# Phylogeny-based delimitation of species boundaries and contact zones in the trilling chorus frogs (*Pseudacris*)

Emily Moriarty Lemmon [a,*], Alan R. Lemmon [a], Joseph T. Collins [b,c],
Julie A. Lee-Yaw [a], David C. Cannatella [a,d]

[a] *Section of Integrative Biology, The University of Texas, 1 University Station C0930, Austin, TX 78712, USA*
[b] *Kansas Biological Survey, University of Kansas, Lawrence, USA*
[c] *Sternberg Museum of Natural History, Fort Hays State University, Hays, KS, USA*
[d] *Texas Memorial Museum, University of Texas, Austin, USA*

## Abstract

Although the trilling chorus frogs (subclade within *Pseudacris*: Hylidae) have been important in studies of speciation, continental patterns of genetic diversity within and among species have not been elucidated. As a result, this North American clade has been the subject of substantial taxonomic debate. In this study, we examined the phylogenetic relationships among the trilling *Pseudacris* and tested previously hypothesized scenarios for speciation using 2.4 kb of mitochondrial 12S and 16S rRNA genes from 253 populations. Bayesian phylogenetic analyses, in combination with published morphological and behavioral data, support recognition of at least nine species, including an undescribed species from the south-central United States. Evidence is presented for substantial geographic subdivision within *P. brachyphona* (northern and southern clades) and *P. feriarum* (coastal and inland clades). Discordance between morphology/behavior and molecular data in several individuals suggests occasional hybridization between sympatric species. These results require major revision of range limits for several taxa, in particular, *P. maculata*, *P. triseriata*, and *P. feriarum*. Hypothesis tests using parametric bootstrapping strongly reject previously proposed scenarios for speciation in the group. The tests also support recognition of the geographically restricted taxon *P. kalmi* as a distinct species. Results of this study provide both a firm phylogenetic basis for future studies of speciation in the trilling *Pseudacris* and a taxonomic framework for conservation efforts.
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*Keywords:* *Pseudacris*; Trilling chorus frogs; Hybridization; Contact zones; Phylogeography; Cryptic species

## 1. Introduction

Deciphering the phylogenetic relationships among taxa and determining how the patterns observed relate to known historical events are important to our understanding of speciation. Although the biogeographic origins and higher-order relationships among hylid treefrogs of North America have been elucidated (Middle American Clade; Smith et al., 2005), additional work is required to understand evolutionary relationships within each of the three main North American genera (*Hyla*, *Acris*, and *Pseudacris*). The trilling chorus frogs form a clade within *Pseudacris* (Moriarty and Cannatella, 2004). The eight putative species (*P. brachyphona*, *P. brimleyi*, *P. clarkii*, *P. feriarum*, *P. kalmi*, *P. maculata*, *P. nigrita*, and *P. triseriata*) generally have parapatric distributions across the continent from northern Mexico to northern Canada and from the East Coast to the Rocky Mountains (Conant and Collins, 1998; Moriarty and Cannatella, 2004). The taxonomic status of half of these species is supported by morphological and behavioral data. The other four taxa (*P. feriarum*, *P. kalmi*, *P. maculata*, *P. triseriata*) were elevated from subspecies to species primarily on the basis of acoustic data,

* Corresponding author. Fax: +1 512 471 3878.
*E-mail address:* chorusfrog@mail.utexas.edu (E.M. Lemmon).

and there is ambiguity with respect to geographic boundaries based on these data (Platz, 1989; Platz and Forester, 1988). To gain insight into the process of speciation in this group, the phylogenetic relationships and range boundaries of these taxa must first be resolved using genetic data.

Although some trilling chorus frog species are distinguishable based on color pattern or advertisement call structure (Brandt and Walker, 1933; Neill, 1949; Smith, 1934; Walker, 1932); a number of putative lineages are more cryptic. Smith and Smith (1952) and Smith (1956) defined the distributions of several trilling *Pseudacris* taxa based on geographic patterns of tibia length to body length ratios. By plotting population means of ratios on a map, they identified morphometric clines, which were then used to define the boundaries between taxa. These authors found a general trend of relatively shorter leg lengths in populations from the northwestern USA and Canada and relatively longer legs in populations in the southeastern USA. These morphological differences translate into behavioral differences as well: short-legged frogs tend to walk rather than hop (Smith and Smith, 1952). Taxonomic designations based on these morphological studies have been generally accepted, (Fig. 1; Conant and Collins, 1998) but recent genetic work cast doubt on the accuracy of these designations (Moriarty and Cannatella, 2004). Molecular data point to a need for wider sampling to identify cryptic lineages and to delineate geographic distributions of species.

The trilling chorus frogs have been the subject of important studies of speciation. Fouquette (1975) and Gartside (1980) independently studied the contact zone between *P. feriarum* and *P. nigrita* and found disparate outcomes of secondary contact in different areas of sympatry. In the Apalachicola River drainage (Florida), the two species show reproductive character displacement of their acoustic signals (Fouquette, 1975). In the Pearl River drainage (Louisiana/Mississippi), they hybridize freely and lack differentiation of calls (Gartside, 1980). Although these apparent differences are extremely interesting from an evolutionary standpoint, what remains unclear is whether these authors examined the same species pair across the contact zone or whether a third species was involved. If a single species pair was studied across the zone, each species, as currently defined, should be monophyletic.

Two scenarios have been put forward to explain the origin of trilling *Pseudacris* in eastern North America. Smith (1957) proposed that following the Wisconsin glaciation (12–110 ka; Denton and Hughes, 1981; Gibbard and Van Kolfschoten, 2004), *P. kalmi* diverged from *P. triseriata* after an eastward expansion of the latter species left relict populations in New Jersey and the Delmarva Peninsula (Fig. 2). These relict populations became what is now called *P. kalmi*. Therefore, *P. kalmi* and *P. triseriata* are predicted to be sister species. Smith (1957) also proposed that when *P. triseriata* expanded eastward, it bisected the range of the widespread *P. feriarum*, leaving populations of *P. feriarum* in the eastern Great Lakes region that were isolated from the main distribution of the species (Fig. 2). Thus, populations in the Great Lakes region are predicted to form a monophyletic group with other *P. feriarum* populations. These scenarios of migration and divergence were based on morphometric data for chorus frogs throughout North America (Smith, 1956; Smith and Smith, 1952), but these hypotheses have not been tested within a genetic framework.

The goals of this study are twofold. First, we elucidate the phylogenetic relationships and establish geographic ranges of the trilling *Pseudacris*, using 2.4 kb of mitochondrial DNA from a dense taxon sample. Second, we test three scenarios for speciation and the associated taxonomic hypotheses, based on assumptions or predictions of previous authors. Detailed tests of timing of speciation and geographic expansion are presented elsewhere (Lemmon et al., in press). The results of this study advance our understanding of the patterns and processes of speciation in this group. In addition, identification of more precise species distributions facilitates efforts to conserve these frogs.

## 2. Methods

### 2.1. Sampling

We sampled chorus frogs from 253 populations (258 total individuals) across North America (Fig. 3; Appendix A). Approximately 30% of the populations were collected by EML; 10% were borrowed from museum or personal tissue collections; the remaining 60% were collected for this project by herpetologists across the continent (Appendix A; see Acknowledgments). Appropriate scientific permits were
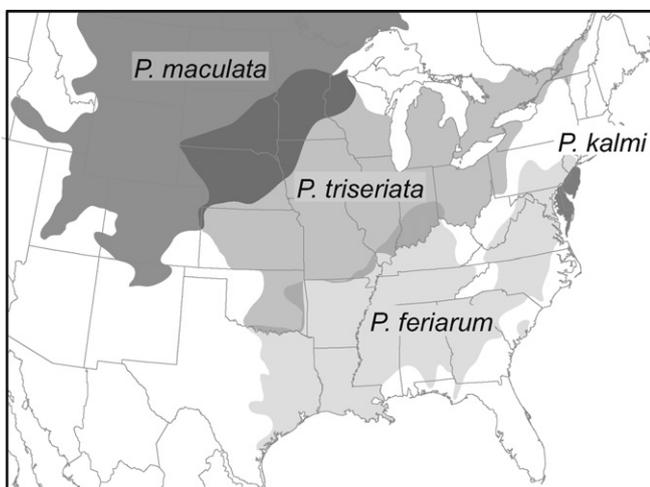


Fig. 1. Distributions of four trilling chorus frog taxa based on earlier nongenetic studies. This figure was modified from Conant and Collins (1998), which was largely based on morphometric data of Smith (1956, 1957), and Smith and Smith (1952). Distributions of *Pseudacris brachyphona*, *P. brimleyi*, *P. clarkii*, *P. nigrita*, and *P.* sp. nov. are not shown because ranges of these species have not changed substantially with the addition of genetic data.
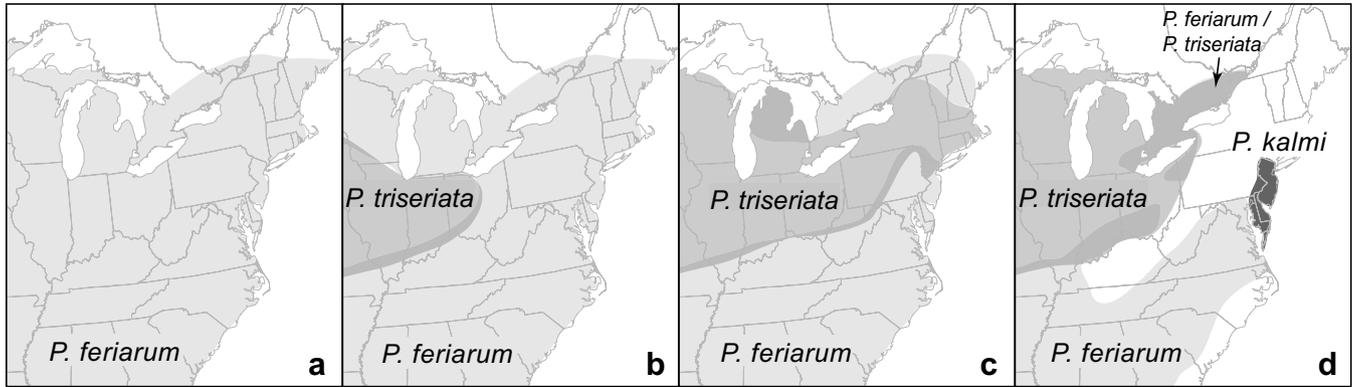
Fig. 2. Scenarios for migration and speciation in the trilling *Pseudacris* proposed by Smith (1957). According to this scenario, the distribution of the wide-ranging *P. feriarum* (a) was bisected by eastward expansion of *P. triseriata* (b), leading to geographic isolation of northern *P. feriarum* populations (c). *Pseudacris triseriata* reached the East Coast where it left relict populations when its range contracted (d). These relict populations are now known as *P. kalmi*.
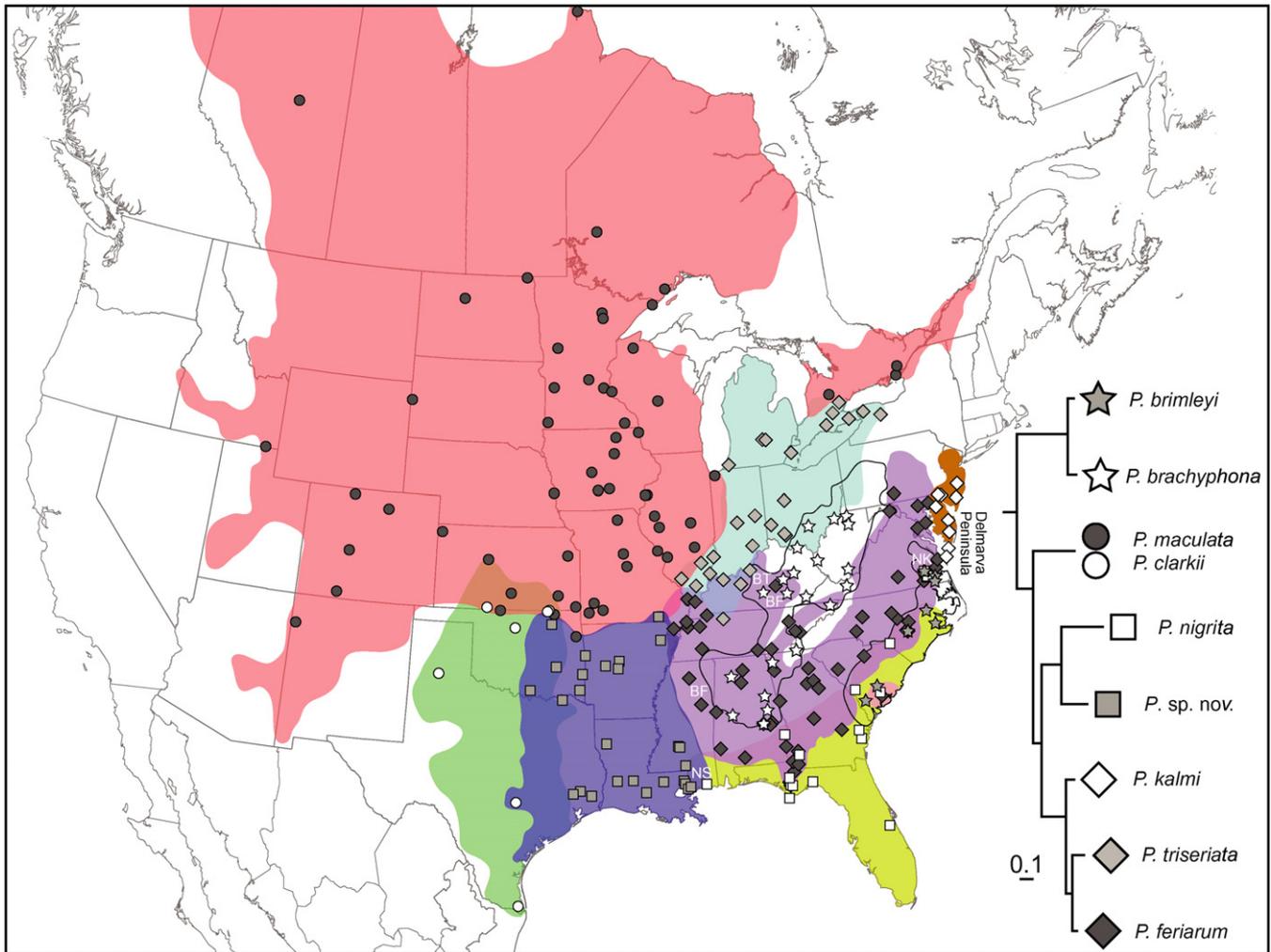


Fig. 3. Updated distributions of all North American Trilling Frogs. Species boundaries are based on the phylogeny (Fig. 4) and county-level taxon records from Lannoo (2005). Markers indicate populations sampled that correspond to species in the phylogeny to the right. Ranges of *Pseudacris brachyphona* and *P. brimleyi* are outlined in black for visual simplicity. Capital letters indicate hybrids and represent the following hybrid combinations: NS–*P. nigrita*–*P.* sp. nov., BF–*P. brachyphona*–*P. feriarum*, BT–*P. brachyphona*–*P. triseriata*, and NK–*P. nigrita*–*P. kalmi*, where the first species listed refers to the behavioral/morphological identity and the second to the mitochondrial DNA identity of the individual. Degree of geographic overlap between species is indicated on map if known; if no overlap is shown between parapatric taxa, then the amount of overlap is unknown.

obtained for collection of specimens. The sample includes 16 basal *Pseudacris* populations (outgroups: *Pseudacris regilla*, *P. cadaverina*, *P. crucifer*, *P. ocularis*, *P. ornata*, *P. streckeri*, and *P. illinoensis*) and 237 trilling *Pseudacris* populations (ingroup: *P. brachyphona*, *P. brimleyi*, *P. maculata*, *P. clarkii*, *P. nigrita*, *P.* sp. nov., *P. kalmi*, *P. feriarum*, *P. triseriata*; Moriarty and Cannatella, 2004). Our sample encompasses all currently described chorus frog species, with the exception of two recently resurrected members of the *P. regilla* species group (Recuero et al., 2006). We focused our sampling efforts on potential contact zones among taxa, particularly along major river drainages and mountain systems, as well as on the edges of species' distributions. Tissues were either frozen in liquid nitrogen or placed in tissue buffer or 95% ethanol and then stored at −80 °C. Specimens were deposited into museums listed in Appendix A.

## 2.2. DNA sequencing and data alignment

Following the methods described in Moriarty and Cannatella (2004), we sequenced eight DNA fragments from a ∼2.4-kb region spanning the 12S, tRNA$^{Val}$, and 16S mitochondrial rRNA genes. Contiguous sequences were constructed using Sequencher 4.5 (GeneCodes). Sequences were aligned in Clustal X 1.8 (Thompson et al., 1997) and the alignment was manually checked in MacClade (4.08; Maddison and Maddison, 2005). Uneven leading and trailing sequence as well as ambiguously aligned regions were identified in MacClade and excluded from further analysis. The 12S, tRNA$^{Val}$, and 16S genes were used as character partitions described below. To maximize the genetic diversity and geographic area sampled, we sequenced 1–2 individuals from many populations rather than several individuals from fewer populations. This sampling minimized the number of redundant haplotypes in the dataset, which were omitted from phylogenetic analyses. All sequences were deposited in Genbank (Appendix A) and the dataset was deposited in TreeBase (www.treebase.org; S1802).

## 2.3. Phylogenetic methods

We used a Bayesian approach to estimate phylogenetic relationships. To determine the appropriate model of evolution for each of the three partitions (12S: GTR + I + G, tRNA$^{Val}$: TRN + I, 16S: GTR + I + G), we employed the Akaike information criterion (Akaike, 1974) as implemented in MODELTEST 3.06 (Posada and Crandall, 1998). Since the TRN + I model is not available in MrBayes 3.1.1 (Ronquist and Huelsenbeck, 2003), we used the more general GTR + I model because overparameterization is less likely to cause bias than underparameterization (Lemmon and Moriarty, 2004). We performed six separate partitioned Bayesian analyses (with four heated chains per analysis) using MrBayes with default prior (prset) and proposal (prop) settings. All parameters were unlinked across partitions except branch lengths, which were not unlinked for two reasons. First, we desired branch lengths that represented the average number of substitutions per site across the entire region sequenced. Second, because recombination among mitochondrial partitions is unlikely, all partitions share a common gene tree.

The posterior probability distribution was estimated using the last 75% of the Markov chain samples. Convergence of the Markov chains on the posterior distribution was assessed by comparing bipartition posterior probability estimates across the six runs. We sampled from the chains every 1000 generations until the *maximum* standard deviation of bipartition posterior probability estimates across runs was less than 0.0625. Running the chains until this level of agreement among the independent runs was reached assured that the runs converged on the posterior distribution and that enough samples were taken to estimate the phylogeny accurately. We also compared distributions for the model parameters across the six runs, which reached stationarity at 3000 samples. A total of 92,682 samples was used to estimate the posterior distribution. A fully resolved tree was obtained by constructing a majority-rule consensus tree from the posterior distribution. Branch lengths were estimated as the average across the 92,682 samples.

## 2.4. Phylogenetic hypothesis testing

We tested three previously proposed taxonomic hypotheses concerning the phylogenetic origin of *Pseudacris* lineages. Hypothesis 1 posits that *P. kalmi* populations from New Jersey and the Delmarva Peninsula are relict populations of *P. triseriata* from an eastward expansion of the latter species. Therefore, *P. kalmi* is predicted to be most closely related to *P. triseriata* (Smith, 1957; Fig. 2). Hypothesis 2 predicts that chorus frogs in southeastern Ontario and New York are relict *P. feriarum* from a northward expansion of the species (Smith, 1957; Fig. 2). Hypothesis 3 states that chorus frogs in Louisiana, Arkansas, and westward are *P. feriarum* (Fouquette, 1975; Gartside, 1980; Smith and Smith, 1952). Following Moriarty and Cannatella (2004), we used a parametric bootstrapping approach to test these hypotheses. The null hypotheses are: (1) *P. kalmi* and *P. triseriata* populations form a monophyletic group, (2) *P. feriarum*, southeastern Ontario, and New York populations form a monophyletic group, and (3) eastern and western *P. feriarum* populations form a monophyletic group. For computational efficiency, we used subsets of the full dataset for each test; each subset included up to 10 geographically dispersed populations of the focal species, 3 populations of each of the other trilling *Pseudacris* species, and 3 representative outgroups. This arrangement produced datasets of 47, 58, and 51 terminals, respectively (see Appendix A for populations included).
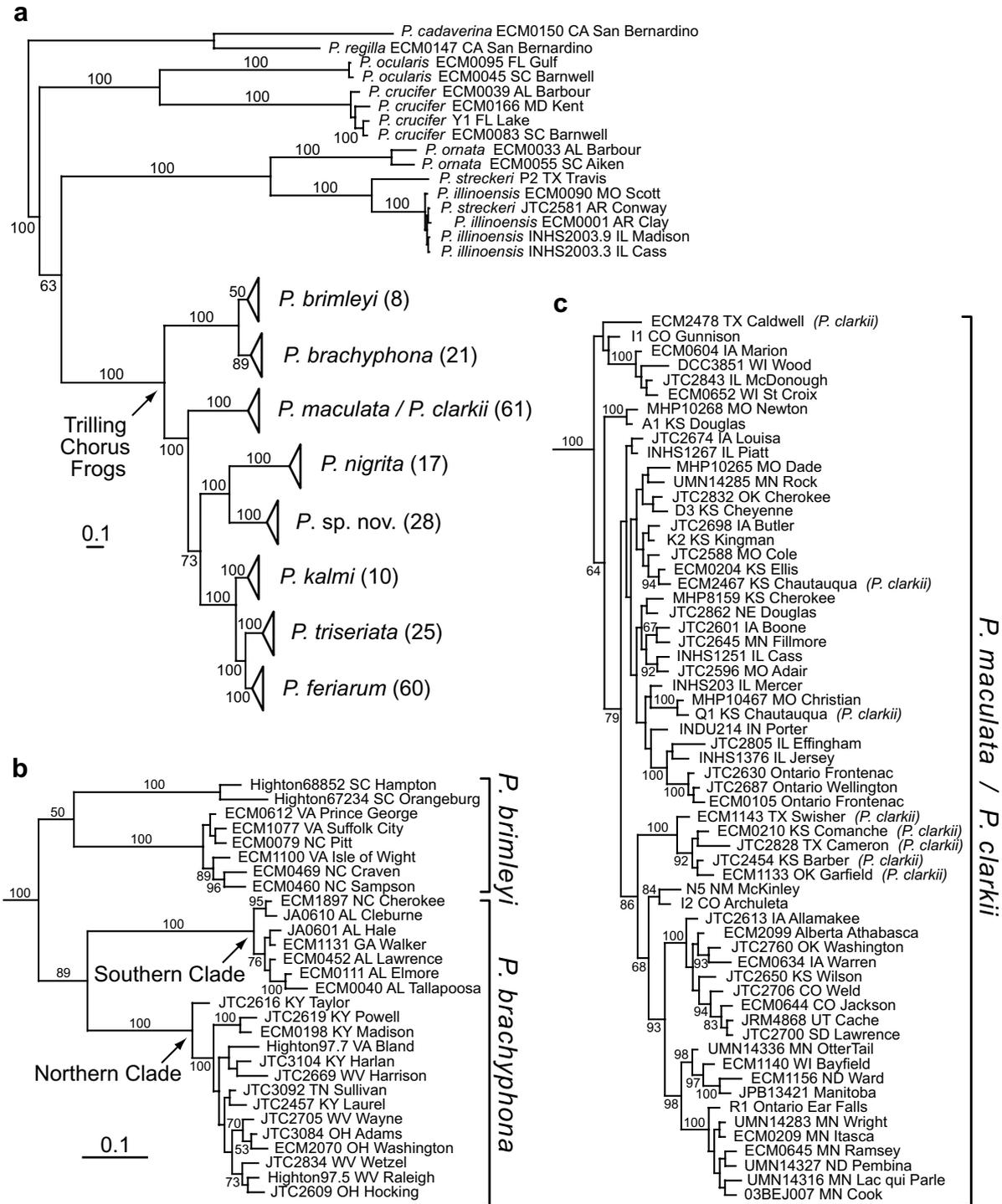
Fig. 4. Bayesian phylogeny of *Pseudacris*. Tree a shows the phylogenetic relationships of the entire genus. Numbers of populations sampled from each species are indicated in parentheses. Trees b–e illustrate the population-level relationships of each subclade. Each tip on the phylogeny is described by a field number, state/province, and county/region of origin. Bayesian posterior probabilities above 50% are located near corresponding branches. Species names in parentheses indicate the morphological/behavioral identity of individuals when this conflicted with the mitochondrial clade identity. Note that the branch length scale for phylogeny a is 25% of the scale for phylogenies b–e.

## 2.5. Designating species

Geographic populations corresponding to haplotype clades were assigned species names based on the inclusion of the type locality within the range of the haplotype clade. Each major haplotype clade contained not more than one type locality, and therefore species designations were straightforward. Type locali-
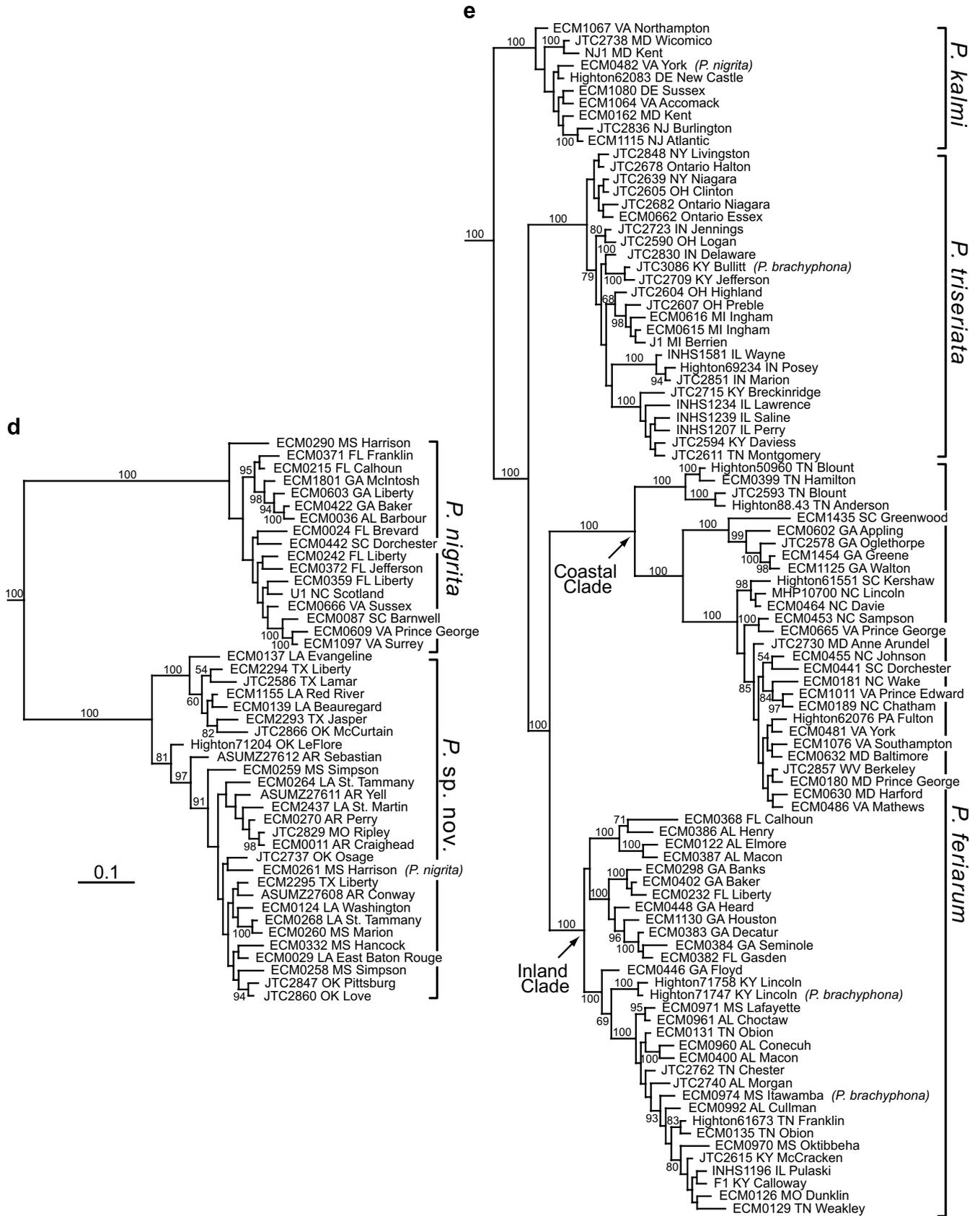
Fig. 4 (*continued*)

ties for the trilling chorus frogs are listed by Frost (2006).

## 3. Results

### 3.1. Phylogenetic relationships

A dataset of 2401 characters was used for the phylogenetic analyses, after exclusion of 164 edge or ambiguously aligned sites; 685 sites were variable and 567 were parsimony-informative. Twelve individuals with redundant haplotypes were identified from the 258 sequences (Appendix A). The phylogeny shows strong support for the monophyly of most currently recognized trilling *Pseudacris* species (Fig. 4a).

*Pseudacris feriarum* is the sister taxon of *P. triseriata*. There is geographic separation of *P. feriarum* into a coastal lineage and an inland lineage, separated roughly by the Altamaha River in eastern Georgia (Fig. 4e; following the nomenclature of Wright and Wright, 1949). The distribution of *P. triseriata* is more restricted than previously described (Smith, 1956, 1957; Smith and Smith, 1952; Figs. 1 and 3). The New Jersey/Delmarva Peninsula native, *P. kalmi*, is the sister taxon of the *P. feriarum* + *P. triseriata* clade (Fig. 4e). *Pseudacris nigrita* is the sister lineage to an undescribed species (*P.* sp. nov.; Fig. 4d), previously thought to be a western extension of *P. feriarum*. *Pseudacris nigrita* + *P.* sp. nov. form the sister clade of *P. triseriata* + *P. feriarum* + *P. kalmi* (Fig. 4a). The *P. maculata*/*P. clarkii* clade (Fig. 4c) is the sister-group of these five species (Fig. 4a). Although *P. maculata* and *P. clarkii* are readily distinguishable in sympatry by morphology and behavior, their mitochondrial gene trees are not reciprocally monophyletic, suggesting recent mitochondrial introgression or incomplete lineage sorting. In concordance with Moriarty and Cannatella (2004), *P. brachyphona* and *P. brimleyi* are sister species (Fig. 4b); this clade is the sister taxon of the remaining trilling *Pseudacris* (Fig. 4a). There is no clear support for reciprocal monophyly of the gene trees of these two species, despite their allopatry and morphological differentiation, suggesting incomplete lineage sorting. Within *P. brachyphona* there is geographic division into a northern lineage and a southern lineage (Fig. 4b).

### 3.2. Phylogenetic hypothesis tests

The parametric bootstrapping results do not support previous hypotheses regarding the biogeographic origin of trilling chorus frogs. In all three tests, the null hypothesis was rejected. Test 1 indicates that populations of *P. kalmi* in New Jersey and the Delmarva Peninsula are not simply relictual *P. triseriata* ($P = 0.028$). In fact, the phylogeny indicates that *P. kalmi* separated from an ancestral lineage of *P. triseriata* and *P. feriarum* prior to the divergence of the latter two species (Fig. 4). Based on this phylogenetic evidence and also previous work on advertisement calls

(Platz and Forester, 1988), we advocate continued recognition of *P. kalmi* as a distinct species. Test 2 shows that southeastern Ontario and New York populations are not derivatives of *P. feriarum* ($P < 0.002$). Rather, these populations encompass a contact zone between *P. maculata* and *P. triseriata* (Figs. 3 and 4). Test 3 indicates that the distribution of *P. feriarum* does not extend from Pennsylvania to Texas ($P < 0.002$). Instead, populations in Louisana, Arkansas, and westward represent a new species of chorus frog, which is the sister taxon of *P. nigrita* (Figs. 3 and 4). This previously unidentified species is currently being described elsewhere (Lemmon et al., unpublished manuscript).

### 3.3. Evidence for hybridization

The phylogeny points to several cases where the mitochondrial clade to which an individual belongs does not correspond to its morphological and behavioral identity. In particular, we found evidence for hybridization between: *P. kalmi*–*P. nigrita* (1 individual), *P. feriarum*–*P. brachyphona* (2), *P. triseriata*–*P. brachyphona* (1), and *P.* sp. nov.–*P. nigrita* (1; Figs. 3 and 4; Appendix A). In each of these cases, the mitochondrial clade to which an individual belongs is listed first and the identity of the individual based on morphological and acoustic data is listed second. The data do not allow us to distinguish between recent hybrids and advanced-generation hybrids. These results support the idea that occasional introgression occurs between trilling *Pseudacris* lineages.

## 4. Discussion

### 4.1. Species diversity within the trilling chorus frogs

Our phylogenetic analysis of mitochondrial lineages and tests of speciation hypotheses support the recognition of at least nine species within the trilling *Pseudacris* clade: *P. brachyphona*, *P. brimleyi*, *P. clarkii*, *P. feriarum*, *P. kalmi*, *P. maculata*, *P. nigrita*, *P.* sp. nov., and *P. triseriata*. These analyses are corroborated by previously published behavioral and morphological data. Revised range distributions of these taxa are shown in Fig. 3. We also found evidence for substantial genetic and geographic structure within *P. brachyphona* (northern and southern clades) and *P. feriarum* (coastal and inland clades), but we refrain from decisions about splitting these taxa until morphological, ecological, or behavioral differentiation between lineages of these species has been demonstrated.

An interesting finding is that *P. maculata* and *P. clarkii* are not genetically differentiated with respect to their mitochondrial DNA. If only the mitochondrial gene trees were considered, *P. clarkii* would be synonymized under *P. maculata*. However, substantial differences in morphology and behavior exist (Smith, 1934; Lemmon et al., unpublished manuscript), suggesting that the mitochon-

drial pattern results from hybridization and/or incomplete lineage sorting. Evidence suggests that both processes may be occurring in this species pair. Fieldwork in the contact zone has yielded individuals with intermediate advertisement calls and morphology between the two species, supporting the hypothesis of hybridization (Lemmon et al., unpublished data). On the other hand, the fact that allopatric *P. clarkii* populations far from the contact zone (west and south Texas) do not form a separate clade from *P. maculata*, is more suggestive of incomplete lineage sorting (Figs. 3 and 4). In this scenario, the two species have undergone rapid morphological and behavioral differentiation relative to molecular divergence, resulting in taxa with distinct phenotypes and acoustic signals that are not, however, reciprocally monophyletic. Data from nuclear markers are needed, however, to determine whether hybridization or incomplete lineage sorting contributed more to the patterns of genetic variation observed. Given the degree of morphological and behavioral divergence between the taxa, we maintain *P. maculata* and *P. clarkii* as separate species, until further data suggest otherwise.

This study provides genetic identification for several marginal populations of uncertain origin including *P. maculata* from Arizona and New Mexico (formerly *P. triseriata*; Smith and Smith, 1952; Platz, 1989), disjunct *P. feriarum* from Berkeley, Charleston, and Dorchester Counties, South Carolina (*P. feriarum*; Schwartz, 1957), disjunct *P. nigrita* from eastern Virginia (new state record; Hobson and Moriarty, 2003), and *P. maculata* and *P. triseriata* from southeastern Ontario (formerly *P. triseriata* only Bleakney, 1959; Cook, 1964; Figs. 3 and 4). In addition, this study provides strong support for recognition of the geographically restricted taxon, *P. kalmi* (formerly *P. feriarum kalmi*, Hedges, 1986) as a distinct species. Furthermore, we have found evidence for a new cryptic species of chorus frog, previously undetected within *P. feriarum* (Smith and Smith, 1952). In fact, this new species is the sister species of *P. nigrita*, with which it forms a narrow hybrid zone in the Pearl River drainage along the boundary between southern Louisiana and Mississippi (Gartside, 1980; Figs. 3 and 4d).

Earlier studies of the contact zone between *P. nigrita* and presumed "*P. feriarum*" in the Apalachicola River drainage of Florida (Fouquette, 1975) and the Pearl River drainage of Louisiana and Mississippi (Gartside, 1980) found disparate outcomes of secondary contact between these taxa. Fouquette (1975) observed a strong pattern of reproductive character displacement between taxa and found no evidence for hybridization, although an allozyme study of Gartside (1980) described a high frequency of hybridization between taxa (center of zone has 60% hybrids). We purposely sampled the majority of the *P. feriarum* populations examined in the Fouquette (1975) and Gartside (1980) studies to ascertain their taxonomic identity and found that all Fouquette (1975) localities are true *P. feriarum* (inland clade) and all Gartside

(1980) localities are a third, cryptic species, *P.* sp. nov. (Figs. 3 and 4). This clarifies why there is a higher incidence of hybridization along the Pearl River (sister taxa) compared to the apparently low incidence along the Apalachicola River (non-sister taxa). Our results indicate that reproductive character displacement occurs between non-sister species in this group (*P. nigrita* and *P. feriarum*; Figs. 3 and 4; Fouquette, 1975).

Results of the parametric bootstrapping tests have important implications for both conservation and speciation in chorus frogs. The tests do not support the biogeographic scenarios proposed by Smith (1957) for speciation in the trilling *Pseudacris* (Figs. 2 and 3). Intriguingly, however, one of the morphological clines identified by Smith and Smith (1952) corresponds very closely to boundaries between mitochondrial lineages. These authors found a steep cline in relative leg length that runs perpendicular to a line stretching from southern Indiana and Illinois (Ohio River drainage), across the boundary between Missouri and Arkansas, and into eastern Oklahoma (Fig. 1). They interpreted this line as the boundary between *P. feriarum* and *P. triseriata*. Our data show that, in fact, four lineages come into contact along this line: *P. feriarum* and *P. triseriata* in the east and *P. maculata/clarkii* and *P.* sp. nov. in the west (Fig. 3). Although Smith and Smith (1952) did not find east–west morphological differentiation at species boundaries, they were able to identify the border between the two north–south species pairs. The congruence between molecular and morphological data provides further support for delineation of these species boundaries.

## 4.2. Evidence for hybridization among species

An interesting pattern that emerges is that most trilling *Pseudacris* lineages hybridize with nearby relatives. Prior to this study, natural hybridization was known only between *P. nigrita* and *P.* sp. nov. (Gartside, 1980) and between *P. clarkii* and *P.* sp. nov. (Michaud, 1964), although laboratory experiments had demonstrated viability of several other hybrid crosses (Mecham, 1965). We show evidence for sporadic mitochondrial introgression in nature between three additional species pairs: *P. kalmi*–*P. nigrita*, *P. feriarum*–*P. brachyphona*, and *P. triseriata*–*P. brachyphona* as well as further evidence for hybridization between *P.* sp. nov–*P. nigrita*. These data suggest that despite large differences in reproductive behaviors (measured by acoustic signals, Lemmon et al., unpublished manuscript), frogs occasionally fail to avoid heterospecific mates. The potential for hybridization can lead to reinforcement (Howard, 1993) and, in some cases, result in differentiation of reproductive signals in sympatry (Fouquette, 1975). This pattern of hybridization underlines the importance of using multiple lines of evidence to delimit species (e.g., morphology, genetics, behavior). It also illustrates the usefulness of mitochondrial genes in identifying areas of genetic admixture.

Future studies should incorporate nuclear markers to establish the utility of mitochondrial DNA in defining species boundaries.

### 4.3. Implications for conservation of Pseudacris

Declining amphibian species have been reported from many regions of North America where *Pseudacris* are found (Gray and Brown, 2005; Reeder et al., 2005; Rorabaugh, 2005). Whereas several other frog taxa (in particular, *Rana* and *Acris*) have experienced declines in parts of the United States, *Pseudacris* populations appear less affected or stable in some areas (Corn et al., 1989; Fisher and Shaffer, 1996). This disparity may be due, in part, to the different natural histories of these taxa. Although *Acris* and *Rana* spend much of their life cycle near their natal ponds, *Pseudacris* disappear from breeding ponds after metamorphosis, dispersing to nearby fields and woods, and returning only for the next year's brief breeding season (Kramer, 1973; Kramer, 1974). Because a number of emerging amphibian diseases are transmitted via water (Daszak et al., 1999; Jancovich et al., 2001; Lips et al., 2006), *Pseudacris* may have an advantage over more aquatic frogs by avoiding bodies of water for the majority of their life cycle.

There are some notable exceptions, however, to the overall pattern of stability in chorus frog populations. Recent field surveys have suggested that several species are declining in parts of the northeastern USA and southeastern Canada (Gibbs et al., 2005; Picard and Desroches, 2004; Pollio and Kilpatrick, 2002; Sias, 2006; Weeber and Vallianatos, 2000; J. Andrews and M. Ferguson, unpublished data; C. Pollio, unpublished data). In addition, several species have been listed by state wildlife agencies as species of conservation concern (*P. feriarum*: Pennsylvania, West Virginia; *P. triseriata*: Pennsylvania; *P. brachyphona*: Pennsylvania; *P. maculata*: Michigan), state threatened (*P. brachyphona*: Maryland), or state endangered (*P. kalmi*: Pennsylvania). In southeastern Ontario and New York, surveys have found that eastern populations have declined but the western populations appear stable (Gibbs et al., 2005; Picard and Desroches, 2004; F. Schueler, unpublished data). Our data indicate that declining eastern populations are *P. maculata* whereas stable western populations are *P. triseriata* (Fig. 3). The apparent declines have been attributed to several factors, including habitat loss, agricultural runoff, and industrial pollution (Gibbs et al., 2005; Pollio and Kilpatrick, 2002; Sias, 2006). Clearly, more focused research is needed to track the causes of these declines. Our study contributes to conservation efforts by defining the taxonomic status and range limits of these taxa. Currently, the trilling *Pseudacris* species that presents the most urgent conservation challenge is the New Jersey Chorus Frog, *P. kalmi*. In this study we have demonstrated that *P. kalmi* is a distinct species. Due to its restricted range, which is located in one of the most densely populated areas of the USA, *P. kalmi* faces extinction particularly through habitat loss. Conservation measures must be undertaken immediately to preserve the remaining populations of this species.

## Appendix A

The appendix uses updated taxonomy for each population. Field number, museum voucher number, and GenBank Accession Numbers are listed. Specimens with the same superscript letter following the field number have identical haplotypes. Putative hybrids are denoted by the following symbols after the field number: **α** (*P. brachyphona* with *P. feriarum* mtDNA), **β** (*P. brachyphona* with *P. triseriata* mtDNA), **ε** (*P. nigrita* with *P.* sp. nov. mtDNA), and **φ** (*P. nigrita* with *P. kalmi* mtDNA). A "n/a" under the museum number header means no voucher specimen is available for that specimen. Vouchers that have not been cataloged are listed as such for respective collections. Superscript numbers following the museum numbers refer to footnotes at the end of the appendix. An asterisk next to a GenBank Number indicates previously published sequences from Moriarty and Cannatella (2004). The test column denotes which taxa were used in each of the three parametric bootstrapping tests (e.g. ECM0041 was included in all three tests). State or province of origin and county, township, or region information is listed for each specimen in addition to GPS coordinates. Museum collection codes are as follows: Arkansas State Museum Herpetology Collection, Jonesboro (ASUMZ), Bell Museum of Natural History, Minneapolis (JFBM), Canadian Museum of Nature, Ottawa (CMN), Cincinnati Museum Center, Museum of Natural History and Science (CMC), Illinois Natural History Survey (INHS), Museum of Vertebrate Zoology, Berkeley (MVZ), North Carolina State Museum of Natural History, Raleigh (NCSM), Royal Ontario Museum, Toronto (ROM), Smithsonian National Museum of Natural History, Washington, D.C. (USNM), Sternberg Museum of Natural History, Fort Hays State University (MHP), Texas Natural History Collection, University of Texas, Austin (TNHC), University of Alabama Herpetology Collection, Tuscaloosa (UAHC), and University of Kansas Museum of Natural History, Lawrence (KU).

List of *Pseudacris* specimens included in this study

| Species | Field no. | Museum no. | GenBank No. | Test | State/Prov. | County/Twshp. | Latitude | Longitude |
|---|---|---|---|---|---|---|---|---|
| *P. brachyphona* | ECM0040 | TNHC62303 | AY291095* | | AL | Tallapoosa | 33.0064 | −85.7603 |
| *P. brachyphona* | ECM0041[A] | TNHC62304 | EF472011 | 1 2 3 | AL | Tallapoosa | 33.0064 | −85.7603 |
| *P. brachyphona* | ECM0111 | TNHC62305 | EF472014 | | AL | Elmore | 32.5175 | −86.0071 |
| *P. brachyphona* | ECM0198 | TNHC62315 | EF472022 | | KY | Madison | 37.6503 | −84.2417 |
| *P. brachyphona* | ECM0452 | TNHC63121 | EF472012 | | AL | Lawrence | 34.3344 | −87.3503 |
| *P. brachyphona* | ECM0974α | TNHC63443 | EF472190 | | MS | Itawamba | 34.1679 | −88.3754 |
| *P. brachyphona* | ECM1131 | n/a | EF472013 | 1 2 3 | GA | Walker | 34.7048 | −85.2819 |
| *P. brachyphona* | ECM1897[A] | NCSM71330 | EF472017 | | NC | Cherokee | 35.0414 | −84.0520 |
| *P. brachyphona* | ECM2070 | n/a | EF472028 | | OH | Washington | 39.5478 | −81.2141 |
| *P. brachyphona* | JA-06-01 | UAHC15645 | EF472016 | | AL | Hale | 32.9222 | −87.4403 |
| *P. brachyphona* | JA-06-10 | UAHC15646 | EF472015 | | AL | Cleburne | 33.5136 | −85.8284 |
| *P. brachyphona* | JTC2457 | TNHC62402 | EF472019 | | KY | Laurel | 37.1333 | −84.1333 |
| *P. brachyphona* | JTC2609 | TNHC63535 | EF472020 | 1 2 3 | OH | Hocking | 39.4161 | −82.6018 |
| *P. brachyphona* | JTC2616 | TNHC63389 | EF472021 | | KY | Taylor | 37.2469 | −85.3197 |
| *P. brachyphona* | JTC2619 | TNHC63387 | EF472023 | | KY | Powell | 37.8169 | −83.6811 |
| *P. brachyphona* | JTC2669 | TNHC66044 | EF472018 | | WV | Harrison | 39.2715 | −80.5192 |
| *P. brachyphona* | JTC2705 | TNHC66046 | EF472026 | | WV | Wayne | 38.1679 | −82.3779 |
| *P. brachyphona* | JTC2834 | TNHC66047 | EF472027 | | WV | Wetzel | 39.5597 | −80.5567 |
| *P. brachyphona* | JTC3084 | CMC10360 | EF472031 | | OH | Adams | 38.7156 | −83.3233 |
| *P. brachyphona* | JTC3086β | MHP12900 | EF472183 | | KY | Bullitt | 37.8636 | −85.6356 |
| *P. brachyphona* | JTC3092 | MHP12896 | EF472030 | | TN | Sullivan | 36.4866 | −82.0717 |
| *P. brachyphona* | JTC3104 | MHP12898 | EF472029 | | KY | Harlan | 36.9279 | −83.2154 |
| *P. brachyphona* | R. Highton71747α | R. Highton uncat.[a] | AY291096* | | KY | Lincoln | 37.4358 | −84.6878 |
| *P. brachyphona* | R. Highton97-5 | n/a | EF472024 | | WV | Raleigh | 37.7489 | −80.9236 |
| *P. brachyphona* | R. Highton97-7 | n/a | EF472025 | | VA | Bland | 37.0372 | −81.1094 |
| *P. brimleyi* | ECM0079 | TNHC62337 | AY291094* | 1 2 3 | NC | Pitt | 35.7006 | −77.4094 |
| *P. brimleyi* | ECM0460 | TNHC63571 | EF472033 | | NC | Sampson | 35.0992 | −78.4772 |
| *P. brimleyi* | ECM0469 | TNHC63573 | EF472036 | | NC | Craven | 35.1892 | −77.0814 |
| *P. brimleyi* | ECM0612 | TNHC63667 | EF472032 | | VA | Prince George | 37.1229 | −77.1094 |
| *P. brimleyi* | ECM1077 | TNHC63669 | EF472035 | 1 2 3 | VA | Suffolk City | 36.6930 | −76.6953 |
| *P. brimleyi* | ECM1100 | TNHC63670 | EF472034 | | VA | Isle of Wight | 36.8666 | −76.6194 |
| *P. brimleyi* | R. Highton67234 | R. Highton uncat. | EF472037 | 1 2 3 | SC | Orangeburg | 33.3227 | −80.4137 |
| *P. brimleyi* | R. Highton68852 | R. Highton uncat. | EF472038 | | SC | Hampton | 32.5594 | −81.2844 |
| *P. cadaverina* | ECM0150 | TNHC62247 | EF472006 | 2 | CA | San Bernardino | 34.1132 | −117.1422 |
| *P. clarkii* | ECM0210 | TNHC63497 | EF472105 | | KS | Comanche | 37.1247 | −99.3258 |
| *P. clarkii* | ECM1133 | TNHC63548 | EF472102 | 1 2 3 | OK | Garfield | 36.3956 | −97.8784 |
| *P. clarkii* | ECM1143 | TNHC63159 | EF472103 | 1 2 3 | TX | Swisher | 34.6464 | −101.5722 |
| *P. clarkii* | ECM2467 | TNHC65044 | EF472107 | | KS | Chautauqua | 37.0401 | −96.1815 |
| *P. clarkii* | ECM2478 | TNHC65763 | EF472106 | | TX | Caldwell | 30.0205 | −97.6946 |

**Appendix A** (*continued*)

| Species | Field no. | Museum no. | GenBank No. | Test | State/Prov. | County/Twshp. | Latitude | Longitude |
|---|---|---|---|---|---|---|---|---|
| *P. clarkii* | JTC2454[E] | TNHC63533 | EF472109 | | KS | Barber | 37.0139 | −98.6492 |
| *P. clarkii* | JTC2455[E] | TNHC63534 | EF472108 | | KS | Barber | 37.0139 | −98.6492 |
| *P. clarkii* | JTC2828 | TNHC63138 | EF472104 | 1 2 3 | TX | Cameron | 26.1809 | −97.5198 |
| *P. clarkii* | Q-1 | KU289035 | AY291093* | | KS | Chautauqua | 37.0044 | −96.2764 |
| *P. crucifer* | ECM0039 | TNHC62210 | AY291099* | | AL | Barbour | 32.0369 | −85.0889 |
| *P. crucifer* | ECM0083 | TNHC62216 | AY291100* | 1 2 3 | SC | Barnwell | 33.3177 | −81.4840 |
| *P. crucifer* | ECM0166 | TNHC62221 | EF472007 | | MD | Kent | 39.3122 | −75.8485 |
| *P. crucifer* | Y-1 | TNHC62369[b] | AY291103* | | FL | Lake | 29.0833 | −81.5833 |
| *P. feriarum* | ECM0122 | TNHC62268 | EF472173 | | AL | Elmore | 32.5175 | −86.0071 |
| *P. feriarum* | ECM0126 | TNHC62380 | EF472189 | 2 3 | MO | Dunklin | 36.2435 | −89.9622 |
| *P. feriarum* | ECM0129 | TNHC62271 | EF472169 | | TN | Weakley | 36.2579 | −88.6676 |
| *P. feriarum* | ECM0131 | TNHC62273 | EF472170 | | TN | Obion | 36.2579 | −89.2597 |
| *P. feriarum* | ECM0135 | TNHC62276 | EF472176 | | TN | Obion | 36.4529 | −89.3035 |
| *P. feriarum* | ECM0180 | TNHC62280 | EF472202 | | MD | Prince George | 38.6909 | −77.0137 |
| *P. feriarum* | ECM0181 | TNHC62385 | EF472206 | | NC | Wake | 35.6238 | −78.8999 |
| *P. feriarum* | ECM0189 | TNHC62287 | EF472205 | | NC | Chatham | 35.8530 | −79.1271 |
| *P. feriarum* | ECM0232 | TNHC63303 | EF472167 | 1 2 3 | FL | Liberty | 30.1626 | −85.0666 |
| *P. feriarum* | ECM0298 | TNHC63326 | EF472196 | | GA | Banks | 34.3322 | −83.5654 |
| *P. feriarum* | ECM0368 | TNHC63322 | EF472175 | | FL | Calhoun | 30.2847 | −85.1073 |
| *P. feriarum* | ECM0382 | TNHC63323 | EF472177 | | FL | Gasden | 30.6591 | −84.8323 |
| *P. feriarum* | ECM0383 | TNHC63358 | EF472172 | | GA | Decatur | 30.9081 | −84.5979 |
| *P. feriarum* | ECM0384 | TNHC63359 | EF472178 | | GA | Seminole | 31.0223 | −84.8292 |
| *P. feriarum* | ECM0386 | TNHC63122 | EF472174 | | AL | Henry | 31.6083 | −85.0710 |
| *P. feriarum* | ECM0387 | TNHC63123 | EF472168 | | AL | Macon | 32.5290 | −85.6016 |
| *P. feriarum* | ECM0399 | TNHC63685 | EF472163 | | TN | Hamilton | 35.1915 | −85.2459 |
| *P. feriarum* | ECM0400 | TNHC63133 | EF472161 | | AL | Macon | 32.4703 | −85.6908 |
| *P. feriarum* | ECM0402 | TNHC63333 | EF472179 | | GA | Baker | 31.3835 | −84.5430 |
| *P. feriarum* | ECM0441 | TNHC63537 | EF472197 | 2 3 | SC | Dorchester | 32.9552 | −80.2613 |
| *P. feriarum* | ECM0446 | TNHC63361 | EF472171 | 1 2 3 | GA | Floyd | 34.4076 | −85.2216 |
| *P. feriarum* | ECM0448 | TNHC63362 | EF472180 | | GA | Heard | 33.2765 | −85.1211 |
| *P. feriarum* | ECM0453 | TNHC63562 | EF472208 | | NC | Sampson | 35.1418 | −78.5562 |
| *P. feriarum* | ECM0455 | TNHC63564 | EF472201 | | NC | Johnson | 35.4392 | −78.3706 |
| *P. feriarum* | ECM0464 | TNHC63567 | EF472212 | 1 2 3 | NC | Davie | 35.8982 | −80.5764 |
| *P. feriarum* | ECM0481 | TNHC63627 | EF472200 | | VA | York | 37.1779 | −76.5007 |
| *P. feriarum* | ECM0486[I] | TNHC63642 | EF472209 | | VA | Mathews | 37.4451 | −76.3424 |
| *P. feriarum* | ECM0601[I] | TNHC63643 | EF472204 | | VA | Mathews | 37.4451 | −76.3424 |
| *P. feriarum* | ECM0602 | TNHC63364 | EF472203 | 2 3 | GA | Appling | 31.9522 | −82.3848 |
| *P. feriarum* | ECM0630 | TNHC63520 | EF472217 | 1 2 3 | MD | Harford | 39.5078 | −76.2195 |
| *P. feriarum* | ECM0632 | TNHC63522 | EF472198 | | MD | Baltimore | 39.4964 | −76.7617 |
| *P. feriarum* | ECM0665 | TNHC63644 | EF472207 | | VA | Prince George | 37.1229 | −77.1094 |
| *P. feriarum* | ECM0960 | TNHC63465 | EF472162 | | AL | Conecuh | 31.3546 | −87.0267 |
| *P. feriarum* | ECM0961[H] | TNHC63466 | EF472188 | | AL | Choctaw | 31.7484 | −88.1277 |
| *P. feriarum* | ECM0969[H] | TNHC63467 | EF472191 | | AL | Pickens | 33.0979 | −88.2033 |
| *P. feriarum* | ECM0970 | TNHC63439 | EF472192 | 1 2 3 | MS | Oktibbeha | 33.4282 | −88.8768 |
| *P. feriarum* | ECM0971 | TNHC63440 | EF472193 | | MS | Lafayette | 34.4114 | −89.3729 |
| *P. feriarum* | ECM0992 | TNHC63468 | EF472186 | 2 3 | AL | Cullman | 34.0928 | −86.8825 |
| *P. feriarum* | ECM1011 | TNHC63645 | EF472214 | | VA | Prince Edward | 37.0973 | −78.4770 |
| *P. feriarum* | ECM1076 | TNHC63652 | EF472216 | | VA | Southampton | 36.7804 | −77.2316 |
| *P. feriarum* | ECM1125 | n/a | EF472218 | | GA | Walton | 33.7948 | −83.7132 |
| *P. feriarum* | ECM1130 | n/a | EF472187 | | GA | Houston | 32.4960 | −83.6077 |
| *P. feriarum* | ECM1435 | TNHC65775 | EF472221 | | SC | Greenwood | 34.1505 | −82.1591 |
| *P. feriarum* | ECM1454 | TNHC65747 | EF472220 | | GA | Greene | 33.5745 | −83.2012 |
| *P. feriarum* | F-1 | KU289227 | AY291084* | | KY | Calloway | 36.6333 | −88.2667 |
| *P. feriarum* | INHS1196 | INHS18810 | EF472181 | | IL | Pulaski | 37.2769 | −89.1833 |
| *P. feriarum* | JTC2578 | TNHC63355 | EF472199 | | GA | Oglethorpe | 33.8628 | −83.4089 |
| *P. feriarum* | JTC2593 | TNHC63686 | EF472165 | 1 2 3 | TN | Blount | 35.7564 | −83.9706 |
| *P. feriarum* | JTC2615 | TNHC63393 | EF472182 | | KY | McCracken | 37.1597 | −88.7972 |
| *P. feriarum* | JTC2730 | TNHC63524 | EF472210 | | MD | Anne Arundel | 38.8283 | −76.5389 |
| *P. feriarum* | JTC2740 | TNHC63134 | EF472194 | | AL | Morgan | 34.5448 | −86.7639 |
| *P. feriarum* | JTC2762 | TNHC63689 | EF472195 | | TN | Chester | 35.4392 | −88.6414 |
| *P. feriarum* | JTC2857 | TNHC66049 | EF472211 | | WV | Berkeley | 39.4927 | −78.2772 |
| *P. feriarum* | MHP10700 | MHP10700 | EF472219 | | NC | Lincoln | 35.4135 | −80.9719 |
| *P. feriarum* | R. Highton50960 | n/a | EF472166 | | TN | Blount | 35.6911 | −83.7989 |
| *P. feriarum* | R. Highton61551 | USNM uncat. | EF472215 | | SC | Kershaw | 34.4782 | −80.8017 |

**Appendix A** (*continued*)

| Species | Field no. | Museum no. | GenBank No. | Test | State/Prov. | County/Twshp. | Latitude | Longitude |
|---|---|---|---|---|---|---|---|---|
| *P. feriarum* | R. Highton61673 | USNM uncat. | EF472184 | | TN | Franklin | 35.2031 | −85.9211 |
| *P. feriarum* | R. Highton62076 | USNM uncat. | EF472213 | | PA | Fulton | 40.0708 | −77.8839 |
| *P. feriarum* | R. Highton71758 | R. Highton uncat. | EF472185 | | KY | Lincoln | 37.4019 | −84.8092 |
| *P. feriarum* | R. Highton88-43 | n/a | EF472164 | | TN | Anderson | 36.1408 | −84.1047 |
| *P. illinoensis* | ECM0001 | TNHC62351 | AY291109* | | AR | Clay | 36.3308 | −90.1090 |
| *P. illinoensis* | ECM0090 | TNHC62346 | AY291110* | | MO | Scott | 37.0667 | −89.5667 |
| *P. illinoensis* | INHS2003.3 | n/a | EF472008 | | IL | Cass | 40.0175 | −90.4242 |
| *P. illinoensis* | INHS2003.9 | n/a | EF472010 | | IL | Madison | 38.7969 | −90.0389 |
| *P. kalmi* | ECM0162 | TNHC62354 | EF472224 | 1 2 3 | MD | Kent | 39.3122 | −75.8485 |
| *P. kalmi* | ECM1064 | TNHC63671 | EF472225 | 1 2 3 | VA | Accomack | 37.7501 | −75.6663 |
| *P. kalmi* | ECM1067 | TNHC63674 | EF472223 | 1 | VA | Northampton | 37.4749 | −75.8583 |
| *P. kalmi* | ECM1080 | TNHC63135 | EF472230 | 1 | DE | Sussex | 38.7459 | −75.3809 |
| *P. kalmi* | ECM1115[J] | TNHC63544 | EF472226 | 1 2 3 | NJ | Atlantic | 39.4765 | −74.7106 |
| *P. kalmi* | JTC2738 | TNHC63403 | EF472227 | 1 | MD | Wicomico | 38.3215 | −75.4499 |
| *P. kalmi* | JTC2836 | TNHC63546 | EF472228 | 1 | NJ | Burlington | 39.9593 | −74.5093 |
| *P. kalmi* | NJ-1 | KU289235 | AY291087* | 1 | MD | Kent | 39.3122 | −75.8485 |
| *P. kalmi* | R. Highton62067[J] | USNM uncat. | EF472229 | 1 | NJ | Salem | 39.6834 | −75.4905 |
| *P. kalmi* | R. Highton62083 | USNM uncat. | EF472222 | 1 | DE | New Castle | 39.7153 | −75.6259 |
| *P. maculata* | 03BEJ007 | TNHC63622 | EF472090 | | MN | Cook | 47.9105 | −90.0075 |
| *P. maculata* | 03EKH001[D] | TNHC63621 | EF472101 | | MN | St. Louis | 47.6988 | −93.0484 |
| *P. maculata* | A-1 | KU224560 | AY291090* | | KS | Douglas | 39.0068 | −95.2233 |
| *P. maculata* | D-3 | KU224558 | AY291092* | | KS | Cheyenne | 39.7722 | −101.7994 |
| *P. maculata* | DCC3851 | n/a | EF472080 | | WI | Wood | 44.4500 | −90.0500 |
| *P. maculata* | ECM0105 | TNHC62324 | AY291081* | | Ontario | Frontenac | 44.2333 | −76.5000 |
| *P. maculata* | ECM0204 | TNHC62296 | EF472123 | | KS | Ellis | 38.8352 | −99.3363 |
| *P. maculata* | ECM0209 | TNHC62389 | EF472078 | | MN | Itasca | 47.5000 | −93.0000 |
| *P. maculata* | ECM0604 | TNHC63365 | EF472088 | | IA | Marion | 41.3875 | −92.9526 |
| *P. maculata* | ECM0634 | TNHC63370 | EF472092 | | IA | Warren | 41.3375 | −93.5570 |
| *P. maculata* | ECM0644 | TNHC65773 | EF472091 | 2 | CO | Jackson | 40.8348 | −106.5705 |
| *P. maculata* | ECM0645 | n/a | EF472089 | | MN | Ramsey | 45.0051 | −93.1011 |
| *P. maculata* | ECM0652 | TNHC65814 | EF472093 | | WI | St. Croix | 44.8614 | −92.6236 |
| *P. maculata* | ECM1140[B] | TNHC65824 | EF472100 | | WI | Bayfield | 46.3941 | −91.2938 |
| *P. maculata* | ECM1156 | TNHC63139 | EF472094 | | ND | Ward | 48.1817 | −101.2924 |
| *P. maculata* | ECM2099 | n/a | EF472132 | | Alberta | Athabasca | 54.6155 | −113.3466 |
| *P. maculata* | I-1 | KU224624 | AY291080* | | CO | Gunnison | 38.8221 | −106.5744 |
| *P. maculata* | I-2 | KU224625 | AY291083* | | CO | Archuleta | 37.2898 | −106.9754 |
| *P. maculata* | INDU214 | n/a | EF472121 | | IN | Porter | 41.6100 | −87.2353 |
| *P. maculata* | INHS1251 | INHS18890 | EF472127 | | IL | Cass | 39.9242 | −90.3904 |
| *P. maculata* | INHS1267 | INHS13035 | EF472115 | | IL | Piatt | 40.0114 | −88.7261 |
| *P. maculata* | INHS1372[F] | INHS13057 | EF472124 | | IL | Madison | 38.8294 | −90.0628 |
| *P. maculata* | INHS1376 | INHS13062 | EF472122 | | IL | Jersey | 39.0778 | −90.5555 |
| *P. maculata* | INHS203 | INHS16769 | EF472113 | | IL | Mercer | 41.1031 | −90.9339 |
| *P. maculata* | JPB13421 | CMN32633 | EF472084 | 2 | Manitoba | Churchill | 58.7667 | −94.1667 |
| *P. maculata* | JPB22607[B] | ROM uncat. | EF472081 | 2 | Ontario | Fraleigh | 48.4500 | −89.2000 |
| *P. maculata* | JRM4868 | TNHC62405 | EF472083 | | UT | Cache | 42.0778 | −111.7222 |
| *P. maculata* | JTC2588 | TNHC63697 | EF472117 | | MO | Cole | 38.5767 | −92.1733 |
| *P. maculata* | JTC2596 | TNHC63699 | EF472126 | | MO | Adair | 40.2540 | −92.5821 |
| *P. maculata* | JTC2600[F] | TNHC63702 | EF472111 | 1 2 3 | MO | Boone | 39.0333 | −92.3333 |
| *P. maculata* | JTC2601 | TNHC63425 | EF472118 | | IA | Boone | 41.9900 | −93.8841 |
| *P. maculata* | JTC2613 | TNHC63423 | EF472099 | | IA | Allamakee | 43.3621 | −91.2264 |
| *P. maculata* | JTC2630 | TNHC63704 | EF472116 | 2 | Ontario | Frontenac | 44.5500 | −76.3333 |
| *P. maculata* | JTC2645 | TNHC63612 | EF472112 | | MN | Fillmore | 43.7208 | −91.9767 |
| *P. maculata* | JTC2650 | TNHC63504 | EF472134 | | KS | Wilson | 37.5667 | −95.7333 |
| *P. maculata* | JTC2674 | TNHC63431 | EF472120 | | IA | Louisa | 41.0997 | −91.0444 |
| *P. maculata* | JTC2687 | TNHC63717 | EF472129 | 2 | Ontario | Wellington | 43.9822 | −80.4039 |
| *P. maculata* | JTC2698 | TNHC63428 | EF472125 | | IA | Butler | 42.6382 | −92.6233 |
| *P. maculata* | JTC2700 | TNHC65819 | EF472079 | 1 2 3 | SD | Lawrence | 44.4060 | −103.9573 |
| *P. maculata* | JTC2706 | TNHC63146 | EF472086 | | CO | Weld | 40.4233 | −104.7086 |
| *P. maculata* | JTC2708[C] | TNHC63430 | EF472098 | | IA | Howard | 43.2130 | −92.4899 |
| *P. maculata* | JTC2760 | TNHC63552 | EF472095 | | OK | Washington | 36.8836 | −95.9259 |
| *P. maculata* | JTC2805 | TNHC63752 | EF472128 | | IL | Effingham | 39.1235 | −88.6194 |
| *P. maculata* | JTC2832 | TNHC63554 | EF472114 | | OK | Cherokee | 36.0895 | −94.8505 |
| *P. maculata* | JTC2843 | TNHC63733 | EF472097 | | IL | McDonough | 40.3325 | −90.6046 |
| *P. maculata* | JTC2862 | TNHC63543 | EF472119 | | NE | Douglas | 41.2586 | −95.9378 |
| *P. maculata* | K-2[F] | n/a | AY291088* | | KS | Kingman | 37.6458 | −98.1133 |

**Appendix A** (*continued*)

| Species | Field no. | Museum no. | GenBank No. | Test | State/Prov. | County/Twshp. | Latitude | Longitude |
|---|---|---|---|---|---|---|---|---|
| *P. maculata* | MHP 8159 | MHP8159 | EF472130 | | KS | Cherokee | 37.1692 | −94.8441 |
| *P. maculata* | MHP10265 | MHP10265 | EF472133 | | MO | Dade | 37.3897 | −93.9138 |
| *P. maculata* | MHP10268 | MHP10268 | EF472131 | | MO | Newton | 36.9416 | −94.1717 |
| *P. maculata* | MHP10467 | MHP10467 | EF472135 | | MO | Christian | 37.0265 | −93.4604 |
| *P. maculata* | N-5 | KU224630 | AY291089* | | NM | McKinley | 36.0023 | −108.8162 |
| *P. maculata* | R-1 | KU290342 | AY291082* | 2 | Ontario | Lac Seul | 50.6333 | −93.2167 |
| *P. maculata* | UMN14283[C] | JFBM14283 | EF472082 | | MN | Wright | 45.3194 | −93.9417 |
| *P. maculata* | UMN14285 | JFBM14285 | EF472110 | | MN | Rock | 43.7917 | −96.2667 |
| *P. maculata* | UMN14316 | JFBM14316 | EF472096 | | MN | Lac qui Parle | 45.0417 | −95.9167 |
| *P. maculata* | UMN14327 | JFBM14327 | EF472087 | | ND | Pembina | 48.9861 | −97.5544 |
| *P. maculata* | UMN14336[D] | JFBM14336 | EF472085 | 1 2 3 | MN | Otter Tail | 46.4583 | −95.7056 |
| *P. nigrita* | ECM0024 | TNHC62364 | AY291079* | 3 | FL | Brevard | 28.2006 | −80.8678 |
| *P. nigrita* | ECM0036 | TNHC62201 | AY291078* | 3 | AL | Barbour | 32.0369 | −85.0889 |
| *P. nigrita* | ECM0087 | TNHC62208 | AY291076* | 1 2 3 | SC | Barnwell | 33.3177 | −81.4840 |
| *P. nigrita* | ECM0215 | TNHC63210 | EF472039 | | FL | Calhoun | 30.4477 | −85.0922 |
| *P. nigrita* | ECM0242 | TNHC63187 | EF472045 | 1 2 3 | FL | Liberty | 30.1626 | −85.0666 |
| *P. nigrita* | ECM0261ε | TNHC63585 | EF472052 | | MS | Harrison | 30.5010 | −88.9084 |
| *P. nigrita* | ECM0290 | TNHC63593 | EF472040 | 3 | MS | Harrison | 30.5010 | −88.9084 |
| *P. nigrita* | ECM0359 | TNHC63191 | EF472050 | | FL | Liberty | 30.1437 | −84.9766 |
| *P. nigrita* | ECM0371 | TNHC63200 | EF472049 | | FL | Franklin | 29.7035 | −85.1901 |
| *P. nigrita* | ECM0372 | TNHC63201 | EF472042 | 3 | FL | Jefferson | 30.1981 | −84.0500 |
| *P. nigrita* | ECM0422 | TNHC63345 | EF472043 | | GA | Baker | 31.2380 | −84.5017 |
| *P. nigrita* | ECM0442 | TNHC63538 | EF472041 | 3 | SC | Dorchester | 33.0956 | −80.3156 |
| *P. nigrita* | ECM0482φ | TNHC62399 | EF472231 | | VA | York | 37.1779 | −76.5007 |
| *P. nigrita* | ECM0603 | TNHC63354 | EF472046 | | GA | Liberty | 31.8470 | −81.5960 |
| *P. nigrita* | ECM0609 | TNHC63658 | EF472044 | 1 2 3 | VA | Prince George | 37.1229 | −77.1094 |
| *P. nigrita* | ECM0666 | TNHC63662 | EF472048 | | VA | Sussex | 36.8921 | −77.0628 |
| *P. nigrita* | ECM1097 | TNHC63664 | EF472047 | | VA | Surrey | 36.9277 | −77.0406 |
| *P. nigrita* | ECM1801 | TNHC65785 | EF472051 | 3 | GA | McIntosh | 31.5343 | −81.5376 |
| *P. nigrita* | FC11452 | MVZ145452[c] | AY291077* | 3 | NC | Scotland | 34.7739 | −79.4631 |
| *P. ocularis* | ECM0045 | TNHC62234 | AY291097* | | SC | Barnwell | 33.1606 | −81.6908 |
| *P. ocularis* | ECM0095 | TNHC62241 | AY291098* | | FL | Gulf | 29.6801 | −85.3287 |
| *P. ornata* | ECM0033 | TNHC62178 | AY291106* | | AL | Barbour | 32.0369 | −85.0889 |
| *P. ornata* | ECM0055 | TNHC62183 | AY291105* | 1 2 3 | SC | Aiken | 33.2167 | −81.7500 |
| *P. regilla* | ECM0147 | TNHC62195 | EF472005 | 1 2 3 | CA | San Bernardino | 34.1132 | −117.1422 |
| *P. sp. nov.* | ASUMZ27608 | ASUMZ27608 | EF472058 | 3 | AR | Conway | 35.1508 | −92.7439 |
| *P. sp. nov.* | ASUMZ27611 | ASUMZ27611 | EF472057 | | AR | Yell | 35.0003 | −93.4167 |
| *P. sp. nov.* | ASUMZ27612 | ASUMZ27612 | EF472056 | | AR | Sebastian | 35.3858 | −94.3983 |
| *P. sp. nov.* | ECM0011 | TNHC62255 | AY291086* | 1 2 3 | AR | Craighead | 35.8546 | −90.6626 |
| *P. sp. nov.* | ECM0029 | TNHC62265 | AY291085* | 1 2 3 | LA | East Baton Rouge | 30.6889 | −90.8894 |
| *P. sp. nov.* | ECM0124 | TNHC62269 | EF472066 | | LA | Washington | 30.6787 | −89.9480 |
| *P. sp. nov.* | ECM0137 | TNHC62277 | EF472064 | | LA | Evangeline | 30.7801 | −92.2819 |
| *P. sp. nov.* | ECM0139 | TNHC62384 | EF472060 | 3 | LA | Beauregard | 30.7821 | −93.0143 |
| *P. sp. nov.* | ECM0258 | TNHC63598 | EF472054 | 3 | MS | Simpson | 31.9682 | −90.1125 |
| *P. sp. nov.* | ECM0259 | TNHC63599 | EF472069 | | MS | Simpson | 31.9274 | −90.0544 |
| *P. sp. nov.* | ECM0260 | TNHC63600 | EF472053 | | MS | Marion | 31.2358 | −89.8228 |
| *P. sp. nov.* | ECM0264 | TNHC63480 | EF472068 | | LA | St.Tammany | 30.5655 | −89.8715 |
| *P. sp. nov.* | ECM0268 | TNHC63483 | EF472059 | | LA | St.Tammany | 30.3840 | −89.7554 |
| *P. sp. nov.* | ECM0270 | TNHC63380 | EF472055 | | AR | Perry | 34.8916 | −92.8044 |
| *P. sp. nov.* | ECM0332 | TNHC63609 | EF472067 | | MS | Hancock | 30.4399 | −89.6576 |
| *P. sp. nov.* | ECM1155 | TNHC63496 | EF472061 | 3 | LA | Red River | 32.1649 | −93.4799 |
| *P. sp. nov.* | ECM2293 | TNHC65744 | EF472075 | | TX | Jasper | 30.2577 | −94.2141 |
| *P. sp. nov.* | ECM2294 | TNHC65745 | EF472076 | | TX | Liberty | 30.4451 | −94.7405 |
| *P. sp. nov.* | ECM2295 | TNHC65746 | EF472077 | 3 | TX | Liberty | 30.3517 | −95.0632 |
| *P. sp. nov.* | ECM2437 | TNHC65022 | EF472074 | | LA | St. Martin | 30.3309 | −91.6964 |
| *P. sp. nov.* | JTC2586 | TNHC63583 | EF472062 | 1 2 3 | TX | Lamar | 33.7803 | −95.5353 |
| *P. sp. nov.* | JTC2737 | TNHC63551 | EF472072 | 3 | OK | Osage | 36.5356 | −96.0507 |
| *P. sp. nov.* | JTC2829 | TNHC63703 | EF472071 | | MO | Ripley | 36.7069 | −90.6938 |
| *P. sp. nov.* | JTC2847 | TNHC63556 | EF472070 | 3 | OK | Pittsburg | 34.9927 | −95.8385 |
| *P. sp. nov.* | JTC2860 | TNHC63557 | EF472065 | | OK | Love | 34.1330 | −97.1062 |
| *P. sp. nov.* | JTC2866 | TNHC63559 | EF472063 | | OK | McCurtain | 34.1405 | −94.6958 |
| *P. sp. nov.* | R. Highton71204 | R. Highton uncat. | EF472073 | | OK | LeFlore | 34.7107 | −94.5497 |
| *P. streckeri* | JTC2581 | TNHC63382 | EF472009 | | AR | Conway | 35.2503 | −92.6833 |
| *P. streckeri* | P-2 | TNHC62317 | AY291108* | | TX | Travis | 30.3218 | −97.8034 |

**Appendix A** (*continued*)

| Species | Field no. | Museum no. | GenBank No. | Test | State/Prov. | County/Twshp. | Latitude | Longitude |
|---|---|---|---|---|---|---|---|---|
| *P. triseriata* | ECM0615 | TNHC63682 | EF472146 | 2 | MI | Ingham | 42.7222 | −84.4275 |
| *P. triseriata* | ECM0616 | TNHC63683 | EF472155 | 1 | MI | Ingham | 42.6890 | −84.2830 |
| *P. triseriata* | ECM0662 | n/a | EF472142 | 2 | Ontario | Essex | 42.1216 | −82.9715 |
| *P. triseriata* | INHS1207 | INHS18840 | EF472138 | | IL | Perry | 38.0188 | −89.4181 |
| *P. triseriata* | INHS1234 | INHS18853 | EF472136 | 2 | IL | Lawrence | 38.7128 | −87.6768 |
| *P. triseriata* | INHS1239 | INHS18857 | EF472153 | 1 | IL | Saline | 37.7358 | −88.6941 |
| *P. triseriata* | INHS1581 | INHS19242 | EF472159 | 2 | IL | Wayne | 38.5257 | −88.3456 |
| *P. triseriata* | J-1 | KU289219 | AY291091* | 1 2 | MI | Berrien | 41.9500 | −86.4167 |
| *P. triseriata* | JTC2590 | TNHC63412 | EF472151 | 1 2 | OH | Logan | 40.4614 | −83.6700 |
| *P. triseriata* | JTC2594 | TNHC63392 | EF472139 | | KY | Daviess | 37.8661 | −87.2855 |
| *P. triseriata* | JTC2604 | TNHC63405 | EF472144 | | OH | Highland | 39.2124 | −83.8362 |
| *P. triseriata* | JTC2605 | TNHC63408 | EF472149 | 2 | OH | Clinton | 39.2599 | −83.8828 |
| *P. triseriata* | JTC2607 | TNHC63410 | EF472148 | 2 | OH | Preble | 39.6478 | −84.5272 |
| *P. triseriata* | JTC2611 | TNHC63687 | EF472137 | 1 3 | TN | Montgomery | 36.4501 | −87.4767 |
| *P. triseriata* | JTC2639 | TNHC63691 | EF472156 | 1 2 | NY | Niagara | 43.1706 | −78.6906 |
| *P. triseriata* | JTC2678[G] | TNHC63708 | EF472141 | 2 | Ontario | Halton | 43.6500 | −79.9167 |
| *P. triseriata* | JTC2679[G] | TNHC63709 | EF472140 | 2 | Ontario | Oxford | 42.9089 | −80.8341 |
| *P. triseriata* | JTC2682 | TNHC63712 | EF472145 | 1 2 3 | Ontario | Niagara R.M. | 43.0085 | −79.5393 |
| *P. triseriata* | JTC2690[G] | TNHC63720 | EF472143 | 1 2 | Ontario | Waterloo R.M. | 43.2984 | −80.3735 |
| *P. triseriata* | JTC2709 | TNHC63394 | EF472147 | 2 | KY | Jefferson | 38.1111 | −85.8703 |
| *P. triseriata* | JTC2715 | TNHC65812 | EF472157 | | KY | Breckinridge | 37.6495 | −86.4241 |
| *P. triseriata* | JTC2723 | TNHC63510 | EF472154 | | IN | Jennings | 38.9848 | −85.6094 |
| *P. triseriata* | JTC2830 | TNHC63517 | EF472150 | 2 | IN | Delaware | 40.0400 | −85.3000 |
| *P. triseriata* | JTC2848 | TNHC63694 | EF472152 | 2 | NY | Livingston | 42.9377 | −77.7739 |
| *P. triseriata* | JTC2851 | TNHC63518 | EF472158 | 1 | IN | Marion | 39.8641 | −86.2904 |
| *P. triseriata* | R. Highton69234 | R. Highton uncat. | EF472160 | 1 2 3 | IN | Posey | 38.1298 | −87.9350 |

[a] R. Highton71747 was labeled as *Pseudacris feriarum* in Moriarty and Cannatella (2004); according to R. Highton (personal communication), who collected the specimen, this specimen is morphologically and acoustically a *P. brachyphona*. However, its mitochondrial DNA suggests that this individual is a hybrid between the two species.

[b] TNHC62369 is from Lake Co. not Ocala Co., as reported in Moriarty and Cannatella (2004).

[c] MVZ145452 was mislabeled in Moriarty and Cannatella (2004) as MVZ11452.

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