Population genomics and geospatial tools to inform management and research priorities for non-marine molluscs

by

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Abstract

Non-marine molluscs comprise a large portion of biodiversity on Earth. However, they are among the most understudied and imperiled groups globally. As such, there is a pressing need to fill knowledge gaps regarding fundamental aspects of their biology and distribution, which can enhance conservation efforts for these animals. In this thesis, I use two different approaches toward studying non-marine molluscs that both improves knowledge of their broad-scale biology and contributes to the development of actionable conservation practices. In chapter one, I use high-resolution genomic data to explore the population genetic patterns and demographic history of a federally endangered freshwater mussel species (*Epioblasma brevidens*). In chapter two, I use geospatial data from museum collections and digital resources to compile a statewide checklist of the terrestrial gastropod fauna associated with the state of Tennessee and to assess the potential for spatial and taxonomic biases in sampling efforts. These two chapters, while different in their methodologies, each serve to accomplish both aforementioned objectives.

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Chapter 1. Introduction

1.1 Overview

Non-marine molluscs comprise tens of thousands of species globally, and broadly molluscs (phylum Mollusca) are the second-most biodiverse group of animals on Earth (Lydeard et al. 2004; Rosenberg 2014). These animals inhabit a myriad of environments, wherein they often provide important ecosystem services (Kappes 2005; Douglas et al. 2013; Vaughn 2018). However, non-marine molluscs have the highest number of recorded extinctions of any other animal group, and most extant species are considered high risk for extinction (Régnier et al. 2015; Cowie et al. 2017).

Anthropogenic pressures have led to the decline or extinction of many non-marine mollusc species. For example, in freshwater ecosystems, modification of rivers or streams through dam construction, navigation, or adjacent land development has led to the majority of freshwater gastropods and mussels being at elevated risk of extinction in the United States and Canada (Williams et al. 1993; Johnson et al. 2013; Haag and Williams 2014). In terrestrial ecosystems, land use change, habitat loss, and invasive species have caused a severe decline for terrestrial gastropods (Régnier et al. 2009; Chiba and Cowie 2016). These threats associated with either freshwater or terrestrial ecosystems are then exacerbated by the generally low vagility and high rate of local endemism associated with many molluscan species (Holland and Cowie 2009; Johnson et al. 2013). Moreover, non-marine mollusc groups can often be difficult to identify or have complicated taxonomy, which leads to poor or inaccurate study (Graf and Cummings 2007; Perez et al. 2020).

Several such approaches for conserving non-marine molluscan groups have been recently outlined by the Freshwater Mollusk Conservation Society (2016), and the strategies and research priorities they outline can be generally applied to non-marine mollusc groups as a whole. Among the broad goals detailed in this document that were associated with various conservation strategies, two specific goals were of interest to me: (1) “Increase fundamental knowledge of the biology of mollusks so managers can more effectively conserve them”, and (2) “Understand the status and trends of mollusk populations to better manage and conserve species” (Freshwater Mollusk Conservation Society 2016).

In relation to these two goals, I identified two broad areas of research that were underdeveloped for non-marine molluscs, despite their importance in effective conservation strategies. First, the use of genomic data has been highly limited in the study of molluscs relative to better studied vertebrate animal groups, despite genomic data having clear benefits for guiding conservation (Hohenlohe et al. 2021). With sequencing technologies rapidly becoming more accessible and cost-efficient, genomics needs to be the forefront of improving our understanding of non-marine mollusc population genetics, demography, evolutionary ecology, and systematics (Supple and Shapiro 2018; Brandies et al. 2019).

Second, although there is a wealth of digital resources associated with determining the geographic associations and biodiversity patterns at various spatial scales, such resources have yet to be implemented for most non-marine mollusc groups. We lack sufficient understanding of how much molluscan biodiversity many geopolitical areas harbor. Furthermore, little information exists about whether past surveys suffered from spatial or taxonomic biases, which can mislead conservation prioritization (Cameron and Pokryszko 2005; Durkan et al. 2013). With the surge of digitization of natural history collections and accessibility of geospatial biodiversity data repositories, these knowledge deficits can also be resolved (Troia and McManamay 2016; Sierwald et al. 2018; Shea et al. 2018)

In this thesis, I aimed to take these two different approaches of studying non-marine molluscs in ways that would contribute to actionable conservation efforts. In the first chapter, I applied next-generation sequencing data (RADseq) to examine population genetic patterns and the demographic histories of three populations of a federally endangered freshwater mussel species (*Epioblasma brevidens*). Population genetic studies in freshwater mussels have been sparse, but those that have been done mostly used mitochondrial or nuclear microsatellite data, which have limited utility in quantifying fine-scale genetic variation or assessing demographic history (e.g., Berg et al. 2007; Elderkin et al. 2008; Inoue et al. 2014; Jones et al. 2015). Thus, the use of high-resolution genomic datasets that allow for more rigorous analyses can be used to enhance knowledge of freshwater mussel biology. Moreover, such studies can contribute to conservation efforts by identifying populations with either low genetic diversity that may be experiencing decline or populations with high genetic diversity that may be ideal candidates for serving as broodstock for future reintroductions (Hohenlohe et al. 2021).

In the second chapter, I used museum materials, digital biodiversity repositories, and scientific literature to quantify the terrestrial gastropod biodiversity fauna of the state of Tennessee. Additionally, I use these resources to determine potential survey biases both across the state (spatial) and across various sub-groupings of terrestrial gastropods (taxonomic). Without a sufficient understanding of what species occupy the different ecoregions of Tennessee, it is difficult to establish areas of conservation priority and individual species that may exhibit local endemism (Cameron 2013; Ovando et al. 2019). Moreover, the identification of spatial and taxonomic biases in sampling of these fauna can guide future survey efforts, which aids in more broadly understanding both biodiversity patterns and potential management practices (Vazquez et al. 2008; Miller et al. 2009).

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Chapter 2. Population Genomics Reveal Low Differentiation and Complex Demographic Histories in a Highly Fragmented and Endangered Freshwater Mussel

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2.1 Abstract

Freshwater mussels are an important element of freshwater biodiversity and provide essential ecosystem services. However, mussels are among the most imperiled groups of organisms on the planet. Although research has increased in recent years, information about range-wide genetic diversity and historical demography of most species is lacking. One such species is Cumberlandian Combshell *Epioblasma brevidens*, which is listed as endangered under the U.S. Endangered Species Act. Genetic diversity of *E. brevidens* was analyzed using a high-resolution RADseq approach and included the previously overlooked Bear Creek population. Hypotheses were tested about population decline, comparative genetic diversity, and population structure with model-based approaches enabled by a genome-scale dataset. Estimates of genetic differentiation among populations of *E. brevidens* were lower than past analyses, suggesting higher historic population connectivity than previously known. Demographic analyses indicate relatively recent splits among *E. brevidens* populations in the late Pleistocene to early Holocene, with clear founder effects in two populations. The Clinch River population has the highest genetic diversity and effective population size, despite demographic analyses revealing decline of this population since the Pleistocene. Analyses of both population structure and migration show evidence of past gene flow, but all populations are currently isolated by anthropogenic barriers.Analyses indicate that populations began declining prior to industrialization, but fragmentation and population extirpation is exacerbated by modern habitat destruction. Relatively high genetic diversity in the Bear Creek population indicates that water quality improvements in the last 20 years has had a positive impact on population viability, offering promise for targeted management actions. In contrast, the Big South Fork population that has been presumed stable showed low genetic diversity and effective population size. Furthermore, genetic structure among sampled populations indicates that reintroduction efforts should use broodstock from as close to the reintroduction site as possible.

2.2 Introduction

Freshwater rivers and lakes are considered among the most imperiled ecosystems globally, and many species have sustained steep declines in response to human demands for water resources (Strayer and Dudgeon 2010). Given that freshwater biota often play important roles in maintaining the function and integrity of lotic and lentic systems, continued loss of freshwater biodiversity is of concern to societal wellbeing (Dudgeon et al. 2006; Reid et al. 2019). Freshwater mussels (order Unionida) are a considerably biodiverse group of freshwater organisms with more than 1,000 described species, and global hotspots of mussel diversity occur in the southeastern United States (Graf and Cummings 2007; Haag and Williams 2014). Freshwater mussels have a unique life cycle that includes a parasitic larval stage (i.e., glochidia). As such, their biology, dispersal capabilities, and demography are tied to their co-distributed freshwater vertebrate hosts, which are primarily fish. Furthermore, mussels provide an array of functional roles and ecosystem services such as nutrient recycling, habitat structuring, and biofiltration (Vaughn 2018). Unfortunately, North American mussel biodiversity is critically imperiled. (Williams et al. 1993). Over 60% of the more than 300 freshwater mussels in the United States and Canada are listed as threatened under NatureServe criteria (NatureServe 2021), and over one-third of all U.S. species are listed as threatened or endangered under the U.S. Endangered Species Act. Among the factors that have led to the decline of freshwater mussels are range fragmentations, extirpations, or extinctions or extirpations in response to dam construction, channelization, and pollution (Haag and Williams 2014).

Active research and management of imperiled freshwater mussels increased in the United States after the development of a national strategy for mussel conservation in the late 1990s (National Native Mussel Conservation Committee 1998; Freshwater Mollusk Conservation Society 2016). However, population genetic studies of freshwater mussels have been limited, despite the importance of such research for informing conservation efforts (but see e.g., Berg et al. 2007; Elderkin et al. 2008; Inoue et al. 2014). Nearly all population genetic studies of freshwater mussels have used allozyme markers or microsatellites, which can display higher error rates during genotyping, have limited capacity to detect fine-scale geographical patterns of genetic differentiation, and possess increased susceptibility to homoplasy compared to next-generation sequencing approaches (Pasqualotto et al. 2007; Jeffries et al. 2016; Garrison et al. 2021). Furthermore, larger sample sizes are generally needed to accurately assess allele frequency and genetic diversity of individual populations when using microsatellites (e.g., Hale et al. 2012), which may be difficult to obtain for endangered species that are difficult to sample. Therefore, high-resolution population genetic studies that use genomic approaches are needed to better quantify geographic patterns of genetic variation, estimate genetic diversity across populations, and examine demographic history, all of which are important to understanding freshwater mussel biology and conservation (Hohenlohe et al. 2021).

Cumberlandian Combshell, *Epioblasma brevidens*, is a freshwater mussel that is native to the Cumberland River and Tennessee River drainages (USFWS 2004). This species was listed as endangered under the U.S. Endangered Species Act in 1997 and Critically Endangered on the International Union for Conservation of Nature (IUCN) Red List in 2000 (USFWS 2004; IUCN 2021). Although *E. brevidens* was once distributed throughout the Tennessee and Cumberland River systems, extensive dam construction and subsequent habitat loss in the last century has led to a range reduction of over 90% (USFWS 2004). Similar range reductions have occurred for other *Epioblasma* species, and the genus has sustained more extinctions than any other freshwater mussel genus in North America (Williams et al. 1993). Only five disjunct populations of *E. brevidens* remain in (i) Bear Creek in northwestern Alabama and northeastern Mississippi, (ii-iii) Buck Creek and Big South Fork Cumberland River in northcentral Tennessee and southcentral Kentucky, and (iv-v) the Clinch River and Powell River in northeastern Tennessee and southwestern Virginia (**Figure 2-1**; USFWS 2004).

Past studies have indicated that the Clinch River maintains the highest census size of *E. brevidens* with estimates typically ranging from several thousand to over ten thousand individuals (Lane et al. 2021). The other four populations are thought to be relatively small, possibly not exceeding a few thousand individuals (McGregor and Garner 2003; Jones and Neves 2011; Ahlstedt et al. 2016). Despite the impacts of adjacent coal mining operations, the

Big South Fork Cumberland River population is considered stable owing to recent surveys finding evidence of reproduction and recruitment (Ahlstedt et al. 2005; USFWS 2019). In contrast, the Bear Creek population is considered of questionable long-term viability because of complete isolation via dam construction and anthropogenic disturbance from impacts such as gold and coal mining, agro-chemical runoff, and sedimentation (McGregor and Garner 2003). Monitoring, propagation, translocation, and reintroduction efforts for *E. brevidens* through the Kentucky Department of Fish and Wildlife Resources, the Virginia Department of Wildlife Resources, and the Tennessee Wildlife Resource Agency have been ongoing since listing (USFWS 2006, 2019; Hubbs 2020). However, such efforts have often not been informed by genetic data, which could limit their utility (Strayer et al. 2019).

Unlike most mussel species in the Tennessee and Cumberland River drainages, *E. brevidens* has been the focus of several demographic and population genetic studies (Jones and Neves 2011; Jones et al. 2012, 2015, 2018; Lane et al. 2021). These studies generally depict that *E. brevidens* has been steadily declining in the Clinch River since the late Pleistocene epoch in response to early human exploitation (Peacock et al. 2005; Jones et al. 2015). Census size (N_c) of *E. brevidens* in the Clinch River is likely lower than other sympatric species, ranging from a few thousand to over 10,000 at individual sites (Jones et al. 2014; Lane et al. 2021). For effective population size (N_e), past estimates in the Clinch River had a 95% confidence interval that spanned 941-2823 individuals (Jones et al. 2015). Jones et al. (2021) also generated N_e estimates for *E. brevidens*, but the confidence intervals spanned infinity and as such the estimates are uninterpretable (Marandel et al. 2019). Compared to the Clinch River, Jones et al. (2021) indicated that *E. brevidens* in the Big South Fork Cumberland River and Powell River had smaller, less genetically diverse populations. Despite concerns regarding the long-term genetic viability of *E. brevidens* from Bear Creek in Alabama and Mississippi (McGregor and Garner 2003), no previous genetic analysis has included individuals from this tributary. Thus, research is needed to assess genetic diversity of the Bear Creek population. Furthermore, high-resolution genomic markers offer promise for finer-scale and more accurate understanding of the molecular ecology of *E. brevidens* which will enable better understanding of population demography and better inform management efforts.

The goals of this study were to use a restriction-site associated DNA sequencing (RADseq) approach to assess population structure, genetic diversity, and demographic history of *E. brevidens* in the Clinch River, Big South Fork Cumberland River, and Bear Creek. Given past population genetic studies on *E. brevidens*, we investigate the hypotheses that (1) *E. brevidens* populations exhibit high genetic structure owing to geographic barriers, and that migration is limited within and across river drainages, (2) the Clinch River population harbors greater genetic diversity and has higher N_e compared to other populations, and (3) each population has undergone steady population decline. Population genetic patterns revealed here, especially regarding the previously unstudied Bear Creek population, will enhance understanding of population genetic patterns across the distribution of *E. brevidens*. Moreover, data from these analyses can be used to guide management and recovery efforts.

2.3 Methods

2.3.1 Study design

2.3.1.1 Sample collection and DNA sequencing

Individuals were collected from three locations: Bear Creek (BEA; N = 29), Clinch River (CLI; N = 14), and Big South Fork Cumberland River (BSF; N = 14) (**Table 2-1**). The sampling locations nearly span the full extent of the remaining range of *E. brevidens* (**Figure 2-1**). For practical purposes, collection sites are referred to as distinct populations. Sites were selected owing to high relative abundance of *E. brevidens*, and prioritization of the previously unsampled BEA population. Mussels were collected from natural populations by hand while snorkeling. All individuals appeared to be adults, but some size variation was observed, indicating multiple age classes. Genetic material was obtained through a non-lethal foot swab with an Isohelix DNA buccal swab. Swabs were immediately placed into Isohelix BuccalFix Stabilization and Lysis Buffer. Individuals were returned to the point of collection after swabbing. All individuals were collected under required state and federal permits.

2.3.1.2 Laboratory methods

DNA was extracted with the Isohelix Xtreme DNA isolation kit, which was previously shown to result in high-quality DNA from mussel foot swabs appropriate for RADseq (Garrison et al. 2021). After DNA extraction, samples were quantified using a Qubit Fluorometer and normalized to 20 ng/µL. Normalized DNA was treated with RNase A (ThermoFisher) by adding 100µg/mL to the sample and incubating the sample at 37°C for 15 minutes. DNA was sent to

Floragenex for single enzyme RADseq. Reduced representation genomic libraries were prepared using the pstI restriction enzyme following Baird et al. (2008). Size selection during library prep ranged from 300-500 bp. Samples were tagged with unique barcode identifiers and sequenced on three Illumina HiSeq 4000 lanes using 150 bp paired-end chemistry.

2.3.2 Data analysis

2.3.2.1 Genomic data assembly

Raw paired-end Illumina reads were demultiplexed and filtered with *process_radtags* in STACKS v2.3 (Rochette et al. 2019). During demultiplexing, barcodes were rescued if they varied by only one nucleotide. Low-quality reads (Phred < 20) and reads without the pstI cut site were discarded. Owing to a lack of a reference genome for *E. brevidens* or any closely related unionid mussel, the *denovo_map.pl* pipeline of STACKS was used for data assembly. Following recommendations from Paris et al. (2017) and Rochette & Catchen (2017), we randomly selected 12 samples to determine optimal STACKS parameters for *de novo* assembly and SNP discovery. Optimal parameters were determined to be a minimum of five reads per locus (-m 5), up to four allowable mismatches within stacks of the same individual (-M 4), and four mismatches between stacks of different individuals (-n 4). These parameters were then used to assemble loci for the full dataset. All other parameters remained at the defaults.

To filter out ‘bad apple’ individuals with a relatively large proportion of missing data, we used a variation of the pipeline described by Cerca et al. (2021). In brief, the *populations* program was run at default settings as a part of the *denovo_map.pl* STACKS pipeline and individual samples were discarded using a missingness threshold of >50%. To visualize and quantify missingness among the samples, we used the R statistical software (R Core Team, 2021) package *vcfR* (Knaus & Grünwald, 2017). After the 'bad apple' individuals were discarded, assembled loci among the samples that remained were re-processed with the *populations* program in STACKS with additional filtering parameters: only loci with a minimum minor allele frequency (MMAF) of 0.025, a maximum heterozygosity of 0.5, and those present in all three populations were retained. We also required all variants within a population be present in at least 90% of individuals.

Two datasets were then created using the STACKS *populations* program: one with multiple single nucleotide polymorphisms (SNPs) per locus (denoted "EpioM") and one that only allowed for single SNPs per locus (denoted "EpioS"). The EpioS dataset was used for downstream analyses that assumed unlinked loci, whereas the EpioM dataset was used for analyses that do not assume SNPs are unlinked. File formats for downstream analyses were output directly by the *populations* program or converted using *PGDSpider* (Lischer and Excoffier 2012).

2.3.2.2 Genetic diversity and effective population size

The number of private alleles, observed heterozygosity, expected heterozygosity, nucleotide diversity, inbreeding coefficients (F_{IS}), and pairwise F_{ST} were calculated for the EpioM dataset in *populations*. The R package *diveRsity* (Keenan et al. 2013) was used to quantify allelic richness of each population. To estimate N_e, the EpioS dataset was used, separated by population with additional filtering steps using *GBS_SNP_Filter* v1.17 (available from http://github.com/laninsky/GBS_SNP_filter) and *BayeScan* v2.1 (Foll and Gaggiotti 2008) to meet assumptions of the linkage disequilibrium method and to lower missing data. First, using *GBS_SNP_Filter*, we only included SNPs present within each population and removed loci that were not in Hardy-Weinberg equilibrium using a *p*-value of 0.05 and an R^2 cutoff of 0.2. Then, to identify loci that may be under selection, we used *BayeScan* v2.1 (Foll and Gaggiotti 2008). *BayeScan* analyses were ran with prior odds of neutrality at 1,000 and 10,000 owing to the large genetic structure expected among populations. All other *BayeScan* parameters were set to defaults. No loci were removed following the use of *BayeScan* as no loci were shown to be under selection. N_e estimates were calculated with the filtered dataset using *NeEstimator* v2 (Do et al. 2014) for each population independently with a minimum minor allele frequency (MMAF) critical value of 0.05.

2.3.2.3 Population structure

Population structure was assessed with several methods. Using the EpioM dataset, analysis of molecular variance (AMOVA; Excoffier et al. 1992) was performed using the *poppr.amova* function in the R package *adegenet* (Jombart 2008; Kamvar et al. 2014). Individuals were stratified by population and whether they were in the Tennessee River drainage or Cumberland River drainage. Significance was tested with a 999-permutation randomization test. Using the EpioS dataset, potential genetic admixture was assessed using the sparse non-negative matrix factorization (sNMF) algorithm in the R package *LEA* (Frichot and François 2015). This program is robust to violations of demographic assumptions of Hardy-Weinberg and linkage equilibrium (Frichot et al. 2014). The number of best-fit clusters (K) was assessed by running 10 replicates of each K value from 1 – 5 and assessing fit with the cross-entropy criterion. The lowest cross-entropy score, averaged among runs for each K, was selected for downstream analysis. Genome ancestry coefficients with the best-fit K were visualized with *LEA* using the run with the lowest cross-entropy.

Discriminant analyses of principal components (DAPC) was performed using the EpioM dataset in *adegenet*, which does not make assumptions of underlying population genetic processes (e.g., linkage disequilibrium). The number of clusters were determined using K-means clustering and Bayesian information criteria (BIC) (**Supplemental Figure S1**). To determine the optimum number of principal components to retain, the *optim.a.score* function in *adegenet* was used (**Supplemental Figure S2**). Genomic co-ancestry among individuals was also assessed using the EpioM dataset and *fineRADstructure* (Malinsky et al. 2018). This method can use linked SNPs, potentially allowing for enhanced assessment of fine-scale patterns of relatedness among individuals (Malinsky et al. 2018). A co-ancestry matrix was inferred using the *RADpainter* script, and clustering was performed using the Markov chain Monte Carlo method with default parameters.

To test for a signature of isolation-by-distance (IBD) a Mantel test of correlation between the F_{ST} values generated by STACKS and the geographical distances between each population was done in the R package *ade4* (Dray and Dufour 2007). To quantify the geographic distance between sites, river paths were measured directly in ArcMap v10.7.1 by ESRI. Significance of the Mantel test was evaluated with 1,000 permutations. A multiple regression on the F_{ST} and geographic matrices was also performed using the MRM function of the *ecodist* R package (Goslee and Urban 2007). Significance of the multiple regression was evaluated with 10,000 permutations. Although MANTEL and MRM analyses may have limited statistical power with only three collection sites, a strong signal of isolation by distance would still be possible to observe.

2.3.2.4 Migration

The coalescence-based program *MIGRATE* v.3.7.2 (Beerli and Palczewski 2010) was used with the EpioM dataset to explicitly test different models of migration and population admixture (see **Table 2-2**). Given large computational requirements of *MIGRATE*, 99 polymorphic loci present in 100% of individuals were randomly selected from the EpioM dataset for use. The script *fasta2genotype* (Maier et al. 2019) was used to generate *MIGRATE* input files. Analyses consisted of 5 independent Metropolis-Hastings Markov chain Monte Carlo (MCMC) runs using the DNA sequence model. Priors were determined based on preliminary analyses to ensure they were not too restrictive; a uniform prior with bounds (0 – 0.1) was used for mutation scaled effective population size (θ) and a uniform prior with bound (0 – 50,000) was used for migration. All other priors were set to default. Each MCMC was Metropolis-coupled with a static heating scheme of four chains (1.00, 1.50, 3.00, 1,000,000.00). Each independent analysis was run for 200,000,000 steps, sampling every 100 steps. The first 50% of steps were discarded as burn-in, resulting in 100,000 steps in the posterior of each MCMC. The best-fit migration model was determined by calculating Bayes factors using the Python utility *BF.py* in *MIGRATE* with the marginal likelihood of each model, which was measured with thermodynamic integration (Berlin and Palczewski 2010).

2.3.2.5 Demographic history

To estimate the demographic history of the three *E. brevidens* populations the ordinary differential equations method of modeling the evolution of allele frequencies was applied in the *moments* software (Jouganous et al. 2017) implemented in the program *GADMA* (Noskova et al. 2020). *GADMA* implements a heuristic genetic algorithm for global optimization of parameter values to automatically infer the best demographic model given the joint allele frequency spectrum, an initial demographic model structure, and a final demographic model structure (Noskova et al. 2020). For *GADMA* analyses, a reduced EpioS dataset was created in *populations* with a 100% missingness threshold and a MMAF of 0.01 because site frequency spectrum methods perform best with information on rare alleles.

The joint allele frequency spectrums were estimated from the EpioS dataset VCF files using *easySFS* (available from https://github.com/isaacovercast/easySFS). A lower projection value of [8, 8, 8] was used when performing demographic analyses to maximize the number of segregating sites among the population with respect to their sample size. The initial population structure given for the analysis was a single ancestral population (P_A) (coded [1, 1, 1]). The final population structure given for the analysis was as follows: P_A followed by a split into two daughter populations (P_1 and P_2), followed by an additional split of P_1 (P_{1a} and P_{1b}), resulting in three descendant populations (coded [2, 1, 1]).

Two demographic models were inferred using the generation times of 4.0 and 6.0 years for *E.* brevidens, respectively. Two generation times were run because of uncertainty in the generation time of *E. brevidens*. The generation time of *E. penita* has been estimated to be 6 years (Jones and Neves 2011), but *E. penita* can reach sexual maturity in three years, at least in captivity (P. Johnson, unpublished data). While using *E. penita* as a proxy, running analyses with a range of generation times allows us to capture uncertainty in generation times for *E. brevidens*. Each demographic model was run with 50 repeats. Although the demographic model produces a time estimate of events, the inherent bias of using variable sites, in addition to not having an accurate estimate of theta (i.e., the expected number of mutations that occur in one chromosome in one generation in the infinite-sites model) available, may cause potential error in absolute timing of demographic events (Noskova et al. 2020). Thus, although the exact time intervals produced by *GADMA* are reported, we only make conclusions in regard to broad geological epochs.

2.4 Results

2.4.1 Genomic data assembly

The 57 sequenced individuals had an average of 25,825,582 reads (range 2,275,874 – 47,770,067) retained of the total ~1.5 billion raw reads. Sequence reads filtered out during demultiplexing comprised ~1.6% of the raw reads. Upon obtaining the initial assembly, five individuals (two from BEA, three from CLI) were removed after not passing the <50% missingness threshold, which left 52 individuals in the final datasets. Filtering implemented in *populations* resulted in 16,556 loci with one SNP in the EpioS dataset. When allowing multiple SNPs per locus in the EpioM dataset, 28,954 SNPs were assembled with an average of 1.42 SNPs per locus.

2.4.2 Genetic diversity and effective population size

The number of private alleles present in each population ranged from 1,331 in CLI to 2,580 in BEA (**Table 2-1**). Observed heterozygosity was lower than expected heterozygosity across all populations ($H_o = 0.15 – 0.17$; $H_e = 0.17 – 0.20$). Average nucleotide diversity was highest in CLI (0.21) and lowest in BSF (0.18), whereas BEA had the highest allelic richness (1.64) and BSF had the lowest (1.52). The separation of each population and additional filtering procedures for estimating N_e resulted in datasets comprising 21,083, 4,249, and 29,073 loci for the BSF, BEA, and CLI populations, respectively. The lowest estimate of N_e was the BSF population at 59 (95% confidence interval 58.6 – 59.3), whereas the N_e estimate of the CLI population was 692 (95% confidence interval 653.2 – 735.5) (**Table 2-1)**.

2.4.3 Population structure

F_{ST} values (**Table 2-3**) were similar (range 0.07 – 0.11) to freshwater mussels with much smaller geographic ranges than *E. brevidens* (e.g., *Margaritifera hembeli*; Garrison et al. 2021). F_{IS} estimates were low (range 0.08 – 0.12), being highest in BEA (**Table 2-1**). AMOVA was significant at all hierarchical levels ($p < 0.001$); 8.41% of genetic variation was explained by river drainage 13.72% of the variation was explained by population, and 64.24% of variation was explained by within individual variability (**Table 2-4**). Both the Mantel test and multiple regression for IBD were not significant ($p > 0.05$), indicating a lack of IBD, but the pattern could be obscured by the low number of sites.

Both sNMF and DAPC analyses indicated that the best-fit number of genetic clusters was three (K = 3) (**Figure 2-2**). However, these methods revealed differing depictions of genetic admixture, and K = 3 fit the data only narrowly better than K = 2 for both analyses (see **Figure 2-2**, **Supporting Information**). DAPC shows three distinct clusters with no evidence of admixture at K = 3, whereas all samples from the Tennessee River drainage are clustered together and distinct from the Cumberland River drainage at K = 2. In contrast, the sNMF analysis shows admixture of approximately 65% between the two Tennessee River drainage populations at K = 2, as well as admixture of up to approximately 35% between BSF and CLI (**Figure 2-2)**. At K = 3, sNMF shows population structure among each site with little to no admixture among populations (**Figure 2-2**). The *fineRADstructure* analyses also inferred three distinct genetic clusters representing each population with greatest genetic similarity between the two Tennessee River drainage populations (**Figure 2-3**).

2.4.4 Migration and demographic history

Among the 12 *MIGRATE* models assessed, the model of no migration among BSF and the other sites coupled with downstream migration from the CLI to BEA was the best fit. The second best-fit model had no migration among BSF and other sites and bidirectional migration in the Tennessee River drainage (**Table 2-2**).

The best demographic models inferred by *GADMA*, for both generation times used, have an initially low N_e beginning in the late Pleistocene followed by a population split between the Tennessee and Cumberland River drainages (**Figure 2-4**). Following the initial split between the two major river drainages, the ancestral population in the Tennessee paleodrainage expanded substantially. Upon the splitting of the two Tennessee River drainage populations, the CLI population maintained a higher N_e followed by constant, linear decline to the present, whereas the BEA population began as a much smaller population followed by gradual increase in N_e to the present. These results were consistent for each generation time model. In contrast, for the 4.0-year generation time model, upon splitting away from the Tennessee River populations, the BSF population showed maintenance of a similarly small N_e to the BEA population, followed by population growth into the early Holocene, followed then by population decline into the present, whereas for the 6.0-year generation time model BSF underwent a decline following the split from the Tennessee River drainage ancestral population that was later followed by gradual population growth beginning in the late Pleistocene. In both models, all three populations were inferred to have gene flow events among each other after splitting (**Figure 2-4)**.

2.5 Discussion

By using high resolution genomic data, novel insights were generated into *E. brevidens* genetic diversity, population differentiation, migration patterns, and demography. Despite high population fragmentation and anthropogenic disturbance, all *E. brevidens* populations sampled exhibit similar or higher levels of genetic diversity compared to other freshwater mussel species for which RADseq data has been generated (Garrison et al. 2021; Kim and Roe 2021; Meyer 2021). However, the CLI population has higher genetic diversity and N_e relative to the other sampled *E. brevidens* populations (**Table 2-1**), corroborating past studies. Compared to previous population genetic analyses of *E. brevidens* with mitochondrial and microsatellite data, estimates of genetic differentiation among populations were lower, suggesting higher historic population connectivity than previously known. Although analyses of both population structure and migration show evidence of past gene flow, contemporary river fragmentation and impoundments have isolated each population. Past studies of the CLI population inferred continual population decline since the Pleistocene epoch (Peacock et al. 2005; Jones et al. 2015), and demographic inferences corroborate these findings (**Figure 2-4)**. Additionally, analysis of the BEA population shows population growth through time, whereas the BSF population was inferred to have either historic population growth followed by more recent decline or the inverse. Analyses done here also indicate a relatively recent split among *E. brevidens* populations in the late Pleistocene to early Holocene, which may contribute to the low genetic differentiation despite high geographic separation.

2.5.1 Genetic diversity and effective population size

Previous studies using mitochondrial and microsatellite data reported that the CLI population of *E. brevidens* had less genetic diversity than other sympatric species (i.e., *Epioblasma capsaeformis*, *Lampsilis fasciola*; Jones et al. 2015; Jones et al. 2021). However, estimates of genetic diversity for all three *E. brevidens* populations were similar, or higher, than other freshwater mussel species (Garrison et al. 2021; Kim and Roe 2021; Meyer 2021). For example, *E. brevidens* exhibits higher allelic richness and heterozygosity than *Cyprogenia stegaria*, *C. aberti*, and *Dromus dromas*, all which have also experienced declines from river fragmentation and human disturbance (Kim and Roe 2021). Furthermore, all three *E. brevidens* populations had higher genetic diversity than any population of *Margaritifera hembeli* analyzed by Garrison et al. (2021). Although the above comparisons do not include other CLI species given difficulties comparing absolute genetic diversity estimates made with microsatellites versus SNPs, genetic diversity of the three populations sampled here suggest that *E. brevidens* may not be as imperiled as other, similarly restricted freshwater mussels. Nevertheless, all three populations had lower observed heterozygosity than expected heterozygosity (**Table 2-1**), suggesting that inbreeding or genetic drift could be having an impact. Yet, estimates of F_{IS} were low for all populations compared to other freshwater mussels (Garrison et al. 2021; Kim and Roe 2021), so inbreeding is likely to be less of a factor for *E. brevidens* than at least some other imperiled mussels.

The CLI population has been the focus of extensive traditional surveys, and N_c estimates range from a few thousand to over 10,000 (Jones and Neves 2011; Lane et al. 2021), but the most recent estimates of N_e with genetic data were potentially inaccurate as they had confidence intervals that were either large or spanned infinity (Jones et al. 2015; Jones et al. 2021). In contrast, precise estimates (95% confidence intervals spanning less than 101) with non-infinite confidence intervals for each sampled population were generated here (**Table 2-1**). That said, the method used to obtain N_e estimates may be downwardly biased because *E. brevidens* has overlapping generations (Waples et al. 2016), and as such the N_e estimates are best interpreted in a comparative, rather than absolute, context. The N_e estimates corroborate past hypotheses that the CLI population is larger than other remaining *E. brevidens* populations (Jones et al. 2021). Furthermore, these estimates provide reliable baseline data that can be used for long-term monitoring in the face of anthropogenic stressors or population improvements as a result of management actions.

2.5.2 Population structure and historical demography

Despite considerable geographic separation among the three *E. brevidens* populations, estimates of population differentiation are similar to freshwater mussels with much smaller ranges. For example, the freshwater mussel *Margaritifera hembeli* had pairwise F_{ST} estimates of 0.043-0.082 among populations separated by a stream-distance of less 105 km (Garrison et al. 2021), whereas *E. brevidens* shows pairwise F_{ST} of 0.071 between the two Tennessee River drainage populations that are separated by a stream distance of ~750 km. This may be attributed to higher historic connectivity among *E. brevidens* populations compared to *M. hembeli* or differences in life history. Even between The Tennessee and Cumberland River drainages, *E. brevidens* shows relatively low pairwise F_{ST} (<0.112 **Table 2-3**).

Population structure analyses generally depict three genetic populations. However, the two clusters model had only a slightly worse fit to the data. When K = 2 there was much higher admixture detected between the BSF and CLI populations compared to BSF and BEA. The K = 2 analyses may therefore suggest historical connectivity among the Upper Tennessee and Cumberland, which is also supported by the historical fluxes of migration from the CLI to BSF inferred by GADMA. Previous studies have suggested that the Powell River (which also maintains a contemporary *E. brevidens* population) was once connected to the Cumberland River before being captured by a CLI tributary and diverting to the Tennessee River drainage (Ross 1972; Starnes and Etnier 1986), which would potentially explain the observed pattern. However, geologic and phylogeographic evidence generally refute the hypothesis that the Upper Tennessee and Cumberland were connected, despite faunal similarities (Ross 1971; Starnes and Etnier 1986; Kozak et al. 2006). Thus, it is unclear how dispersal events occurred for *E. brevidens* between river drainages following the evolution of the contemporary Tennessee River drainage beyond fish migration through the geographically proximate mouths of both the Tennessee and Cumberland Rivers at the confluence of the Ohio River. Historical connectivity among populations may also be obscured by the inability to sample extirpated populations, and routes of connectivity between other parts of the Tennessee and Cumberland River drainages (e.g., between Duck River and Harpeth or Collins Rivers) may have existed. Owing to the limited study of freshwater invertebrate phylogeography in the Cumberland and Tennessee River drainages, we advocate for greater taxon sampling and the use of similarly high-resolution data to address these questions.

Dispersal capability of *E. brevidens* is directly related to that of its host fish, so fish movement patterns may be driving the downstream-biased migration inferred by *MIGRATE*. However, movement studies of putative *E. brevidens* host fish in the genera *Percina* and *Etheostoma* do not indicate significant differences in up- and downstream dispersal (Schwalb et al. 2011; Roberts et al. 2016). Other studies have shown limited upstream migration bias in *Cottus* sculpins (McCleave 1964; Breen et al. 2009; Lamphere and Blum 2012), which *E. brevidens* can use as a host (Yeager and Saylor 1995). *Percina* are likely the primary host used by *E. brevidens* in the wild, as the violent capture event that *E. brevidens* employs to parasitize fish will often kill *Etheostoma* spp. and *Cottus* heads may be too wide to be easily captured (Barnhart et al. 2008). As such, past migration was likely tied to *Percina* movement patterns.

The stepping-stone model of dispersal (Kimura and Weiss 1964) has been shown to best explain population structure in other freshwater mussels, including in *E. triquetra* (Berg et al.

2007; Beaver et al. 2019). Movement by host fish among suitable habitat patches for *E. brevidens* in the Tennessee River prior to dam construction may have similarly facilitated stepping-stone migration of *E. brevidens*. However, such a pattern would be obscured as mainstem Tennessee River and Cumberland River populations of *E. brevidens* have been extirpated. Thus, extirpated populations (i.e., ‘ghost’ populations) could be biasing migration inference (Beerli 2004; Delser et al. 2019) and also obscuring a historical pattern of IBD. Nevertheless, both analyses of genetic structure and migration analyses indicate population fragmentation, and if migration between sites is currently occurring, it must be rare.

Demographic modeling suggests a geologically recent division between *E. brevidens* in the Tennessee and Cumberland River drainage that occurred in the late Pleistocene (**Figure 2-4**). This finding corroborates previous molecular clock estimates for *E. brevidens* and mirrors patterns seen in some fish (Berendzen et al. 2008; Hollingsworth and Near 2009; Jones et al. 2015). Notably, both BEA and BSF populations had a founder effect followed by gradual population growth after splitting. BEA was inferred to continue expansion until present day in both *GADMA* models, which may not accurately reflect recent decline in the last century (McGregor and Garner 2003). Recent and severe decline may, in fact, also be why BEA is inferred to have such a small population size since the split of CLI and BEA. The concurrent decline or limited population growth of the CLI and BSF populations beginning in the late Pleistocene to early Holocene is possibly related to the advent of using freshwater mussels as a food resource by indigenous peoples (Peacock et al. 2005; Jones et al. 2015). Inference from relic shell deposits show that freshwater mussels, including *Epioblasma*, have sustained deleterious impacts from early civilizations manipulating river-adjacent landscapes for agricultural development and from use as food resources and shell harvesting (Peacock et al. 2005). Continued anthropogenic pressure through the present has only worsened the conditions for remaining *E. brevidens* (McGregor and Garner 2003; USFWS 2006; Ahlstedt et al. 2016), which is reflected by the inferred sustained decline of N_e to present day in CLI and BSF (**Figure 2-4)**.

2.5.3 Conservation implications

Epioblasma brevidens is at high risk of extinction given recent extirpations and the persistence of only a few fragmented populations. However, data generated here indicate that genetic diversity is maintained within remaining populations. These analyses also provide important information about relative population sizes, particularly for the previously understudied BEA population. Such information is essential for long-term monitoring, conservation prioritization, and planning on-the-ground management actions. The generation of genomic data for *E. brevidens* will provide reliable comparative material for other freshwater mussel species to better identify species of conservation concern and broadly enhance the understanding of population genetic patterns in freshwater mussels. Moreover, although the following information is relevant for guiding management for *E. brevidens*, the methods used in this study and their implications can be applied to other freshwater fauna of conservation concern.

Captive propagation is an increasingly common conservation tool for freshwater mussels, but care must be taken that reintroductions accomplish recovery goals without unintended consequences (Strayer et al. 2019). Many freshwater species display an IBD pattern, leading to recommendations that source populations for reintroduction through captive propagation or translocation come from as close to the reintroduction site as possible (IUCN/SSC 2013; Strayer et al. 2019; Garrison et al. 2021). Although IBD was not detected in *E. brevidens*, we also recommend that source populations come from as close to reintroduction sites as possible because *E. brevidens* has strong genetic structure and a pattern of IBD may be obscured by the inability to sample extirpated populations.

A recent status review of *E. brevidens* reported that the BSF population is stable and potentially growing (USFWS 2019), but the conservation genomic analyses indicate that BSF has the least genetic diversity and smallest N_e of sampled populations. The immediate cause for this lower genetic diversity is unknown. Demographic analysis showed that the BSF population had dramatic reductions in N_e starting around the beginning of the Holocene, but this is also true for the CLI population. The F_{IS} estimate for BSF was the lowest among all sampled populations suggesting that inbreeding is likely not causing reduction of genetic diversity. Nevertheless, data indicate that the BSF population may be of greatest concern for decline. In addition to monitoring efforts within the BSF drainage by the Kentucky Department of Fish and Wildlife Resources, more than 2,000 individuals have been propagated and released back into the BSF since 2015, and we advocate for continued propagation with broodstock from the Cumberland River drainage. Data generated here should also serve as a baseline goal for genetic diversity at reintroduction sites. Inferred genetic diversity and N_e suggest that the BEA population is in better shape than previously indicated (McGregor and Garner 2003), which could be a result of recent improvements to water quality in Bear Creek (ADEM 2014). Given these findings, we suggest that propagation efforts could begin using the BEA population as a genetically diverse broodstock to re-establish mussel beds in other portions of Bear Creek drainage in Alabama and Mississippi and possibly other geographically proximate sites within the historical range of *E. brevidens*.

The Clinch River has been referred to as “arguably the most important river for freshwater mussel conservation in the United States” (Zipper et al. 2014), and the high genetic diversity of the CLI population indicates that the population is essential for long-term survival and maintaining evolutionary potential of *E. brevidens*. The Virginia Department of Wildlife Resources and the Tennessee Wildlife Resource Agency have worked to monitor existing populations as well as propagate and release thousands of *E. brevidens* CLI broodstock back into several sites within the Clinch River (USFWS 2019; Hubbs 2020). CLI-sourced individuals have also been used in reintroduction efforts at several other historical sites, including in the Duck River, Elk River, and Nolichucky River (USFWS 2006; Hubbs 2020). The use of the most genetically diverse possible broodstock, which would come from the Clinch River, has potential advantages for long-term survival of *E. brevidens*. However, the Duck and Elk Rivers are more geographically proximate to the BEA population, and the use of BEA broodstock probably should have been prioritized under widely accepted best practices for introductions (IUCN/SSC 2013; McMurray and Roe 2017). Future efforts should consider whether using CLI or BEA broodstock is ideal for the target reintroduction site based upon geographic proximity between the reintroduction sites and potential broodstock source.

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Table 2-1. Population summary statistics of the *E. brevidens* populations, including: sample size after filtering (N), private alleles (PA), allelic richness (AR), observed heterozygosity (H_o), expected heterozygosity (H_e), nucleotide diversity (Π), coefficient of inbreeding (F_{IS}), and effective population size (N_e). SD = standard deviation, CI = 95% confidence interval.

Population	**N**	**PA**	**AR (SD)**	**H_o (SD)**	**H_e (SD)**	**Π (SD)**	**F_{IS} (SD)**	**N_e (CI)**
Big South Fork	14	1454	1.516 (0.458)	0.150 (0.178)	0.171 (0.183)	0.178 (0.190)	0.082 (0.248)	59 (58.6 – 59.3)
Bear Creek	27	2580	1.637 (0.400)	0.164 (0.154)	0.195 (0.169)	0.198 (0.172)	0.120 (0.241)	274.3 (262.9 – 286.6)
Clinch River	11	1331	1.612 (0.438)	0.174 (0.179)	0.201 (0.179)	0.211 (0.188)	0.104 (0.284)	692.0 (653.2 – 735.5)

Table 2-2. Models assessed by *MIGRATE*, sorted by model fit. Log Bayes factors were used to rank models.

Description	**Log Marginal Likelihood**	**Log Bayes Factor**	**Rank**
Downstream migration from Clinch River to Bear Creek	-81372.79	0	1
Bidirectional migration from Bear Creek and Clinch River	-81607.48	-234.69	2
Bear Creek and Clinch River panmictic; migration from the Tennessee River drainage to the Cumberland River drainage	-82013.84	-641.05	3
Bear Creek and Clinch River panmictic; migration from the Cumberland River drainage to the Tennessee River drainage	-82035.62	-662.83	4
Bidirectional migration from Bear Creek and Clinch River; migration from Big South Fork to Clinch River	-82059.7	-686.91	5
Downstream migration from Clinch River to Bear Creek; migration from Big South Fork to Clinch River	-82078.01	-705.22	6
Downstream migration from Clinch River to Bear Creek; migration from Clinch River to Big South Fork	-82090.79	-718	7
Bidirectional migration from Bear Creek and Clinch River; migration from Clinch River to Big South Fork	-82112.96	-740.17	8
Upstream migration from Bear Creek to Clinch River	-82120.23	-747.44	9
Bidirectional migration from Bear Creek and Clinch River; bidirectional migration from Clinch River and Big South Fork	-82146.37	-773.58	10
Downstream migration from Clinch River to Bear Creek; bidirectional migration from Clinch River and Big South Fork	-82157.31	-784.52	11
Full migration	-82201.93	-829.14	12

Table 2-3. Pairwise F_{ST} (above diagonal) and geographic distance between populations in km (below diagonal).

	Big South Fork, KY	**Bear Cr, AL**	**Clinch R, TN**
Big South Fork, KY	-	0.09762	0.1125
Bear Cr, AL	1,064.30	-	0.07144
Clinch R, TN	1,779.70	747.8	-

Table 2-4. Analysis of molecular variance (AMOVA) showing genetic variation among and within populations nested by river basin.

Source of variation	**df**	**Sum sq**	**% total variation**	**Φ-statistic**	***p*-value**
Between River Basin	1	36645.91	8.41%	0.0841	0.001
Between Population within River Basin	1	21638.22	13.72%	0.1498	0.001
Between Samples within Population	49	186370.51	13.63%	0.1751	0.001
Within Samples	52	138852.15	64.24%	0.3576	0.001

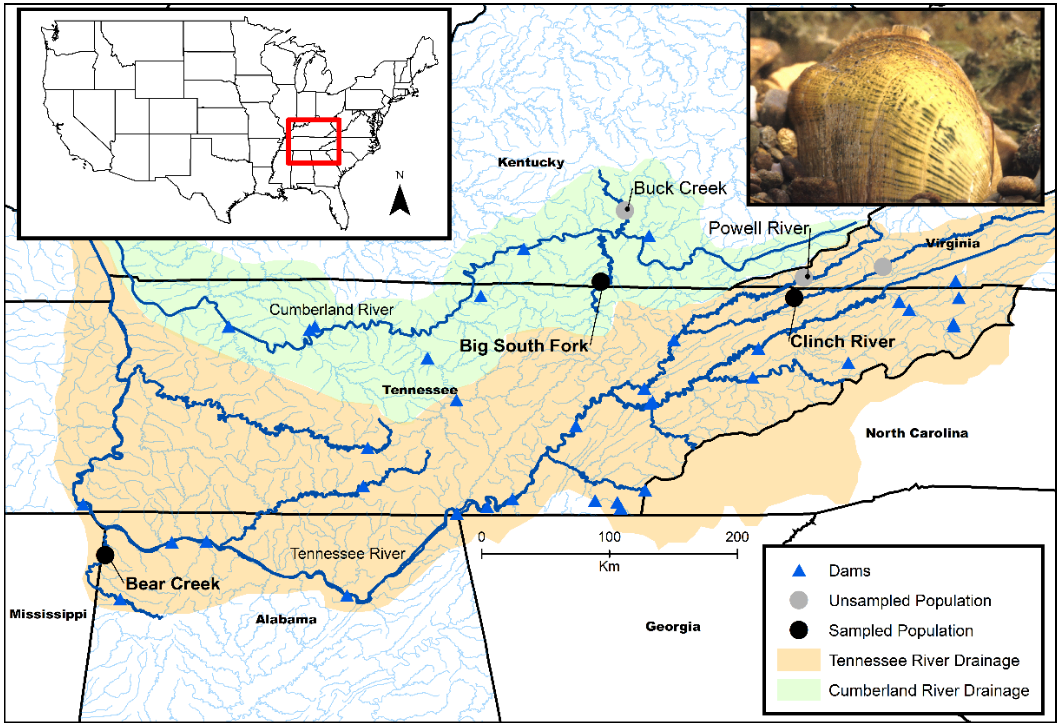


Figure 2-1. Geographic range of remaining *E. brevidens* populations and photograph of *E. brevidens* in the wild. Sampled populations, unsampled populations, dams, and river drainages are delineated by color.

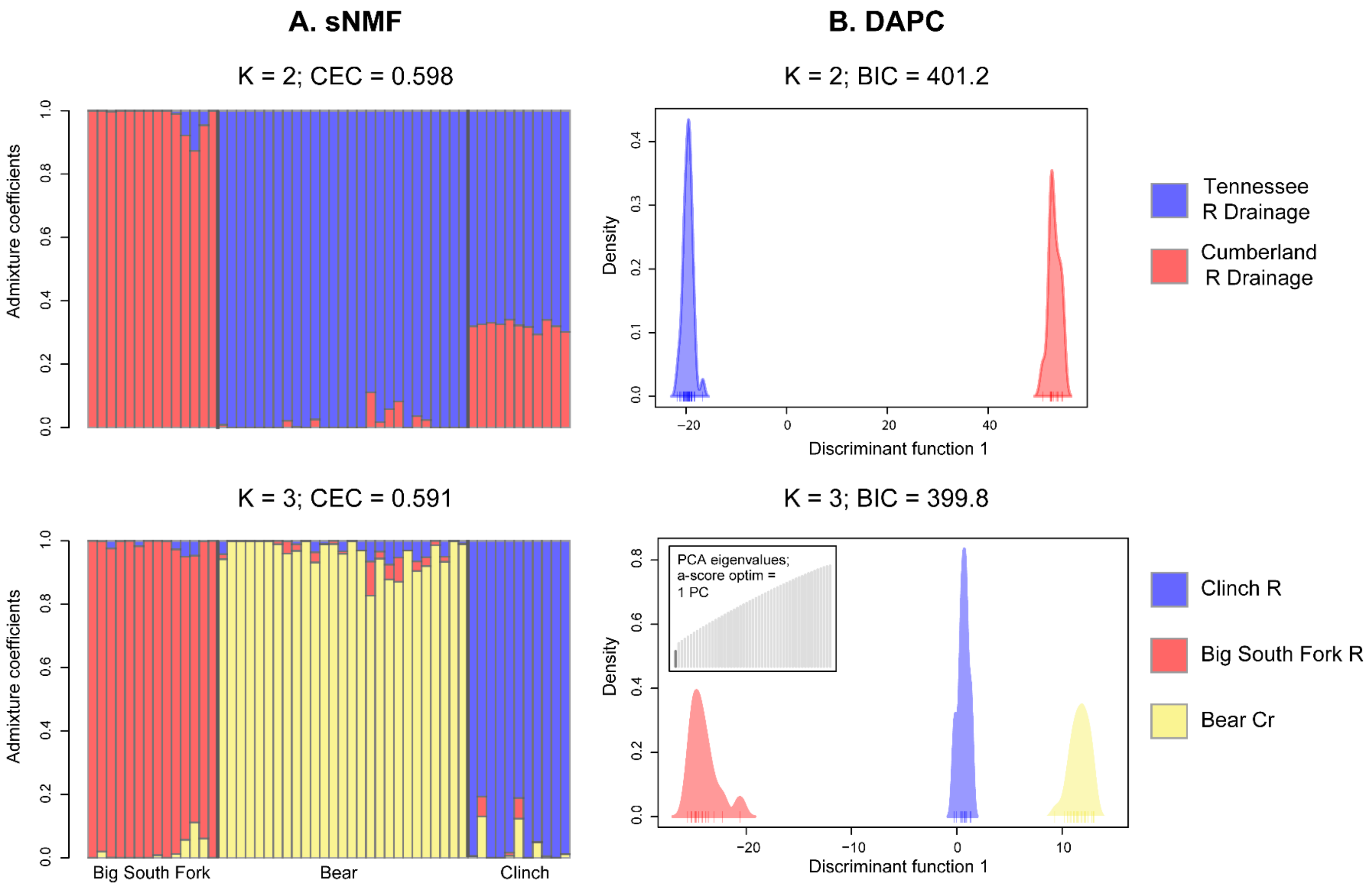


Figure 2-2. A. Discriminant analysis of principal components (DAPC) and B. genetic admixture inferred by the sparse non-negative matrix factorization (sNMF) algorithm implemented in *LEA*. Individuals are colored by population. Top: sNMF and DAPC using the second best K (K = 2). Bottom: sNMF and DAPC using the best fit K (K = 3).

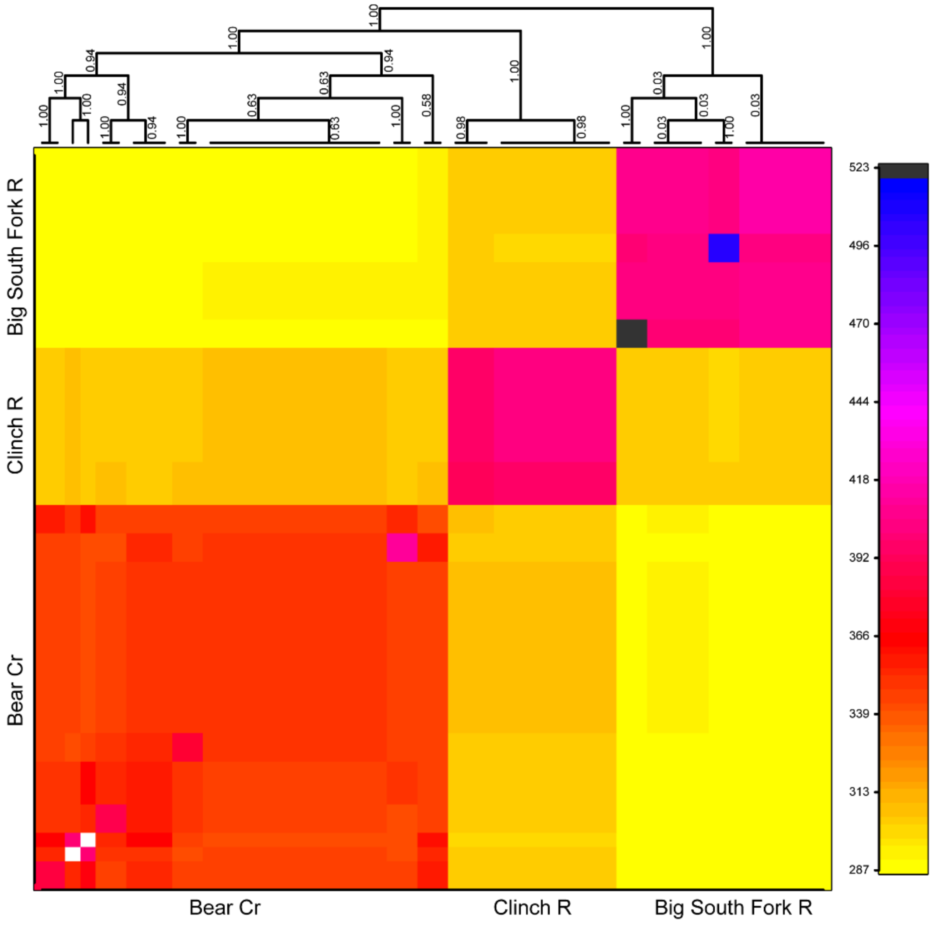
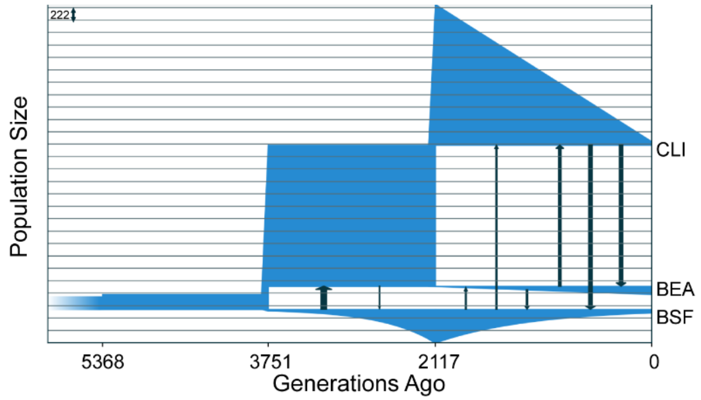


Figure 2-3. Hierarchical heatmap and inferred simple tree generated by *fineRADstructure* of all individuals from the three sampled populations. Colors represent relative co-ancestry values. Numerical values next to each branch of the tree are posterior population assignment probabilities.


A. Four Year Generation Time Demographic Model

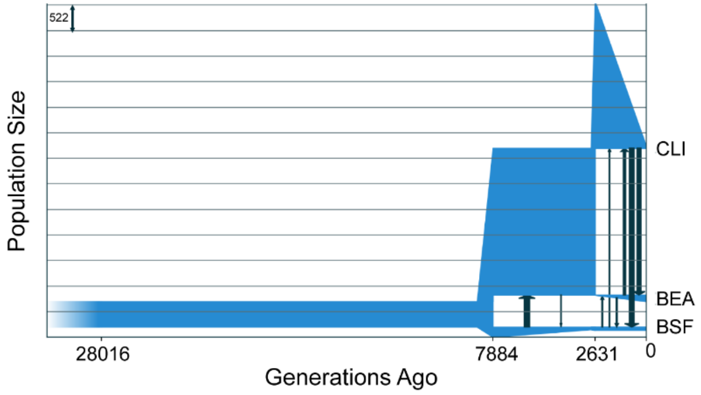

B. Six Year Generation Time Demographic Model

Figure 2-4. Demographic history for the three *E. brevidens* populations inferred by *GADMA*. Values on y-axis correspond to inferred effective population size; value on x-axis correspond to generation time before present. Thickness of black arrows between populations indicates migration rates. A. four-year generation time model. B. six-year generation time model.

Chapter 3. The Land Snails and Slugs of Tennessee, USA: Taxonomic Composition, Distribution, and an Evaluation of State-Wide Spatial and Taxonomic Survey Biases

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3.1 Abstract

The increased availability of publicly available, digitally accessioned biodiversity data and scientific literature presents the opportunity to reassess geographical associations of understudied taxonomic groups. Using these resources, in addition to occurrence data managed by the National Park Service at the Great Smoky Mountains National Park and private collections, we have assembled an updated taxonomic checklist and robust geographic dataset of all records of terrestrial gastropods (land snails and slugs) within the state of Tennessee. Additionally, we quantified spatial and taxonomic biases in survey coverage and overall survey quality for terrestrial gastropods at two spatial resolutions: ecoregion and county. Our state checklist includes 301 species of terrestrial gastropods that have at least one occurrence record, which increases the number of species by 40 from previous assessments of terrestrial gastropod biodiversity in Tennessee. Spatial analyses revealed < 33% of ecoregions and < 18% of counties to be sufficiently sampled for any terrestrial gastropod group as inferred by accumulation curves and the ratio between number of records to number of genera within each spatial unit. Significant biases in survey coverage in the state were revealed at both spatial scales, and a disproportionate

number of occurrence records were found in the eastern portion of the state near urban centers, universities, and the Great Smoky Mountains National Park. Taxonomic diversity estimates were similarly biased. Moreover, larger snails comprise the vast majority of occurrence records compared to snails less than 5 mm in shell diameter and slugs. From a conservation perspective, the addition of many newly reported species requires updating status assessments, and the spatial and taxonomic bias reported here should act as an incentive for future targeted gastropod survey effort, particularly in western Tennessee.

3.2 Introduction

Terrestrial gastropods (*i.e.*, land snails and slugs) are an important component of terrestrial ecosystems worldwide and play an integral role in influencing decomposition of organic matter, soil detoxification, and nutrient cycling to larger vertebrate fauna (Kappes 2005; Pearce 2008; Douglas et al. 2013). Terrestrial gastropods can also serve as bioindicators for various environmental perturbations and play an important role in conservation planning (Clements et al. 2008; Druart et al. 2011; Douglas et al. 2013). Despite their importance, many groups are understudied owing to their cryptic ecology or complicated taxonomy, and terrestrial mollusks are one of the most globally imperiled taxonomic groups (Lydeard et al. 2004; Cowie et al. 2017). A significant hindrance to their effective conservation is insufficient understanding of biodiversity patterns at multiple spatial scales, which is foundational for identifying areas of conservation priority (Cameron 2013; Ovando et al. 2019). Furthermore, conservation initiatives are typically overseen by state governments, so documenting state-level biodiversity patterns within the context of ecologically-relevant bioregions can provide crucial information for guiding management efforts (Vazquez et al. 2008; Miller et al. 2009).

Terrestrial gastropods of the eastern United States are exceptionally diverse with over 500 species documented (Hubricht 1985). Within the eastern U.S., Tennessee has the highest terrestrial mollusk biodiversity recorded with over 260 known species (Perez et al. 2008; **Figure 3-1**). The high number of species in Tennessee is likely attributable to the karst-rich topography and temperate climate of the state (Martin and Sommer 2004; Pearce and Örstan 2006; Niemiller and Zigler 2013). Tennessee spans six major physiographic provinces, which can be further partitioned into eight primary ecoregions based on geology, topography, soil chemistry, and vegetative communities (**Figure 3-2**; Omernik and Griffith 2014). Terrestrial gastropods in Tennessee have been documented from a variety of habitats at both state- and county-levels (Lutz 1950; Bogan et al. 1982; Coney et al. 1982; Hubricht 1973, 1985; Douglas et al. 2010; Dourson 2013; Dinkins and Dinkins 2018). The most concerted survey efforts have focused on the molluscan biodiversity within and adjacent to the Great Smoky Mountains National Park (GSMNP), an international biosphere reserve of approximately 522,427 acres that is divided almost evenly between North Carolina and Tennessee (Pilsbry 1900; Sharkey 2001; Dourson and Langdon 2012; Dourson 2012, 2013; Douglas et al. 2014).

While research efforts in the last two decades have improved our knowledge of the diversity and distributions of terrestrial gastropods in Tennessee (e.g., Dinkins and Dinkins 2018; Hodges and McKinney 2018), there has never been a state-wide assessment of the fauna. Perez et al. (2008) wrote an unpublished guide for terrestrial gastropod identification for a workshop held by the American Malacological Society that included the most recent synthesis of terrestrial gastropods known from Tennessee. Perez et al. (2008) reported 18 families, 55 genera, and 261 species from Tennessee, although finer scale location data such as county occurrences were not included. Thus, geographic ranges, spatial patterns of state-wide species composition, and potential spatial biases in survey effort have not been assessed, which can hinder accurate interpretation of biodiversity patterns (Cameron and Pokryszko 2005; Triantis et al. 2008). For example, terrestrial gastropod surveys may favor shelled specimens over those lacking external shell structures (i.e., slugs). Moreover, the majority of terrestrial gastropod species in eastern North America are micro-mollusks (i.e., shell diameter < 5 mm), and surveys are generally biased towards collecting macro-mollusks (i.e., shell diameter > 5 mm) (Dourson 2007; Nekola and Coles 2010; Dinkins and Dinkins 2018; Perez et al. 2020). Consequently, not only is there a need to update our understanding of all terrestrial gastropod groups within Tennessee, there is also a need to quantify the extent to which these biases have impacted our understanding of biodiversity patterns to better identify and resolve knowledge gaps.

The increasing development and improvement of open access, digital databases of museum collections provide an important resource for reassessing the terrestrial mollusk biodiversity of Tennessee, as well as quantifying potential spatial and taxonomic biases. However, there have been valid criticisms about data quality associated with large data aggregates (e.g., Troia and McManamay 2016; Bayraktarov et al. 2019), and fine-scale inferences of biodiversity patterns from such databases may not always represent on-the-ground realities (Perez et al. 2020). Specifically, for invertebrate collections such as terrestrial mollusks, difficultly in species identification or lack of informative locality descriptions may lead to many erroneous records that require direct verification before proper usage (Nekola et al. 2019). Despite limitations, these data can still be used for broad assessments such as the development of a state checklist or analyses of spatial bias at regional or county scales where precise locality information is not required. Moreover, collections data sourced from digital repositories can be directly acquired via museum loans to verify species identifications or analyzed at broader taxonomic scale (e.g., family, genus). In this way, assessments of taxonomic biases among broad groups of organisms (i.e., macro- versus micro-mollusks can be performed regardless of incomplete taxonomic certainty.

Here we reassess the taxonomic composition and distribution of the terrestrial gastropods in Tennessee and provide the first evaluation of the impact that spatial and taxonomic biases have on our understanding of their distributional and diversity patterns. To accomplish this, we compiled a comprehensive dataset of occurrence records from publicly available digital resources, the All Taxa Biodiversity Inventory at Great Smoky Mountains National Park through the National Park Service, and the private collection of R. Wayne Van Devender for land snails and slugs in the state. Then, using this dataset, we (1) provide an updated state checklist of all known terrestrial gastropods in Tennessee, (2) evaluate total survey quality of genus-level occurrence records at regional and county scales, and (3) compare the quality of survey data between micro-mollusks, slugs, and all other terrestrial gastropods.

3.3 Methods

3.3.1 Dataset assembly and filtering procedures

The Tennessee terrestrial gastropod dataset we compiled is comprised of occurrence records from: (1) primary scientific literature, (2) the InvertEBase digitized museum collection repository (Invert Base 2020), which provides access to over 20 major invertebrate zoological collections across North America, (3) the McClung Museum Malacology Collection at the University of Tennessee, Knoxville, (4) the Tennessee-specific records within the All Taxa Biodiversity Inventory database associated with the Great Smoky Mountains National Park (accessed with permit #GRSM-2020-SCI-2130 through the U.S. National Park Service) and (5) the personal collection of R. Wayne Van Devender (Appalachian State University, retired). To the best of our knowledge, the dataset compiled here represents the largest compilation of terrestrial gastropod occurrence records within Tennessee to date.

Two primary datasets were created by filtering occurrence records from the above sources: (1) the ‘full dataset’ that includes all occurrence records with and without georeferences (latitude, longitude) and (2) the ‘georeferenced dataset’ that is a subset of the full dataset that only includes records with georeferences. Filtering procedures to create the full dataset included removing records that did not contain at least species-level taxonomic rank, were reported as fossils, or did not have a locality description to at least county level. Additionally, to increase quality assessment of our full dataset, we acquired museum loans to verify species identifications and locality information for occurrence records based on two criteria: (1) if a species had less than or equal to five records in our initially filtered dataset excluding type materials, or (2) if a species was previously unknown from Tennessee as of Perez et al. (2008). We diagnosed loaned specimens using original species descriptions or authoritative works on terrestrial gastropods, including Pilsbry (1940, 1946, 1948), Burch (1962), Dourson (2010, 2013), and Nekola and Coles (2010). All materials from the Field Museum of Natural History were personally verified by the malacological collections manager (Jochen Gerber, personal communication). If the species identification was found to be incorrect, the record was corrected and retained for later analysis or removed if unidentifiable. If the locality description could not be verified, the record was removed. If a species previously unknown from Tennessee was identified in the dataset from literature, we contacted the original specimen collectors to verify the identification by determining diagnostic characters, we note however that the majority of novel records from literature were published previously by the authors of this paper (BJD, GRD, NSG) or were a part of collections made with the assistance of at least one of us (e.g., Hodges and McKinney 2018) and were considered accurate. All other literature used in this study were authored by taxonomic experts (e.g., Hubricht 1985; Dourson 2013; Bogan 1982).

The full dataset was used to assess survey quality at county resolution and to compile an updated state-wide checklist. We followed the taxonomy of MolluscaBase when cataloging taxonomic identifications in our full dataset (available at www.molluscabase.org), accordingly, the genus and species designation of any entity from source material that did not follow current taxonomy was changed to reflect MolluscaBase synonymies. We report all taxonomic and locality information with references (museum catalog numbers or literature citation) for each record in the full dataset. Further, we also cataloged all ‘non-native’ species found within our full dataset. We categorized species as ‘non-native’ if they were not known to be indigenous to North America, or not previously reported from a two-state buffer around Tennessee. When the checklist was completed, a subsequent search for the full, previously known state range of all new native species was conducted through NatureServe (NatureServe 2021) and for the conservation status of all taxa within the checklist at the global and state levels.

From the full dataset, the georeferenced dataset was generated as a subset. Georeferences were screened to ensure coordinates matched the location description, and those found to be of low-quality (e.g., coordinates falling outside of the location description or falling in the centroid of a large city, county, or state) were not used. If a record contained a georeference (i.e., longitude and latitude) that did not match the county description, we deferred to the county description and this record was not included. In the absence of a georeference or if the georeference was found to be of low-quality, an effort was made to generate a georeference for each occurrence record based upon the accompanying location description (e.g., "entrance to Cumberland State Park, 6.5 mi S of Crossville") using the web application GEOLocate (available at www.geo-locate.org/). Owing to the broad geographic scales assessed in this study, location descriptions were deemed possible to georeference if the total uncertainty radii associated with the centroid of the described area was less than 0.2 decimal degrees as calculated in GEOLocate. We also recognize the possible inaccuracies associated with georeferenced data obtained from digitized repositories (e.g., user estimation, translation error, incorrect search of location, etc.). Therefore, we do not assess spatial bias beyond the ecoregion level.

The georeferenced dataset was used for all ecoregion analyses below. Only georeferenced records were used for ecoregion analyses because although many counties fall directly within a single ecoregion, there are several which include two ecoregions, thereby enabling positional bias to occur when using county centroid data as the location. For data reporting purposes, all georeferenced data sourced from the All Taxa Biodiversity Inventory from the Great Smoky Mountains National Park were excluded and only reported by county affiliation in accordance with the data user agreement with the U.S. National Park Service.

3.3.2 Spatial analyses of survey quality

Although species-level identifications were assessed for all new or rarely found species found in Tennessee to generate the state checklist, the goal of our study was not to critically evaluate the species identifications for all museum materials. Therefore, owing to the high rate of terrestrial mollusk species misidentification in previous studies of museum collections databases (e.g., Nekola et al. 2019; Perez et al. 2020), we opted to use genus-level identifications for all analyses of spatial or taxonomic bias. However, the dataset provided will still report all species-level identifications, such that this data can be used to potentially identify gaps in species-specific ranges in the state or potential misidentifications among museum materials. Spatial analyses were carried out at two resolutions: (1) Level III ecoregion (Omernik and Griffith 2014, data available at ftp://newftp.epa.gov/EPADataCommons/ORD/Ecoregions/) and (2) the 2019 geopolitical Tennessee county boundaries (TIGER/Line® shapefiles, available at https://www.census.gov/geographies/mapping-files/time-series/geo/tiger-line-file.html). All coordinate data were projected or estimated in the World Geodetic Survey 1984 (WGS84) coordinate system and all shapefiles were projected to WGS84 for analyses of survey quality.

To assess geographical patterns and potential biases of past terrestrial gastropod surveys in Tennessee, we used the *KnowBR* R package (Lobo et al. 2018), which generates spatial summary statistics for each spatial unit (i.e., county or ecoregion) and corresponding maps. Specifically, at each spatial scale, we calculated: (1) number of occurrence records, (2) observed genus richness, (3) slope of the relationship between occurrences and genus richness, (4) ratio between the number of records and observed genus richness (R/G ratio), and (5) survey completeness. Survey completeness was estimated using the Clench (1979) species accumulation convex curve model implemented in *KnowBR*, which is robust to overfitting and critical richness underestimation. This model is also appropriate for the assessment of large or heterogenous sampling areas of understudied taxa (Soberón and Llorente 1993; Hortal et al. 2004). All analyses were conducted on the following data subsets of the full species dataset to identify potential taxonomic bias in survey effort: (1) all data, (2) land snails, (3) slugs, (4) macro-mollusks, and (5) micro-mollusks.

Overall survey quality was also assessed using each taxonomic subset at county and ecoregion scale based upon slope values of the accumulation curves, the completeness values, and the R/G ratio. These values provide an unbiased estimate of total occurrences recorded within a spatial unit against how many genera are recorded. Specifically, we followed Pelayo-Villamil et al. (2018) to partition survey quality into 'sufficient' (slope < 0.02, completeness $> 90\%$, R/G ratio > 15) or 'insufficient' (values under the thresholds just given). The survey quality operations were executed for the county and ecoregion spatial resolutions using the *SurveyQ* function within *KnowBR*. All *KnowBR* code used in this study is publicly available at https://github.com/ngladstone/TN-Terrestrial-Gastropods.

3.4 Results

3.4.1 Data collection

The complete Tennessee terrestrial gastropod dataset after filtering includes 27,899 occurrence records. The dataset contains 15,956 records from 12 museum collections from the

InvertEbase web portal, 350 records from the McClung Museum, 9,759 records from literature, 713 records from the Van Devender collection, and 1,120 records from the All Taxa Biodiversity Inventory of the Great Smoky Mountains National Park. The total number of both county and georeferenced records associated with each of the five data subsets was heavily skewed towards macro-mollusks (**Table 3-1**). From all records, 10,412 contained viable georeferences and another 5,572 records contained sufficient locality descriptions to facilitate georeferencing by the authors (15,984 georeferenced records total or ~57% of total dataset).

The checklist of Tennessee terrestrial gastropods includes 29 families, 68 genera, and 301 species (**Supplemental Table S1**), increasing the number of species known from Tennessee by 40 from the checklist of Perez et al. (2008) and by 63 from the checklist of Hubricht (1985). Of all the materials verified, 23 misidentifications were discovered and corrected to formerly known species from the state or removed. Additionally, three species from Perez et al. (2008) were not included in our final dataset: *Daedalochila dorfeuilliana* (one record was located from the Carnegie Museum but lacked at least county location), *Mesomphix perfragilis* (no records found), and *Succinea greerii* (synonymized with *Succinea grosvenorii*). This results in Tennessee having the highest known terrestrial gastropod biodiversity in the U.S., with California having the second highest diversity (279 species, Roth and Sadeghian 2006).

3.4.2 Updated species checklist

Among the newly reported species to the state, we classified 16 as non-native owing to their native range being distant from Tennessee or outside of North America altogether (see **Supplemental Table S1**). We categorized *Euglandina rosea* as non-native because while it occurs in the bordering states of Alabama and Georgia, its native range is subtropical. The only occurrence of this species in Tennessee is in the Nashville metropolitan area (Irwin et al. 2016). Among the non-native species, seven were macro-snails, three were micro-snails, and seven were slugs.

Of the remaining 32 newly reported species, all but two (*Daedalochila auriculata* and *Paravitrea bellona*) have been previously recorded in one or more bordering states, suggesting that historical survey efforts in Tennessee may not have been sufficiently robust to discover populations of these species. Among the newly reported native species, ten were micro-mollusks, and three were slugs. There was considerable variability in the previously known geographic range of the new native species in our checklist, 11 were previously known from over ten states, whereas 11 were known from two or fewer states (see **Supplemental Table S1**).

3.4.3 Spatial coverage and survey quality

3.4.3.1 County dataset

The full dataset with all county occurrences has records from all but one county (Henderson, **Figure 3-3**). Knox County had the highest density of both occurrence records and observed genus richness (2,979 records, 62 genera), likely owing to increased survey effort (Dinkins and Dinkins 2018) Several other eastern counties with large metropolitan areas or universities had disproportionately more records and observed genus richness (e.g., Chattanooga: Hamilton County [791 records, 41 genera] and Marion County [945 records, 41 genera], Franklin County [1,532 records, 38 genera]). Counties partially within or near the boundaries of the Great Smoky Mountains National Park also had a relatively high number of records (Blount County:

2,378 records, 46 genera, Cocke County: 512 records, 40 genera, Monroe County: 940 records, 43 genera, Polk County: 1,142 records, 35 genera, Sevier County: 2,041 records, 40 genera, see Fig. 3). In the central part of Tennessee, only Davidson County, which is part of the Nashville metropolitan area, held more than 250 records (658 records, 46 genera). Overall, high-to-low inventory completeness values exhibit a general east-to-west gradient along counties when considering all genera (**Figure 3-3**), suggesting there has been a comparatively greater effort spent surveying for terrestrial gastropods in the eastern portion of the state.

When estimating total survey quality, only 17 of 95 counties were assessed as sufficient survey quality for the macro-mollusk subset, and only three counties (Knox, Sevier, Blount) was sufficient for the micro-mollusks and combined taxonomic dataset (**Figure 3-4**). The vast majority of counties were assessed as insufficient survey quality for all taxonomic subsets particularly slugs for which only one county were assessed as sufficient (Knox). Most notably for micro-mollusks, Knox County contained over 3x more occurrence records than all bordering counties, despite environmental similarity. This comparably large sampling effort in Knox can be attributed to the recent survey of the county (Dinkins and Dinkins 2018). Micro-mollusks account for only ~17% of occurrence records in the total county dataset (4,777 records), despite this group being particularly diverse in the eastern United States (Hubricht 1985). Moreover, slugs, though far less diverse than land snails in North America, represent only ~2% of the total county-level dataset (569 records).

3.4.3.2 Ecoregion dataset

The georeferenced dataset used to evaluate spatial bias at the ecoregion level revealed additional sampling biases at a larger scale. The Blue Ridge (5,059 records, 49 genera), Ridge and Valley (4,254 records, 61 genera), and Interior Plateau (3,026 records, 56 genera) ecoregions held the highest number of total records and greatest observed genus richness (**Figure 3-5**). These three ecoregions comprise portions of two major karst regions, the Appalachians and Interior Low Plateau, that span much of the southeastern United States. As such, the high number of occurrence records and observed genus richness is likely attributable to the calcium-rich karst topography within these regions (Pearce and Örstan 2006). The Central and Southwestern Appalachians ecoregions (informally the Cumberland Plateau) provide a natural break between the aforementioned karst regions, and our findings show a coinciding gap of both records and observed genus richness in this area. Similar to the county dataset, the number of records and genus richness decreases west of the Interior Plateau.

Compared to the county-level dataset, a greater geographical area was assessed as sufficiently sampled at the ecoregion level. Three of eight ecoregions, Blue Ridge, Valley and Ridge, Interior Plateau, were assessed as sufficient for macro-mollusks and the combined taxonomic dataset. One ecoregion, Valley and Ridge, was assessed as sufficient for micro-mollusks (**Figure 3-4**). All ecoregions were assessed as insufficient for slugs, including two ecoregions, Mississippian Alluvial Plain and Southwestern Appalachians, that contained zero occurrence records for slugs. For micro-mollusks, less than 25 records were associated with the three ecoregions west of the Interior Plateau combined.

3.5 Discussion

3.5.1 Spatial biases of terrestrial gastropod surveys in Tennessee

We compiled nearly 28,000 occurrence records within the state, and our findings indicate that the terrestrial gastropod fauna of Tennessee includes more species than previously understood. Our findings increased the known terrestrial molluscan fauna of Tennessee by ~18% compared to Perez et al. (2008). Although there is a long history of collecting terrestrial gastropods in the state as evidenced by the museum materials compiled, collections within the last two decades have uncovered many species previously unknown from the state (e.g., Dinkins and Dinkins 2018, Hodges and McKinney 2018). Moreover, the increased accessibility of museum materials through digital aggregation assisted us in uncovering new state records. For instance, more than 10 new state records were collected or deposited in museums more than 25 years ago, including several from over 100 years ago. Clearly, museums records are essential to understanding biodiversity. We also advocate for our approach being replicated for other states.

Despite the substantial increase in the number of species known to Tennessee, there are notable geographical biases in our understanding of the state's terrestrial gastropod biota. Using various biodiversity metrics, we showed that there is a paucity of survey effort in the western portion of the state for all taxonomic subgroups of terrestrial gastropods, but particularly for slugs and micro-mollusks. While it is likely that there may be comparably less terrestrial gastropod biodiversity in the western portion of the state owing to more optimal habitat within the Appalachian Mountains and surrounding areas (Hotopp 2002; Dourson 2013), there are many counties and ecoregions that have apparently not seen any survey efforts for some taxonomic groups (see **Figure 3-3** and **Figure 3-5**). Several sampling epicenters such as Knox County or the Great Smoky Mountains National Park have the highest number of records, observed genus richness, and inventory completeness. Yet, at the county scale, spatial coverage of terrestrial mollusk surveys is comparably reduced in areas surrounding these epicenters as a byproduct of individual sampling endeavors favoring specific areas (e.g., Pilsbry 1900; Douglas et al. 2010; Dinkins and Dinkins 2018) and a lack of systematic survey efforts throughout the state.

Ecoregions considered sufficiently surveyed occur predominantly in the eastern-most extent of Tennessee apart from the Interior Plateau ecoregion in the center of the state. However, at the smaller, county scale, our results show that most areas are insufficiently surveyed for terrestrial gastropods. Regarding taxonomic biases present in the compiled dataset, there is a substantial inequality in the number of records for micro-mollusks and slugs compared to all other taxonomic subsets. These two taxonomic subsets comprised only a fraction of the total dataset (**Table 3-1**), likely owing to the lack of systematic survey efforts that include leaf-litter sampling (Coppolino 2010; Durkan et al. 2013). The paucity of slug specimens is likely due to their lack of a shell which makes properly preserving them difficult, further biasing our dataset towards land snails. Moreover, there are comparatively far fewer studies of slugs in the U.S., and as such less targeted research or survey efforts have been completed.

Understanding biases in sampling effort and quality at a variety of resolutions is necessary to outline priority areas in need of further sampling or to inform management efforts (Ferrier 2002; Huber et al. 2010). However, only ~57% of records were georeferenced by the original data source or had sufficient locality information for georeferencing. Thus, only slightly more than half of all Tennessee records were useful for spatial bias assessments at the ecoregion level. Georeferenced data is becoming increasingly important to identify spatial patterns at high resolutions and to highlight conservation priorities (Clements et al. 2008; Ovando et al. 2019). Therefore, we wish to stress the importance of assigning accurate georeferences to past collections and urge future collectors to diligently record detailed locality data when depositing terrestrial gastropod material.

3.5.2 New state records

Our study provides an updated checklist of terrestrial gastropods from Tennessee, including the first comprehensive list of non-native taxa found within the state (**Supplemental Table S1**). While cataloging the native fauna of Tennessee is important for understanding biodiversity patterns and guiding conservation efforts, monitoring the dispersal and prevalence of non-native species is important to both ecosystem integrity and human wellbeing (Cowie et al. 2009; Gladstone et al. 2020). Most of the non-native species are known primarily from point occurrences in urban areas, but several are known to negatively impact native fauna and agricultural activities. For example, *Euglandina* spp. and *Oxychilus* spp. are carnivorous land snails that, where introduced, have the potential to predate on smaller, native species (Proschwitz 1994; Cowie et al. 2009; Curry and Yeung 2013; Gerlach et al. 2020). Additionally, *Arion* spp. and *Deroceras* spp. are widespread invasive slug species and known agricultural pests that can cause significant economic impacts (Frank 1998; Robinson 1999; McDonnell et al. 2008).

Among native terrestrial gastropods that are new in our checklist, approximately one third had a previously known, widespread geographic distribution which included multiple states near Tennessee. However, ten new species are of notable conservation concern as denoted by their global NatureServe status and have few known occurrences. Four are considered Imperiled (G2): *Carychium riparium*, *Dryachloa dauca*, *Patera sargentiana*, and *Stenotrema brevipila*. Six are considered Critically Imperiled (G1): *Fumonelix orestes*, *Fumonelix roanensis*, *Inflectarius verus*, *Paravitrea bellona*, *Paravitrea lacteodens*, and *Philomycus batchi*. Although several of these species were discovered from recent surveys (Dinkins and Dinkins 2018, Van Devender collection), others had been deposited into museum collections several decades ago (see **Supplemental Table S1**). It was only when digital access to these older collection records became available that these species were 'rediscovered' within Tennessee. Thus, these occurrence records reported here now expand their known distributions and this may warrant reassessment of their respective conservation statuses. In particular, newly reported species of conservation concern may warrant protection at the state level now that they are known to occur in Tennessee.

3.5.3 Biodiversity patterns

The underlying karst topography of the Interior Plateau and the eastern ecoregions (particularly the Valley and Ridge) may provide a natural explanation for the higher number of records and observed genus richness in these areas (Pearce and Örstan 2006; Niemiller and Zigler 2013). Terrestrial mollusks need calcium-rich resources in their local environment to facilitate shell growth (Pearce and Örstan 2006). However, the Cumberland Plateau, which provides the notable break between the genus richness of the eastern regions and the Interior Plateau, appears to have seen far fewer incidences of rigorous sampling (**Figure 3-5**). As seen at the county scale, many areas within the Cumberland Plateau have had highly limited sampling

(**Figure 3-5**). This may be owing to the predisposition of land snail survey efforts to target karst-rich areas because of the expectation of finding more genus diversity. Therefore, we cannot conclusively determine if this east-to-west gradient of taxonomic richness and abundance of terrestrial mollusks (taken as a surrogate for number of records) is a natural phenomenon, a sampling artifact, or a combination of both.

3.6 Conclusions

Owing to the ongoing decline of non-marine mollusks in general (Lydeard et al. 2004; Johnson et al. 2013; Cowie et al. 2017), there is increased importance to maintaining accurate state checklists for terrestrial mollusks. Combining the available wealth of digital information with online databases allowed us to uncover previously unreported records of terrestrial mollusk species in Tennessee. With our new synthesized Tennessee checklist, local, state and federal wildlife managers can initiate conservation assessments of species that are newly reported to the state with respect to their known regional distribution and initiate regionally focused surveys and conservation activities. Further, our study has shown there is a regional bias to sampling within Tennessee and we strongly recommend expanded sampling efforts in the western part of the state.

3.7 References

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Table 3-1. Number of genera and species for each terrestrial gastropod category assessed in the study, and the number of records associated with each data subset used in spatial analyses.

Subset	**TN Genera**	**TN Species**	**County Records**	**Georeferenced Records**
Land Slugs	7	20	566	248
Land Snails	61	281	27,333	15,736
Micro-species	18	74	4,777	3,233
Macro-species	50	227	23,122	11,631
Total	**68**	**301**	**27,899**	**15,984**

Figure 3-1. Select terrestrial gastropod species from Tennessee. A.*Stenotrema stenotrema* (L. Pfeiffer, 1842), B. *Strobilops labyrinthicus* (Say, 1817), C. *Philomycus carolinianus* (Bosc, 1802), D. *Lobosculum pustuloides* (Bland, 1858), E. *Haplotrema concavum* (Say, 1821), F. *Patera perigrapta* (Pilsbry, 1894), G. *Pallifera fosteri* F.C. Baker, 1939, H. *Glyphyalinia praecox* (H. B. Baker, 1930), I. *Ventridens demissus* (A. Binney, 1843), *Mesomphix capnodes* (W. G. Binney, 1857), K. *Zonitoides arboreus* (Say, 1817), L. *Discus patulus* (Deshayes, 1832). White line in bottom right corner of each photo is equivalent to 1 mm. Photo credits: Nicholas Gladstone.

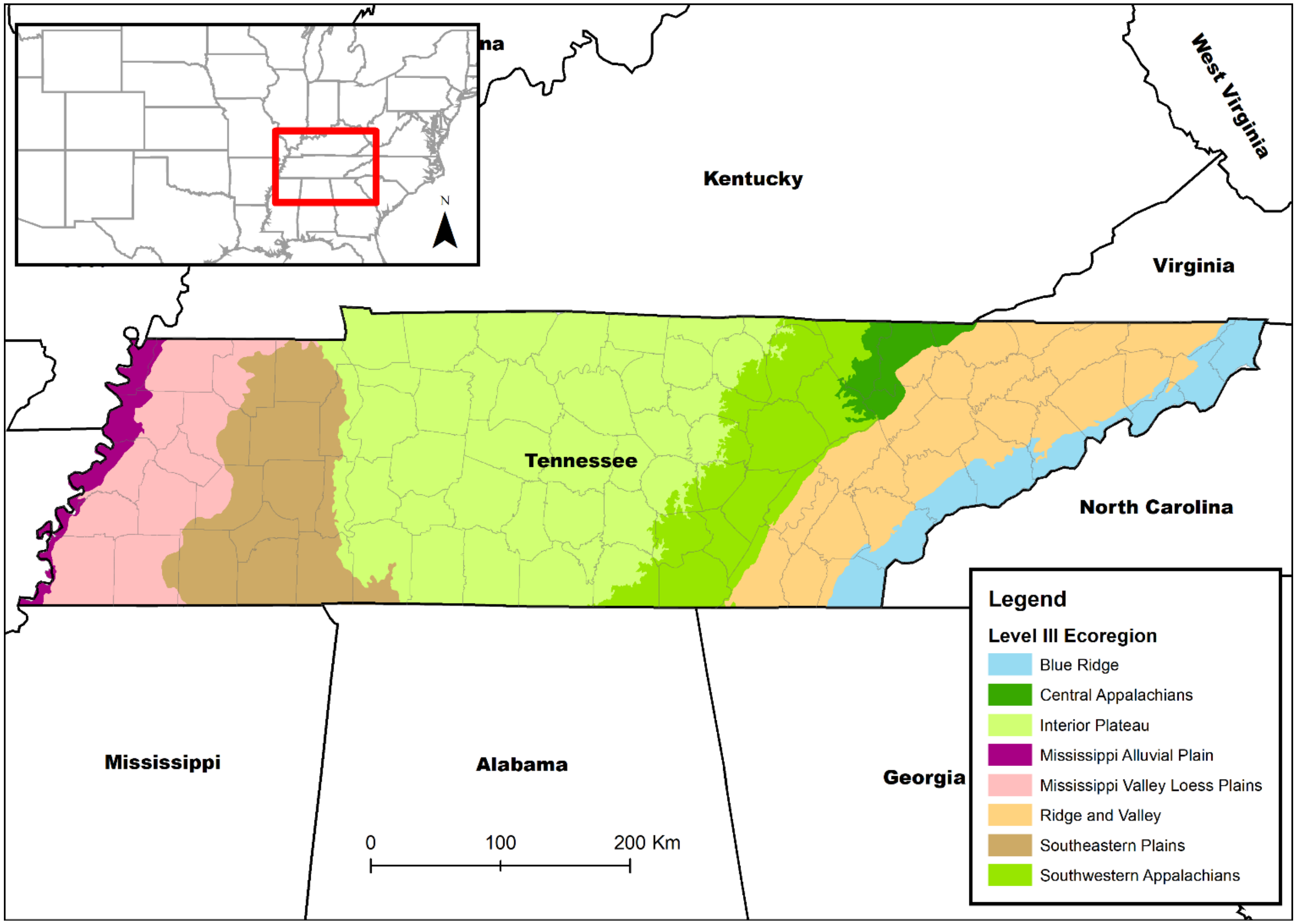


Figure 3-2. Map of Tennessee with county outlines. Colored portions of the state represent all Level III ecoregions (Omernik and Griffith 2014).

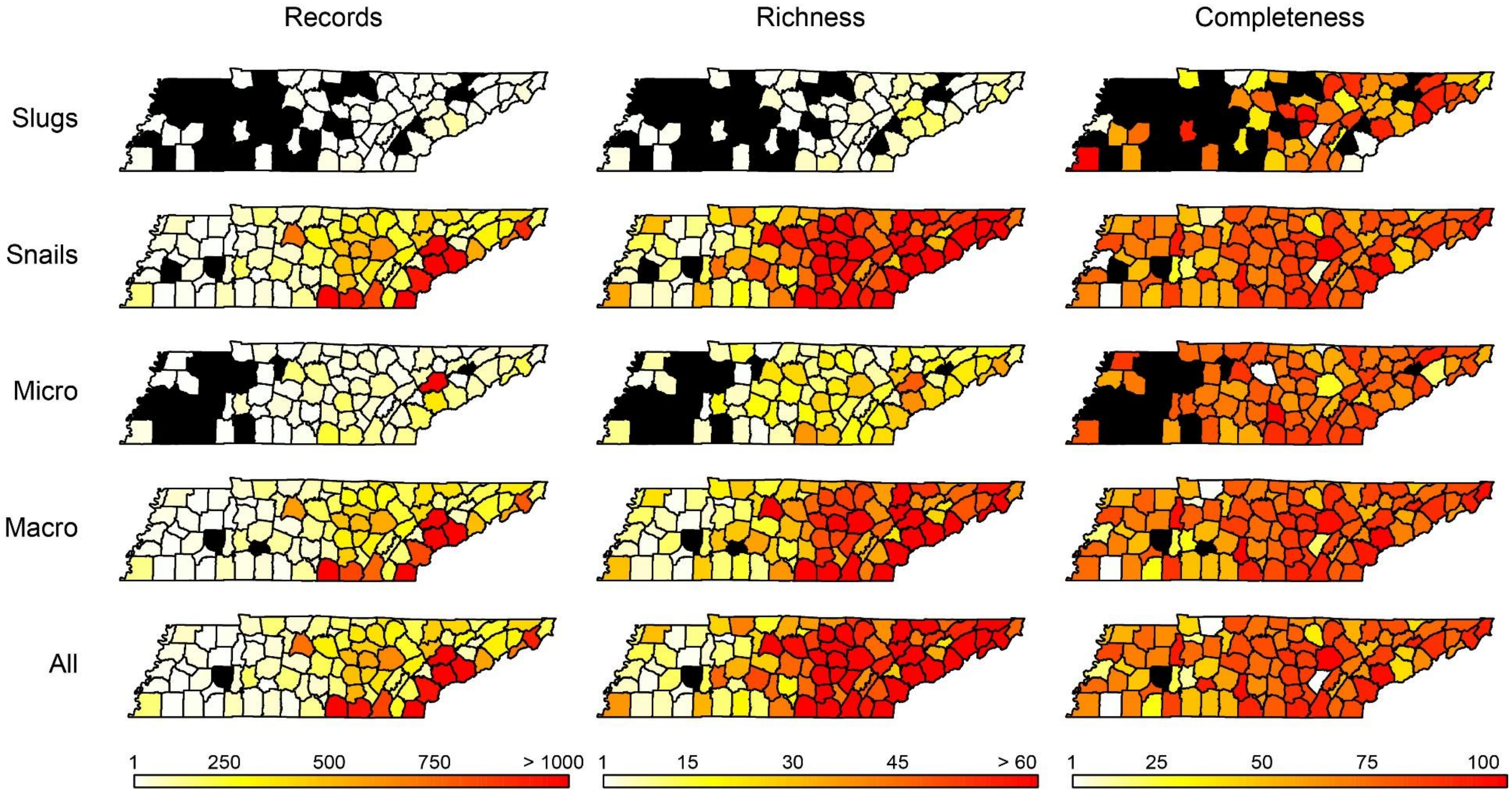


Figure 3-3. Geographical coverage of terrestrial mollusk surveys at the county spatial scale as number of records, observed genus richness, and overall inventory completeness generated using the *KnowBR* R package (Lobo et al. 2018). Areas colored in black indicate no available records.

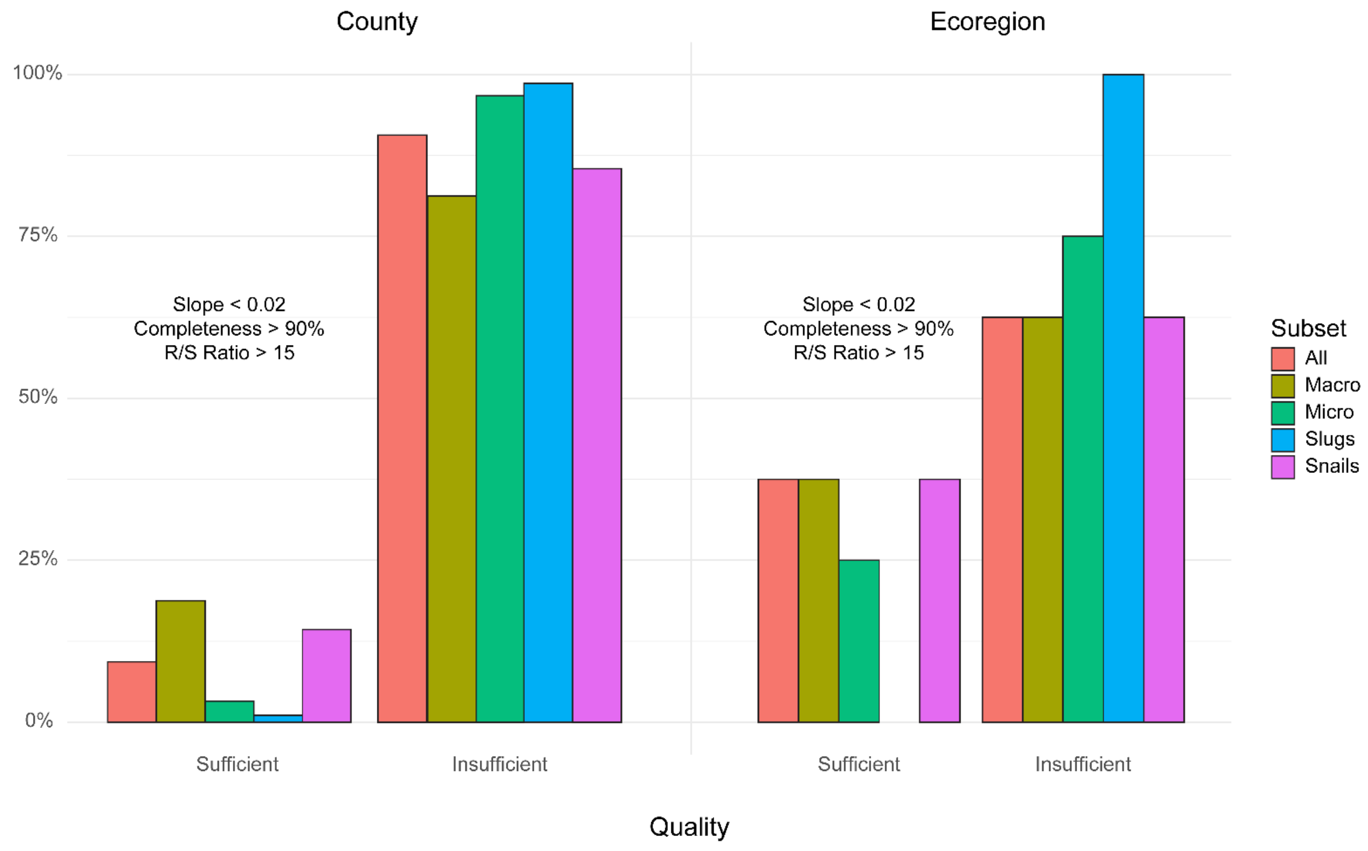


Figure 3-4. Proportion of spatial units assessed as sufficient or insufficient among taxonomic subsets when assessing standardized survey quality for the county and ecoregion spatial scale using the *KnowBR* R package (Lobo et al. 2018). All proportions among each survey quality category sum to 100% for each spatial scale, respectively.

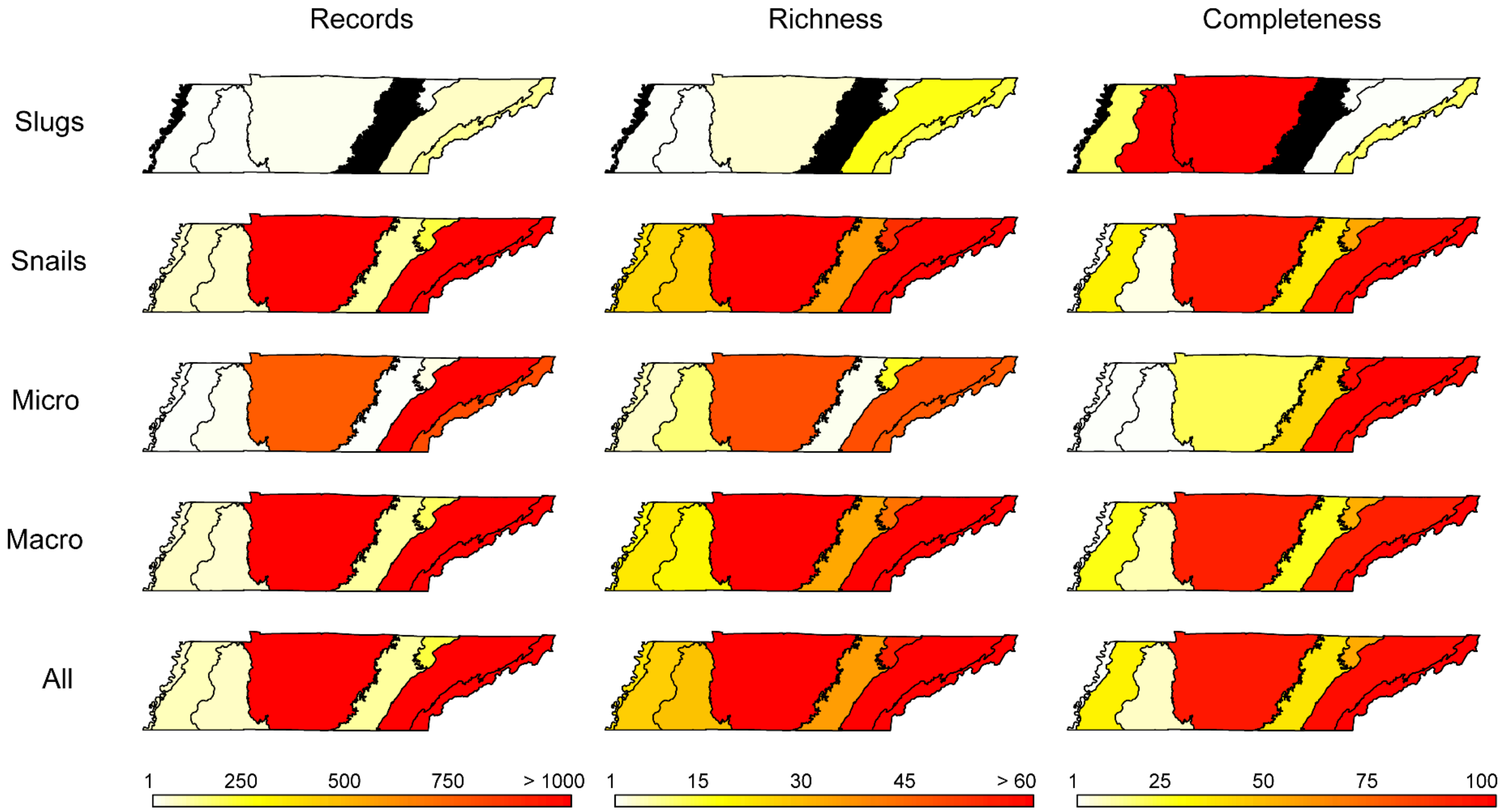


Figure 3-5. Geographical coverage of terrestrial mollusk surveys at the ecoregion spatial scale as number of records, observed genus richness, and overall inventory completeness generated using the *KnowBR* R package (Lobo et al. 2018). Areas colored in black indicate no available records.

Appendices and Supplementary Materials

Supplementary Table S1. Checklist of Tennessee terrestrial gastropods.

Scientific Name	Common Name	Nature Serve	State Status	Reference for New State Records	Previously Known Range of New Native Species (NatureServe)
Achatinidae Swainson, 1840					
Allopeas H. B. Baker, 1935					
Allopeas clavulinum (Potiez & Michaud, 1838)*	Spike Awlsnail			Dinkins and Dinkins (2018)	
Subulina H. Beck, 1837					
Subulina octona(Bruguière, 1789)*	Miniature Awlsnail			North Carolina Science Museum (#46932)	
Opeas Albers, 1850					
Opeas pyrgula Schmacker & O. Boettger, 1891*	Sharp Awlsnail			Hubricht (1973), Dinkins and Dinkins (2018), Hodges and McKinney (2018), McKinney et al. (2019)	
Agriolimacidae H. Wagner, 1935					
Deroceras Rafinesque, 1820					
Deroceras laeve (O. F. Müller, 1774)	Meadow Slug				
Deroceras reticulatum (O. F. Müller, 1774)*	Gray Fieldslug			Dinkins and Dinkins (2018), Van Devender Collection	
Arionidae Gray, 1840					
Arion A. Férussac, 1819					

Arion hortensis A. Férussac, 1819*	Garden Arion Slug			Dinkins and Dinkins (2018)	
Arion intermedius Normand, 1852*	Hedgehog Arion Slug			Dinkins and Dinkins (2018)	
Arion subfuscus (Draparnaud, 1805)*	Dusky Arion Slug			Field Museum of Natural History (#294956, #294958), Dinkins and Dinkins (2018), Van Devender Collection	
Bulimulidae Tryon, 1867					
Rabdotus Albers, 1850					
Rabdotus dealbatus (Say, 1821)	Whitewashed Rabdotus				
Camaenidae Pilsbry, 1895					
Bradybaena Beck, 1837					
Bradybaena similaris (Férussac, 1822)*	Asian Trampsnail			Dinkins and Dinkins (2018), McKinney et al. (2019)	
Cochlicopidae Pilsbry, 1900 (1879)					
Cochlicopa A. Férussac, 1821					
Cochlicopa lubrica (O. F. Müller, 1774)	Glossy Pillar Snail	G5	SNA		
Cochlicopa lubricella (Porro, 1838)	Thin Pillar Snail	G5		Dinkins and Dinkins (2018), Hodges and McKinney (2018)	CT, DE, IA, IL, IN, KS, KY, MA, MD, ME, MI, MN, MO, NJ, NY, OH, PA, RI, SD, TX, VA, VT, WI, WV, WY
Cochlicopa morseana (Doherty, 1878)	Appalachian Pillar Snail	G5	S4S5		
Discidae Thiele, 1931 (1866)					
Anguispira Morse, 1864					

Anguispira alabama (Clapp, 1920)	Alabama Tigersnail	G2	S2
Anguispira alternata (Say, 1817)	Flamed Tigersnail	G5	S5
Anguispira cumberlandiana (I. Lea, 1840)	Cumberland Tigersnail	G3	S3
cumberlandiana columba (Clapp, 1920)			
cumberlandiana cumberlandiana (I. Lea, 1840)		G3	S3
Anguispira jessica Kutchka, 1938	Mountain Disc	G3G4	S3
Anguispira knoxensis (Pilsbry, 1901)	Rustic Tigersnail	G1G2	S1S2
Anguispira mordax (Shuttleworth, 1852)	Appalachian Tigersnail	G4	S4
Anguispira picta (Clapp, 1920)	Painted Tigersnail	G1	S1
Anguispira strongylodes (L. Pfeiffer, 1855)	Southeastern Tigersnail	G5	SNR
Discus Fitzinger, 1833			
Discus bryanti (Harper, 1881)	Sawtooth Disc	G3	S1S2
Discus clappi (Pilsbry, 1924)	Channeled Disc	G1	S1
Discus nigrimontanus (Pilsbry, 1924)	Black Mountain Disc	G4	S4
Discus patulus (Deshayes, 1832)	Domed Disc	G5	S5

Discus whitneyi (Newcomb, 1864)	Forest Disc Snail	G5		Harvard Museum of Comparative Zoology (#216966), Florida Museum of Natural History (#382287, #403263), Hodges and McKinney (2018)	AK, AR, AZ, CA, CT, DE, IA, ID, IL, IN, KS, KY, MA, MD, ME, MI, MN, MO, MT, NC, ND, NE, NJ, NM, NY, OH, OK, SD, TX, UT, VA, VT, WA, WI, WV, WY
Ellobiidae L. Pfeiffer, 1854 (1822)					
Carychium O. F. Müller, 1773					
Carychium clappi Hubricht, 1959	Appalachian Thorn	G5	S4S5		
Carychium exiguum (Say, 1822)	Obese Thorn Snail	G5	S3		
Carychium exile H. C. Lea, 1842	Ice Thorn Snail	G5	S3		
Carychium mexicanum Pilsbry, 1891	Southern Thorn	G5	S3?		
Carychium nannodes Clapp, 1905	File Thorn Snail	G5	S4S5		
Carychium riparium Hubricht, 1978	Floodplain Thorn Snail	G2		Dinkins and Dinkins (2018)	IN, KY
Carychium stygium Call, 1897	Cave Thorn	G3	S2		
Euconulidae H. B. Baker, 1928					
Dryachloa F. G. Thompson & H. G. Lee, 1980					
Dryachloa dauca Thompson & Lee, 1980	Carrot Glass	G2		Dinkins and Dinkins (2018)	AL, FL, LA
Euconulus Reinhardt, 1883					

Euconulus chersinus (Say, 1821)	Wild Hive Snail	G5	S5
Euconulus dentatus (Sterki, 1893)	Toothed Hive	G5	S4S5
Euconulus fulvus (O. F. Müller, 1774)	Brown Hive Snail	G5	S4
Euconulus trochulus (Reinhardt, 1883)	Silk Hive	G5	S5
Guppya Mörch, 1867			
Guppya sterkii (Dall, 1888)	Brilliant Granule Snail	G5	S4S5
Gastrocoptidae Pilsbry, 1918			
Gastrocopta Wollaston, 1878			
Gastrocopta armifera (Say, 1821)	Armed Snaggletooth Snail	G5	S5
Gastrocopta clappi (Sterki, 1909)	Bluegrass Snaggletooth	G4	S4S5
Gastrocopta contracta (Say, 1822)	Bottleneck Snaggletooth Snail	G5	S5
Gastrocopta corticaria (Say, 1817)	Bark Snaggletooth Snail	G5	S4?
Gastrocopta holzingeri (Sterki, 1889)	Lamda Snaggletooth Snail	G5	SNA
Gastrocopta pentodon (Say, 1822)	Comb Snaggletooth Snail	G5	S5

Gastrocopta procera (Gould, 1840)	Wing Snaggletooth Snail	G5	S4S5	Illinois Natural History Survey (#74231)	IA, IL, IN, KS, KY, MI, MN, MO, ND, NY, OH, OK, PA, SD, WI, WY
Gastrocopta rupicola (Say, 1821)	Tapered Snaggletooth	G4			
Gastrocopta similis (Sterki, 1909)	Great Lakes Snaggletooth Snail	G5			
Gastrocopta tappaniana (C. B. Adams, 1841)	White Snaggletooth Snail	G5	S4?		
Gastrodontidae Tryon, 1866					
Gastrodonta Albers, 1850					
Gastrodonta fonticula Wurtz, 1948	Appalachia Bellytooth	G3G4	S2S3		
Gastrodonta interna (Say, 1821)	Brown Bellytooth	G5	S5		
Glyphyalinia E. von Martens, 1892					
Glyphyalinia carolinensis (Cockerell, 1890)	Spiral Mountain Glyph	G4	S3S4		
Glyphyalinia cryptomphala (Clapp, 1915)	Thin Glyph	G5	S5		
Glyphyalinia cumberlandiana (Clapp, 1919)	Hill Glyph	G4	S4		
Glyphyalinia indentata (Say, 1822)	Carved Glyph Snail	G5	S5		
Glyphyalinia junaluskana (Clench & Banks, 1932)	Dark Glyph	G2G3	S2		
Glyphyalinia lewisiana (Clapp, 1908)	Pale Glyph	G4	S3S4		

Glyphyalinia luticola Hubricht, 1966	Furrowed Glyph Snail	G4G5	S3
Glyphyalinia ocoae Hubricht, 1978	Blue-gray Glyph	G1	S1
Glyphyalinia pentadelphia (Pilsbry, 1900)	Pink Glyph	G1G3	S2
Glyphyalinia praecox (H. B. Baker, 1930)	Brilliant Glyph	G4	S4
Glyphyalinia rhoadsi (Pilsbry, 1899)	Sculpted Glyph Snail	G5	S3
Glyphyalinia rimula Hubricht, 1968	Tongued Glyph	G3	S2S3
Glyphyalinia sculptilis (Bland, 1858)	Suborb Glyph	G4	S4
Glyphyalinia solida (H. B. Baker, 1930)	Imperforate Glyph	G5	SNR
Glyphyalinia specus Hubricht, 1965	Hollow Glyph	G4	S3
Glyphyalinia wheatleyi (Bland, 1883)	Bright Glyph Snail	G5	S5
Mesomphix Rafinesque, 1819			
Mesomphix andrewsae (Pilsbry, 1895)	Mountain Button	G3G4	S3S4
Mesomphix anurus Hubricht, 1962	Frog Button	G4G5	S5
Mesomphix capnodes (W.G. Binney, 1857)	Dusky Button	G5	S4S5
Mesomphix cupreus (Rafinesque, 1831)	Copper Button Snail	G5	S5
Mesomphix friabilis (W.G. Binney, 1857)	Brittle Button	G5	S3?
Mesomphix globosus (MacMillan, 1940)	Globose Button	G5	S4?

Mesomphix inornatus (Say, 1822)	Plain Button Snail	G5	S3S4		
Mesomphix latior (Pilsbry, 1900)	Broad Button	G3G4	S3		
Mesomphix perlaevis (Pilsbry, 1900)	Smooth Button	G4G4	S4		
Mesomphix pilsbryi (Clapp, 1904)	Striate Button	G4		Carnegie Museum (#140964), Delaware Museum of Natural History (#150479), Florida Museum of Natural History (#139733, 27859), North Carolina Science Museum (#44998, #41317, #41740), Van Devender Collection	AL, FL, GA, MS, NC, SC
Mesomphix rugeli (W.G. Binney, 1879)	Wrinkled Button	G4	S3		
Mesomphix subplanus (A. Binney, 1842)	Flat Button	G3G4	S3S4		
Mesomphix vulgatus H. B. Baker, 1933	Common Button	G4	S4		
Nesovitrea C. M. Cooke, 1921					
Nesovitrea electrina (Gould, 1841)	Amber Glass Snail	G5			
Striatura Morse, 1864					
Striatura exigua (Stimpson, 1850)	Ribbed Striate Snail	G5	S1		
Striatura ferrea Morse, 1864	Black Striate Snail	G5	S3	Dourson (2013)	KY, MA, MD, ME, MI, MN, NC, NH, NY, OH, PA, TN, VA, VT, WI, WV

Striatura meridionalis (Pilsbry & Ferriss, 1906)	Median Striate	G5	S5		
Striatura milium (Morse, 1859)	Fine-ribbed Striate Snail	G5		Carnegie Museum (#110201, #62.20923, #100788), Florida Museum of Natural History (#108135)	CT, DE, IA, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, NE, NJ, NY, OH, OK, PA, SD, VA, VT, WI, WV
Ventridens W. G. Binney & Bland, 1869					
Ventridens acerra (Lewis, 1871)	Glossy Dome	G4	S4		
Ventridens arcellus Hubricht, 1976	Golden Dome	G4	S3S4		
Ventridens coelaxis (Pilsbry, 1899)	Bidentate Dome	G3	S2S3		
Ventridens collisella (Pilsbry, 1896)	Sculptured Dome	G4	S4		
Ventridens decussatus (Walker & Pilsbry, 1902)	Crossed Dome	G3	S2?		
Ventridens demissus (A. Binney, 1843)	Perforate Dome Snail	G5	S4S5		
Ventridens eutropis Pilsbry, 1946	Carinate Dome	G2G3	S2S3		
Ventridens gularis (Say, 1821)	Throaty Dome	G5	S4S5		
Ventridens intertextus (A. Binney, 1840)	Pyramid Dome Snail	G5	S5		
Ventridens lasmodon (Phillips, 1841)	Hollow Dome	G4	S3		
Ventridens lawae (W.G. Binney, 1883)	Rounded Dome	G4	S4		
Ventridens ligera (Say, 1821)	Globose Dome Snail	G5	S5		
Ventridens percallosus (Pilsbry, 1898)	Tennessee Dome	G3	S3		

Ventridens pilsbryi Hubricht, 1964	Yellow Dome	G4	S4		
Ventridens suppressus (Say, 1829)	Flat Dome Snail	G5			
Ventridens theloides (Walker & Pilsbry, 1902)	Copper Dome	G4G5	S4S5		
Ventridens virginicus (Vanatta, 1936)	Split-tooth Dome	G4		Carnegie Museum (#127269, #127271)	IL, MD, NY, PA, VA, WV
Vitrinizonites W.G. Binney, 1879					
Vitrinizonites latissimus (Lewis, 1875)	Glassy Grapeskin	G4	S4		
Zonitoides Lehmann, 1862					
Zonitoides arboreus (Say, 1817)	Quick Gloss Snail	G5	S5		
Zonitoides elliotti (Redfield, 1858)	Green Gloss	G4	S4		
Zonitoides lateumbilicatus (Pilsbry, 1895)	Striate Gloss	G3G4	S2?		
Zonitoides patuloides (Pilsbry, 1895)	Appalachian Gloss	G3	S2		
Haplotrematidae H. B. Baker, 1925					
Haplotrema Ancey, 1881					
Haplotrema concavum (Say, 1821)	Gray-foot Lancetooth Snail	G5	S5		
Haplotrema kendeighi Webb, 1951	Blue-foot Lancetooth	G2G3	S2		
Helicidae Rafinesque, 1815					
Cepaea Held, 1838					
Cepaea nemoralis (Linnaeus, 1758)*	Grovesnail			Hodges and McKinney (2018)	

Helicinidae Férussac, 1822			
Helicina Lamarck, 1799			
Helicina orbiculata (Say, 1818)	Globular Drop	G5	S5
orbiculata orbiculata (Say, 1818)	Globular Drop	G5	S5
orbiculata tropica L. Pfeiffer, 1850			
Hendersonia A. J. Wagner, 1905			
Hendersonia occulta (Say, 1831)	Cherrystone Drop	G4	S3
Helicodiscidae Pilsbry, 1927			
Helicodiscus Morse, 1864			
Helicodiscus aldrichianus (Clapp, 1907)	Burrowing Coil	G3	S2S3
Helicodiscus barri Hubricht, 1962	Raccoon Coil	G3G4	S3
Helicodiscus bonamicus Hubricht, 1978	Spiral Coil	G1	S1
Helicodiscus enneodon Hubricht, 1967	Bluff Coil	G3G4Q	S4S5
Helicodiscus fimbriatus Wetherby, 1881	Fringed Coil	G4	S3
Helicodiscus hadenoecus Hubricht, 1962	Cricket Coil	G3	S3
Helicodiscus hexodon Hubricht, 1966	Toothy Coil	G1	S1
Helicodiscus multidens Hubricht, 1962	Twilight Coil	G3	S1
Helicodiscus notius Hubricht, 1962	Tight Coil	G5	S5
notius notius Hubricht, 1962	Tight Coil	G5	S5

notius specus Hubricht, 1962	Hollow Coil	T2	S1?	
Helicodiscus parallelus (Say, 1821)	Compound Coil Snail	G5	S5	
Helicodiscus punctatellus Morrison, 1942	Punctate Snail	G1	S1	
Lucilla R. T. Lowe, 1852				
Lucilla scintilla (R. T. Lowe, 1852)	Oldfield Coil	G4	S4	
Lucilla singleyana (Pilsbry, 1889)	Smooth Coil	G5	SNA	
Limacidae Lamarck, 1801				
Ambigolimax Pollonera, 1887				
Ambigolimax valentianus (A. Férussac, 1821)*	Threeband Gardenslug			Dinkins and Dinkins (2018), Van Devender Collection
Limax Linnaeus, 1758				
Limax maximus Linnaeus, 1758*	Giant Gardenslug			Lutz (1950), Field Museum of Natural History (#213942, #209876, #213658)
Oxychilidae Hesse, 1927 (1879)				
Oxychilus Fitzinger, 1833				
Oxychilus cellarius (O. F. Müller, 1774)*	Cellar Glass-snail			Hodges and McKinney (2018)
Oxychilus draparnaudi (H. Beck, 1837)*	Dark-bodied Glass-snail			Dinkins and Dinkins (2018)
Pilsbryna H. B. Baker, 1929				
Pilsbryna aurea H. B. Baker, 1929	Ornate Bud	G1	S1	
Pilsbryna castanea H. B. Baker, 1931	Prominent Bud	G2	S2	

Pilsbryna nodopalma Slapcinsky & Coles, 2004	Oar Tooth Bud	G1G2	S1		
Pilsbryna quadrilamellata Slapcinsky & Coles, 2004	Four Blade Bud	G1	S1		
Pilsbryna vanattai (Walker & Pilsbry, 1902)	Honey Bud	G2G3	S2		
Philomycidae Gray, 1847					
Megapallifera Hubricht, 1956		G5	S4S5		
Megapallifera mutabilis (Hubricht, 1951)	Changeable Mantleslug	G2G3	S2		
Megapallifera wetherbyi (W.G. Binney, 1874)	Blotchy Mantleslug				
Pallifera Morse, 1864					
Pallifera dorsalis (A. Binney, 1842)	Pale Mantleslug	G5		Dourson (2013)	IA, IL, IN, KY, MD, ME, MI, NC, NY, OH, PA, VA, WV
Pallifera fosteri F.C. Baker, 1939	Foster Mantleslug	G5	S5		
Pallifera hemphilli (W.G. Binney, 1885)	Black Mantleslug	G4	S2S3		
Pallifera secreta (Cockerell, 1900)	Severed Mantleslug	G4	S4		
Philomycus Rafinesque, 1820					
Philomycus batchi Branson, 1968	Dusky Mantleslug	G1		Florida Museum of Natural History (#520648)	KY, OK, VA, WV
Philomycus carolinianus (Bosc, 1802)	Carolina Mantleslug	G5	S5		
Philomycus flexuolaris Rafinesque, 1820	Winding Mantleslug	G5	S3?		

Philomycus sellatus Hubricht, 1972	Alabama Mantleslug	G2G3	S2		
Philomycus togatus (Gould, 1841)	Variable Mantleslug	G5	S4?		
Philomycus venustus Hubricht, 1953	Brown-spotted Mantleslug	G4	S4?		
Philomycus virginicus Hubricht, 1953	Virginia Mantleslug	G3	S2		
Polygyridae Pilsbry, 1895					
Allogona Pilsbry, 1939					
Allogona profunda (Say, 1821)	Brown-banded Forestsnail	G5	S4		
Appalachina Pilsbry, 1940					
Appalachina chilhoweensis (Lewis, 1871)	Queen Crater	G4	S4		
Appalachina sayana (Pilsbry & Ferriss, 1906)	Spike-lip Crater Snail	G5	S3		
Daedalochila H. Beck, 1837					
Daedalochila auriculata (Say, 1818)	Ocala Liptooth	G3		McClung Museum (#11818)	FL, LA
Daedalochila auriformis (Bland, 1859)	Rockpile Liptooth	G4	S1		
Daedalochila fatigiata (Say, 1829)	New Harmony Liptooth	G3	S3		
Daedalochila leporina (Gould, 1848)	Gulf Coast Liptooth	G4G5	S4?		
Daedalochila plicata (Say, 1821)	Cumberland Liptooth	G4	S4		
Daedalochila troostiana (I. Lea, 1838)	Nashville Liptooth	G4	S5		
Euchemotrema Archer, 1939					

Euchemotrema fasciatum (Pilsbry, 1940)	Mountain Pillsnail	G3	SNA		
Euchemotrema fraternum (Say, 1824)	Upland Pillsnail	G5	S4S5		
fraternum fraternum (Say, 1824)	Upland Pillsnail	G5	S4S5		
fraternum montanum (Archer, 1939)	a pillsnail				
Euchemotrema leaii (A. Binney, 1840)	Lowland Pillsnail	G5	SNA		
leaii leaii (A. Binney, 1840)	Lowland Pillsnail	G5	SNA		
leaii aliciae (Pilsbry, 1893)	a pillsnail				
Fumonelix Emberton, 1991					
Fumonelix archeri (Pilsbry, 1940)	Ocoee Covert	G1	S1		
Fumonelix christyi (Bland, 1860)	Glossy Covert	G3	S2		
Fumonelix jonesiana (Archer, 1938)	Big-tooth Covert	G1	S1		
Fumonelix langdoni Dourson, 2012	Talus Covert	GNR	SNA	Dourson (2013)	NC, TN
Fumonelix orestes (Hubricht, 1975)	Engraved Covert	G1		Van Devender Collection	NC
Fumonelix roanensis Dourson, 2012	Roan Mountain Covert	G1		Dourson (2013)	
Fumonelix wetherbyi (Bland, 1874)	Clifty Covert	G2	S2S3		NC
Fumonelix wheatleyi (Bland, 1860)	Cinnamon Covert	G4	S3		
wheatleyi wheatleyi (Bland, 1860)	Cinnamon Covert	G4	S3		

wheatleyi clingmanicus (Pilsbry, 1904)	Clingman Covert	T2	S2		
Inflectarius Pilsbry, 1940					
Inflectarius downieanus (Bland, 1861)	Dwarf Gobelet	G3	S3		
Inflectarius ferrissi (Pilsbry, 1897)	Smoky Mountain Covert	G2	S2		
Inflectarius inflectus (Say, 1821)	Shagreen Snail	G5	S5		
Inflectarius kalmianus (Hubricht, 1965)	Brown Gobelet	G3	S3		
Inflectarius rugeli (Shuttleworth, 1852)	Deep-tooth Shagreen	G5	S5		
Inflectarius smithi (Clapp, 1905)	Alabama Shagreen	G2	S2		
Inflectarius subpalliatus (Pilsbry, 1893)	Velvet Covert	G2G3	S2		
Inflectarius verus (Hubricht, 1954)	Fuzzy Covert	G1		Dourson (2013)	NC, SC
Lobosculum Pilsbry, 1930					
Lobosculum pustuloides (Bland, 1858)	Tiny Liptooth	G4	S4		
Mesodon A. Férussac, 1821					
Mesodon altivagus (Pilsbry, 1900)	Wandering Globe	G2G3	S2		
Mesodon andrewsae (W.G. Binney, 1879)	Balsam Globe	G3	S2S3		
Mesodon clausus (Say, 1821)	Yellow Gobelet Snail	G5	SNA		
Mesodon elevatus (Say, 1821)	Proud Globe	G5	S5		
Mesodon mitchellianus (I. Lea, 1838)	Sealed Gobelet Snail	G4	S2		

Mesodon normalis (Pilsbry, 1900)	Grand Globe	G5	S4		
Mesodon sanus (Clench & Archer, 1933)	Squat Gobelet	G3	S2S3		
Mesodon thyroidus (Say, 1817)	White-lip Globe Snail	G5	S5		
Mesodon zaletus (A. Binney, 1837)	Toothed Globe	G5	S5		
Neohelix Ihering, 1892					
Neohelix albolabris (Say, 1817)	Whitelip Snail	G5	S5		
Neohelix alleni (Wetherby in Sampson, 1883)	Western Whitelip	G5	S3?		
alleni alleni (Wetherby in Sampson, 1883)	Western Whitelip	G5	S3?		
alleni fuscolabris (Pilsbry, 1903)					
Neohelix divesta (Gould, 1851)	Ozark Whitelip	G3G4		Field Museum of Natural History (#267391), Van Devender Collection	AR, KS, LA, MO, OK, TX
Neohelix major (A. Binney, 1837)	Southeastern Whitelip	G4G5	S4S5		
Patera Albers, 1850					
Patera appressa (Say, 1821)	Flat Bladetooth Snail	G5	S4		
Patera clarki (I. Lea, 1858)	Dwarf Proud Globe	G3	S2S3		
Patera laevior (Pilsbry, 1940)	Smooth Bladetooth	G4	S4		
Patera pennsylvanica (Green, 1827)	Proud Gobelet	G4			

Patera perigrapta (Pilsbry, 1894)	Engraved Bladetooth	G5	S5		
Patera sargentiana (C.W. Johnson & Pilsbry, 1892)	Grand Bladetooth	G2		Carnegie Museum (14 lots, but see #98377), Delaware Museum of Natural History (#57435), Field Museum of Natural History (#375142)	AL
Polygyra Say, 1818					
Polygyra cereolus (Megerle von Mühlfeldt, 1818)	Southern Flatcoil	G4		Dinkins and Dinkins (2018), Hodges and McKinney (2018), McKinney et al. (2019)	AL, FL, GA, HI, LA, MS, SC, TX
Praticolella E. von Martens, 1892					
Praticolella lawae (J. Lewis, 1874)	Appalachian Scrubsnail	G3	S2		
Stenotrema Rafinesque, 1819					
Stenotrema altispira (Pilsbry, 1894)	Highland Slitmouth	G3	S2		
Stenotrema angellum Hubricht, 1958	Kentucky Slitmouth	G4	S3		
Stenotrema barbatum (Clapp, 1904)	Bristled Slitmouth Snail	G5	S3		
Stenotrema barbigerum (Redfield, 1856)	Fringed Slitmouth	G3	S3		
Stenotrema brevipila (Clapp, 1907)	Talledega Slitmouth	G2		Van Devender Collection	AL, GA
Stenotrema calvescens Hubricht, 1961	Chattanooga Slitmouth	G3	S3		
Stenotrema cohuttense (Clapp, 1914)	Cohutta Slitmouth	G2	S2		

Stenotrema deceptum (Clapp, 1905)	Monte Sano Slitmouth	G3G4	S3		
Stenotrema depilatum (Pilsbry, 1895)	Great Smoky Slitmouth	G2	S2		
Stenotrema edgarianum (I. Lea, 1841)	Sequatchie Slithmouth	G2G3	S2		
Stenotrema edvardsi (Bland, 1856)	Ridge-and-valley Slitmouth	G4G5	S4		
Stenotrema exodon (Pilsbry, 1900)	Alabama Slitmouth	G2	S2		
Stenotrema hirsutum (Say, 1817)	Hairy Slitmouth Snail	G5	S5		
Stenotrema labrosum (Bland, 1862)	Ozark Slitmouth	G3G4		Illinois Natural History Survey (#74103)	AR, IA, LA, MO, OK
Stenotrema macgregori Dourson, 2011	Fraudulent Slitmouth	GNR		Florida Museum of Natural History (9 lots, but see #520669)	KY, WV
Stenotrema magnifumosum (Pilsbry, 1900)	Appalachian Slitmouth	G4	S3		
Stenotrema morosum Hubricht, 1978	a slitmouth snail	GH			
Stenotrema pilula (Pilsbry, 1900)	Pygmy Slitmouth	G3G4	S3		
Stenotrema spinosum (I. Lea, 1831)	Carinate Slitmouth	G4	S4		
Stenotrema stenotrema (L. Pfeiffer, 1842)	Inland Slitmouth	G5	S5		
Stenotrema waldense Archer, 1938	Doaks Creek Slitmouth	G2	S2		
Triodopsis Rafinesque, 1819					

Triodopsis alabamensis (Pilsbry, 1902)	Alabama Threetooth	G4	S3		
Triodopsis anteridon Pilsbry, 1940	Carter Threetooth	G3	S1		
Triodopsis claibornensis Lutz, 1950	Claiborne Threetooth	G2	S2		
Triodopsis complanata (Pilsbry, 1898)	Glossy Threetooth	G2	S2		
Triodopsis discoidea (Pilsbry, 1904)	Rivercliff Threetooth	G3		Illinois Natural History Survey (#73920, #73977)	IL, IN, KY, MO, OH
Triodopsis fallax (Say, 1825)	Mimic Threetooth	G5	S3		
Triodopsis fraudulenta (Pilsbry, 1894)	Baffled Threetooth	G4			
Triodopsis hopetonensis (Shuttleworth, 1852)	Magnolia Threetooth	G4			
Triodopsis juxtidens (Pilsbry, 1894)	Atlantic Threetooth	G5		Carnegie Museum (#62.33860, #62.32894, #123566), Chicago Academy of Science (#6726)	DC, GA, MD, ME, NC, NJ, NY, PA, SC, VA, WV
Triodopsis tennesseensis (Walker & Pilsbry, 1902)	Budded Threetooth	G4	S4		
Triodopsis tridentata (Say, 1817)	Northern Threetooth Snail	G5	S5		
Triodopsis vulgata Pilsbry, 1940	Dished Threetooth Snail	G5	S5		
Webbhelix Emberton, 1988					

Webbhelix multilineata (Say, 1821)	Striped Whitelip	G5	S2		
Xolotrema Rafinesque, 1819					
Xolotrema caroliniense (I. Lea, 1831)	Blunt Wedge	G4	S4S5		
Xolotrema denotatum (Férussac, 1823)	Velvet Wedge Snail	G5	S5		
Xolotrema fosteri (F.C. Baker, 1932)	Bladetooth Wedge	G5	S3?		
Xolotrema obstrictum (Say, 1821)	Sharp Wedge	G4	S5		
Pristilomatidae Cockerell, 1891					
Hawaiia Gude, 1911					
Hawaiia alachuana (Dall, 1885)	Southeastern Gem Snail	G4G5Q	S4?		
Hawaiia minuscula (Binney, 1841)	Minute Gem Snail	G5	S5		
Paravitrea Pilsbry, 1898					
Paravitrea alethia Hubricht, 1978	Goddess Supercoil	G1	S1		
Paravitrea andrewsae (W.G. Binney, 1879)	High Mountain Supercoil	G2	S2		
Paravitrea bellona Hubricht, 1978	Club Supercoil	G1		Hubricht (1973), Field Museum of Natural History (#268135)	WV
Paravitrea blarina Hubricht, 1963	Shrew Supercoil	G3	S3		
Paravitrea calcicola H. B. Baker, 1931	Pearl Supercoil	G1	S1		
Paravitrea capsella (Gould, 1851)	Dimple Supercoil	G4	S4		

Paravitrea clappi (Pilsbry, 1898)	Mirey Ridge Supercoil	G2G3	S2S3		
Paravitrea lacteodens (Pilsbry, 1903)	Ramp Cove Supercoil	G1		Carnegie Museum (7 lots, but see #62.21039), Florida Museum of Natural History (#195263), Van Devender Collection	NC
Paravitrea lamellidens (Pilsbry, 1898)	Lamellate Supercoil	G2	S2		
Paravitrea lapilla Hubricht, 1965	Gem Supercoil	G2	S2		
Paravitrea metallacta Hubricht, 1963	Caneyfork Supercoil	G3	S3		
Paravitrea multidentata (A. Binney, 1840)	Dentate Supercoil Snail	G5	S4S5		
Paravitrea petrophila (Bland, 1883)	Cherokee Supercoil	G4	S4		
Paravitrea pilsbryana (Clapp, 1919)	Translucent Supercoil	G2	S2		
Paravitrea placentula (Shuttleworth, 1852)	Glossy Supercoil	G3	S2S3		
Paravitrea reesei Morrison, 1937	Round Supercoil	G3	S1S2		
Paravitrea seradens Hubricht, 1972	Barred Supercoil	G3		Field Museum of Natural History (#187069), Van Devender Collection	VA, WV
Paravitrea significans (Bland, 1866)	Domed Supercoil	G3	S1S2		
Paravitrea subtilis Hubricht, 1978	Slender Supercoil	G2	S2		

Paravitrea tantilla Hubricht, 1963	Teasing Supercoil	G3	S3		
Paravitrea ternaria Hubricht, 1978	Sculpted Supercoil	G1G2	S1S2		
Paravitrea tridens Pilsbry, 1946	Whitefoot Supercoil	G2	S2		
Paravitrea umbilicaris (Ancey, 1887)	Open Supercoil	G3?	S2?		
Paravitrea variabilis H. B. Baker, 1929	Variable Supercoil	G2G3	S2S3		
Paravitrea varidens Hubricht, 1978	Roan Supercoil	G1G2	S1S2		
Punctidae Morse, 1864					
Paralaoma Iredale, 1913					
Paralaoma servilis (Shuttleworth, 1852)*	Pinhead Spot			Field Museum of Natural History (#381453), Hodges and McKinney (2018)	
Punctum Morse, 1864					
Punctum blandianum Pilsbry, 1900	Brown Spot	G4	S4		
Punctum minutissimum (I. Lea, 1841)	Small Spot Snail	G5	S5		
Punctum smithi Morrison, 1935	Lamellate Spot	G4	S4		
Punctum vitreum H. B. Baker, 1930	Glass Spot Snail	G5	S3		
Pupillidae W. Turton, 1831					
Pupilla J. Fleming, 1828					
Pupilla muscorum (Linnaeus, 1758)	Widespread Column Snail	G5		Carnegie Museum (#62.39769)	CO, IA, IL, KS, KY, MA, MD, ME, MI, MN, MO, MT, ND, NE, NJ, NM, NY, OH, OK, PA, SD, TX, UT, VA, VT, WV, WY
Pupoides L. Pfeiffer, 1854					

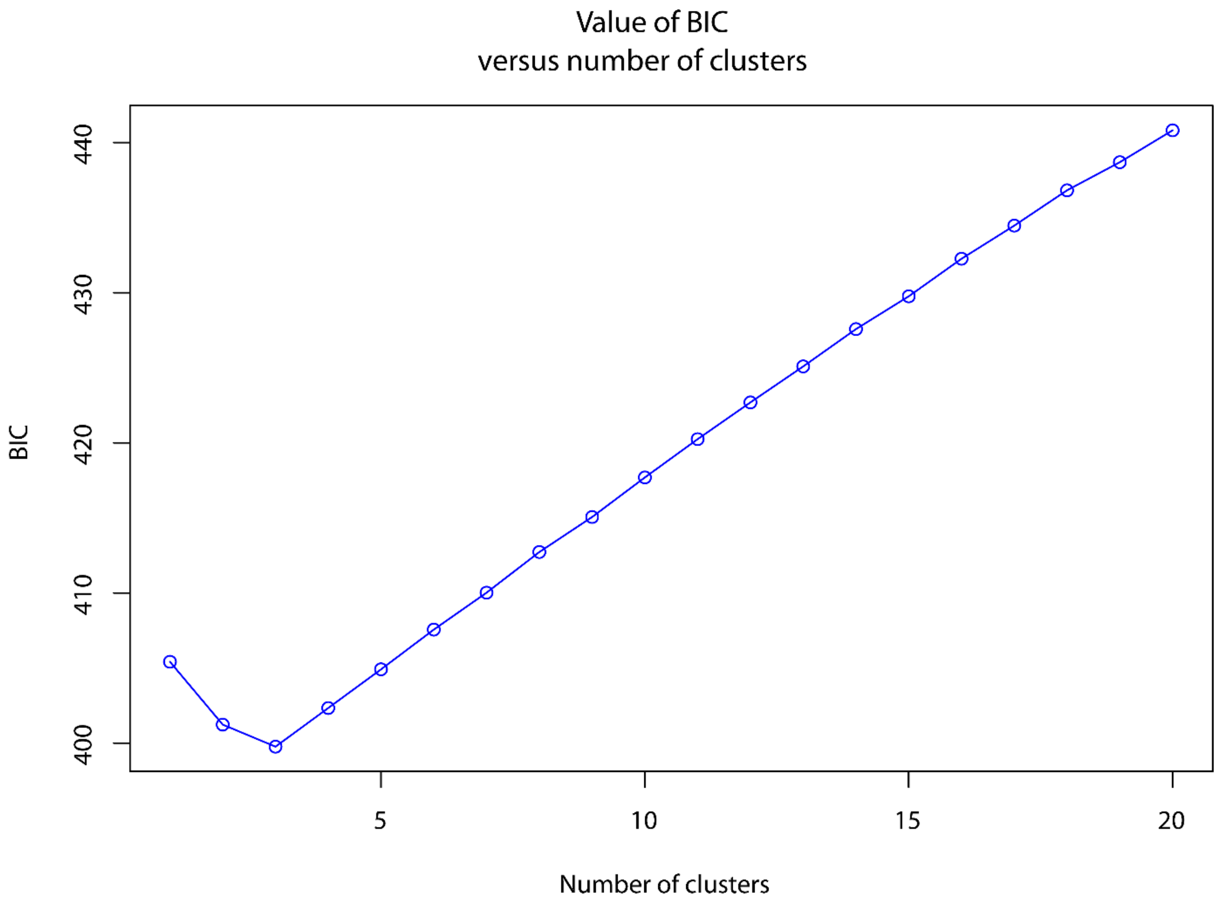
Pupoides albilabris (C. B. Adams, 1841)	White-lip Dagger Snail	G5	S5		
Spiraxidae H. B. Baker, 1939					
Euglandina Crosse & P. Fischer, 1870					
Euglandina rosea (Férussac, 1821)*	Rosy Wolfsnail	G5		Irwin et al. (2016)	AL, GA, FL*
Strobilopsidae Wenz, 1915					
Strobilops Pilsbry, 1893					
Strobilops aeneus Pilsbry, 1926	Broze Pinecone Snail	G5	S5		
Strobilops labyrinthicus (Say, 1817)	Maze Pinecone Snail	G5	S5		
Strobilops texasianus Pilsbry & Ferriss, 1906	Southern Pinecone	G5	S4		
Succineidae Beck, 1837					
Mediappendix Pilsbry, 1948					
Mediappendix oklahomarum (Webb, 1953)	Detritus Ambersnail	G5	S4S5		
Mediappendix texana (Hubricht, 1961)	an ambersnail	G1Q			
Mediappendix vermeta (Say, 1829)	Suboval Ambersnail	G5	S5		
Novisuccinea Schileyko & Likharev, 1986					
Novisuccinea ovalis (Say, 1817)	Oval Ambersnail	G5	S5		
Oxyloma Westerlund, 1885					
Oxyloma retusum (I. Lea, 1834)	Blunt Ambersnail	G5			

Oxyloma salleanum (L. Pfeiffer, 1850)	Louisiana Ambersnail	G3	S2S3		
Succinea Draparnaud, 1801					
Succinea concordialis Gould, 1848	Spotted Ambersnail	G4	S3		
Succinea grosvernorii Lea, 1864	Santa Rita Ambersnail	G5	SNR		
Testacellidae Gray, 1840					
Testacella Lamarck, 1801					
Testacella haliotidea Draparnaud, 1801*	Earshell Slug			Dinkins and Dinkins (2018)	
Truncatellinidae Steenberg, 1925					
Columella Westerlund, 1878					
Columella edentula (Draparnaud, 1805)	Toothless Column Snail	G5	S4		
Columella simplex (Gould, 1840)	High-spire Column Snail	G5	S4?		
Valloniidae Morse, 1864					
Vallonia Risso, 1826					
Vallonia costata (O. F. Müller, 1774)	Costate Vallonia Snail	G5		Dinkins and Dinkins (2018)	CA, DC, DE, IA, IL, IN, KY, MA, MD, ME, MI, MN, NE, NJ, NY, OH, PA, SD, VA, VT, WI, WV
Vallonia excentrica Sterki, 1893	Iroquois Vallonia Snail	G5	S3?		
Vallonia perspectiva Sterki, 1893	Thin-tip Vallonia Snail	G4G5	S3?		
Vallonia pulchella (O. F. Müller, 1774)	Lovely Vallonia Snail	G5		Dinkins and Dinkins (2018)	CA, CT, DE, IA, ID, IL, IN, KY, MA, MD, ME, MI, MN, MO, MT, NC, NE, NJ, NY, OH,

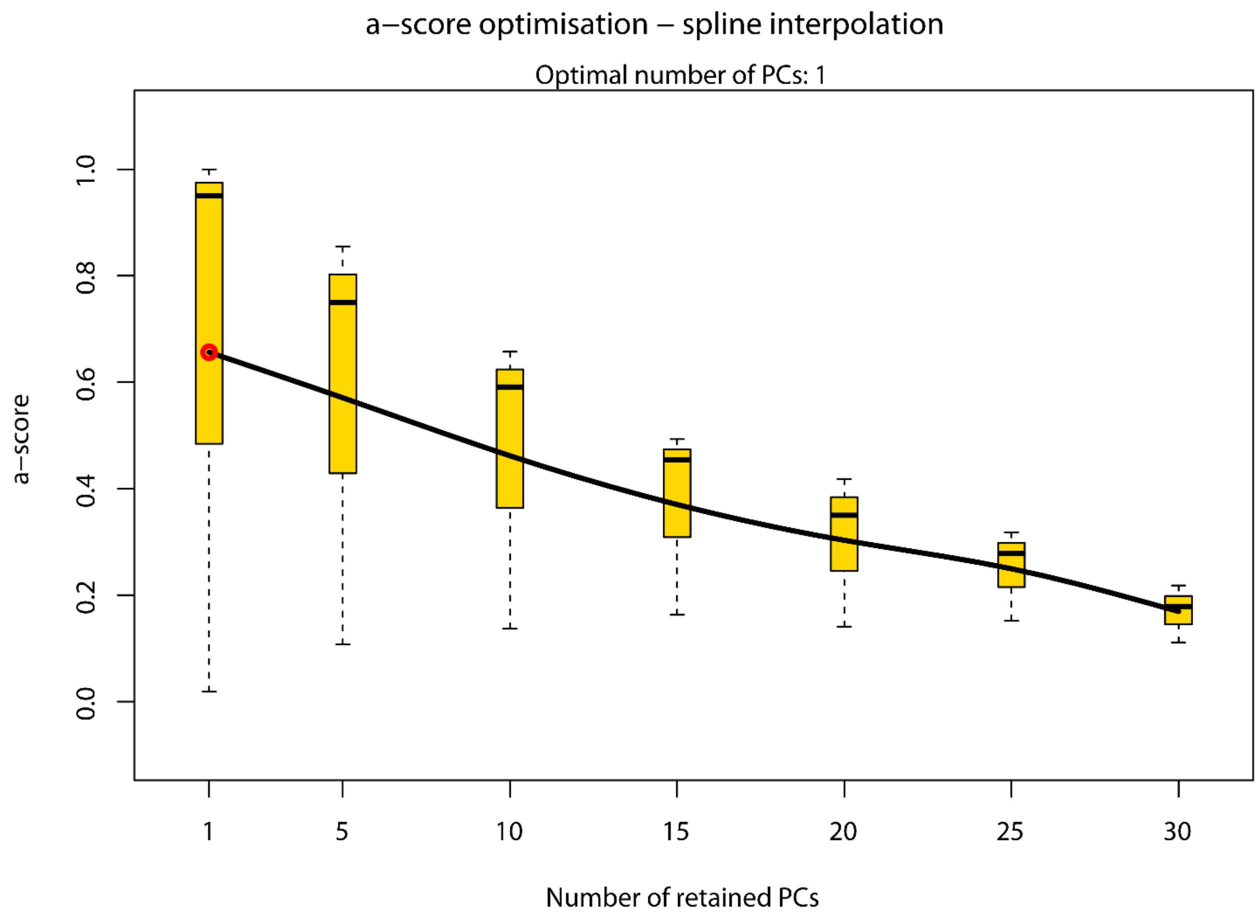
					PA, SD, TX, UT, VA, VT, WA, WI, WV
Vertiginidae Fitzinger, 1833					
Vertigo O. F. Müller, 1773					
Vertigo bollesiana (Morse, 1865)	Delicate Vertigo Snail	G4G5	S2		
Vertigo clappi Brooks & Hunt, 1936	Cupped Vertigo Snail	G1G2	S1		
Vertigo gouldii (Binney, 1843)	Variable Vertigo Snail	G5	S4		
Vertigo milium (A. A. Gould, 1840)	Blade Vertigo Snail	G5	S3S4		
Vertigo oralis Sterki in Pilsbry, 1898	Palmetto Vertigo	G5		Florida Museum of Natural History (#401733), McClung Museum (#11816, #11832)	AL, AR, FL, GA, LA, MD, MS, NC, SC, TN, TX, VA
Vertigo oscariana Sterki, 1890	Capital Vertigo	G4	S4		
Vertigo ovata Say, 1822	Ovate Vertigo Snail	G5	S4?		
Vertigo parvula Sterki, 1890	Smallmouth Vertigo	G3	S2S3		
Vertigo pygmaea (Draparnaud, 1801)	Pygmy Vertigo Snail	G5	S1		
Vertigo rugosula Sterki, 1890	Striate Vertigo	G4	S2		
Vertigo teskeyae Hubricht, 1961	Swamp Vertigo	G5	S1		

Vertigo tridentata Wolf, 1870	Honey Vertigo Snail	G5	S3?
Vertigo ventricosa (Morse, 1865)	Five-tooth Vertigo Snail	G5	

Supplementary Figure S1. Bayesian information criteria (BIC) scores associated with the number of clusters (K) that the discriminant analysis of principal components (DAPC) analysis inferred for our dataset.



Supplementary Figure S1. Bayesian information criteria (BIC) scores associated with the number of clusters (K) that the discriminant analysis of principal components (DAPC) analysis inferred for our dataset.



Supplementary Figure S2. A-score optimization plot indicating the optimal number of principal components to include in the discriminant analysis of principal components (DAPC) analysis.