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CONSERVATION GENETICS OF TWO ENDANGERED UNIONID BIVALVE SPECIES, *EPIOBLASMA FLORENTINA WALKERI* AND *E. CAPSAEFORMIS* (UNIONIDAE: LAMPSILINI)

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ABSTRACT

The genus *Epioblasma* consists of 20 species and eight subspecies, of which 15 species and four subspecies are presumed or probably extinct. Remaining taxa are considered endangered or threatened largely due to habitat destruction or modification. We conducted a molecular genetic study on all known extant populations of two federally endangered freshwater *Epioblasma* species: the tan riffleshell, *E. florentina walkeri* (Wilson & Clark, 1914), and oyster mussel, *E. capsaeformis* (Lea, 1834), to determine the extent of genetic variation within and among populations of the two species. Mitochondrial DNA sequence data from two protein-coding genes (COI and ND1) failed to differentiate *E. capsaeformis* from *E. f. walkeri*. Molecular variation within and between the two species does not exceed limits observed within *Epioblasma brevidens*, although substantial genetic differences are observed among *E. brevidens*, *E. triquetra* and *E. capsaeformis* + *E. florentina walkeri*. Although data do not support the recognition of the two taxa as separate phylogenetic species, they still warrant endangered status due to the fact that few reproducing populations are known. Life history and population biology studies have already been conducted on the single known extant reproducing population of nominal *E. f. walkeri*. However, similar comparative studies should be conducted on the different mantle-pad morphs of nominal *E. capsaeformis* populations to provide valuable data for more effective management of the recovery of the phylogenetic species and to further test our hypothesis.

INTRODUCTION

Unionid bivalves are one of the most imperiled groups of animals in the world, with 70% of the recognized species in North America considered extinct, endangered, threatened or of special concern (Williams, Warren, Cummings, Harris & Neves, 1993; Neves, Bogan, Williams, Ahlstedt & Hartfield, 1997; Master, Flack & Stein, 1998). One of the centres of greatest diversity in North America is the Tennessee-Cumberland basin of the southeastern United States (Parmalee & Bogan, 1998). Historically, 111 unionid taxa were found in the two river systems, including 35 endemics (Starnes & Bogan, 1988). The genus *Epioblasma* was once widely distributed across the Tennessee and Cumberland systems, but 15 species and four subspecies are now presumed extinct due to habitat destruction or modification (Williams *et al.*, 1993; Neves *et al.*, 1997). Currently consisting of 20 species and eight subspecies (Turgeon, Quinn, Bogan, Coan, Hochberg, Lyons, Mikkelsen, Neves, Roper, Rosenberg, Roth, Scheltema, Thompson, Vecchione & Williams, 1998), the genus is noted for the presence of often extreme sexual dimorphism in shell shape, which is filled by a fleshy, soft, mantle pad apparently used as a fish-host attractant. Little is known about the life histories and habitat requirements of the remaining extant *Epioblasma* taxa, and all but one (*E. triquetra*) are federally listed as endangered. *Epioblasma triquetra* is considered imperiled, but not federally listed.

Many taxonomic questions remain unanswered due to the uncertainty of whether shell variation represents a response to environmental conditions or genetically-based diagnostic characteristics (e.g. Williams & Mulvey, 1994). The vast majority of currently recognized unionid species are based on interpretations of how to partition qualitative shell differences, although recent studies have coupled genetic data along with shell characteristics (Mulvey, Lydeard, Pyer, Hicks, Brim-Box, Williams & Butler, 1997; Hoeh, Bogan, Cummings & Guttman, 1998; King, Eackles, Gjetvaj & Hoeh, 1999; Lydeard, Minton & Williams, 2000; Hoeh, Bogan & Heard, 2001). Whether species are deemed valid or not alters our current views of conservation status, distribution and general biodiversity assessment (Lydeard *et al.*, 2000). This, in turn, may influence the management or active recovery plan for federally or state listed species (Lydeard & Roe, 1998).

We conducted a molecular genetic study on populations of two federally endangered freshwater *Epioblasma* species, the tan riffleshell, *E. florentina walkeri* (Wilson & Clark, 1914), and oyster mussel, *E. capsaeformis* (Lea, 1834). *Epioblasma capsaeformis* (Lea, 1834) was formerly found throughout the Tennessee and Cumberland River systems in Virginia, Tennessee, northern Alabama and Kentucky. Currently, it persists at extremely low numbers in three locales: (1) the Nolichucky River, Tennessee (extremely rare, only three specimens found since 1980); (2) the Clinch River (now rare in Virginia, but still relatively common in Tennessee), and (3) the Duck River, Tennessee. It is listed as federally endangered [United States Fisheries and Wildlife Service (USFWS), 1997], but has no approved recovery plan. *Epioblasma f. walkeri* (Wilson & Clark, 1914) is considered the Tennessee basin, headwater form of *E. f. florentina*, which is now presumed extinct (Parmalee & Bogan, 1998). *Epioblasma f. walkeri* has also been reported historically throughout the Cumberland drainage, including the Stones, Red and Harpeth rivers (USFWS, 1984). Four extant populations of *Epioblasma f. walkeri* are known: (1) Big South Fork, Cumberland River, Tennessee and Kentucky (reproducing populations); (2) Indian Creek (tributary to upper Clinch River, Virginia); (3) Hiwassee River, Tennessee (extremely rare, one live female found in

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1999); and (4) Middle Fork Holston River, Virginia (extremely rare, one live male found in 1997). One other relatively recent population of *E. f. walkeri* from the Clinch River below Indian Creek, was extirpated in 1999 from a chemical spill. *Epioblasma florentina curtisi* is presumably a member of the same species, but is distributed allopatrically in the Spring River system of Arkansas and Missouri. It is listed federally as endangered and, although a recovery plan has been developed for the taxon (USFWS, 1986), it is now probably extinct (S. Ahlstedt, personal communication).

The objective of this study was to determine the degree of genetic differentiation among extant populations of *Epioblasma florentina walkeri* and *E. capsaeformis*. We chose to focus our efforts on mitochondrial DNA (mtDNA) sequence data because mtDNA haplotypes have a smaller effective population size and, therefore, coalesce four times more rapidly than nuclear markers (Moore, 1995; Wiens & Penkrot, 2002). In addition, we were interested in testing the validity of the two recognized morphospecies using the Phylogenetic Species Concept (*sensu* Mishler & Theriot, 2000). A phylogenetic species is the smallest monophyletic group deemed worthy of formal recognition, because of the amount of support for monophyly and/or because of its importance in biological processes operating on the lineage in question (Mishler & Theriot, 2000).

MATERIALS AND METHODS

Specimens and vouchers

Permission was obtained to collect a limited number of *Epioblasma capsaeformis* ($n = 4$), *E. f. walkeri* ($n = 3$), *E. brevidens* ($n = 4$) and *E. triquetra* ($n = 1$) specimens or remove tissue clips from the edge of the mantle without killing the animal from extant populations, under the auspices of federal collecting permits SA96-31 to Paul Hartfield and C. Lydeard, and SA00-14 to C. Lydeard. Given the rarity or endangered status of the study taxa, sample sizes were extremely limited. Specimens of *Lampsilis siliquoidea*, *Villosa taeniata* and *V. villosa* were included as out-group lampsiline taxa (Lydeard, Mulvey & Davis, 1996; Graf & ÓFoighil, 1999). Table 1 lists the taxa, localities and tissue sources included in the present study. Voucher material of *Epioblasma* specimens are deposited at the University of Alabama Unionid Collection (UAUC).

DNA processing sequence procurement, alignment and analysis

Whole genomic DNA was extracted from mantle tissue from specimens using standard phenol/chloroform extraction methods followed by ethanol precipitation as described in Roe & Lydeard (1998). Initially, a 650-base pair (bp) region of the cytochrome oxidase C subunit I (COI) gene was amplified using primers LCO1490 and HCO2198 (Folmer, Hoeh, Black & Vrijenhoek, 1994), and sequenced using standard amplification parameters described in Roe & Lydeard (1998) and Lydeard *et al.* (2000). Our pilot study examining one *Epioblasma capsaeformis*, two *E. f. walkeri*, one *E. brevidens*, one *E. triquetra* and two *Lampsilis siliquoidea* specimens revealed little variation within a 449-bp aligned data matrix of COI (see results below), therefore a 700-bp region of the 5′-end of the first subunit of the NADH dehydrogenase (ND1) gene was amplified using primers Leu-uurF (5′-TGGCAGAAAAGTGCATCAGATTAAAGC-3′) and NIJ-12073 (5′-TCGGAATTCTCCTTCTGCAAAGTC-3′). ND1 and ND1-flanking primers were designed based on examination of the complete mitochondrial genome sequence of *Lampsilis ornata* (J. M. Serb & C. Lydeard, unpublished data). Leu-uurF was designed from an alignment of Leu-tRNA, which included sequence of *Lampsilis ornata*, *Drosophila melanogaster* and various molluscan mt genomes available on GenBank (Benson, Karsch-Mizrachi, Lipman, Ostell, Rapp & Wheeler, 2000). NIJ-12073 was modified from N1-N-12051 (Simon, Frati, Beckenbach, Crespi, Liu & Flook, 1994). Reactions were amplified for 34 cycles at 94°C for 40 s, 50°C for 60 s and 68°C for 90 s. Sequencing of all specimens was conducted using Big Dye (Perkin Elmer) terminator cycle sequencing, and the products were visualized using an ABI373 or ABI3100 automated sequencer.

Sequences were initially entered into the software program XESEE (Ver. 3.0, Cabot & Beckenbach, 1989). A visual alignment was constructed by eye. The aligned data matrices are available electronically from the authors, and individual sequences have been submitted to GenBank (see Table 1 for accession codes). Parsimony analysis was performed by using version 4.0b5 of PAUP* (Swofford, 2001) with ACCTRAN, MULPARS and TBR options. Branch-and-Bound searches were conducted for both the COI and ND1 sequence data separately. Characters were treated as unordered and equal weight for the phylogenetic analyses due to the presumably close phylogenetic affinity of the in-group taxa (Lydeard *et al.*, 1996). Bootstrap values (1000 replicates) using the FAST step-wise addition option of PAUP* (Felsenstein, 1985) and decay indices/Bremer support values (Bremer, 1988, 1994) using the Decay Index option of MacClade 4.0 (Maddison & Maddison, 2000) in conjunction with PAUP* were calculated to assess support for the individual nodes of the resulting phylogenetic hypotheses.

RESULTS

Sequence data

Alignment of the COI sequences resulted in a data matrix of 449 bp. The two *Epioblasma f. walkeri* DNA sequences were identical with the exception of an ambiguously scored (n) nucleotide for one of the sequences. One of the two (UAUC 1717) *E. f. walkeri* sequences differed from the *E. capsaeformis* by a single nucleotide (the other *E. f. walkeri* was ambiguous at this site). In contrast, interspecific genetic distances (uncorrected p-distances) ranged from 6.95 to 7.15% for *E. capsaeformis/f. walkeri* versus *E. brevidens*; 4.26–4.46% for *capsaeformis/f. walkeri* and *E. triquetra*; and 5.79% for *E. brevidens* and *E. triquetra*. The in-group *Epioblasma* taxa differed from the *Lampsilis siliquoidea* out-group specimens ($n = 2$) from 7.35 to 8.49%. The aligned data matrix including all taxa yielded 19 variable/parsimony-uninformative characters and 37 parsimony informative characters.

Alignment of the mitochondrial ND1 sequences resulted in a data matrix of 610 bp. Intraspecific genetic distances ranged from 0 to 0.984% for *E. capsaeformis*, 0.492% for *E. f. walkeri*, and 0–0.164% for *E. brevidens*. Interspecific differences between *E. capsaeformis* and *E. f. walkeri* are within the range of intraspecific values observed for *E. brevidens* (0.492–0.984%). Interspecific uncorrected p-distance values ranged from 6.557 to 7.541% for *E. capsaeformis/f. walkeri* versus *E. brevidens*; 6.23–7.213% for *E. capsaeformis/walkeri* versus *E. triquetra* and 6.885–7.049% for *E. triquetra* versus *E. brevidens*. The out-group lampsiline taxa differed from the *Epioblasma* from 10.328 to 14.562%. The aligned data matrix including all taxa yielded 79 variable/parsimony-uninformative characters and 77 parsimony informative sites. The number of parsimony informative sites for each codon position of the mtDNA *ND1* gene is 1st = 12, 2nd = 3, 3rd = 62, which follows patterns described for other mitochondrial protein coding genes in molluscs (Roe & Lydeard, 1998).

Phylogenetic analysis

Maximum-parsimony analysis of the *COI* and *ND1* genes was conducted by treating each character transformation as

Table 1. Taxa, locality, collector and GenBank accession number of material used in this study

Species	UAUC #	Locality, collector, and voucher material	GenBank sequences
Epioblasma brevidens (Lea, 1831)	509	Kyles Ford, Clinch River, Tennessee River drainage, Hancock Co., TN; J. Khym; mantle clip	COI = AF156527
	2012	Station Camp Creek, Big South Fork, Cumberland River drainage, Scott Co., TN; S. Ahlstedt; no shell, whole tissue	ND1 = AY094378
	2729	Parch Corn Creek, Big South Fork, Cumberland River drainage, Scott Co., TN; S. Ahlstedt; whole animal	ND1 = AY094376
	2778	Frost Ford, Clinch River, Tennessee. River drainage, Hancock Co., TN; S. Ahlstedt; whole animal	ND1 = AY094377
E. capsaeformis (Lea, 1834)	1527	Lillard Mill Dam, Duck River, Tennessee River drainage, Marshall Co., TN; L. Koch; adductor muscle clip	COI = AY094372 ND1 = AY094381
	2006	Nolichucky River, Tennessee River, drainage, Hamblen Co., TN; S. Ahlstedt & S. Fraley; mantle clip	ND1 = AY094382
	2720	Venable Spring, Duck River, Tennessee. River drainage, Marshall Co., TN; S. Ahlstedt & C. Hobbs; whole animal	ND1 = 094379
	2776	Frost Ford, Clinch River, Tennessee River drainage, Hancock Co., TN; S.Ahlstedt; whole animal	ND1 = 094380
E. florentina walkeri (Wilson & Clark, 1914)	1690	Cedar Bluff of Indian Creek, upstream of confluence with Clinch River, Tennessee River drainage, Tazewell Co., VA; C. Kane & L. Koch; whole animal	COI = AY094373 ND1 = AY094383
	1717	Cedar Bluff of Indian Creek, downstream from railroad bridge, Tennessee River drainage, Tazewell Co., VA; L. Koch; whole animal	COI = AY094374
	2777	Big South Fork below Parchcorn Creek, Cumberland River drainage, Scott Co., TN; S. Ahlstedt; whole animal	ND1 = AY094384
E. triquetra (Rafinesque, 1820)	2779	Frost Ford, Clinch River, Tennessee. River drainage, Hancock Co., TN; S. Ahlstedt; whole animal	ND1 = AY094375
		UMMZ 265716	COI = AF156528
Lampsilis siliquoidea (Barnes, 1823)	882	South Fishtail Bay, Douglas Lake, Great Lakes drainage, Cheboygan Co., MI; A.G.A. Pinoswka; whole animal	ND1 = AY094386
		UMMZ 265709	COI = AF156521
		UMMZ 265709	COI = AF156522
Villosa taeniata (Conrad, 1834)	2718	Venable Spring, Duck River, Tennessee. River drainage, Marshall Co., TN; S. Ahlstedt & C. Hobbs; whole animal	ND1 = AY094385
V. villosa (Wright, 1898)	652	Suwannee River at campground near Fanning Springs, Suwannee River drainage, Dixie Co., FL; D. Ruessler; whole animal	ND1 = AY094387

unordered and of equal weight due to the presumably close relationship among the study taxa (Lydeard *et al.*, 1996). Parsimony analysis of the COI data resulted in three equally parsimonious trees [consistency index (CI) = 0.953; homoplasy index (HI) = 0.0465; retention index (RI) = 0.9636; total length = 65]. The strict consensus tree is shown in Fig. 1. All three parsimonious trees support the monophyly of *Epioblasma*, with *E. capsaeformis* and *E. f. walkeri* forming an unresolved clade. *Epioblasma brevidens* is the most basal species. *Epioblasma triquetra* is the sister taxon to an *E. capsaeformis/f. walkeri* clade.

Phylogenetic analysis of the mitochondrial ND1 data resulted in five equally parsimonious trees (CI = 0.679; HI = 0.321; RI = 0.754; total length = 218). A strict consensus tree of the five most parsimonious trees and a phylogram of one of the five most parsimonious trees is shown in Figure 2A and B, respectively. The topology is similar to that of the COI-based tree, with the genus *Epioblasma* being monophyletic. In addition, support is found for the monophyly of *E. brevidens* and *E. capsaeformis/E. f. walkeri*; however, as in the COI-based tree, *E. capsaeformis* and *E. f. walkeri* are not reciprocally monophyletic. Unlike the COI-based tree, the strict consensus tree reveals an unresolved trichotomy between the *E. brevidens*, *E. triquetra* and *E. capsaeformis/f. walkeri* clades.

DISCUSSION

Epioblasma f. walkeri and *E. capsaeformis* are medium-sized species, rarely exceeding 60–70 mm in length, and each species possesses a dull green periostracum and bluish-white nacre (USFWS, 1984; Parmalee & Bogan, 1998). Isaac Lea described nearly half of the currently recognized species of *Epioblasma* (Turgeon *et al.*, 1998). After examining two or three of his own specimens, and an unknown number from Mr Cooper's cabinet, Lea described *E. capsaeformis* as a distinct species (Lea, 1837). Lea (1857) described *Epioblasma florentina* from which a less inflated, medium-sized subspecies, *E. f. walkeri*, was described (Wilson & Clark, 1914). Johnson (1978) reviewed the genus *Epioblasma* noting that *E. capsaeformis* most closely resembled *E. florentina* (he did not recognize *E. f. walkeri* as a distinct species or subspecies), with the male *E. capsaeformis* being longer, lower and less swollen than that of *E. florentina*. He noted that female *E. capsaeformis* has a darker marsupial swelling than the rest of the shell, while in *E. florentina* the periostracum is a uniform honey yellow or yellowish brown.

The mtDNA sequence data reveals that *E. f. walkeri* and *E. capsaeformis* are genetically indistinguishable from one another, forming a single, non-exclusive clade: Failure to distinguish

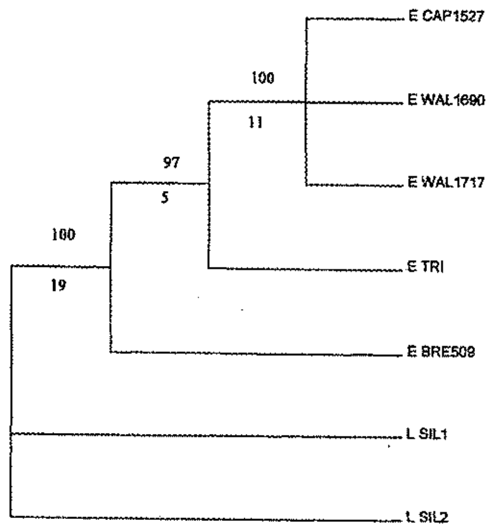
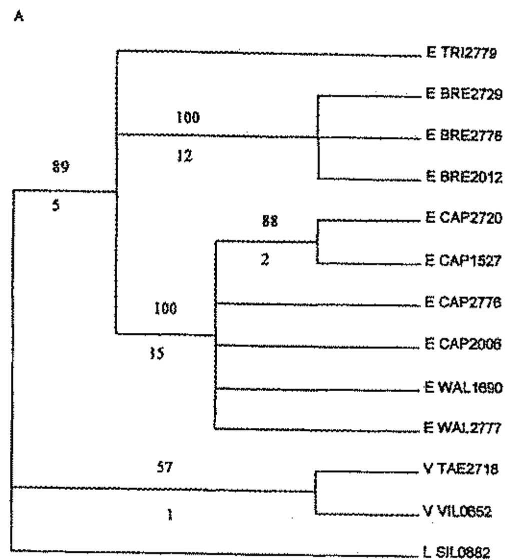


Figure 1. A strict consensus tree of three equally most-parsimonious trees (TL = 65; CI = 0.953) based on a maximum-parsimony analysis of 449 bp of the mitochondrial *COI* gene. Numbers above the nodes are bootstrap values (1000 replicates) and numbers below the nodes are Bremer support values. Specimen numbers = UAUC identification numbers as shown in Table 1. E cap = *Epioblasma capsaeformis*, E wal = *E. florentina walkeri*, E tri = *E. triquetra*, E bre = *E. brevidens* and L sil = *Lampsilis siliquoidea*.



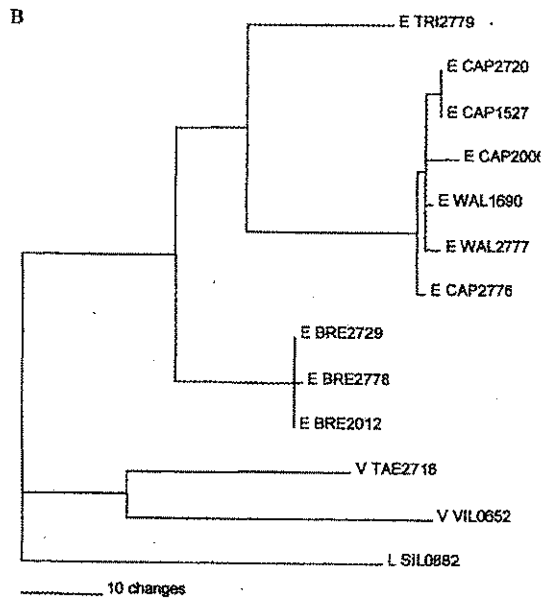


Figure 2. A strict consensus tree (A) of five equally most-parsimonious trees and (B) phylogram of one of the five equally most-parsimonious trees (TL = 218; CI = 0.679) based on an analysis of 610 bp of the mitochondrial *ND1* gene. Numbers above the nodes are bootstrap values (1000 replicates) and numbers below the nodes are Bremer support values. Specimen numbers = UAUC identification numbers as shown in Table 1. E cap = *Epioblasma capsaeformis*, E wal = *E. florentina walkeri*, E tri = *E. triquetra*, E bre = *E. brevidens*, L sil = *Lampsilis siliquoidea*; V tae = *Villosa taeniata*; V vil = *V. villosa*.

E. capsaeformis and *E. florentina walkeri* as mutually exclusive or monophyletic indicates the two taxa are not valid phylogenetic species (de Quieroz & Donoghue 1988; Baum & Donoghue, 1995; Mishler & Theriot, 2000; Wiens & Penkrot, 2002). Admittedly, one cannot prove the null hypothesis that genetic differences between the putative taxa are absent; however, management decisions should be based on the best available scientific information (see Avise, 1994, 2000, for other similar conservation genetic studies). The vast majority of unionid species have been described based implicitly on a morphospecies concept. Although considerable intraspecific shell variation within unionids and hyriids appears to be a response to the hydro-dynamics of living in large versus small river or stream environments (e.g. Walker, 1981; Watters, 1994), there was a tendency for taxonomists of the late nineteenth and early twentieth centuries to describe each morph as a unique species. From a monograph by Simpson (1900, 1914) to a check list by Turgeon *et al.* (1998), the taxonomy of *Epioblasma* has exhibited instability and subjective synonomy. Many biologists fail to understand that our view of currently recognized unionid species represents hypotheses that are subject to testing and may not adequately reflect species that actually exist in nature. For example, based on Johnson (1978) the Mobile River system of Alabama, Tennessee, Georgia and Mississippi would have only one *Epioblasma* species, compared with three recognized by Turgeon *et al.* (1998). Table 2 provides a comparison of the three primary classification schemes and current conservation status of *Epioblasma* taxa.

One intriguing feature that warrants further scrutiny, is the significance and mechanism explaining population variation in mantle pad colour. Ortmann (1924) reported geographic variation in the mantle pad colour of nominal *E. capsaeformis* with individuals from the Clinch, Powell and Nolichucky rivers possessing an iridescent bluish-white, while individuals in the Duck River possessing a slate-greyish almost black colour. Although some specimens from the Duck have been observed to have a small brown mottling along the outer edge of the mantle, the remaining part of the mantle is always grey or almost black in colour. In contrast, *E. f. walkeri* is mottled brownish/tan in Indian Creek and Big South Fork of the Cumberland River (S.

Table 2. Currently recognized species of *Epioblasma* according to a popular identification manual by Burch (1975), a monographic review by Johnson (1978) and a compiled checklist by Turgeon *et al.* (1998). The conservation status of each species is provided in the Turgeon *et al.* (1998) column as follows: X = presumed extinct; X* = probably extinct (based on recent survey data from S. Ahlstedt, W. Haag, P. Hartfield, P. Johnson, J. Williams); E = federally endangered + date listed; I = imperiled, not federally listed.

Burch 1975[a] (17 species; 2 subspecies)	Johnson 1978 (18 species)	Turgeon *et al.* 1998 (20 species; 8 subspecies)
arcaeformis (Lea, 1831)	*arcaeformis*	*arcaeformis* X
biemarginata[b] (Lea, 1857)	*biemarginata*	*biemarginata* X
brevidens (Lea, 1834)	*interrupta* (Rafinesque, 1820)	*brevidens* E,1997
capsaeformis (Lea, 1834)	*capsaeformis*	*capsaeformis* E,1997
flexuosa (Rafinesque, 1820)	*flexuosa*	*flexuosa* X
		florentina curtisii (Frierson & Utterback) E,1986/X*
florentina (Lea, 1857)	*florentina*	*florentina florentina* E,1976/X
		florentina walkeri (Wilson & Clark, 1914) E,1977
haysiana (Lea, 1833)	*haysiana*	*haysiana* X
lenior (Lea, 1842)	*lenior*	*lenior* X
lewisi (Walker, 1910)	= *flexuosa*	*lewisii* X
metastriata (Conrad, 1840)	= *penita*	*metastriata* E,1993/X*
modicella (Lea, 1859)	= *penita*	= *penita*
penita (Conrad, 1834)	*penita*	*penita* E,1987
personata (Say, 1829)	*personata*	*personata* X
sampsonii[d] (Lea, 1861)	*sampsonii*	*sampsonii* X
stewardsonii (Lea, 1852)	*stewardsonii*	*stewardsonii* X
sulcata (Lea, 1829)	*obliquata*	*obliquata obliquata* (Rafinesque, 1820) E,1990
		obliquata peroblique (Conrad, 1836) E,1976
		torulosa gubernaculum[c] (Reeve, 1865) E,1976/X
		torulosa rangiana[e] (Lea, 1865) E,1993
torulosa torulosa (Rafinesque, 1820)	*torulosa*	*torulosa torulosa* E,1976/X
torulosa propinqua (Lea, 1857)	*propinqua*	*propinqua* X
triquetra (Rafinesque, 1820)	*triquetra*	*triquetra* I
turgidula (Lea, 1858)	*turgidula*	*turgidula* E,1976/X
		othcaloogensis (Lea, 1857) E,1993/X*

[a]Burch (1975) largely followed the first monographic treatment of *Epioblasma* (= *Truncilla*) by Simpson (1900) with minor exceptions (e.g. Burch synonymized *E. compacta* and *E. othcaloogensis* with *E. metastriata*.
[b]Burch (1975) hypothesized that *E. biemarginata* was a large river form of *E. turgidula*.
[c]Burch (1975) considered *E. torulosa gubernaculum* as an upper Tennessee River form of *E. torulosa torulosa*.
[d]Burch (1975) hypothesized that *E. sampsonii* might be a Wabash River form of *E. torulosa torulosa*.
[e]Bogan (1997) and Parmalee & Bogan (1998) replaced *E. torulosa rangiana* with *E. biloba* (Rafinesque 1831), because the name had priority over *rangiana*.

Ahlstedt & J. Jones, personal communication). Although the two nominal *E. capsaeformis* specimens from the Duck River, which possess black mantle pads, are monophyletic, the remaining specimens did not form monophyletic groups associated with mantle pad colour. It is possible that other more rapidly evolving neutral genetic markers will delineate other phylogenetic species within the *E. capsaeformis* + *E. f. walkeri* (e.g. the Duck River black mantle pad morph) clade, which warrant recognition. However, our data refute the recognition of the currently recognized species boundaries based on shell morphology. Alternatively, mantle pad colour may be a response to some unknown environmental parameter or represent intraspecific allelic variation.

Although there is a desire for some biologists to accept and protect all currently recognized taxonomic units (e.g. Berg & Berg, 2000), we believe it is more important to test and determine species boundaries using rigorous scientific methods and principles, preferably prior to developing conservation recovery plans, particularly when advocating augmentation or transplanting of populations (Mulvey & Lydeard, 2000). Admittedly, sometimes decisions must be made on limited data due to the imperiled status of many unionids, particularly when faced with the complete loss of the putative species. We believe the Phylogenetic Species Concept, based on the recognition of minimal monophyletic groups (Mishler & Donoghue, 1982; Mishler & Brandon, 1987; de Queiroz & Donoghue, 1988, 1990), offers an objective and rigorous approach to test the validity of problematic unionid species complexes. This method has been applied to other unionid taxa (Mulvey *et al.*, 1997; Roe & Lydeard 1998; King *et al.*, 1999; Lydeard *et al.*, 2000) and should eventually result in a sound classification and understanding of unionid biodiversity and evolution. Our interpretation of the mtDNA sequence data, however, should be treated as an hypothesis subject to further testing.

Given that so few reproducing populations of *E. capsaeformis* and *E. f. walkeri* exist, combining the two into a single phyloge-

netic species does not alter their conservation status. Life history and population biology studies have already been conducted on the single known extant reproducing population of *E. f. walkeri* (Rogers, Watson & Neves, 2001). However, similar comparative studies should be conducted on remaining nominal *E. capsaeformis* populations with the intent of further testing our hypothesis that the two taxa are one phylogenetic species and to provide valuable data to manage more effectively the recovery of the species.

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