



Species limits and phylogeography of North American cricket frogs (*Acris*: Hylidae)

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ABSTRACT

Cricket frogs are widely distributed across the eastern United States and two species, the northern cricket frog (*Acris crepitans*) and the southern cricket frog (*A. gryllus*) are currently recognized. We generated a phylogenetic hypothesis for *Acris* using fragments of nuclear and mitochondrial genes in separate and combined phylogenetic analyses. We also used distance methods and fixation indices to evaluate species limits within the genus and the validity of currently recognized subspecies of *A. crepitans*. The distributions of existing *A. crepitans* subspecies, defined by morphology and call types, do not match the distributions of evolutionary lineages recovered using our genetic data. We discuss a scenario of call evolution to explain this disparity. We also recovered distinct phylogeographic groups within *A. crepitans* and *A. gryllus* that are congruent with other codistributed taxa. Under a lineage-based species concept, we recognize *Acris blanchardi* as a distinct species. The importance of this revised taxonomy is discussed in light of the dramatic declines in *A. blanchardi* across the northern and western portions of its range.

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1. Introduction

Named taxa are implicitly assumed to represent distinct evolutionary lineages (de Quieroz, 2005). Advances in phylogenetics, particularly the use of DNA sequence data, have allowed biologists to test the validity of taxonomic nomenclature against phylogenetic hypotheses. A number of recent studies have shown that many subspecies designations do not in fact represent valid evolutionary lineages (Burbrink et al., 2000; Starkey et al., 2003; Zink, 2004). Amphibian systematics and taxonomy in particular has benefited from detailed molecular studies that identified morphologically “cryptic” species and highlighted incongruence between morphology-based taxonomies and evolutionary lineages (Chek et al., 2001; Hanken, 1999; Pauly et al., 2007; Lemmon et al., 2007a). For example, recent molecular phylogenies of taxa within the genus *Pseudacris* (Hylidae) demonstrated that named subspecies of *Pseudacris crucifer* and *Pseudacris nigrita* did not correspond to recognizable evolutionary lineages (Austin et al., 2002; Moriarty and Cannatella, 2004). These studies and others have recommended sweeping taxonomic changes to reflect historical lineages.

Mitochondrial DNA (mtDNA), because of its faster substitution rate and small effective population size, will typically coalesce faster than nuclear DNA (Palumbi et al., 2001; Hudson and Coyne,

2002). Coalescent mtDNA lineages offer diagnostic characters that satisfy the requirements of lineage-based species definitions (de Quieroz, 1998; Wiens and Penkrot, 2002), and mtDNA has been widely used to recover species relationships and to delimit species. However, as gene tree/species tree conflicts continue to be identified in mtDNA analyses, nuclear DNA (nDNA) is being used more frequently to answer questions at and below the species level (Ballard and Whitlock, 2004; Hare, 2001; Howes et al., 2006; Weisrock et al., 2006). The use of nDNA to resolve species relationships incurs new problems related to operational species definitions and the interpretation of conflicts among datasets, with little consensus on their resolution in the systematics literature (Baker and DeSalle, 1997; Huelsenbeck et al., 1996; Moore, 1995). The lack of phylogenetic information often present in nDNA phylogenies, due to incomplete lineage sorting, raises the question of how nDNA information can be incorporated into a lineage-based species concept. Two strategies may be employed. First, nDNA can be used in a combined phylogenetic analysis with mtDNA (e.g. Egge and Simons, 2006; Rokas et al., 2003). However, given the low resolution found in many nuclear-gene trees, this is often tantamount to relying on mtDNA results alone (Spinks and Shaffer, 2005). The second strategy is to use nDNA to test for gene flow, or a lack thereof, between mitochondrially diagnosed lineages using fixation indices and distance methods (e.g. Sota and Sasabe, 2006).

We address these issues by examining North American cricket frogs in the genus *Acris* (Hylidae). Cricket frogs are widely

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distributed across the eastern United States and consist of two currently recognized species, the northern cricket frog (*Acris crepitans*) and the southern cricket frog (*A. gryllus*; Conant and Collins, 1998). The northern cricket frog is currently separated into three subspecies. The eastern cricket frog, *A. c. crepitans* occurs from southeastern New York south to the Florida Panhandle and west to eastern Texas, generally east and south of the Appalachian/Central Highlands. Blanchard's cricket frog, *A. c. blanchardi* occurs in the Midwest and Great Plains from South Dakota to west Texas and east to the Appalachian/Central Highlands. The coastal cricket frog, *A. c. paludicola*, occupies a limited area along the Gulf Coast from Houston, Texas to central Louisiana (Fig. 1; Gray et al., 2005). The southern cricket frog is separated into two subspecies, both of which occur in coastal habitats below the Fall Line along the Atlantic and Gulf Coasts. The Coastal Plain cricket frog, *A. g. gryllus* occupies the southeastern US from the Mississippi River to the Atlantic coast as far north as Virginia, but excluding peninsular Florida. Replacing it in peninsular Florida is the Florida cricket frog, *A. g. dorsalis* (Jensen, 2005).

Acris crepitans and *A. gryllus* are morphologically similar and have previously been treated both as distinct species and as conspecific subspecies, leading to taxonomic and systematic confusion within the genus (Chantell, 1967; Harper, 1947; Mecham, 1964; Wright and Wright, 1949; McCallum and Trauth, 2006; Frost, 2007). Call data support the species status of *A. crepitans* and *A. gryllus* as well as the geographic distribution of currently described subspecies within *A. crepitans* (Nevo and Capranica, 1985). Allozyme data show a different geographic pattern within *A. crepitans*, which Dessauer and Nevo (1969) divided into: a "Plains group" containing individuals from north of the Ohio River and west of the lower Mississippi River; a "Delta group" consisting of individuals from southern Louisiana, which shared many of the same proteins as the "Plains group" but differed at a few key polypeptides; and an "Appalachian group" that consisted of frogs from Alabama and Georgia northeast to New York and considered more divergent

from the "Plains group" than is the "Delta group". Additionally, the morphological data used to differentiate *A. c. crepitans* from *A. c. blanchardi* fail to adequately discriminate the two forms (McCallum and Trauth, 2006). Given the taxonomic confusion within *Acris* and the precipitous declines of *A. c. blanchardi* across the northern and western portions of its range (Gray and Brown, 2005), we undertook a broad-scale genetic analysis of the complex to help clarify the evolutionary distinctiveness and relationships among currently recognized taxa.

We conducted a phylogenetic analysis of the genus *Acris* with the goal of determining how many species occur in the genus and whether currently recognized subspecies within *A. crepitans* represent distinct evolutionary lineages. Molecular systematists increasingly view single gene trees as tentative hypotheses of organismal lineages and their interrelationships, and mtDNA in particular can show quite different evolutionary relationships compared to that of the actual organisms (Ballard and Rand, 2005; Funk and Omland, 2003; Weisrock et al., 2006). Therefore, we examined the phylogenetic relationships within *Acris* using four distinct loci: a fragment of the mitochondrial cytochrome *b* gene; protein-coding fragments of the nuclear genes tyrosinase and proopiomelanocortin (POMC); and nuclear intron 4 of beta-crystallin (*cryB*). Analyses of these data provide a novel hypothesis regarding the species boundaries in *Acris*. We use this hypothesis to reinterpret aspects of the biogeography, behavioral evolution, taxonomy and conservation of *Acris*.

2. Materials and methods

2.1. Material examined

We examined 64 specimens of *A. crepitans* and *A. gryllus* from 42 localities including all three described *A. crepitans* subspecies and both *A. gryllus* subspecies (Fig. 2; Table 1). Tissues were frozen in liquid nitrogen or stored in 95% ethanol. Sequence data from *A.*

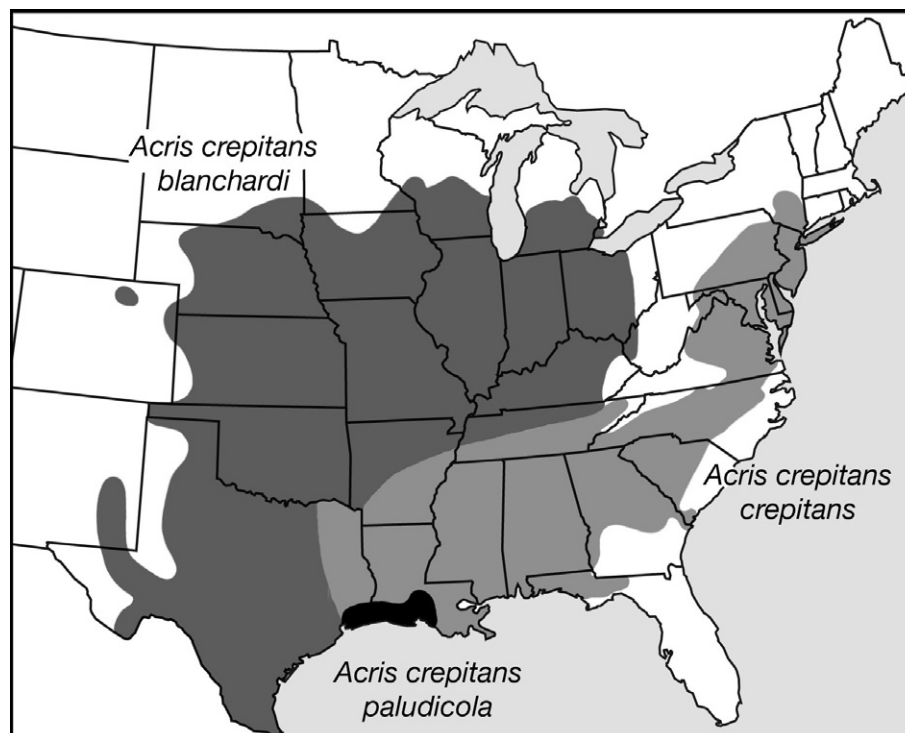


Fig. 1. Map of eastern North America showing the approximate geographic range of *Acris crepitans* subspecies, modified from Conant and Collins (1998) and Gray et al. (2005).

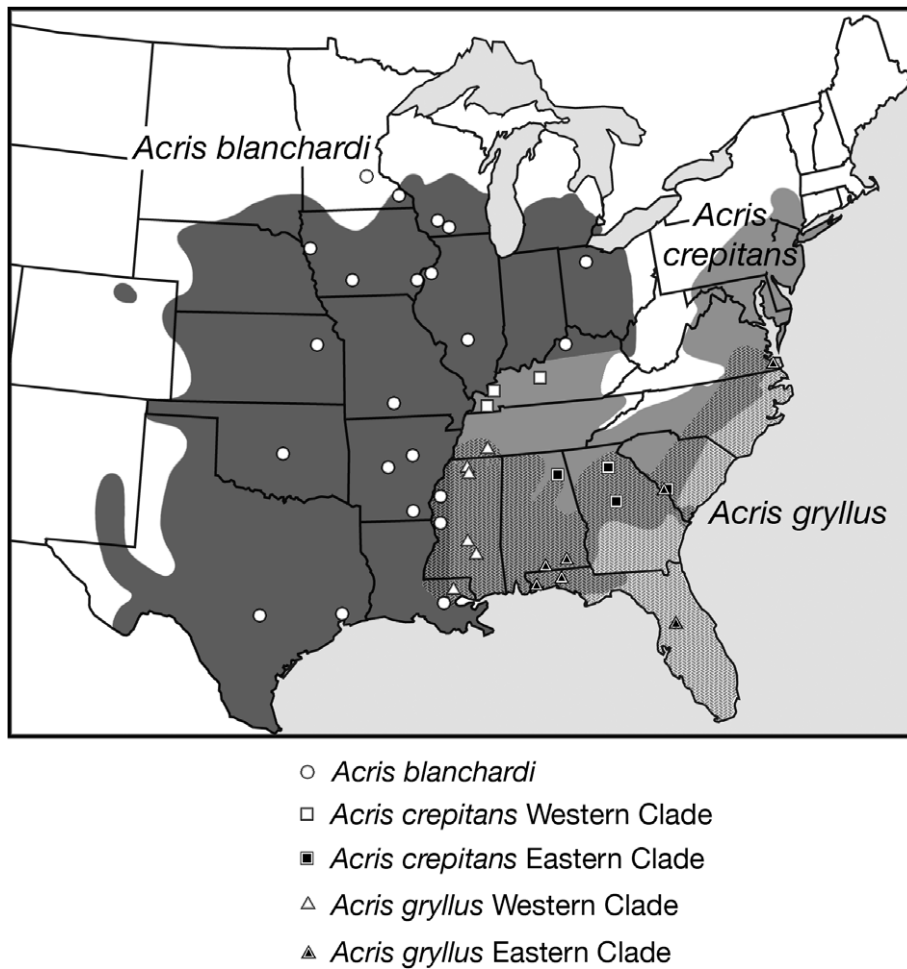


Fig. 2. Map of eastern North America showing the approximate geographic range of *Acris* species as delimited here (distributions modified from Conant and Collins, 1998; Gray et al., 2005; Jensen, 2005) and localities of specimens used for the molecular analyses. Symbols represent major clades recovered in the combined analyses. *Acris blanchardi* is shown in dark gray, *A. crepitans* in light gray, and *A. gryllus* in stippling.

crepitans (LSUMZ H-2164; De Kalb Co., Alabama) was obtained from GenBank. Based on recent phylogenetic analyses, we used *Pseudacris crucifer* and *P. maculata* as outgroups (Faivovich et al., 2005; Wiens et al., 2005).

2.2. DNA sequencing

We extracted genomic DNA from liver, thigh muscle, or tail tips (in the case of larvae) using QIAamp™ tissue extraction kits (Qiagen, Valencia, CA, USA) following manufacturer's recommendations. Positive PCR products were purified using the QIAquick PCR Purification (Qiagen) or *ExoI/SAP* digestion. Sequences were checked for accuracy of base determination and assembled using the computer program Sequencher 4.0 (Gene Codes Corp., Ann Arbor, MI, USA). All sequence data have been deposited in GenBank (Table 1). Primers are listed in Table 2.

2.3. Phylogenetic analyses

We performed parsimony analyses of cytochrome *b* data, nDNA, and combined mtDNA and nDNA datasets using MP criteria with heuristic searches, 1000 random addition sequence replicates, and tree-bisection-reconnection (TBR) with all bases equally weighted (PAUP*; Swofford, 2001). Trees were rooted using *Pseudacris crucifer* and *P. maculata* in all cases. Multistate data were treated as polymorphisms and gaps were treated as a fifth base. Parsimony trees were evaluated using summary values reported

by PAUP*. Support for the resultant phylogeny was evaluated using the bootstrap (Felsenstein, 1985) with 100 replicates, full heuristic search, simple step-wise addition option, and TBR as implemented in PAUP* (Swofford, 2001).

Likelihood analyses were performed using GARLI 0.951 (Zwickl, 2006). Model choice was based on the AIC (Posada and Buckley, 2004) using the software MrModeltest 2.2 (Nylander, 2004). Analyses were terminated after 10,000 generations without an improvement in the overall tree topology. Two likelihood analyses were performed to ensure convergence. Support was evaluated using 100 bootstrap repetitions (Felsenstein, 1985), with each repetition terminated after 5000 generations without a topology improvement.

We conducted Bayesian analyses using MrBayes 3.0b4 (Ronquist and Huelsenbeck, 2003) on the following data sets: cytochrome *b*, partitioned by codon; nDNA data with exons partitioned by gene and by codon with a separate partition for the cryB intron; and combined mtDNA and nDNA with protein-coding gene fragments partitioned by gene and codon, and a separate partition for the cryB intron. CryB indels were coded as present/absent and included as a separate partition using the MK model (Lewis, 2001). Model choice was based on the AIC (Posada and Buckley, 2004) using MrModeltest 2.2 (Nylander, 2004). Bayesian settings included random starting trees and default priors except the rate prior, which was set to "variable". Markov chain Monte Carlo was run with four chains for 2,000,000 generations. Trees were sampled every hundred gener-

Table 1
Specimens examined, locality, museum voucher number, and GenBank number

Species	Subspecies	Voucher and locality	GenBank Accession Nos			
			Cytochrome <i>b</i>	Tyrosinase	Beta-crystallin	POMC
<i>Acris blanchardi</i>	Acb	JFBM 14485; Ozark Co., Missouri	EF988127	EF988300	EF988191	EF988242
<i>Acris blanchardi</i>	Acc	HBS 575; Bolivar Co., Mississippi	EF988144	EF988316	EF988208	EF988256
<i>Acris blanchardi</i>	Acc	HBS 576; Bolivar Co., Mississippi	EF988145	EF988317	EF988209	EF988257
<i>Acris blanchardi</i>	Acc	HBS 612; Issaquena Co., Mississippi	EF988143	EF988315	EF988207	EF988255
<i>Acris blanchardi</i>	Acc	HBS 613; Issaquena Co., Mississippi	EF988142	EF988314	EF988206	EF988254
<i>Acris blanchardi</i>	Acb	INHS 201; Rock Co., Illinois	EF988109	EF988283	EF988174	EF988229
<i>Acris blanchardi</i>	Acb	INHS 73; Fayette Co., Illinois	EF988108	EF988282	EF988173	EF988228
<i>Acris blanchardi</i>	Acb	JLC (2); Louisa Co., Iowa	EF988099	EF988273	EF988164	—
<i>Acris blanchardi</i>	Acb	JLC (3); Louisa Co., Iowa	EF988100	EF988274	EF988165	EF988223
<i>Acris blanchardi</i>	Acb	JFBM 14472; Madison Co., Iowa	EF988097	EF988271	EF988162	EF988221
<i>Acris blanchardi</i>	Acb	JFBM 14478; Woodbury Co., Iowa	EF988098	EF988272	EF988163	EF988222
<i>Acris blanchardi</i>	Acc	LSUMZ H-2674; Ascension Pa., Louisiana	EF988117	—	EF988181	EF988235
<i>Acris blanchardi</i>	Acc	LSUMZ H-2676; Ascension Pa., Louisiana	EF988119	EF988292	EF988183	EF988236
<i>Acris blanchardi</i>	Acp	JFBM 14501; Chambers Co., Texas	EF988122	EF988295	EF988186	EF988239
<i>Acris blanchardi</i>	Acp	JFBM 14502; Chambers Co., Texas	EF988121	EF988294	EF988185	EF988238
<i>Acris blanchardi</i>	Acb	JFBM 14546; Travis Co., Texas	EF988120	EF988293	EF988184	EF988237
<i>Acris blanchardi</i>	Acb	JFBM 14454; Carroll Co., Kentucky	EF988133	EF988306	EF988197	EF988247
<i>Acris blanchardi</i>	Acb	JFBM 14511; Cleveland Co., Oklahoma	EF988140	EF988312	EF988204	EF988253
<i>Acris blanchardi</i>	Acb	JFBM 14504; Cleveland Co., Oklahoma	EF988141	EF988313	EF988205	—
<i>Acris blanchardi</i>	Acb	JFBM 14377; Douglas Co., Kansas	EF988114	EF988288	EF988179	EF988233
<i>Acris blanchardi</i>	Acc	JFBM 14482; Ashley Co., Arkansas	EF988106	EF988280	EF988171	EF988226
<i>Acris blanchardi</i>	Acc	JFBM 14484; Ashley Co., Arkansas	EF988107	EF988281	EF988172	EF988227
<i>Acris blanchardi</i>	Acb	JFBM14489; Perry Co., Arkansas	EF988102	EF988276	EF988167	—
<i>Acris blanchardi</i>	Acb	JFBM 14490; Perry Co., Arkansas	EF988101	EF988275	EF988166	EF988224
<i>Acris blanchardi</i>	Acb	JFBM 14462; White Co., Arkansas	EF988104	EF988278	EF988169	EF988225
<i>Acris blanchardi</i>	Acb	JFBM 14461; White Co., Arkansas	EF988105	EF988279	EF988170	—
<i>Acris blanchardi</i>	Acb	JFBM 14459; White Co., Arkansas	EF988103	EF988277	EF988168	—
<i>Acris blanchardi</i>	Acb	No voucher; Iowa Co., Wisconsin	EF988115	EF988289	—	EF988234
<i>Acris blanchardi</i>	Acb	No voucher; Lafayette Co., Wisconsin	EF988116	EF988290	EF988180	—
<i>Acris blanchardi</i>	Acb	No voucher; Hennepin Co., Minnesota	EF988139	EF988311	EF988203	EF988252
<i>Acris blanchardi</i>	Acb	No voucher; Winona Co., Minnesota	EF988138	EF988310	EF988202	EF988251
<i>Acris blanchardi</i>	Acb	UMFS 11155; Wood Co., Ohio	EF988137	EF988309	EF988201	EF988250
<i>Acris crepitans</i>	Acc	HBS 35340; Jasper Co., Georgia	EF988113	EF988287	EF988178	EF988232
<i>Acris crepitans</i>	Acc	LSUMZ H-2164; De Kalb Co., Alabama	AY843782	AY844019	—	—
<i>Acris crepitans</i>	Acc	JFBM 15172; Monroe Co., Georgia	EF988110	EF988284	EF988175	EF988230
<i>Acris crepitans</i>	Acc	JFBM 15173; Monroe Co., Georgia	EF988112	EF988286	EF988177	—
<i>Acris crepitans</i>	Acc	JFBM 14540; Banks Co., Georgia	EF988111	EF988285	EF988176	EF988231
<i>Acris crepitans</i>	Acb	JJDE 04-65 (A); Graves Co., Kentucky	EF988131	EF988304	EF988195	EF988245
<i>Acris crepitans</i>	Acb	JJDE 04-65 (C); Graves Co., Kentucky	EF988130	EF988303	EF988194	EF988244
<i>Acris crepitans</i>	Acb	JJDE 04-65 (D); Graves Co., Kentucky	EF988132	EF988305	EF988196	EF988246
<i>Acris crepitans</i>	Acb	JFBM 14475; Larue Co., Kentucky	EF988128	EF988301	EF988192	EF988243
<i>Acris crepitans</i>	Acb	JFBM 14467; Livingston Co., Kentucky	EF988129	EF988302	EF988193	—
<i>Acris crepitans</i>	Acc	JFBM 14433; Marion Co., South Carolina	EF988126	EF988299	EF988190	EF988241
<i>Acris gryllus</i>	Agg	HBS 271; Santa Rosa Co., Florida	EF988149	EF988321	EF988213	EF988259
<i>Acris gryllus</i>	Agg	HBS 315; Walton Co., Florida	EF988151	EF988323	EF988215	—
<i>Acris gryllus</i>	Agg	HBS 317; Walton Co., Florida	EF988148	EF988320	EF988212	EF988258
<i>Acris gryllus</i>	Agd	JFBM 14428; Sumpter Co., Florida	EF988150	EF988322	EF988214	EF988260
<i>Acris gryllus</i>	Agg	JC 31; Coffee Co., Alabama	EF988135	EF988308	EF988199	EF988248
<i>Acris gryllus</i>	Agg	RM 0411; Covington Co., Alabama	EF988136	—	EF988200	EF988249
<i>Acris gryllus</i>	Agg	JC 103; Covington Co., Alabama	EF988134	EF988307	EF988198	—
<i>Acris gryllus</i>	Agg	JFBM 14451; McNairy Co., Tennessee	EF988159	—	—	EF988268
<i>Acris gryllus</i>	Agg	LSUMZ H-1594; Tangipahoa Pa., Louisiana	EF988118	EF988291	EF988182	—
<i>Acris gryllus</i>	Agg	JFBM 14493; Norfolk Co., Virginia	EF988152	EF988324	EF988216	EF988261
<i>Acris gryllus</i>	Agg	JFBM 14494; Norfolk Co., Virginia	EF988153	EF988325	EF988217	EF988262
<i>Acris gryllus</i>	Agg	JFBM 14436; Barnwell Co., South Carolina	EF988123	EF988296	EF988187	—
<i>Acris gryllus</i>	Agg	JFBM 14439; Barnwell Co., South Carolina	EF988124	EF988297	EF988188	EF988240
<i>Acris gryllus</i>	Agg	JFBM 14442; Barnwell Co., South Carolina	EF988125	EF988298	EF988189	—
<i>Acris gryllus</i>	Agg	HBS 392; Madison Co., Mississippi	EF988146	EF988318	EF988210	—
<i>Acris gryllus</i>	Agg	HBS 393; Madison Co., Mississippi	EF988147	EF988319	EF988211	—
<i>Acris gryllus</i>	Agg	TG00015; Marshall Co., Mississippi	EF988154	EF988326	—	EF988263
<i>Acris gryllus</i>	Agg	TG00016; Lafayette Co., Mississippi	EF988155	EF988327	EF988218	EF988264
<i>Acris gryllus</i>	Agg	TG00017; Lafayette Co., Mississippi	EF988156	EF988328	—	EF988265
<i>Acris gryllus</i>	Agg	TG00029; Smith Co., Mississippi	EF988157	EF988329	—	EF988266
<i>Acris gryllus</i>	Agg	TG00030; Smith Co., Mississippi	EF988158	EF988330	—	EF988267
<i>Pseudacris crucifer</i>	—	JFBM 14294; Fillmore Co., Minnesota	EF988160	EF988331	EF988219	EF988269
<i>Pseudacris maculata</i>	—	JFBM 14310; Yellow Medicine Co., Minnesota	EF988161	EF988332	EF988220	EF988270

Subspecies designations follow Conant and Collins (1998); Acb = *Acris crepitans blanchardi*, Acc = *A. c. crepitans*, Acp = *A. c. paludicola*, Agg = *A. gryllus gryllus*, Agd = *A. g. dorsalis*. HBS (H. Bradley Shaffer field number), INHS (Illinois Natural History Survey), JFBM (Bell Museum of Natural History), JJDE (Jacob Egge field number), JLC (Jeff LeClere), LSUMZ (Louisiana State University Museum of Zoology), RM & JC (University of Alabama, uncataloged tissues), TG (Tony Gamble field number), UMFS (University of Michigan field series).

ations. Branch lengths of sampled trees were saved, and burn-in determined by plotting the log-likelihood scores of sampled

trees against generation time with a visual assessment of stationarity.

Table 2
Primers used in this study

Primer name	Primer sequence (5'-3')	Source
Cytochrome <i>b</i>		
MVZ16-H	AAA TAG GAA RTA TCA YTC TGG TTT RAT	Moritz et al. (1992)
MVZ15-L	GAA CTA ATG GCC CAC ACW WTA CGN AA	Moritz et al. (1992)
Tyrosinase, exon 1		
Fx3	TCA TCT CCC GYC AYC TTC TGG AT	Vences et al. (2003)
1G	TGC TGG GCR TCT CTC CAR TCC CA	Bossuyt and Milinkovitch (2000)
POMC, exon 3		
POMC1	GAA TGT ATY AAA GMM TGC AAG ATG GWC CT	Wiens et al. (2005)
POMC-R	GGG TCA TGA ATC CTC CRT ATC T	This study
CRYB, intron 4		
CRYB1Ls	CGC CTG ATG TCT TTC CGC C	Dolman and Phillips (2004)
CRYB2Ls	CCA ATG AAG TTC TCT TTC TCA A	Dolman and Phillips (2004)

We used Partitioned Bremer Support to calculate the relative contribution of each gene to a given clade on the combined phylogeny (Baker and DeSalle, 1997). Partitioned Bremer Support was calculated using TreeRot (Sorenson, 1999) with data partitioned by gene.

2.4. Hypothesis testing

To test whether current subspecies designations were supported by our data, we tested subspecies validity within *A. crepitans* by comparing the maximum likelihood tree from the combined data against trees constrained to reflect monophyly of *A. c. crepitans*, *A. c. blanchardi*, and *A. c. paludicola* (subspecific assignment is listed in Table 1). Because morphological characteristics used to define *A. crepitans* subspecies perform poorly at differentiating subspecies (McCallum and Trauth, 2006) we assigned individuals to subspecific taxa based on locality using the map from Conant and Collins (1998). An additional tree was constrained to reflect two lineages within *A. crepitans* taking into account the recent findings of Rose et al. (2006) that synonymizes *A. c. paludicola* with *A. c. blanchardi*. Alternative phylogenetic hypotheses were constructed using the constraint function in GARLI 0.951 (Zwickl, 2006) which finds the maximum likelihood tree given a particular constraint. The Shimodaira–Hasegawa test (SH test, Shimodaira and Hasegawa, 1999) was implemented in PAUP* with 1000 Resampling Estimated Log Likelihood bootstraps (Kishino et al., 1990).

2.5. Genetic divergence and species limits

We generated rooted phylograms of each nuclear gene data partition using neighbor-joining (NJ) in PAUP*. Viewing the nuclear gene data as NJ networks allows clustering of alleles with respect to the mtDNA lineages, but does not necessarily represent the true phylogeny (Sota and Sasabe, 2006).

Net between-group mean distances between all of the major mtDNA lineages were determined using the formula: $\delta = \delta_{xy} - (\delta_x + \delta_y)/2$ where δ_x and δ_y are the mean distances within groups *x* and *y* and δ_{xy} is the average distance between groups *x* and *y* (Nei and Li, 1979). This correction is important for recently diverged lineages to ensure that divergences are not overestimated (Edwards, 1997). Mean sequence divergence within each major clade was calculated using MEGA3 (Kumar et al., 2004). Distances and standard error, using 500 bootstrap replicates, were calculated using the Kimura 2-parameter (K2P) model in MEGA3.

Population subdivision (F_{ST}) among mtDNA lineages was estimated using pairwise distances (Reynolds et al., 1983) under the K2P model (Arlequin 2.0, Schneider et al., 2000). We tested the null hypotheses of no population differentiation among the major

mtDNA lineages for all four loci separately ($F_{ST} = 0$) using the permutation test. Significant F_{ST} values provide evidence of reduced gene flow regardless of monophyly, and can thus provide additional insight into the validity of non-monophyletic groups as distinct evolutionary lineages (Hudson et al., 1992).

3. Results

3.1. Phylogenetic analyses

The cytochrome *b* fragment (725 base pairs in length) was sequenced for all 64 *Acris* individuals and *Pseudacris* outgroups and had 281 variable sites of which 199 were parsimony informative; within *Acris*, 225 characters were variable and 48 were parsimony informative. Models of sequence evolution, as determined by the AIC were: k80+I (1st codon); F81+I (2nd codon); and GTR+G (3rd codon). The tyrosinase gene fragment (446 base pairs) was sequenced for 61 *Acris* individuals and outgroups and had 104 variable sites of which 55 were parsimony informative; within *Acris*, 49 characters were variable and 29 were parsimony informative. Models of sequence evolution, as determined by the AIC were: SYN+I (1st codon); K80+I (2nd codon); and HKY+G (3rd codon). The POMC gene fragment (487 base pairs) was sequenced for 48 *Acris* individuals and outgroups. A total of 60 sites were variable of which 27 were parsimony informative; within *Acris*, 19 characters were variable and 11 were parsimony informative. Models of sequence evolution, as determined by the AIC were: F81 (1st codon); HKY (2nd codon); and GTR+G (3rd codon). The cryB intron (263 base pairs) was sequenced for 57 *Acris* individuals and outgroups and had 123 variable sites of which 76 were parsimony informative; within *Acris*, 18 characters were variable and 12 were parsimony informative. The model of sequence evolution, as determined by the AIC was: HKY+G. The model of sequence evolution, as determined by the AIC, for the combined dataset was: GTR+I+G.

Cytochrome *b*: The consensus Bayesian tree (harmonic mean $-\ln L = 3643.8930$), the maximum likelihood tree ($-\ln L = 3322.5241$), and parsimony trees (>10,000 equally parsimonious trees, TL = 550, CI = 0.675, RI = 0.940, RC = 0.634) all recovered a monophyletic *Acris* and reciprocally monophyletic *A. gryllus* and *A. crepitans* with additional genetic structure within each of those clades (Fig. 3). All analyses recovered three clades within *A. crepitans*: the *A. blanchardi* clade, distributed west of the Mississippi River and north of the Ohio River with several populations in western Mississippi and one individual from northern Kentucky that appear on the southeastern side of this tentative boundary; *A. crepitans* Western clade, composed of specimens from southwestern Kentucky; and *A. crepitans* Eastern clade, containing specimens from Alabama, Georgia, and South Carolina. The *A. blanchardi* clade was the sister taxon to the *A. crepitans* clade in the Bayesian and



Fig. 3. Bayesian phylogeny of the frog genus *Acris*, using mitochondrial cytochrome *b* data. Nodal support, parsimony and likelihood bootstrap (100 replicates) and Bayesian posterior probabilities (BPP < 0.50 not shown), are indicated. Clade names are shown on the right.

parsimony analyses but this relationship was not recovered with the maximum likelihood analysis. Within *A. gryllus* we recovered Eastern and Western clades. The Eastern *A. gryllus* clade contained specimens from South Carolina, Virginia, Alabama, and Florida. The Western *A. gryllus* clade was composed of specimens from Louisiana, Mississippi, and Tennessee.

Nuclear gene data: The combined nuclear gene analyses (Fig. 4) recovered a monophyletic *Acris* and well-supported *A. gryllus* (in terms of Bayesian posterior probabilities and maximum likelihood bootstrap values), but otherwise recovered little structure among the parsimony (>10,000 equally parsimonious trees; TL = 363, CI = 0.791, RI = 0.915, RC = 0.724), maximum likelihood ($-\ln L$ 3286.5774), and Bayesian analyses (harmonic mean $-\ln L$ 3294.6410). The topology was characterized by a relatively undifferentiated collection of *Acris blanchardi* samples with no evidence of monophyly, a monophyletic, but weakly supported *A. crepitans*, and a monophyletic and well-supported *A. gryllus*. The strong geographic structure observed in cytochrome *b* data within *A. crepitans* and *A. gryllus* was not observed in the nDNA data. However, a Georgia-plus-South Carolina clade of *A. crepitans* was similar to the *A.*

crepitans Eastern mtDNA clade. The two *A. gryllus* clades, consisting of samples from Mississippi plus Tennessee, and from Alabama, Florida, Louisiana, South Carolina, and Virginia were similar in content to the *A. gryllus* Western and Eastern clades, respectively, (with the POMC NJ network, discussed below, showing a clustering of samples from the *A. gryllus* Western clade).

Combined data: The parsimony trees (>10,000 equally parsimonious trees, TL = 1058, CI = 0.741, RI = 0.928, RC = 0.688), the maximum likelihood tree ($-\ln L$ 7019.2932), and the consensus Bayesian tree (harmonic mean $-\ln L$ = -7073.93) recovered well-supported (Figs. 5 and 6), reciprocally monophyletic *A. gryllus* and *A. crepitans* + *A. blanchardi*. Overall, the topology was similar to the cytochrome *b* phylogenies (Fig. 3), although for a few clades (Eastern and Western *A. gryllus*, and *A. crepitans*) support levels increased with the addition of the nuclear data. The primary difference between parsimony + maximum likelihood topologies and the Bayesian analysis was the placement of the *A. gryllus* from Sumpter County in peninsular Florida. The Bayesian analyses place the specimen from peninsular Florida as the sister taxon to a clade consisting of

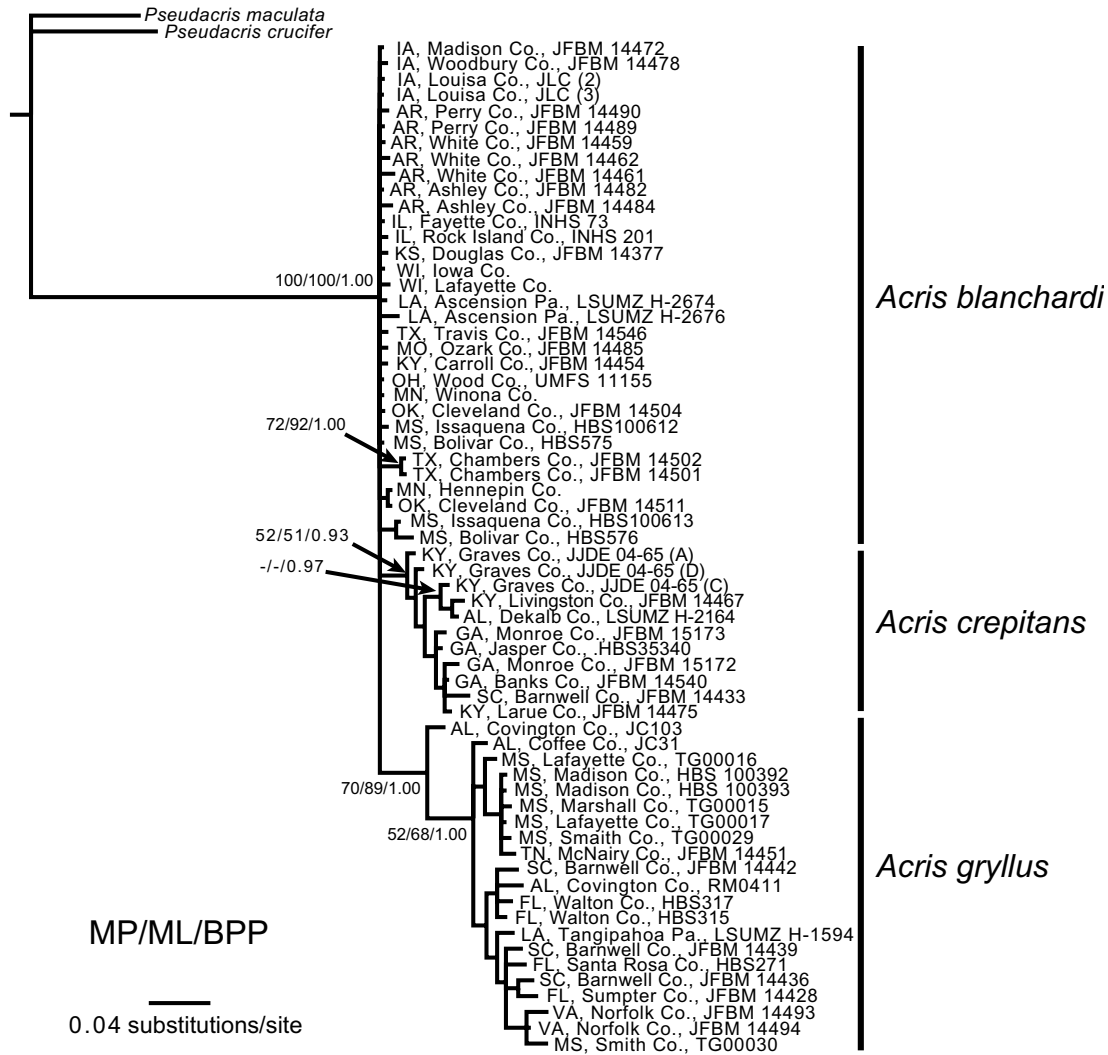


Fig. 4. Bayesian phylogeny of the frog genus *Acris*, from a combined analysis of the nuclear gene fragments: tyrosinase, POMC, and beta-crystallin. Nodal support, parsimony and likelihood bootstrap (100 replicates) and Bayesian posterior probabilities (BPP < 0.50 not shown), are indicated. Clade names are shown on the right.

Western + Eastern *A. gryllus* specimens, whereas the parsimony and maximum likelihood analyses grouped the Sumpter County, Florida specimen with the eastern *A. gryllus* as in the mtDNA analyses. Additionally, the model-based methods (maximum likelihood and Bayesian analyses) had no nodal support for the relationship between *A. crepitans* and *A. blanchardi*. Only the combined parsimony analyses produced strong bootstrap values supporting the sister-group relationship between these two clades.

Partitioned Bremer Support values (Fig. 7) indicate overall support provided by each gene for a given node. The sum of partitioned support values at a node from each partition equals the Bremer support value from the combined analysis at that node (Baker and DeSalle, 1997). Negative values indicate conflicting support at a node and values of zero indicate neither support nor conflict for a node. Overall, the nuclear loci provided strong support for the monophyly of *Acris* (node A, Fig. 7) and limited support or conflict for all other groups, with the strongest support for the monophyly of *A. gryllus* Western and Eastern clades. Across all clades, POMC often supports the mtDNA results, while the other two nuclear genes are frequently in conflict. Cytochrome *b* data provide strong support for most clades, although support was low for Western and Eastern *A. gryllus* clades.

3.2. Hypothesis testing

The constrained *A. crepitans* subspecies trees had significantly lower likelihood scores than the unconstrained combined-data maximum likelihood tree (3 subspecies: difference in $-\ln L = 165.75637$, $P = < 0.001$; 2 subspecies: difference in $-\ln L = 133.28955$, $P = < 0.001$). The SH test strongly rejected the hypothesis that each *A. crepitans* subspecies, *sensu* Conant and Collins (1998) represent a distinct evolutionary lineage.

3.3. Genetic divergence and species limits

Neighbor-joining networks of nuclear loci (Fig. 8) show patterns indicating incomplete lineage sorting as evidenced by their lack of monophyly among mtDNA-defined clades. Tyrosinase and POMC show individual clusters largely congruent with the *A. gryllus*, *A. crepitans*, and *A. blanchardi* clades. The cryB network indicated exclusivity between *A. gryllus* and *A. crepitans* + *A. blanchardi* clades but not between the *A. crepitans* and *A. blanchardi* clades. Tyrosinase and cryB analyses recover no structure between Eastern and Western *A. gryllus* clades, while analyses of POMC recovered each clade as exclusive. When all three nuclear genes are considered, there are no shared alleles between the Eastern and Western *A.*

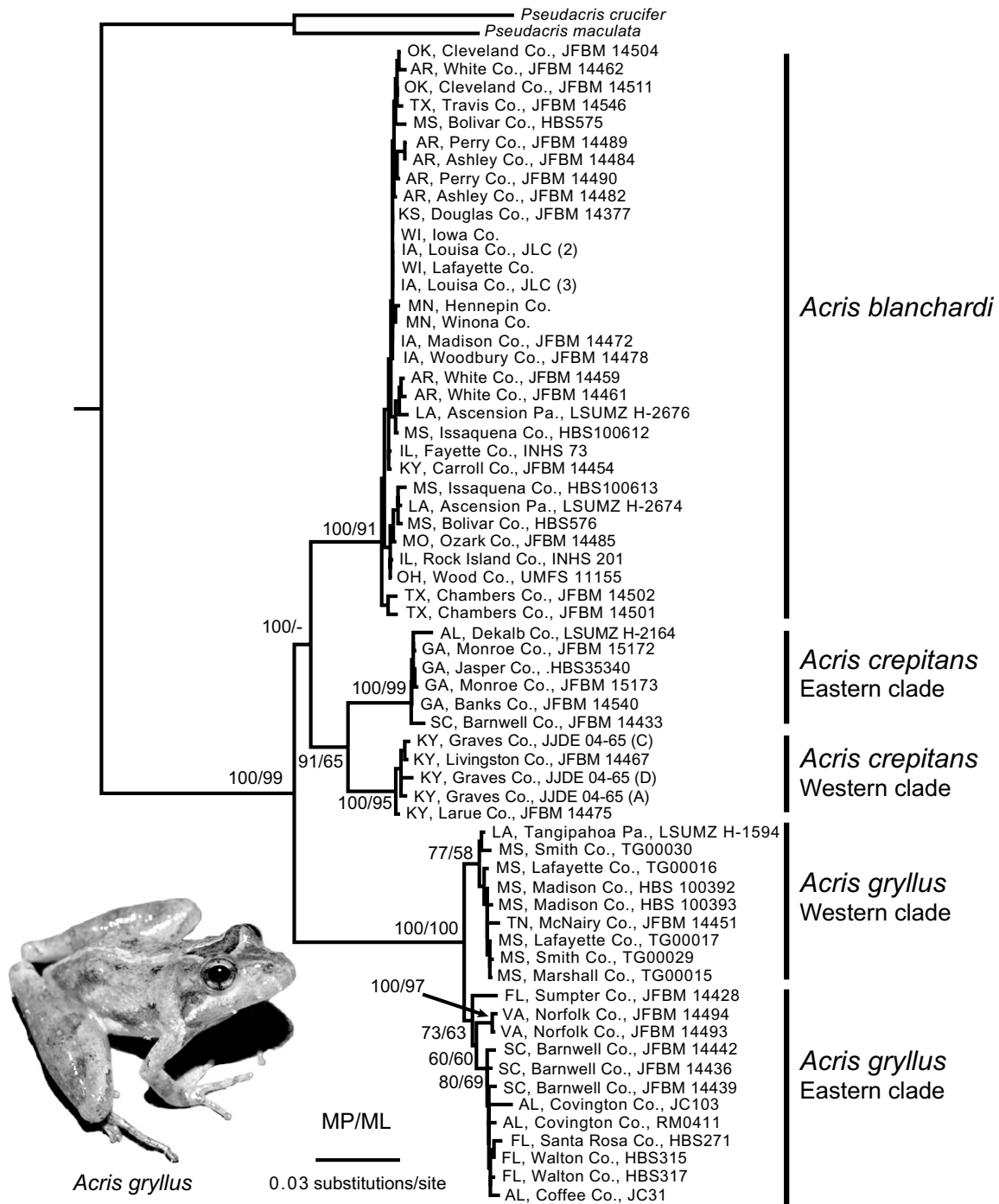


Fig. 5. Maximum likelihood phylogeny of the frog genus *Acris*, from a combined analysis of the nuclear genes tyrosinase, POMC, and cryB and the mitochondrial gene cytochrome *b*. Nodal support, parsimony and likelihood bootstrap (100 replicates), is indicated. Clade names are shown on the right. Photo by T. Gamble.

gryllus clades whereas Western and Eastern *A. crepitans* clades shared alleles at all nuclear loci.

Net between-group mean sequence divergences for all loci between major clades are provided in Table 3. Sequence divergences not shown in Table 3 included the net between-group mean sequence divergences using the K2P model for cytochrome *b* between the *A. crepitans* + *blanchardi* clade and *A. gryllus* (0.129, SE = 0.014) and between the *A. crepitans* clade and *A. blanchardi* (0.097, SE = 0.012). Population subdivision, measured as pairwise F_{ST} , was significantly different from zero in all pairwise comparisons with the exception of Eastern *A. crepitans* × Western *A. crepi-*

tans for the tyrosinase dataset and Eastern *A. gryllus* × Western *A. gryllus* for the cryB dataset (Table 3).

4. Discussion

4.1. Phylogenetic analyses

Our combined analyses provide a robust phylogeny for the genus *Acris*. The combined data topology was strongly concordant with the cytochrome *b* topology, which is not unexpected given the strong phylogenetic signal from the mtDNA (Fig. 7). Differences in

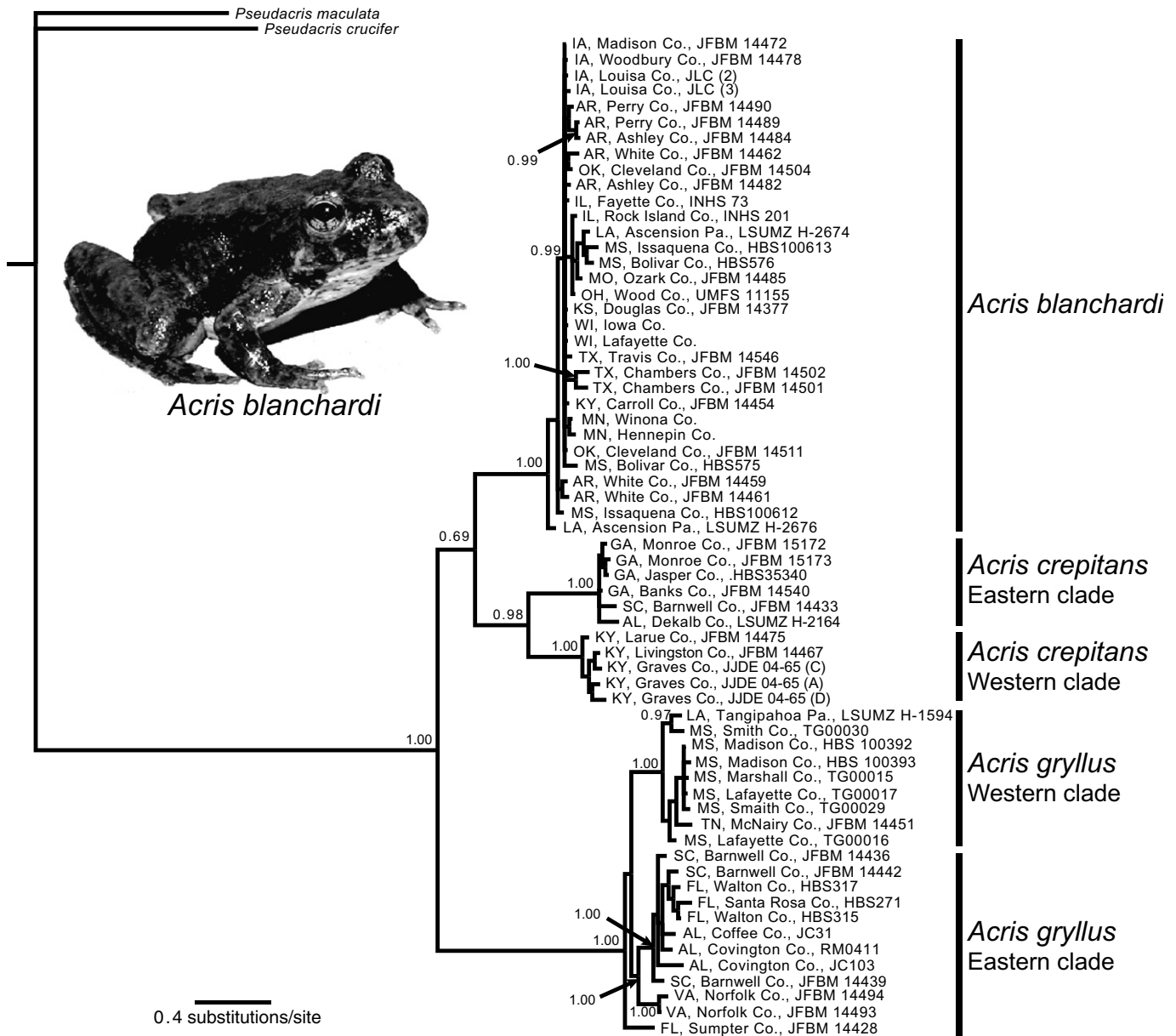


Fig. 6. Bayesian phylogeny of the frog genus *Acris*, from a combined analysis of the nuclear genes tyrosinase, POMC, and cryB and the mitochondrial gene cytochrome *b*. Bayesian posterior probabilities (BPP < 0.50 not shown) are indicated. Clade names are shown on the right. Photo by T. Gamble.

resolution seen between the nuclear and mitochondrial datasets seemed to be due to incomplete lineage sorting and/or slower substitution rates in the nDNA, rather than any obvious conflict between the two data partitions. In some studies, positively different results have been found between nuclear and mitochondrial datasets, which are often interpreted as indicating introgressive hybridization or natural selection on mtDNA. These conflicting signals have led many authors to advocate the use of nuclear genes to provide independent estimates of phylogeny (e.g. Dettman et al., 2003; Gaines et al., 2005; Giannasi et al., 2001; Prytchitko and Moore, 1997). Here, we saw little conflict, and some increase in resolution with the addition of nDNA (Cunningham, 1997; Gaines et al., 2005; Rokas et al., 2003; Wiens, 1998). The posterior probability for the node connecting *A. crepitans* to *A. blanchardi* decreased in the combined Bayesian analyses (0.69) compared to the mtDNA only Bayesian analyses (0.96). This decrease in nodal support may be related to conflict among the data partitions at that node, particularly from the tyrosinase gene (Fig. 8). *Post hoc* exam-

ination of NJ networks and F_{ST} values were consistent with the combined phylogenetic analyses, and the combination of NJ networks and F_{ST} methods provide an intuitive means of evaluating data conflict and interpreting results.

4.2. Species limits

The phylogenetic hypothesis recovered in the present analysis identifies several lineages within what is called *A. crepitans*, but their geographic distributions do not precisely match the recognized *A. crepitans* subspecies (*sensu* Conant and Collins, 1998). The primary division of *Acris* among *A. crepitans*, *A. blanchardi*, and *A. gryllus* clades is well-supported. Cytochrome *b* distances between all three lineages are comparable to distances between other recognized anuran sister species, which can vary from 7% to 15% (Austin et al., 2002; García París and Jockusch, 1999; Shaffer et al., 2004; Vences et al., 2005). A complete synonymy for the genus *Acris* was presented in

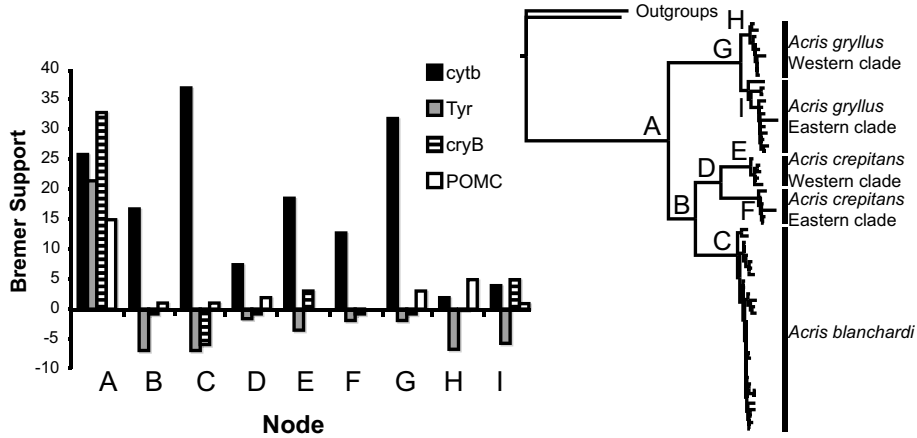


Fig. 7. Partitioned Bremer Support for nodes delimiting major *Acris* clades. Nodes are indicated on the maximum likelihood phylogram of the combined data to the right. The topology of the phylogram is the same as in Fig. 5.

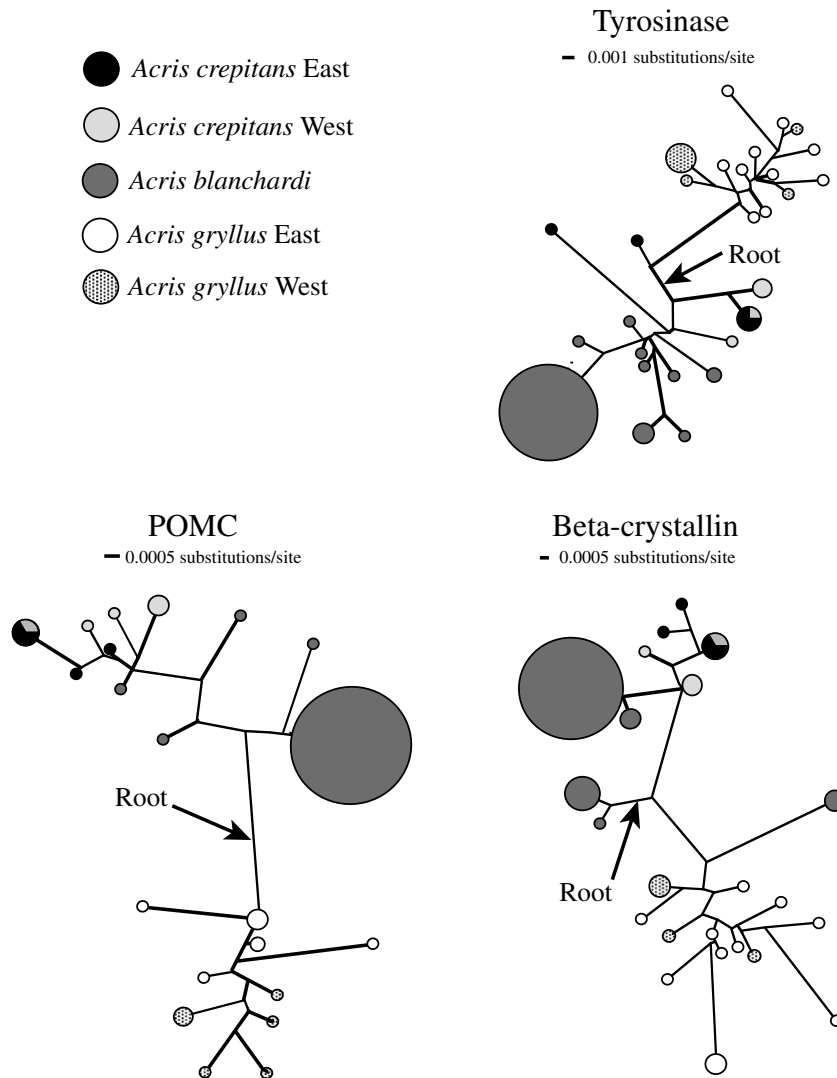


Fig. 8. Unrooted NJ networks of individual nuclear genes within the frog genus *Acris*. Circle shade represents an individual's membership in the major clades from the combined and cytochrome *b* analyses. Circle size indicates the relative number of individuals sharing a particular haplotype. The root, as determined from outgroup analysis, is indicated by the arrow.

Frost (2007), and the oldest name available for populations west of the Mississippi River is *A. blanchardi*. Based on our

combined mitochondrial and nuclear analyses we therefore propose to elevate *A. blanchardi* (Harper, 1947) to species status

Table 3On diagonals (in **bold**) are within group average genetic distances [and standard error] for each locus

Gene	Species/Clade	<i>A. blanchardi</i>	<i>A. crepitans</i> West	<i>A. crepitans</i> East	<i>A. gryllus</i> East	<i>A. gryllus</i> West
Cytochrome <i>b</i>	<i>A. blanchardi</i>	0.008 [0.001]	0.95055	0.95908	0.9571	0.97149
	<i>A. crepitans</i> West	0.114 [0.014]	0.009 [0.002]	0.93569	0.94119	0.96997
	<i>A. crepitans</i> East	0.130 [0.014]	0.093 [0.011]	0.006 [0.002]	0.9437	0.98104
	<i>A. gryllus</i> East	0.170 [0.017]	0.194 [0.020]	0.173 [0.018]	0.013 [0.002]	0.71532
	<i>A. gryllus</i> West	0.141 [0.014]	0.161 [0.018]	0.144 [0.016]	0.019 [0.004]	0.041 [0.004]
Tyrosinase	<i>A. blanchardi</i>	0.009 [0.002]	0.62716	0.60459*	0.77626	0.7997
	<i>A. crepitans</i> West	0.012 [0.004]	0.007 [0.002]	−0.05721*	0.71749	0.79144
	<i>A. crepitans</i> East	0.011 [0.004]	0.000 [0.000]	0.009 [0.003]	0.67791	0.74873
	<i>A. gryllus</i> East	0.026 [0.007]	0.020 [0.006]	0.019 [0.006]	0.009 [0.002]	0.25161
	<i>A. gryllus</i> West	0.027 [0.007]	0.022 [0.007]	0.020 [0.006]	0.003 [0.001]	0.007 [0.003]
beta-crystallin	<i>A. blanchardi</i>	0.010 [0.005]	0.30475	0.53033	0.6664	0.66466
	<i>A. crepitans</i> West	0.003 [0.002]	0.001 [0.001]	0.62547	0.7104	0.87716
	<i>A. crepitans</i> East	0.008 [0.006]	0.002 [0.002]	0.000 [0.000]	0.75946	0.90369*
	<i>A. gryllus</i> East	0.016 [0.006]	0.019 [0.008]	0.023 [0.009]	0.015 [0.004]	0.21285
	<i>A. gryllus</i> West	0.020 [0.009]	0.022 [0.010]	0.027 [0.011]	0.003 [0.002]	0.004 [0.004]
POMC	<i>A. blanchardi</i>	0.002 [0.001]	0.8612	0.91014	0.83973	0.91168
	<i>A. crepitans</i> West	0.005 [0.003]	0.002 [0.001]	0.53307	0.75871	0.87342
	<i>A. crepitans</i> East	0.007 [0.003]	0.001 [0.001]	0.001 [0.001]	0.83565	0.94602
	<i>A. gryllus</i> East	0.005 [0.003]	0.010 [0.004]	0.012 [0.004]	0.003 [0.001]	0.48158
	<i>A. gryllus</i> West	0.009 [0.004]	0.013 [0.005]	0.014 [0.005]	0.002 [0.002]	0.001 [0.001]

Below each diagonal are net between group average genetic distances [and standard error] between populations. Above each diagonal are pairwise F_{ST} , based on pairwise distance (all values significantly different from zero at $P = 0.05$, except those marked with an *). All distances estimated using the K2P model.

and retain *A. crepitans* for frogs in the eastern and central portions of the range (see Fig. 2).

Previous allozyme, ecological, and mate-choice data provide additional support for this taxonomic decision. Dessauer and Nevo (1969) found that four of 20 proteins surveyed showed population substructure within *A. crepitans sensu lato* with western and eastern subgroups that approximately correspond to our *A. crepitans* and *A. blanchardi* lineages. An examination of habitat preferences and mate choice (Nevo and Capranica, 1985), indicated that *A. c. crepitans* and *A. c. blanchardi* may be incipient ecological species although the proposed geographic distributions better match *A. crepitans* subspecies (*sensu* Conant and Collins, 1998) because of reliance on call data.

Morphological data, which have historically been used to define *A. crepitans* subspecies, provide a somewhat mixed signal with respect to variation within *A. crepitans*. Although originally used to delimit subspecies, a recent analysis indicates that the traditionally-used morphological features do not adequately differentiate lineages within *A. crepitans sensu lato*. Thus, McCallum and Trauth (2006) found the morphological characters used to diagnose *A. c. blanchardi*: “greater bulk”; “somewhat more extensive webbing of the toes”; and “the more extensive dusky area on the posterior face of the femora in the vicinity of the vent” (Harper, 1947), were not well defined and did not consistently discriminate between specimens of *A. c. blanchardi* and *A. c. crepitans*. Based on their analyses, McCallum and Trauth (2006) recommended synonymizing *A. c. blanchardi* and *A. c. crepitans*. While there has been no morphological reanalysis of *A. c. paludicola*, the coastal subspecies was similarly defined by qualitative characters including color, pattern, toe disk size, and extent of toe webbing (Burger et al., 1949). However, recent mtDNA analysis (Rose et al., 2006), consistent with our mtDNA results, indicates that *A. c. paludicola* is nested within *A. c. blanchardi* and does not warrant subspecific status.

Reciprocal monophyly with mtDNA satisfies the requirements of lineage-based species concepts (de Queiroz, 1998; Wiens and Penkrot, 2002), as long as the true species tree is reflected in mtDNA gene trees. The inclusion of nuclear gene data allowed us to test the validity of identified mitochondrial lineages as defensible species. Monophyly or exclusivity at a majority of nuclear genes is not necessarily a reasonable assumption in recently and/or rapidly radiating lineages (Hudson and Coyne, 2002), where

the time to monophyly of nuclear loci (coalescence) is expected to be great, and a strict reliance on monophyly may often overlook recently-derived species (Shaffer and Thomson, 2007). Reciprocal monophyly, at least with mtDNA, for *A. blanchardi*, *A. crepitans*, and *A. gryllus* suggests that these taxa represent good lineage-based species. Shared alleles at all the nuclear loci between Eastern and Western *A. crepitans* clades suggest either incomplete lineage sorting or continued gene flow between populations. In either case, Western and Eastern *A. crepitans* lineages are distinct based on F_{ST} /genetic distance approaches (Table 3), but additional sampling and preferably, call analyses, are needed to determine if they warrant species status. The same is true for Eastern and Western *A. gryllus* groups, which showed significant F_{ST} values for mtDNA and two of three nuclear loci (Table 3). Lack of structure between Eastern and Western *A. gryllus* for beta-crystallin as well as shallow mtDNA divergence suggests, as with *A. crepitans*, additional sampling and call analyses are needed before a firm taxonomic decision can be reached.

4.3. Biogeography

The biogeographic pattern evident in the *A. crepitans* + *A. blanchardi* group (western and eastern clades) is consistent with many other co-distributed vertebrate species such as ratsnakes (Burbrink et al., 2000), spring peepers (Austin et al., 2002), chorus frogs (Lemmon et al., 2007a,b), painted turtles (Starkey et al., 2003), and short-tailed shrews (Brant and Orti, 2003). The Mississippi and Ohio Rivers form the primary eastern boundary for *A. blanchardi* with *A. crepitans* found south of the Ohio River and east of the lower Mississippi River. The confluence of the Mississippi and Ohio Rivers is an area where a number of taxonomic groups have multi-lineage contact (Austin et al., 2002; Starkey et al., 2003; Lemmon et al., 2007a,b). The southern Mississippi River has been shown to be a prominent barrier to gene flow in numerous species including fish, ratsnakes, spiny lizards, and shrews (Berendzen et al., 2003; Brant and Orti, 2003; Burbrink et al., 2000; Leache and Reeder, 2002; Mayden, 1988; Moriarty and Cannatella, 2004) although it has not been absolute and there are several taxa where eastern haplotypes occur on the west side of the river or vice versa (Burbrink et al., 2000; Shaffer and McKnight, 1996; Starkey et al., 2003; Lemmon et al., 2007a,b). In our dataset, for example, cricket

frogs from Issaquena and Bolivar counties in Mississippi, east of the Mississippi River, are genetically part of *A. blanchardi*. Further sampling is needed to determine if these populations are the result of a 'leaky' barrier to gene flow due to the dynamic boundaries of rivers, or a more widespread distribution of *A. blanchardi* haplotypes east of the Mississippi River. Incomplete geographic sampling in the south-eastern US made it difficult to ascertain precisely where the boundary occurred between Western and Eastern *A. crepitans* clades. The Mobile Bay and Tombigbee River, like the Mississippi River, have been proposed to be a significant biogeographic boundary (Gill et al., 1993; Lawson, 1987) and may be a potential boundary between Eastern and Western *A. crepitans* clades.

Additional phylogeographic structure was found within *A. gryllus*. Most obvious is the split between Eastern and Western haplotypes, which appear to be separated by the Mobile basin. The position of the Sumpter County, Florida frog as either part of the Eastern *A. gryllus* clade or sister taxon to the Eastern + Western *A. gryllus* clades suggests that additional sampling from peninsular Florida is needed. The existence of a third *A. gryllus* clade from peninsular Florida would not be too surprising given Florida's biogeography and the morphological distinctness of Florida specimens (Conant and Collins, 1998).

4.4. Call evolution in the genus *Acris*

Nevo and Capranica (1985) grouped cricket frogs into three distinct groups based on multivariate analysis of 16 call variables. The distinction among calls was attributed to environmental and acoustic differences in the three broad habitat types occupied by cricket frogs: grasslands (*A. c. blanchardi*, sensu Conant and Collins, 1998), deciduous woodlands (*A. c. crepitans*, sensu Conant and Collins, 1998), and meadows within pine forests (*A. gryllus*). These different call types were used as evidence for subspecific boundaries within *A. crepitans sensu lato* (Nevo and Capranica, 1985). In a more detailed analysis, Ryan and Wilczynski (1991) found a similar pattern in cricket-frog calls across a longitudinal environmental gradient in east Texas, with habitat type (forest or open habitats) having the greatest influence on call characteristics.

Based on our new phylogenetic results, we asked whether call variation within *Acris* now appears to reflect lineages, habitats, or both. Although we did not record any call data ourselves, Nevo and Capranica's (1985) data indicate that both *A. gryllus* and *A. crepitans* have unique calls that correspond with, and help to diagnose these genetic lineages. However, *A. blanchardi*, as diagnosed by our DNA analyses, contains both the "grassland" (*A. c. blanchardi sensu Conant and Collins, 1998*) and "deciduous woodland" (*A. c. crepitans sensu Conant and Collins, 1998*) call types identified by these authors. Calls of cricket frogs from the forested areas of east Texas and Louisiana, part of the *A. blanchardi* clade, are of particular interest. Calls from cricket frogs in this region show similarities with calls from cricket frogs further east from Alabama, Georgia, New Jersey, and New York, which comprise the *A. crepitans* clade in our analysis. This apparent polymorphism of *A. blanchardi* calls was the source of much of the taxonomic confusion in the genus. At this point, we cannot say whether the two call types found within *A. blanchardi* represent distinct, but very recently-derived evolutionary lineages or a true within-species polymorphism.

4.5. Conservation

We used mtDNA monophyly along with nuclear gene F_{ST} data and the presence of private nuclear alleles to support our recognition of *A. blanchardi* as a full species. Such taxonomic decisions, resulting from phylogenetic analyses, have consequences outside the realm of systematic biology. Conservation decisions are often made based on the assumption that named taxonomic units repre-

sent evolutionary lineages (Mayden and Wood, 1995). The failure to diagnose biological diversity can hamper conservation efforts, as well as basic scientific inquiry (Mayden and Wood, 1995; Metcalf et al., 2007). The northern cricket frog (*A. blanchardi*) has exhibited dramatic population declines in the northern portion of its range (Baker, 1997; Gray and Brown, 2005; Hammerson and Livo, 1999; Hay, 1998; Lannoo, 1998; Lehtinen and Skinner, 2006). This phenomenon first came to light in the 1970s, and has continued to the present (Hay, 1998; Lehtinen, 2002; Vogt, 1981). These declines have been characterized by the disappearance of cricket frogs from apparently suitable habitat with no concurrent decline in populations of other amphibian species (Lannoo, 1998). Possible causes include climate (Hay, 1998; Irwin, 2005), habitat alteration (Lannoo, 1998), pollution (Reeder et al., 2005), and habitat fragmentation (Hay, 1998). Understanding the biological diversity within northern cricket frogs is an essential step in the deeper understanding of the patterns, causes, and reversal of these declines. Although we cannot condone the recognition of so-called "conservation species" (Gamauf et al., 2005), the recognition of valid, defensible cryptic species diversity within an already-recognized declining taxon implies that each of the new species' distributions will be smaller than that of the formerly recognized species. In that sense, each newly recognized species must be at greater risk than was formerly considered for the more inclusive taxon. Recent work on the severely-declining flatwoods salamander, *Ambystoma cingulatum/bishopi*, is a case in point (Pauly et al., 2007). Recognizing *A. blanchardi*, where its northerly range encompasses the majority of the most severe *Acris* declines, highlights the delicate status of this distinct evolutionary lineage.

5. Conclusions

Our data indicate that genetic diversity within the genus *Acris* is not reflected in its current taxonomy. As a result, we recognize three distinct species within the genus: *A. blanchardi*, *A. crepitans*, and *A. gryllus*. Additional sampling across the southeastern US will be needed to determine the extent of the diversity and geographical range of *Acris* species. Further work is also needed to evaluate the genetic diversity within *A. crepitans* and the status of *A. gryllus* from peninsular Florida. A reexamination of morphology and call data in light of results presented here would provide additional insight into the diversity and evolution of this genus.

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