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Molecular phylogenetics and morphological variation reveal recent speciation in freshwater mussels of the genera *Arcidens* and *Arkansia* (Bivalvia: Unionidae)

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Intra- and interspecific morphological variation due to both phenotypic plasticity and evolutionary convergence hinder the work of taxonomists and lead to over- and underestimates of species richness. Nevertheless, most species on Earth are recognized solely based on morphological characters. We used molecular phylogenetic and morphometric techniques to examine two freshwater mussel species. One is common and widespread, while the other is imperiled and endemic to the Interior Highlands of the USA. Phylogenetic and molecular clock analyses revealed that divergence of *Arcidens confragosus* and *Arkansia wheeleri* is small and relatively recent. Divergence in these and other taxa is probably due to isolation of streams in the Interior Highlands. Morphometric analyses showed distinct shell shapes using traditional morphometrics, but not through geometric morphometrics. Outlined shell shapes are indistinguishable; geometric morphometrics could not capture a three-dimensional component. Our analyses support the validity of these two species as congeners, with the nomen *Arcidens* (Simpson 1900) having priority. Because shell morphologies are both heritable and environmentally determined, our study emphasizes the importance of considering both molecular and morphometric analyses for identification of freshwater molluscs of conservation concern. *Biological Journal of the Linnean Society*, 2014, **112**, 535–545.

ADDITIONAL KEYWORDS: divergence time – Interior Highlands – ITS1 – mitochondrial DNA – molecular clock – post-Pleistocene diversification – traditional and geometric morphometrics.

INTRODUCTION

Most species on Earth are recognized solely on the basis of morphological characters; however, morphology is complex and non-static, and phenotypic variation within a single species is greatly influenced by environment, biological interactions and genotypes (*sensu* phenotypic plasticity; Via *et al*., 1995). At the same time, morphology can be very similar between two or more distinct species, leading to misclassification as a single species due to similarity of appearance (*sensu* cryptic species; Bickford *et al*., 2006). Phenotypic plasticity and cryptic morphology are more common in sessile and relatively immobile organisms because their lifestyles require coping with ambient conditions (Schlichting, 1986). This is particularly true in bivalves and gastropods, which possess external shells to protect their fragile bodies from the surrounding environment. Shell morphologies are commonly used as primary characters to describe and identify species, and most type collections are limited to shells alone. A combination of cryptic species and phenotypic plasticity makes morphology-based identification difficult and leads to both over- and underestimates of total species diversity in such taxa. This morphological variation has been the subject of many evolutionary studies; recently, these have increased greatly due to increasing availability of DNA sequences (Bickford *et al*., 2006).

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Phenotypic plasticity in morphological variation led early taxonomists to describe more than 4000 freshwater mussel species, whereas ~840 species are currently recognized (Haas, 1969; Graf & Cummings, 2007). Intraspecific morphological variation of the shell was recognized in the early 20th century (Utterback, 1917; Ortmann, 1920; Ball, 1922; Mackie & Topping, 1988; see Haag, 2012 for a summary), and such studies reported that single species expressed clinal morphological variation within river drainages (Utterback, 1917; Ortmann, 1918; Zieritz & Aldridge, 2009). Ortmann (1920) termed this phenomenon the Law of Stream Distribution, where gradual changes in shell morphologies occur from upstream to downstream within a single river. Because shell morphologies are highly influenced by environmental factors, evolutionary convergence in general shell shape is relatively common among freshwater mussel taxa (see Haag, 2012 for a summary). However, species descriptions based on shell characteristics alone sometimes fail to distinguish cryptic species, leading to underestimation of species. Advanced morphometric analyses and molecular techniques have uncovered such cryptic species (e.g. Gangloff, Williams & Feminella, 2006; Serb, 2006; Williams, Bogan & Garner, 2009). Thus, the primary use of morphology for investigating taxonomy can result in under- or overestimates (Inoue *et al*., 2013) of species richness. Resolving taxonomic and systematic inconsistencies becomes especially crucial when developing conservation strategies for threatened and endangered species, a status that applies to ~70% of all currently recognized unionoid mussel species in North America (Lydeard *et al*., 2004).

In this study, we examined phylogenetics and morphology of two freshwater mussel species, *Arcidens confragosus* (Say 1829), rock pocketbook, and *Arkansia wheeleri* (Ortmann and Walker 1912), Ouachita rock pocketbook (Fig. 1), following the nomenclature of Turgeon *et al*. (1998). No genetic information for these species has been reported to date. They have been variously aligned in different genera (Turgeon *et al*., 1998) or subgenera (Clarke, 1981), and as congeners (Clarke, 1981; Graf & Cummings, 2007). These generic assignments are based on morphological and anatomical characteristics. *Arcidens* is distinguished by the absence of a lunule; by the presence of pyramidal, dorso-ventrally compressed pseudocardinal teeth that are not curved; by heavy sculpturing over nearly the entire shell surface and very heavy beak sculpturing as two radial rows of raised loops (in the form of v-shaped tubercles); and by outer demibranch external membranes that are not openly porous (Clarke, 1981). *Arkansia* is distinguished by the presence of a lunule; by an anterior pseudocardinal tooth in the left valve and a pseudocardinal tooth in the right valve, which are both curved and parallel to the lunule; by heavy sculpturing only on the posterior shell half and barely perceptible beak sculpturing; and by outer demibranch external membranes that are openly porous, like a loosely woven net (Clarke, 1981). Some observers have reported that shell morphology of *Ark. wheeleri* is similar to *Arc. confragosus* (Howells, Neck & Murray, 1996). Ortmann and Walker (1912) suggested the genera were closely related based on similar shell morphologies.

Neither species exhibits well-defined sexual dimorphism. Gravid *Arc. confragosus* and *Ark. wheeleri* females were observed in late autumn to winter (Seagraves, 2006; Haggerty *et al*., 2011). Clarke (1981) provided a detailed analysis of *Arc. confragosus* glochidia and described them as pyriform in shape, asymmetrical, and with each apical stylet covered with approximately 75 major microstylets. Clarke (1981) and Hoggarth (1999) both produced SEM photographs of *Arc. confragosus* glochidia. Although no detailed glochidial description for *Ark. wheeleri* has been published, Seagraves (2006) reported that they were asymmetrical, pyriform in shape and possessed large hooks or stylets, which at least superficially appeared very similar to glochidia of *Arc. confragosus* as described by Clarke (1981).

Arcidens confragosus occurs widely in the Mississippi Basin and coastal drainages of the Gulf of Mexico (Williams, Bogan & Garner, 2008), but *Ark. wheeleri* is thought to be restricted to a small portion of the Interior Highlands in Arkansas and Oklahoma (Howells *et al*., 1996; U.S. Fish and Wildlife Service, 2004). The range of *Ark. wheeleri*, the Interior Highlands, contains rich aquatic communities with large numbers of endemic species (Robison, 1986). Current species distributions are believed to have been shaped by pre-Pleistocene vicariance (Mayden, 1985; Wiley & Mayden, 1985; Mayden, 1988), geographical isolation during the Pleistocene glaciations (Near, Page & Mayden, 2001) and post-glacial dispersal (Inoue *et al*., 2014). Although there are no records of the two species occurring syntopically, they are sympatric in some drainages.

We evaluated the phylogenetic relationships of these two species and other North American freshwater mussels and quantified shell morphologies of the two species in order to better understand their evolutionary history and taxonomic status. Because *Ark. wheeleri* is listed as 'critically endangered' on the IUCN Red List and 'endangered' under the U.S. Endangered Species Act and *Arc. confragosus* is thought to be its closest relative (Clarke, 1981; Graf & Cummings, 2007), understanding the phylogenetic relationship and taxonomic status of these two taxa is important for developing conservation strategies

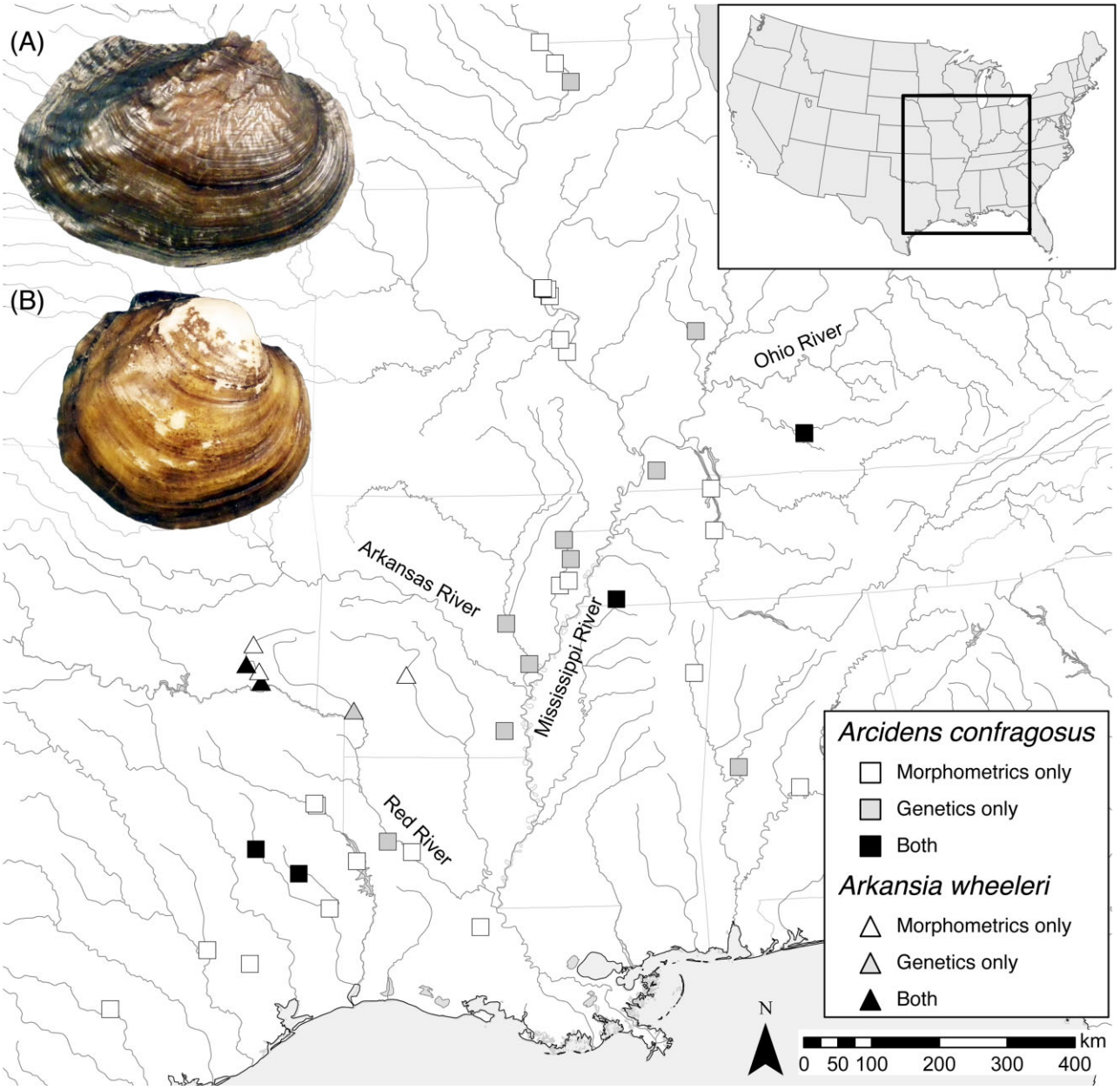

Figure 1. Collection sites in the United States. *Arcidens confragosus* (A) is shown in squares and *Arkansia wheeleri* (B) is shown in triangles. Colours represent morphometrics-only specimens (white), genetics-only specimens (grey) and specimens used for both analyses (black).

and implementing the species recovery plan for *Ark. wheeleri*.

METHODS

For genetic analysis, we obtained specimens either by collecting for this study or from museum collections. Because of its conservation status, all *Ark. wheeleri* tissues were collected via either mantle clips or swabs to minimize harm. A few *Arc. confragosus* were collected as whole specimens, which were separated into soft tissues and shells. Shells were scrubbed inside and out to remove excess material. Soft tissue samples were preserved in absolute ethanol and kept in a −20 °C freezer. We obtained *Arc. confragosus* from the following river drainages: Gulf Coast drainages (Pearl River, MS; Angelina and Neches rivers, TX), Mississippi River drainage (St. Francis River, AR; Obion Creek, KY; Wolf River, TN; Mississippi River, WI), Mobile River drainage (Black Warrior

River, AL; Tombigbee River, MS), Ohio River drainage (Fox River, IL; Green River, KY), Ouachita River drainage (Bayou Bartholomew, AR), Red River drainage (Bayou Pierre, LA) and White River drainage (Black and White rivers, AR). We obtained *Ark. wheeleri* from two drainages: the Ouachita River drainage (Ouachita River, AR) and the Red River drainage (Little River, AR; Kiamichi River, OK). Detailed museum/locality information is provided in Supporting Information Table S1. Due to the condition of historical museum specimens and our use of non-destructive sampling methods, we were not able to use the same specimens for both molecular phylogenetic and morphometric analyses, except for some *Arc. confragosus* collected for this study (see Results).

We extracted whole genomic DNA using standard CTAB/chloroform extraction followed by ethanol precipitation (Saghai-Maroof *et al*., 1984). We amplified two mitochondrial (mtDNA) genes, cytochrome *c* oxidase subunit I (COI) and NADH dehydrogenase subunit 1 (ND1), and one nuclear DNA fragment, internal transcribed spacer 1 (ITS1), following the specifications included with *Taq* DNA polymerase (Promega). We used the COI and ND1 primers and PCR conditions described by Campbell *et al*. (2005), and ITS1 primers and PCR conditions described by Correa *et al*. (2010). Because of difficulty amplifying DNA in museum specimens, internal primers for the two mtDNA genes were designed for this study, amplifying 200–300 bp. PCR products were purified on agarose gels followed by cycle sequencing using BigDye Terminator and an ABI3130 genetic analyser (Life Technologies). DNA sequences were assembled, edited and aligned by eye using the program DNADYNAMO (Blue Tractor Software), and an open-reading frame for the two mtDNA genes was verified. Ambiguous sequences of both the 3′- and the 5′-ends were trimmed.

We estimated number of haplotypes, mean number of base pair differences (k) and mean nucleotide diversity (π) within each species from concatenated mtDNA sequences and ITS1 sequences in DNASP v5.10 (Librado & Rozas, 2009). Because ITS1 contained alignment gaps, we treated such gaps as a fifth nucleotide state for estimating genetic diversity. Genetic divergence between *Arc. confragosus* and *Ark. wheeleri* was estimated using the maximum composite likelihood method in MEGA v5.2.1 (Tamura *et al*., 2011).

We separately analysed phylogenetic relationships of mtDNA (COI and ND1) and nuclear DNA (ITS1) sequences using Bayesian analysis. We concatenated the two mtDNA sequences and used only unique haplotypes for the analyses. For the mtDNA dataset, we included 13 species in the tribe Anodontini to determine the phylogenetic relationship of *Arc. confragosus* and *Ark. wheeleri*. In addition, we included a species from each tribe of the family Unionidae and *Cumberlandia monodonta* (Say 1829; family Margaritiferidae) as outgroups. For the ITS1 dataset, we were able to include only five species in the tribe Anodontini due to a limited number of published sequences in GenBank, and we used *Amblema plicata* (Say 1817; tribe Amblemini) for the outgroup. We estimated best-fit models of nucleotide substitution for each dataset using KAKUSAN4 (Tanabe, 2011). We used the Bayesian information criterion (BIC), corrected for number of base pairs, to determine best-fit models. Bayesian analysis was performed with MRBAYES v3.1.2 (Huelsenbeck & Ronquist, 2001) by Markov chain Monte Carlo simulation. Two simultaneous Markov chains were run for two million generations with trees sampled every 1000 generations, yielding 2001 trees for each chain in the initial samples. We assessed burn-in by plotting the log likelihood scores for each sampling point using TRACER v1.5 (Rambaut & Drummond, 2009), and we considered the Markov chains as stationary when likelihood values reached a plateau. Therefore, we discarded the first 501 trees (25%) as burn-in for each run, and the remaining 1500 trees were calculated using the 50% majority rule consensus trees. The most credible inferences of relationships were confined to nodes where Bayesian posterior probabilities were > 0.85.

We estimated divergence time between taxa using a molecular clock method implemented in BEAST v1.7.4 (Drummond *et al*., 2012). We used only the mtDNA dataset. A UPGMA starting tree was estimated under the HKY+G model with empirical base frequencies. A strict clock model and constant size coalescent model were used. We calibrated the clock using COI substitution rates ranging from 0.67 to 1.21% substitutions per million years obtained from a marine bivalve (Marko, 2002). Analysis was run for 10 million generations with sampling every 1000 generations and a burn-in of 25% of the total saved trees.

We conducted two morphometric analyses: traditional morphometrics and geometric morphometrics. Because many museum specimens are shell-only, individuals are not necessarily the same as those used in the genetic analyses. For traditional morphometrics, we measured three shell characters [maximum length (anterior to posterior), height (dorsal to ventral) and width (right to left valve)] to the nearest 0.1 mm for each individual using digital calipers. To standardize the variables for size, we calculated ratios of the height/length, width/length and width/height and normalized using an arcsine-transformation. We verified a normal distribution for each ratio using Shapiro–Wilk tests (Sokal & Rohlf,

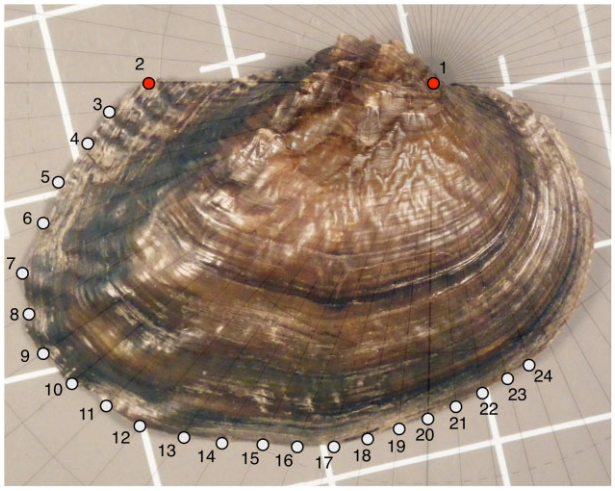

Figure 2. A right valve with the 24 landmarks used for geometric morphometric analyses. Homologous landmarks are indicated with red circles; pseudo-landmarks are indicated with white circles.

1995). For geometric morphometrics, we used the right valve of each specimen. Photographs of external views were taken with a digital camera, and then radial lines were extended every 5° in a circle extending from the anterior end of the umbo using PHOTOSHOP v9.0 (Adobe Systems). The shell was placed such that a horizontal line extended from the anterior of the umbo to the posterior end of the hinge ligament. Twenty-four shell landmarks were placed at the intersection of the shell margin and lines extending below the horizontal line using TPSDIG v2.10 (Rohlf, 2003; Fig. 2). We used Procrustes transformation to remove size variables from landmark coordinates, and therefore geometric morphometric analysis was based solely on outlined shell shape.

For both morphometric analyses, we analysed morphological variation within and among species through principal components analysis (PCA), which simplifies descriptions of variation among individuals and requires no *a priori* assumptions to group individuals. Additionally, Hotelling's T^2 test was utilized for pairwise comparisons between groups assigned by species, and discriminant function analysis (DFA) was conducted for the pair of species to determine how frequently PC scores correctly distinguished between species. All statistical analyses were performed using the software PAST (Hammer, Harper & Ryan, 2001).

RESULTS

We examined 42 specimens of *Arc. confragosus* and 14 specimens of *Ark. wheeleri* for genetic analyses. However, due to the condition of historical museum specimens (collected in the early 1900s), we were unsuccessful in sequencing some of these specimens. As a result, we obtained data from 34 specimens of *Arc. confragosus* and seven specimens of *Ark. wheeleri* for phylogenetic analyses.

We obtained 18 haplotypes for *Arc. confragosus* ($k = 2.01$, $\pi = 0.00193$) and five haplotypes for *Ark. wheeleri* ($k = 1.48$, $\pi = 0.00122$) from concatenated mtDNA sequences. Sequences were submitted to GenBank (accession numbers KJ716925–KJ716966). From ITS1, we obtained two genotypes each for *Arc. confragosus* ($k = 2.07$, $\pi = 0.00154$) and *Ark. wheeleri* ($k = 0.50$, $\pi = 0.00074$). Genetic divergence between the two species was similar for both markers: 0.0075 for mtDNA and 0.0061 for ITS1. Phylogenetic analyses showed reciprocally monophyletic sister clades for *Arc. confragosus* and *Ark. wheeleri* with high posterior probability support, while species within the genus *Lasmigona* were next most closely related to both of these species [white healsplitter *Lasmigona complanata* (Barnes 1823) for the mtDNA phylogeny and green floater *Lasmigona subviridis* (Conrad 1835) for the ITS1 phylogeny; Fig. 3]. Trees indicated that *Arc. confragosus* and *Ark. wheeleri* had very shallow branches for the mtDNA phylogeny (Fig. 3A) with an estimated divergence time of 5860 years ago (95% CI: 2850–10 190 years ago), the early to mid-Holocene. Both *Arc. confragosus* mtDNA haplotypes and ITS1 genotypes obtained from the Ouachita and Red River drainages (Ac06 and Ac07 for mtDNA and Ac20 for ITS1; Fig. 3) were distinct from *Ark. wheeleri* haplotypes and genotypes from the same drainages. One *Arc. confragosus* mtDNA haplotype (Ac06) is the same as a sample from the type locality (Fox River, IL), and the Ac20 ITS1 genotype was shared by *Arc. confragosus* from throughout the range.

We analysed 65 individuals identified morphologically as *Arc. confragosus* and 24 individuals identified morphologically as *Ark. wheeleri*. For traditional morphometric analysis, PCA yielded two distinct eigenvalues and described > 99% of the total variability between species; the PC1 axis described 65.16% and the PC2 axis described 34.82% of the total variation (Fig. 3A). The PCA with group assigned by species showed distinct morphological separation between the species primarily due to ratios of height/length and width/length (Fig. 4A). Morphological differences were statistically significant ($T^2 = 262.93$, $F = 85.629$, $P < 0.001$), and the DFA scores between the species revealed 98.92% of individuals were assigned to the correct species. For geometric morphometric analysis, PCA yielded 14 distinct eigenvalues and described > 99% of the total variability between species; the PC1 axis described 64.38% and the PC2 axis described 13.06% of the total variation (Fig. 4B). In contrast to traditional

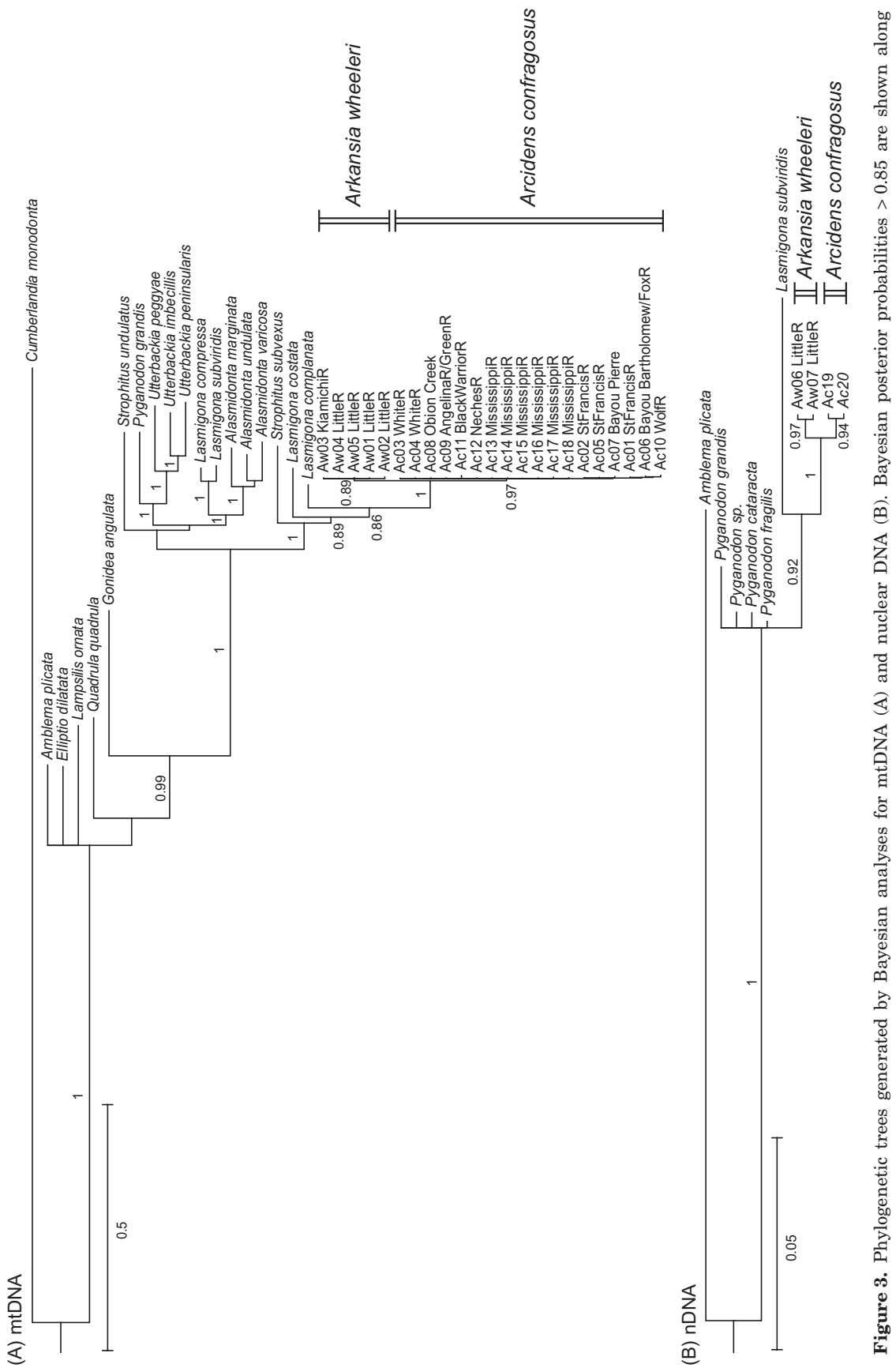

Figure 3. Phylogenetic trees generated by Bayesian analyses for mtDNA (A) and nuclear DNA (B). Bayesian posterior probabilities > 0.85 are shown along branches.

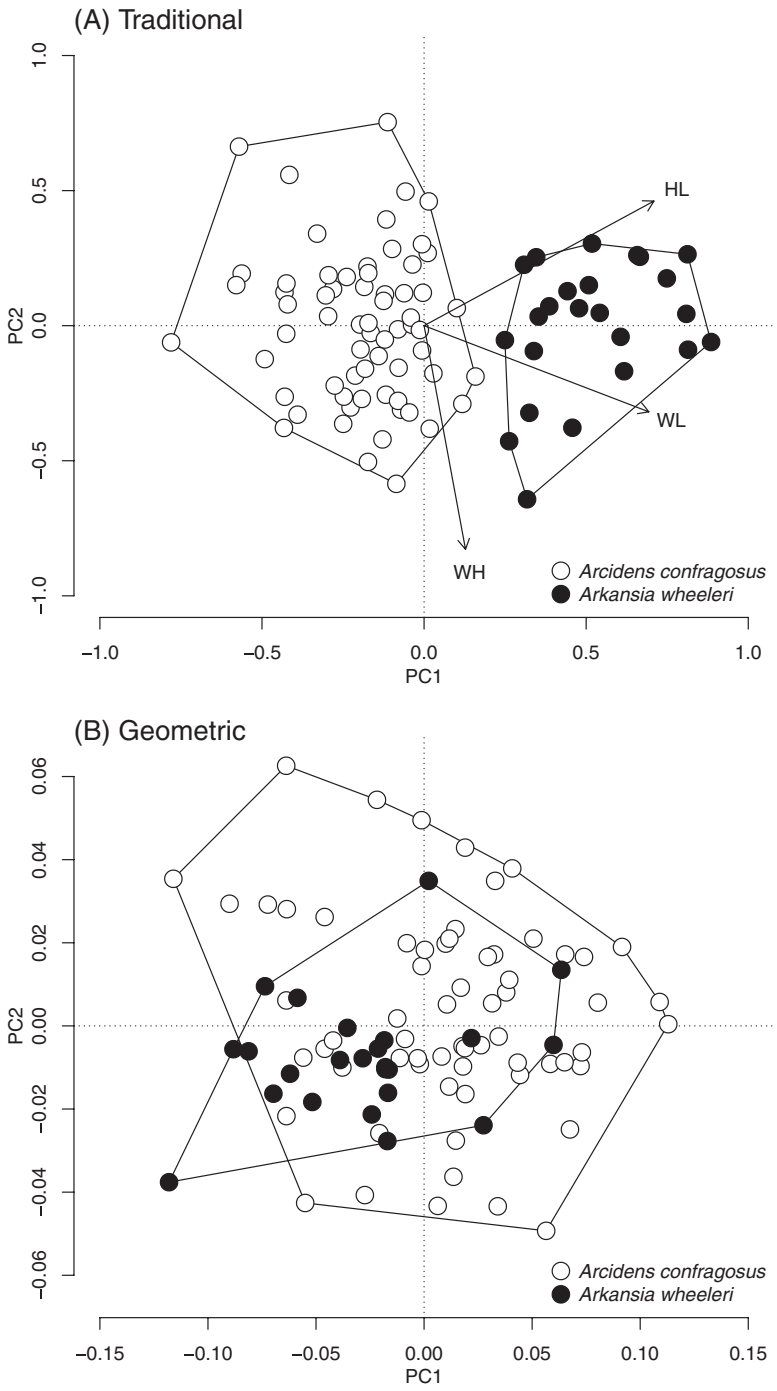

Figure 4. Scatter plots of traditional morphometrics (A) and geometric morphometrics (B) from principal component analysis (PCA) of *Arcidens confragosus* (white circles; $n = 65$) and *Arkansia wheeleri* (black circles; $n = 24$). Polygons enclose convex hulls of each species. Arrows show biplots of variables on PCA plots (HL, height/length; WL, width/length; WH, width/height).

morphometrics, the species clusters overlapped; 92% of *Ark. wheeleri* individuals were within the boundaries of the *Arc. confragosus* cluster. Despite this morphological overlap, there was a statistically significant difference between the species ($T^2 = 378.44$, $F = 4.350$, $P < 0.001$) and the DFA scores revealed that 98.88% of individuals were correctly assigned to species.

DISCUSSION

Neither mtDNA haplotypes nor ITS1 genotypes were shared between the two species and both formed reciprocally monophyletic clades, suggesting that no genetic exchange occurs between the species. We observed relatively small genetic divergence between the species compared with other pairs of closely related freshwater mussel species (Roe & Lydeard, 1998; Serb, Buhay & Lydeard, 2003; Inoue *et al*., 2013). This is probably due to very recent speciation; molecular clock analysis indicated that divergence occurred in the early Holocene.

Arkansia wheeleri occurs only in smaller tributary rivers of the Ouachita Highlands, whereas *Arc. confragosus* occurs throughout the Mississippi River Basin (Clarke, 1981; Howells *et al*., 1996; U.S. Fish and Wildlife Service, 2004; Williams *et al*., 2008). Recent diversification and segregation of the species' distributional ranges indicate that *Ark. wheeleri* probably adapted to the environment of upland streams in the Ouachita Highlands and was subsequently isolated by the Mississippi Embayment. Rivers in the Mississippi Embayment are considered to be major dispersal barriers to aquatic organisms, particularly those species inhabiting clear upland streams, due to turbid and sprawling river characteristics (Robison, 1986). Molecular phylogeographical studies have revealed that the Mississippi Embayment and Mississippi River are dispersal barriers for endemic fishes (Mayden, 1985; Berendzen, Simons & Wood, 2003), crayfishes (Crandall & Templeton, 1999) and mussels (Elderkin *et al*., 2008; Inoue *et al*., 2013, 2014) in the Interior Highlands. The fact that *Ark. wheeleri* utilizes widely distributed centrarchids [e.g. *Lepomis cyanellus* (Rafinesque, 1819), *L. megalotis* (Rafinesque, 1820) and *L. gulosus* (Cuvier, 1829)] as hosts (Seagraves, 2006) suggests that restricted distribution is due to isolation by environment in the Ouachita Highlands. In contrast, *Arc. confragosus* occurs in medium-sized to large rivers with reduced or slow current and a substrate of mud and fine sand (Parmalee & Bogan, 1998). It tolerates lentic conditions (Howells *et al*., 1996; Haag, 2012), suggesting that this species is able to survive environmental conditions in Mississippi Embayment drainages.

The Mississippi Embayment was formed by rising sea levels and floods of glacial meltwater during Pleistocene interglacial periods. During these periods, however, rivers draining into the Mississippi Embayment may not have been hostile to highland organisms because meltwater created swifter and clearer streams with coarser sediment (Robison, 1986). Thus, the most recent common ancestor of *Arc. confragosus* and *Ark. wheeleri* presumably occurred throughout the Mississippi River Basin. However, since the last glaciation, the Mississippi River and rivers in the Mississippi Embayment developed their present-day environmental conditions, and consequently, the two species were segregated into two physiographical regions. The divergence time of the segregation is congruent with another widely distributed mussel species, *Cumberlandia monodonta* (Say, 1829), that exhibits genetic isolation between the Ouachita River population and other populations found throughout the Mississippi River Basin (Inoue *et al*., 2014).

Intraspecific genetic diversities for mtDNA and nuclear DNA were similar, but very low, in both *Arc. confragosus* and *Ark. wheeleri*. Unlike other widespread freshwater mussels (Burdick & White, 2007; Elderkin *et al*., 2008; Inoue *et al*., 2014), *Arc. confragosus* did not show phylogeographical patterns relative to its overall distribution, indicating that dispersal rates are relatively high throughout the range of the species. High levels of gene flow may be due to *Arc. confragosus* utilizing diverse host fish species that include highly migratory American eel *Anguilla rostrata* (Lesueur, 1817) (Howells, 1997; Williams *et al*., 2008; Hove *et al*., 2011; Ward *et al*., 2011). Population genetic studies of *Arc. confragosus* using more variable genetic markers, such as microsatellites, along with similar studies of its host species are needed to elucidate gene flow and connectivity among populations, because population genetic structure of mussel species and their hosts may be broadly congruent (Zanatta & Wilson, 2011).

We found relatively similar shell morphologies between the species through geometric morphometrics but not traditional morphometrics. PCA from traditional morphometrics indicated that *Arc. confragosus* shells are short in height and compressed in width relative to length, whereas *Ark. wheeleri* has tall and inflated shells. These observations are not congruent with Ortmann's Law of Stream Distribution, where mussels downstream in large rivers have short and inflated shells, while elongated and compressed shells occur among individuals from headwaters of the same river (Ortmann, 1920). Baker (1928) noted that *Arc. confragosus* from small streams tend to be more compressed than those from large rivers.

Shell sculpturing is substantially different and diagnostic in these species. *Arcidens confragosus* often has heavy sculpturing over the entire shell surface especially in young individuals; however, Clarke (1981) noted variation in the strength and extent of sculpturing from heavily sculptured (Pigeon Creek, IN) to almost smooth (Mississippi River, WI). Shell sculpturing has been thought to stabilize shell position in river substrates; sculptured taxa are generally found in hard substrates in large rivers

(Watters, 1994). Furthermore, sculptured taxa tend to show heavier shell sculpturing in individuals from large rivers compared with individuals from headwaters (Ortmann, 1920). Although no record was found of the two species occurring syntopically, the two species co-occur in the same drainages (e.g. Ouachita and Red River drainages) where *Arc. confragosus* occupies the downstream portion of rivers.

Geometric morphometric analysis showed relatively similar morphology between the species. This indicates that outlined shell morphologies between the species are indistinguishable without shell width adding a third dimension to measures of shell shape. Overlooking such subtle characteristics among species and among individuals within a species can lead to inaccurate estimates of total species richness.

Based on phylogenetic and morphometric analyses, we confirmed these as two distinct species. However, because of relatively shallow branches between the two species compared with the *Alasmidonta* or *Utterbackia* clades (Fig. 2), our results support the assertion that *Arc. confragosus* and *Ark. wheeleri* are congeners (Clarke, 1981; Graf & Cummings, 2007). Thus, *Ark. wheeleri* should be recognized as *Arcidens wheeleri* (Ortmann and Walker, 1912) based on the priority of the genus nomen *Arcidens*. This taxonomic change will not affect current conservation and management of *Arc. wheeleri*. The distribution of *Arc. wheeleri* is restricted to small portions of the Red River and Ouachita River systems; recent surveys recovered this species from only a few locations (Galbraith, Spooner & Vaughn, 2008; Harris *et al.*, 2010). The restricted distribution and rarity of occurrence suggest maintaining its current conservation status.

Phenotypic characteristics are a principal means for discovering, describing and identifying species; however, phenotypic variation may hinder the work of taxonomists by creating morphological conundrums. Freshwater mussels in particular evolve their shell morphologies to adapt to the environment (Watters, 1994; Haag, 2012), in contrast to other bivalves where predation pressure has been proposed to be a primary force leading to evolution of shell morphology (Vermeij, 1980). Such phenotypic variation may not only lead to misidentification of species, but also cause inaccurate estimates of total biodiversity on earth. Because the current biodiversity crisis has accelerated rates of extinction, accurate estimates of biodiversity are urgently needed (Scheffers *et al.*, 2012). Our study emphasizes the importance of considering both molecular and morphometric analyses for identification of species relationships, while also suggesting that such identification may have significant conservation implications.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. List of specimen information used in the study.