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PHYLOGEOGRAPHY OF THE WABASH PIGTOE, *FUSCONAIA FLAVA* (RAFINESQUE, 1820) (BIVALVIA: UNIONIDAE)

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ABSTRACT

Reconstructing the phylogeographic patterns of widely distributed and common freshwater mussel species (Bivalvia: Unionidae) may provide insight into unionid evolution and speciation. The Wabash pigtoe, *Fusconaia flava*, is currently recognized as a single, polytypic species that is widely distributed and common throughout the Mississippi River drainage and parts of the Canadian Interior, Great Lakes and Gulf Coast drainages. Sequence analysis of the mitochondrial COI gene revealed two divergent (3.43%) clades. Clade A consisted of specimens located throughout the upper and lower Mississippi River drainage and in the Red River (Canada) and Lake Erie drainages and all *F. cerina* specimens. All haplotypes within clade A differed by three (0.55%) or fewer nucleotide substitutions from the most widely distributed and abundant haplotype, F1. Clade B, consisting of specimens located in the far western portion of the species' range, may comprise an undescribed species. There was no evidence of genetic differentiation among *F. flava* inhabiting headwater and intermediate-sized river localities of the Muskingum River system and large river localities of the nearby Ohio River. The divergence among *F. flava* haplotypes comprising clade A (0.18–1.10%) was similar to the divergence between the *F. cerina* haplotypes and the *F. flava* haplotypes comprising clade A (0–1.10%). This study illustrates the importance of accessing genetic diversity across the distribution of a polytypic species. Additional analyses based on a combination of morphology and genetics are needed to determine the taxonomic status of clade B and to strengthen our understanding of the relationship between *F. flava* and *F. cerina*.

INTRODUCTION

North America has the world's greatest diversity of freshwater mussels (Bivalvia: Unionidae) (Lydeard, Mulvey & Davis, 1996). This unique group of bivalves has a life cycle that involves an obligate, parasitic stage on the gills or fins of host fish species during early development (Hoggarth, 1999). The nearly 250 species that comprise the subfamily Ambleminae are particularly diverse in their morphology, habitat requirement and life history (Campbell *et al.*, 2005). Phylogenetic analyses are being used to complement morphology-based taxonomic classifications because of the tendency for evolutionary convergence and phenotypic plasticity in unionids (Lydeard *et al.*, 1996). Although there has been support for higher taxonomic levels (e.g. tribes), most of the genera in the subfamily Ambleminae, as currently defined, are polyphyletic (Campbell *et al.*, 2005).

Phylogenetic studies that incorporate a large number of taxa strengthen our understanding of unionid systematics as well as identify problematic taxa that warrant immediate attention. However, our current views of conservation status, distribution and general biodiversity assessment are ultimately tied to our understanding of unionids at the species level (Lydeard, Minton & Williams, 2000). Most of the studies that have examined genetic diversity at or below the species level in unionids have focused on imperiled species (Mulvey *et al.*, 1997; King *et al.*, 1999; Roe, Hartfield & Lydeard, 2001; Buhay *et al.*, 2002; Grobler *et al.*, 2006; Serb, 2006). Although data from these analyses can guide critical conservation efforts (Lydeard & Roe, 1998), reconstructing the large-scale phylogeographic patterns of widely distributed and common species may provide additional insight into the evolution and speciation of unionids (Mulvey *et al.*, 1997). For example, identifying the current level and distribution of intraspecific genetic variation in widespread species may reveal the extent to which past large-scale events (e.g. Pleistocene glaciations) impacted unionid evolution (Elderkin *et al.*, 2007).

The Wabash pigtoe, *Fusconaia flava* (Rafinesque, 1820), is currently recognized as a single, polytypic species that is widely distributed and common throughout the Mississippi River drainage and parts of the Canadian Interior, Great Lakes and Gulf Coast drainages (Oesch, 1984). *Fusconaia flava*, a member of the subfamily Ambleminae, is characterized by its strong posterior ridge and angulation. Shell obesity (shell width/shell length) has been associated with stream position; a less obese (compressed) form is found in headwater streams whereas a more obese (swollen) form is found in larger rivers (Watters, 1994). The compressed form enables a mussel to quickly burrow in a variety of substrata, a particularly advantageous strategy for the less stable conditions associated with headwater streams. The obese form enables a mussel to anchor itself into the more stable substrate of strong-flowing large rivers (Watters, 1994). Historically, these forms were thought to have comprised two distinct species: *F. flava* (headwater forms) and *F. undata* (larger river forms). However, a morphological cline of the compressed form downstream to the obese form was observed and the two species were united and recognized as a single species under the name of *flava* (Stansbery, 1983). This upstream–downstream variation in shell morphology conforms to Ortmann's Law of Stream Position (Ortmann, 1920). The genetic relationship among the various morphological forms of *F. flava* is unknown.

The Gulf pigtoe, *F. cerina* (Conrad, 1838), has been reported from the Gulf Coastal Plain streams and rivers in Alabama, Mississippi and Louisiana and persists in stable populations throughout its range (Williams *et al.*, 1993; Haag & Warren, 2003). *Fusconaia cerina* is currently recognized as the sister species to *F. flava* (Williams

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Table 1. Collection localities, sample sizes and mitochondrial COI haplotypes for *Fusconaia flava*, *F. cerina* and outgroup species.

Species	Locality number	River	Locality	OSU Museum number	Observed haplotype	Geographic coordinate (decimal degrees)	
F. flava	1	Muskingum River	Muskingum Co., OH	1980:0034	F1 ($n = 4$)	40.0917	−82.0136
–	2	Ohio River	Scioto Co., OH	1981:0111	F1 ($n = 2$), F10 ($n = 1$)	38.6242	−83.2240
–	3	Muskingum River	Washington Co., OH	1966:0067	F1 ($n = 2$)	39.5235	−81.5043
–	4	Walhonding River	Coshocton Co., OH	1967:0126	F1 ($n = 5$)	40.3598	−82.0879
–	5	Wills Creek	Guernsey Co., OH	1987:0464	F1 ($n = 3$)	40.1597	−81.5753
–	6	Ohio River	Clermont Co., OH	1984:0296	F1 ($n = 2$)	39.0207	−84.3168
–	7	Little Muskingum River	Monroe Co., OH	1987:0741	F1 ($n = 2$)	39.6205	−81.1607
–	8	Stillwater Creek	Tuscarawas Co., OH	1987:0710	F1 ($n = 4$)	40.3887	−81.3468
–	9	Little Scioto River	Scioto Co., OH	1987:0213	F1 ($n = 1$)	38.8536	−82.7886
–	10	Killbuck Creek	Wayne Co., OH	FC 2003	F1 ($n = 4$), F3 ($n = 1$)	40.8658	−82.0083
–	11	Tennessee River	Benton Co., TN	1993:0003	F1 ($n = 1$), F3 ($n = 1$)	36.0602	−87.9915
–	12	Salt River	Spencer Co., KY	1980:0103	F1 ($n = 2$)	38.0237	−85.4311
–	13	Osage River	Miller Co., MO	1964:0250	F9 ($n = 1$)	38.2323	−92.4566
–	14	Neosho River	Lyon Co., KA	1977:0001	F8 ($n = 2$)	38.3843	−96.1805
–	15	Strawberry River	Lawrence Co., AR	1980:0064	F1 ($n = 2$)	35.9862	−91.2818
–	16	Wolf River	Benton Co., MS	1998:0021	F5 ($n = 2$)	34.9058	−89.1450
–	17	Brown Creek	Rapides Parish Co., LA	1982:0023	F4 ($n = 2$)	31.2894	−92.7015
–	18	East Fork Chippewa River	Sawyer Co., WI	2000:0077	F3 ($n = 1$), F6 ($n = 1$)	45.8970	−91.0506
–	19	East Fork White River	Lawrence Co., IN	FC 2003	F7 ($n = 1$)	38.8250	−86.5146
–	20	Grand River	Ashtabula Co., OH	1968:0128	F2 ($n = 1$)	41.6495	−80.8694
–	21	Assiniboine River	Manitoba, Canada	FC 2002	F3 ($n = 1$), F13 ($n = 1$)	49.6994	−98.9005
–	22	Coal River	Kanawha Co., WV	1969:0242	F1 ($n = 2$)	38.3751	−81.8592
–	23	Spring River	Lawrence Co., AR	1978:0249	F1 ($n = 1$)	36.2054	−91.1719
–	24	Clear Creek	Fayette Co., TN	1999:0080	F5 ($n = 1$)	35.0150	−89.3719
–	25	Black River	Lawrence Co., AR	1980:0066	F11 ($n = 1$), F12 ($n = 1$)	36.1034	−91.0956
–	26	Mississippi River	Allamakee Co., IA	1981:0144	F3 ($n = 1$)	43.1470	−91.1774
–	27	White River	Jackson Co., AR	1980:0062	F12 ($n = 1$)	35.4740	−91.3608
F. cerina	28	Sipsey River	Green Co., AL	1986:0055	C1 ($n = 1$)	33.0539	−88.0392
–	29	Yellow Creek	Lowndes Co., MS	2000:0047	C2 ($n = 1$)	33.6206	−88.2674
–	30	Sipsey River	Tuscaloosa Co., AL	2000:0009	C2 ($n = 1$)	33.2733	−87.7544
F. ebena	–	Tombigbee River	Marengo Co., AL	1999:0070	–	–	–
L. ornata	–	–	–	GenBank	–	–	–

Specimens were collected from the Ohio State University Museum of Biological Diversity, Division of Molluscs (OSU) or field (FC).

Table 2. Taxa analysed and GenBank accession numbers for partial mitochondrial COI sequences.

Species	Accession no.	Reference
Elliptio crassidens (Lamarck, 1819)	DQ383428	D.C. Campbell *et al.*, unpubl.
Elliptio dilatata (Rafinesque, 1820)	AF156507	Graf & O'Foighil (2000)
Fusconaia barnesiana (Lea, 1838)	AY613822	Campbell *et al.* (2005)
Fusconaia cerina 1 (Conrad, 1838)	AY049522	Roe & Lydeard (1998)
Fusconaia cerina 2	AY613823	Campbell *et al.* (2005)
Fusconaia cor (Conrad, 1834)	AY654997	Campbell *et al.* (2005)
Fusconaia cuneolus (Lea, 1840)	AY654998	Campbell *et al.* (2005)
Fusconaia escambia (Clench & Turner, 1956)	AF232816	Lydeard *et al.* (2000)
Fusconaia ozarkensis (Call, 1887)	-	D.C. Campbell, pers. comm.
Fusconaia masoni (Conrad, 1834)	EF619921	C. Morrison, pers. comm.
Fusconaia subrotunda (Lea, 1831)	AY613824	Campbell *et al.* (2005)
Plethobasus cyphus (Rafinesque, 1820)	AY613828	Campbell *et al.* (2005)
Pleurobema beadleanum (Lea, 1861)	DQ383429	D.C. Campbell *et al.*, unpubl.
Pleurobema chattanoogaense 1 (Lea, 1858)	AY613829	Campbell *et al.* (2005)
Pleurobema chattanoogaense 2	DQ383430	D.C. Campbell *et al.*, unpubl.
Pleurobema clava (Lamarck, 1819)	AF231754	Hoeh & Bogan (2000)
Pleurobema collina (Conrad, 1834)	AY613830	Campbell *et al.* (2005)
Pleurobema cordatum 1 (Rafinesque, 1820)	AY613831	Campbell *et al.* (2005)
Pleurobema cordatum 2	EF619917	C. Morrison, pers. comm.
Pleurobema cordatum 3	EF619918	C. Morrison, pers. comm.
Pleurobema decisum (Lea, 1831)	AY613832	Campbell *et al.* (2005)
Pleurobema furvum (Conrad, 1834)	AY613833	Campbell *et al.* (2005)
Pleurobema georgianum (Lea, 1841)	AY613834	Campbell *et al.* (2005)
Pleurobema gibberum (Lea, 1838)	AY613835	Campbell *et al.* (2005)
Pleurobema hanleyianum (Lea, 1852)	AY613836	Campbell *et al.* (2005)
Pleurobema oviforme (Conrad, 1834)	AY613837	Campbell *et al.* (2005)
Pleurobema perovatum (Conrad, 1834)	AY613838	Campbell *et al.* (2005)
Pleurobema plenum 1 (Lea, 1840)	EF619919	C. Morrison, pers. comm.
Pleurobema plenum 2	EF619920	C. Morrison, pers. comm.
Pleurobema pyriforme (Lea, 1857)	AY613839	Campbell *et al.* (2005)
Pleurobema rubellum (Conrad, 1834)	AY613840	Campbell *et al.* (2005)
Pleurobema rubrum (Rafinesque, 1820)	AY613841	Campbell *et al.* (2005)
Pleurobema sintoxia 1 (Rafinesque, 1820)	AF156508	Graf & O'Foighil (2000)
Pleurobema sintoxia 2	AF156509	Graf & O'Foighil (2000)
Pleurobema stabile (Lea, 1861)	AY613842	D.C. Campbell *et al.*, unpubl.
Pleurobema strodeanum (Wright, 1898)	AY613843	Campbell *et al.* (2005)
Pleurobema taitianum (Lea, 1834)	AY613844	Campbell *et al.* (2005)
Quincuncina berkei 1 (Walker, 1922)	AF232804	Lydeard *et al.* (2000)
Quincuncina berkei 2	AF232802	Lydeard *et al.* (2000)

RESULTS

Nucleotide sequence data of 547-bp in length were obtained for a fragment of the COI gene for all *Fusconaia flava*, *F. cerina*, '*F.*' *ebena* and *Lampsilis ornata* specimens. There were 113 variable sites and 45 parsimony-informative sites. Of the 113 variable sites, 10 were first position, none were second position and 103 were third position. The transition to transversion substitution ratio was 4.9. No insertions or deletions were observed. Translation of codons into amino acids resulted in three substitutions, one in *F. cerina* (locality 28) and two in '*F.*' *ebena*. Representative *F. flava*, *F. cerina* and '*F.*' *ebena* sequences were deposited in GenBank (DQ298524–DQ298539).

Thirteen haplotypes were identified among the 58 *F. flava* specimens (Fig. 1). The F1 and F3 haplotypes were the most widely distributed. All *F. flava* haplotypes differed from F1 by three (0.55%) or fewer nucleotide substitutions, with the exception of F8, F9 and F10. The F8 and F9 haplotypes were found in the Neosho River, KS (Arkansas River drainage; locality 14) and Osage River, MO (Missouri River drainage; locality 13), respectively. The F8 and F9 haplotypes differed from F1 by 19 (3.47%, SE = 0.0077) and 16 (2.93%, 0.0072) substitutions. The F10 haplotype was found in one specimen from the Ohio River (locality 2). This haplotype differed from F1 by 25 nucleotide substitutions (4.57%, 0.0084). The two *F. cerina* haplotypes (C1 and C2) differed from the F1 haplotype by three (0.55%, 0.0031) and two (0.37%, 0.0025) nucleotide substitutions, respectively. The '*F.*' *ebena* and *L. ornata* haplotypes differed from *F. flava* (F1) by 68 (12.43%, 0.0135) and 64 (11.70%, 0.0133) nucleotide substitutions, respectively.

Fusconaia flava *in the Muskingum River system and the Ohio River*

There was little detectable COI variation among the *F. flava* specimens inhabiting the headwater and intermediate-sized river localities of the Muskingum River system (localities 1, 3, 4, 5, 8 and 10) and nearby large river localities of the Ohio

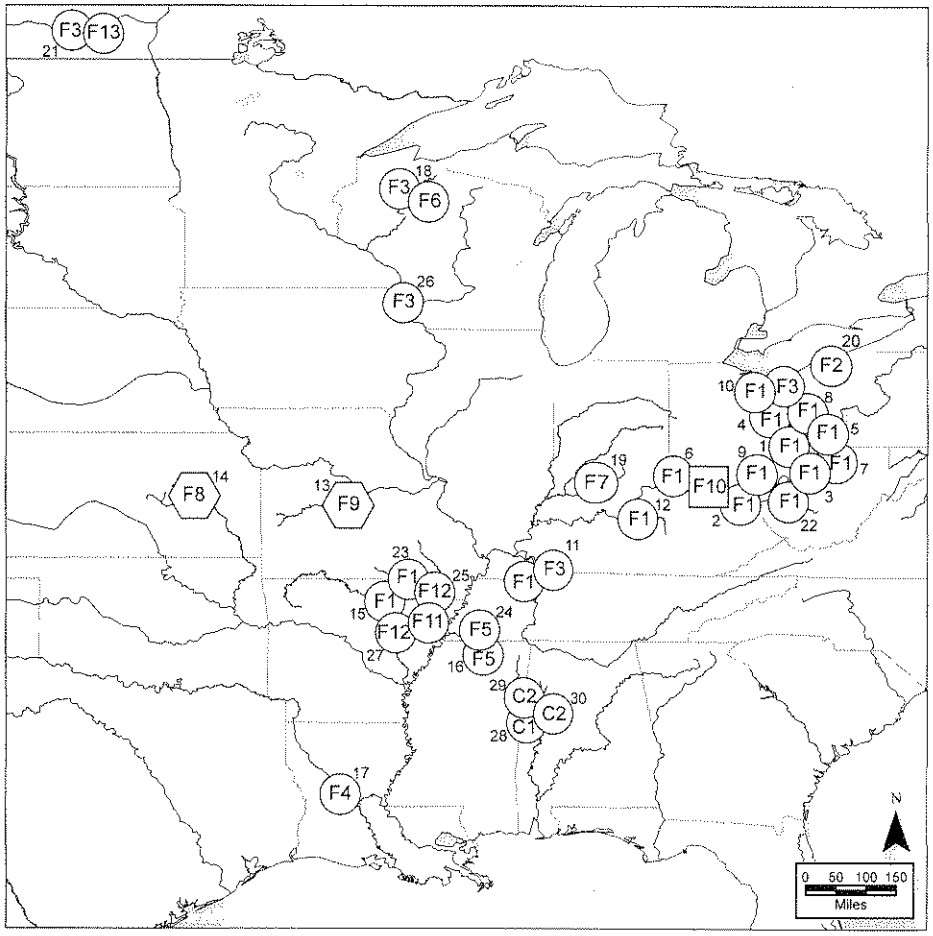

Figure 1. Map of *Fusconaia flava* (F) and *F. cerina* (C) mitochondrial COI haplotypes and localities. The major clades are represented by the following symbols: circles, clade A; hexagons, clade B; squares, clade C. The locality number is located next to each haplotype symbol. See Table 1 for details on localities.

River (localities 2 and 6). The AMOVA revealed the molecular variance attributed to differences among drainage groups (% total variation = 16.13; $\Phi_{CT} = 0.16133$) was relatively small and not significant ($P = 0.067$). One specimen from the Killbuck Creek locality (headwater stream of the Muskingum River system) contained the F3 haplotype. All other specimens in the Killbuck Creek locality and all other localities of the Muskingum River system contained the F1 haplotype. The F1 haplotype was also found in both of the Ohio River localities.

Phylogenetic analysis

The neighbour-joining (NJ) analysis based on 547-bp sequence data supports three clades within *F. flava*. The results of this analysis are not shown since the same *F. flava* clades were recovered in the NJ analysis that was based on 484-bp sequence data (see below). Clade A contains haplotypes F1, F2, F3, F4, F5, F6, F7, F11, F12, F13 and the *F. cerina* haplotypes C1 and C2, clade B contains haplotypes F8 and F9, and clade C contains haplotype F10. There was more differentiation among the haplotypes comprising clade B (1.28%, 0.0046) than among the haplotypes comprising clade A (0.18–1.10%). An AMOVA revealed that a large proportion of molecular variance was attributable to differences among clades (% total variation = 83.10; $\Phi_{CT} = 0.83101$; $p = 0.005$). The variance attributed to differences among population within clades (% total variation = 4.08%; $\Phi_{SC} = 0.24121$, $P = 0.001$) and within populations (% total variation = 12.82; $\Phi_{ST} = 0.87177$, $P = 0.001$), although comparatively low, was also significant. Similar to the NJ analysis, the MSN indicated three major groups within the *F. flava* samples (Fig. 2). The genetic distance values calculated for clades A and B (3.43%, 0.0069), B and C (4.57%, 0.0084) and A and C (4.66%, 0.0084) were relatively high.

The NJ analysis of the *F. flava* and *F. cerina* haplotypes with COI sequences from other Pleurobemini species indicates the uniqueness of the F8 and F9 haplotypes (Fig. 3). The F10 haplotype was most similar to two *P. cordatum* COI sequences. The NJ tree closely follows the phylogenetic trees presented in Campbell *et al.*, 2005 in regards to the species groupings, particularly within *Fusconaia* and *Pleurobema*. The low support for the basal nodes is not surprising given the small sequence data set. The results suggest *Pleurobema* is paraphyletic, where the *P. sintoxia*, *P. rubrum* and *P. cordatum* group share a common ancestor with the major *Fusconaia* group. In addition, mean pairwise comparisons of the haplotypes comprising clades A, B and C to the *Q. burkei* and *F. escambia* sequences revealed sequence divergence comparable to the interspecific genetic divergence reported for unionids (13.09%, Roe & Lydeard, 1998; 3.81–16.42%, King *et al.*, 1999; 3.65–15.35%, Serb *et al.*, 2003): clade A to *Q. burkei* (4.40%, 0.0089) and *F. escambia* (8.36%, 0.0140), clade B to *Q. burkei* (4.39%, 0.0087) and *F. escambia*

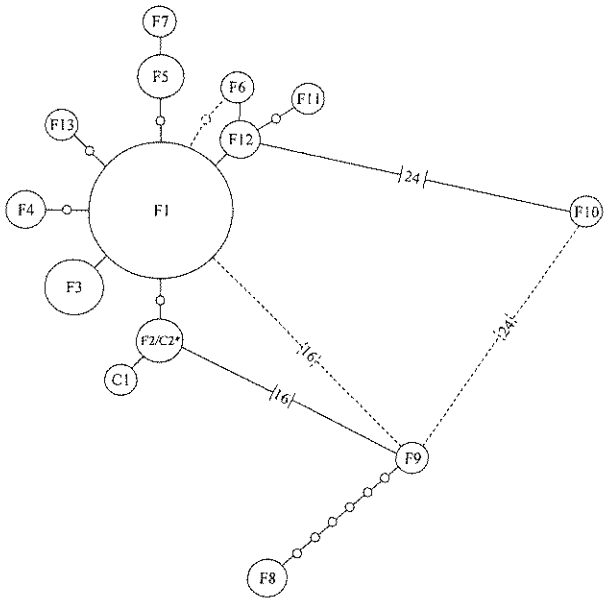

Figure 2. Minimum-spanning network based on *Fusconaia flava* (F) and *F. cerina* (C) mitochondrial COI haplotypes. Small circles indicate intermediate haplotypes. Lines connecting two circles represent a single base-pair difference between the two haplotypes, except when indicated by numbers. Dashed lines indicate alternative most parsimonious connections. *The F2 haplotype was identical to the C2 haplotype.

(8.48%, 0.0141), clade C to *Q. burkei* (6.14%, 0.0115) and *F. escambia* (9.65%, 0.0155).

DISCUSSION

Fusconaia flava *in the Muskingum River system and the Ohio River*

Based on sequence analysis of 547-bp from the mitochondrial COI gene, there was no evidence of genetic differentiation among *Fusconaia flava* inhabiting headwater and intermediate-sized localities of the Muskingum River system and large river localities of the Ohio River. The AMOVA results suggest that these *F. flava* are not genetically isolated. All but two specimens possessed the F1 haplotype (Table 1). The use of microsatellites or a faster evolving mitochondrial gene could be used to further examine the genetic relationship between *F. flava* specimens inhabiting headwater and large river localities within the same river drainage. Male mitochondrial DNA, which evolves much faster than female mitochondrial DNA (Liu, Mitton & Wu, 1996), may also be utilized to reconstruct within drainage phylogeographic structure (Curole, 2004; Krebs, 2004).

Phylogeography of Fusconaia flava

Clade A consisted of specimens found throughout the upper and lower Mississippi River drainage and in the Lake Erie and Red River drainages (Fig. 1). The two most widely distributed and most abundant haplotypes in clade A, F1 and F3, differed by only one substitution (0.18%, 0.0018). The level of divergence among haplotypes comprising clade A (0.18–1.10%) was higher than what was documented for *Amblema plicata* (0.3% COI divergence, Elderkin *et al.*, 2007). Moreover, all haplotypes within clade A differed by three (0.55%) or fewer mutations from F1 (Fig. 2). This starburst pattern is suggestive of widespread species having originated from a small number of

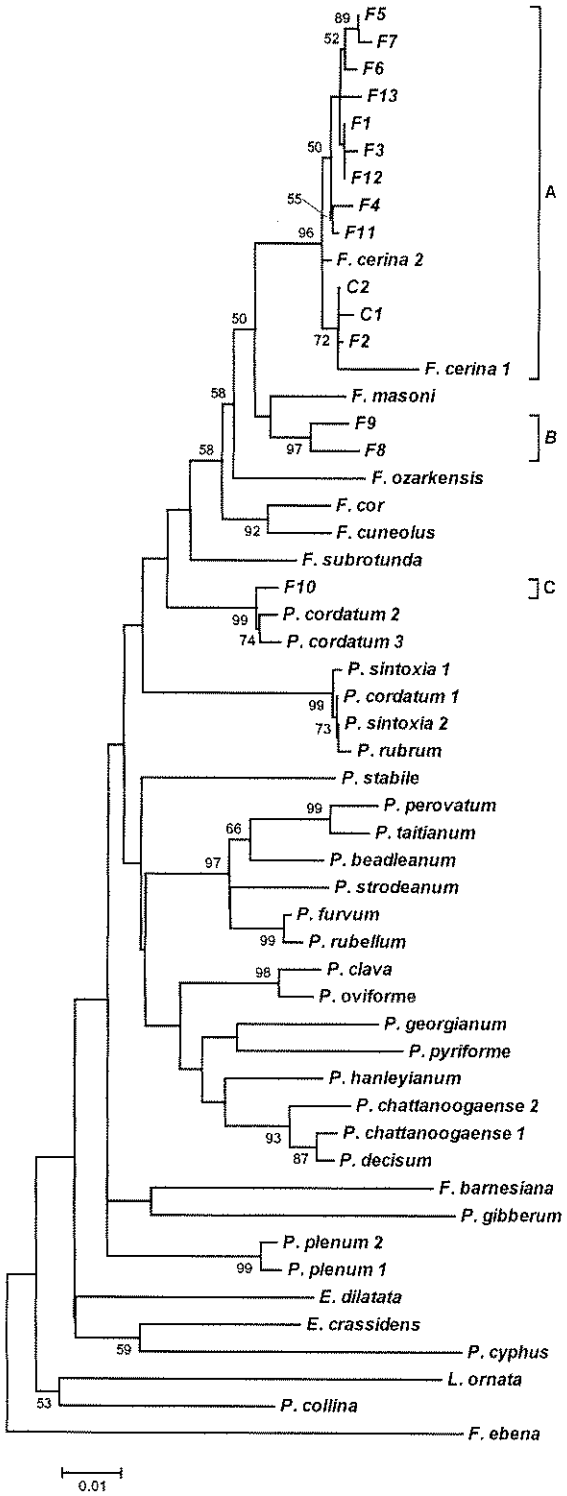

Figure 3. Neighbour-joining tree based on Kimura-two-parameter distances among mitochondrial COI haplotypes (484 nucleotides) for *Fusconaia flava* (F), *F. cerina* (C) and other Pleurobemini species. The major *F. flava* clades are represented by A, B and C. *Lampsilis ornata* and '*F.*' *ebena* were used as outgroup species. Numbers after the species name reflects multiple individuals of some of the species. The bootstrap values (reported as a percentage of 10,000 replicates) supporting each node are indicated; only values greater than 50% are shown. Scale bar represents genetic distance. See Table 2 for species information.

founding individuals (Avise, 2000). Although many phylogenetic studies of North American aquatic species have indicated that speciation events occurred prior to the Pleistocene glaciations (Mayden, 1988; Strange & Burr, 1997; Near *et al.*, 2003), the Pleistocene glaciations are still thought to have significantly impacted intraspecific phylogeographic structuring through a combination of geographic isolation of fragmented populations and reducing genetic variation (Hewitt, 1996). The presence of these *F. flava* haplotypes in localities that were not directly impacted by glaciation (Tennessee, Kentucky and Arkansas) and areas that were (Southern Canada, Wisconsin, Iowa and parts of Ohio) (Strange & Burr, 1997) suggests a postglacial expansion northwards from southern refugia. The repeated recolonization of previously glaciated areas may have reduced genetic variation in the northern species of black basses (genus *Micropterus*) from repeated founder-flush cycles (Near *et al.*, 2003). This model describes the phylogeographic pattern of the more northern populations of *F. flava*. Similarly, *A. plicata* exhibited lower genetic diversity within populations inhabiting previously glaciated regions than within populations inhabiting regions unaffected by the Pleistocene glaciers (Elderkin *et al.*, 2007).

The F3 and F13 haplotypes were found in the only locality representing the Red River drainage (northern United States/southern Canada; locality 21). The F3 haplotype is widespread whereas the F13 haplotype is unique to the Assiniboine River locality. Temporary postPleistocene connections between the Red River and the upper Mississippi River drainages have been proposed (Graf, 1997). Given that the F3 haplotype is also found outside of the Red River drainage, the presence of F3 in this locality indicates a relatively recent migration from the upper Mississippi River drainage.

Two substitutions (0.37%, 0.0025) distinguished the Lake Erie drainage haplotype (F2) from F1, the most widely distributed haplotype in this study. Johnson (1980) suggested that melt waters produced from wasting glaciers during the latter part of the Wisconsinan episode allowed for the migration of unionoids (via host fish) upstream to the Great Lakes drainage from southern refugia. Once the water levels fell after the glacial retreats, the basins took on their present configurations and consequently limited the dispersal of aquatic organisms across major divides. A refugium from which ancestors of the Grand River haplotype originated may have existed during the Pleistocene in the central and/or southern Mississippi River drainage, since these areas were not directly impacted by glacial events (Strange & Burr, 1997). The expansion of *F. flava* into the Great Lakes drainages was likely a continuation of its postglacial expansion from the northern Mississippi River drainage, as evidence by its low divergence from other northern haplotypes F1 and F3. Limited genetic variation in Lake Erie drainage populations of unionids has been documented (Krebs, Vlasceanu & Tevesz, 2003; Elderkin *et al.*, 2007).

The specimens in Brown Creek (Louisiana), the most southern *F. flava* locality in this study (Fig. 1), contained a haplotype (F4) that differed by two substitutions (0.37%, 0.0025) from the more northern distributed F1. Brown Creek has only recently become part of the Gulf Coast system. Recent construction of levees in the Red River (circa 70 years ago) diverted the Brown Creek drainage (via Bayou Rapides) from the Red River drainage to the Bayou Boeuf drainage (Gulf Coast system) (Johnson & Brown, 2000). Although F4 is unique to the Brown Creek locality, it is difficult to estimate the origin of *F. flava* into this particular locality because of the recent change in drainage patterns. Individuals located further upstream in the Red River drainage or even in nearby Gulf Coast drainages may also possess the F4 haplotype. The specimens in the two Wolf River drainage localities (lower Mississippi River drainage) contained a haplotype (F5) that was closely related to F7, a haplotype found in the East Fork of the White River locality (upper Mississippi River drainage). Haplotypes similar to F5 and F7 may be distributed throughout the Mississippi River drainage.

The haplotypes comprising clade B, F8 and F9, are distinct from the *F. flava* haplotypes and the other *Fusconaia* species (Fig. 3). The divergence between the western clade B and the more eastern clade A (3.43%) indicates the presence of a long-term genetic barrier. The lack of glaciation in the western portion of the species' range (Oesch, 1984) during the Pleistocene and the relatively unchanged river systems in this region since the Pliocene, specifically the Old Missouri River (Strange & Burr, 1997), indicates the potential for a western glacial refugium for *F. flava*. Moreover, the two most western localities in this study are not directly connected; locality 14 (Neosho River) is in the Arkansas River drainage whereas locality 13 (Osage River) is in the Missouri River drainage. Two possible scenarios can explain the presence of two related, yet divergent (1.28%, 0.0048) haplotypes in river systems that are not currently connected; either past connections existed between these two drainages or they shared a common ancestor that inhabited the Mississippi River and migrated to their current positions. Pflieger (1971) reviewed the ancestral drainage patterns of the central plains. The Flint Hills of Kansas comprised a major divide, where streams west of the divide flowed southward into Oklahoma and streams east of the divide flowed eastward through Missouri. During Kansan time (~0.7–0.13 Ma), a temporary meltwater connection resulting from the western lobe of the Kansas ice sheet was established across the divide. This historical connection would have allowed the migration of *F. flava* (via host fish) across the divide. A similar pattern of divergence was observed between the Eastern Highlands and Ouachita Highlands populations of the crystal darter, *Crystallaria asprella* (Morrison *et al.*, 2006).

Similar to the haplotypes comprising clade B, the haplotype comprising clade C, F10, is distinct from the *F. flava* haplotypes and the other *Fusconaia* species (Fig. 3). However, the F10 haplotype closely resembles two *P. cordatum* COI sequences. The most likely explanation for the divergence of clade C from the other *F. flava* clades is that the specimen containing the F10 haplotype is actually *P. cordatum*. Identifying unionids can be difficult given the tendency for evolutionary convergence and phenotypic plasticity (Lydeard *et al.*, 1996). A second possibility is that there is cryptic diversity within *F. flava* and that the specimen containing the F10 haplotype represents a distinct evolutionary lineage (i.e. species). Analyses utilizing life history or ecology would be needed to explore the possibility that this specimen comprises a separate species.

Fusconaia flava *and* F. cerina

These data suggest *F. flava*, as currently recognized, is not monophyletic (Fig. 3). The sequence divergence among *F. flava* haplotypes comprising clade A (0.18–1.10%) was similar to the divergence observed between the *F. cerina* haplotypes and the *F. flava* haplotypes comprising clade A (0–1.10%). These values fall in between the intraspecific genetic distance values that have been reported for the same fragment of COI for other unionid species (2.62% for *Potamilus* species, Roe & Lydeard, 1998; 0.17–0.35% for *Lasmigona subviridis* populations, King *et al.*, 1999; 0.3% for *A. plicata*, Elderkin *et al.*, 2007) and well below the interspecific values that have been documented for unionids (13.09%, Roe & Lydeard, 1998; 3.81–16.42%, King *et al.*, 1999; 3.65–15.35%, Serb *et al.*, 2003). The low genetic divergence between the *F. cerina* haplotypes and the *F. flava* haplotypes comprising clade A is interesting given that the *F. flava* complex in the Mobile-Alabama-Tombigbee

system is currently recognized as *F. cerina* (Williams *et al.*, 1993). Based on the minimal genetic divergence observed between the *F. cerina* haplotypes and the *F. flava* haplotypes comprising clade A, these *F.* '*cerina*' specimens in the Yellow Creek (Mississippi) and Sipsey River (Alabama) localities may actually be *F. flava*.

The presence of a historical connection between the Tennessee River drainage and the Mobile Basin via an Appalachian river has been debated (Starnes & Etnier, 1986). Recent geological analysis indicates that, if such a connection occurred, it was formed sometime during the Paleozoic and was severed by the Early Cenozoic (Mills & Kaye, 2001). However, the low genetic divergence between the *F. cerina* haplotypes and the nearby *F. flava* haplotypes supports a Pleistocene origin for the *F. cerina* specimens in this study. Perhaps geologically recent stream capture of a tributary transferred *F. flava* from the lower Tennessee River drainage or the lower Mississippi River drainage to the nearby river systems of the Gulf Coastal Plains. The migration of *F. flava* to the Gulf Coast systems may have also been possible during the sea level fluctuations of the Pleistocene, a mechanism that has been proposed for the migration of other unionid species between Gulf Coast drainages (Roe *et al.*, 2001). More recently, *F. flava* could have migrated from the lower Tennessee River to the Tombigbee River drainage via the Tennessee–Tombigbee waterway.

CONCLUSION

Because of the tendency for morphological convergence and phenotypic plasticity in unionids, an assessment of genetic diversity in currently recognized polytypic species might reveal highly divergent populations or even cryptic species. A relatively high level of divergence in a fragment of the mitochondrial COI gene for *F. flava* has been described in the present work. Future studies that utilize analyses based on a combination of morphology and genetics (nuclear and mitochondrial DNA) are needed to determine the taxonomic status of clade B and to strengthen our understanding of the phylogeographic structure of *F. flava*. In addition, low levels of genetic variation between *F. cerina* and *F. flava* have been described. Specimens of *F. cerina* representing localities throughout its range and *F. flava* from nearby drainages should be analysed to determine if *F. cerina* might actually be *F. flava*.

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