closer affinities with

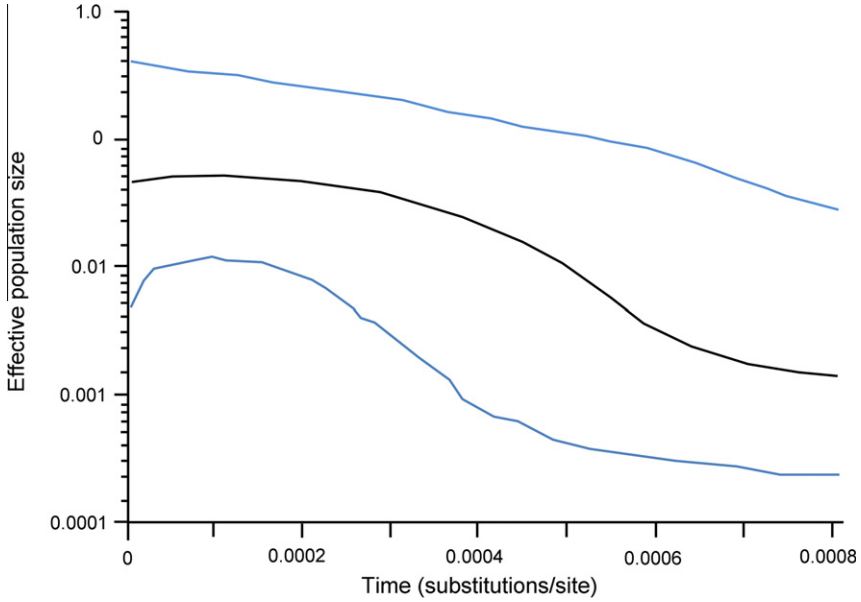

Fig. 6. Bayesian Skyline plot constructed from mitochondrial sequences of 98 *Gastrophryne olivacea* from Mexico and the United States. Flanking blue lines indicate upper and lower 95% highest posterior density estimates. Values on the *X* and *Y*-axes are equivalent to (1) mutations/site and (2) population size vs. mutation rate, respectively.

Table 2
Mean mitochondrial (16S rRNA subunit [16S]) and nuclear (rhodopsin exon 1 [Rho]) sequence divergence levels (uncorrected "*p*") between the enigmatic taxon *Gastrophryne usta* (*Hypopachus ustum*) and representative groups of the genera *Gastrophryne*, *Hypopachus*, *Elachistocleis*, and *Chiasmocleis*. *Hypopachus ustum* pictured (UTA A-61716) is from Michoacán, Mexico.

	Gastrophryne usta (*Hypopachus ustum*)	
	16S	Rho
Hypopachus (*barberi, pictiventris, variolosus*)	0.069	0.012*
Gastrophryne (*olivacea, carolinensis, elegans, mazatlanensis*)	0.085	0.017
Elachistocleis bicolor	0.790	0.040
Chiasmocleis spp. (albopunctata [16S], ventrimaculata [Rho])	0.121	0.076

Hypopachus than with *Gastrophryne* (Table 1). Furthermore, nodal support in trees (Greenbaum et al., 2011; this study, Fig. 2) and parsimony network patterns (Fig. 3) place *G. pictiventris* and *G. usta* in a clade with *Hypopachus*. Consequently, we place *G. pictiventris* and *G. usta* in the genus *Hypopachus* (also see discussion below regarding relationships within *Hypopachus*) to avoid a paraphyletic *Gastrophryne*. We elect to move *G. usta* and *G. pictiventris* into *Hypopachus* as opposed to collapsing *Hypopachus* into *Gastrophryne* to maintain taxonomic stability (Pauly et al., 2009) and to emphasize the separate evolutionary history of each clade. Our taxonomic rearrangement of these genera renders *Gastrophryne* with four species (*G. carolinensis*, *G. elegans*, *G. mazatlanensis*, and *G. olivacea*) and *Hypopachus* with four species (*H. barberi*, *H. pictiventris*, *H. ustum*, and *H. variolosus*).

Morphological diagnosis for these two genera relies mostly on osteological characters. The appendicular skeleton of *Hypopachus* possesses both clavicles and procoracoids, whereas these elements are absent in *Gastrophryne* (Carvalho, 1954). However, Tyson (1987) studied in detail the pectoral girdle of *G. carolinensis* and interpreted the clavicles to be ossified and present as small lateral remnants. She also identified procoracoids in *G. carolinensis*, a small, medial and calcified extension of the fused epicoracoids. These findings make the previous osteological definition equivocal. Thus, all morphological characters used to distinguish the two genera need to be reexamined. Species of *Gastrophryne* have a single metatarsal tubercle whereas *Hypopachus* have two metatarsal tubercles (Parker, 1934; Carvalho, 1954) except for *H. pictiventris* which possess only an inner metatarsal tubercle (Parker, 1934). *Hypopachus* possess more extensive toe webbing, although this is often a sexually dimorphic character, than *Gastrophryne*, where toe webbing is rudimentary or absent (Parker, 1934).

4.2. Phylogenetic relationships of North American microhylids

Previous studies have identified a well-supported sister-group relationship between *Hypopachus* and *Gastrophryne* using a fairly robust molecular sampling of mitochondrial and nuclear data (Frost et al., 2006; van der Meijden et al., 2007; Greenbaum et al., 2011). These genera are contained in the subfamily Gastrophryninae, which also contains seven other genera from North and South America (*Chiasmocleis*, *Ctenophryne*, *Dasypops*, *Dermatonotus*, *Elachistocleis*, *Hamptophryne*, and *Nelsonophryne* [Frost, 2011]). Based on our current sampling, the phylogenetic patterns observed in species of the genera *Hypopachus* and *Gastrophryne* are remarkably different in that all four extant lineages of *Gastrophryne* appear to possess low levels of intraspecific genetic diversity, whereas several *Hypopachus* species contain far more variation (Makowsky et al., 2009; Greenbaum et al., 2011). These patterns may suggest differences in life-history traits related to range-expansion capability and should be explored in the future. Our taxonomic suggestions related to the genus *Hypopachus* warrant some additional comment in the regard that, unlike *Gastrophryne*, this genus contains diversity that may be related to a somewhat ancient adaptive burst (i.e., a fast initial radiation of the ancestral lineage that led to *H. barberi*, *H. pictivientris*, *H. variolosus*, and *H. ustum*). This process is thought to produce patterns similar to the sequence divergence levels and branching events that we observed for our current sampling of *Hypopachus* (Fig. 2; Table 1). In light of this, our system for defining the genus

Hypopachus may require modification when a more thoroughly sampled dataset is available for the Gastrophryninae.

The last thorough investigation of *Gastrophryne* (Nelson, 1972) used morphology to identify five species contained within the genus (*G. usta*, *G. elegans*, *G. pictiventris*, *G. olivacea*, and *G. carolinensis*). Nelson (1972) also discussed that hybridization between *G. olivacea* and *G. carolinensis* occasionally occurs (Blair, 1955). In relation to these hybridization events, other research (Loftus-Hills and Littlejohn, 1992) has identified putative character displacement in the advertisement calls of *G. olivacea* and *G. carolinensis* where they occur syntopically in the United States (e.g., Texas and Louisiana). The phylogenetic relationships that we herein propose for the genus *Gastrophryne* raise some interesting issues related to these previous investigations. Perhaps most notable is that *G. carolinensis*, the only *Gastrophryne* endemic to the United States, is the sister taxon to the rest of the genus and is not the closest living relative of *G. olivacea* (Fig. 2). Furthermore, our related discovery that *G. olivacea* and *G. elegans* are likely sister taxa has several interesting implications for the reported hybridization described between *G. olivacea* and *G. carolinensis*. Specifically, the existence of these hybridization events may indicate the maintenance of conserved life history and reproductive traits across a fairly large amount of evolutionary time. To identify whether members of *Gastrophryne* have a highly conserved breeding ecology, future examinations should be performed in a comparative context that include data from the tropical species *G. elegans*. Regarding extant levels of genetic variability, our results for *G. olivacea* are remarkably similar to those reported by Makowsky et al. (2009) for *G. carolinensis* and may indicate some phylogenetically related elements of dispersal capability in the genus.

4.3. Range expansion in Gastrophryne olivacea

Collectively, our 16S analyses for *G. olivacea* are consistent with a scenario of a genetic bottleneck followed by population size expansion. Estimates of haplotype (h), per site nucleotide diversity (π), Tajima's D and Fu's F support this narrative by matching the expected patterns associated with expanding population size. Our mismatch distribution and Bayesian Skyline plot describe a similar scenario of consistent population expansion in recent times (Figs. 5 and 6). The observed data from our mismatch distribution contained a single deviation (at two pairwise differences) from the expected patterns associated with sudden population expansion (Fig. 5). However, we interpret this dip as noise rather than a significant conflict with the predicted result because the goodness of fit test statistics obtained from this analysis (sum of squared deviations [0.011, $P = 0.550$] and Harpending's raggedness index [0.035, $P = 0.700$]) indicate a relatively good fit of our *Gastrophryne* data to the model. The levels of genetic variation observed in *G. olivacea* are indicative of a young lineage that has experienced constant and recent population growth. Additionally, the wide-spread geographic distribution of this genetically shallow group suggests a fairly rapid range expansion (Fig. 4). These range dynamics are similar to those observed in other vertebrates of the Great Plains region including other amphibians (Moriarty Lemmon et al., 2007), reptiles (Fontanella et al., 2008; Pyron and Burbrink, 2009), birds (Johnson, 2008), and fishes (Kreiser et al., 2001).

We suspect that the rapid expansion of *G. olivacea* may have been facilitated by their ecological association with open habitats. Specifically, *G. olivacea* breed in temporary bodies of water produced by seasonal rains (Fitch, 1956b), produce clutch sizes of around 650 eggs per mating event (Livezey and Wright, 1947), and have rapid larval development lasting between 24 and 50 days (Fitch, 1956a,b; Stuart and Painter, 1996). These natural history traits of *G. olivacea* are similar to those observed in other anurans that are thought to have experienced rapid and recent geographic radiations facilitated by open habitat ecologies (Van Bocxlaer et al., 2010). Thus, the recent radiation of *G. olivacea* described herein represents an ideal opportunity to investigate how small terrestrial vertebrates can rapidly expand their geographic distribution despite limited vagility.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.05.020.

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