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Conservation implications of a morphometric comparison between the Illinois Chorus Frog (*Pseudacris streckeri illinoensis*) and Strecker's Chorus Frog (*P. s. streckeri*) (Anura: Hylidae) from Arkansas, Illinois, Missouri, Oklahoma, and Texas

JOY B. TRAUTH[1,4], RONALD L. JOHNSON[2], & STANLEY E. TRAUTH[3]

Department of Biological Sciences, Arkansas State University, State University, AR 72467. E-mail: [1]jtrauth@astate.edu; [2]rlj@astate.edu; [3]strauth@astate.edu
[4]Corresponding author

Abstract

Much uncertainty exists regarding the taxonomic status of the Illinois Chorus Frog (*Pseudacris streckeri illinoensis* Smith; ICF) relative to Strecker's Chorus Frog (*P. s. streckeri*, Wright & Wright; SCF) of the southcentral United States (US). Molecular analyses have been inconsistent in providing taxonomic insight, and no formal morphological comparisons have been previously performed. Each taxon possesses a wide range of background colors. We undertook morphometric analyses to help clarify their taxonomic relationship. Tibia length and mass were compared for live Arkansas (AR) specimens and snout-vent, head and tibia lengths were measured from preserved vouchered specimens. Tibia length and mass were significantly greater for living ICFs versus SCFs in AR. Among preserved specimens, tibia, snout-vent and head lengths were significantly greatly for AR ICFs relative to most intraspecific groups, and Texas (TX) SCFs were significantly smaller than most other groups. Principal components analysis was largely consistent with univariate analyses, although Missouri (MO) ICFs also partitioned distinctly from other sample groups. These data provide morphological evidence of geographic (clinal) variation within a species, but do not provide support for the taxonomic elevation of the ICF to species status. Our data do provide evidence of distinct population segments of *P. streckeri*. As ICF habitat suitable for reproduction has dramatically declined in Arkansas as have population numbers, we recommend the listing of AR ICFs as a distinct population segment under the Endangered Species Act.

Key words: amphibian, coloration, conservation, morphometrics

Introduction

Much uncertainty exists regarding the taxonomic status of the Illinois Chorus Frog (*Pseudacris streckeri illinoensis* Smith 1951; ICF). For instance, some authors (Conant & Collins 1998; Johnson 2000; Phillips *et al*. 1999; Trauth *et al*. 2004; Lannoo 2005) currently classify the ICF and the nominate race of the species (*P. s. streckeri* Wright & Wright 1933; SCF) as separate subspecies, whereas others (Collins 1997; Collins & Taggart 2002; Hedges 1986; Moriarity & Cannatella 2004) have proposed that the ICF should be classified as a distinct species. Although both taxa exhibit very similar morphologies, Smith (1951) used subtle color differences and a slightly larger body size in the ICF as primary features by which to differentiate the two taxa.

The ranges of the ICF and SCF are currently discrete with SCF being common and broad-ranging throughout much of Oklahoma (OK) and eastern TX (Fig. 1; see also Lannoo 2005). In AR, SCF is restricted to the western half of the AR Valley (Conant & Collins 1998; Trauth *et al*. 2004; Lannoo 2005) from just west of Little Rock westward to the Fort Smith area. The ICF, however, inhabits a very small and fragmented

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range. In AR, it is found only in the extreme northeastern corner of the state (Trauth *et al*. 2004) in the Mississippi Alluvial Plain (Robison & Buchanan 1988). The ICF also occurs throughout the bootheel of southeastern MO and in disjunct populations along the Mississippi and Illinois rivers in Illinois (IL) (Conant & Collins 1998; Briggler 2001). The populations of ICFs and SCFs in AR represent the closest (at least 250 km apart) and, presumably, the most recent biogeographic connection between the two taxa (McCallum & Trauth 2002).

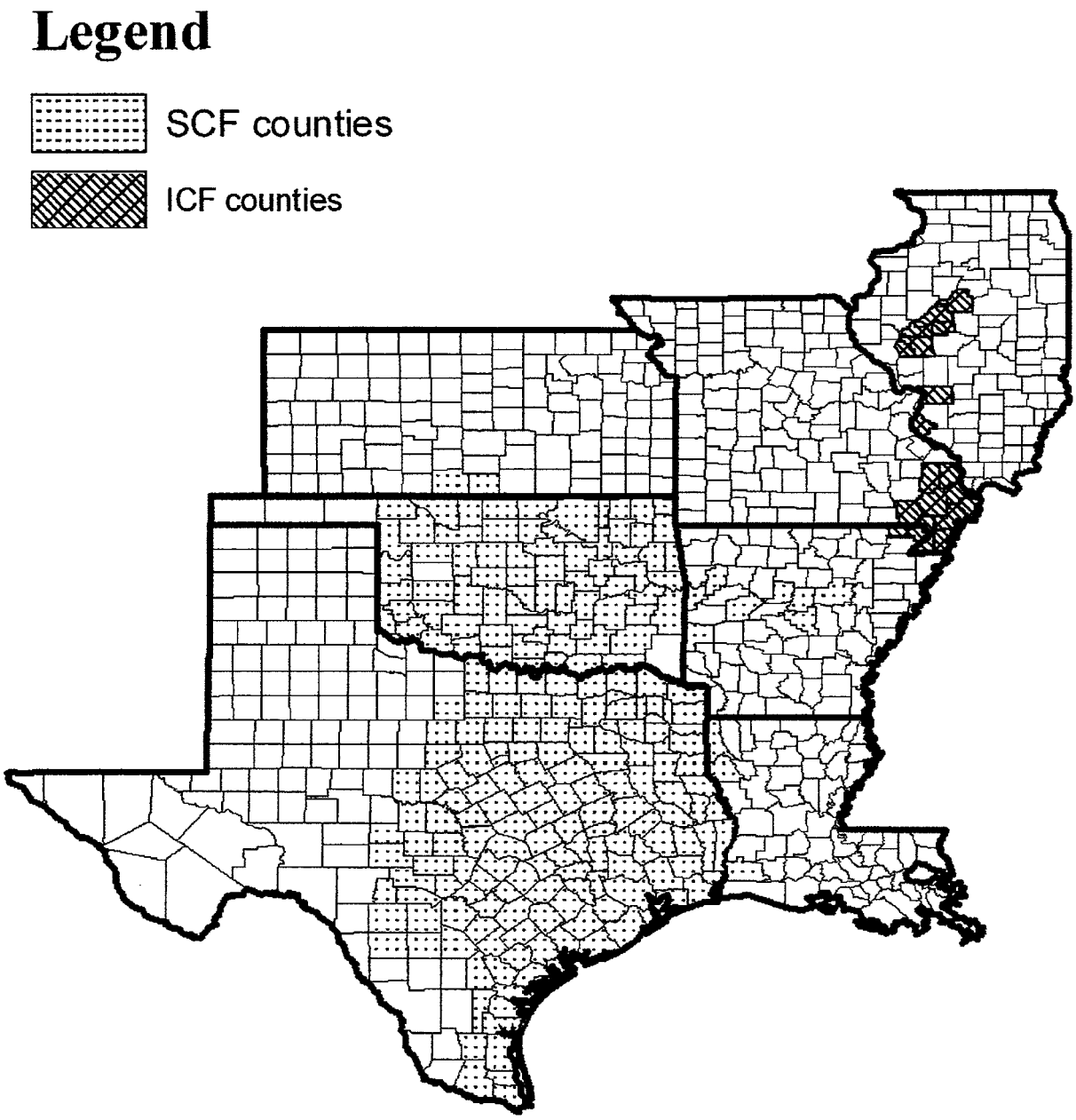

FIGURE 1. Counties with documented populations of ICFs and SCFs.

Smith (1951) suggested that SCF and the ICF share a broad-ranging ancestor that once occupied a wider and more easterly range during expansion of the Prairie Peninsula (Smith 1957) approximately 4000 years ago. With the recession of the Prairie Peninsula and the growth of forests, many prairie species such as *P. streckeri* survived only in small pockets of suitable habitat (Smith 1957). Conversely, Axtell and Haskell (1977), based upon current ICF habitat requirements of sandy substrate and floodplain topography, proposed a derivation from more western SCFs followed by northern range expansion for the ICF from the western seg-

ment of the Arkansas River floodplain along the Mississippi Embayment into central Illinois. Isolation of small populations of *P. streckeri* in Illinois, Missouri, and Arkansas (now classified as *P. s. illinoensis* based on geographic separation) may have resulted in morphometric and genetic divergence of different populations.

Molecular analyses have been inconclusive in providing taxonomic insight. For example, Hedges (1986) used allozyme analysis of 33 presumed loci to examine phylogenetic relationships between 30 taxa of Holarctic hylid frogs, including *Pseudacris*. He found genetic distance between the ICF and SCF (D = 0.13) to be similar to distances between some other recognized species (range of 0.02 to 0.16 for 6 other species comparisons), yet much lower than for most species comparisons. Hedges (1986) suggested that *P. s. illinoensis* probably should be recognized as a full species, but that more detailed studies were required. Conversely, Moriarty & Cannatella (2004) examined the phylogenetic relationships among North American chorus frogs by sequencing 2.4 kb of the 12S and 16S mtDNA genes. They reported the ICF (*n* = 1 each from AR and MO) sequences to be "nested" within SCF (*n* = 1 each from KS and TX) sequences. They also recommended further taxonomic study.

Smith (1951) noted a larger size for the IL ICF relative to SCF, yet he provided no statistical evidence. Although no formal morphological comparisons have been performed on these two subspecies, Smith (1957, 1966) and Conant & Collins (1998) have described the ICF as being pale relative to SCF, contrary to what we have observed in AR populations (Fig. 2). We have noted a wide range in background colors, including light reddish tan, greenish brown, light brown, and dark brown, for both taxa, with SCFs in general having the paler background color (Fig. 2). Color changes associated with temperature changes have been observed by us for both taxa with colder temperatures inducing darker coloration. These temperature-related color changes are common for amphibian taxa (Duellman & Trueb 1986). Many anurans show considerable conspecific variation in color (Conant & Collins 1998), making coloration unsuitable as a distinguishing trait.

Due to the inconclusiveness of molecular data and a paucity of previous morphological studies, we conducted a narrow-scale morphometric analysis of the ICF and SCF to help clarify the taxonomic status of the ICF. Historically, morphological analyses have been an integral component in studying anuran systematics (e.g., see Gaudin 1974; Trueb 1977; Duellman & Trueb 1986). Morphological differences generally provide supportive evidence of underlying genetic differentiation interacting with environmental factors in reproductively-isolated populations. Genetic differences are, thus, inferred from the more obvious phenetic differences, which they cause (Scott 2005).

Material and methods

Snout-vent (head-body) length, head length, and tibia length are the three primary external morphological characters measured for anurans (Peters 1964; Conant & Collins 1998). These three standard measurements of head, body, and limb dimensions are also patently osteological in nature (Trueb 1977). Of these three characters, we determined that only tibia length could be reliably measured in live specimens (*n* = 117 ICFs and 23 SCFs in Arkansas).

All live ICFs were collected in Clay County, AR, during the early spring breeding seasons (February and March) of 2003 and 2004, and all frogs were returned to their respective breeding pools after data collection. All live SCFs were collected in Conway and Yell counties during the spring breeding season of 2003 (March). After data collection, the SCFs were sacrificed and deposited in the AR State University Museum of Zoology (ASUMZ) herpetological collection. Mass was also measured for live male specimens. Males only were sampled to minimize seasonal reproductive effects on biomass, and all specimens were collected over a short time span in early spring soon after emergence.

FIGURE 2. On the left are three representative ICFs from Clay County, AR. On the right are three representative SCFs from Yell and Conway counties, AR.

The three characters were analyzed in preserved specimens of each subspecies collected from sites within their respective ranges in AR, IL, MO, OK, and TX (Table 1). Tibia lengths, snout-vent lengths, and head lengths were measured to 0.01 mm using a Grobet Vigor electronic digital caliper. Tibia length was measured from the convex surface of the heel to the convex surface of the knee (Goin & Netting 1940; Peters 1964). Snout-vent length was measured from the anterior margin of the snout to the posterior margin of the cloacal opening/end of the body (Peters 1964; Lee 1982). Head length was measured from the snout to the posterior border of the tympanum (Cochran 1955; Peters 1964). To minimize inter-observer error (Lee 1982; Hayek *et al*. 2001), JBT performed all morphological measurements.

TABLE 1. Specimen collection localities for preserved specimens.

Taxon	State	County	*n* males	*n* females
ICF	AR	Clay	140	12
ICF	IL	Cass	36	5
ICF		Madison	10	0
ICF	MO	Scott	4	1
ICF		Pemiscot	3	0
ICF		Mississippi	1	0
ICF		Dunklin	2	0
ICF		New Madrid	2	0
SCF	AR	Conway	16	8
SCF		Yell	15	1
SCF		Faulkner	4	0
SCF		Pope	2	0
SCF	TX	Travis	27	27
SCF	OK	Bryan	12	0

Kolmogorov-Smirnov normality tests were performed on all data sets to test for normal distribution of characters within samples and Levene's test was used to check for equal variance among samples. Parametric analyses (two-sample *t*-tests and ANOVA) were performed when samples were normally distributed and showed equal variance. A two-sample *t*-test was used to compare tibia lengths for living male ICFs and SCFs from AR. The variables of snout-vent length and head length of males were log transformed (log x + 1), as one sample for snout-vent length was not normally distributed, and samples for head length lacked equal variance. One-way ANOVA was used for each character to compare separately preserved male and female specimens of differing subspecies and states, with Tukey's pairwise comparisons revealing differences between groups showing significant differences. A non-parametric test (Mann-Whitney) was used to compare mass of living male ICFs and SCFs.

Even though individuals were collected from several counties in some states, these counties represented limited fragmentary geographic ranges; therefore, samples were grouped by state and subspecies for statistical analyses. To validate homogeneity between state samples, two-sample *t*-tests were performed between county populations having sufficient sample size ($n > 9$) for morphological characters for male ICFs in IL (Cass and Madison counties) and for male SCFs in AR (Yell and Conway counties). There was no significant difference between the county samples for any character. Average *p*-value was 0.578 with a range from 0.149 to 0.910.

Because of the significant gender bias in collecting (only male frogs call), our samples contained few female frogs, with no females present from OK and only one from MO (Table 1). The Mann-Whitney test was used to compare snout-vent length for preserved specimens between genders for AR ICFs and SCFs and TX ICFs, and two-sample *t*-tests were used to compare head length and tibia length. Males and females were significantly different for all three characters (*p*-values were < 0.001, = 0.002, and = 0.002, respectively) for TX ICFs, contrary to results reported by Lee (1982). However, no AR ICF gender differences were identified for snout-vent length, head length, and tibia length (*p*-values = 0.594, 0.215 and 0.175, respectively). Two-sample *t*-tests of AR SCFs yielded inconsistent results with *p*-values = 0.036 for snout-vent length, 0.069 for head length, and 0.369 for tibia length. Data from male and female frogs were, therefore, analyzed separately due to the occurrence of some sexual dimorphism.

As significant short term reductions were identified in tibia lengths following preservation for AR ICF of the present study ($p < 0.001$; yet not AR SCF, $p = 0.313$), data from living and preserved specimens were analyzed separately.

All confidence intervals given are 95% group confidence intervals for the difference between the two taxa for the trait. Alpha levels for all statistical tests were set at $\alpha = 0.05$. Multivariate ordination of morphologic data was also determined for sample groups using principal components analysis (PCA) [PC-Ord; MJM Software].

Results

Tibia length (*t*-test, $p = 0.008$) and mass (Mann-Whitney, $p < 0.001$) were significantly greater for living male ICFs versus SCFs in AR (Table 2). ANOVA also demonstrated significant differences among male sample groups for snout-vent length, head length, and tibia length of preserved specimens ($F_{5,268} = 40.42$, $p < 0.001$; $F_{5,259} = 13.98$, $p < 0.001$; $F_{5,230} = 21.87$, $p < 0.001$), with Tukey's pairwise comparisons indicating the following: For snout- vent length AR ICF > IL ICF = MO ICF = AR SCF = OK SCF > TX SCF; for head length AR ICF > IL ICF = MO ICF = AR SCF = TX SCF; for tibia length AR ICF = IL ICF = OK SCF > MO ICF = TX SCF (Tables 3–4).

TABLE 2. Descriptive statistics and morphometric comparison for live male ICFs and live male SCFs from AR including sample means, medians, standard errors, *p*-values, and 95% confidence intervals (CI) for the difference between taxa for tibia length (mm) and mass (g).

	Tibia Length		Mass	
	ICF	SCF	ICF	SCF
n	105	23	105	23
Mean	15.493	14.976	5.317	4.107
SE Mean	0.073	0.165	0.081	0.095
Median	15.570	15.080	5.180	3.960
95% CI	(0.140, 0.830)		(0.850, 1.480)	
p-value	= 0.008		= 0.001	

The AR ICFs were the largest of the chorus frogs, whereas the TX SCFs were the smallest. Illinois Chorus Frogs sampled were generally larger than SCFs for all traits sampled**.** When all preserved male ICFs were compared to all preserved male SCFs sampled, Mann-Whitney tests indicated ICFs were significantly larger than SCFs for all three traits (*p*-values < 0.001 for each).

ANOVA demonstrated no significant differences between female ICFs from AR and IL and female SCFs from AR and TX in snout-vent length (*p*-value = 0.105) or head length ($p = 0.320$). ANOVA ($F_{3,49} = 3.61$; $p = 0.020$) with Tukey noted only one difference among female chorus frogs for tibia length: AR ICF > TX SCF (CI = (0.0330, 1.4955).

Principal components analysis of the combined data for snout-vent length, tibia length, and head length is largely consistent with individual trait analyses (Fig. 3). The AR ICFs and TX SCFs partition distinctly from other groups in opposing planes with a clustering of ICFs from IL with SCFs from AR and OK. However, unlike that observed in univariate analyses, the MO ICFs also partitioned distinctly from other sample groups. Axes 1 and 2 explained 92 % of the sample variance (Eigenvalues of 2.404 and 0.333, 80 and 11 % of the variance explained, respectively).

TABLE 3. Descriptive statistics for preserved male Illinois Chorus Frogs and male Strecker's Chorus Frogs from Arkansas, Illinois, Missouri, Oklahoma, and Texas including sample means, standard errors, and medians for snout-vent length, tibia length, and head length in mm.

	n	Mean	SE Mean	Median
Snout-Vent Length				
AR ICF	140	36.938	0.138	36.935
IL ICF	46	35.002	0.266	35.045
MO ICF	12	35.432	0.539	35.650
AR SCF	37	34.475	0.306	34.390
OK SCF	12	34.923	0.432	34.995
TX SCF	27	32.584	0.292	32.170
Tibia Length				
AR ICF	105	14.919	0.063	14.870
IL ICF	44	14.768	0.083	14.835
MO ICF	12	14.143	0.155	14.205
AR SCF	36	14.730	0.119	14.750
OK SCF	12	15.106	0.174	15.090
TX SCF	27	13.593	0.126	13.800
Head Length				
AR ICF	132	11.508	0.034	11.480
IL ICF	45	11.170	0.070	11.150
MO ICF	12	10.878	0.126	10.890
AR SCF	37	11.215	0.096	11.150
OK SCF	12	11.126	0.106	11.160
TX SCF	27	10.902	0.093	10.970

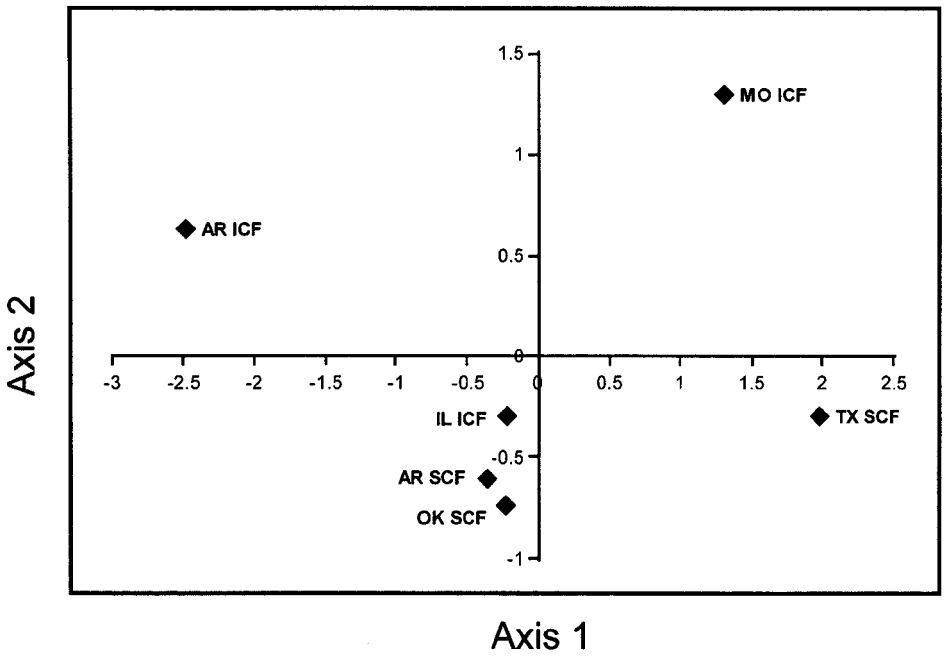

FIGURE 3. Results of principal components analysis of the combined data for snout-vent length, tibia length, and head length for all state samples of ICFs and SCFs.

TABLE 4. Morphometric comparisons between state samples of male ICFs and male SCFs from AR, IL, MO, TX, and OK with a 95% group confidence level. Where a significant difference exists between state samples, the 95% CI for the difference between taxa for each variable is given (column–row). All one-way ANOVA *p*-values were ≤ 0.001. Where no significant difference exists, NSD is stated.

	ICF AR	ICF IL	ICF MO	SCF AR	SCF OK
Snout-Vent Length					
ICF IL	(1.117, 2.756)				
ICF MO	(0.056, 2.957)	NSD			
SCF AR	(1.572, 3.355)	NSD	NSD		
SCF OK	(0.565, 3.467)	NSD	NSD	NSD	
SCF TX	(3.341, 5.368)	(1.249, 3.587)	(1.174, 4.521)	(0.670, 3.111)	(0.665, 4.012)
Tibia Length					
ICF IL	NSD				
ICF MO	(0.221, 1.330)	(0.032, 1.217)			
SCF AR	NSD	NSD	NSD		
SCF OK	NSD	NSD	(-1.705, -0.220)	NSD	
SCF TX	(0.933, 1.718)	(0.730, 1.619)	NSD	(0.674, 1.600)	(0.881, 2.144)
Head Length					
ICF IL	(0.120, 0.556)				
ICF MO	(0.249, 1.011)	NSD			
SCF AR	(0.058, 0.528)	NSD	NSD		
SCF OK	NSD	NSD	NSD	NSD	
SCF TX	(0.339, 0.873)	NSD	NSD	NSD	NSD

Discussion

Our data provide morphological evidence of geographic (clinal) variation within a species, but do not provide support for the taxonomic elevation of the ICF to *Pseudacris illinoensis*, separate from *Pseudacris streckeri.* The larger size identified for ICF versus SCF is consistent with Smith (1951). This is essentially because AR ICFs are significantly larger than all other frogs tested, and TX SCFs are significantly smaller than all other frogs tested. The other populations of frogs differ little in body size.

Our morphometric analyses are consistent with the molecular data of Moriarty and Cannatella (2004). Illinois Chorus Frog habitat suitable for reproduction has dramatically declined in Arkansas from an estimated range of 59 km^2 in 1992 to a current range of 23 km^2 as have ICF population numbers (Trauth *et al*. 2006). Prior to 1992, breeding choruses were sufficiently large that individual calling frogs could not be distinguished in the loud and continuous choruses (S.E. Trauth, AR State University, State University, AR., unpubl. data); during the 2004 breeding season, choruses consisted of fewer than eight calling frogs per site (Trauth *et al*. 2006). Illinois Chorus Frogs were documented in seven counties in IL prior to 1980, but have not been found in Tazewell and Menard counties since that time (Phillips *et al*. 1999). The range of ICFs in Madison County, IL, has also contracted significantly from that of the 1970s (Tucker 1998), and breeding choruses have become smaller with more recent choruses consisting of <10 males (Tucker & Philipp 1993). However, Briggler (2001) found 178 ICF breeding sites in six counties in southeast Missouri. He stated that the ICF appeared to be doing well in areas of increased development, and he predicted that the population should remain stable in southeast Missouri if some suitable habitat could be acquired and set aside for the ICF.

TABLE 5. Descriptive statistics for preserved female Illinois Chorus Frogs and female Strecker's Chorus Frogs from Arkansas, Illinois, and Texas including sample means, standard errors, and medians for snout-vent length, tibia length, and head length in mm.

	n	Mean	SE Mean	Median
Snout-Vent Length				
AR ICF	12	37.205	0.466	37.295
IL ICF	5	36.770	1.370	36.700
AR SCF	8	37.169	0.976	37.520
TX SCF	27	35.264	0.509	34.960
Tibia Length				
AR ICF	12	15.098	0.111	15.065
IL ICF	5	15.074	0.346	15.150
AR SCF	9	14.958	0.214	15.070
TX SCF	27	14.334	0.183	14.300
Head Length				
AR ICF	11	11.701	0.143	11.490
IL ICF	5	11.628	0.128	11.690
AR SCF	9	11.691	0.216	11.660
TX SCF	27	11.390	0.114	11.400

Populations at the edge of a species' range often have characteristics that differ from those populations in the interior of the species' range due to the greater challenges associated with life at the edge of a habitat (Hunter 1996). All populations of ICFs in AR are fragmentary and dynamic (Trauth *et al.* 2006). All remaining populations of ICFs in AR exist only on agricultural land, which has been largely precision leveled.
The ICF in AR is significantly larger than other ICFs and SCFs. In PCA of the combined morphological data, AR ICFs, MO ICFs, and TX SCFs all partition separately as distinct populations within the broad range of *P. streckeri* (Fig. 3). The three populations of *P. streckeri* that partition together include two populations of SCFs (MO and AR) and one population of ICFs (IL). This again supports geographic variation within a species due to differing environmental conditions and does not support species status for the ICF. We, therefore, recommend designation and listing of the ICF in AR for protection as a distinct population segment under the Endangered Species Act.

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