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Using morphometrics to identify glochidia from a diverse freshwater mussel community

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Abstract. We measured shell length, hinge length, and height of glochidia from 21 freshwater mussel species occurring in the Sipsey River, Alabama, to test our ability to identify species based on these glochidial morphometrics. Glochidial size and shape differed widely among species; for all 3 dimensions, mean values for the largest species were 5 to 7× greater than for the smallest species. Within-species variation in glochidia size was low for all species with the exception of *Pleurobema decisum*, which was represented by 2 glochidial morphotypes; variation within each morphotype was similar in magnitude to variation within other species. We were able to classify 72 to 79% of total glochidia (n = 870 or 750, respectively) to the correct species with discriminant function analysis. Percentage of correct classification ranged from 40 to 100% for individual species. Misclassifications were caused by overlap in shell dimensions between some species, but even species with poor classification success were confounded with an average of only 2.2 to 3.0 other species. Unlike previous studies, we found that glochidia of closely related species were not necessarily more similar to each other than to glochidia of more distantly related species. For example, species in the tribe Quadrulini were widely divergent in glochidium size and represented some of both the smallest and largest glochidia in our study. These 3 shell measurements and subsequent application of discriminant function analysis can be useful for identification of unknown glochidia or for rapidly narrowing the range of potential species identifications to smaller groups of species with similar glochidia.

Key words: freshwater mussels, Unionidae, glochidia, fish-host relationship, drift, discriminant function.

Freshwater mussels in the families Unionidae and Margaritiferidae (superfamily Unionacea) have a unique reproductive life cycle in which larvae (glochidia) must attach to a fish host for a brief period before becoming free-living juveniles (Neves et al. 1997). Host use varies among mussel species from generalists that use a wide array of fish species to specialists that are able to complete metamorphosis to the juvenile life stage on a few, closely related fish species (Haag and Warren 1997). Host identity, mode of glochidial transmission to hosts, and other features of the host relationship have a strong influence on freshwater mussel ecology and evolution (Haag and Warren 1998, Vaughn and Taylor 2000).

Fish-host relationships based on artificial infection trials in the laboratory have been reported for a large number of North American species (Hoggarth 1992, Watters 1994). In contrast, patterns of fish-host use in the wild and seasonal patterns of glochidia abundance in stream drift are less well known. Because glochidia of many species are similar, most field studies have taken place in headwater streams or lakes with low mussel diversity (Tedla and Fernando 1969, Trdan 1981, Threlfall 1986, Jansen and Hanson 1991, Hove and Neves 1994), or have focused on a single species with distinctive glochidial morphology (Zale and Neves 1982a, Cummings and Mayer 1993, Baird 2000). The few studies of glochidia in large streams with diverse mussel assemblages often have been able to identify glochidia only to genus or subfamily (Neves and Widlak 1988, Weaver et al. 1991, Weiss and Layzer 1995, Baird 2000). Consequently, species-level patterns of glochidia occurrence on wild fishes or in stream drift in diverse mussel communities are poorly known. These patterns represent an important information need for freshwater mussel ecology and conservation.

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A number of morphometric and qualitative characters have been used to identify glochidia with varying degrees of precision (Surber 1912, Hoggarth 1988, 1999, O'Brien et al. 2003). Scanning electron microscopy (Rand and Wiles 1982, Waller et al. 1988) and molecular genetic techniques (White et al. 1996) have proven successful in identifying similar glochidia to species, but may be too time-consuming and expensive for routine applications. A combination of 3 standard measurements (length, height, and hinge length, see Hoggarth 1988, 1999) is used commonly for identifying glochidia to subfamily or genus but, in many cases, this combination has not allowed identification to species because of overlap in glochidia size (e.g., Zale and Neves 1982b, Bruenderman and Neves 1993). However, most studies that have used these 3 measurements have only qualitatively compared unknown glochidia with glochidia measurements from a known reference library; therefore, the potential utility of this suite of measurements to quantitatively discriminate mussel glochidia has not been explored fully.

We tested the efficacy of using these 3 commonly and easily measured shell morphometrics to identify glochidia to species within a diverse, large-stream mussel assemblage in the Sipsey River, Alabama. We examined sources and magnitude of variation in glochidia size within and among species, and examined the ability of discriminant function analysis to correctly classify glochidia to species based on standard shell measurements.

Methods

The Sipsey River is a major southeastern tributary of the Tombigbee River in northwestern and west-central Alabama and currently supports the most intact remaining large-stream mussel assemblage in the Mobile Basin. A total of 42 mussel species is known from the river basin (McCullagh et al. 2002), but mussel assemblages in the lower and middle river are characterized by the regular occurrence of about 25 species (Haag 2002).

We collected glochidia from 21 mussel species, including all common constituents of mussel assemblages in the Sipsey River (Haag 2002). We collected as many species as possible from the Sipsey River but used specimens from other streams as necessary (Table 1). These species represent a wide range of higher-level phylogenetic diversity within the family Unionidae. Because widely used taxonomic classifications are based on polyphyletic groupings (Lydeard et al. 2000), we used a contemporary classification scheme to portray higher-level relationships among species (Graf 2002, Campbell et al. 2005).

For most species, we extracted glochidia from gravid gills of preserved female mussels. Because of their federal conservation status, we collected glochidia of *Hamiota perovalis*, *Medionidus acutissimus*, and *Pleurobema decisum* in a nondestructive manner (see Haag and Warren 2003) and released females alive. We measured shell length, hinge length, and shell height (Fig. 1; see Hoggarth 1988) to the nearest 1 μm for 10 mature glochidia each from 1 to 9 female mussels/species (Table 1). We made all measurements using a binocular microscope and digital camera interfaced with video-imaging software. Because glochidium size was distributed bimodally in *P. decisum*, we treated the 2 morphotypes as distinct entities, denoted PDEC1 and PDEC2, for computation of mean measurements and data analysis.

We evaluated sources of variation in glochidium shell measurements among species using an unbalanced, nested multivariate analysis of variance (MANOVA), with glochidia from different females nested within species. We $\log_{10}(x+1)$-transformed all measurements to satisfy assumptions of normality and homogeneity of variance (Johnson and Wichern 2002). *Tritogonia verrucosa* was excluded from the MANOVA because glochidia from >1 female were not available. We tested for correlations among morphometrics using Pearson's correlation coefficient.

We examined the ability of shell measurements to separate glochidia by species in 3 ways. First, we constructed bivariate plots of shell measurements using grand means (± 2 SE) for each species to examine relative differences in glochidia size among species. Second, we constructed 2-tailed 95% ranges for glochidium size for each species by excluding glochidia that were below the 2.5^{th} percentile or above the 97.5^{th} percentile for any dimension. We used these truncated ranges to assess the degree of discrimination possible by simple comparison of variation in shell dimensions among species without the confounding effects of extreme values. Third, we tested the ability of discriminant

TABLE 1. Grand means (±1 SE) and ranges (in parentheses) for 3 glochidial shell dimensions of 22 mussel taxa. Data included fell within the 2-tailed 95% range of observations (see methods). Sample size (*n*) refers to the number of female mussels from which glochidia were measured; 10 glochidia were measured from each female. Species are sorted by increasing mean glochidial shell length.

Species (code, tribe)	*n*	Shell length (μm)	Hinge length (μm)	Shell height (μm)	Source
Leptodea fragilis (LFRA, Lampsilini)	4	71 ± 1 (60–88)	37 ± 1 (27–48)	87 ± 1 (74–99)	St. Francis River, Cross County, Arkansas
Quadrula rumphiana (QRUM, Quadrulini)	4	78 ± 1 (65–90)	39 ± 1 (27–50)	85 ± 1 (69–96)	Sipsey River, Pickens County, Alabama
Tritogonia verrucosa (TVER, Quadrulini)	1	94 ± 1 (88–98)	47 ± 1 (42–50)	111 ± 2 (104–119)	St. Croix River, Chisago County, Minnesota
Pleurobema decisum (PDEC1, Pleurobemini)	4	141 ± 1 (131–158)	99 ± 3 (66–137)	136 ± 2 (106–155)	Sipsey River, Pickens County, Alabama
Fusconaia cerina (FCER, Pleurobemini)	3	143 ± 1 (132–155)	113 ± 1 (97–125)	162 ± 2 (149–182)	Sipsey River, Pickens County, Alabama
Obovaria unicolor (OUNI, Lampsilini)	5	171 ± 1 (158–183)	91 ± 1 (77–104)	216 ± 1 (196–228)	Sipsey River, Pickens County, Alabama
Lampsilis teres (LTER, Lampsilini)	3	173 ± 3 (149–198)	106 ± 2 (89–123)	216 ± 3 (190–250)	St. Francis River, Cross County, Arkansas
Lampsilis ornata (LORN, Lampsilini)	5	189 ± 1 (173–206)	104 ± 1 (86–125)	241 ± 2 (223–266)	Sipsey River, Pickens County, Alabama
Obliquaria reflexa (OREF, Lampsilini)	5	191 ± 2 (161–214)	108 ± 3 (87–133)	203 ± 3 (173–237)	Sipsey River, Pickens County, Alabama
Potamilus purpuratus (PPUR, Lampsilini)	3	193 ± 2 (177–213)	105 ± 1 (92–116)	343 ± 2 (321–361)	St. Francis River, Cross County, Arkansas
Villosa lienosa (VLIE, Lampsilini)	3	196 ± 2 (181–210)	103 ± 1 (96–111)	275 ± 1 (268–286)	Sipsey River, Pickens County, Alabama
Medionidus acutissimus (MACU, Lampsilini)	5	196 ± 2 (175–215)	89 ± 1 (77–102)	250 ± 4 (187–280)	Sipsey River, Pickens County, Alabama
Lampsilis straminea (LSTR, Lampsilini)	2	201 ± 1 (193–209)	105 ± 1 (94–113)	266 ± 2 (254–278)	Sipsey River, Pickens County, Alabama
Amblema plicata (APLI, Amblemini)	7	203 ± 2 (177–224)	131 ± 2 (92–165)	218 ± 2 (191–242)	Sipsey River, Pickens County, Alabama
Pleurobema decisum (PDEC2, Pleurobemini)	5	203 ± 3 (181–265)	135 ± 3 (106–171)	198 ± 3 (166–249)	Sipsey River, Pickens County, Alabama
Elliptio arca (EARC, Pleurobemini)	5	227 ± 3 (189–256)	146 ± 2 (119–172)	234 ± 2 (201–265)	Sipsey River, Pickens County, Alabama
Quadrula asperata (QASP, Quadrulini)	6	232 ± 3 (207–290)	102 ± 2 (85–137)	289 ± 8 (259–322)	Sipsey River, Pickens County, Alabama
Hamiota perovalis (HPER, Lampsilini)	3	241 ± 2 (220–258)	114 ± 1 (101–128)	298 ± 2 (279–323)	Brushy and Rush Creeks, Lawrence County, Alabama
Villosa vibex (VVIB, Lampsilini)	3	249 ± 2 (234–265)	121 ± 1 (105–133)	312 ± 2 (298–329)	Shoal Creek, Cleburne County, Alabama
Megalonaias nervosa (MNER, Quadrulini)	5	259 ± 2 (235–277)	153 ± 2 (135–179)	331 ± 1 (310–349)	Tennessee River, Colbert County, Alabama
Strophitus subvexus (SSUB, Anodontini)	3	332 ± 3 (294–357)	235 ± 3 (194–262)	355 ± 2 (331–378)	Brushy Creek, Lawrence County, Alabama
Pyganodon grandis (PGRA, Anodontini)	3	359 ± 3 (338–385)	258 ± 4 (215–294)	369 ± 3 (346–397)	Pond, Lafayette County, Mississippi

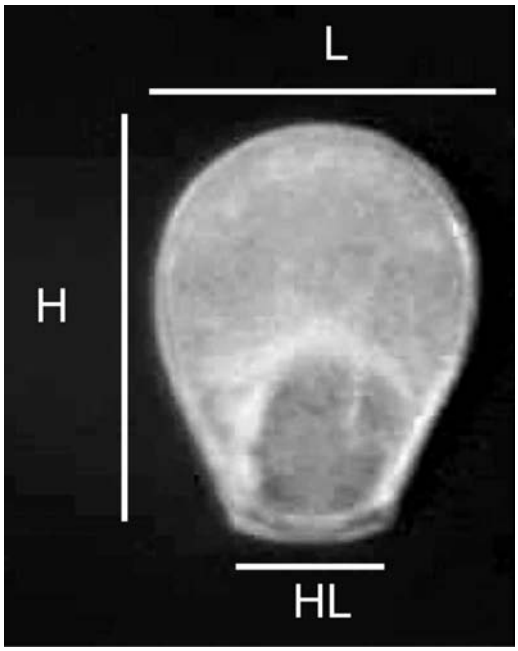

FIG. 1. Glochidium of *Medionidus acutissimus* showing shell dimensions measured in this study. L = length, HL = hinge length, H = height.

function analysis (DFA) to correctly classify glochidia to species based on $\log_{10}(x+1)$-transformed shell measurements (SAS, version 8, SAS Institute, Cary, North Carolina). We derived quadratic discriminants for each species because variance–covariance matrices of shell dimensions for species groups were unequal (χ^2 test: $\chi^2 = 893.3$, df = 126, $p < 0.0001$; Morrison 1976, Khattree and Naik 2000). For each individual glochidium, we solved functions for all species using the observed measurements of that glochidium, and determined putative species identity based on the function yielding the highest *Q*. We computed identification success using cross-validation scores because these estimates are nearly unbiased (Lachenbruch 1975), and we reported results for each species as the % of the total number of measured glochidia identified correctly. We applied DFA to both the full data set and the truncated 95% range of values for each species to compare discrimination success with and without the influence of extreme values. Discriminant function coefficients and variance–covariance matrices are available at the URL http://www2.srs.fs.fed.us/cbhr/db/scdet.asp?Initials=wrh&Site=OX.

Results

Glochidium size varied widely among the 22 mussel taxa (21 species, *Pleurobema decisum* with 2 morphotypes). For all 3 dimensions, mean values for the largest species were 5 to 7× greater than mean values for the smallest species (Table 1). Hinge length was highly correlated with both length ($r = 0.90$, $p < 0.0001$) and height ($r = 0.77$, $p < 0.0001$); length also was highly correlated with height ($r = 0.92$, $p < 0.0001$). Bivariate plots depicted a broadly scattered range of glochidium sizes among species, but standard errors around mean size and 95% ranges of observations overlapped for some species (Table 1, Fig. 2). In contrast, glochidium size varied little within most species. Variation among species accounted for 94 to 97% of total variation in glochidium size for all 3 dimensions, but variation within species accounted for only 1 to 2% of total variation (MANOVA, length: among-species $R^2 = 0.96$, $F_{20,774} = 260.06$, within-species $R^2 = 0.01$, $F_{65,774} = 5.42$; hinge length: among-species $R^2 = 0.94$, $F_{20,774} = 192.99$, within-species $R^2 = 0.02$, $F_{65,774} = 3.78$; height: among-species $R^2 = 0.97$, $F_{20,774} = 322.84$, within-species $R^2 = 0.01$, $F_{65,774} = 5.25$; all variables significant at $p < 0.0001$).

The single exception to low within-species variability in glochidium size was *Pleurobema decisum* in which size was bimodally distributed as 2 morphotypes (PDEC1 and PDEC2). Size of PDEC2 glochidia was 27 to 31% greater than PDEC1 for all 3 dimensions (Table 1). All glochidia measured from a single female were of a single morphotype and, among females with similar morphotypes, variation was low and similar in magnitude to variation seen within all other species (Table 1). Females that produced different morphotypes did not differ with respect to length (mean lengths and range, mm: PDEC 1 = 52.8, 49.2–56.4; PDEC 2 = 50.8, 48.8–52.3), date of collection (PDEC1 = 1998, 2000, 2001; PDEC 2 = 1998, 2000; late May–early June for all specimens), overall fecundity (PDEC 1 = 40,000, $n = 1$; PDEC 2: mean = 40,268, range = 20,500–69,553, $n = 3$; data from Haag and Staton 2003), female body color, or conglutinate color (orange for both morphotypes). Both morphotypes were found at the same sample sites.

DFA based on all values correctly classified 72% of the 870 glochidia in the total data set, but correct-classification percentages ranged

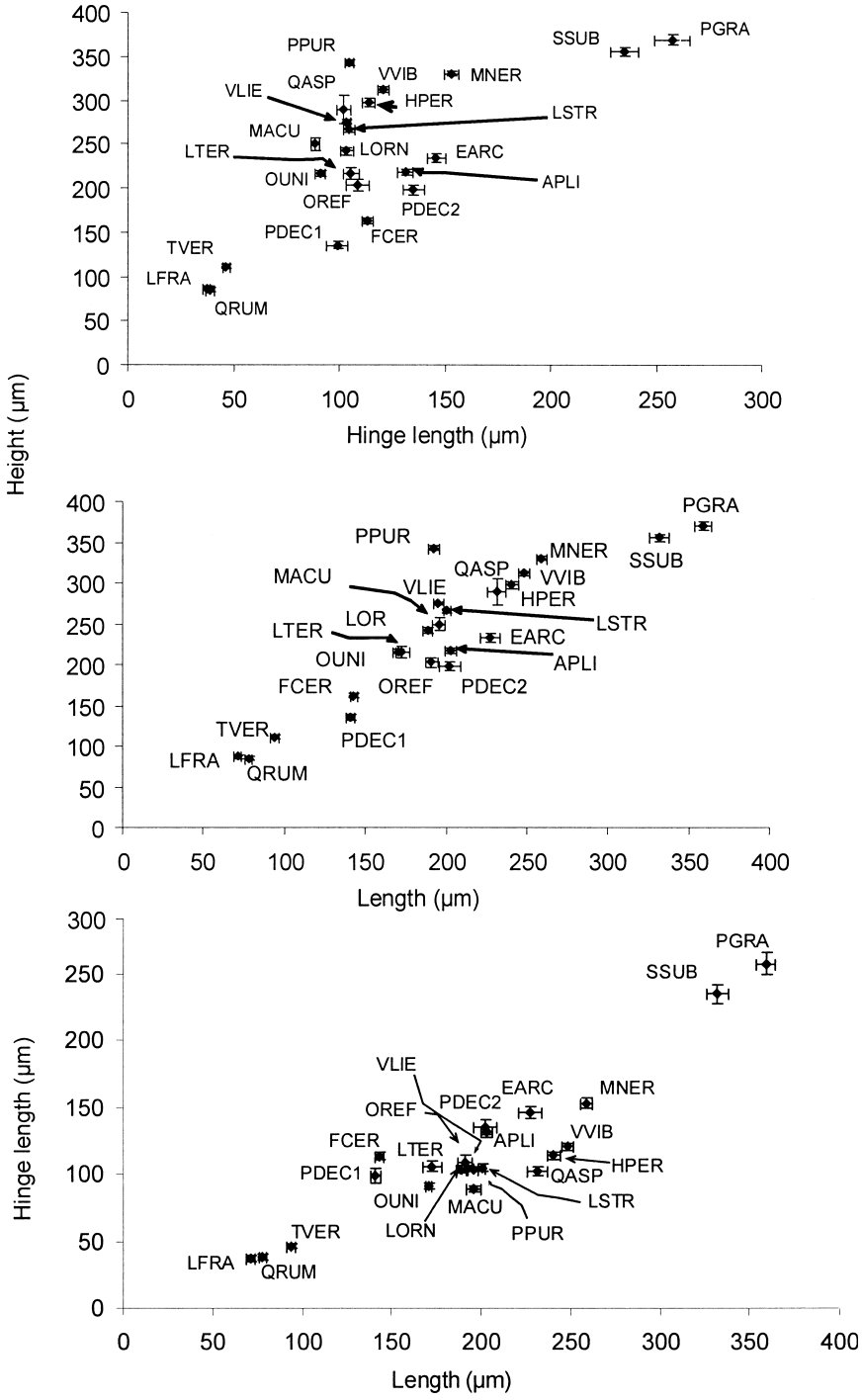

FIG. 2. Bivariate plots of glochidial dimensions for 22 mussel taxa. Points represent the grand mean (±2 SE) for all observations of a particular species. See Table 1 for species codes.

from 40 to 100% among taxa (Table 2). Correct classification was ≥80% for 8 taxa, and <75% for 10 taxa. Glochidia of each taxon were misclassified as 0 to 7 other taxa (mean = 3.0). For all misclassified glochidia combined, 46% were classified into the correct tribe and 7% were classified into the correct genus. For each taxon, the % of misclassified glochidia that were classified into the correct tribe ranged from 0 to 100% (mean = 59%).

DFA based on the 95% range for glochidial measurements correctly classified 79% of the 750 glochidia retained in the data set, and correct classification percentages ranged from 54 to 100% among taxa (Table 2). Correct classification was ≥80% for 13 taxa and <75% for 8 taxa. Glochidia of each taxon were misclassified as 0 to 6 other taxa (mean = 2.2). Individual taxa overlapped with 1 to 11 other taxa (mean = 5.3) on ≥1 shell dimension using qualitative comparisons of 95% ranges (Table 1).

Discussion

Utility of DFA and morphometrics for identifying glochidia

With application of DFA, these 3 commonly and easily measured shell dimensions can be useful for identification of mussel glochidia, even in diverse communities. For the faunal assemblage we considered, we were able to identify glochidia of >25% of the species with an error rate of <10% and >50% of the species with an error rate of ≤20% after removal of extreme values. The improvement in identification success using the 95% range demonstrated the sensitivity of DFA to outliers. Nevertheless, DFA performed poorly in discriminating some species with similar glochidia, especially those species that plotted consistently near the midpoint of the data scatter (e.g., *A. plicata*, *Lampsilis* spp., PDEC 2, and *O. reflexa*). Simple qualitative comparison of overlap in 95% ranges of glochidial measurements among species resulted in discrimination that was similar, though coarser than DFA. However, qualitative comparison of overlap in 95% ranges is very laborious and time consuming and is, therefore, impractical for application to a large number of specimens.

One of the primary values of using DFA in glochidial identification is to narrow rapidly the range of potential species identifications for unknown glochidia. Using DFA, most glochidia could be placed in groups representing ≤3 species. The level of discrimination within these groups using our 3 standard measurements varied widely, but the search for additional diagnostic characters will be simplified greatly by focusing only within these groups of similar species. Furthermore, although we approximated normal distributions by $\log_{10}(x+1)$-transforming shell measurements, species with similar glochidia may be classified better using distribution-free models that use inherent distributional geometries of data clouds rather than assuming linear or quadratic functions (Ghosh and Chaudhuri 2005). Nevertheless, some species may remain indistinguishable using characters visible under light microscopy (e.g., *Lampsilis* spp., Waller et al. 1988; *Villosa* spp., Zale and Neves 1982c). These cases may require the use of techniques such as scanning electron microscopy (Rand and Wiles 1982, Waller et al. 1988) and molecular genetics (White et al. 1996).

Variability among related taxa and among populations of the same species

Previous studies consistently have found that glochidia of closely related species are similar morphologically and often indistinguishable, but identifications of glochidia to higher taxonomic level (e.g., genus or subfamily) often can be made based on glochidial characteristics common to the group (Weaver et al. 1991). In our study, the 2 species of Anodontini (*Pyganodon grandis* and *Strophitus subvexus*) were similar in size and shape and were easily distinguished from all other species. In contrast, for all other species, we found little phylogenetic basis for glochidial size or shape, and misclassified glochidia were about equally likely to be identified as glochidia from closely related species (same tribe) as species from another tribe. For example, the tribe Quadrulini contained species with some of the smallest glochidia (*Quadrula rumphiana* and *Tritogonia verrucosa*) as well as some of the largest glochidia (*Megalonaias nervosa*). Glochidia of the 2 species of *Quadrula* did not resemble each other. *Quadrula rumphiana* was small and most closely resembled a member of the Lampsilini (*Leptodea fragilis*) and another member of the Quadrulini (*T. verrucosa*); *Q. asperata* was larger and closely resembled several species of Lampsilini (*Lampsilis* spp., *Medionidus*

TABLE 2. Identification success for glochidia of 22 mussel taxa using cross-validation scores of quadratic discriminant functions for all glochidia (all) and for glochidia within 95% ranges of observations (95%). Numbers in parentheses are % of glochidia misclassified as the given species. *n* = number of glochidia included in the analysis. See Table 1 for species codes.

Species	Data set	% correct	*n*	Misclassified as
Amblema plicata	All	75.7	70	PDEC2 (8.6), OREF (7.2), EARC (4.3), MACU (1.4), OUNI (1.4), PDEC1 (1.4)
	95%	74.2	62	OREF (9.6), EARC (8.1), PDEC2 (8.1)
Elliptio arca	All	54.0	50	APLI (36.0), PDEC2 (10.0)
	95%	83.3	42	APLI (14.3), LTER (2.4)
Fusconaia cerina	All	96.7	30	PDEC1 (3.3)
	95%	96.2	26	LTER (3.8)
Hamiota perovalis	All	43.3	30	QASP (26.7), VVIB (26.7), MNER (3.3)
	95%	54.2	24	VVIB (29.1), QASP (16.7)
Lampsilis ornata	All	70.0	50	MACU (10.0), OUNI (10.0), LSTR (4.0), EARC (2.0), LTER (2.0), OREF (2.0)
	95%	72.1	43	MACU (11.6), OUNI (7.0), LSTR (4.7), LTER (2.3), OREF (2.3)
Lampsilis straminea	All	60.0	20	VLIE (25.0), LORN (10.0), QASP (5.0)
	95%	61.1	18	VLIE (16.7), LORN (11.1), MACU (11.1)
Lampsilis teres	All	40.0	30	LORN (20.0), OUNI (16.7), APLI (10.0), OREF (10.0), LSTR (3.3)
	95%	53.9	26	LORN (19.2), OREF (11.5), OUNI (11.5), APLI (3.9)
Leptodea fragilis	All	75.0	40	QRUM (25.0)
	95%	67.6	37	QRUM (32.4)
Medionidus acutissimus	All	68.0	50	LORN (10.0), QASP (10.0), OREF (6.0), OUNI (4.0), VLIE (2.0)
	95%	81.8	44	LORN (9.1), OUNI (4.5), OREF (2.3), QASP (2.3)
Megalonaias nervosa	All	96.0	50	QASP (2.0), VVIB (2.0)
	95%	100	40	
Obliquaria reflexa	All	48.0	50	APLI (18.0), PDEC2 (10.0), LTER (8.0), MACU (6.0), OUNI (6.0), EARC (2.0), LORN (2.0)
	95%	57.1	42	APLI (11.9), LTER (9.5), PDEC2 (9.5), OUNI (4.8), MACU (4.8), LORN (2.4)
Obovaria unicolor	All	82.0	50	LTER (8.0), LORN (4.0), OREF (4.0), APLI (2.0)
	95%	90.7	43	LTER (4.7), LORN (2.3), OREF (2.3)
Pleurobema decisum (PDEC 1)	All	92.5	40	FCER (5.0), OREF (2.5)
	95%	97.1	35	FCER (2.9)
Pleurobema decisum (PDEC 2)	All	50.0	50	APLI (26.0), OREF (12.0), EARC (10.0), FCER (2.0)
	95%	65.8	41	APLI (19.5), OREF (9.8), EARC (4.9)
Potamilus purpuratus	All	100	30	
	95%	100	26	
Pyganodon grandis	All	83.3	30	SSUB (16.7)
	95%	80.0	25	SSUB (20.0)
Quadrula asperata	All	76.6	60	HPER (6.7), MACU (6.7), EARC (3.3), VLIE (3.3), MNER (1.7), VVIB (1.7)
	95%	88.6	53	HPER (3.8), MACU (3.8), EARC (1.9), VVIB (1.9)
Quadrula rumphiana	All	70.0	40	LFRA (30.0)
	95%	75.7	37	LFRA (24.3)
Strophitus subvexus	All	80.0	30	PGRA (16.7), MNER (3.3)
	95%	80.0	25	PGRA (20.0)
Tritogonia verrucosa	All	70.0	10	QRUM (30.0)
	95%	100	9	
Villosa lienosa	All	83.4	30	LSTR (10.0), MACU (3.3), QASP (3.3)
	95%	84.6	26	LSTR (11.5), QASP (3.9)
Villosa vibex	All	76.7	30	HPER (13.3), QASP (6.7), MNER (3.3)
	95%	84.6	26	HPER (15.4)

acutissimus, *Villosa* spp.). Our results show that glochidial morphology is less taxonomically conserved than suggested by previous studies. Consequently, in streams with diverse mussel assemblages, coarse glochidial morphology may be of limited usefulness in identifying glochidia to genus or higher taxonomic levels.

The utility of identifying glochidia using simple morphometrics would be increased if published size information or discriminant functions for glochidia of a variety of species were generally available. General availability of such information could alleviate the time-consuming task of building a glochidial reference library for a particular study area. For published size information and discriminant functions to be useful, the degree to which glochidial size and shape varies within and among populations must be known. No studies have evaluated variation in glochidial size among populations across the range of a mussel species, but several studies have found low within-population variability in glochidial measurements (e.g., Zale and Neves 1982c, Bruenderman and Neves 1993). Our results corroborate this low within-population variability for the species we studied, with the exception of *Pleurobema decisum* for which glochidial size differed among subsets of individuals in the population. Such intraspecies variation in glochidial size would severely confound attempts to identify glochidia using morphometrics and DFA if not recognized and accounted for. This case underscores the need to assess sources of glochidial shell variation in the study area carefully.

Glochidial morphotypes of Pleurobema decisum

The existence of 2 distinct glochidial morphotypes of *P. decisum* is surprising. One other species of *Pleurobema*, *P. perovatum*, occurs in the Sipsey River (McCullagh et al. 2002). We were unable to obtain mature glochidia of *P. perovatum*, but this species is easily differentiated from *P. decisum* by distinctive shell morphology, habitat affinity, and conglutinate shape (Haag 2004, WRH, unpublished data). Divergent glochidial morphology could be explained by the existence of an unrecognized, cryptic species subsumed within *P. decisum*. We consider this explanation unlikely because there is no published or observational evidence of polytypy in shell morphology for this species in the western Mobile Basin despite extensive collecting and conchological investigation in the region during the last 100 y (J. D. Williams, US Geological Survey, personal communication). Likewise, we have seen no consistent patterns of shell variation among thousands of *P. decisum* examined during 10 y of field work in the Sipsey River. Furthermore, host use did not differ among a subset of individuals representing each morphotype (primary host = *Cyprinella venusta*, $n = 2$ for each morphotype, see Haag and Warren 2003). Within-population variation in *P. decisum* in the Sipsey River exists for body tissue and conglutinate color (orange and white morphotypes, Haag and Warren 2003) and fecundity (higher fecundity in larger females and differences in mean fecundity among sites, Haag and Staton 2003). However, within the small sample we examined, variation in these traits was not concordant with the occurrence of glochidial morphotypes.

To our knowledge, the existence of 2 distinct glochidial morphotypes has not been reported for any other mussel species. Bimodal distribution of conglutinate size or shape among individuals within a population has been reported for *Ptychobranchus fasciolaris* (Watters 1999) and *P. greeni* (Hartfield and Hartfield 1996, Haag and Warren 1997) and may be an adaptation for targeting different subsets of the host resource (Watters 1999). The ecological or phylogenetic significance of glochidial variation in *P. decisum* is unknown and its elucidation will require an expanded set of observations from the Sipsey River and across the range of this species.

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