Descriptions of a new species and a new subspecies of freshwater mussels, *Epioblasma ahlstedti* and *Epioblasma florentina aureola* (Bivalvia: Unionidae), in the Tennessee River drainage, USA

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ABSTRACT

A new species and a new subspecies of *Epioblasma* are described from the Tennessee River drainage, USA. *Epioblasma ahlstedti* (Duck River Dartersnapper) currently is restricted to the Duck River in west-central Tennessee (TN). However, museum collections indicate that the species likely occurred in the Buffalo River, TN, a tributary to the Duck River, and in the Tennessee River at Muscle Shoals, Alabama (AL), and lower Shoal Creek, AL. The following diagnostic morphological characteristics of *E. ahlstedti* are based on the female: (1) pronounced posterior-ventral shell expansion of the adult female shell; (2) slate-gray to dark-purple mantle-pad; (3) spongy texture of the mantle-pad; and (4) display of a single, tan-colored micro-lure that moves slowly side-to-side. *Epioblasma florentina aureola* (Golden Riffleshell) currently is restricted to Indian Creek, a tributary to the upper Clinch River, Virginia. Historically, the species occurred in numerous tributaries in the Tennessee River drainage downstream at least to the Duck River. The following diagnostic morphological characteristics of *E. florentina aureola* are based on the female: (1) gray mantle-pad with a black mottled background; and (2) mantle-pad is pustuled but the pustules are rounded. The genus *Epioblasma* represents the most endangered group of freshwater mussels in North America; 18 of the recognized 25 species or subspecies are already extinct. Likewise, these newly described species and subspecies are critically endangered and despite being listed as endangered under the Endangered Species Act remain in need of focused conservation to prevent their extinction.

Additional keywords: Endangered, molecular DNA markers, phylogenetic analysis, Tennessee River basin

INTRODUCTION

The Tennessee River and its tributaries support the most species-rich mussel assemblage in North America (NA) (Figure 1), with 102 species known historically from the system (Parmalee and Bogan, 1998). Unfortunately, pollution and hydrological modifications (e.g., dams) to the river system over the past 100 years have reduced mussel diversity to approximately 80 extant species (Parmalee and Bogan, 1998; Williams et al., 2008). The decline of species belonging to the genus *Epioblasma* was the most severe (Johnson, 1978). Of the 18 species and subspecies known from the system, only four remain, and of those lost, most are considered extinct and one is extirpated but remains in a small isolated population in Ohio. Species in this genus have specialized reproductive traits, including species with shell morphologies and mantle-lures that can attract and capture their fish hosts to facilitate infestation by the glochidia (Jones et al., 2006; Barnhart et al., 2008). *Epioblasma* shells are characterized by small to medium sizes (~30–70 mm) and sexual dimorphism. The posterior-ventral end of the female shell is expanded, to form a distinct protrusion, or an area of the shell herein termed, the *shell expansion* (Figures 2, 3, 5, 8). This distinctive feature of female *Epioblasma* was considered by Walker (1910) to be the "highest expression of unionid development." The shell expansion houses a modified portion of the mantle, known as the mantle lure that functions to attract host fish. The focal species of the current study belong to the *Epioblasma* subgenus *Torulosa* (Johnson, 1978), which have a prominent shell expansion and a mantle-lure that contains a mantle-pad and micro-lure (Figures 7, 9–14). The mantle-pad is a folded and articulated portion of the mantle, and the micro-lure is a modified and innervated incurrent aperture papilla that moves to attract fish hosts, seemingly mimicking aquatic insect larvae (Jones et al., 2006; Barnhart, 2008).

Such morphological and life history specialization may have contributed to the decline of *Epioblasma* species, as changes in environmental conditions over the 19th and 20th centuries may have disrupted this complex life cycle. Unfortunately, the loss of numerous taxa in the genus has prevented a more complete understanding of the life history and taxonomy of the group, based on modern diagnostic methods using DNA and mantle-lure displays. Since only 7 of the 25 species and subspecies in NA

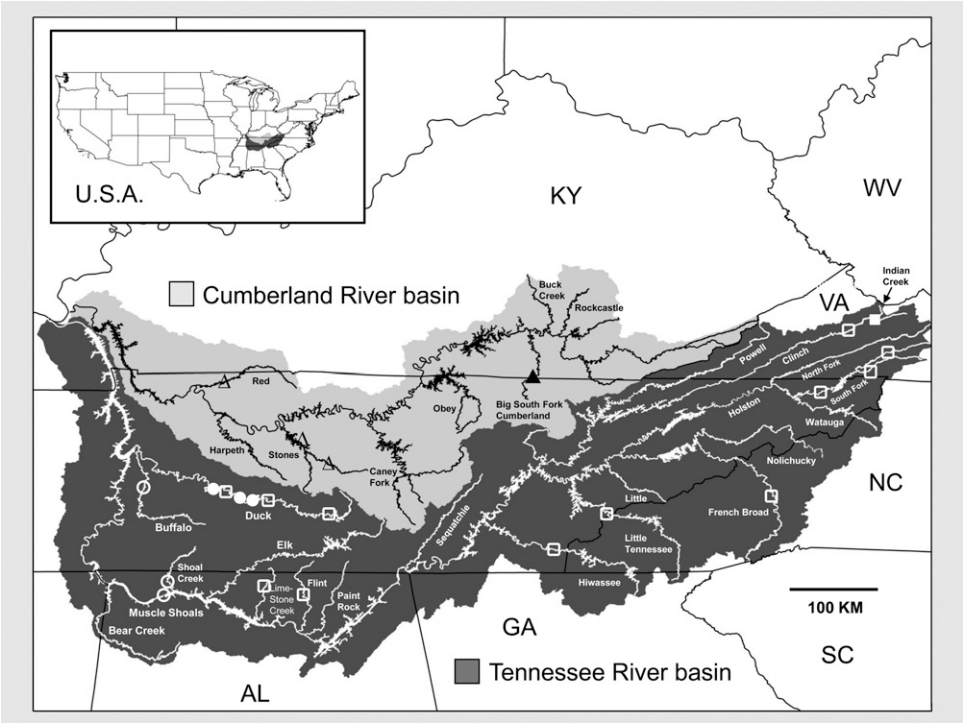

Figure 1. Distribution of *Epioblasma ahlstedti* (current ●, historic ○) and *E. florentina aureola* (current ■, historic □) in the Tennessee River system, USA. Also shown is the distribution of *E. florentina walkeri* (current ▲, historic △) in the Cumberland River system.

remain (Williams et al., 1993), it is possible that additional taxa were never identified before their extinction, while other described taxa may have been phenotypic variants of the same species or subspecies.

The diverse phenotypic variation within *Epioblasma*, especially the varied shell morphologies and mantle-lure displays of females, has allowed for a more comprehensive approach to understanding the taxonomy of extant species within the group, one that includes both traditional phenotypic characters and molecular markers. The study conducted by Jones et al. (2006) showed that the population of *Epioblasma capsaeformis* in the Duck River, Tennessee (TN) was distinct from the population in the Clinch River, TN and Virginia (VA), and that the population of *Epioblasma florentina walkeri* in Indian Creek, VA, a tributary to the upper Clinch River, was distinct from the population in the Big South Fork Cumberland River, TN and Kentucky (KY). Based on extensive phenotypic data (e.g., shell morphology, mantle-lures, fish host specificity) and molecular data (e.g., mitochondrial DNA, nuclear DNA microsatellites), these authors recommended the reclassification of the Duck River and Indian Creek populations of *Epioblasma*, respectively. However, the study did not formally describe and provide taxonomic recognition to these populations. Thus, the purpose of this paper is to present formal descriptions and provide scientific and common names for the new species and new subspecies of freshwater mussel in the Tennessee River system.

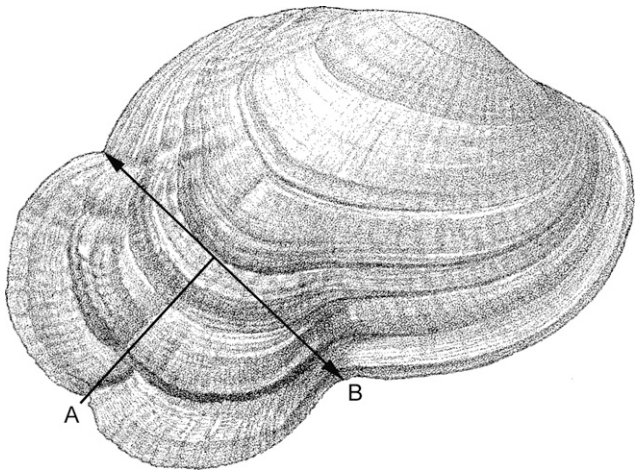

Figure 2. The height (axis-A) and length of the base (axis-B) of the shell expansion are shown. The arrows on axis-B point to the articulation points of the posterior-ventral shell expansion with the main body of the shell. The figure was modified from Burch (1975) and with permission from Dr. J.B. Burch.

MATERIALS AND METHODS

Type specimens, other shell material, and collection records for *Epioblasma ahlstedti*, *E. capsaeformis*, *E. florentina aureola*, *E. florentina florentina* and *E. florentina walkeri* were examined at the following museums: Academy of

Figures 3–4. **3.** Holotype (OSUM 68523) of *Epioblasma ahlstedti* (female), 50.7 mm long and 37.8 mm high; **4.** Paratype (OSUM 82238) of *Epioblasma ahlstedti* (male), 45.8 mm long and 32.0 mm high. Photos by G. Thomas Watters.

Natural Sciences of Philadelphia, Pennsylvania (ANSP); Carnegie Museum, Pittsburgh, Pennsylvania (CM); Florida Museum of Natural History, University of Florida (UF), Gainesville, Florida; Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts (MCZ); Ohio State University Museum of Biological Diversity, Columbus, Ohio (OSUM); and National Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM) [see Jones (2004) for all examined lots]. Type specimens provided standard references for comparing shell material from various rivers, and collection records were used to construct species distributions. Museum specimens of *E. ahlstedti* were identified using only female shells.

To assess differences in the shell expansion of adult female shells among populations, simple linear regression equations of total length (*x*-axis) versus the height (*y*-axis) of the shell expansion were computed for each population. The height of the shell expansion was measured from its base, which is the length between the two articulation points with the main body of the shell (Figure 2). Digital calipers were used to measure shell dimensions to the nearest 0.1 mm. To test for differences, the slopes of the fitted-lines of each regression equation were compared among populations using the homogeneity of regression coefficients test statistic.

Fecundity was estimated by counting the number of glochidia from each of 6–10 females per population. Although not a diagnostic trait in this study, it helped to demonstrate quantitative differences among species.

Live female mussels for fecundity analysis were collected from the following river locations: (1) *E. ahlstedti*, Duck River, Lillard Mill [River Kilometer (RKM) 287.7], Marshall County (Co.), TN; (2) *E. capsaeformis*, Clinch River between Horton Ford (RKM 321) and Swan Island (RKM 277), Hancock Co., TN; (3) *E. florentina aureola*, Indian Creek, a tributary to the upper Clinch River at RKM 518.2, Tazewell Co., Virginia (VA); (4) *E. f. walkeri*, Big South Fork Cumberland River, Station Camp Creek, Scott Co., TN, downstream to Bear Creek, McCreary Co., KY (Jones et al. 2006).

Figures 5–6. **5.** Holotype (OSUM 82239) of *Epioblasma florentina aureola* (female), 44.1 mm long and 34.8 mm high; **6.** Paratype (OSUM 82240) of *Epioblasma florentina aureola* (male), 48.1 mm long and 33.4 mm high. Photos by G. Thomas Watters.

Fecundity was compared using analysis of variance (ANOVA). All statistical analyses of shells and glochidia were conducted in MINITAB 14 Statistical Software (Minitab, Inc., State College, PA).

Samples of mantle tissue from live mussels were collected from the above river locations and, additionally, individuals of *E. torulosa rangiana* were collected from the Allegheny River, Venango Co., Pennsylvania (PA) (Jones et al., 2006). Other subspecies were not included in the study because they are presumed extinct, i.e., *E. florentina florentina*, *E. florentina curtisi*, and *E. t. torulosa*. A small piece of mantle tissue (20–30 mg) was collected non-lethally from 8–20 live mussels from each population (Naimo et al., 1998).

Sequences of three regions of mitochondrial DNA (mtDNA) and one region of nuclear DNA (nDNA) were amplified by polymerase chain reaction (PCR) using primers and conditions reported in: (1) *16S*, ribosomal RNA (Lydeard et al., 1996) (2) *ND1*, first subunit of NADH dehydrogenase (Buhay et al., 2002; Serb et al., 2003), (3) *cytochrome-b* (Merritt et al., 1998; Bowen and Richardson, 2000), and (4) *ITS-1* (King et al., 1999). All PCR products were sequenced with a Big Dye Terminator Cycle Sequencing kit with Ampli*Taq* DNA Polymerase (Applied Biosystems). Cycle sequence reactions were purified using a Qiagen DNA Purification kit (Qiagene), and subjected to electrophoresis and sequencing using an Applied Biosystems 3100 automated sequencer [detailed PCR methods are available in Jones (2004) and Jones et al. (2006)].

Phylogenetic analysis of DNA sequences and morphological characters were conducted to infer genealogical relationships among *Epioblasma* spp. Sequences from mtDNA and nDNA were combined, and six morphological characters (Table 1) were included in the character matrix for a total evidence analysis (Kluge, 1989). DNA sequences were edited and aligned using the program SEQUENCHER (version 3.0, Gene Codes Corporation), and phylogenetic analysis was performed using PAUP* (version 4.0b10, Swofford, 1998).

Figures 7–14. Mantle-pad displays of female *Epioblasma ahlstedti*, *E. florentina aureola* and congeners. **7.** Mantle-pad and micro-lure of *E. ahlstedti*, Duck River, Marshall Co., TN. **8.** Protruded shell expansion of female *E. ahlstedti*. **9.** *E. capsaeformis* mantle-pad, Clinch River, Hancock Co., TN. **10.** Double micro-lure display of *E. capsaeformis*; **11.** *E. florentina aureola* mantle-pad, Clinch River, Tazewell Co., VA. **12.** Micro-lure of *E. florentina aureola*. **13.** *E. florentina walkeri* mantle-pad and micro-lure, Big South Fork Cumberland River, Scott County, TN. **14.** Pustuled mantle surface of *E. florentina walkeri*. The gaps between shell valves of female mussels are approximately 2 cm. The arrows are pointing at microlures. This figure was previously published in Jones et al. (2006) but updated in this study to include the new species and new subspecies scientific names; photograph in Figure 9 has not been previously published. Photographs of the mantle-pad display of *E. torulosa rangiana* are available in Jones (2004).

Table 1. Matrix and coding for shell and mantle-lure characters for *Epioblasma* species. Character states were determined from direct observation and from those reported in Jones et al. (2006). A gap ('–') indicates the character was not applicable to the species.

Species	CHARACTER MATRIX							
	1	2	3	4	5	6	7	8
Epioblasma ahlstedti	1	1	0	0	0	–	1	0
Epioblasma capsaeformis	1	1	0	1	1	–	2	1
Epioblasma florentina aureola	1	1	1	2	2	0	1	0
Epioblasma florentina walkeri	1	1	1	3	2	1	1	0
Epioblasma torulosa rangiana	1	0	2	4	1	–	0	2
Epioblasma brevidens	0	–	–	–	–	–	–	–
Epioblasma triquetra	0	–	–	–	–	–	–	–

CHARACTERS:

(1) Prominent posterior-ventral shell expansion of adult female. 0=absent or diminutive; 1=present.
(2) Denticulations present along margin of posterior-ventral shell expansion. 0=absent; 1=present.
(3) Periostracum color and ray pattern. 0=yellow-green periostracum with irregularly spaced green rays; 1=honey-yellow periostracum with fine green rays evenly spaced over entire shell surface; 2=brown periostracum with irregularly spaced green rays.
(4) Mantle-pad color. 0=dark purple to slate gray; 1=blue to bluish-white; 2=gray with mottled black background; 3=brown with mottled tan background; 4=white.
(5) Mantle-pad texture. 0=spongy; 1=smooth; 2=pustules.
(6) Mantle-pad pustules. 0=rounded; 1=pointed.
(7) Number of micro-lures prominently displayed. 0=0; 1=1; 2=2.
(8) Mantle-pad is invaginated where it meets incurrent aperture. 0=yes; 1=no; 2=incomplete.

Phylogenetic trees were evaluated using the maximum parsimony (MP) criterion because the extent of sequence divergence was low among in-group taxa (Nei and Kumar, 2000; Felsenstein, 2004). Characters were treated as unordered and of equal weight for the analysis due to in-group taxa being closely related (Nei and Kumar, 2000). The tree search was conducted using the branch-and-bound method with ACCTRAN and TBR options; insertions and deletions were treated as missing data. Bootstrap analyses (10,000 replicates) were conducted using the FAST step-wise addition option of PAUP* to assess support for the individual nodes of each phylogenetic tree (Felsenstein, 1985). An additional phylogenetic analysis was conducted using Bayesian inference in MrBayes v3.0b4 (Huelsenbeck and Ronquist, 2001), where the DNA and morphological data sets were combined following the approach of Nylander et al. (2004). MrBayes was run for 1 million generations, sampling trees every 100 generations and posterior probabilities were computed after a burn-in of 40,000 generations. In-group taxa were *E. ahlstedti*, *E. capsaeformis*, *E. florentina aureola*, *E. florentina walkeri*, and *E. torulosa rangiana*. Because of significant differences in morphology and DNA sequences, the Cumberland Combshell (*Epioblasma brevidens*) and Snuffbox (*Epioblasma triquetra*) were designated as out-group taxa. The study by Jones et al. (2006) showed that DNA sequences from these two species are diverged from the in-group taxa by ~5%, and based on obvious differences in shell morphology, Johnson (1978) classified *E. brevidens* and *E. triquetra* into different *Epioblasma* subgenera, *Plagiola* and *Truncillopsis*, respectively. Furthermore, previous phylogenetic studies have demonstrated the monophyly of the *Epioblasma* among North American unionids and that *E. brevidens* and *E. triquetra* are basal to the in-group taxa (Campbell et al., 2005; Zanatta and Murphy, 2006). Thus, available morphological and genetic data justify use of these two species as appropriate out-group taxa.

Photographs of the mantle-pad and micro-lures of live female mussels were taken using a Nikonos V underwater camera with 28 or 35 mm macro-lenses and Kodak 200 Ektachrome film. Female mussels were held in temperature-controlled water in recirculating artificial streams with gravel-filled bottoms. This setup allowed females to display their mantle-pad and behavioral observations of micro-lure movements to be recorded under controlled conditions [photographic methods were previously published in Jones et al. (2006)].

Epioblasma ahlstedti new species
Duck River Dartersnapper
Figures 3, 4, 7, 8

Diagnosis: The following diagnostic morphological characteristics of *Epioblasma ahlstedti* are based on the female and are summarized in Table 2: (1) pronounced posterior-ventral shell expansion of the adult shell; (2) slate-gray to dark purple mantle-pad; (3) spongy texture of the mantle-pad; and (4) display of a single, tan-colored micro-lure that moves slowly side-to-side. In young individuals, the base of the shell expansion is constricted, appearing narrow but distinctly protruded. However, as the female shell grows, the shell expansion becomes extremely protruded and enlarged, compared to the main body of the shell. The shell expansion of *E. ahlstedti* is distinguishable from that of *E. capsaeformis* using the following criteria: (1) length of the shell expansion base in young individuals (~3–5 y), ranging in size from ~35–45 mm, typically appears constricted, being ~5–10 mm narrower basally than those of female *E. capsaeformis* of similar age and size, and (2) mean height (9.6 mm) and maximum height (24.6 mm) of the shell expansion of adult females is significantly greater than that of female *E. capsaeformis* (Table 3; Figure 15).

Description: Length of the female shell can reach 60 to 70 mm, with mean length ~42 mm (Table 3). Male shell lengths are similar. The shell outline of males is typically elliptical, appearing pointed at the posterior end (Figure 4), whereas the shell outline of females is more sub-oval and rounded, with a very enlarged and protruded posterior-ventral shell expansion (Figures 3, 8). The mean height of the shell expansion of the female is ~10 mm or ~23% of shell length, but maximum height is

Table 2. Diagnostic morphological and molecular genetic characters for *Epioblasma ahlstedti*, *E. florentina aureola* and closely related taxa. Data are summarized from Jones et al. (2006).

Species or subspecies	Morphological characters	DNA regions and base-pair site positions	
		mtDNA	nuclear DNA
Epioblasma ahlstedti	• shell expansion of female: large and protruded in adults • mantle-pad color: dark purple to slate-gray • mantle-pad texture: spongy • micro-lure display: 1 lure prominent; rotates clockwise sweeping side-to-side	• *16S*: 2 • *cytochrome-b*: 43, 208, 211, 214 • *ND1*: 281, 564	• *ITS*-1: 152, 153
Epioblasma capsaeformis	• mantle-pad color: blue to bluish-white • mantle-pad texture: smooth • micro-lure display: 2 together; the left lure rotates clockwise, while the right lure rotates counter-clockwise • posterior portion of the mantle-pad is not invaginated where it meets incurrent aperture	• *16S*: 179 • *cytochrome-b*: 13, 87, 148, 184, 327 • *ND1*: 282, 444	• *ITS*-1: 467, 468
Epioblasma florentina aureola	• mantle-pad color: light gray with mottled black background • mantle-pad texture: rounded pustules • micro-lures: 1 lure prominent; rotates clockwise sweeping side-to-side	• *cytochrome-b*: 252 • *ND1*: 405	
Epioblasma florentina walkeri	• mantle-pad color: brown with mottled tan background • mantle-pad texture: pointed pustules • micro-lures: 1 lure prominent; rotates clockwise sweeping side-to-side	• *cytochrome-b*: 52, 96 • *ND1*: 249, 423	• *ITS*-1: 215
Epioblasma torulosa rangiana	• denticulations: absent from posterior-ventral shell expansion • mantle-pad color: white • micro-lures: absent	• *16S*: 167, 452 • *cytochrome-b*: 99, 172 • *ND1*: 30	• *ITS*-1: 44, 127, 385, 386, 387, 388, 511

Table 3. Mean shell length, mean height of shell expansion, and linear regression equations of shell expansion height (y-axis) to total length (x-axis) of adult female mussels. Pairwise comparisons of regression equation slopes were significantly different ($p<0.001$), except equations A vs D, and C vs D. *The p-value indicates the significance level of the slope for each regression equation.

Mussel species	N	Mean length of shell (mm) (range)	Mean height of shell expansion (mm) (range)	Regression equations A–D	R^2	p-value*
Epioblasma ahlstedti	62	41.9 (31.0–68.9)	9.6 (3.1–24.6)	A. $y = -19.8 + 0.701x$	0.76	$p<0.001$
Epioblasma capsaeformis	63	40.7 (30.4–58.4)	8.3 (2.8–14.6)	B. $y = -4.92 + 0.324x$	0.54	$p<0.003$
Epioblasma florentina aureola	55	40.0 (30.7–46.1)	5.7 (1.0–10.2)	C. $y = -16.7 + 0.561x$	0.85	$p<0.001$
Epioblasma florentina walkeri	20	40.5 (30.3–45.2)	8.5 (2.0–14.1)	D. $y = -19.7 + 0.696x$	0.51	$p<0.007$

~25 mm (Table 3), and it is dark green, sometimes appearing almost black. Denticulations occur along the margin of the shell expansion and are typically ~0.5 to 1 mm long and spaced ~0.5 to 1 mm apart. The periostracum of adults is yellowish green, becoming more yellowish at the anterior end. The shell surface contains distinct broad to fine green rays that are typically irregularly spaced. The male shell is short and high with a shallow sulcus. Nacre color is white, but hues of blue and salmon may be present, especially near the beak cavities.

The color of the foot and gills of *E. ahlstedti* is dull white. In females, only the two outer gills are marsupial (i.e., the outer water-tubes contain and brood glochidia when gravid). Located at the distal end of each marsupial water-tube is a pore, which allows for release of glochidia. The mantle-pad is slate-gray to dark purple and has a spongy texture, and the micro-lure is tan (Figure 7). The posterior portion of the mantle-pad is invaginated where it meets the incurrent aperture, so the attachment points of the micro-lures cannot be seen when the female is displaying (Jones et al., 2006).

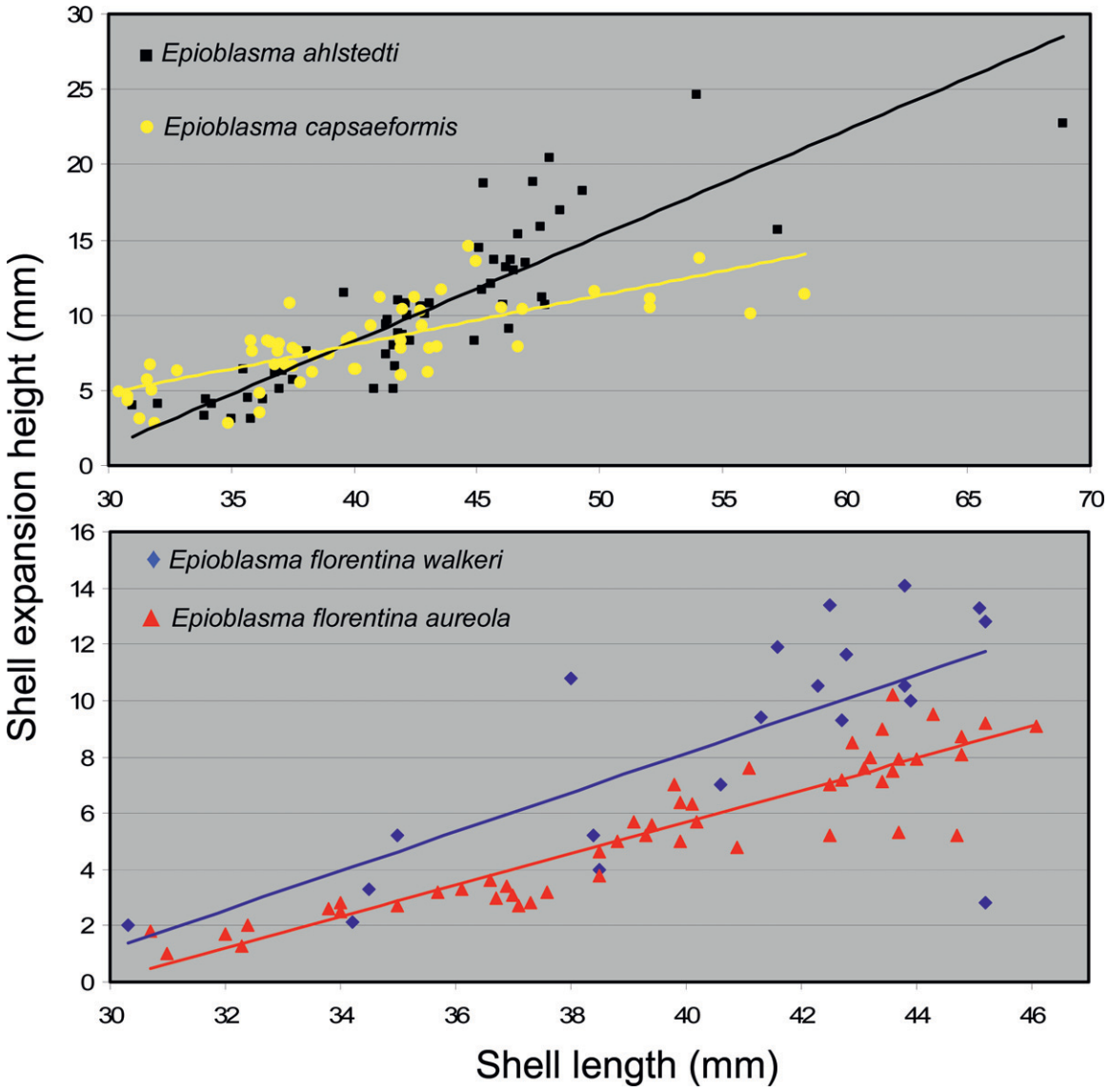

Figure 15. Relationship of posterior-ventral shell expansion height versus shell length of female *Epioblasma ahlstedti* and *E. capsaeformis*, and of female *E. florentina aureola* and *E. florentina walkeri*. Pairwise comparison of regression equation slopes was significantly different ($p<0.001$) between *Epioblasma ahlstedti* and *E. capsaeformis* but not significantly different between *E. florentina aureola* and *E. florentina walkeri*. However, the slope of each regression equation is significant for all four taxa (see Table 3).

Type Material: Holotype: Designated herein as OSUM 68523, collected by H.D. Athearn, from type locality, 30 Sep. 1956. **Paratypes:** CM 61.11669, Duck River, Lillard Mill, Marshall Co., TN, collected by A.E. Ortmann, 25 Aug. 1923; MCZ 236214, Duck River, Lillard Mill, Marshall Co., TN, collected by H.D. Athearn, 30 Sep. 1956; OSUM 52509, Duck River, Lillard Mill, Marshall Co., TN, collected by S.A. Ahlstedt, 1 Oct. 1982; OSUM 82238, Duck River, Lillard Mill, Marshall Co., TN, collected by S.A. Ahlstedt, 1 Oct. 1999 (see Appendix for other material examined); OSUM 82241, Duck River, Lillard Mill, Marshall Co., TN, collected by H.D. Athearn, 30 Sep. 1956.

Type Locality: Duck River, Lillard Mill, Marshall Co., TN, 35°35′09.08″ N; 86°47′14.07″ W.

Comparison with Similar Species: The shell of adult *Epioblasma capsaeformis* (*sensu stricto*) in the Clinch River is of small to medium length (~30–50 mm). The shell surface contains distinct broad to fine green rays that are irregularly spaced, and very similar to those of *E. ahlstedti*. However, the following diagnostic, morphological characteristics of female *E. capsaeformis* distinguish it from *E. ahlstedti*: (1) bluish-white mantle-pad (Figure 9), (2) smooth texture of the mantle-pad, and (3) simultaneous display of two micro-lures that move

Table 4. Fecundity estimates of female *Epioblasma*.

Mussel species	Number of females (*N*)	Mean length (mm)	Length range (mm)	Mean number of glochidia/female	Range
Epioblasma ahlstedti	6	45.4	35.8–56.44	18,757*	6,668–38,716
Epioblasma capsaeformis	10	41.5	36.7–46.4	13,008	7,780–16,876
Epioblasma florentina aureola	7	42.2	40.6–45.7	7,602	3,261–12,558
Epioblasma florentina walkeri	6	42.8	40.5–45.2	9,606	1,828–16,921

*Mean fecundity for *Epioblasma ahlstedti* is significantly different ($p<0.05$) from the other taxa.

synchronously in a circular motion; the left micro-lure moves clockwise, and the right micro-lure moves counterclockwise (Figure 10). The dorsal margin of the mantle-pad is black, forming a discrete uniform band ~2–3 mm wide. The posterior portion of the mantle-pad is not invaginated where it meets the incurrent aperture, so the attachment points of the micro-lures can be seen when the female is displaying. The denticulations along the margin of the shell expansion of *E. capsaeformis* are typically finer and more closely spaced than those of *E. ahlstedti*. The shell expansion is greenish, but not as dark as that of *E. ahlstedti*.

Life History: The typical habitat of *E. ahlstedti* is gravel shoals in medium to large rivers. It is a long-term brooder, gravid from late summer to the following spring and early summer. In the Duck River, at least some females will emerge from the substrate in early spring (e.g., March–April) to display their mantle-pad lure to attract host fishes, while others emerge later in the spring and summer (e.g., May–July). Mean fecundity of females was 18,757 glochidia, and ranged from 6,668 to 38,716 (Table 4). The largest females were not examined; hence maximum fecundity is unknown but likely exceeds 50,000 glochidia. Known fish hosts for *E. ahlstedti* include three darter species, the greenside darter (*Etheostoma blennioides*), fantail darter (*Etheostoma flabellare*), and redline darter (*Etheostoma rufilineatum*) (Jones et al., 2006). However, tested hosts were not collected from the Duck River, but from the North Fork Holston River, VA, in the upper Tennessee River drainage. Although all three darters are widely distributed and common in Duck River, the native Duck River hosts remain uncertain.

Molecular DNA Markers and Phylogenetic Analysis: The Duck River population of *E. ahlstedti* contains presumably diagnostic nucleotides at three mitochondrial DNA gene regions [*16S* (n=1), *cytochrome-b* (n=4), and *ND*1 (n=2)] and at one non-coding nuclear DNA region [*ITS*-1 (n=2)] (Table 2). Based on analysis of 10 nuclear DNA microsatellite loci, the population is moderately diverged (F_{ST}=0.12) from the Clinch River population of *E. capsaeformis* (Jones et al., 2006). The MP and Bayesian phylogenetic analyses showed high statistical support for the *E. ahlstedti* clade (Figures 16, 17); a finding previously demonstrated by Jones et al. (2006) using only the DNA sequences. Furthermore, the MP tree in Jones et al. (2006) shows *E. ahlstedti* as the basal member of the in-group taxa, whereas the MP tree (Figure 16) reported in this study shows *E. torulosa rangiana* as basal. However, several interior nodes in both MP trees are weakly supported and collapse in the respective consensus trees (not shown). A 50% majority-rule Bayesian consensus tree depicting a more conservative topology is given in Figure 17.

Distribution: *Epioblasma ahlstedti* is currently restricted to 48.3 RKM in the Duck River from Lillard Mill (RKM 286.5) downstream to the backwaters of the Old Columbia Dam reservoir (RKM 238.2) in Marshall Co. and Maury Co., west-central Tennessee (Figure 1). Based on shells, the species likely occurred historically in the Buffalo River, TN (Parmalee and Bogan, 1998), a tributary to the Duck River, and the Tennessee River at Muscle Shoals and Shoal Creek, Lauderdale Co., AL (Jones, 2004).

Conservation Status: The oyster mussel (*Epioblasma capsaeformis*) was listed as endangered under the Endangered Species Act (ESA) in 1997, to include the Duck River population. Now that *E. ahlstedti* has been designated a separate species, it is restricted to only the Duck River population. Being linearly distributed to 48 river kilometers and susceptible to a stochastic impact, this species should be considered critically endangered and continue to receive full protection under the ESA.

Etymology: The species name is given in honor of biologist Steven A. Ahlstedt, U.S. Geological Survey (retired), Knoxville, TN, who has dedicated over 30 years of service to freshwater mussel conservation in the United States. The common name denotes the snapping behavior of many female *Epioblasma* species (i.e., displaying females will quickly close their shells when touched, which can capture a host fish to facilitate infestation with glochidia on the host) (Jones et al., 2006).

Epioblasma florentina aureola new subspecies
Golden Riffleshell
Figures 5, 6, 11, 12

Diagnosis: The following diagnostic morphological characteristics of *E. florentina aureola* are based on the female and are summarized in Table 2: (1) gray mantle-pad with a black mottled background, and (2) mantle-pad has rounded pustules.

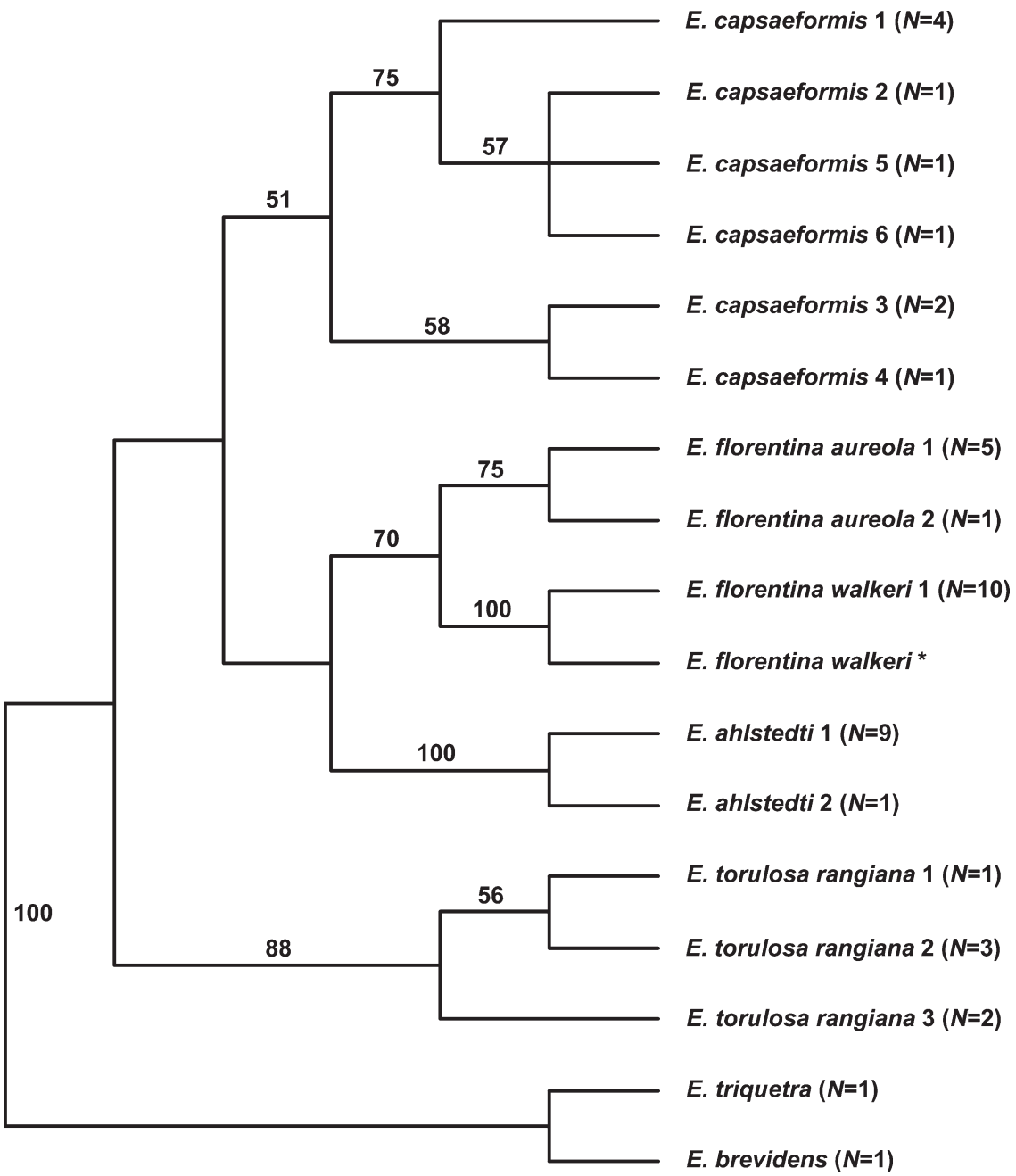

Figure 16. Cladogram showing phylogenetic relationships among *Epioblasma ahlstedti*, *E. florentina aureola* and congeners, inferred from the combined mitochondrial DNA regions of *16S* (468 bp), *cytochrome-b* (360 bp), *ND1* (568 bp), the nuclear DNA region *ITS-1* (515 bp) and eight morphological characters (see Table 1) using maximum parsimony (MP) (31 equally parsimonious trees were resolved; length=195 steps; CI=0.92; RI=0.89). Numbers above the branches (MP) represent bootstrap support (10,000 replicates); only values >50% are shown. All *E. florentina walkeri* were identical; however, to demonstrate the monophyly of this population, an additional sequence was added to the analysis. Out-group taxa are *E. triquetra* and *E. brevidens*.

Description: Length of the female shell can reach 46 mm in Indian Creek and the upper Clinch River, with mean length of 40 mm (Table 3). Shell lengths of males are similar. The shell outline of females is sub-oval and rounded with a moderately protruded shell expansion (Figure 5). The mean height of the female shell expansion is 5.7 mm or ~14% of shell length, but maximum height can reach ~10 mm (Table 3; Figure 15). Denticulations occur along the margin of the shell expansion and are fine and narrowly spaced ~0.5 mm apart. The shell outline of males is elliptical, appearing pointed at the posterior end (Figure 6), and may have a shallow sulcus, especially older individuals. The periostracum of adults can range from golden honey-yellow, to tan and

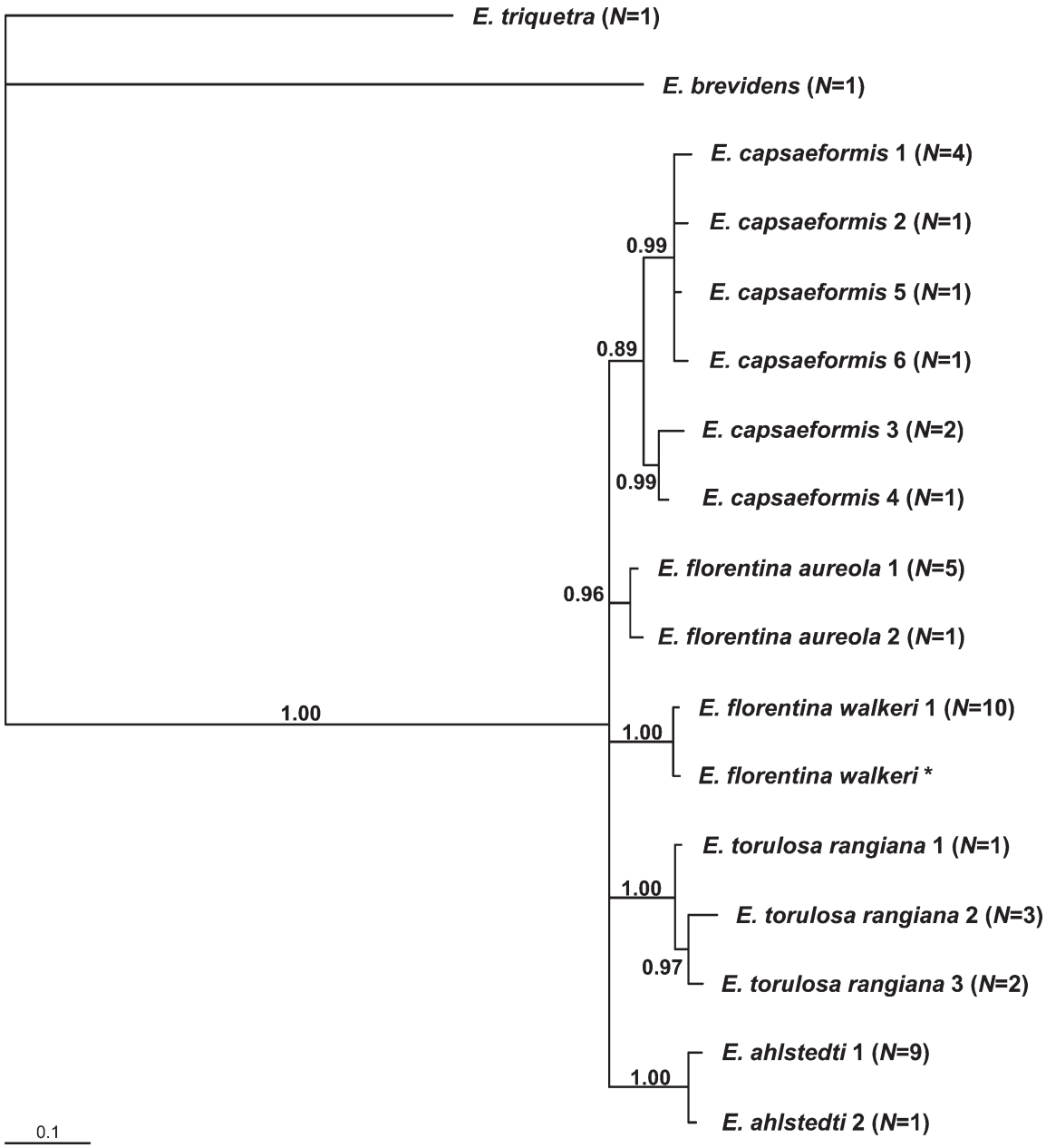

Figure 17. Phylogenetic relationships. **17.** A 50% majority-rule consensus tree (phylogram) showing phylogenetic relationships among the ingroup taxa. The tree was produced from the same DNA sequences and morphological characters listed above and evaluated using Bayesian inference. The analysis was run for 1 million generations with a burn-in of 40,000 generations (mean log likelihood=-3,667). Numbers at the nodes are calculated posterior probabilities (>50%) indicating proportion of trees containing the inferred nodes. Numbers in parentheses at the end of each taxonomic name represent the number of observed DNA haplotypes. *All *E. florentina walkeri* haplotypes were identical; however, to demonstrate the monophyly of this population, an additional sequence was added to the analysis. Out-group taxa are *E. triquetra* and *E. brevidens*.

brown, but coloration is usually evenly distributed over the shell, although occasionally the shell expansion of the female is tinted green. The periostracum contains distinct fine green rays that are evenly spaced over the shell. Nacre color is white, but hues of blue and salmon may be present.

The color of the foot and gills of *E. florentina aureola* is dull white. In females, only the two outer gills serve as marsupia. Each marsupial water-tube contains a distal pore to allow for release of glochidia. The mantle-pad is gray with a black-mottled background and with rounded pustules (Figures 11, 12). The dorsal margin of the mantle-pad is tan, forming a discrete, uniform band ~2–3 mm wide. Micro-lures are darkly colored, and only a single micro-lure is prominently displayed,

which moves slowly side-to-side in a sweeping motion (Figure 12). The posterior portion of the mantle-pad is invaginated where it meets the incurrent aperture, so attachment points of the micro-lures cannot be seen when the female is displaying. The undisplayed microlure is obscured from view inside the invaginated area of the mantle pad (Figure 12).

Type Material: Holotype: Designated herein as OSUM 82239, collected by Leroy Koch, from type locality, 1 Sep. 1998. **Paratypes:** OSUM 16266, Clinch River, Route 460 Bridge, Cedar Bluff, Tazewell Co., VA, collected by D.H. Stansbery and J.J. Jenkinson, 6 Oct. 1965; OSUM 42321, Clinch River, Route 460 Bridge, Cedar Bluff, Tazewell Co., VA, collected by C.R. Ciola, 1 July 1978; OSUM 42434, Clinch River, below railroad bridge, Cedar Bluff, Tazewell Co., VA, collected by C.R. Ciola and G. Wargowsky, 10 Aug. 1978; OSUM 43294, Clinch River, below railroad bridge, Cedar Bluff, Tazewell Co., VA, collected by J.M. Condit and C.R. Ciola, 15 July 1978; OSUM 53252, Clinch River, Cedar Bluff, Tazewell Co., VA, collected by R. Taylor, 10 July 1983; OSUM 82240, Clinch River, Route 460 Bridge, Cedar Bluff, Tazewell County, VA, collected by Leroy Koch, 1 Sep. 1998. (See Appendix for other material examined.)

Type Locality: Clinch River, Route 460 Bridge, Cedar Bluff, Tazewell County, VA, 36°05′16.13″ N; 81°46′05.98″ W.

Comparison with Similar Species: The shell of adult *E. florentina walkeri* (*sensu stricto*) in the Big South Fork Cumberland River is of small to medium length (~30–45 mm) (Table 3), and nearly indistinguishable in shape and color to those of *E. florentina aureola*. The mean height (8.5 mm) and maximum height (14.1 mm) of the shell expansion of adult females is slightly larger than that of female *E. florentina aureola*, but it is not significantly greater (Table 3; Figure 15). The periostracum color of both is honey-yellow, to tan and brown, with fine green rays evenly spaced across the shell. However, the following diagnostic morphological characteristics of female *E. florentina walkeri* distinguish it from *E. florentina aureola*: (1) mantle-pad is brown with a tan-mottled background (Figure 13); and (2) while the mantle-pad also is pustuled, the pustules are pointed and not rounded (Figure 14). The denticulations along the margin of the shell expansion of the female shell are larger and more widely spaced ~1 mm apart, compared to those of *E. florentina aureola*. Similarities include display of a single micro-lure, which moves slowly side-to-side, and the dorsal margin of the mantle-pad is tan, forming a distinctive band ~2–3 mm wide. Similarly, the posterior portion of the mantle-pad is invaginated where it meets the incurrent aperture, so the attachment points of the micro-lures cannot be seen when the female is displaying.

Life History: The typical habitat of *E. florentina aureola* is stable sand and gravel substrates in headwater reaches of rivers and creeks. The subspecies is a long-term brooder, gravid from late summer to the following spring and early summer (Rogers et al., 2001). In Indian Creek, females will emerge in spring and summer (April–July) to display their mantle-pad lure to attract host fishes. Based on an estimate from a single female, maximum fecundity is at least 20,000 glochidia (Rogers et al., 2001). However, mean fecundity based on six females during this study was 7,602 glochidia per female, ranging from 3,261 to 12,558 glochidia (Table 4). Known fish hosts for *E. florentina aureola* based on laboratory trials include: greenside darter (*Etheostoma blennioides*), fantail darter (*E. flabellare*), redline darter (*E. rufilineatum*), snubnose darter (*E. simoterum*), black sculpin (*Cottus baileyi*), mottled sculpin (*C. bairdi*), and banded sculpin (*C. carolinae*) (Jones and Neves, 2001; Rogers et al., 2001).

Molecular DNA Markers and Phylogenetic Analysis: The Indian Creek and upper Clinch River population of *E. florentina aureola* contains presumably diagnostic nucleotides at two mitochondrial DNA gene regions [*cytochrome-b* (n=1), and *ND*1 (n=1)] (Table 2). Further, based on analysis of 10 nuclear DNA microsatellite loci, the population is diverged (F_{ST}=0.39) from the Big South Fork Cumberland River population of *E. florentina walkeri* (Jones et al., 2006). The MP and Bayesian phylogenetic analyses showed high statistical support for the *E. florentina aureola* clade (Figures 16, 17), a finding previously reported by Jones et al. (2006).

Distribution: *Epioblasma florentina aureola* is currently restricted to the lower ~1.6 KM of Indian Creek in southwestern Virginia (Figure 1). Historically, this subspecies presumably occurred in numerous tributary streams of the middle and upper Tennessee River system downstream to the Duck River. However, all of these historical populations are considered extirpated. Therefore, the color and texture of the mantle pad and other traits of those populations are unknown. Thus, it is unknown whether these historical populations represented *E. florentina aureola* or other undescribed taxa.

Conservation Status: The Tan Riffleshell (*E. florentina walkeri*) was listed as endangered under the ESA in 1977. Now that *E. florentina aureola* has been designated herein as a separate subspecies, the subspecies only occurs as a single small population in the lower reach of Indian Creek, VA. This subspecies is one of the most critically endangered populations of freshwater mussel in the United States being linearly distributed to a short stream section and highly susceptible to a stochastic event. This subspecies should continue to receive maximum protection under the ESA.

Etymology: The subspecies name *aureola* is the diminutive form of the Latin adjective for golden, and is here chosen to denote the honey-yellow to occasionally golden color of the shell.

Remarks: The anatomical characteristics of females, such as the distal pores, mantle-pad, and micro-lure,

and the shell expansion with denticulations along the margin of the shell are considered advanced traits among unionids, based on their complexity and uniqueness to *Epioblasma* (Jones et al., 2006). The prominent shell expansion of female *E. ahlstedti* is a defining trait first recognized around the turn of the 20th century by the American malacologist Bryant Walker (1856–1936). The following undated letter was written by him and found in a small box in the ANSP shell collection (ANSP 100538). The letter was a hand-written note to a physician and to our knowledge has never been published. The handwriting was difficult to read but was deciphered exactly as is by JWJ, with assistance from Paul Callomon, Elana Benamy, and Earle Spamer of ANSP, on 8 Jan. 2003:

> "I also send some *Truncillas* [*Epioblasmas*] that may be of interest. The Shoal Creek form is typical *capsaeformis* as I understand it. The male of the Duck R. form is very similar, but the females have invariably the enormous expansion of the specimen sent. In the Clinch, on the other hand, the females are quite typical in form, but the males are usually decidedly more elongated. The Duck R. form has been generally called "*turgidulus*" but it is not. Lea's *turgidulus* is the male of *deviata* as I proved to my own satisfaction, at least, while I was in Washington."

> "P.S. The element of uncertainty in *capsaeformis* matter is the fact that we don't know what the ♀ form of the Cumberland is. The ♀ shell I had at Phila. & which agreed best with Lea's figure of the type was from the Duck R., variety *expansa*. The only Cumberland R. ♀ I have seen is an immature shell belonging to Ferris, and it is apparently like the Tenn. R. & Shoal Crk. form. If the ♀ Cumberland R. form is *expansa* like the Duck R. shell, that would be typical *capsaeformis* & *expansa* could not be used".

These observations by Bryant Walker indicate that the taxonomic position of the Duck River population was being questioned nearly 100 years ago, and that the expansion of the female shell was seen as a diagnostic trait when compared to specimens collected from other rivers in the region, including shells from the Clinch River. In Walker's view, the size of the shell expansion of adult females is a signature phenotypic character, reaching a mean and maximum height that is greater than those of other species and subspecies belonging to the *Epioblasma* subgenus *Torulosa* [*sensu* Johnson (1978)]. Thus, the combination of key phenotypic traits, especially the unique shell morphology and mantle-lure display of females, unambiguously define *E. ahlstedti* as a valid species.

The population of *Epioblasma florentina aureola* in the upper Clinch River watershed is not deserving of a separate species designation because of several shared traits with *E. florentina walkeri* in the Big South Fork Cumberland River: (1) honey-yellow to tan-colored periostracum, (2) similar fish host specificity, (3) pustuled mantle-pads, and (4) preference for headwater stream habitats. These two populations also have similar-sized glochidia, similar fecundity, and are closely related phylogenetically. In addition, the periostracum color of the nominal species *E. florentina florentina* is also yellow and was thought to simply represent clinal variation; i.e., the large river form of the subspecies complex. Ortmann (1918; 1924; 1925) considered the two forms as merely clinal variants, a claim supported by his observation that the big river form appeared to grade into the headwater form as one progressed upstream. However, based on the shell material examined in Jones (2004) and Jones et al. (2006), it is uncertain whether *E. florentina florentina* merely represents clinal variation, a subspecies or perhaps even a separate species for the following reasons: (1) large distances of seemingly unoccupied habitat commonly occurred between mainstem and headwater populations (e.g., Clinch and Holston rivers >200 RKM), and ecological conditions between mainstem and headwater locations are substantial (e.g. distribution of host fishes, water temperature, stream size, etc.); (2) a transitional series of shells representing a continuously distributed population of this species from mainstem to headwaters *does not exist*; and (3) because most populations are extirpated, additional genetic, morphological and life history data are unobtainable. For example, available specimens of *E. florentina florentina* collected from the lower Clinch River near its mouth are short (~30–35 mm), solid, and thick-shelled, and are quite distinctive from the larger-sized headwater form *E. florentina aureola* (Jones, 2004). However, because *E. florentina florentina* is extinct, adequate comparisons of the mantle-lure and DNA cannot be made with the other subspecies. Therefore, since each nominally described subspecies occurred or occurs in distinct geographic regions and habitats, we believe *E. florentina* ssp. minimally was a polytypic species complex and best categorized by the current trinomial designations.

The current taxonomy of *Epioblasma* recognizes 20 species and 5 subspecies (Turgeon et al., 1998; Williams et al., 1993). Thus, with the description of *E. ahlstedti* and *E. florentina aureola*, 21 species and 6 subspecies now are recognized in the genus. The historical distributions of *E. florentina aureola* and *E. florentina walkeri* are unclear because most populations are extirpated and only shell material and collection records are available to assess the distribution of each subspecies. Furthermore, the anatomy of live individuals cannot be compared among historical populations. Therefore, a practical approach to delineating the distribution of each subspecies is to consider all records of *E. florentina walkeri* in the Tennessee River drainage as *E. florentina aureola*, and all respective records in the Cumberland River drainage as *E. florentina walkeri*. This approach would simplify the management and recovery of each subspecies within their respective river drainages.

ACKNOWLEDGMENTS

Financial support for this project was provided by the U.S. Fish and Wildlife Service (USFWS) and the Tennessee Wildlife Resources Agency. We thank Brett Ostby, Virginia Tech University, for assistance in preparing the distribution map. We especially thank Robert Butler, USFWS, Jim Williams, U.S. Geological Survey (Retired), Daniel Graf, University of Alabama, and an anonymous reviewer for their respective peer reviews of this work, which greatly improved the manuscript. We also gratefully acknowledge G. Thomas Watters and Clarissa Lawlis at the Museum of Biological Diversity, Ohio State University for cataloging and photographing the shell holotypes and paratypes, and José H. Leal, The Bailey-Matthews Shell Museum, for his assistance in preparing the figures. The views expressed in this publication are the authors and do not necessarily represent those of the USFWS.

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APPENDIX 1. Shell collection lots of *Epioblasma ahlstedti* and *E. florentina aureola* examined during this study are provided below, where NA=Not Available, H=holotype, P=paratype.

The following lots are of *E. ahlstedti*:

Museum	Lot number	Type	Location	Collector	Date
ANSP	100504		Duck River, TN	H.H. Smith	NA
ANSP	100538		Shoal Creek, Lauderdale Co., AL	H.H. Smith	NA
ANSP	391133		Duck River, Hardison Mill, Maury Co., TN	S. Ahlstedt	1/16/1986
CM	61.646		NA	Hartman Collection	NA
CM	61.4491		Shoal Creek, Lauderdale Co., AL	B. Walker	11/2/1909
CM	61.6498		Shoal Creek, Lauderdale Co., AL	A.E. Ortmann	NA
CM	61.7696		Tennessee River, Florence, Lauderdale Co., AL	A.E. Ortmann	NA
CM	61.7697		Shoal Creek, Lauderdale Co., AL	A.E. Ortmann	NA
CM	61.11267		Duck River, Maury Co., TN	A.E. Ortmann	8/26/1921
CM	61.11497		Duck River, Maury Co., TN	A.E. Ortmann	9/6/1922
CM	61.11669	P	Duck River, Lillard Mill, Marshall Co., TN	A.E. Ortmann	8/25/1923
CM	61.11670		Duck River, Wilhoite, Marshall Co., TN	A.E. Ortmann	8/27/1923
CM	61.11672		Duck River, Leftwich, Maury Co., TN	A.E. Ortmann	9/3/1923
UF	64238		Tennessee River, Florence, Lauderdale Co., AL	A.A. Hinkley	1904
UF	64241		Duck River, Wilhoite, Marshall Co., TN	NA	NA
UF	64242		Duck River, Hardison Mill, Maury Co., TN	NA	NA
UF	64245		Tennessee River, Muscle Shoals, Lauderdale Co., AL	H.H. Smith	11/1/1909
UF	64250		Shoal Creek, Lauderdale Co., AL	H.H. Smith	10/1/1909
UF	226003		Duck River, TN	NA	NA
UF	269045		Duck River, Columbia, Maury Co., TN	A.A. Hinkley	NA
UF	269049		Duck River, TN	Marsh	NA
UF	269058		Duck River, Columbia, Maury Co., TN	B. Walker	NA
MCZ	6210		Duck River, Columbia, Maury Co., TN	R.E. Call	NA
MCZ	29828		Shoal Creek, Lauderdale Co., AL	B. Walker Collection	NA
MCZ	83995		Duck River, Wilhoite, Marshall Co., TN	Goodrich	NA
MCZ	83441		Shoal Creek, Lauderdale Co., AL	H.H. Smith	NA
MCZ	236214	P	Duck River, Lillard Mill, Marshall Co., TN	H.D. Athearn	9/30/1956
MCZ	236718		Tennessee River, Muscle Shoals, Lauderdale Co., AL	B. Walker Collection	NA
MCZ	272794		Duck River, Maury Co., TN	P. Yokley	9/3/1965
OSUM	8628		Duck River, TN	NA	1800's
OSUM	12246		Duck River, 431 Bridge, Maury Co., TN	C.B. Stein	7/19/1964
OSUM	14496		Duck River, Sowell Ford, Maury Co., TN	P. Yokley	7/7/1965
OSUM	14864		Duck River, Milltown, Marshall Co., TN	D.H. Stansbery	9/5/1964
OSUM	15149		Duck River, Wilhoite Mill, Marshall Co., TN	D.H. Stansbery	9/8/1964
OSUM	16229		Duck River, Sowell Ford, Maury Co., TN	P. Yokley B. Isom	9/2/1965
OSUM	16238		Duck River, Columbia, Maury Co., TN	P. Yokley B. Isom	9/1/1965
OSUM	33341		Duck River, Milltown, Marshall Co., TN	D.H. Stansbery W.J. Clench	10/14/1972
OSUM	33922		Duck River, Wilhoite Mill, Marshall Co., TN	P. Yokley B. Isom	9/3/1965
OSUM	33959		Duck River, Milltown, Marshall Co., TN	P. Yokley B. Isom	9/3/1965
OSUM	34074		Duck River, Leftwich, Maury Co., TN	P. Yokley B. Isom	9/3/1965
OSUM	50107		Duck River, TN	NA	Prior 1931
OSUM	50108		Duck River, TN	NA	Prior 1928
OSUM	52509	P	Duck River, Lillard Mill, Marshall Co., TN	S. Ahlstedt	10/1/1982
OSUM	57291		Duck River, TN	Wheatley	Prior 1882
OSUM	67899		Duck River, Shelbyville, TN	Call	1885

(Continued)

APPENDIX
(Continued.)

Museum	Lot number	Type	Location	Collector	Date
OSUM	68523	H	Duck River, Lillard Mill, Marshall Co., TN	H.D. Athearn	9/30/1956
OSUM	82238	P	Duck River, Lillard Mill, Marshall Co., TN	S.A. Ahlstedt J. Powell	10/1/1999
OSUM	82241	P	Duck River, Lillard Mill, Marshall Co., TN	H.D. Athearn	9/30/1956
USNM	510913		Duck River, Columbia, TN	NA	NA
USNM	521353		Duck River, Columbia, TN	NA	NA

The following lots are of *E. florentina aureola*:

Museum	Lot number	Type	Location	Collector	Date
ANSP	103740		Flint River, Maysville, AL	H.H. Smith	NA
ANSP	103903		Hurricane Creek, Gurley, AL	H.H. Smith	NA
ANSP	103997		Flint River, Gurley, AL	H.H. Smith	NA
CM	61.6765		South Fork Holston River, Washington Co., VA	A.E. Ortmann	NA
CM	61.6767		South Fork Holston River, Emmitt, Sullivan Co., TN	A.E. Ortmann	NA
CM	61.11668		Duck River, Wilhoite, Marshall Co., TN	A.E. Ortmann	NA
UF	269050		Flint River, Madison, Co., AL	B. Walker	NA
UF	269057		Tennessee	C.T. Simpson	NA
UF	269059		Tennessee	NA	NA
MCZ	276026		Middle Fork Holston River, Route 91 Bridge, Smyth Co., VA	Wilson & Clark	8/21/1911
MCZ	293653		Middle Fork Holston River, Chilhowie, Smyth Co., VA	R.E. Winters	9/2/1977
OSUM	15150		Duck River, Wilhoite Mills, Marshall Co., TN	D.H. Stansbery	9/8/1964
OSUM	16266	P	Clinch River, Route 460 Bridge, Cedar Bluff, Tazewell Co., VA	D.H. Stansbery & J.J. Jenkinson	10/6/1965
OSUM	16344		Clinch River, Route 80 Bridge, Russell Co., VA	D.H. Stansbery & J.J. Jenkinson	10/6/1965
OSUM	24342		Middle Fork Holston River, Route 91 Bridge, Smyth Co., VA	D.H. Stansbery & W.J. Clench	9/16/1968
OSUM	25330		Middle Fork Holston River, Route 638 Bridge, Smyth Co., VA	D.H. Stansbery	8/28/1970
OSUM	29072		Duck River, Route 65 Bridge, Maury Co., TN	S.A. Ahlstedt	4/26/1988
OSUM	34943		Middle Fork Holston River, Chilhowie, Smyth Co., VA	D.H. Stansbery	10/16/1973
OSUM	42198		Middle Fork Holston River, Route 638 Bridge, Smyth Co., VA	D.H. Stansbery & F.L. Kokai	7/24/1978
OSUM	42321	P	Clinch River, Route 460 Bridge, Cedar Bluff, Tazewell Co., VA	C.R. Ciola	7/1/1978
OSUM	42434	P	Clinch River, below railroad bridge, Cedar Bluff, Tazewell Co., VA	C.R. Ciola & G. Wargowsky	10/8/1978
OSUM	43294	P	Clinch River, below railroad bridge, Cedar Bluff, Tazewell Co., VA	J.M. Condit & C.R. Ciola	7/15/1978
OSUM	53252	P	Clinch River, Cedar Bluff, Tazewell Co., VA	R. Taylor	7/10/1983
OSUM	57118		French Broad River, Asheville, Buncombe Co., NC	J.F. Hardy	NA
OSUM	82239	H	Clinch River, Route 460 Bridge, Cedar Bluff, Tazewell Co., VA	L. Koch	9/1/1998
OSUM	82240	P	Clinch River, Route 460 Bridge, Cedar Bluff, Tazewell Co., VA	L. Koch	9/1/1998
USNM	29898		French Broad River, Asheville, NC	NA	NA