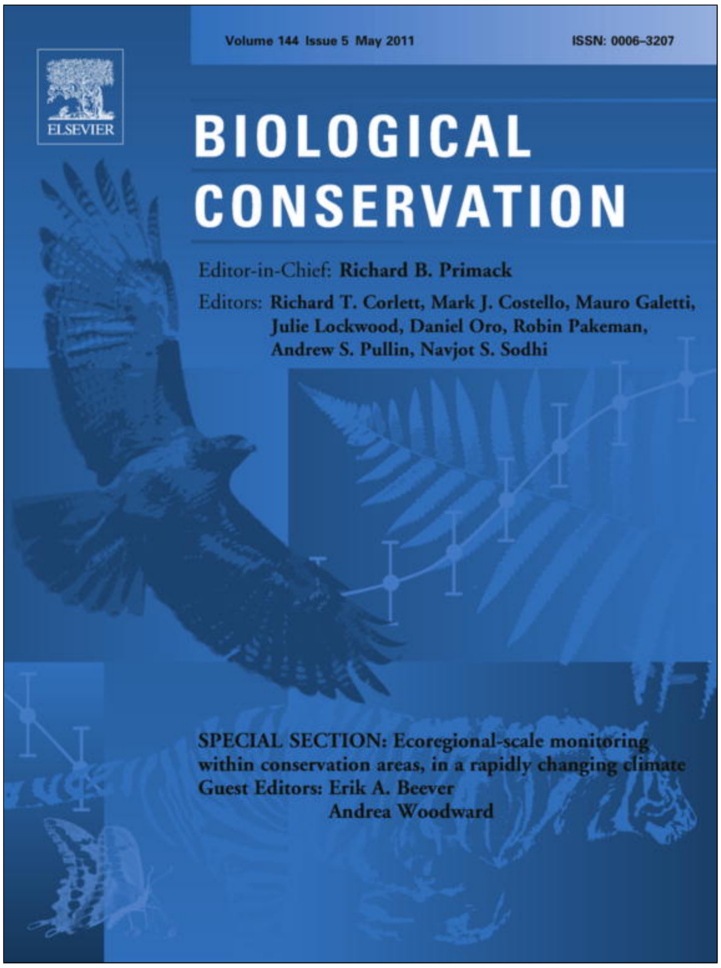
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Combining genetic structure and ecological niche modeling to establish units of conservation: A case study of an imperiled salamander

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ABSTRACT

Identification of conservation units below the species level has been difficult for researchers and conservation planners. Methods reliant solely on genetics to identify conservation units are widely used but have limited scope. Additionally, methods used to assess ecological distinctness are typically difficult to interpret, and hence not broadly applicable. Here, we attempt to reconcile these problems by defining conservation units utilizing both genetic and ecological methods. This study suggests a framework to evaluate discreteness and significance among populations for assessment of distinct population segments (DPSs). Specifically, we highlight a methodology that incorporates genetic analyses and niche-based distribution modeling to identify conservation units. As a case study, we sought to determine whether populations of an imperiled salamander (*Notophthalmus perstriatus*), appearing to exist in two regions separated by 125 km, exhibited genetic and ecological distinctness such that the regions demarcate separate conservation units. Using mtDNA (*cyt-b*), we found that haplotypes were shared between localities within each region but none were shared between regions. Niche-based distribution modeling revealed significant differences in the ecological setting between the two regions. In combination, the absence of evidence for recent genetic exchange and model-based support for differing ecological conditions utilized by newts between regions provides evidence that eastern and western populations are both distinct and significant. This study formalizes a method to assess DPS distinctness and significance providing general utility for this methodology as a conservation tool for many species.

1. Introduction

Conservation focused solely at the species level has the potential to overlook population-level diversity important to a species' evolutionary trajectory. The classification of taxa into units of conservation is a tool used to identify populations requiring integrative conservation planning. Assignment of conservation units facilitates efficient monitoring and management of targeted populations of imperiled species at the appropriate geographic scale (Moritz, 1994a; Fraser and Bernatchez, 2001). However, identifying the smallest meaningful unit of conservation has presented a challenge for biologists and conservation planners. Despite this difficulty, the theory of such operational units has been considered among the most revolutionary ideas resulting from phylogeographic studies at the microevolutionary level (Avise, 2004).

The Endangered Species Act (hereafter the Act), established in 1966, was designed to protect imperiled wildlife at the species level in the United States. In 1978, the Act was amended to include "distinct population segments" (DPS) providing legal protection below the species level to populations of terrestrial vertebrate species and fish (not including Pacific northwest salmonids). The sub-specific DPS classification facilitates efficient management of populations of conservation concern, allowing for application of management only within populations where such actions are necessary (Pennock and Dimmick, 1997). A sub-specific taxonomic unit must be recognized as a DPS in order for legal conservation action to be taken.

According to the Act, DPSs are assessed based on three criteria: discreteness, significance, and status relative to other populations of the same species. Assessment of discreteness is well-established using morphological or genetic methodology. Indeed, many proposed conservation units exclusively depend on genetic data (i.e., reciprocal monophyly of mtDNA sequence data *and* statistically significant differentiation at nuclear loci; Moritz, 1994b; Zink, 2004). Although genetic data are important to consider in the assessment of conservation units, studies strictly based on genetics may be limited in scope and legal support. With increasing recognition that both genetics and ecology influence evolutionary trajectories, many widely supported conservation units, including DPS,

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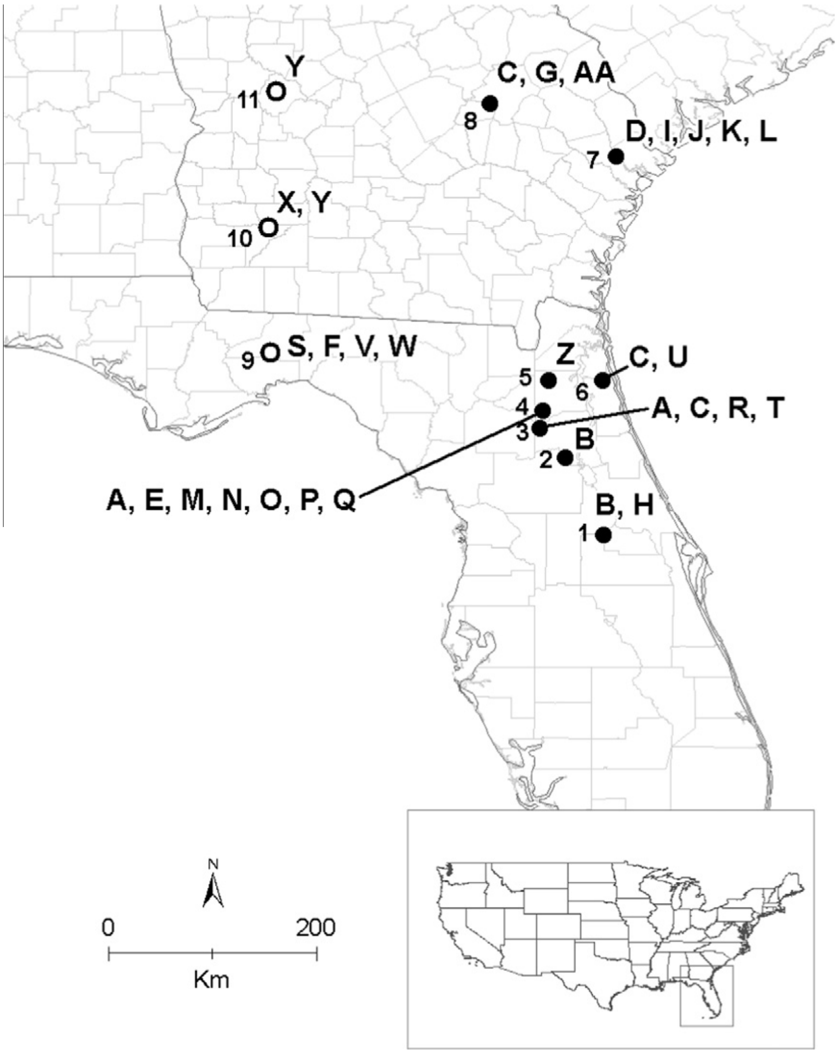

Fig. 1. Map of sample localities for genetic analyses. Site IDs (numbers) correspond to IDs in Supplementary Table 1. Note that western region populations are denoted with open circles and that eastern region populations are denoted with closed circles. Letters designate haplotypes found within each population.

encompass a more integrated approach (Crandall et al., 2000; Fraser and Bernatchez, 2001; Moritz, 2002).

While genetic data are traditionally used to identify discreteness among population segments, significance remains difficult to assess. Some factors considered when assessing significance include: inhabitance of an ecological setting that is atypical for the taxon, extinction of the segment would result in a break in the species distribution, or a population segment represents the only natural occurrence of a species within its native range (Policy regarding recognition of DPS, 1996). There are several methods possible to assess the ecological component of significance. Rader et al. (2005) proposed methods to test for ecological exchangeability based on observed trait differences as a measure of local adaptation (e.g., reciprocal transplants, common-garden experiments). However, this method may be difficult to conduct for many species, especially those that are difficult to collect due to rarity or high vagility. Moreover, conducting reciprocal transplants with imperiled species elicits ethical issues. The DPS designation of "atypical ecological setting" provides an opportunity to assess the ecology of proposed population segments without requiring the manipulation of the species of interest. In this paper, we use a non-invasive technique, niche-based distribution modeling, to assess significance between two disjunct regions of an imperiled newt. Recently, niche-based distribution modeling has become widely used to model geographic distributions and to compare aspects of the ecological niche for a species between regions and/or continents (Guisan and Thuiller, 2008; Mau-Crimmins et al., 2006; Medley, 2010). While such models clearly don't include every aspect of the niche, they can be used to test hypotheses about differences in environments used by species between regions. In addition, newly developed randomization techniques facilitate the interpretation of observed differences in distribution models. These techniques test whether differences between distribution models (a proxy for the ecological niche) are a result of differences in the overall environment available to the species or if they result from differences in the niche that populations utilize (Warren et al., 2008).

The overall objective of the study was to provide a framework for DPS assessment that efficiently identifies both discreteness and significance and can be implemented across taxa. We combine genetic analyses with niche-based distribution modeling to assess discreteness and significance among populations of a rare salamander species, the striped newt (*Notophthalmus perstriatus*). The striped newt is a poorly understood species endemic to sandhill and scrub habitats of north-central Florida and southern Georgia, USA. This species has a complex multi-stage life cycle comprised of obligately aquatic larvae (Johnson, 2005). Adults either remain aquatic (i.e. are neotenic) or they metamorphose and move to terrestrial upland habitats (Johnson, 2005). Terrestrial adults disperse hundreds of meters from their natal pond spending much of their adult lives in the uplands from where they occasionally disperse to non-natal ponds to breed (Johnson, 2005). Their complex life history makes striped newts vulnerable to threats at breeding ponds (e.g., ditching and draining of temporary ponds) as well as in the surrounding uplands (e.g., silviculture practices, fire suppression). Previous studies suggest population persistence is declining throughout its range, owing largely to habitat loss and limited habitat connectivity (Dodd and LaClaire, 1995; Franz and Smith, 1999; Johnson, 2005). Populations of this species appear to occur in two geographic regions (separated by approximately 125 km) with one region consisting of populations located in eastern Florida and Georgia and the other region consisting of populations located in western Florida and Georgia (Dodd and LaClaire, 1995; Franz and Smith, 1999). It appears that *N. perstriatus* does not occur in the geographic area between eastern and western regions: to date, censuses have not identified *N. perstriatus* within this region (Dodd and LaClaire, 1995; Franz and Smith, 1999). Lack of locality data in combination with extensive habitat loss and fragmentation (Dodd and LaClaire, 1995) throughout this geographic area suggest that *N. perstriatus* is likely absent from this region.

Based upon the apparent physical isolation between eastern and western regions, we predicted that the subsequent restricted gene flow would have resulted in genetic divergence and differences in niche utilization between regions. In addition, we predicted that genetic diversity would be lower within western populations than within eastern populations because of low population number and relative isolation in the west. To test these *a priori* predictions, we sought to: (1) determine whether eastern and western regions of *N. perstriatus* populations exhibited genetic differentiation using traditional population genetics techniques (mtDNA sequence analysis) and (2) assess ecological differences between regions using niche-based distribution modeling. Our results are discussed with regard to whether populations in eastern and western regions meet the criterion for DPS assessment. We conclude by discussing the conservation implications of our technique for assessing conservation units in general and how our results impact the conservation status of striped newts.

2. Methods

2.1. Genetic analyses

2.1.1. Sample collection

We collected 92 samples from 11 localities throughout the range of *N. perstriatus* (Supplementary Table 1; Fig. 1). Samples were collected from March 1999 through March 2000. Additionally, samples from the TAY site were collected in March 2009. We approximated the site locality for one area on private property in St. John's County (site 6, Fig. 1) because the owner wishes the location to remain undisclosed. We captured newts with dip nets, seine nets or wire-screen funnel traps. We removed a small section from the distal end of each individual's tail using sterilized scissors, placed the tissue in saturated salt buffer (NaCl; 25 mM EDTA, pH 7.5; 20% DMSO; protocol modified from Amos and Hoelzel, 1991), or in DrieRite® Desiccant, and released individuals after the samples were taken.

2.1.2. DNA isolation and sequencing

We isolated total genomic DNA from each sample using standard phenol/chloroform extraction followed by ethanol precipitation and storage in Tris/EDTA buffer (Hillis et al., 1996) or using a Qiagen DNeasy kit. For genetic analysis we used Polymerase Chain Reaction (PCR) to amplify a 585 base-pair fragment of the mitochondrial (mtDNA) cytochrome b (cyt-*b*) gene using primers H14447 (Edwards et al., 1991) and MVZ15 (Moritz et al., 1992). Amplifications were carried out in 25-μl reactions containing: 1× buffer, 3 mM $MgCl_2$, 200 μM each dNTP, 0.25 μM each primer, 1 U Taq polymerase (Sigma–Aldrich Corp., St. Louis, MO), and 2–5 ng of template DNA. PCR conditions were as follows: initial denaturation of 94°C for 3 min, 35 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 1 min; and a final extension for 7 min. Positive and negative controls were used in each round of PCR. PCR products were purified with 30,000 MW filters (Millipore, Inc., Bedford, MA) or via ExoSAP-IT (USB Corp., Cleveland, OH). Samples were sequenced at either University of Florida's DNA Sequencing Core or by Nevada Genomics Center on ABI sequencers. We checked chromatograms for base calling and edited sequences using Sequencher version 3.1 (Genes Codes Corp., Ann Arbor, MI).

2.1.3. Data analysis

We used ClustalW implemented in the software *MEGA* version 4.0 (Tamura et al., 2007) to align the 585 bp fragment of mtDNA cyt-*b* for 92 samples. Genbank sequences for *Notopthalmus viridescens* and *N. meridonalis* were used as outgroups (accession numbers EU88032 and AY691731). We inferred phylogeny using MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003) using default priors. We tested three different partitioning strategies: no partitioning, first and second codon positions separate from third positions, and all codon positions separate. We used MrModeltest version 2.3 with the Akaike Information Criterion (AIC) (Nylander, 2004) to determine the best evolutionary model for each partition. We ran two Markov-chain Monte Carlo (MCMC) runs with five million generations. Samples were taken every 500th generation. Using Tracer 1.4 (Rambaut and Drummond, 2007), we checked for stationarity and eliminated the first 1,000,000 generations as burnin. We estimated summary statistics and consensus phylograms with nodal posterior probability support from the combination of both runs.

Additionally, we constructed a 95% parsimony haplotype network using TCS version 1.2 (Clement et al., 2000). Haplotype networks enable visualization of the relationship among haplotypes including internal nodes and are often useful in resolving intraspecific haplotype relationships (Hoffman and Blouin, 2004). For all populations with sample size $\geqslant 5$ we calculated within-population genetic diversity statistics including number of haplotypes, haplotype diversity (h) and nucleotide diversity (π) using DnaSP ver. 5 (Librado and Rozas, 2009). To assess differences between eastern and western regions in haplotype and nucleotide diversities we implemented a Welch's two sample *t*-test in the statistical program R. Finally, we evaluated the effect of sample size on the cumulative number of haplotypes detected for each population using an accumulation curve (i.e. species–area curve) in PC-ORD v5.0. If the shape of the curve flattens, additional samples are unlikely to yield new haplotypes.

2.2. Ecological analyses

2.2.1. Modeling approach

We estimated climatic and habitat aspects of the niche for *N. perstriatus* by generating niche-based distribution models for eastern and western regions using a maximum entropy algorithm implemented in MAXENT software (Phillips et al., 2006). Maximum entropy is a machine-learning technique that predicts species distributions using detailed climatic and environmental datasets together with species occurrence data, and generally performs better than other algorithms in tests of model performance (Elith et al., 2006; Ortega-Huerta and Peterson, 2008). Maximum entropy uses presence-only data to predict the suitability of habitat, and is quite robust to spatial errors in occurrence data (Phillips et al., 2006; Graham et al., 2008).

We calibrated models using known occurrences and six environmental datasets. In addition to using occurrence data from our genetic analyses, we obtained occurrence data for *N. perstriatus* from the Georgia Department of Natural Resources and the Smithsonian National Museum of Natural History Collection (http://collections.nmnh.si.edu), resulting in a total of ten occurrence points for the western region and 47 points for the eastern region. This occurrence data included all known occurrence points for the western region. Initially, we calibrated models using nine environmental variables. For the final models, we removed three environmental datasets that provided no contribution to the preliminary models, resulting in six environmental variables for calibration: maximum temperature of the warmest month, temperature seasonality, annual precipitation, precipitation of the driest month, and precipitation seasonality from the WORLDCLIM database (Hijmans et al., 2005), and 2001 land cover from the Multi-Resolution Land Characteristics Consortium (29 landcover categories, http://mrlc.gov). We chose a subset of the 19 available WORLDCLIM variables because some of the variables are correlated with one another (Kozak and Weins, 2006; Rissler and Apodaca, 2007; Shepard and Burbrink, 2008). Thus, we selected variables that represented mean values and also included extremes for both temperature and precipitation because extreme values tend to limit population persistence for the study species. We obtained WORLDCLIM data at 30 arc-second resolution ($\sim$1 km^2 per pixel); land-cover data were obtained at 30 m^2 resolution and resampled to 30 arc-second resolution ($\sim$1 km^2) in ArcGIS version 9.2, (Environmental Systems Research Institute, Redlands CA, USA). We clipped all environmental layers to the study extent and exported them as ASCII grids for use in model development. We used 10,000 points to determine the background distribution, a regularization parameter of 1 for the eastern model and 0.80 for the western model, and a convergence threshold of 1.90.

We used spatial statistics to ensure independence of occurrence locations prior to calibrating models. We first calibrated models with all available occurrence points and calculated spatial autocorrelation on model residuals at multiple distance classes using Moran's I in SAM v3.0 software (Rangel et al., 2006). Significant spatial autocorrelation was calculated using permutation tests. The distance class at which spatial autocorrelation ceased (i.e. became non-significant), was $\sim$35 km. Thus, we randomly omitted occurrence points from the complete occurrence dataset that were nearer than 35 km from one another. The remaining points were used to calibrate final distribution models (7 points for western region, 10 points for eastern region). This is a substantial reduction from the number of available points in the east, but recent work by Costa et al. (2010) revealed that MAXENT produces accurate predictions with small sample sizes. Moreover, because of the imperiled status of this newt and few known western localities, reducing the number of localities for model training in the eastern region balances analyses between regions.

To determine the optimal extent at which to calibrate models, we ran a suite of models at extents increasing in size from the extent of either eastern or western occurrence points (regional extent) to the extent of all occurrence points combined (full extent). Models calibrated at the regional extent produced models with AUC values $\leqslant$0.75, even when we reduced the regularization modifier below 1 to improve fit. Thus, final models were calibrated at the full extent. Because random background points are used as pseudo-absences, it is assumed that all occurrence points within the calibration extent that are not included in calibration (e.g. western points for the eastern model) could be a pseudo-absence. However, calibrating in this way also guards against projecting models onto "non-analog climate", a problem recently discussed in the literature as one that can incorrectly predict occurrence probability when projecting models that are calibrated using existing combinations of climate and habitat onto areas having potentially different combinations of such variables (i.e. extrapolation error, Fitzpatrick and Hargrove, 2009).

We evaluated model accuracy in two ways. For the eastern model, we used the 10 occurrence points that were >35 km apart for calibration and used the remainder of all known occurrences (37) to test the model using binary tests of omission (Phillips et al., 2006). We calculated omission rates as the proportion of test points that were not predicted at a threshold probability equaling the minimum probability of any pixel containing an occurrence point. Because of the limited number of populations in the western region, seven of ten occurrence points were used to generate the model and an omission test on the three remaining points was not useful (i.e. a test using three occurrence points has little power). Thus, we also evaluated model accuracy using the threshold-independent area under the curve (AUC) of the receiver operating characteristic (ROC) plot. AUC is a composite measure of model performance, and compares model fit to that of a random prediction. AUC values range 0–1, where 1 is a perfect fit. Useful models produce AUC values of 0.7–0.9, and models with "good discriminating ability" produce AUC values above 0.9 (Swets, 1988).

2.2.2. Ecological comparisons between regions

We evaluated regional ecological differences in two ways. The first compared entire distributions (our estimate of the regional niche) using niche overlap statistics and the second compared environmental variables at occurrence points using multivariate procedures. We used both procedures because while the multivariate procedures provide an intuitive interpretation, evaluating differences only at known occurrences can bias environmental values towards sampling locations. Comparisons between entire distributions provide a broader estimate of the environment utilized by populations. To compare entire distributions, we used two randomization procedures to compare "niche overlap" between a pair of real models to that between models generated using either randomly generated occurrence points (background similarity) or by randomly assigning identity (eastern or western) to occurrence points (niche identity). Niche identity tests whether models generated for the eastern and western populations are significantly different. For this procedure, random models are generated by randomly assigning identity (eastern or western) to occurrence points. Background similarity evaluates whether differences detected between models can be explained by underlying environmental differences between regions (i.e. the "background"). This test generates models using points that are randomly generated from all the available pixels in the study area (eastern or western region). We calculated "niche overlap" between all pairs of models using the metric I (Warren et al., 2008), which ranges from 0 (no overlap) to 1 (identical). This metric compares probability values for individual pixels between two niche models. For both randomization procedures, 100 overlap values (I) were calculated between random models and compared to overlap (I) between real models to assess significance. If niche identity indicates a significant difference between models, a significant difference in background similarity would indicate differences in the models were due to differences in the overall environment between regions. Alternatively, if niche identity is significant and background similarity is not significant, then the differences in the models are not due to underlying environmental differences; rather, the differences are due to differences in the niche utilized by populations in each region.

As a supplement to distributional comparisons, we used Principle Components Analysis (PCA) to compare environmental data at occurrence points between regions using PC-ORD 5 (MjM Software, Gleneden Beach, Oregon, USA). We extracted environmental data at each occurrence point in ArcGIS and used Monte Carlo simulations to test whether the PCA ordination was different from a random configuration. We conducted Multi-Response Permutation Procedures (MRPP) in PC-ORD 5 (McCune and Grace, 2002) to test the null hypothesis that environmental data at occurrence points were no different between eastern and western regions. The test statistic for MRPP is A, which quantifies the amount of within-group agreement. A values range from negative values to 1; 1 indicates all localities within regions are identical; 0 indicates heterogeneity within groups is no different from that expected by chance, and values <0 indicate within-group heterogeneity is less than a random expectation. We evaluated environmental variable importance by correlating each variable with axis scores from the PCA ordination (continuous variables) or using logistic regression (categorical data) as appropriate.

3. Results

3.1. Genetic analyses

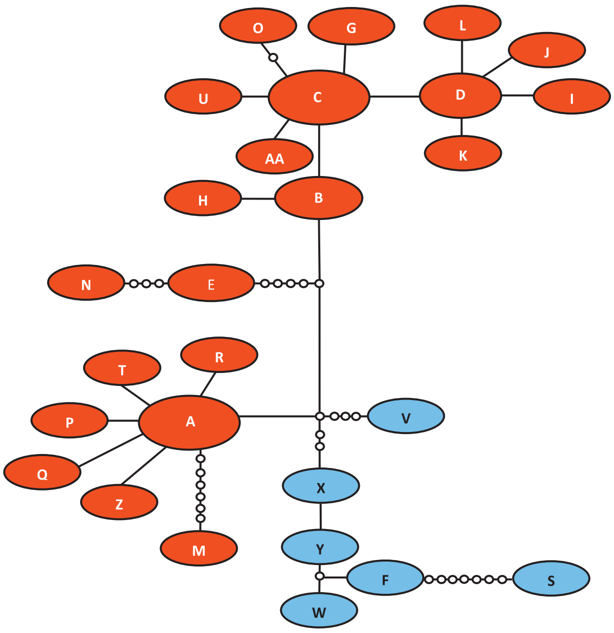

Fig. 3. Relationships among haplotypes for *N. perstriatus* populations. Haplotype network was inferred by statistical parsimony. Circles labeled with white letters represent sampled haplotypes from the eastern region and circles labeled with black letters represent sampled haplotypes from the western region. The letters correspond to the phylogeny in Fig. 2. The size of each circle represents the relative frequency of that haplotype among all samples.

We aligned a 585 bp fragment of the mtDNA cyt-*b* gene for 92 samples of *N. perstriatus* collected throughout the species range and identified 27 unique haplotypes (Figs. 2 and 3). Haplotype sequences generated from this study have been submitted to GenBank (accession numbers AF380362, AF380363, and HM804253–HM804277). Our calculations of cumulative haplotype estimates using PC-ORD support that identified haplotypes are representative of those present within each site even though some sites may have been under-sampled (Supplementary Table 2). For phylogenetic reconstruction, the best fit model identified by AIC for the unpartitioned scheme was HKY + I. The best fit model for the two partition scheme was GTR + G for first and second position and GTR for third position. The best fit models chosen for the three partition scheme were K80 + I for position 1, F81 for position 2, and GTR for position 3. Across all models, Bayes factors (Kass and Raftery, 1995) provided very strong support (2 lnB10 > 10) for the two partition model as the best-fit to the dataset and this model was used for phylogeny reconstruction. While the Bayesian phylogeny (Fig. 2) provided support for the *N. perstriatus* samples as a monophyletic group, intraspecific relationships among *N. perstriatus* re-

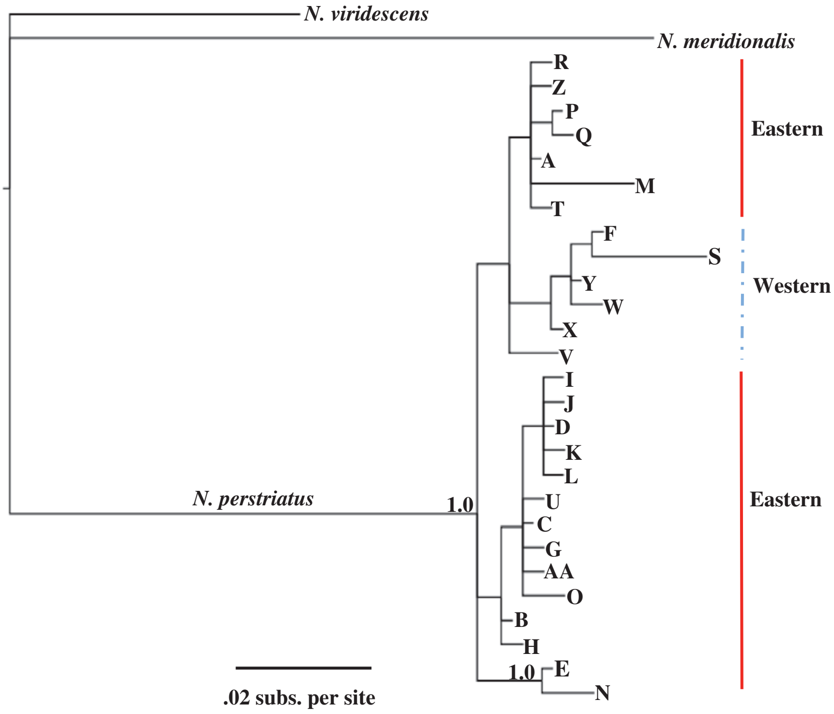

Fig. 2. Bayesian phylogeny of *N. perstriatus* samples. Letters correspond to haplotypes. Numbers represent posterior probabilities at major nodes greater than 0.95. All nodes having posterior probabilities less than 0.5 have been collapsed.

gions remained unresolved. We did not recover reciprocal monophyly among eastern and western populations.

The 95% statistical parsimony haplotype network generated detailed information regarding relationships among individual haplotypes and haplotype occurrence information per population (Fig. 3). Haplotype C (found only in eastern populations) was identified as the most likely ancestral haplotype given the greatest value for outgroup weight (Clement et al., 2000). Haplotype C was also the most widespread, shared among three of the eleven locations (all eastern region, sites 3, 6, and 8; Fig. 1, Supplementary Table 1), and occurred with the greatest frequency (62.5%) at site 8 in Georgia. Haplotype A was the most abundant haplotype and was found at sites 3 and 4, both in north-central Florida (eastern region). The geographic distribution of the 27 haplotypes (Fig. 1) illustrates the limited number of haplotypes shared among populations throughout the species range. Most sample localities contained endemic haplotypes, and only haplotypes A, B, C, and Y were found at more than one site. Interestingly, shared haplotypes only occurred within regions; that is, we did not uncover any haplotypes shared between western and eastern regions (Fig. 1). Furthermore, the haplotype network (Fig. 3) revealed a pattern suggestive of limited gene flow between regions in that eastern and western haplotypes were not intermingled.

Table 1
Estimates of *cyt-b* genetic diversity in *Notophthalmus perstriatus*. Descriptive statistics comparing genetic variation among sampled populations (SD = standard deviation).

Site	No. samples	No. haplotypes	Nucleotide diversity, π (SD)	Haplotype diversity, *h* (SD)
Eastern region				
ORA	5	2	0.00103 (0.00030)	0.600 (0.175)
MAR	8	1	0	0
PUT	24	4	0.00165 (0.00081)	0.308 (0.118)
CL1	11	7	0.01324 (0.00215)	0.873 (0.089)
CL2	1	1	na	na
STJ	2	2	na	na
BRY	13	4	0.00127 (0.00037)	0.628 (0.143)
EMM	9	4	0.00123 (0.00032)	0.639 (0.126)
Western region				
LEO	8	2	0.00757 (0.00316)	0.643 (0.184)
BAK	5	4	0.00103 (0.00030)	0.600 (0.175)
TAY	6	1	0	0

Maximum sequence divergence between haplotypes was 3%; haplotypes S and M were the most divergent differing at 22 nucleotide positions. Estimated average sequence divergence between regions was 0.8%, whereas average sequence divergence within regions was 0.3%. For comparison, the estimated sequence divergence between *N. perstriatus* and *Notophthalmus meridionalis* was 12.9% and between *N. perstriatus* and *N. viridescens* was 9.3%. Estimates of within population nucleotide diversity (π) ranged from 0 to 0.01324 in the east and from 0 to 0.00757 in the west. Estimates of haplotype diversity (h) ranged from 0.3 to 0.873 in the east and 0 to 0.643 in the west (Table 1). Moreover, regional comparisons of genetic diversity revealed that diversity harbored within populations was not significantly different between regions (for π: $t = 0.0649$, $df = 5$, $p = 0.9508$; for h: $t = 0.3863$, $df = 4$, $p = 0.7213$).

3.2. Ecological setting

MAXENT produced models with "good discriminating ability" (AUC = 0.91 and 0.93, respectively; test omission rate for eastern model = 0.03) and predicted different patterns of probability of occurrence between eastern and western regions (Fig. 4). The eastern model predicted high probabilities in the eastern region and into central and south Florida, and an area of low probability flanking western populations (Fig. 4). The western model predicted high probabilities of occurrence over the western region and in a patch along the east Florida coast, and lower probabilities in the eastern

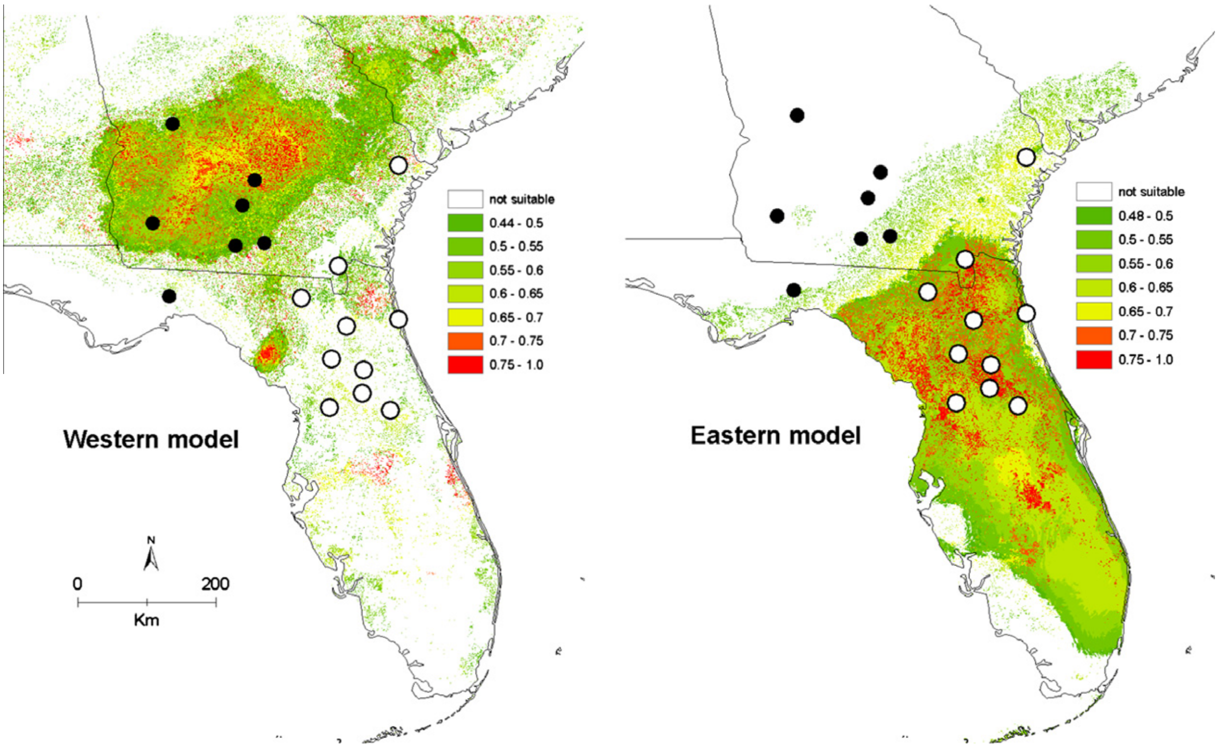

Fig. 4. Niche-based distribution models for *N. perstriatus*. Eastern model calibrated using open circles; Western model calibrated using filled circles. Higher probability of occurrence is indicated by warm colors while lower probability of occurrence is represented by cool colors. Areas shown as "not suitable" had a probability of occurrence less than the minimum probability assigned to any occurrence point used to train each model.

region and beyond (Fig. 4). Temperature seasonality was the most important variable for the eastern model (63% of variation explained) followed by precipitation seasonality (16% of variation) and land cover (11% of variation, Table 2). Land cover was the most important variable for the western model (33% of variation explained), followed by precipitation of the driest month (24% of variation) and maximum temperature of the warmest month (23% of variation, Table 2).

Table 2
Relationship between environmental variables and regional estimates of niche-related characteristics. Pearson correlation coefficients between environmental variables and PCA axis scores (see Fig. 5), and percent contributions of each environmental variables to model output shown. * indicate environmental variables that were removed from final models. ** Logistic regression revealed no significant relationship between land cover and PCA axis 1 ($z = -0.9$, $p = 0.37$) or PCA axis 2 ($z = 1.6$, $p = 0.10$).

Variable	Correlation with PCA		Contribution to model	
	Axis 1	Axis 2	West	East
Annual mean temperature*	−0.95	0.14	0	0
Temperature seasonality	0.97	−0.14	0	63.4
Maximum temperature of warmest month	0.16	−0.35	22.8	6.3
Mean temperature of coldest quarter*	−0.97	0.13	0	0
Annual precipitation	−0.50	−0.78	0	0.5
Precipitation of wettest month*	−0.88	−0.33	0	0
Precipitation of driest month	0.07	−0.91	24.1	2.2
Precipitation seasonality	−0.94	0.23	20.0	15.4
Land cover	**	**	33.2	11.2

Table 3
Results from randomization tests evaluating environmental differences between regions. Randomizations were compared to niche overlap (I) between eastern and western models ($I = 0.79$). Randomization tests showed the background environment available to newts in east and west regions is similar (background similarity), but that the niche that they utilize differs significantly between regions (niche identity).

Statistic	Comparison	*p*-value
Background similarity	East to West (10 pts.)	0.64
	West to East (7 pts.)	0.10
Niche identity	East vs. West	<0.001

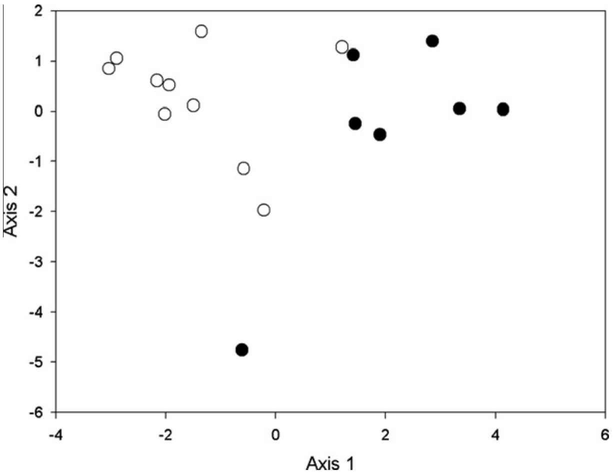

Fig. 5. Principal Components Analysis (PCA) ordination of environmental data at *N. perstriatus* occurrences used for model calibration. Populations in the Eastern region represented by open circles and populations in the Western region represented by filled circles. Two axes were significant (Axis 1 = 53% of overall variance; Axis 2 = 25% of variance). Environmental data at occurrence points were significantly different between regions (MRPP: T = −6.5, A = 0.33, $p = 0.0007$). Statistical relationships between environmental variables and PCA axes are provided in Table 3.

Ecological comparisons revealed distinct differences between regions. Niche overlap (I) between eastern and western models was 0.79, and randomization procedures revealed differences between regions were largely due to differences in the environment utilized by newts. Niche identity procedures revealed eastern and western distribution models were significantly different from one another (i.e. niche overlap was higher than random, $p < 0.001$; Table 3). Background similarity tests showed no significant difference in the overall environment between eastern and western regions (background similarity, $p_{\text{east\_west}} = 0.64$, $p_{\text{west\_east}} = 0.10$; Table 3).

Principal components analysis revealed an eastern and a western group with minimal overlap based upon environmental data at occurrences (Fig. 5). Two axes were significant (Axis 1 = 53% of overall variance; Axis 2 = 25% of overall variance). Multiple Response Permutation Procedures revealed significant environmental differences between regions (A = 0.33, $p = 0.0007$). Correlations between PCA Axis 1 and climatic variables revealed strong associations between mean annual temperature, temperature seasonality, minimum temperature of the coldest quarter, and precipitation seasonality (Table 2). Logistic regression between PCA axis scores and land cover showed no significant associations (Table 2).

4. Discussion

4.1. Conservation unit assessment

This study employed a combined approach utilizing genetic analyses and distribution modeling to evaluate discreteness and significance among population segments as a framework to evaluate DPS assessment. This methodology has broad conservation utility as a combined approach that can provide valuable information regarding the feasibility of translocation and is applicable to many taxa. Additionally, niche-based distribution models serve as multifunctional tools, useful not only for evaluating environmental conditions but also assessing habitat factors including suitability which are important for management of endangered taxa. Additionally, this framework has potentially broad applications as a tool for conservation unit assessment in many other species. One highlight of our framework is that it links DPS assessment (a legal, political and scientific term) with contemporary definitions of Evolutionarily Significant Units (ESUs; e.g. Crandall et al., 2000) a largely scientific term by assessing both genetic distinctness and ecological significance which are the primary criteria for identifying both ESUs and DPSs. Our study provides an alternative to ecological exchangeability (i.e. ecological setting) for assessing the ecological component of conservation unit assessment. Ecological exchangeability can be difficult to assess, given the threatened nature of populations undergoing conservation assessment. Use of niche-based distribution modeling provides a non-invasive alternative, and the randomization techniques we employed can serve as a proxy for measuring ecological exchangeability. Moreover, while ESU designation is a widespread and useful conservation tool for identifying populations of concern, ESUs do not provide legal protection under the Act [except as adopted by National Marine Fisheries Service which has defined the ESU as their criterion for DPS (Pennock and Dimmick, 1997)]. Although semantic differences between DPS and ESU are problematic only in the United States, we seek to remove international confusion that arises from the disjunction between intraspecific units described in the literature and the legal policy associated with those units (Haig et al., 2006). The methodology employed in this study bridges the gap between DPSs and ESUs, broadening the scope of conservation with the flexibility to address both legal and scientific concerns.

How does our methodology work in our case study? Our *a priori* expectation was that restricted gene flow would have resulted in genetic divergence and differences in niche utilization between putatively isolated regions of the striped newt (*N. perstriatus*). In contrast to our expectations, reciprocal monophyly was not recovered between eastern and western haplotypes. Despite the fact that no clear historical phylogenetic patterns were revealed using the mtDNA *cyt-b* gene, our genetic analysis supports a lack of recent genetic exchange as evidenced by the distribution of haplotypes among populations within and between regions. Namely, haplotypes were shared within regions but eastern and western haplotypes were not intermingled and no haplotypes were shared between regions. Therefore, *N. perstriatus* population segments comprising eastern and western regions meet the discreteness criteria of DPS assessment based on a lack of contemporary gene flow. Distribution models were significantly different between regions, confirming significant climatic and environmental differences between regions. Thus, the ecological analyses support that *N. perstriatus* population segments within eastern and western regions inhabit different ecological settings and are thereby ecologically significant. Based on our conclusions, population segments in eastern and western regions are both discrete and significant. Therefore, a conservation status assessment should be conducted for each region to determine their current conservation status.

4.2. Evolutionary ecology of N. perstriatus

The combination of genetic and distribution model analyses used in our study provides unique insight into the evolution and ecology of *N. perstriatus.* Genetic analyses did not recover reciprocal monophyly between eastern and western regions. This is likely due to lack of time since populations began to diverge. However, the absence of intermingled haplotypes between regions suggests that recent gene flow among populations does not occur. As one of the fastest-evolving genes, *cyt-b* often provides sufficient information to resolve historical divergences (e.g. Wagner et al., 2005; Canestrelli et al., 2006; Miller et al., 2006; Sotiropoulos et al., 2008). Our genetic results suggest that observed genetic divergence was recent and not historic. In order to obtain more accurate estimates of contemporary gene flow among populations, different molecular markers (e.g. microsatellites) are likely needed.

Our genetic results were not surprising in light of previous population genetic studies of salamanders that typically reveal patterns of genetic structuring among populations. Studies investigating genetic divergence between populations of salamanders occurring in different regions typically lead to the designation of separate conservation units including ESUs (Canestrelli et al., 2006; Miller et al., 2006; Sotiropoulos et al., 2008) and Management Units (Lecis and Norris, 2004; Pabijan et al., 2005; Wagner et al., 2005; Eastman et al., 2007). Indeed, results of other studies have suggested discordance among current taxonomic classification and genetic data identifying cryptic species (Shaffer et al., 2004; Sotiropoulos et al., 2007). Furthermore, population differentiation may reflect historical climatic and geological processes (Kuchta and Tan, 2006) leading to isolation and eventually speciation between some salamander populations (Shepard and Burbrink, 2008). Moreover, natural barriers such as streams, altitude, topography (Marsh et al., 2007; Giordano et al., 2007) in addition to anthropogenic barriers such as roads (Marsh et al., 2008) have been found to limit dispersal resulting in divergence between populations. One aspect of our results that did differ from many of the previously mentioned studies is that we found relatively low levels of differentiation between geographic regions based on mtDNA (*cyt-b*). However, like our study, some salamander populations have managed to retain genetic diversity despite occupation of fragmented habitat (Purrenhage et al., 2009).

While our study identified ecological differences between regions, we did not find sufficient genetic divergence to support splitting eastern and western regions into separate species. In contrast, two recent studies have investigated genetic divergence in combination with ecological differentiation in other species of salamanders and determined that populations comprise multiple species (Kozak and Weins, 2006; Rissler and Apodaca, 2007). Although we identified a lack of shared haplotypes between regions suggesting a lack of contemporary gene flow, the lack of genetic divergence between regions suggests that further corroborating evidence (e.g. based on behavior) would need to be confirmed before eastern and western regions of *N. perstriatus* should be considered separate cryptic species. Moreover, estimates of mean pairwise sequence divergence within *N. perstriatus* were low compared to maximum diversity estimates described within other species (e.g. 9% sequence divergence within *Taricha torosa* (Tan and Wake, 1995)) versus 3% sequence divergence between haplotypes for *N. perstiatus* (this study).

Distribution models revealed significantly different ecological settings between regions. Niche-based distribution modeling is often used to map out species ranges (e.g. Zhu et al., 2007), but comparisons between models allow hypothesis testing about differences in aspects of the niche between species (e.g., Graham et al., 2004) or between regions or seasons for the same species (e.g. Fitzpatrick et al., 2007; Suárez-Seaone et al., 2008; Medley, 2010). Often, comparisons between distributions are made by extracting data from occurrence points and evaluating overlap in multidimensional space (e.g., Gebremedhin et al., 2009), or by assessing how well one model predicts species occurrences in a different region (Pearman et al., 2008; Broennimann et al., 2007). Our approach went further by evaluating whether differences between models resulted from overall differences in the environment or were a result of differences in the environment utilized by newts. In this way, we were able to show that even while the environment between eastern and western regions was not significantly different, the environment utilized by newts in eastern and western regions was significantly different. These results suggest that transplants between regions to supplement populations may be risky and thus eastern and western regions require separate conservation efforts. In addition, the niche-based distribution models may provide information regarding the suitability of habitat found in the geographic area between regions. When the eastern and western distribution models were overlaid on a single map (Supplementary Fig. 1), it was apparent that this gap region does contain areas of suitable habitat. However, these areas of suitable habitat have a very limited and patchy distribution as compared to the large expanses of continuous suitable habitat found in the eastern and western regions. It is our hypothesis that this patchy distribution of suitable habitat within this gap area might make dispersal between regions difficult possibly contributing to the apparently disjunct distribution of *N. perstriatus*.

4.3. Conservation implications for N. perstriatus

How does identifying discreteness and significance between eastern and western regions relate to the conservation of striped newts? In recent years, *N. perstriatus* populations have declined throughout their range (Dodd and LaClaire, 1995; Franz and Smith, 1999; Johnson, 2005). Habitat loss, fire suppression, and the naturally patchy distribution of upland habitats (i.e., sandhill and scrub communities) have likely resulted in the fragmented and patchy distribution of the species. Additionally, a complex life history makes striped newts vulnerable to threats at breeding ponds (e.g., ditching and draining of temporary ponds) as well as in the surrounding uplands (e.g., silviculture practices, fire suppression). Densities of striped newts are very low at most sites where they persist (Dodd and LaClaire, 1995; Franz and Smith, 1999). For example, historically large populations in the Florida panhandle have been documented as declining with less than five newts caught from one of the largest clusters of known *N. perstriatus* breeding ponds within the past ten years (1999–2008; R. Means, personal communication). Because of historical declines and current low population densities, the striped newt is currently protected in Georgia as a "threatened" species. Furthermore, it is currently listed as a species of concern in Florida (Christman and Means, 1992) although it has no legal status in the state. The biological status of this species is currently under review by the US Fish and Wildlife Service.

In light of the known conservation concerns for *N. perstriatus*, our study identifies an intensified need for conservation action to help preserve the remaining populations. A conservation status assessment should be conducted for both regions and may result in subsequent legal action (listing as separate DPSs). Specifically, western populations are at extreme risk as only nine locations have been recently identified as containing active newt populations. Interestingly, despite the isolation and low number of localities in the west, our analysis of genetic diversity supports that haplotype and nucleotide diversities within populations are not significantly different between regions. High genetic diversity in the western region may be a result of the longevity (Johnson, 2005) of *N. perstriatus* individuals. As such, the recent demographic decline is not reflected in the genetic data owing to insufficient time for genetic drift to impact diversity. Thus, it is important that conservation actions be taken in the immediate future to conserve this genetic diversity before it declines. Knowledge of niche characteristics can guide conservation efforts. For example, given the differing niche for each region, individuals translocated between eastern and western regions may produce maladapted offspring (Kirkpatrick and Barton, 1997) and is thus not recommended. Understanding population genetic structure and species ecology additionally aid translocation efforts ensuring that genetically similar individuals are moved between areas with similar environmental conditions to encourage successful establishment. Further research into the population genetics, natural history and ecology of this species is urgently needed to effectively manage and conserve this species.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biocon.2011.01.013.

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