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We are submitting herewith a dissertation written by Christopher Eugene Skelton entitled "Phylogenetic Systematics and Zoogeography of the Nearctic Members of the Minnow Genus *Phoxinus* (Cyprinidae: Cypriniformes) with a Description of a New Species from the Tennessee River Drainage, Tennessee". We have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

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PHYLOGENETIC SYSTEMATICS AND ZOOGEOGRAPHY OF THE NEARCTIC MEMBERS OF THE MINNOW GENUS *PHOXINUS* (CYPRINIDAE: CYPRINIFORMES) WITH A DESCRIPTION OF A NEW SPECIES FROM THE TENNESSEE RIVER DRAINAGE, TENNESSEE

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ABSTRACT

Questions regarding the taxonomy and systematics of the Nearctic *Phoxinus* (Cyprinidae: Cypriniformes) were investigated in this study. First, a new member of the genus, Laurel Dace, was described from the Tennessee River drainage, Tennessee. This distinctive minnow is one of seven described North American *Phoxinus* species and one of four that occurs in Tennessee. It differs from all congeners by having the combination of two uninterrupted black lateral stripes, and development of black pigment on the underside of the head of nuptial males. Based on coloration and tuberculation patterns, the Laurel Dace is hypothesized to be part of a monophyletic group comprised of *P. oreas*, *P. tennesseensis*, and *P. cumberlandensis*. The Laurel Dace appears to be very rare; it is currently known from only six localities on the Walden Ridge portion of the Cumberland Plateau. Human activities such as coal mining, agriculture, and timbering threaten its future existence.

A second objective was to assess the phylogenetic relationships of the Nearctic *Phoxinus*. Five data sets were employed to this end. The first consisted of allele frequencies calculated from gene products resolved with starch gel electrophoresis, and the second of 30 morphological characters. The third was a subset of the morphological data set exclusive of all characters construed as being related to feeding. The fourth and fifth data sets employed a combination of the allele frequency data and the two morphological data sets, respectively. There was little congruence between results generated with morphological data sets and the discretely coded allozyme data set. The

latter data set produced poorly resolved results and was not useful for phylogenetic analysis. However, a UPGMA phenogram based on Rogers genetic similarity was highly congruent with a tree produced from the morphological data set exclusive of feeding characters. The relationships indicated by the UPGMA phenogram and the morphological data set were both considered viable hypotheses. Strong evidence supported a clade consisting of *P. oreas*, *P. tennesseensis*, *P. cumberlandensis*, and Laurel Dace. Of the remaining Nearctic *Phoxinus*, *P. erythrogaster* is sister to this clade or sister to *P. eos*. *Phoxinus neogaeus* is the basal member of the genus. Distributions of *Phoxinus* species are hypothesized to be influenced in large part by Pleistocene glaciation and stream capture events.

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PART 1: INTRODUCTION

GENERAL BACKGROUND

In North America, the cyprinid genus *Phoxinus* consists of six formally described species and at least one undescribed form. The genus is hypothesized to have members in Eurasia (Etnier and Starnes, 1993), the only cyprinid genus with this distinction. The Eurasian Minnow (*Phoxinus phoxinus*) is hypothesized to be the sister species to all of the Nearctic members of the genus (Joswiak, 1980). The following discussion will relate to the Nearctic region unless otherwise stated.

Phoxinus species are widespread throughout the central United States and southern Canada, and as far south as Mississippi. With few exceptions, *Phoxinus* species inhabit waters that eventually flow into the Mississippi River. However, *Phoxinus oreas* lives in Atlantic Slope drainages in Virginia, and a few populations of *P. neogaeus* inhabit rivers that flow to the Arctic Ocean and Hudson Bay. The six named species are *Phoxinus cumberlandensis*, *P. eos*, *P. erythrogaster*, *P. neogaeus*, *P. oreas*, and *P. tennesseensis*. A seventh species, the Laurel Dace, has not been formally described, but is widely accepted as a valid species (Skelton, 1995; Warren et al., 2000).

The daces, as these minnows are commonly called, are small colorful fishes that reach a maximum length of about 90 mm. Most species in the genus live in cool headwater streams, but the northern forms, *P. neogaeus* and *P. eos*, thrive in lentic waters such as beaver ponds and boggy lakes. Spawning typically takes place in late spring and early summer (April-June). All of the southern forms preferentially spawn over the nests of other minnows (Etnier and Starnes, 1993; Jenkins and Burkhead, 1994). These nests,

built by such species as Stoneroller (*Campostoma* spp.), River Chub (*Nocomis* spp.), and Creek Chub (*Semotilus atromaculatus*), provide clean, well aerated substrate for egg development. Because *P. neogaeus* and *P. eos* primarily live in lentic waters, they spawn in filamentous algae, under logs, and in woody debris (Stasiak, 1972, 1977). Some *Phoxinus* individuals mature by their first spring and all are mature by year two. Three year classes are typically found within a given population.

Except for *P. neogaeus* and the Laurel Dace, all of the North American *Phoxinus* feed primarily on vegetable matter. Common items found in *Phoxinus* intestines are diatoms, filamentous algae, and sand grains (Settles and Hoyt, 1976; Starnes and Starnes, 1981). The main food sources of *Phoxinus neogaeus* appear to be aquatic insects and molluscs (Stasiak, 1972), whereas Laurel Dace prefer aquatic insects.

Phoxinus erythrogaster, *P. eos*, *P. oreas*, and *P. neogaeus* were described as species in the 1800s (Rafinesque, 1820; Cope, 1861, 1867, 1868). *Phoxinus cumberlandensis* and *P. tennesseensis* were described fairly recently (Starnes and Starnes, 1978; Starnes and Jenkins, 1988), and the Laurel Dace was only recognized as a new species in 1994 (Skelton, 1995). Laurel Dace were first collected by Tennessee Valley Authority biologists in 1976 (C. Saylor, TVA, pers. comm.), but were not thought to be unique until 1990 when Dr. D. Etnier (University of Tennessee ichthyologist) examined the only extant specimens (UT 44.4789). Breeding individuals collected in 1994 exhibited a unique combination of colors, thus providing strong evidence that the fish was undescribed. Because the Laurel Dace was suspected to be very rare, a status survey was funded by the US Fish and Wildlife Service to determine the extent of its range.

Collection of data to be used in a formal description of the this fish was also initiated.

Studies of relationships among some of the Nearctic *Phoxinus* plus the Eurasian Minnow (*Phoxinus phoxinus*) have been conducted over the past 25 years (eg. Mahy, 1975; Joswiak, 1980; Howes, 1985). Most of these studies utilized osteological characters to elucidate relationships; however, Joswiak (1980) employed starch gel electrophoresis. These works supported the hypothesis that the Nearctic members of the genus are derived from Eurasian stock. Recent osteological work by Coburn and Cavender (1992) and genetic studies by Simons and Mayden (1998) provide further support that Eurasian and Nearctic *Phoxinus* are closely related. Although the above cited studies support a close relationship between the Eurasian and Nearctic *Phoxinus*, there is no published work that uses genetic data to investigate interspecific relationships among the Nearctic members of the genus. Two new *Phoxinus* species have been discovered since Joswiak (1980) completed his dissertation, and gaining an understanding their relatedness to the remainder of the genus provides additional evidence regarding the evolution of the fish fauna of the upper Tennessee River drainage.

OBJECTIVES

Part 2 of this dissertation presents a description of the Laurel Dace. Portions of the work involved with the description included extensive surveys to determine the species range, identification of critical habitats, and suggestions for actions to recover the species. In Part 3, I examine the relationships of the seven Nearctic *Phoxinus* using

traditional morphological characters and characters derived from starch gel electrophoresis. Analyses were performed on each data set independently and then on data sets combining morphological and genetic characters. Using the phylogeny (ies) generated with these methods, I present hypotheses regarding the geographic distribution of the Nearctic *Phoxinus*. Part 4 is a summary of the findings presented in Parts 2 and 3.

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PART 2: A NEW DACE OF THE GENUS *PHOXINUS* (CYPRINIDAE: CYPRINIFORMES) FROM THE TENNESSEE RIVER DRAINAGE, TENNESSEE

INTRODUCTION

The North American members of the cyprinid genus *Phoxinus* are widely distributed throughout the Mississippi and Ohio river basins of the northeastern, central, and southeastern United States (Etnier and Starnes, 1993). The most northerly species, *Phoxinus neogaeus*, can be found as far north as the Mackenzie River system of the Northwest Territories (Scott and Crossman, 1973) and a single species, *P. oreas*, inhabits rivers and streams draining to the Atlantic Ocean.

There are six described North American *Phoxinus* species (Robins et al., 1991), and at least one undescribed species (Skelton, 1995). Of the six named species, *P. erythrogaster, P. tennesseensis*, and *P. cumberlandensis* are native to Tennessee (Etnier and Starnes, 1993). The wide ranging *P. erythrogaster* is found throughout much of Middle and West Tennessee, whereas *P. tennesseensis* and *P. cumberlandensis* are confined to upper portions of the Tennessee and Cumberland river drainages, respectively. The last two species have restricted ranges, and *P. cumberlandensis* is federally listed as threatened.

A new *Phoxinus* was first collected in July 1976 by the Tennessee Valley Authority (TVA) in Horn and Laurel branches of Rock Creek on the Walden Ridge portion of the Cumberland Plateau (C. Saylor, TVA, pers. comm.). Because non-breeding individuals of the new species look like the common *P. erythrogaster*, the collection remained in storage at TVA until 1990. At that time, the specimens were

donated to The University of Tennessee Research Collection of Fishes (UT). When Dr. David Etnier (UT) examined the specimens, he remarked that the dace probably represented an undescribed species (D. Etnier, pers. comm.). Subsequent collections of breeding individuals revealed a unique combination of breeding colors that provided strong evidence the fish was undescribed (Skelton, 1995). Because the form was known from only two localities, a status survey was funded by US Fish and Wildlife Service (USFWS) to determine the species' distribution. During the next three years, over 100 sites on Walden Ridge and surrounding areas were sampled. As a result of these surveys, four additional populations were discovered.

Herein, the new dace is formally described. The new species has nuptial coloration characteristics intermediate between its geographically proximate congeners, *P. tennesseensis* and *P. erythrogaster*, and is thus compared with and differentiated from them.

MATERIALS AND METHODS

Specimens were collected using a backpack electroshocker, minnow seines (1.8 mm mesh), and galvanized minnow traps baited with cat food. One hundred twelve localities were collected between 1993 and 1997 on or near the Walden Ridge portion of the Cumberland Plateau (Figure 2-1*, Appendix 2-B). Scale counts were made on

*All tables and figures in the appendix.

specimens from all known localities. Morphometric measurements were taken from representatives from all populations except Moccasin Creek. Methods of counts and measurements generally follow Hubbs and Lagler (1964) except that gape width was measured at the corner of the mouth, and the fleshy margin of the operculum was excluded from head length. Additionally, transverse scale rows were counted diagonally from the third anal ray to the third dorsal ray because of the difficulty met when counting the very small scales near the origin of the dorsal and anal fins. In order to design a truss (Humphries et al., 1981), several measurements that were not described by Hubbs and Lagler (1964) were used in this analysis: occiput to pectoral fin origin, dorsal fin origin to pectoral fin origin, dorsal fin origin to pelvic fin origin, dorsal fin origin to anal fin origin, end of dorsal fin base to pelvic fin origin, end of dorsal fin base to anal fin origin, end of dorsal fin base to anal fin end, end of dorsal fin base to caudal fin base, anal fin origin to pelvic fin origin, pelvic fin origin to pectoral fin origin, pectoral fin origin to isthmus, snout to isthmus, pectoral fin origin to pectoral fin origin (breast width), body width below dorsal fin origin, and body width above anal fin origin. Measurements were made using a needle-point digital caliper and were estimated to the nearest 0.1 mm. In order to reduce the confounding effects of sexual dimorphism, among-species shape comparisons were made using only adult males. Overall size effects were minimized using sheared principal components analysis (SPCA, Bookstein et al., 1985; Humphries et al., 1981) with a program written for SAS by D. Swofford (privately distributed). SPCA was also used to assess shape dimorphism between non-breeding males and females and

intraspecific shape variation among males. A two sample t-test (PROC TTEST) was used to assess sexual dimorphism of distance measurements and ANOVA was used to assess among species differences of distance measurements. Statistical analyses were performed with SAS (vers 6.12, 1996, Cary, NC). Institutional abbreviations follow Leviton et al. (1985) and Leviton and Gibbs (1988).

Vertebral counts were made from cleared and stained specimens with the Weberian apparatus counted as four elements and the hypural as one element. Pharyngeal tooth formulae, shape of the pharyngeal arch, and basioccipital shape were described from skeletal material prepared by macerating specimens in water.

RESULTS

Phoxinus sp. new species

Laurel Dace

Figure 2-2

Phoxinus sp. cf. *erythrogaster* Etnier and Starnes, 1993, 246

Phoxinus sp. cf. *tennesseensis* Warren et al., 1997, 146

Holotype.—UMMZ 236817, ex. UT 44.7710, a nuptial male, 50.5 mm SL, Bumbee Creek at only road crossing, 10.0 air km wsw of Spring City, Rhea County, Tennessee, 35°39'46''N, 84°58'09''W, 6 June 1997, R.D. Bivens, B.D. Carter, C.E. Skelton, C.E. Williams.

Allotype.—UMMZ 236818, ex. UT 44.7709, female, 46.5 mm SL, same locality as holotype, 15 March 1997, C.E. Skelton.

Paratopotypes.—UT 44.7710 (5, 45.0–54.0 mm SL), collected with holotype; UT 44.7293 (6, 22.0–38.0 mm SL), 10 May 1996, C.E. Skelton; UT 44.7305 (5, 42.0–50.0 mm SL), 24 October 1996, C.E. Skelton; USNM 360019 (2, 43.4–47.2 mm SL), OSUM 90895 (2, 43.9–44.7 mm SL), CU 81096 (2, 39.5–45.0 mm SL), TU 189962 (2, 44.4–46.4 mm SL), UAIC 12364.01 (2, 44.0–44.2 mm SL), and NLU 76157 (2, 42.5–44.3 mm SL), 23 May 1998, C.E. Skelton.

Paratypes.—**Tennessee River Drainage, Tennessee, Bledsoe County,** INHS 53769 (2, 43.6–53.2 mm SL), Cupp Creek, ca. 14.6 air km w of Dayton, 27 June 1996, ex. UT 44.7300; NCSM 28473 (2, 47.2–48.1 mm SL) and JFBM 32313 (2, 45.7–48.3 mm SL), Soddy Creek below Wolf Branch Road, ca. 25.7 air km s of Pikeville, 4 December 1993, ex. UT 44.7340; UT 44.4789 (47, 29.0–47.0 mm SL), Horn Branch at only road crossing, 21.9 air km ssw of Pikeville, 29 July 1976; CAS

210943 (2, 40.8–42.9 mm SL) and AMNH 229529 (2, 40.6–46.8 mm SL), ex. UT 44.4789; UT 44.7295 (7, 25.0–43.0 mm SL). **Rhea County,** Moccasin Creek at first Summer City Road crossing, 1.6 km sw of Milo and 19.6 air km nw of Pikeville, 14 May 1996; UT 44.7320 (9, 39.0–54.0), Youngs Creek, 4 July 1996; SIUC 38000 (2, 44.5–51.7) ex. UT 44.7320.

***Non-type Material.*—Tennessee River Drainage, Tennessee, Bledsoe County,** UT 44.5884 (23), Horn Branch at only road crossing, 21.9 air km ssw of Pikeville, 19 June 1991; UT 44.7304 (2), same locality, 31 May 1994; UT 44.7306 (1) same locality, 10 June 1994; UT 44.7339 (21), same locality, 29 August 1993; UT 44.7341 (2), Cupp Creek, ca. 0.8 km nne of intersection of Hendon and Brayton roads, ca. 14.6 air km w of Dayton, 31 March 1995; UT 44.7300 (6), same locality, 27 June 1996; UT 44.6003 (30), Soddy Creek below Wolf Branch Road, ca. 25.7 air km s of Pikeville, 19 November 1993; UT 44.7294 (2), same locality, 1 June 1996; UT 44.7303 (2), same locality, 31 May 1994; UT 44.7307 (2), same locality, 10 June 1994; UT 44.7340 (18), same locality, 4 December 1993. **Rhea County,** UT 44.7322 (1), Bumbee Creek at only road crossing, 10.0 air km wsw of Spring City, 5 June 1996; UT 44.7301 (2), same locality, 1 June 1996; UT 44.7292 (2), same locality, 23 May 1996; UT 44.7902 (3), Youngs Creek 0.6 km upstream of confluence with Moccasin Creek, ca. 11.7 air km sw of Spring City, 16 May 1997; UT 44.7705 (2) same locality, 15 March 1997.

Diagnosis.—Coloration and tuberculation patterns align the new species with the genus *Phoxinus*. Differs from all congeners by combination of two uninterrupted black lateral stripes and black pigment covering breast and underside of head of nuptial males. Further differentiated from all congeners (except *P. erythrogaster* and *P. eos* occasionally) by 0,5-4,0 pharyngeal tooth formula; from all congeners except *P. neogaeus* by s-shaped (or loosely coiled) gut configuration and winged rather than rounded basioccipital pharyngeal pad.

Description.—General shape and coloration of new species shown in Figure 2-2. The largest specimen examined was a female, 62 mm SL. Pectoral fins of non-breeding males broadly rounded; fins of females narrow and taper to broad point. Nuptial males develop distinctly thickened pectoral rays and distal edges of these fins appear almost straight. Anterior rays of the dorsal and anal fins extend slightly past the posterior rays when depressed. Rear margins of dorsal and anal fins slightly convex and dorsal fin inserted just behind pelvic fin origin. Modal number of dorsal, anal, and pelvic rays 8. Number of pectoral rays (Table 2-1) modally 16 (14–17).

Entire body except head covered with tiny cycloid scales. Scales in lateral series (Table 2-2) 72–90 ($\bar{x}$ =78.5, n=55); transverse scale rows (Table 2-3) usually 24–26 ($\bar{x}$ =25.2, n=55); belly scales somewhat embedded, but outline visible with magnification (40x); breast scales very small (ca. 0.5 mm in diameter) and deeply embedded. Lateral line incomplete with 13–36 pored scales ($\bar{x}$ =24, n=47). All portions of cephalic lateralis

system usually incomplete with varying numbers of external pores. Supratemporal pores broadly interrupted at midline of nape. Infraorbital canal pores, 14(3 specimens), 15(2), 16(2), 17(7), 18(5), 19(1); preoperculomandibular canal pores, 9(1), 10(3), 11(5), 12(8), 13(3); supraorbital pores, 9(6), 10(6), 11(5), 12(1); supratemporal pores, 3 + 2(8), 3 + 3(7), 3 + 4(3), 2 + 4(1), 4 + 4(2).

Pharyngeal tooth formulae 0,5-4,0 (n=22). Pharyngeal arch moderately heavy and teeth laterally compressed. The fifth tooth (ventral) on the left arch one-third to one-half the length of other teeth, blade-like, and tapering abruptly to sharp point. Remaining teeth hooked with poorly developed grinding surfaces. Instead, they have shallow angled furrows with sharp edges. Total gill rakers on first arch 7(2 specimens), 8(11), 9(11), 10(5), 11(1) and total vertebrae 36(1), 37(1), 38(8), 39(4). Gut is s-shaped (or nearly so) and peritoneum light brown or gray with scattered melanophores.

Coloration.—As with three other species in the genus (*P. erythrogaster*, *P. eos*, *P. tennesseensis*), Laurel Dace have two black lateral stripes. In life, these stripes vary from dark black to barely discernible. The upper stripe originates near the junction of the occiput and the posterodorsal portion of the operculum. Anteriorly the stripe is approximately three scale rows deep and gradually narrows caudally. This stripe differs from the upper stripe of other *Phoxinus* species because it is usually persistent from beginning to end rather than breaking into discrete spots on the caudal peduncle. The lower stripe begins on the snout and passes through the eye and onto the body. It angles

downward from the shoulder girdle toward the belly, and often dips slightly but abruptly over the anal fin, creating a small notch. The stripe then narrows somewhat and continues uninterrupted to the base of the caudal fin. There is usually a continuation of diffuse black pigment down the middle of the caudal fin. At the caudal fin base there is often a small black spot or narrow bar about the same depth as the lower black stripe.

A black line of varying completeness and intensity runs along the midline of the dorsum, and a row of small (smaller than the pupil) irregular spots lies on either side of the midline. The number of these spots varies from less than five to approximately 20.

The dorsum of Laurel Dace varies from dark olive to a very pale tan. The area between the lateral stripes has a silvery-white sheen. The lower caudal peduncle is typically the same color as the dorsum. The belly, breast, and lower half of the head are whitish-silvery. At any time during the year, however, Laurel Dace may develop red coloration below the lower lateral stripe from the insertion of the pectoral fins to the base of the caudal fin.

As seen in other species of the genus (Etnier and Starnes, 1993), nuptial males may acquire brilliant colors during the breeding season (Figure 2-2). The two lateral stripes become intensely black as does the entire underside of the head and anterior portion of the breast. Occasionally, additional black pigment develops on the breast and/or belly.

The entire ventral portion of the body becomes intense scarlet and this color is contiguous with the lower black stripe and black breast. However, a narrow strip of red

pigment passes obliquely through the lower black stripe from the insertion of the pectoral fin and follows the gill opening to the upper attachment of the operculum. The red pigment is most intense on the caudal peduncle. Except for the first ray, the basal 20–30 percent of the dorsal fin turns red. Red pigment develops around the black caudal spot (bar) and radiates out toward the fin edges. The lips also become red.

The cheek, the area between the two lateral stripes, and the upper and lower halves of the operculum become metallic gold as do the upper and lower portions of the iris. This color is most intense in the area between the lateral stripes. The bases of the paired fins develop a small pearly-white patch of pigment. The pigment at the base of the pectoral fin is about the size of the eye, while the spot at the base of the pelvic fin is about the size of the pupil. The paired fins become bright yellow and dorsal (above basal red band) and anal fins somewhat less so. The caudal fin acquires a yellow wash. Females develop all of these colors but they are less intense. A diffuse widening of the anterior portion of the upper lateral stripe is sometimes present on females.

In preservative, all of the red, white, yellow, and iridescent colors fade quickly. Black pigment persists after preservation but fades slightly. Fins become translucent and areas that were white or yellowish become cream colored. The dorsum color varies from light brown to pale tan.

Tuberculation.—Male Laurel Dace develop nuptial tubercles on the head and the posterior edge of every scale except for the belly and breast scales, where tubercles are

reduced or absent. Most tubercles are uniconic on the front half of the body but become increasingly multiconic posteriad. Multiconic tubercles (up to six or seven points) are most evident around the anal fin and the ventral half of the caudal peduncle. As with other species in the genus (Howes, 1985), Laurel Dace develop 7–10 rows of comb-like breast tubercles anterior to each pectoral fin. The dorsal portion of pectoral rays two to five or two to six develop tubercles on the distal half of the fin (ca. 10-12 ray segments). The five or six most distal segments have two tubercles while the segments proximal to those usually have one tubercle.

Head tubercles are small and uniconic and are dispersed over the entire surface. They appear to be randomly distributed except for a dense patch of retrorse tubercles on the posterodorsal portion of the opercle. Additionally, a row of tubercles is sometimes present along the posterior edges of the opercle and subopercle. Female tubercles are not as well developed but have a similar pattern.

In addition to tubercles, there is another apparently cornified structure found on Laurel Dace that develops during the breeding season. To the naked eye, they appear to be small raised dots. They are found in the center of each scale, along the dorsal, pelvic, anal, and caudal rays and scattered randomly all over the head. They are probably some type of sensory structure.

Variation.—SPCA of morphological measurements of Laurel Dace males indicates broad overlap of body shape among the five populations examined (Figure 2-3). There is no

apparent separation along SPC3 and limited separation along SPC2. Based on the SPC2 loadings (Table 2-4), it appears that the variation described by this axis is related primarily to body width. Other measurements that loaded heavily on SPC2 were anal fin length and distance from pectoral fin origin to isthmus. The heaviest loadings on the SPC3 axis were dorsal fin base length, upper jaw length, and gape width.

Gut length is consistently s-shaped in all populations except for Soddy Creek. The gut configuration of this population varies from a nearly double looped configuration like *P. erythrogaster* to a simple s-shape.

Sexual dimorphism in Laurel Dace is similar to that seen in other *Phoxinus* species (Smith, 1908; Starnes and Starnes, 1978). Secondary sexual characteristics such as coloration and tuberculation patterns develop on both males and females, however, these characteristics are almost always more fully developed in males. Proportional measurements indicate that males have longer fins and a wider breast than females (Table 2-5). The broadly rounded pectoral fins of males vs. broadly pointed fins of females allows for easy separation of male and female Laurel Dace at any time during the year. Sheared PCA of specimens from Soddy Creek and Horn Branch shows complete separation of non-breeding males and females along the SPC2 axis (Figure 2-4). The heaviest loadings on the SPC2 axis are eye diameter, gape width, dorsal fin length, and pelvic fin length (Table 2-6). There is wide overlap between males of both populations and Horn Branch females on the SPC3 axis. Females from Soddy Creek are almost completely separated from all three groups. Heaviest loadings for SPC3 are snout length,

distance between pectoral fin origin and pelvic fin origin, orbit diameter, pectoral fin origin to isthmus, and pectoral fin origin to pectoral fin origin (Table 2-6).

Comparisons.—The color pattern of nuptial Laurel Dace is unique among all North American cyprinids. The combination of two uninterrupted black lateral stripes and the development of black pigment on the underside of the head of breeding males separates Laurel Dace from all congeners. It is sometimes difficult to differentiate small Laurel Dace from the Creek Chub (*Semotilus atromaculatus*) in the field, but they are easily separated using magnification.

Laurel Dace have significantly fewer scales in lateral series than either *P. erythrogaster* or *P. tennesseensis* (Table 2-2). There is considerable overlap in number of transverse scale rows; however, Laurel Dace usually have one or two fewer rows than the other species (Table 2-3).

Laurel Dace differ significantly from *P. erythrogaster* and *P. tennesseensis* in several morphometric measurements (Table 2-7). The scatterplot of SPCA scores shows some separation along the SPC2 axis between Laurel Dace and the other two species (Figure 2-5). Heaviest loadings on this axis were body width, gape width, pectoral fin length, and distance from anal fin origin to pelvic fin origin (Table 2-8). All three species overlap broadly on the SPC3 axis.

DISCUSSION

Relationships and Biogeography.—Starnes and Jenkins (1988) hypothesized that *P. tennesseensis* and *P. oreas* formed a closely related pair based on three putative synapomorphies, an interrupted and decurved lower black lateral stripe, red pigment on the lower operculum, and black pigment covering the underside of the head of nuptial males. They further suggested that this pair was sister to *P. cumberlandensis* based on opercular tuberculation, dorsolateral speckling, and shape of the opercular bone. Laurel Dace shares with *oreas* + *tennesseensis* the hypothesized synapomorphy of black pigment on the underside of the head of nuptial males and, with all three species, a dense triangular patch of breeding tubercles on the posterodorsal portion of the operculum. Laurel Dace, *P. tennesseensis*, *P. oreas*, and *P. cumberlandensis* are thus hypothesized to form a monophyletic group within the Nearctic *Phoxinus.* Based solely on morphological characters, placement of Laurel Dace within this group is problematic. However, recent genetic evidence suggests that Laurel Dace is a distinctive taxon sister to *P. cumberlandensis* and that this pair is sister to *oreas* + *tennesseensis* (Figure 2-6A, R. Mayden, Univ. Alabama, pers. comm.). An alternative hypothesis places Laurel Dace as sister to the hypothesized sister pair of *P. eos* and *P. erythrogaster*. These three species possess two uninterrupted lateral black stripes. If this character is a synapomorphy, these three species may be sister to *P. cumberlandensis* plus the sister pair of *P. oreas* + *P.*

tennesseensis. *Phoxinus neogaeus* is the hypothesized sister to the entire group (Figure 2-6B).

An hypothesized biogeographic scenario explaining the distributions of *P. tennesseensis, P. oreas*, and *P. cumberlandensis* was described in detail by Starnes and Jenkins (1988). They suggested that the ancestor (similar to *P. oreas*) to these three species was widespread in the upper Teays River drainage. *Phoxinus cumberlandensis* was likely derived from a portion of this stock that dispersed into the upper Cumberland River drainage through a capture of upper Teays drainage tributaries (Starnes and Starnes, 1978). The *oreas*-like form may have then dispersed into the upper Tennessee River (Holston), giving rise to Laurel Dace. *Phoxinus tennesseensis* would have then been derived from a second invasion into the Tennessee River system from the upper New River by an *oreas*-like form. *Phoxinus oreas* has subsequently dispersed into several Atlantic Slope drainages (Starnes and Jenkins, 1988)

Distribution.—Laurel Dace are known to occur in six streams on the Walden Ridge portion of the Cumberland Plateau in Tennessee (Figures 2-7, 2-8). Walden Ridge extends from approximately Lake City, TN, southwest to the Tennessee River near Chattanooga. Beginning near Pikeville, TN, the ridge is set off from the Cumberland Plateau by the Sequatchie Valley, thus forming a finger-like projection. This part of Walden Ridge is approximately 99 km in length, averages 14.5 km in width, and rises 300 m from the surrounding valleys.

The major drainages on Walden Ridge are comprised of eastward flowing, meandering streams that drop abruptly off the edge of the plateau and then enter the Tennessee River. The streams where Laurel Dace have been collected are found in three independent systems on Walden Ridge: the Soddy Creek system, the Sale Creek system, and the Piney River system. In the Soddy Creek system, one population is known from Soddy Creek proper. In the Sale Creek system, there are populations in Horn Branch of Rock Creek and Cupp Creek. In the Piney River system, populations are known from Youngs, Moccasin, and Bumbee creeks (Figure 2-8).

Laurel Dace are fairly common to abundant where they occur, but known populations are extremely localized. All populations except in Cupp Creek inhabit reaches of only one to three km (Skelton, 1997). The Cupp Creek population also appears to be sharply localized but further sampling is required for a confident assessment.

Behavior, Ecology, and Life History.—Although a detailed life history study of Laurel Dace has not been completed, the species appears to have ecological requirements similar to those of other species in the genus. Laurel Dace are most often collected in pools or slow runs from undercut banks or beneath slab boulders. The creeks that they inhabit are first or second order, clear and cool (max. ca. 26° C), and typically have a substrate consisting of a mixture of cobble, rubble, and boulders. Most streams have a dense riparian zone, of which mountain laurel (*Kalmia* sp.) is often the main component.

Three year classes are found in some collections, although it is often difficult to find young-of-year fish. Nuptial individuals have been collected from late March until mid-June.

Several studies have documented *Phoxinus* spp. spawning over the nests of nest building minnows (e.g. Raney, 1947; Starnes and Starnes, 1981). This phenomenon is widespread within Cyprinidae and is termed nest association. I witnessed behavior of Laurel Dace that suggests it is also a nest associate where syntopic with a nest-building minnow species. In late May 1994, I observed a school of approximately 20 Laurel Dace moving over a Largescale Stoneroller (*Campostoma oligolepis*) nest in Soddy Creek. The males were at peak coloration and tuberculation, and I expected to witness spawning; however, they were periodically burying their noses between gravel in the nest, possibly trying to eat eggs. Soddy Creek is the only locality where a nest-building minnow has been collected with Laurel Dace. In Horn Branch and Bumbee Creek, behavior I assumed to be associated with spawning was observed, but again, spawning did not occur. At both sites, several nuptial Laurel Dace were moving in and out of a shallow riffle that contained small gravel. Unfortunately, the fish were disturbed on both occasions and further observations were not made. Smith (1908) observed *P. erythrogaster* spawning in a shallow gravel riffle.

In March 1997, I collected nuptial Laurel Dace from Bumbee Creek for the purpose of making color photographs. The fish were taken to the Tennessee Wildlife Resources Agency (TWRA) office at Buffalo Springs and placed in aquaria. They were

photographed several days later and then preserved. Subsequently, larval fish were discovered in the aquarium indicating that spawning had occurred. The larvae were taken to Conservation Fisheries Incorporated (CFI) to be reared for possible propagation efforts. A series of these larvae was illustrated to assist with identification of larval Laurel Dace (Figure 2-9).

Qualitative analysis of intestinal tracts (n=12) indicates that Laurel Dace eat a mixture of food items but rely heavily on animal material. A variety of benthic invertebrates including larvae from the insect orders Trichoptera, Plecoptera, and Diptera were found. Plant material and sand grains were present in some intestines. The animal-based diet of Laurel Dace reflects its trophic morphology. Relative to congeners, they have a large mouth, short digestive tract, reduced number of pharyngeal teeth, and a primitively shaped basioccipital bone. During the spring I have observed them in loose schools picking at insects and surface materials.

Conservation Status.—The Laurel Dace is a former Category 2 candidate as recognized by the US Fish and Wildlife Service (USFWS). Although Laurel Dace are currently afforded no federal protection, they are considered endangered by the State of Tennessee (P. Shute, TVA Nat. Heritage, pers. comm.).

Laurel Dace were first collected in Horn and Laurel branches of Rock Creek in 1976 during a TVA rotenone survey (C. Saylor, TVA, pers. comm.). The species was not collected again until 1991 when C. Saylor revisited Horn and Laurel branches.

Additional specimens were collected in Horn Branch; however, they have not been taken again in Laurel Branch, and I suspect that they are now extirpated.

Other than the apparent extirpation of the Laurel Branch population, it is difficult to say whether or not Laurel Dace populations have declined in recent years. Examination of range maps in Etnier and Starnes (1993) indicates that little collecting occurred on Walden Ridge prior to that publication; thus, there is no historical information to draw from. Agriculture, coal mining, and timbering have been widespread on Walden Ridge, and it is likely that additional populations may have existed before human activities. Extensive timber harvesting and some agriculture are ongoing in the vicinity of some Laurel Dace populations. A large area surrounding the headwaters of Horn Branch of Rock Creek was recently clearcut, as was an approximately 200 m stretch along Bumbee Creek adjacent to the type locality. Further cutting is proposed near the type locality in the next four years (W. Boyd, Bowater Newsprint, pers. comm.). Increased siltation from these activities is already evident and has the potential of destroying available spawning areas.

It is possible that this species has never been very widespread. It is nearly sympatric with *P. tennesseensis* in the Piney River system (Figure 2-8) which could possibly inhibit dispersal. The harsh nature of the environment on Walden Ridge is another factor. Many of the smaller creeks dry up completely during the summer, and some of the larger ones are reduced to isolated pools. Also, Walden Ridge is very near the southern boundary of the range of the entire genus (Etnier and Starnes, 1993). Only

two or three of the heartiest fish species (e.g. *Semotilus atromaculatus*, *Rhinichthys atratulus*, *Catostomus commersoni*, and *Lepomis cyanellus)* were typically captured at sites where Laurel Dace occur.

It is encouraging that Laurel Dace seem to be fairly tolerant of a wide range of physical conditions. They are apparently tolerant of low pH values (lowest recorded was 5.4 in Horn Branch) and presumably tolerant of some siltation. Most of the areas adjacent to streams where Laurel Dace occur have been clearcut at one time or another and at least some of the populations have survived. Currently, populations appear to be relatively secure. However, since there are only six known populations, a single catastrophic event could significantly reduce the species' range. Based solely on the limited number of populations of Laurel Dace, this species warrants federal protection, minimally at the threatened level.

Because of the isolated nature of many of the streams on Walden Ridge, it would be difficult for Laurel Dace to re-colonize former habitats should one population be eliminated. Evidence of this is the fact that Laurel Dace have probably been extirpated from Laurel Branch since at least 1991. Although the mouth of Laurel Branch is close to the Horn Branch population, Laurel Dace have not re-colonized the creek. Other creeks with known populations also have no source population for re-colonization should they be extirpated (i.e. Cupp Creek, Soddy Creek). The increase of human activities on Walden Ridge provides a scenario conducive to severe reduction of the species range.

In order to preserve this species, the known populations should be monitored on a regular basis and surveys should be conducted to determine if additional populations exist. Also, artificial propagation protocols should be established in the event that populations must be brought into captivity. Propagated individuals from Horn Branch could be used as a source to re-establish a population in Laurel Branch.

MATERIAL EXAMINED

Phoxinus tennesseensis—Tennessee River Drainage, Tennessee: UT 44.6784 (6), Duskin Creek at Shut-In Gap Rd., ca. 22.2 air km nw of Pikeville, Bledsoe County, 14 May 1996; UT 44.7708 (3), Duskin Creek at Walden Mt. Rd., ca. 7.6 air km w of Spring City, Rhea County, 6 June 1997; UT 44.6779 (4), Sandy Creek, 2 mi. upstream of Possum Trot Rd., ca. 6.0 air km sw of Ozone, Cumberland County, 8 April 1996. Holston River system, UT 44.5266 (19), Brice Branch adjacent to Idumea Rd., ca 0.4 km n of TN Hwy. 11W, Knox County, 20 March 1991; UT 44.2693 (1), Beaverdam Creek, TN Hwy. 133 at Backbone Rock, Johnson County, 24 June 1982; UT 44.5820 (3), R. Prong Hatcher Creek along Ryder Church Rd., Sullivan County, 9 April 1992. Clinch River system, UT 44.3037 (11), Flatfork Creek, trib. to Emory River at Frozen Head State Park Headquarters, Morgan County, 7 September 1983; UT 44.5748 (18), Pinhook Branch of E. Fork Poplar Creek, at rd. 3.2 km e of jct. TN Hwys. 95 & 58, Roane Co., 6 June 1990. Little Tennessee River system, UT 44.4045

(8), Tributary to Nine-mile Creek at Porter Rd., 1.4 rd. km n of Six-mile Rd., Blount County, 2 July 1987. Sequatchie River system, UT 44.7119 (3), Little Brush Creek above TN Hwy. 111, Sequatchie County, 31 May 1996. French Broad River system, UT 44.5450 (2), Cove Creek at road to fire station, Wears Cove, Sevier County, 4 November 1991. Little River system, UT 44.1070 (1), Reed Creek 100 m below junction of Double Branch, Blount County, 13 April 1975.

Phoxinus erythrogaster—Tennessee River Drainage, Tennessee, Elk River system: UT 44.7882 (3), Dry Creek near Richland Creek, 16 May 1994, and (2), Anderson Creek 12.1 air km ne of Pulaski, 5 May 1994, (1), Dry Weakly Creek km 0.2, 4 May 1994, Giles Co. Duck River system, UT 44.7110 (4), Bobo Creek off Short Springs Rd., Coffee Co., 30 May 1996; UT 44.6064 (2), Anderton Branch, 61.0 m upstream from confluence with Thompson Creek, Bedford Co., 27 May 1993. Cumberland River Drainage, Rockcastle River system, Kentucky, UT 44.7790 (10) Clear Creek at co. rd. 1789, ca. 1.6 km sw of Dispunta, Rockcastle Co., KY, 6 May 1997; UT 44.7796 (2), Dry Fork of Skeggs Cr., at co. rd. 1249, 6.4 km s of Mt. Vernon, Rockcastle Co., KY, 6 May 1997. Harpeth River system Tennessee, UT 44.7607 (7), Kelly Creek, 0.8 mi. above Blowing Springs Branch, 20.6 air km wsw of Franklin, Williamson Co., 8 May 1997. Caney Fork River system: UT 44.1513 (8), Big Indian Creek, 4.3 air km wnw of Buffalo Valley, 6.8 air km nne of Silver Point, off co. rd. 6330, Putnam Co., 9 July 1976; UT 44.6897 (9), Indian Creek at Indian Creek Rd. and Leftwich Hollow Rd., ca. 10.4 air km w of Baxter, Putnam Co., 23 May 1996.

Ohio River Drainage, Barren River system, Tennessee, UT 44.5745 (4), Little Salt Lick Creek and trib. 0.4 km e of TN 56, 4.8 air km nne of Red Boiling Springs, Macon Co., 16 April 1991; UT 44.5756 (11), Trammel Creek at Pop Branch, off Williams Rd. 1.9 km s of KY state line, Macon Co., 16 April. Hocking River system, Ohio, UT 44.5571 (6), Wolf Pen Creek at OH Hwy. 50 near Coolville, Athens Co., 26 May 1980. Kentucky River system, Kentucky, UT 7784 (11), Long Branch above Chestnut Ridge Rd., ca. 1.6 km n of Brodhead, Rockcastle Co., 6 May 1997; UT 7788 (7), unnamed trib. to Boone Fork, at KY Hwy. 1326, Rockcastle Co., 6 May 1996; UT 44.7706 (8), Red Lick Creek at intersection of US Hwy. 421 & KY Hwy. 21, ca. 18.5 air km sse of Richmond, Madison Co., 22 May 1997. UT 44.7598 (35), same locality, 6 May 1997. UT 44.7602 (9), Possum Run at Possum Run Rd., ca. 6.4 km NE Irvine, Estill Co., 6 May 1997.

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APPENDICES

Appendix 2-A. Tables and Figures.

Table 2-1. Frequency Distribution of Pectoral Fin Rays for Laurel Dace, *Phoxinus tennesseensis*, and *Phoxinus erythrogaster*.

	No. Rays							
Species	13	14	15	16	17	n	$\bar{x}$	SD
Laurel Dace		1	13	28	13	55	16.0	0.75
Phoxinus tennessensis		3	18	23	4	48	15.6	0.74
Phoxinus erythrogaster	1	6	41	9	4	61	15.1	0.75

Table 2-2. Frequency Distribution of Scales in Lateral Series for Laurel Dace, *Phoxinus tennesseensis*, and *Phoxinus erythrogaster*.

Species	No. scales																																		n	$\bar{x}$	SD
	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105			
Laurel Dace	2	3	2	3	9	4	8	5	3	5	2	4	1	2	—	—	1	—	1																55	78.5	3.9
P. tennesseensis				1	3	1	2	3	1	5	4	7	4	2	—	4	2	3	1	—	2	2	—	1											48	83.7	4.9
P. erythrogaster		1	—	—	1	1	—	1	2	1	3	4	1	2	1	8	7	5	5	2	—	6	2	—	—	1	1	—	1	1	—	—	1	1	61	88.3	6.2

Table 2-3. Frequency Distribution of Transverse Scale Rows for Laurel Dace, *Phoxinus tennesseensis*, and *Phoxinus erythrogaster*.

	No. Scales																
Species	21	22	23	24	25	26	27	28	29	30	31	32	33	34	n	$\bar{x}$	SD
Laurel Dace		1	3	13	15	15	6	1		1					55	25.2	1.4
Phoxinus tennesseensis			4	7	11	10	13	2	1						48	25.6	1.5
Phoxinus erythrogaster	1		4	7	10	12	7	2	7	4	3		2	2	61	26.9	2.9

Table 2-4. Sheared Principal Component Scores for Male Laurel Dace from Soddy Creek, Horn Branch, Cupp Creek, Youngs Creek, and Bumbee Creek.

Measurement	Sheared PC 2	Sheared PC 3
Standard length	0.084	-0.092
Pectoral fin length	0.166	-0.009
Pelvic fin length	0.139	0.000
Dorsal fin length	0.213	0.019
Anal fin length	0.273	-0.004
Interorbital distance	0.035	-0.018
Head width	-0.102	0.231
Body width at dorsal fin origin	-0.371	0.135
Body width at anal fin origin	-0.461	0.224
Snout length	-0.203	0.109
Head length	0.146	0.077
Snout-Occiput	0.067	-0.072
Occiput-Dorsal origin	0.180	0.015
Dorsal fin base	0.041	-0.288
Dorsal end-Caudal base	0.034	-0.186
Caudal peduncle depth	-0.107	0.009
Anal end-Caudal base	0.042	-0.188
Anal fin base	-0.107	-0.037
Anal origin-Pelvic origin	0.244	-0.259
Pectoral origin-Pelvic origin	-0.067	0.064
Snout-Isthmus	0.039	-0.060
Gape width	0.129	0.509
Upper jaw length	0.058	0.336
Head depth	0.028	0.009
Occiput-Pectoral origin	-0.025	-0.055
Dorsal origin-Pectoral origin	0.042	-0.006
Dorsal origin-Pelvic origin	-0.171	-0.200
Dorsal origin-Anal origin	-0.075	-0.210
Dorsal end-Pelvic origin	-0.108	-0.154
Dorsal end-Anal origin	-0.218	-0.125
Dorsal end-Anal end	-0.137	-0.144
Orbit diameter	0.233	0.223
Pectoral origin-Isthmus	0.271	0.175
Pectoral origin-Pectoral origin	-0.076	0.132

Table 2-5. Means and Ranges of Proportional Measurements (Expressed in Thousandths of Standard Length) for Male and Female Laurel Dace from Horn Branch of Rock Creek and Soddy Creek. Significant differences marked with an asterisk.

	Males (n=15)		Females (n=19)	
Measurement	$\bar{x}$	Range	$\bar{x}$	Range
Standard length	43.9	39.7-48.5	44.3	39.8-50.9
Pectoral fin length*	211	195-225	192	175-200
Pelvic fin length*	170	153-180	148	139-161
Dorsal fin length*	237	223-259	213	200-228
Anal fin length	208	191-217	196	189-206
Interorbital distance	91	87-96	92	85-99
Head width	147	136-160	150	138-165
Body width at dorsal origin	129	120-137	126	108-141
Body width at anal origin	97	89-102	96	90-104
Snout length*	74	69-81	78	68-87
Head length	262	246-289	266	247-285
Snout–occiput*	211	194-221	206	196-220
Occiput–dorsal origin	355	340-377	362	322-383
Dorsal fin base*	104	93-117	96	82-108
Dorsal base end–hypural*	374	360-389	370	342-383
Caudal peduncle depth	121	118-124	118	106-128
Anal base end–hypural	247	233-266	235	217-251
Anal fin base	102	97-111	103	83-109
Anal origin–pelvic origin	180	164-192	184	168-201
Pectoral origin–pelvic origin*	215	200-230	233	218-253
Snout–isthmus	170	166-181	168	152-181
Gape width*	68	61-72	72	64-83
Upper jaw length	81	74-90	84	67-97
Head depth	174	163-183	171	148-186
Occiput–pectoral origin*	173	161-187	164	154-181
Dorsal origin–pectoral origin*	339	324-367	349	334-369
Dorsal origin–pelvic origin*	222	208-246	214	192-240
Dorsal origin–anal origin	227	216-242	221	201-237
Dorsal base end–pelvic origin	241	220-255	235	216-264
Dorsal base end–anal origin	164	152-174	161	147-179
Dorsal base end–anal base end	183	169-194	180	167-198
Orbit diameter*	74	64-87	80	65-96
Pectoral origin–isthmus*	137	123-146	122	105-134
Pectoral origin–pectoral origin*	161	149-173	141	125-150

Table 2-6. Sheared Principal Component Scores for Male and Female Laurel Dace from Horn Branch of Rock Creek and Soddy Creek.

Measurement	Sheared PC 2	Sheared PC 3
Standard length	0.050	-0.048
Pectoral fin length	-0.217	0.091
Pelvic fin length	-0.334	0.233
Dorsal fin length	-0.277	0.093
Anal fin length	-0.114	0.075
Interorbital distance	0.084	0.093
Head width	0.170	0.195
Body width at dorsal fin origin	-0.075	-0.211
Body width at anal fin origin	0.031	-0.070
Snout length	0.202	-0.279
Head length	0.152	0.177
Snout-Occiput	-0.020	-0.023
Occiput-Dorsal origin	0.086	-0.060
Dorsal fin base	-0.182	-0.053
Dorsal end-Caudal base	0.006	-0.064
Caudal peduncle depth	-0.009	0.079
Anal end-Caudal base	-0.099	-0.028
Anal fin base	0.019	-0.151
Anal origin-Pelvic origin	0.102	-0.210
Pectoral origin-Pelvic origin	0.227	-0.289
Snout-Isthmus	0.019	0.060
Gape width	0.278	0.087
Upper jaw length	0.154	-0.067
Head depth	0.008	0.024
Occiput-Pectoral origin	-0.089	0.053
Dorsal origin-Pectoral origin	0.128	-0.131
Dorsal origin-Pelvic origin	-0.088	-0.172
Dorsal origin-Anal origin	-0.007	0.047
Dorsal end-Pelvic origin	-0.063	-0.229
Dorsal end-Anal origin	0.018	0.005
Dorsal end-Anal end	0.049	0.089
Orbit diameter	0.425	0.558
Pectoral origin-Isthmus	-0.239	0.313
Pectoral origin-Pectoral origin	-0.376	-0.035

Table 2-7. Means and Ranges of Proportional Measurements (Expressed in Thousandths of Standard Length) for the Holotype and Males of Laurel Dace, *Phoxinus tennesseensis*, and *Phoxinus erythrogaster*. Holotype not included in mean. Significant differences marked with an asterisk.

	Holotype	Laurel Dace	(n=41)	*Phoxinus tennesseensis*	(n=43)	*Phoxinus erythrogaster*	(n=50)
Measurement		$\bar{x}$	Range	$\bar{x}$	Range	$\bar{x}$	Range
Standard length	50.5	45.7	39.7-54.6	49.53	42.3-60.7	49.6	44.3-56.4
Pectoral fin length	205	206	185-225	201	181-231	232	207-266
Pelvic fin length	177	168	153-180	170	158-193	162	146-178
Dorsal fin length*	219	230	216-259	216	195-254	220	193-243
Anal fin length*	209	203	186-217	196	174-223	194	180-208
Interorbital distance*	83	90	82-100	83	75-91	83	74-96
Head width*	147	151	132-171	136	118-153	141	122-155
Body width at dorsal fin origin	140	138	120-165	130	106-162	137	101-173
Body width at anal fin origin	116	107	89-133	99	82-128	104	78-127
Snout length*	77	78	69-88	75	66-90	75	62-86
Head length*	262	258	245-289	247	236-270	248	229-274
Snout-Occiput	205	211	192-271	207	193-219	203	181-224
Occiput-Dorsal origin*	340	350	314-377	334	318-359	342	316-365
Dorsal fin base*	104	106	93-120	109	93-126	110	100-131
Dorsal end-Caudal base*	360	375	358-395	385	338-421	382	358-411
Caudal peduncle depth	127	126	118-138	110	97-131	122	110-136
Anal end-Caudal base*	234	249	233-266	260	229-277	255	232-278
Anal fin base	110	107	97-118	106	86-117	106	96-115
Anal origin-Pelvic origin*	170	174	155-192	185	163-215	181	154-205
Pectoral origin-Pelvic origin*	216	218	197-239	212	188-234	228	210-250

Table 2-7, cont.

Snout-Isthmus	175	172	154-183	169	130-198	168	155-183
Gape width*	65	67	54-74	57	41-77	59	47-73
Upper jaw length*	82	82	70-93	67	60-77	73	63-85
Head depth*	164	174	163-189	164	152-182	167	153-182
Occiput-Pectoral origin*	182	175	155-189	168	158-181	170	154-186
Dorsal origin-Pectoral origin	327	341	324-367	332	306-364	345	326-372
Dorsal origin-Pelvic origin	231	230	208-256	220	194-272	234	207-264
Dorsal origin-Anal origin	234	233	216-248	229	196-253	243	223-263
Dorsal end-Pelvic origin	243	248	220-269	243	216-272	249	228-277
Dorsal end-Anal origin	171	173	152-190	164	145-192	176	162-198
Dorsal end-Anal end	193	189	169-205	180	161-202	190	175-210
Orbit diameter*	67	71	62-87	66	58-78	67	60-81
Pectoral origin-Isthmus*	141	132	116-146	121	107-139	112	100-126
Pectoral origin-Pectoral origin*	166	167	149-186	147	127-172	149	123-166

Table 2-8. Sheared Principal Component Scores for Males of Laurel Dace, *Phoxinus tennesseensis*, and *Phoxinus erythrogaster*.

Measurement	Sheared PC 2	Sheared PC 3
Standard length	-0.144	0.057
Pectoral fin length	-0.304	-0.183
Pelvic fin length	-0.145	0.183
Dorsal fin length	-0.067	0.116
Anal fin length	-0.095	0.188
Interorbital distance	0.054	0.080
Head width	0.201	0.000
Body width at dorsal fin origin	0.278	-0.300
Body width at anal fin origin	0.352	-0.306
Snout length	0.078	0.137
Head length	-0.025	0.159
Snout-Occiput	-0.060	0.148
Occiput-Dorsal origin	-0.111	0.080
Dorsal-fin base	-0.180	0.019
Dorsal end-Caudal base	-0.222	-0.009
Caudal peduncle depth	0.111	-0.176
Anal end-Caudal base	-0.252	0.027
Anal fin base	-0.026	0.038
Anal origin-Pelvic origin	-0.318	0.105
Pectoral origin-Pelvic origin	-0.076	-0.153
Snout-Isthmus	-0.037	0.154
Gape width	0.360	0.230
Upper jaw length	0.230	0.244
Head depth	0.028	0.036
Occiput-Pectoral origin	0.016	0.039
Dorsal origin-Pectoral origin	-0.087	-0.048
Dorsal origin-Pelvic origin	-0.037	-0.235
Dorsal origin-Anal origin	-0.128	-0.168
Dorsal end-Pelvic origin	-0.069	-0.124
Dorsal end-Anal origin	-0.019	-0.220
Dorsal end-Anal end	-0.054	-0.160
Orbit diameter	-0.022	0.219
Pectoral origin-Isthmus	0.129	0.432
Pectoral origin-Pectoral origin	0.285	0.056

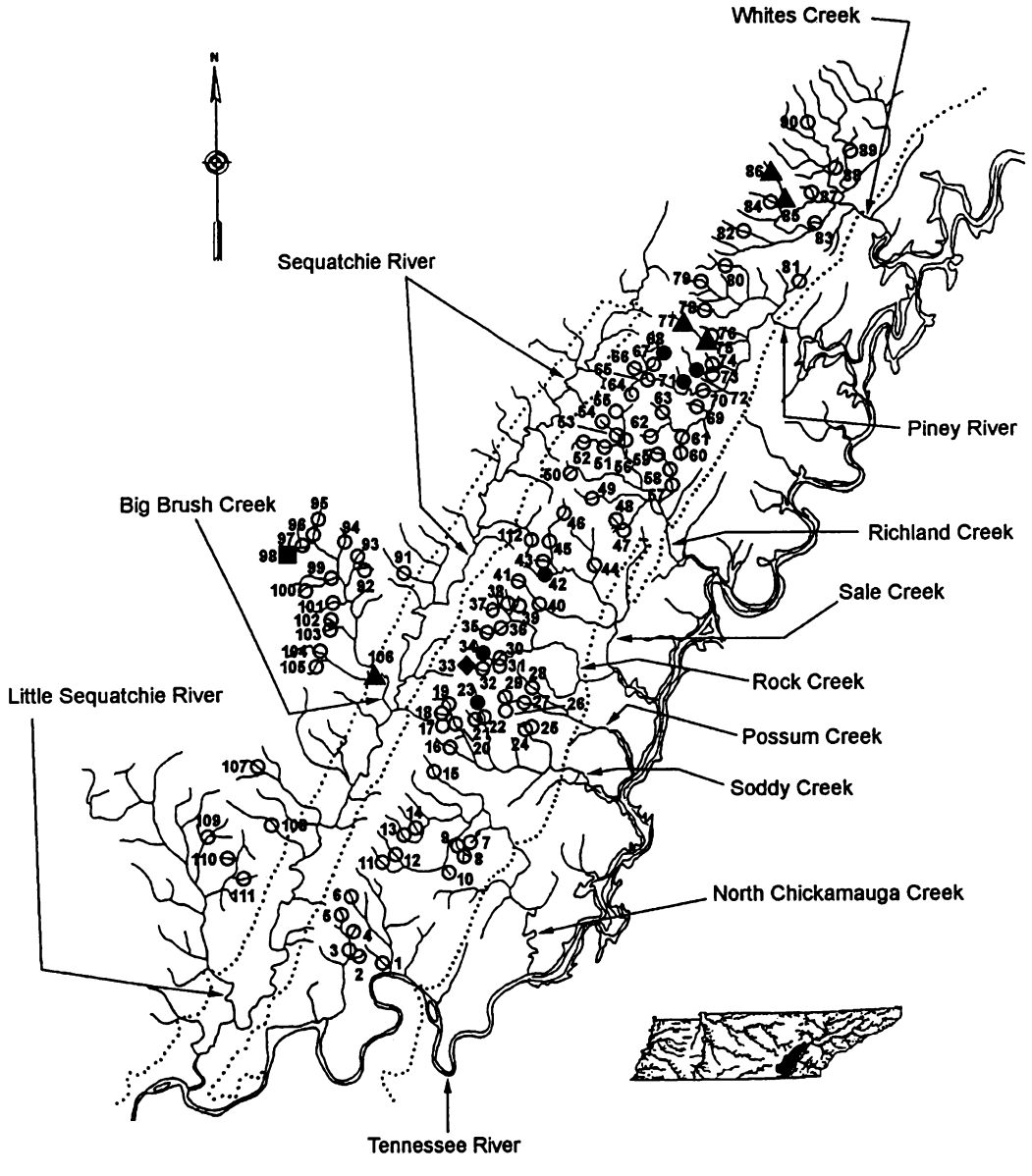


Figure 2-1. Status survey localities for Laurel Dace from 1993-1997. Open circles represent no *Phoxinus* spp. found; closed circles are Laurel Dace; closed triangles are *Phoxinus tennesseensis*; closed square is *Phoxinus erythrogaster*; closed diamond is extirpated population of Laurel Dace. Numbered locations listed in Appendix 2-B.



Figure 2-2. Nuptial male Laurel Dace, 50.5 mm SL, Bumbee Creek, Rhea County, Tennessee. Photo by C. Williams.

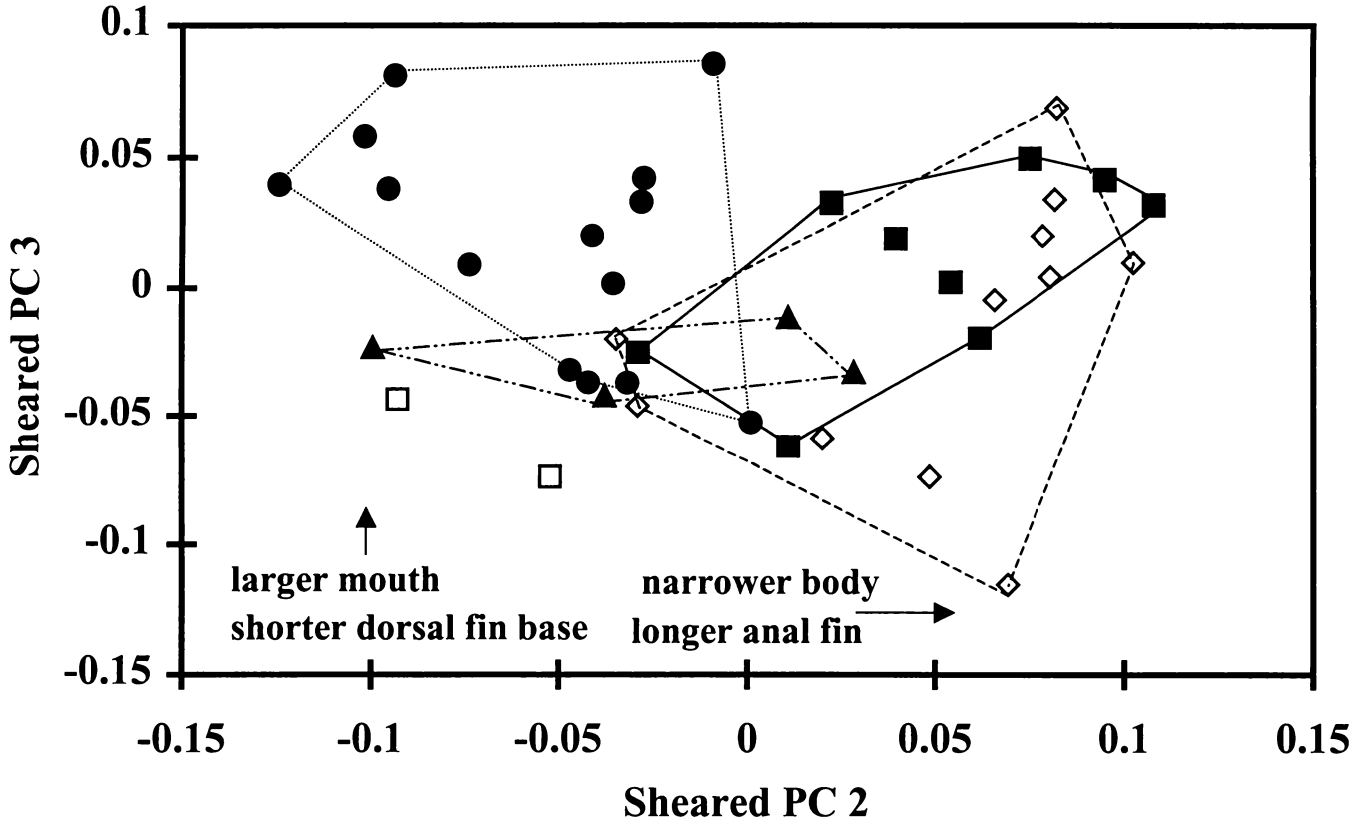


Figure 2-3. Sheared principal component scores for male Laurel Dace from Horn Branch of Rock Creek (open diamonds), Soddy Creek (closed squares), Bumbee Creek (closed circles), Cupp Creek (open squares), and Youngs Creek (closed triangles), Bledsoe and Rhea counties, Tennessee.

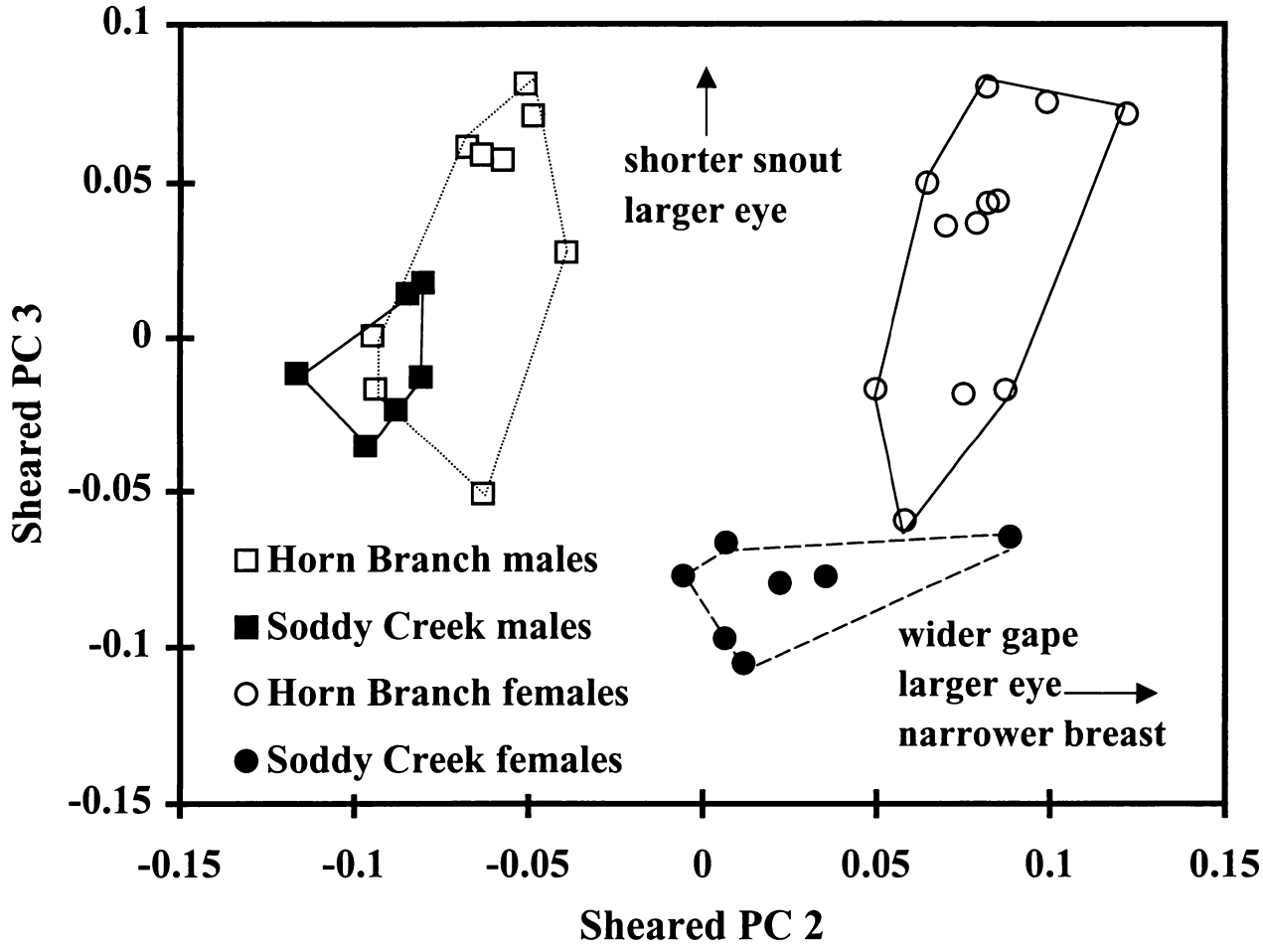


Figure 2-4. Sheared principal component scores for non-breeding male and female Laurel Dace from Horn Branch of Rock Creek and Soddy Creek, Bledsoe County, Tennessee.

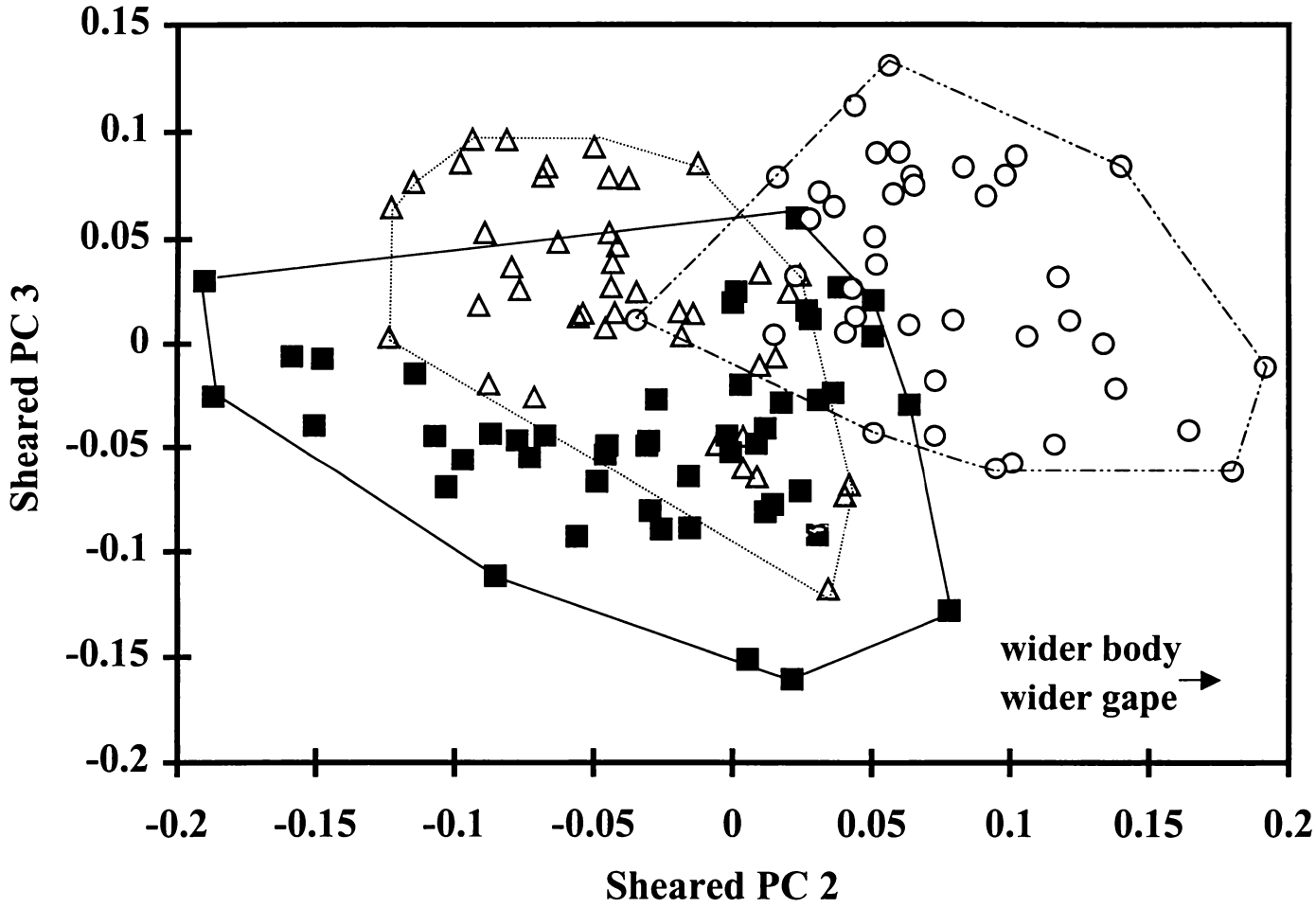


Figure 2-5. Sheared principal component scores for males of Laurel Dace (open circles), *Phoxinus tennesseensis* (open triangles), and *Phoxinus erythrogaster* (closed squares).

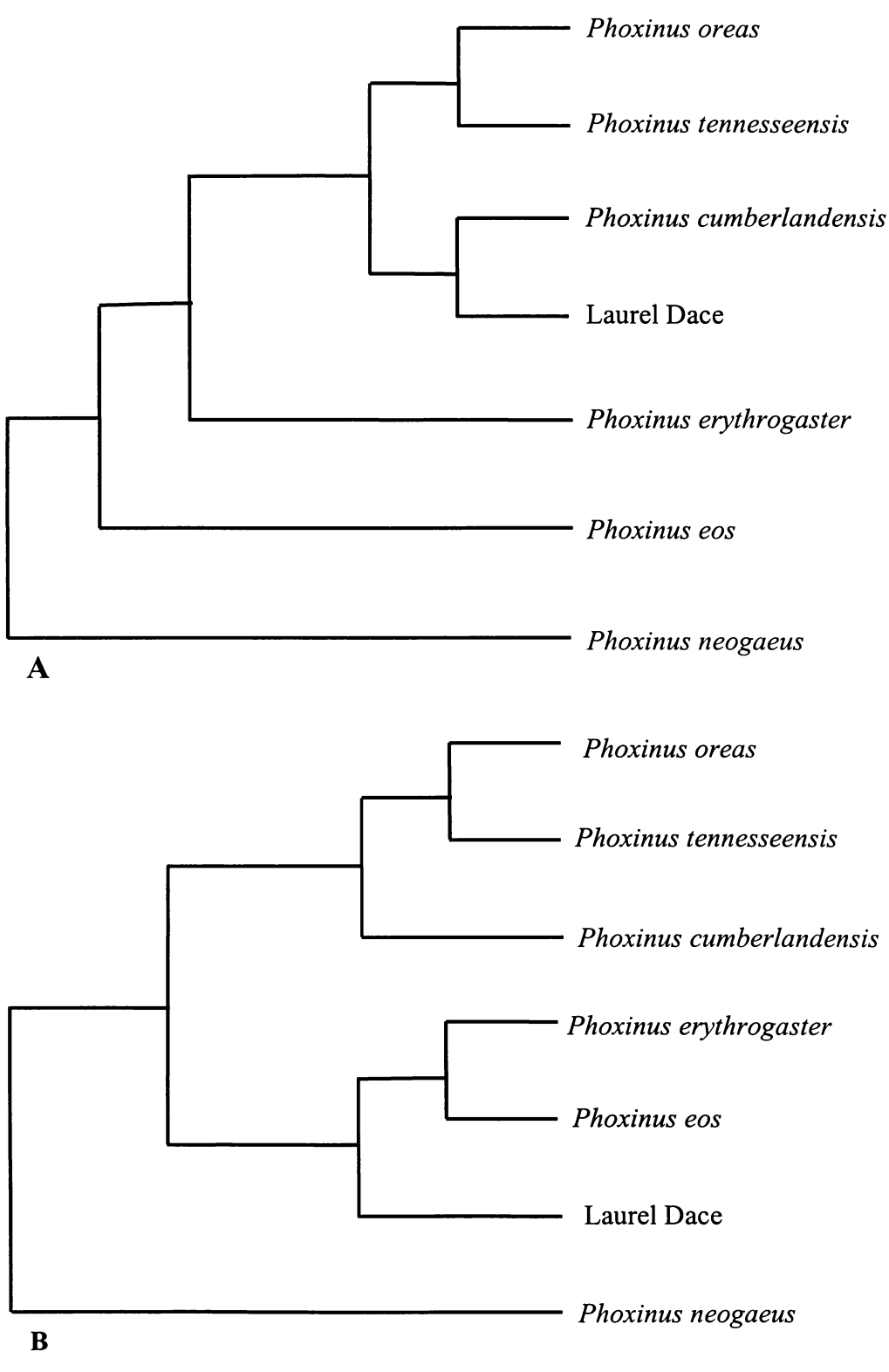


Figure 2-6. Hypothesized relationships of Laurel Dace to the Nearctic *Phoxinus*. A) Tree based on shared nuptial characteristics; B) Tree based on possession of two continuous lateral stripes.

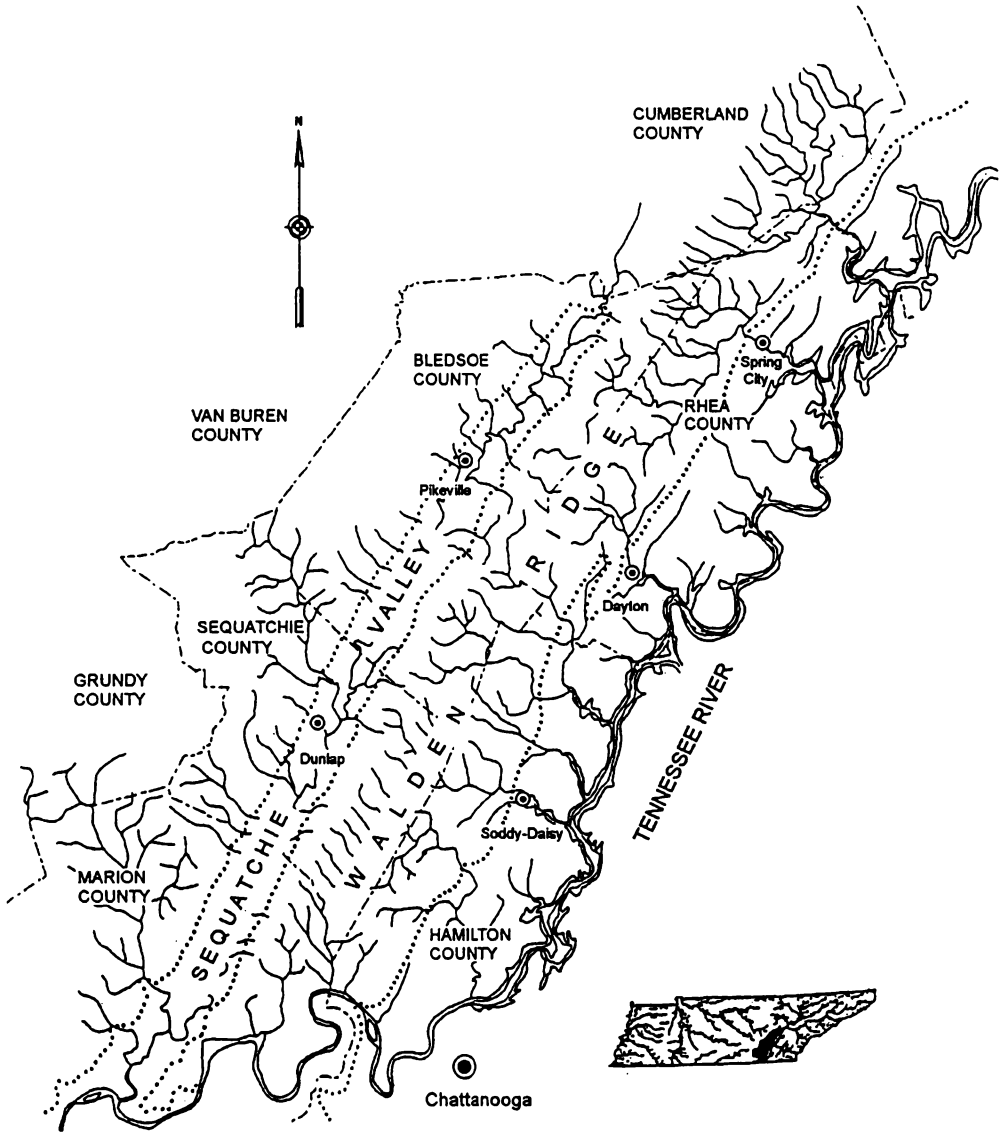


Figure 2-7. Political boundaries and names of cities in the study area. The dotted line represents the Cumberland Escarpment.

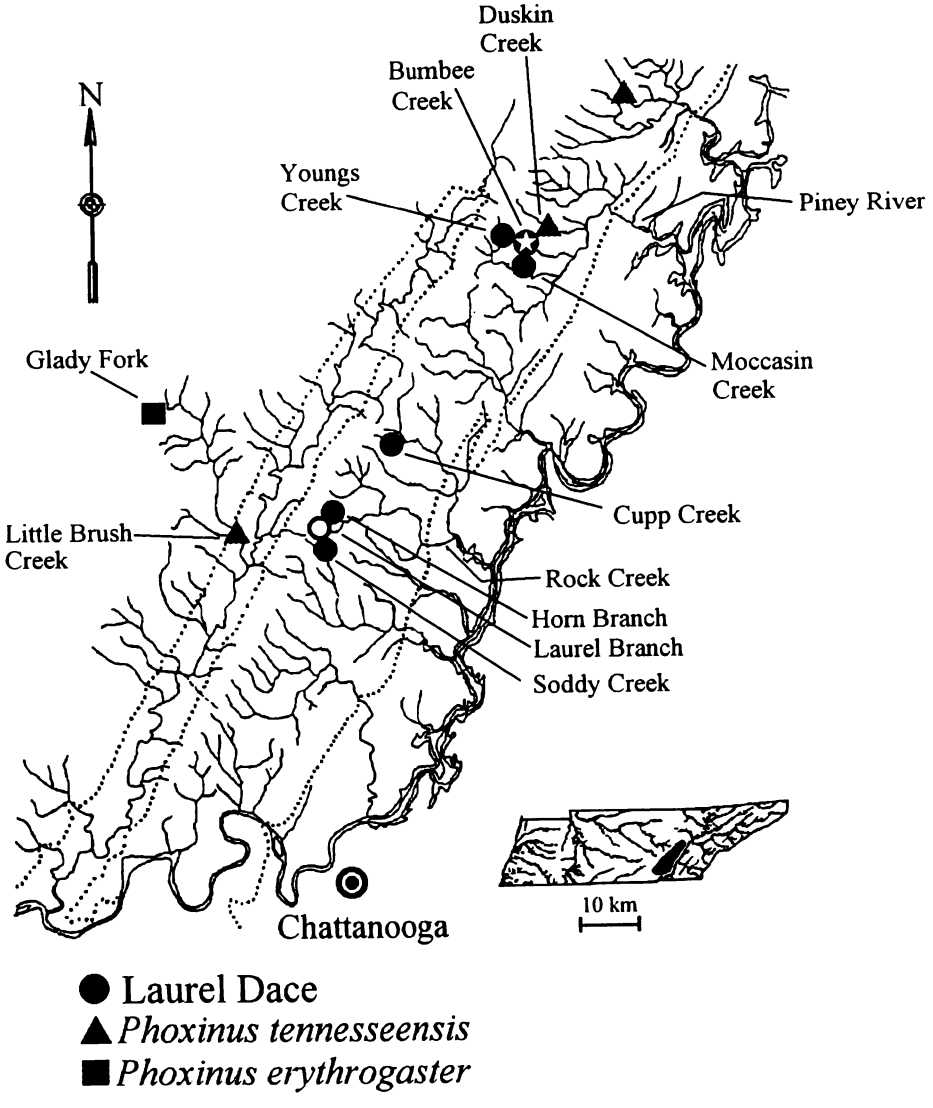


Figure 2-8. Known locations of Laurel Dace and proximate populations of *Phoxinus tennesseensis* and *Phoxinus erythrogaster*. The open circle represents an extirpated population of Laurel Dace, the star is the type locality for Laurel Dace and the dotted line represents the Cumberland Escarpment

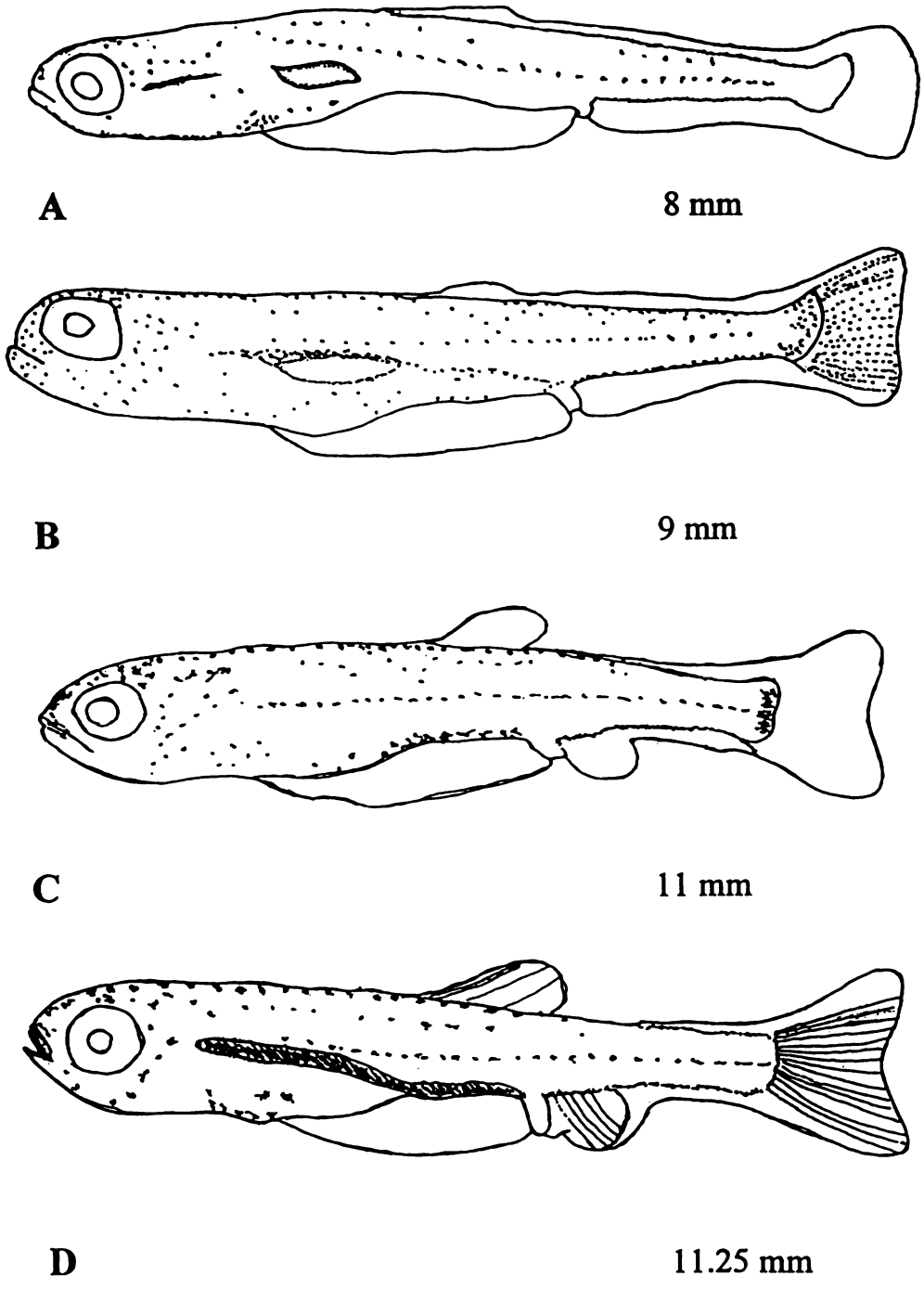


Figure 2-9. Larval Laurel Dace.

Appendix 2-B. Locality data for all sites surveyed for Laurel Dace from 1993-1997 (Figure 2-1).

1. Sulpher Creek, 1.1 mi. upstream of confluence with Suck Creek, Prentice-Cooper State Forest ca. 9 air mi. SE Whitwell, Marion Co., TN. 10 March 1995.
2. Short Creek at only road crossing, ca. 1.3 mi. upstream of confluence with Mullens Creek, ca. 7 air mi. se Whitwell, Prentice-Cooper State Forest, Marion Co. TN. 10 March 1995.
3. Mullens Creek at Persimmon Branch Rd., Prentice-Cooper State Forest, ca. 0.5 air mi. downstream of confluence with Shelton Creek, ca. 5.7 air mi. se Whitwell, Marion Co., TN. 10 March 1995.
4. Hurricane Creek at northern boundary of Prentice-Cooper State Forest, ca. 5.8 air mi. ese Whitwell, Marion Co., TN. 10 March 1995.
5. Northernmost branch of Mullens Creek, off of Dixie Lane, ca. 8 air mi. ese Whitwell, Marion Co., TN. 10 March 1995.
6. North Suck Creek just downstream of Johnson Spring ca. 5.8 air mi. ene Whitwell, Marion Co., TN. 9 March 1995.
7. Boston Branch, ca. 0.2 mi. upstream of Boston Branch Lake, above and below powerline crossing, ca. 14.6 air mi. nw of Chattanooga, Hamilton Co., TN. 9 March 1995.
8. Second unnamed tributary to Hixson Branch looking downstream, ca. air 12.5 mi. nw Chattanooga, Hamilton Co., TN. 9 March 1995.
9. Hixson Branch, 0.75 mi. upstream of confluence with N. Chickamauga Creek, ca. 14 air mi. nw Chattanooga, Hamilton Co., TN. 9 March 1995.
10. Unnamed tributary to N. Chickamauga Creek. It is the next nw flowing stream up from Hixson Branch, ca. 12.4 air mi. nw Chattanooga, Hamilton Co., TN. 9 March 1995.
11. Standifer Creek, ca. 2 air mi. upstream of its confluence with Brimer Creek, ca. 9.8 air mi. s Dunlap, Sequatchie Co., TN. 9 March 1995.
12. Brimer Creek, ca. 1.8 mi. upstream of its confluence with Standifer Creek, ca. 8.5 air mi. s Dunlap, Sequatchie Co., TN. 9 March 1995.
13. Frederick Creek at Frederick Creek road crossing, off of Patton Rd., ca. 7.8 air mi. sse Dunlap, Sequatchie Co., TN. 17 March.
14. McGrew Creek ca. 0.7 mi. upstream of confluence with Frederick Creek, ca. 8.6 air mi. se Dunlap, Sequatchie Co., TN. 17 March 1995.
15. Cooper Creek off of Pete Lewis Road, ca. 5.2 air mi. s Dunlap, Sequatchie Co., TN.

23 March 1995.
16. Sawmill Creek above Henson Gap Rd., ca. 5 air mi. ese Dunlap, Sequatchie Co., TN. 23 March 1995.
17. Unnamed tributary to Gray Creek at Lewis Chapel Rd., near the intersection of Lewis Chapel and Snyder Loop, ca. 4.9 air mi. ese Dunlap, Sequatchie Co., TN. 7 April 1995.
18. Unnamed tributary to Gray Creek ca. 0.6 air mi. upstream of Lewis Chapel Rd., ca. 5.5 air mi. e Dunlap, Sequatchie Co., TN. 22 July, 1996.
19. Gray Creek ca. 1.1 air mi. upstream of Lewis Chapel Rd. and ca. 5.7 air mi. e Dunlap, Sequatchie Co., TN. 22 July 1996.
20. Gray Creek above Lewis Chapel Rd., ca. 5.6 air mi. e Dunlap, Sequatchie Co., TN. 7 April 1995.
21. Unnamed tributary to Soddy Creek ca. 2 mi. downstream of Lewis Chapel Rd., ca. 8.5 air mi. e of Dunlap, Sequatchie Co., TN. 18 July, 1996.
22. Soddy Creek above TN Hwy 111, ca. 9 air mi. e Dunlap, Sequatchie Co., TN. 25 August 1996.
23. Soddy Creek above and below Wolf Branch road, ca. 16 air mi. s Pikeville, Bledsoe Co. TN.
24. Board Camp Creek just upstream from TN Hwy 111, ca. 6.7 air mi. nnw Soddy-Daisy, Hamilton Co., TN. 25 August 1996.
25. Right unnamed tributary to Board Camp Creek just upstream from TN Hwy 111, ca. 6.7 air km nnw Soddy-Daisy, Hamilton Co., TN. 25 August 1996.
26. Unnamed tributary to Big Possum Creek, downstream from Hughes Rd., ca. 16.8 air mi. s Pikeville, Bledsoe Co., TN. 29 June 1994.
27. Big Possum Cr. at road crossing 0.9 mi. n Jones Gap Road. The road the stream crosses is about 50 m e of Flat Top Church, Hamilton Co., ca. 7.3 air mi. nnw of Soddy-Daisy, Hamilton Co., TN. 31 March 1995.
28. Little Possum Creek at Hughes Branch, ca. 3.4 mi. upstream of confluence with Big Possum Creek, Hamilton Co., ca. 7.5 air mi. nnw Soddy-Daisy, TN. 23 March 1995.
29. Big Possum Creek ca. 1 mi. upstream of Hamilton-Bledsoe Co. line, ca. 4.1 air mi. nw of Soddy-Daisy, Hamilton Co., TN. 7 April 1995.
30. Rock Creek above confluence with Stewart Branch ca. 14.1 air mi. s Pikeville, Bledsoe Co., TN. 7 March 1995.
31. Stewart Branch of Rock Creek downstream of Blane Smith Rd., ca. 14.5 air mi. ssw Pikeville, Bledsoe Co., TN. 7 March 1995.
32. Laurel Branch of Rock Creek above Blane Smith Road, ca. 14.8 air mi. ssw

Pikeville, Bledsoe Co., TN. 7 March 1995.
33. Laurel Branch of Rock Creek above Hendon Road, ca. 14.8 air mi. ssw Pikeville, Bledsoe Co., TN. 16 March 1995.
34. Horn Branch of Rock Creek above Hendon Road, ca. 13.6 air mi. ssw Pikeville, Bledsoe Co., TN. 16 March 1995.
35. Suzanne Branch above and below Hendon Road, ca. 12.5 ssw Pikeville, Bledsoe Co., TN. 16 March 1995.
36. Suzanne Branch above Suzanne Rd., ca. 12.4 air mi. ssw Pikeville, Bledsoe Co., TN. 30 March 1995.
37. Skiles Creek just above confluence with Reel Creek, ca. 10.6 air mi. ssw Pikeville, Bledsoe Co., TN. 24 March 1995.
38. Smith Creek above confluence with Hunt Branch, ca. 10.2 air mi. ssw Pikeville, Bledsoe Co., TN. 2 August 1994.
39. Hunt Branch above confluence with Smith Creek, ca. 10.6 air mi. ssw Pikeville, Bledsoe Co., TN. 2 August 1994.
40. McGill Creek ca. 0.7 mi. upstream from Hendon Rd. crossing, Bledsoe Co., ca. 10.3 air mi. wsw of Dayton, Rhea Co., TN. 31 March 1995.
41. Unnamed tributary to McGill Creek at Graysville Rd. crossing, Bledsoe Co., ca. 10.4 air mi. w of Dayton, Rhea Co., TN. 30 March 1995.
42. Cupp Creek at rear of Jessie Sullivan property, ca. 0.5 mi. nne of Hendon and Brayton Rd. intersection, Bledsoe Co., ca. 9.1 air mi. W of Dayton, Rhea Co., TN. 31 March 1995, 27 June 1996.
43. Unnamed tributary to Roaring Creek adjacent to New Harmony Rd., Bledsoe Co., TN., ca. 9 air mi. w Dayton, Rhea Co., TN. 26 June 1996.
44. Brush Creek at Ogden Road, ca. 5.7 air mi. w Dayton, Rhea Co., TN. 27 June 1996.
45. Roaring Creek at New Harmony Rd., ca. 6.2 air mi. se Pikeville, Bledsoe Co., TN. 27 August 1993.
46. Roaring Creek at Wooden Loop Rd., ca. 5 air mi. se Pikeville, TN. 12 December 1993.
47. Morgan Creek at Jewel Rd., ca. 3.9 air mi. wnw Dayton, Rhea Co., TN. 12 December 1993.
48. Walkertown Branch at Walkertown Rd., ca. 5 air mi. wnw Dayton, Rhea Co., TN. 12 December 1993.
49. Tigues Creek at Riggs Rd., ca. 6.2 air mi. nw Dayton, Rhea Co., TN. 12 December 1993.
50. Polebridge Creek at Summer City Rd. near junction with TN Hwy 30, ca. 3.9 air mi. ese of Pikeville, Bledsoe Co., TN. 17 November 1995.

51. Lowry Creek at Summer City Road, 6.4 air mi. e Pikeville, Bledsoe Co., TN. 27 June 1996.
52. Coalbank Br. ca. 5.1 air mi. e Pikeville, Bledsoe Co., TN. 4 September 1994.
53. Henderson Creek just above confluence with Double Branch, ca. 7.3 air mi. e Pikeville, Bledsoe Co., TN. 27 June 1996.
54. Henderson Creek at logging rd. crossing ca. 0.3 mi. above confluence with Mitts Creek, ca. 5.5 air mi. ene Pikeville, Bledsoe Co., TN.
55. Unnamed tributary to Double Branch ca. 0.6 mi. downstream of Frazier Spring, ca. 7.4 air mi. e Pikeville, Bledsoe Co., TN. 5 June 1996.
56. Henderson Creek at Liberty Hill Road, on the Rhea-Bledsoe Co. line, ca. 7.8 air mi. nnw Dayton, Rhea Co., TN. 27 June 1996.
57. Unnamed tributary to Laurel Creek ca. 3 mi. downstream of Sinclair Lake, ca. 4.1 air mi. n Dayton, Rhea Co., TN. 5 June 1996.
58. Laurel Creek ca. 2.4 mi. downstream of Sinclair Lake, ca. 4.6 air mi. n Dayton, Rhea Co., TN. 5 June 1996.
59. Laurel Creek at logging rd. 0.5 mi. downstream of Sinclair Lake, ca. 6 air mi. nnw Dayton, Rhea Co., TN. 3 March 1996.
60. Bonine Creek at Liberty Hill Rd., ca. 5.6 air mi. n Dayton, Rhea Co., TN. 4 September 1994.
61. Bonine Creek at Bill Vaughn Rd., ca. 7.1 air mi. n Dayton, Rhea Co., TN. 3 March 1996.
62. Piney Creek at Nelson-Harrison Rd., ca. 7.4 air. mi. n Dayton, Rhea Co., TN. 3 March 1996.
63. McSherley Branch at logging rd. crossing, ca. 9.1 air mi. sw Spring City, Rhea Co., TN. 23 May 1996.
64. Cooper Branch at Cooper Branch Rd., ca. 9.4 air mi. ne Pikeville, Bledsoe Co., TN. 23 August 1996.
65. Mocassin Creek at 3rd Summer City Road crossing, ca. 10.3 air mi. nw Pikeville, Bledsoe Co., TN. 14 May 1996.
66. Unnamed tributary to Mocassin Creek at Fire Tower Rd., ca. 10.3 air mi. ne Pikeville, Bledsoe Co., TN. 14 May 1996.
67. Mocassin Creek at 2nd Summer City Road crossing, ca. 11 air mi. nw Pikeville, Bledsoe Co., TN. 16 November 1996.
68. Mocassin Creek at 1st Summer City Road crossing, ca. 1 mi. sw Milo and ca. 12.2 air mi. NW Pikeville, Bledsoe Co., TN. 14 May 1996.
69. Maple Branch of Piney Creek, at logging road crossing, ca. 7.5 air mi. sw Spring City, Rhea Co., TN. 23 May 1996.

70. Mocassin Creek at logging road crossing, ca. 1 mi. upstream of confluence with Piney Creek, ca. 6.7 air mi. sw Spring City, Rhea Co., TN. 23 May 1996.
71. Youngs Creek ca. 0.4 mi. upstream of confluence with Mocassin Creek, ca. 7.3 air mi. sw Spring City, Rhea Co., TN. 4 July 1996.
72. Bumbee Cr. at logging rd., ca. 6.2 air mi. wsw Spring City, Rhea Co., TN. 10 May 1996.
73. Bumbee Creek above confluence with Maplelush Creek, ca. 5.9 air mi. wsw Spring City, Rhea Co., TN. 16 November 1996.
74. Maplelush Creek above confluence with Bumbee Creek, ca. 5.9 air mi. wsw Spring City, Rhea Co., TN. 16 November 1996.
75. Duskin Creek at Walden Mountain Rd., ca. 4.7 air mi. w Spring City, Rhea Co., TN. 10 May 1996.
76. Newby Branch at Newby Branch Forest Camp, Forest Camp Rd., ca. 5.3 air mi. w Spring City, Rhea Co., TN. 10 May 1996.
77. Duskin Creek at Shut-in Gap Road, ca. 13.8 air mi. nw Pikeville, Bledsoe Co., TN. 14 May 1996.
78. Stinging Fork ca. 1 mi. upstream of Stinging Fork Falls, ca. 4.3 mi. wnw Spring City, Rhea Co., TN. 27 June 1996.
79. Soak Creek, just above confluence with Sweeney Branch, ca. 16.5 air mi. ne Pikeville, Bledsoe Co., TN. 28 June 1996.
80. Dunlap Creek just below confluence of Jewett Branch and Pond Cove Creek ca. 18.1 air mi. neE Pikeville, Bledsoe Co., TN. 5 July 1996.
81. Little Piney River at TN Hwy. 68 crossing, ca. 3.8 air mi. n Spring City, Rhea Co., TN. 5 July 1996.
82. Coal Creek upstream of TN Hwy. 68, ca. 13.7 air mi. se Crossville, Cumberland Co., TN. 5 July 1996.
83. Whites Creek at Possum Trot Rd., ca. 7.5 air mi. nne Spring City, Rhea Co., TN. 17 February 1996.
84. Tom Harris Branch at Alloway Rd., ca. 10.8 air mi. se Crossville, Cumberland Co., TN. 17 February 1996.
85. Sandy Creek at Alloway Rd., ca. 10.9 air mi. se Crossville, Cumberland Co., TN., 17 February 1996.
86. Sandy Creek at mouth of unnamed tributary, ca. 1.5 mi. upstream of Alloway Rd. crossing, 12.1 air mi. se Crossville, Cumberland Co., TN., April 1996.
87. Licklog Branch at Dogwood Rd., ca. 4.1 air mi. s Ozone, Cumberland Co., TN. 8 April 1996.
88. Day Branch upstream of confluence with Fall Cr., ca. 2.9 air mi. sse of Ozone,

Cumberland Co., TN. 8 April 1996.
89. Mammys Creek adjacent to US Hwy. 70, ca. 1.5 air mi. ese Ozone, Cumberland Co., TN. 8 April 1996.
90. Fall Creek between US Hwy. 70 and I-40, Ozone, Cumberland Co. TN. 8 April 1996.
91. Pond Creek downstream of logging rd. crossing, ca. 10.5 air mi. sw Pikeville, Bledsoe Co., TN. 17 July, 1996.
92. Rattlesnake Branch above confluence with Flatrock Branch, ca. 12.8 air mi. sw Pikeville, Bledsoe Co., TN. 17 July 1996.
93. Flatrock Branch above and below confluence with Rattlesnake Branch, ca. 12.8 air mi. sw Pikeville, Bledsoe Co., TN. 17 July 1996.
94. Blair Creek just above confluence with Big Brush Creek, ca. 8.6 air mi. sw Pikeville, Bledsoe Co., TN. 4 July 1996.
95. Big Brush Creek just downstream of Van Buren Co. line, ca 7.6 air mi. n Dunlap, Sequatchie Co., TN. 16 July 1996.
96. Big Brush Creek ca. 0.4 mi. upstream of confluence with Glady Fork, ca. 9.9 air mi. nnw Dunlap, Sequatchie Co., TN. 16 July 1996.
97. Glady Fork above and below confluence with Spring Branch, ca. 10 air mi. nnw Dunlap, Sequatchie Co., TN. 16 July 1996.
98. Glady Fork downstream of TN Hwy. 111, ca. 11.5 air mi. nnw Dunlap, Sequatchie Co., TN. 3 July 1996.
99. Long Fork just above confluence with Big Brush Creek, ca. 7.7 air mi. n Dunlap, Sequatchie Co., TN. 4 July 1996.
100. Bird Fork ca. 0.4 mi. upstream from confluence with Long Fork and ca. 7.7 air mi. nnw Dunlap, Sequatchie Co., TN. 4 July 1996.
101. Kelly Creek ca. 1.7 mi. upstream from confluence with Big Brush Creek, ca. 7 air. mi. n Dunlap, Sequatchie Co., TN. 4 July 1996.
102. Unnamed tributary to Reynolds Creek which is the next upstream tributary from Hurricane Branch, ca. 5 air mi. nnw Dunlap, Sequatchie Co., TN. 16 July 1996.
103. Reynolds Creek below confluence with unnamed tributary and ca. 1.4 mi. upstream of Hurricane Branch, ca. 5 air mi. nnw Dunlap, Sequatchie Co., TN. 16 July 1996.
104. Little Brush Creek above and below confluence with Roberson Fork, ca. 5 air mi. nw Dunlap, Sequatchie Co., TN. 3 July 1996.
105. Roberson Fork above confluence with Little Brush Creek, ca. 5 air mi. nw Dunlap, Sequatchie Co., TN. 3 July 1996.
106. Little Brush Creek above TN Hwy. 111, ca. 2.4 air mi. n Dunlap, Sequatchie Co., TN. 22 July 1996.

107. Kelley Creek just above confluence with Dicks Branch, ca. 8.8 air mi. wsw Dunlap, Sequatchie Co., TN. 17 July, 1996.
108. Griffith Creek ca. 4 air mi. n Whitwell, Marion Co., TN. 23 July 1996.
109. Grays Creek at logging rd. ca. 3.1 air mi. above confluence with Little Sequatchie River, ca. 5.7 air mi. ne Whitwell, Marion Co., TN. 23 July 1996.
110. Johns Branch at Pocket Creek Rd. 3.5 air mi. nw Whitwell, Marion Co., TN. 23 July 1996.
111. Bridge Creek at Owl Hollow Rd. 2.5 air mi. ne Whitwell, Marion Co., TN. 23 July 1996.
112. Skillern Cr. at Yeargan Rd., New Harmony, ca. 4 air mi. se Pikeville, Bledsoe Co., TN. 17 November, 1995.

PART 3: PHYLOGENETIC SYSTEMATICS AND ZOOGEOGRAPHY OF THE NEARCTIC MEMBERS OF THE MINNOW GENUS *PHOXINUS* (CYPRINDAE: CYPRINIFORMES)

INTRODUCTION

Relationships among members of the cyprinid genus *Phoxinus* have been examined by several researchers over the past 25 years. A key goal of most of these studies was to determine whether the Nearctic members of the genus (previously *Chrosomus*) should be synonymized with the Eurasian *Phoxinus*. This synonymy was first proposed by Banarescu (1964). His study was criticized (eg. McPhail and Lindsey, 1970; Scott and Crossman, 1973) for lack of evidence, but was adopted by the American Fisheries Society Names Committee (Bailey et al., 1970; Robins et al., 1980). In all recent studies (Mahy, 1975; Howes, 1985; Coburn and Cavender, 1992; Simons and Mayden, 1998), Banarescu's (1964) synonymy was supported.

Most of the above cited studies relied on osteological characters to infer relationships; however, Joswiak (1980) used allozyme techniques to examine this question. In his study, the Eurasian *Phoxinus phoxinus* was sister to the Nearctic *P. neogaeus* and that pair was sister to the remainder of the North American members of the genus. In the time since Joswiak completed his study, two new North American *Phoxinus* species have been discovered. *Phoxinus tennesseensis* was described by Starnes and Jenkins (1988) and the Laurel Dace (*Phoxinus* sp.) is recognized as being a new species (Skelton, 1995, 2001; Warren, 2000). Understanding the relationships of these two species to the remainder of the genus provides additional evidence regarding the evolution of the fish fauna of the upper Tennessee River drainage. Also, elucidation of the relatedness of the Laurel Dace to the remainder of the genus may assist with

conservation efforts for this rare species. Laurel Dace are known from only six populations, are imperiled, and in need of management. One method of insuring the continued existence of a species is to breed it in captivity and release the progeny into known or presumed historic localities. Instead of testing techniques on a rare species, it makes sense to learn propagation techniques from a closely related surrogate. This method is currently employed by Conservation Fisheries Incorporated for propagation of rare fishes, and the Southeast Aquatic Research Institute for rare mussels (P. Johnson, SARI, pers. comm.). Thus, a phylogenetic systematics study of Laurel Dace relative to the Nearctic *Phoxinus* species is needed to allow informed decisions regarding captive propagation and possible introductions of Laurel Dace.

MATERIALS AND METHODS

Morphological and biochemical methods were used to assess phylogenetic relationships of the Nearctic *Phoxinus* and three outgroup taxa. Five data sets were employed to this end. The first consisted of allele frequencies calculated from gene products resolved with starch gel electrophoresis, and the second of 30 morphological characters. The third was a subset of the morphological data set exclusive of all characters construed as being related to feeding. The fourth and fifth data sets employed a combination of the allele frequency data and the two morphological data sets, respectively.

Outgroup Choice.—Outgroup comparison is widely used in phylogenetic analysis to assist with determinations of character polarity (Maddison et al., 1984). In the ideal situation, an outgroup is the sister group to the group being studied (ingroup). Determining the sister group of the genus *Phoxinus* is problematic. Two recent studies assessed generic level relationships among the North American Cyprinidae, but their results were not in agreement concerning the placement of *Phoxinus*.

Coburn and Cavender (1992) used lepidological and osteological characters to examine relationships among North American cyprinids. Their analysis revealed three major clades: the shiner clade, the western clade, and the chub clade (Figure 3-1A). They hypothesized that the chub and western clades are sister to one another and the shiner clade is sister to that pair. Their study indicated that *Phoxinus* is one of the basal members of the chub clade along with the northeast Asian genera *Tribolodon* and *Rhynchocypris*.

Simons and Mayden (1997, 1998) investigated cyprinid generic relationships using molecular markers. Their studies suggest that *Phoxinus* is the basal member of the western clade (sensu Coburn and Cavender, 1992, Figure 3-1B). The western clade was sister to the open posterior myodome (OPM) clade (= shiner clade of Coburn and Cavender, 1992) plus the chub clade. Because the genus *Notropis* was determined in both studies to be well outside of *Phoxinus*, I used a member of that genus as an outgroup (*N. telescopus*). When a single outgroup taxon is used, there is a risk that one or more characters will suggest a false relationship between the ingroup and outgroup. Inclusion of additional outgroup taxa reduces this chance. Thus, I included the genus *Luxilus*

which is thought to be closely related to *Notropis*. *Semotilus atromaculatus* was also included in these analyses because the relationship of *Phoxinus* to *Semotilus* differs in the above cited studies. Use of this taxon allowed further tests of its placement among North American cyprinids.

Allozyme methods.—Representatives of each species of *Phoxinus* and the three outgroup taxa were collected from at least one locality within their respective ranges (Appendix 3-B). Because *P. cumberlandensis* is Federally Listed as threatened, captively reared specimens of this species were acquired from Conservation Fisheries Incorporated, Knoxville, Tennessee. All other specimens were collected from the wild with minnow traps, seines, or electrofishing gear. Choice of localities for a given *Phoxinus* species was driven primarily by what was readily available. In general, this worked fine because the only questions of substantial intraspecific variation involve *Phoxinus erythrogaster*. This species is recognized as being variable across its range (Robison and Buchanan, 1988), particularly in aspects of nuptial coloration, and is likely a species complex (Etnier and Starnes, 1993). Because of this potential problem, I used *P. erythrogaster* from as close to the type locality as possible for the phylogenetic analysis. In the original description of *P. erythrogaster*, Rafinesque (1820) stated that he "saw it in the Kentucky River". This vague statement leaves some question as to the exact location from which the fish was described. Thus, I used specimens from Red Lick Creek, a tributary to Station Camp Creek, which empties into the Kentucky River. As part of the allozyme study, I included specimens from the Tennessee River drainage to get an idea of variation that might exist

among populations of the species. Except for specimens collected in Canada (*P. eos and P. neogaeus*), all fish were transported alive to the laboratory and euthanized with an overdose of tricaine methanesulfate (MS-222). Fish were immediately dissected and samples of eye, muscle, and liver tissue were collected. Canadian fish were wrapped alive in aluminum foil, placed in liquid nitrogen, and dissected upon return to the laboratory. An equal volume (ml/g) of grinding buffer was added to each tissue sample and then each sample was ground by hand until it became a viscous liquid. Samples were centrifuged for 3-5 minutes at 14,000 rpm, and the supernatant was stored at –80 C until it was to be used for electrophoresis.

Methods of starch gel electrophoresis generally follow Selander et al. (1971) and McCracken and Wilkinson (1988). Supernatant fractions were absorbed onto 6 x 9 mm Whatman #1 paper wicks and subjected to electrophoresis in 12% starch gels. Samples were screened for the products of 21 presumptive gene loci coding for 14 enzymes. Staining procedures were as described by Selander et al. (1971) and Harris and Hopkinson (1976) and locus designations follow Buth (1984). Loci examined, tissue types, and buffer systems are presented in Table 3-1. Electromorphs were assigned lowercase letters by increasing mobility from the origin and are applicable only to this study. Mean number of alleles per locus and percentages of polyallelic loci were determined with the BIOSYS-2 computer program. This program is a slightly modified version of BIOSYS-1 (Swofford and Selander, 1981).

Phylogenetic Methods.—Because phylogenetic analysis of allozyme data is somewhat controversial (Swofford et al., 1996), a variety of methods was used to assess species relationships (Wood, 1996). First, distance matrices were calculated from genotype frequencies with the BIOSYS-2 computer program. The distance algorithms used were arc and chord of Cavalli-Sforza and Edwards (1967), Rogers (1972), modified Rogers (Wright, 1978), and Prevosti (Wright, 1978). The distances were used to build Wagner trees (Farris, 1972) which were then tested against the allozyme frequency data with the FREQPARS computer program (Swofford and Berlocher, 1987). FREQPARS uses the principle of maximum parsimony to minimize the amount of allele frequency change necessary to build a given tree. All Wagner trees were input into FREQPARS with the USERTREE option. A similarity matrix using Rogers (1972) method was also generated with BIOSYS-2. Using this matrix, a phenogram was created with the UPGMA algorithm of Sneath and Sokal (1973).

Allozyme data were also coded discretely with each locus considered a character and allelic combinations as character states (Table 3-2B, Appendix 3-D; Buth, 1984). This method has been criticized because of the lack of homology information available for polarization of characters. To reduce this problem, Mabee and Humphries (1993) recommend using stepmatrices. With this method, a cost of one is assigned for the loss or gain of an allele at a given locus. The stepmatrices were subjected to the principle of maximum parsimony using PAUP 3.1 (Swofford, 1993). The PAUP program used for analysis of the discrete allozyme data is displayed in Appendix 3-E.

Two morphological data sets were subjected to the principle of maximum parsimony using PAUP. Most of the characters used were secondary sexual characteristics exhibited by males in nuptial condition (Figure 3-2). The first analysis used 30 morphological characters (Table 3-2A; Appendix 3-C). In the second analysis, seven characters construed as being associated with feeding were removed. Feeding characters have the potential to evolve quickly and may not be useful for analysis of relationships. All of the tree topologies generated with PAUP were examined with FREQPARS against the background allele frequency information as described above.

Finally, the allozyme and morphological data sets were combined and subjected to the principle of maximum parsimony in PAUP. As above, all of the trees derived with these data sets were tested against the allele frequency information with FREQPARS.

RESULTS

Allozyme analyses.—Genotypic arrays of the 21 loci examined by population are displayed in Appendix 3-F. Levels of heterogeneity and percentages of polymorphic loci were highly variable within *Phoxinus* (Table 3-3). Mean heterozygosity from direct counts ranged from 0.0 in Laurel Dace to 0.111 for *P. eos*. Populations of *Phoxinus erythrogaster* also had a high numbers of heterozygotes (0.050-0.110). Species with the lowest levels of genetic variability were Laurel Dace, *P. cumberlandensis*, and *P. neogaeus*.

Of the 21 loci examined, Gapdh-A and Idh-A were monoallelic across all taxa. Further, the Lap-A and Ldh-A loci were monoallelic for all *Phoxinus*. The hypothesized closely related pair of *P. tennesseensis* and *P. oreas* exhibited fixed differences at Ck-A (*P. tennesseensis*, Ck-A^{aa}; *P. oreas* Ck-A^{bb}) and Sod-A (*P. tennesseensis*, Sod-A^{gg}; *P. oreas,* Sod-A^{ff}), corroborating the recent elevation of *P. tennesseensis* to species status (Starnes and Jenkins, 1988). The undescribed Laurel Dace was fixed for alternate alleles from all other *Phoxinus* spp. at four loci (Aat-A^{dd}, Gpi-B^{bb}, Pep-A^{gg}, and Sod-A^{dd}), also corroborating its hypothesized specific distinctiveness.

Variation between populations of the same species was minimal (Appendix 3-F). Exceptions to this were populations of Laurel Dace and *P. erythrogaster*. In Laurel Dace, the Bumbee Creek population showed a fixed allelic difference at the Ldh-B^{cc} locus from all other *Phoxinus* populations examined. The Bumbee Creek population further differs by showing polymorphism at the Mdh-B locus. These conclusions must be qualified because only five specimens were examined from Bumbee Creek and a total of only eight from Soddy Creek and Horn Branch.

Notable variation was observed among the populations of *P. erythrogaster*. A Tennessee River sample from Glady Fork, a tributary to the Sequatchie River, was fixed for the Idh-A^{ee} genotype. Only one of 22 individuals examined from the Kentucky River system had this genotype. Unfortunately, the other Tennessee River sample (Knob Creek) was not resolved at this or six other loci. The Pgdh-A locus was also polymorphic for the species. The Knob Creek population was the only one with the Pgdh-A^{ee} genotype out of all samples examined. The Gpi-A locus was interesting in that Redlick

Creek was fixed for the Gpi-A^{bb} genotype and Knob Creek was fixed for the Gpi-A^{ee} genotype. All other populations had combinations of the b and e alleles. The Glady Fork population was the only one possessing the GPI-A^{c} allele. Finally, all *P. erythrogaster* populations examined were fixed for the Mdh-C^{dd} genotype except for Knob Creek which was nearly fixed for an alternate allele (Mdh-C^{a}, Appendix 3-F)

Phylogenetic analyses.—A total of 18 strictly bifurcating trees was generated with the distance Wagner procedure in BIOSYS-2. Two were derived with the arc distance of Cavalli-Sforza and Edwards, three with the chord of Cavalli-Sforza and Edwards, four with Rogers, four with modified Rogers, and five with Prevosti. When these trees were tested against the background allele frequency data with FREQPARS, tree lengths ranged from 129.406-133.823. *Phoxinus* was monophyletic in all trees generated; however, groupings within the genus were variable. The most consistent grouping placed *P. cumberlandensis* sister to *P. oreas*. *Phoxinus neogaeus* was the basal member of the genus in two-thirds of the trees and sister to the sister pair of *eos-erythrogaster* in all remaining trees except one. This exception was a tree generated with the Prevosti distance, which turned out to the shortest tree examined (FREQPARS length = 129.406, Figure 3-3A). In that tree, *P. neogaeus* is sister to *P. eos*, with that pair sister to the remainder of the genus. The second shortest tree (FREQPARS length = 129.910) was found by all distance methods used (Figure 3-3B). This tree differed from the shortest tree by placing *P. erythrogaster* sister to *P. eos* and that pair sister to *P. neogaeus*. These

three species were sister to the remaining members of the genus, thus dividing the genus into two major clades.

Forty-two phylogenetic trees were generated with the other data sets using PAUP. A single most parsimonious tree (Figure 3-4A) was found with the complete morphological data set (PAUP length = 63; FREQPARS length = 135.04). This was the longest FREQPARS distance of any tree examined. Bootstrap values for this tree indicate strong support for a clade containing *P. oreas*, *P. tennesseensis*, and *P. cumberlandensis* (Figure 3-4B). This grouping was found in 16 of 18 trees generated with the distance methods, although in those trees, *cumberlandensis* was sister to *oreas* rather than to *tennesseensis*. *Phoxinus neogaeus* received strong support as the sister species to the entire genus whereas placement of *P. eos*, *P. erythrogaster*, and Laurel Dace is less certain. When the feeding characters were removed from the data set, two most parsimonious trees were found (Figures 3-5A, 3-5B, PAUP length = 49). These trees differed only in their placement of Laurel Dace and *P. cumberlandensis*. FREQPARS lengths for these two trees were 131.732 and 133.506, respectively. Bootstrap values indicated strong support for a monophyletic *Phoxinus* with *P. neogaeus* the sister species to the remainder of the genus (Figure 3-6).

The discretely coded allozyme data set resulted in five most-parsimonious trees, but only four were strictly bifurcating (PAUP length = 147). In all four trees, Laurel Dace was the basal member of a monophyletic *Phoxinus*. Additionally, *P. oreas* and *P. cumberlandensis* were sister in three of four trees. When these trees were run against the allozyme frequency data, two trees were shorter than the shortest tree derived from the

distance methods (FREQPARS length = 128.681 and 128.682). The bootstrap 50% majority rule consensus tree (Figure 3-7) supports a monophyletic *Phoxinus* but shows no resolution within the genus. There was also strong support for *Semotilus* as the sister to *Phoxinus*.

Each of the combined data sets produced 17 most-parsimonious trees. These trees indicated no resolution within *Phoxinus*, nor between *Phoxinus* and the outgroup species.

The UPGMA phenogram based on a matrix derived from Rogers (1972) genetic similarity was similar to a tree produced with the morphological data set minus the feeding characters (Table 3-4; Figure 3-5A). The phenogram differed by placing *P. erythrogaster* and *P. eos* as sister taxa rather than *P. eos* basal relative to *P. erythrogaster* (Figure 3-8).

The analyses based on genetic data are in disagreement concerning the placement of *Semotilus*. The consensus tree generated from the discrete allozyme data set indicates strong support for *Semotilus* as sister to *Phoxinus* (Figure 3-7), whereas the UPGMA phenogram places *Semotilus* closer to the other outgroup taxa (Figure 3-8).

DISCUSSION

Allozyme analyses.—Reduced genetic variability found in Laurel Dace and *P. cumberlandensis* may be a reflection of their relative rarity. Laurel Dace in particular, are restricted to only short stretches in a few isolated streams, and population sizes appear very small. The *Phoxinus cumberlandensis* specimens that I analyzed were F2 progeny

of captive individuals. This may partly explain lower levels of heterozygosity observed in this analysis. Because of the small sample sizes, however, these results are tentative.

Phylogenetic Analyses.—A total of 60 bifurcating trees generated with the above methods was analyzed against the background allele frequency information with the FREQPARS program. The tree with the shortest FREQPARS length (128.681) came from the discretely coded allozyme data set. This was one of five shortest trees generated in PAUP (lengths = 147). These trees are considered uninformative because of the lack of resolution indicated by the bootstrap 50% majority rule consensus tree (Figure 3-7).

The results of the combined data sets also indicated no resolution within *Phoxinus* or the outgroups. Seventeen trees were produced from each data set with no visible consistency, and are also considered uninformative.

The most perplexing result found in the study was the consistent sister grouping of *P. neogaeus* and *P. eos* as indicated by the discrete allozyme data. The shortest tree derived from distance methods (Prevosti, 129.406) also placed *P. neogaeus* sister to *P. eos*. The two loci that unite *P. neogaeus* and *P. eos* are Ck-A and Ck-B. At both loci, these fish were fixed for the Ck-A^{cc} and Ck-B^{cc} genotypes (Appendix 3-F). No other *Phoxinus* species shared these alleles; however, *Semotilus atromaculatus* was fixed for Ck-A^{cc} and Ck-B^{cc} and *Luxilus chrysocephalus* was fixed for Ck-A^{cc}. The sister relationship between *P. neogaeus* and *P. eos* contradicts hypotheses suggested by all previous studies (Mahy, 1975; Joswiak, 1980) and further clouds the debate concerning this enigmatic pair. Although they previously have not been considered sister species,

they hybridize freely where they are syntopic (Joswiak et al., 1982; Dawley et al., 1987). Apparently, the hybrids are all female and typically outnumber both parental species. Studies by Dawley et al. (1987) suggest that the hybrids reproduce gynogenetically. In this study, *Phoxinus neogaeus* and *P. eos* specimens were collected syntopically from each of the two localities where specimens were obtained. I examined all of the specimens prior to dissection to ensure I had no hybrids. Many hybrids were found in the collections from the Lake Saganagons tributary. These were removed prior to electrophoresis, and I ran what I thought were pure *P. eos* and *P. neogaeus*. However, during scoring of gels, I found more specimens that were hybrids. These were easily differentiated with the Pgm, and Sod diagnostic loci as reported by Joswiak et al. (1982). My observations and those made by Joswiak et al. (1982) indicate that intermediacy of morphological traits is not a reliable way of detecting *P. eos* x *P. neogaeus* hybrids.

In the analyses of morphological characters, *P. tennesseensis* and *P. oreas* received strong support as sister species and *P. neogaeus* was consistently the sister to the entire genus. Placement of Laurel Dace varied in trees produced with the morphological data sets (Figures 3-4, 3-5). When all of the morphological characters were used, Laurel Dace was basal to all *Phoxinus* species exclusive of *P. neogaeus*. This is because it possesses several feeding characteristics that are considered primitive (gut shape, shape of pharyngeal pad, reduced pharyngeal teeth, preferred food items). When the feeding characters were omitted, Laurel Dace was part of a clade consisting of *P. oreas*, *P. tennesseensis*, and *P. cumberlandensis* (Figure 3-5). This result makes the most sense in light of several probable synapomorphies found in these species. The black pigment on

the underside of the head of nuptial male *P. tennesseensis* and *P. oreas* was considered a synapomorphy by Starnes and Jenkins (1988). Laurel Dace exhibit this characteristic, thus indicating a close relationship to that pair. Similar tuberculation patterns of nuptial males are shared by these three species and *P. cumberlandensis*. The broken lower lateral stripe shared by *P. oreas* and *P. tennesseensis* is likely a synapomorphy as is the pearly white spot at the insertion of the dorsal fin of nuptial males (Figure 3-2). The latter character is also shared by *P. cumberlandensis*. The distribution pattern of the genus also suggests a close relationship between Laurel Dace, *P. cumberlandensis*, *P. oreas*, and *P. tennesseensis*. There is a problem with the resolution of *P. cumberlandensis* and Laurel Dace in these trees. In one tree, *P. cumberlandensis* is sister to *oreas*, *tennesseensis*, and Laurel Dace (Figure 3-5B), and in the second tree, the Laurel Dace holds the basal position of that clade (Figure 3-5A). Additional evidence supporting the topology in Figure 3-5A is seen in the UPGMA phenogram generated from Rogers (1972) genetic similarity matrix. In the phenogram, Laurel Dace is also basal to a clade consisting of *P. cumberlandensis*, *P. tennesseensis*, and *P. oreas* (Figure 3-8). Regarding *Phoxinus*, the only difference between Figure 3-8 and Figure 3-5A is the placement of *P. erythrogaster* and *P. eos* as sister species rather than *P. eos* basal relative to *P. erythrogaster*. Because of the partial congruency between these two trees, I consider both Figure 3-5A and 3-8 to represent viable hypotheses of the relationships among the Nearctic *Phoxinus*.

Zoogeography.—Several independent studies support the sister relationship between the Eurasian and Nearctic *Phoxinus* (Howes, 1985; Coburn and Cavender, 1992; Simons and Mayden, 1998). The most likely entry into North America by the ancestral *Phoxinus* was across the Beringia land connection between the Oligocene and the beginning of the Pleistocene (Lindsey and McPhail, 1986). This connection was apparently present for extended periods in the Tertiary, and also during Pleistocene glaciation events (Briggs, 1986).

The Nearctic *Phoxinus* are now widely distributed throughout the northern and central portion of North America with patterns partially explained by Pleistocene glaciation. The following is a description of the current distribution of the genus and hypotheses based on phylogenetic evidence that support the distributions.

Phoxinus cumberlandensis is endemic to the upper Cumberland River drainage of Tennessee and Kentucky (Figure 3-9; Starnes and Starnes, 1978) and is fairly widespread above Cumberland Falls. Based on historical records, O'bara (1985) found *P. cumberlandensis* was present in 41 streams throughout the upper Cumberland drainage. The number of extant populations is now known to be closer to 70. However, these populations are still considered to be remnants of populations that ranged in suitable habitat all over the upper Cumberland drainage above the falls. The reduced numbers are likely the result of extensive strip mining which occurs throughout the upper Cumberland drainage (Starnes and Starnes, 1978). An additional 10 populations are known from below the falls and the downstream limit of the species is near the mouth of the Rockcastle River (Burr and Warren, 1986). A single population was recently discovered

in the upper Powell River system in Virginia. This is the only known Tennessee River drainage population.

Phoxinus eos is found in boggy streams, lakes, and ponds in the northern United States and southern Canada. It is distributed from the Peace-Mackenzie drainage in British Columbia and Northwest Territory south to the upper Missouri drainage; east to Nova Scotia including all of the Great Lakes basins except the Lake Erie drainage of Ohio. Relict populations can be found in Massachusetts and New Jersey and the Sand Hills of Nebraska (Figure 3-10; Scott and Crossman, 1973; Stasiak, 1980a).

Phoxinus erythrogaster inhabits small upland streams and is widely distributed throughout the central and eastern United States (Figure 3-10). Its range extends from Iowa and southern Minnesota to the Ohio River drainages of Pennsylvania and West Virginia; south on either side of the Mississippi River to the Tennessee River system in Tennessee and Northern Alabama, and to the northern part of the Ozarks in northern Arkansas and northeast Oklahoma. The range of this species includes several disjunct relict populations. In the Missouri River drainage there are several populations in northeast Kansas and three from southwestern Minnesota/northwestern Iowa. There are also a few populations in the Mississippi River basin, Mississippi. The westernmost populations are found in the Mora River system of New Mexico (Sublette et al., 1990). In Arkansas, disjunct populations are known from Crowley's Ridge (Robison and Buchanan, 1988). In Tennessee, records from the Sequatchie River may be erroneous. There are no extant specimens and targeted survey efforts have failed to turn up reported populations. There is however an interesting population in Glady Fork; a small tributary

to Big Brush Creek which empties into the Sequatchie River (Figure 3-11). The farthest upstream Tennessee River locality was recently discovered near Chattanooga (E. Crews, pers. comm.)

Phoxinus neogaeus is widely distributed from the Arctic circle in the Mackenzie River drainage in northwestern Canada to the Atlantic drainages of New Brunswick, Maine, New Hampshire, and New York. Its distribution roughly mirrors that of *P. eos*. It is found north of the lower Great Lakes in Southern Ontario, Michigan, Wisconsin, and Minnesota (Figure 3-10). This fish is similar to *P. eos* and different from all other Nearctic species of *Phoxinus* in that it occurs in small boggy streams and lakes. Populations considered to be relicts are located in the Sand Hills of Nebraska and the Black Hills of South Dakota (Hubbs and Lagler, 1964; Stasiak, 1980b).

Phoxinus oreas is found in small streams in the mountains and piedmont of the central Atlantic slope from the Shenandoah system of Virginia, south to the Neuse drainage, North Carolina, and in adjacent mountains of the New drainage, North Carolina, Virginia, and rarely West Virginia (Jenkins and Burkhead, 1994). It is found in the extreme upper Tennessee drainage of Virginia, however these populations are thought to be of "bait bucket" origin (Figure 3-9). Stauffer et al. (1995) offer no explanation of a single outlying population in western West Virginia (Figure 3-9); however, I suspect it was also introduced by humans.

Phoxinus tennesseensis typically occurs in small, spring influenced streams of the Ridge and Valley province of eastern Tennessee and southeastern Virginia. The southern limit of the species appears to be near Chattanooga, Tennessee and its northern limit is

tributaries to the North, Middle, and South Fork Holston rivers (Figure 3-9). This species has been considered threatened by the State of Tennessee but many new populations have been discovered since its description. The most interesting records are near the species southern limit where it is parapatric with the Laurel Dace in the Piney River system (Figure 3-11)

Laurel Dace are known from only six streams on the Walden Ridge portion of the Cumberland Plateau in southeastern Tennessee (Figure 3-11). However, two specimens of a dace that may represent Laurel Dace were collected in the upper Clinch River system of Virginia in the spring of 1999 (Figure 3-9). When I examined the specimens, they had remnants of black pigment on the underside of the head and two lateral black stripes like Laurel Dace. However, their gut morphology and fin shape resembled that of *P. erythrogaster*. A second population was discovered in November 1999 and an attempt to collect nuptial individuals was made in April 2000. Unfortunately, nuptial individuals were not found. In order to make a confident taxonomic decision, additional specimens are needed, particularly nuptial males. Although the taxonomic status of the Virginia populations is unresolved, they are either Laurel Dace or a very close relative.

Joswiak (1980) hypothesized that *Phoxinus* invaded North America from Eurasia across the Beringia land connection approximately 13 mya. The current distribution of *P. erythrogaster* provides evidence that *Phoxinus* entered North America well before the Pleistocene, corroborating Joswiak's hypothesis. Prior to Pleistocene glaciation, the Mississippi River was thought to have been on the same level as adjacent uplands (Robison, 1986). Subsequent downcutting by the Mississippi River in present-day

eastern Arkansas and Missouri created a significant topographic feature known as Crowley's Ridge. The downcutting occurred on the western side of the ridge. During the Pleistocene, the Mississippi River cut through Crowley's Ridge and now flows on its eastern side (Fenneman, 1938). That *P. erythrogaster* remains on the ridge is evidence that this species was widespread prior to the Pleistocene.

Joswiak (1980) suggested that *P. erythrogaster* was widespread in the upper Teays River drainage (Figure 3-12). This would help explain speciation events that gave rise to the southeastern forms. The vicariance event that split the genus was probably the onset of the Nebraskan glaciation. This glacial advance apparently dammed the Teays River and stopped its western flow (Ray, 1974, cited in Hocutt and Wiley, 1986). The population east of the dam differentiated into the ancestor of Laurel Dace, *P. cumberlandensis*, *P. oreas*, and *P. tennesseensis* and the population west of the dam evolved into *P. erythrogaster*. Most of the remaining speciation events within the genus were likely the result of stream capture; a phenomenon that is widely accepted as a means of dispersal for upland fishes (Gilbert, 1976).

Based on the preferred phylogenetic hypotheses (Figure 3-5A, 3-8), the first species to evolve from the ancestral Teays River stock was the Laurel Dace. A stream capture event between an upper Teays tributary (possibly New River) and an upper Tennessee River tributary (Holston) was suggested by Starnes and Jenkins (1988) as the route for *P. tennesseensis* into the Tennessee River drainage. This was likely the route that Laurel Dace used to enter the Tennessee River drainage. It is possible that Laurel Dace were once widespread in the upper Tennessee River drainage. Laurel Dace are

currently known only from the Walden Ridge portion of the Cumberland Plateau; however, the recent discovery of two populations from the upper Clinch River system that represent Laurel Dace or a very close relative suggest that Laurel Dace may have been distributed throughout the upper Tennessee River drainage. Laurel Dace are now found only in remote upland areas along the eastern edge of the Cumberland Plateau. A possible reason for this relictual distribution pattern is that they could not persist in a warmer post-glacial climate and were forced into these cooler upland streams. A second possibility is that the other Tennessee River *Phoxinus*, *P. tennesseensis*, was able to displace them from the central portion of their range.

A stream capture may also have permitted dispersal of an upper Teays *Phoxinus* into the Cumberland River drainage. The upper Teays River included present-day Ohio River tributaries. According to Starnes and Etnier (1986) virtually all of the fish fauna inhabiting the Cumberland River drainage originated in the Teays River, which would include the ancestor to *P. cumberlandensis* (Starnes and Starnes, 1978). Starnes and Starnes (1978) suggested that this ancestor invaded the Cumberland River above Cumberland Falls via the Rockcastle or Big Sandy rivers. Strange and Burr (1995) evaluated the population genetics of *P. cumberlandensis* to assist with management of the species. Their work suggests that the center of dispersal of *P. cumberlandensis* was the Rockcastle River system in southeastern Kentucky which corroborates hypotheses suggested by Starnes and Starnes (1978) and Starnes and Etnier (1986).

Cumberland Falls has been fairly effective in preventing the downstream migration of *P. cumberlandensis* (Starnes and Starnes, 1978) as it is found in only 10

localities below the falls. Starnes and Starnes (1978) hypothesized that *P. cumberlandensis* circumvented this barrier by lateral stream capture. They noted strong geological and faunal evidence in support of this hypothesis. These stream capture events would likely have occurred as Cumberland Falls eroded headward. It is thought that Cumberland Falls has moved upstream approximately 75 m from its origin (O'bara, 1985).

The recently discovered population of *P. cumberlandensis* in the upper Powell River system in Virginia is not easily explained by natural events. This population is within two miles of the Tennessee-Cumberland rivers divide along the Kentucky-Virginia border. The divide is very steep on the Cumberland River side and it seems unlikely that a stream capture event was responsible for the faunal exchange. It is more probable that the population was introduced by humans. Further collecting along the ridge is required to determine the extent of this introduction. Genetic analysis may also provide insight as to the origin of this population.

Phoxinus oreas inhabits several Atlantic Slope drainages as well as the upper New River system. The New River is a remnant of the ancient Teays River, and because *P. oreas* remains there, it likely evolved in the New. It subsequently was able to disperse onto the Atlantic Slope and became widely distributed throughout what is now Virginia. This species apparently tolerates warmer conditions and can be found in much larger streams than other *Phoxinus* species. It is thought that *P. oreas* has been introduced into several drainages outside of its native range (Jenkins and Burkhead, 1994) including the upper Tennessee River drainage (Holston system).

Phoxinus tennesseensis is the hypothesized sister species to *P. oreas* (Starnes and Jenkins, 1988; this study). Starnes and Jenkins (1988) suggested a possible dispersal route from the Atlantic Slope through an upper Tennessee River tributary (probably the Holston River). As mentioned above, it is possible that the ancestor to *P. tennesseensis* entered the Tennessee River drainage after the Laurel Dace was already widespread. The *tennesseensis*-form was already adapted to a milder climate on the Atlantic Slope, and once in the Tennessee drainage, was able to thrive in lowland areas. Subsequently, Laurel Dace may have been competitively excluded by *P. tennesseensis* and pushed into remote fringes of their former range. This hypothesis may unfold in the Piney River system. Both Laurel Dace and *P. tennesseensis* occur in the system and are currently separated by only 2-3 km of stream with no apparent structural barriers (Figure 3-11). Although the current thinking is that *P. tennesseensis* prefers very small streams (< 2 m), the species has been collected in large numbers in the lower portion of Duskin Creek (ca. 10 m wide). Thus, they may be able to move down into the Piney River and up Bumbee Creek.

In addition to the Duskin Creek population, *P. tennesseensis* was also recently collected in the lower portion of Little Brush Creek just above its confluence with the Sequatchie River. I suspect that this population was introduced. There is an account of *P. tennesseensis* from northeastern Alabama (Gilbert, 1891) but this was discounted by Starnes and Jenkins (1988) as *P. erythrogaster*.

Phoxinus erythrogaster is hypothesized to have evolved from the *erythrogaster*-like stock that was widely distributed in the Teays River drainage. As mentioned above,

it was probably isolated by the damming of the Teays River during the Nebraskan glacial period. The range map (Figure 3-10) depicting the sites where *P. erythrogaster* lives at present shows a distribution pattern consistent with this hypothesis. It seems that *P. erythrogaster* arose in the Mississippi basin and dispersed northward up the major tributaries after the retreat of the Wisconsinan glacier. The disjunct populations in Mississippi, Arkansas, Nebraska, and Iowa probably represent relicts and indicate that this species once had a wider range. They were subsequently trapped in suitable habitat and have been unable to disperse further. Starnes and Jenkins (1988) postulated that the New Mexico population is probably an introduction, while Sublette et al. (1990) suggest that they are glacial relicts. The population in Glady Fork in the Sequatchie River system is puzzling. I suggest that this population entered the Tennessee River drainage via a stream capture from the upper Caney Fork River system. This stream is right on the divide and also harbors a population of a rare crayfish (*Cambarus pristinus*) previously known only from the upper Caney Fork system (Hobbs, 1965).

Distribution of *P. eos* reflects a possible association with glacial retreat. Joswiak (1980) suggested that this species was most closely related to *P. erythrogaster* and probably diverged from this species during the Pleistocene. One noticeable difference between the two species is that *P. erythrogaster* prefers stream situations (Phillips, 1968; Trautman, 1981) while *eos* prefers boggy lakes, creeks, and ponds (Hubbs and Lagler, 1964; Scott and Crossman, 1973). Phillips (1968) went on to say that where they occur together in Minnesota, *P. eos* is rare.

Joswiak (1980) postulated that glacial events may have isolated populations of *P. erythrogaster* and that some of these populations could have adapted to bog conditions and evolved to *P. eos*. Because of wet conditions associated with icemelt, this bog adapted species would then have been able to disperse northward behind the receding glaciers. This hypothesis is supported by the relationships determined with Rogers (1972) genetic similarity in this study (Figure 3-8). An alternate hypothesis determined in this study places *P. eos* basal to *P. erythrogaster* (Figure 3-5A). If this is the correct hypothesis, then *P. erythrogaster* would have evolved from a *P. eos* ancestor prior to the Pleistocene.

When looking at the present distribution of *P. eos*, the disjunct nature of the western-most populations is apparent (Figure 3-10). During the Wisconsinan, *P. eos* was probably pushed into the Missourian and Mississippian refuges. Dispersal from these two areas would have created the more or less eastern and western populations.

Joswiak (1980) postulated that *P. neogaeus* differentiated from the ancestral Eurasian *Phoxinus* after a second invasion by the genus into North America. He suggested that this dispersal occurred about 8 mya, much later than the initial invasion that gave rise to the other Nearctic forms. Because I did not have material from any Eurasian *Phoxinus*, I cannot support or refute this hypothesis. *Phoxinus neogaeus* was the basal member to the Nearctic members of the genus; a placement which would allow for Joswiak's hypothesis. If Joswiak was correct, I think that this new form distributed itself across northern North America much as the previous invader did. Based on the congruency of the ranges of *neogaeus* and *eos*, it appears that Pleistocene glaciation

forced this fish into the same Missourian and Mississippian refuges. Similarly, *P. neogaeus* followed the retreating glaciers to its present distribution.

MATERIAL EXAMINED

Phoxinus cumberlandensis—UT 44.5577 (9) unnamed trib. to Capuchin Creek, at unnumbered gravel road crossing, ca. 13.3 air km w of Jellico, Scott County, Tennessee, 20 March 1979; UT 44.5754 (9), Locality wrong on bottle- see WCS 1761.

Phoxinus eos—BMNH? (3) Prairie Creek, Koochiching County, Minnesota, 6 July 1983.

Phoxinus erythrogaster—UT 44.7706 (8) and UT 44.7598 (35) Red Lick Creek at intersection of US 421 and KY 21, ca. 19.2 air km sse Richmond, Madison Co., Kentucky, 22 May 1997 and 6 May 1997.

Phoxinus neogaeus—BMNH 25414 (3) Goose Lake, Camp Ripley, Morrison County, Minnesota, 22 June 1992.

Phoxinus oreas—REJ 327 (10) Back Creek at Poages Mill, Roanoke County, Virginia, 25 May 1969; REJ 1260 (10) Little River at rt. 642, ca. 3 air km. se of Check, Virginia.

Phoxinus tennesseensis—UT 44.6784 (6) Duskin Creek at Shut-in Gap Rd., ca. 23 air km nw of Pikeville, Bledsoe County, Tennessee, 14 May 1996; UT 44.5266 (18) Brice Branch adjacent to Idumea Rd, ca. 0.4 km from TN 11W, Knox County, Tennessee, 20 March 1991-1 January 1998.

Laurel Dace—UT 44.7710 (6) Bumbee Creek at only road crossing 10.0 air km wsw of Spring City, Rhea County, Tennessee, 6 June 1997; UT 44.7341 (2) Cupp Creek, c. 0.8

km nne of intersection of Hendon and Dayton roads, ca. 14.6 air km w of Dayton, Bledsoe County, Tennessee; UT 44.7303 (2) Soddy Creek below Wolf Branch Road, ca. 25.7 air km s of Pikeville, Bledsoe County, Tennessee, 31 May 1994; UT 44.7304 (2) Horn Branch of Rock Creek, above only road crossing, ca. 21.9 air km ssw of Pikeville, Bledsoe County, Tennessee, 31 May 1994.

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APPENDICES

Table 3-1. Enzyme Systems, Tissues, and Electrophoretic Conditions Used in Allozyme Electrophoresis Study of the Nearctic *Phoxinus* and Three Outgroup Taxa.

Enzyme (EC Number)	Locus	Tissue	Electrophoretic Conditions*
Asparate Aminotransferase (EC 2.6.1.1)	Aat-A	Muscle	A
	Aat-B		
Creatine Kinase (EC 2.7.3.2)	Ck-A	Eye	A,C
	Ck-B	Eye	
Esterase (nonspecific)	Est-1	Liver	B
General Protein (nonspecific)	Gp-1	Muscle	A,C
Glucose-6-Phosphate Isomerase (EC 5.3.1.9)	Gpi-A	Muscle	C
	Gpi-B	Muscle	
Glyceraldehyde-3-Phosphate Dehydrogenase (EC 1.2.1.12)	Gapdh-A	Eye	A,C
Isocitrate Dehydrogenase (EC 1.1.1.42)	Idh-A	Eye	A,C
	Idh-B	Eye	
Leucine Aminopeptidase	Lap-A	Muscle	A
L-Lactate Dehydrogenase (EC 1.1.1.27)	Ldh-A	Muscle	A
	Ldh-B	Muscle	
Malate Dehydrogenase (EC 1.1.1.37)	Mdh-A	Muscle	A,C
	Mdh-B	Muscle	
	Mdh-C	Muscle	
Dipeptidase (EC 3.4.13.11)	Pep-A	Muscle	A
Phosphoglucomutase (EC 5.4.2.2)	Pgm-A	Muscle	A,C
Phosphogluconate Dehydrogenase (EC 1.1.1.44)	Pgdh-A	Muscle	A
Superoxide Dismutase (EC 1.15.1.1)	Sod-A	Liver	B

*A: Tris-Citrate II pH 8.0 (Selander et al. 1971); B: Tris-Citrate/Borate pH 8.7, "Poulik", (Selander et al. 1971); C: Tris-Citrate pH 7.5 (Stein et al. 1985).

Table 3-2. Character State Arrays Used in Phylogenetic Analysis of the Nearctic *Phoxinus* and Three Outgroup Taxa. A) Characters and character state codes for the morphological data set as defined in Appendix 3-C. B) Allozyme characters and character state codes corresponding to numbered loci and allelic combinations in Appendix 3-D.

A.

	Characters																												
Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
P. oreas	1	1	3	1	0	1	3	1	4	2	1	3	2	2	2	1	2	2	1	1	1	1	3	2	2	2	1	1	1	1
P. tennesseensis	1	1	3	1	0	1	3	1	4	2	2	3	2	2	2	1	2	2	1	1	1	1	3	2	2	2	1	1	1	1
P. cumberlandensis	1	1	3	1	0	1	3	1	4	2	0	1	1	1	1	2	1	1	1	1	0	1	3	2	2	2	1	1	1	1
Laurel Dace	0	0	2	0	0	1	3	1	4	2	2	2	1	2	1	2	1	1	1	1	0	0	3	1	0	2	1	0	1	1
P. erythrogaster	1	0	3	0	2	1	3	0	4	1	2	2	1	1	1	1	1	1	1	1	0	0	3	2	1	2	1	1	1	1
P. eos	1	0	3	0	0	1	3	0	5	1	2	2	0	0	1	2	1	1	1	0	0	0	1	2	1	2	1	1	0	1
P. neogaeus	0	0	0	0	0	1	0	0	3	0	0	2	0	0	0	0	0	0	1	0	0	0	2	1	0	2	1	0	0	1
S. atromaculatus	0	0	0	0	0	0	1	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	1
L. chrysocephalus	0	0	1	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0
N. telescopus	0	0	1	0	1	0	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0

B.

	Characters																	
Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
P. oreas	4	1	2	0	1	2	4	2	2	2	4	1	4	3	4	5	4	6
P. tennesseensis	4	2	3	2	1	4	3	5	2	2	4	1	4	4	2	4	4	5
P. cumberlandensis	4	1	2	0	1	3	3	2	2	2	4	1	4	5	2	4	4	3
Laurel Dace	6	1	3	0	0	2	1	6	2	3	4	2	4	6	2	4	4	4
P. erythrogaster	5	1	2	0	1	4	1	4	2	2	4	1	5	2	5	4	4	3
P. eos	5	1	1	1	1	2	5	3	2	2	4	1	6	7	6	4	3	3
P. neogaeus	3	1	1	1	2	2	3	3	2	2	3	1	3	2	3	3	2	2
S. atromaculatus	2	0	1	1	0	1	2	2	1	2	2	0	2	1	2	2	0	1
L. chrysocephalus	1	0	1	0	1	0	1	1	1	1	1	0	1	0	1	1	1	0
N. telescopus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3-3. Summary of Genetic Statistics for the Nearctic *Phoxinus* and Three Outgroup Taxa.

Sample	Mean sample size per locus	Mean No. of alleles per locus	Percentage of loci polyallelic*	Mean Heterozygosity Direct Count**
Phoxinus oreas				
Population 1	6.5 ± 0.2	1.1 ± 0.1	14.3	0.052 ± 0.034
Population 2	6.5 ± 0.2	1.2 ± 0.1	23.8	0.063 ± 0.026
Phoxinus tennesseensis				
Population 1	5.5 ± 0.2	1.1 ± 0.1	14.3	0.048 ± 0.026
Population 2	4.8 ± 0.1	1.1 ± 0.1	14.3	0.038 ± 0.022
Phoxinus cumberlandensis	9.1 ± 0.4	1.0 ± 0.0	4.8	0.021 ± 0.021
Laurel Dace				
Population 1	4.5 ± 0.1	1.1 ± 0.1	4.8	0.024 ± 0.024
Population 2	5.7 ± 0.1	1.0 ± 0.0	0.0	0.000 ± 0.000
Soddy Creek	2.8 ± 0.1	1.0 ± 0.0	0.0	0.000 ± 0.000
Phoxinus erythrogaster				
Population 1	6.2 ± 0.3	1.2 ± 0.1	14.3	0.050 ± 0.031
Population 2	9.1 ± 0.3	1.2 ± 0.1	19.0	0.068 ± 0.036
Population 3	4.6 ± 0.2	1.2 ± 0.1	19.0	0.110 ± 0.058
Population 3	7.3 ± 0.3	1.3 ± 0.1	23.8	0.105 ± 0.046
Phoxinus eos				
Population 1	6.3 ± 0.2	1.2 ± 0.2	14.3	0.066 ± 0.040
Population 2	5.3 ± 0.2	1.3 ± 0.1	28.6	0.111 ± 0.050
Phoxinus neogaeus				
Population 1	2.7 ± 0.1	1.1 ± 0.1	9.5	0.016 ± 0.016
Population 2	6.7 ± 0.1	1.1 ± 0.1	9.5	0.020 ± 0.020

Table 3-3, cont.

Sample	Mean sample size per locus	Mean No. of alleles per locus	Percentage of loci polyallelic	Mean Heterozygosity Direct Count
Semotilus atromaculatus				
Population 1	4.3 ± 0.40	1.1 ± 0.1	9.5	0.048 ± 0.048
Population 2	3.6 ± 0.02	1.0 ± 0.0	4.8	0.048 ± 0.048
Notropis telescopus	7.3 ± 0.30	1.3 ± 0.1	28.6	0.127 ± 0.060
Luxilus chrysocephalus				
Population 1	3.0 ± 0.00	1.0 ± 0.0	4.8	0.048 ± 0.048
Population 2	7.2 ± 0.00	1.1 ± 0.1	14.3	0.099 ± 0.061

* A locus was considered polyallelic if more than one allele was detected.
** Based on a direct count of heterozygotes.

Table 3-4. Rogers (1972) Genetic Similarities Calculated From Allele Frequencies.

Species	1	2	3	4	5	6	7	8	9	10
Phoxinus oreas	-----									
Phoxinus cumberlandensis	0.799	-----								
Phoxinus erythrogaster	0.624	0.612	-----							
Phoxinus tennesseensis	0.696	0.675	0.521	-----						
Laurel Dace	0.600	0.570	0.559	0.615	------					
Phoxinus eos	0.454	0.476	0.709	0.497	0.450	-----				
Phoxinus neogaeus	0.402	0.368	0.377	0.412	0.343	0.528	-----			
Semotilus atromaculatus	0.201	0.222	0.112	0.196	0.163	0.262	0.269	-----		
Luxilus chrysocephalus	0.124	0.097	0.189	0.059	0.151	0.130	0.097	0.265	-----	
Notropis telescopus	0.109	0.124	0.164	0.096	0.193	0.121	0.083	0.262	0.575	-----

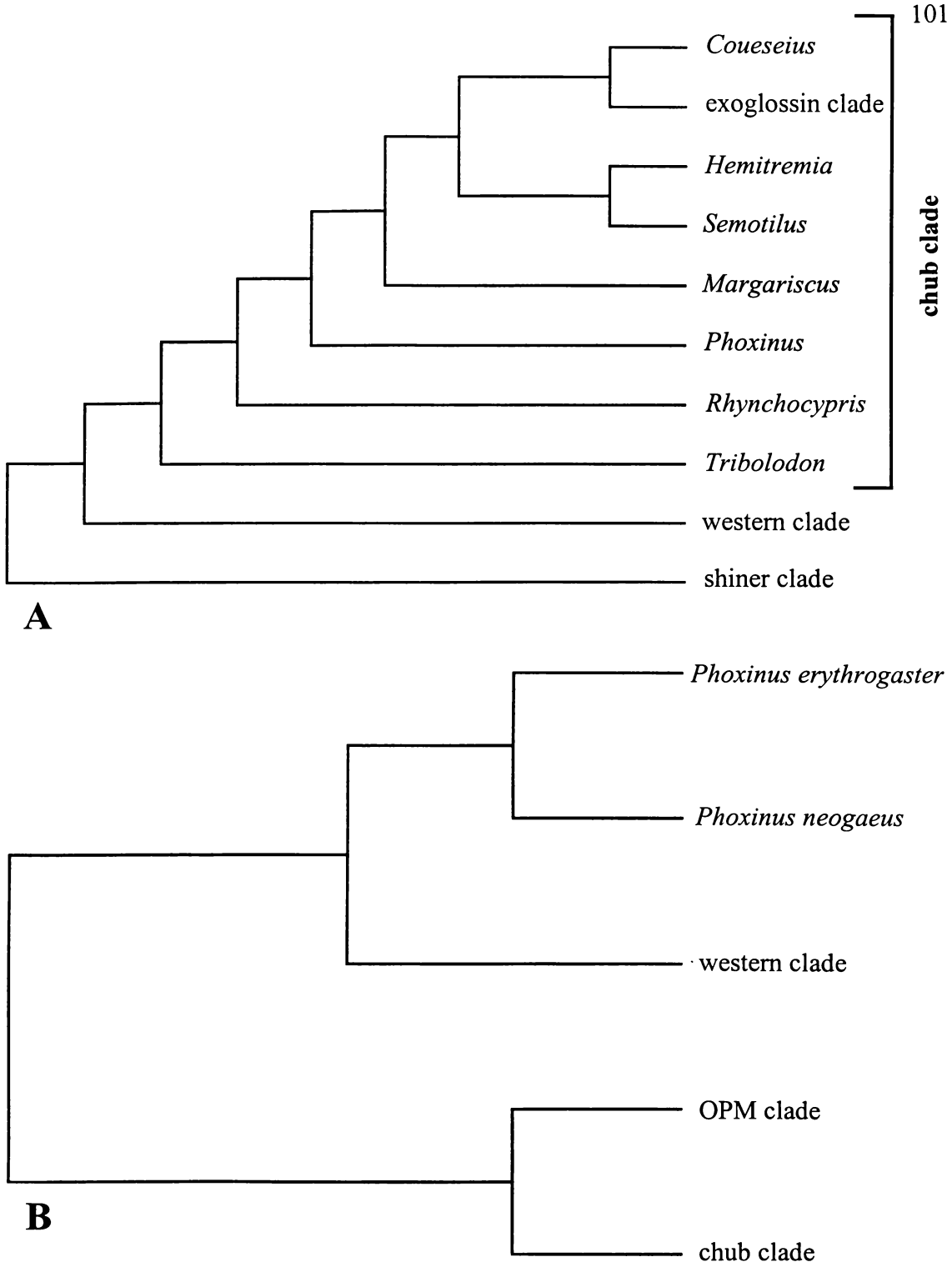


Figure 3-1. Hypothesized generic relationships of the North American Cyprinidae. A) modified from Coburn and Cavender (1992); B) modified from Simons and Mayden (1998).

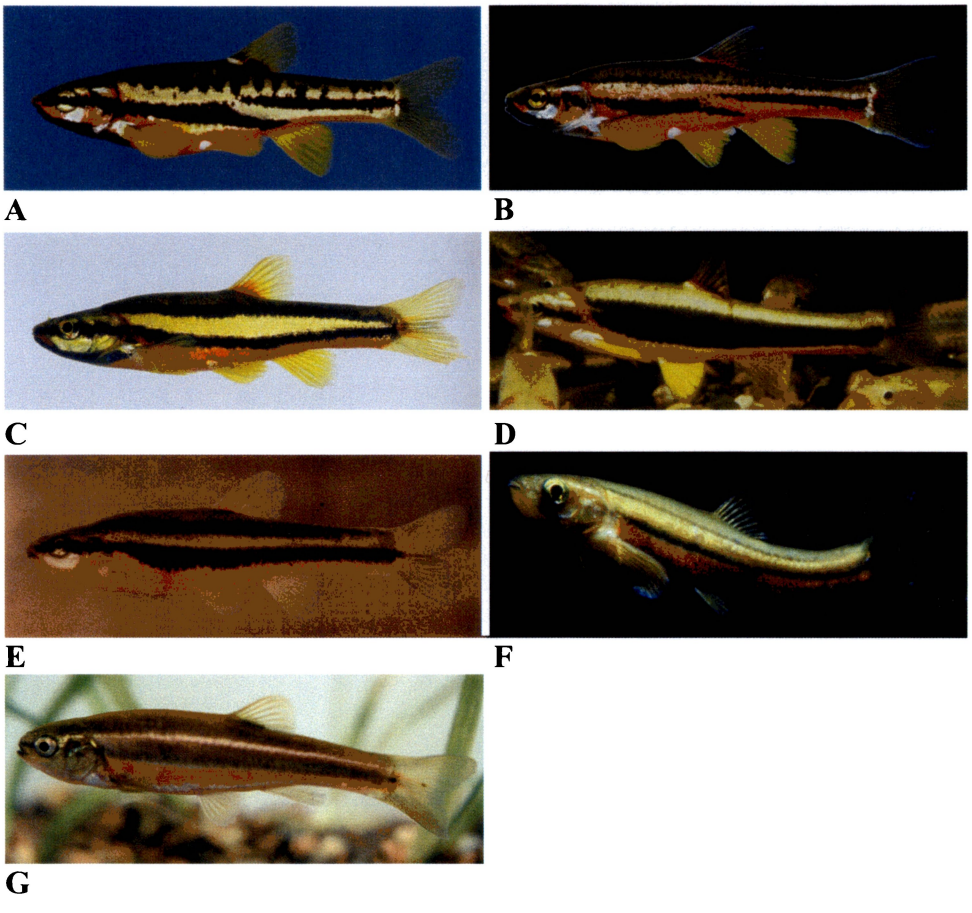


Figure 3-2. Male *Phoxinus* species in nuptial coloration. A) *P. oreas*, photo by N.M. Burkhead; B) *P. tennesseensis*, photo by R.T. Bryant; C) Laurel Dace, photo by C.E. Williams; D) *P. cumberlandensis*, photo by J.R. Shute; E) *P. erythrogaster*, photo by B.H. Bauer; F) *P. eos*, photo by K. Schmidt; G) *P. neogaeus*, photo by K. Schmidt.

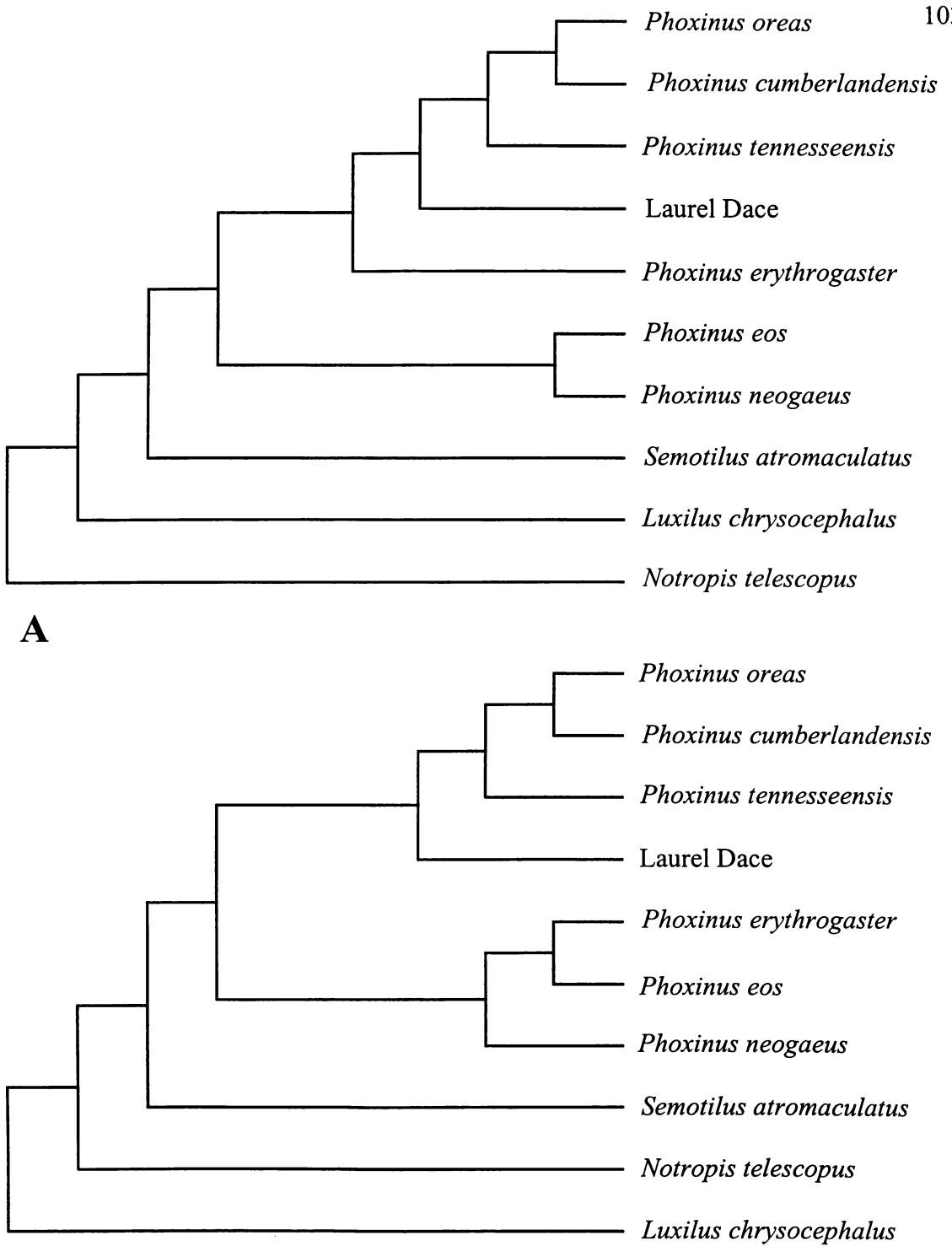


Figure 3-3 Wagner trees generated from distance matrices indicating relationships of the Nearctic *Phoxinus* and three outgroup taxa. A) Tree with shortest FREQPARS length (= 128.406) found with the Prevosti distance; B) Tree with the second shortest FREQPARS length (= 129.910) found with all distance methods.

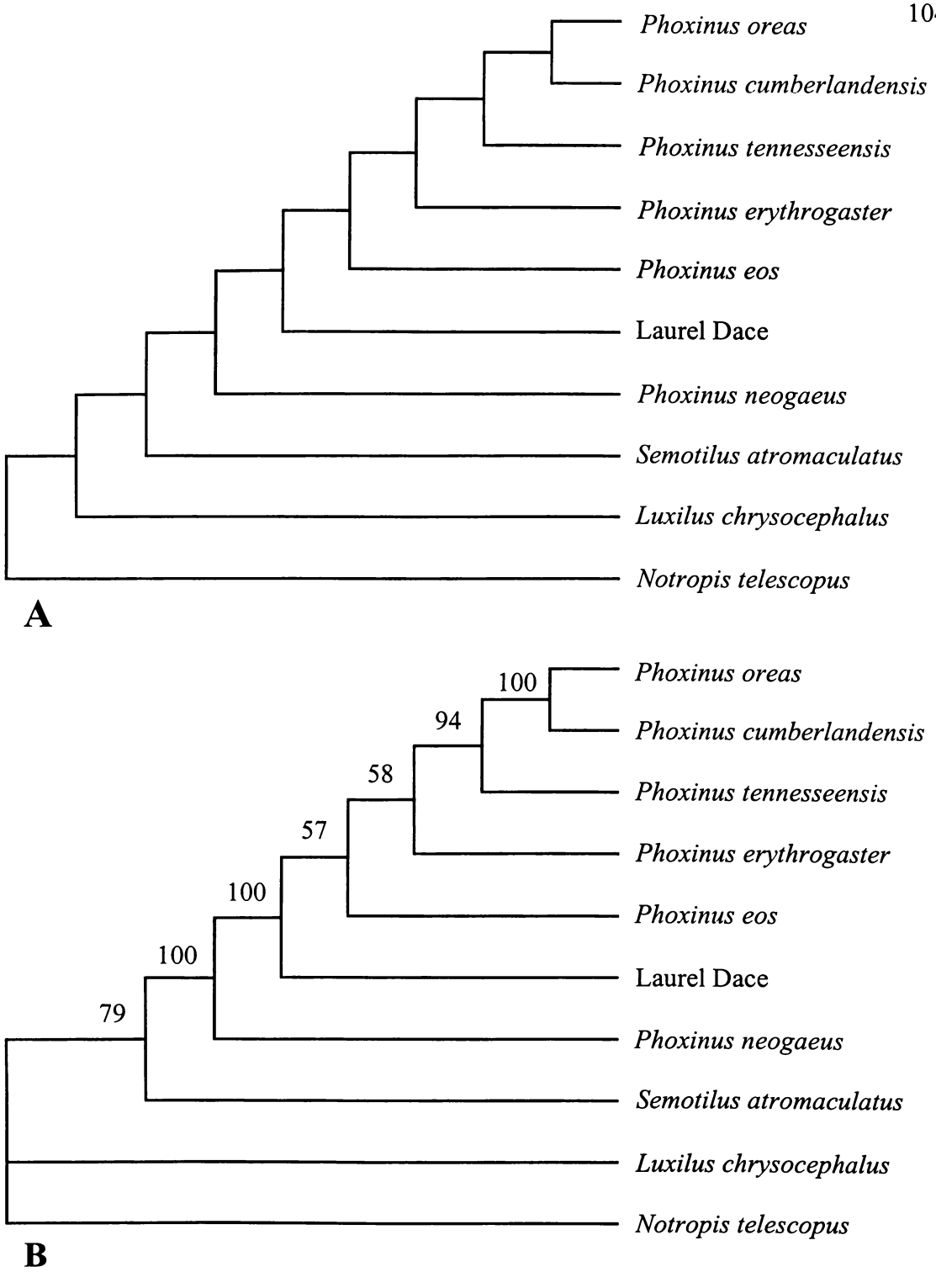


Figure 3-4. Phylogenetic trees derived from PAUP analysis of 30 morphological characters indicating relationships of the of Nearctic *Phoxinus* and three outgroup taxa. A) Tree with shortest PAUP length (= 63 steps); B) Bootstrap 50% majority rule consensus tree.

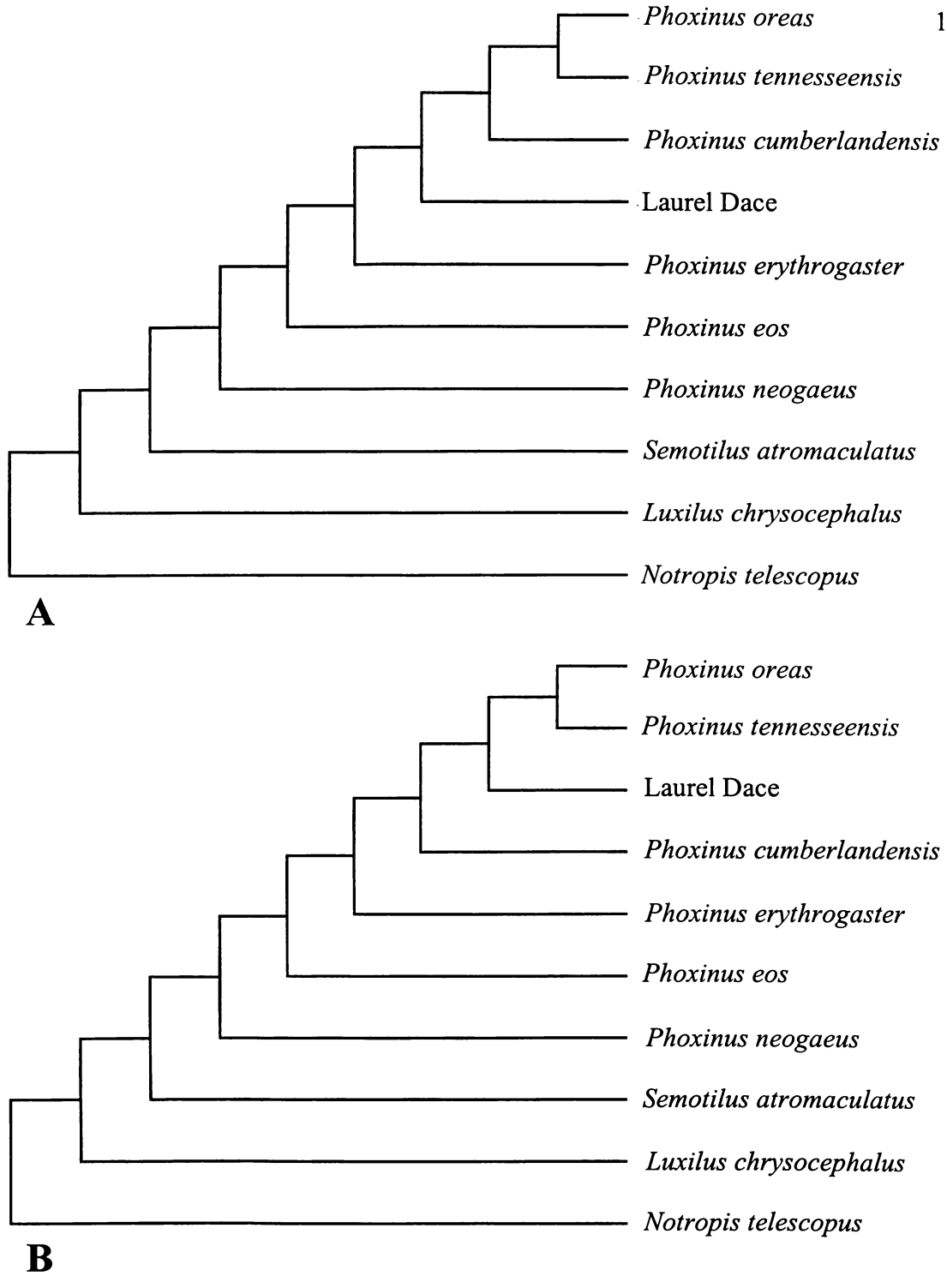


Figure 3-5. Two most-parsimonious trees derived from PAUP analysis of 23 morphological characters indicating relationships of the Nearctic *Phoxinus* and three outgroup taxa. PAUP length = 49 steps.

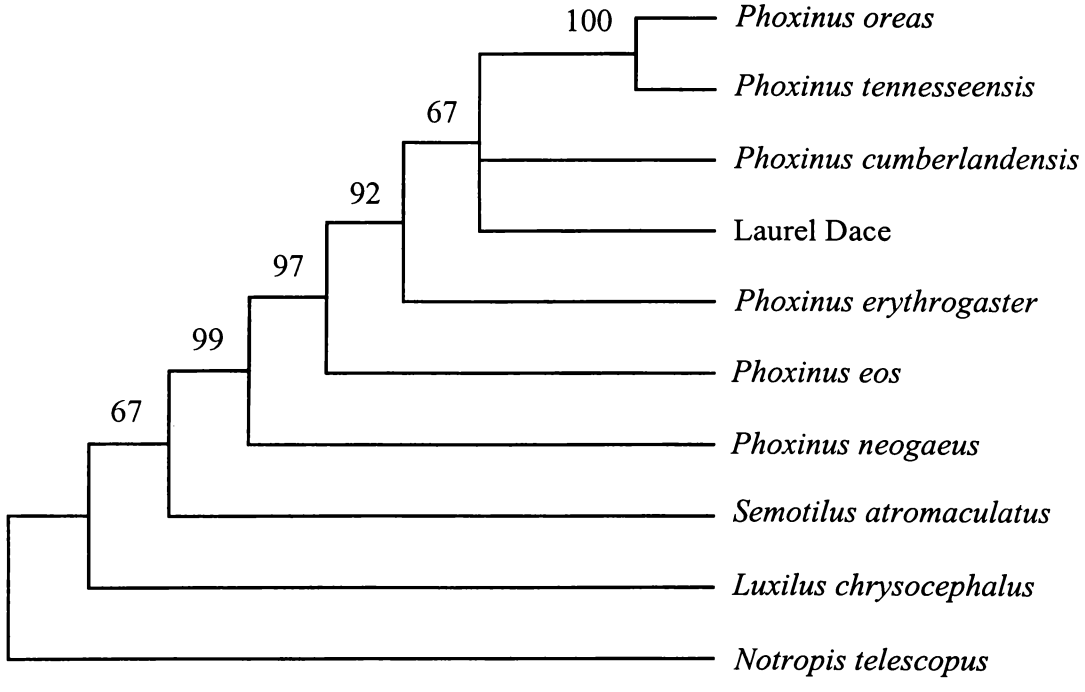


Figure 3-6. Bootstrap 50% majority rule consensus tree indicating relationships of the Nearctic *Phoxinus* and three outgroup taxa. Tree based on two most-parsimonious trees generated from PAUP analysis of 23 morphological characters.

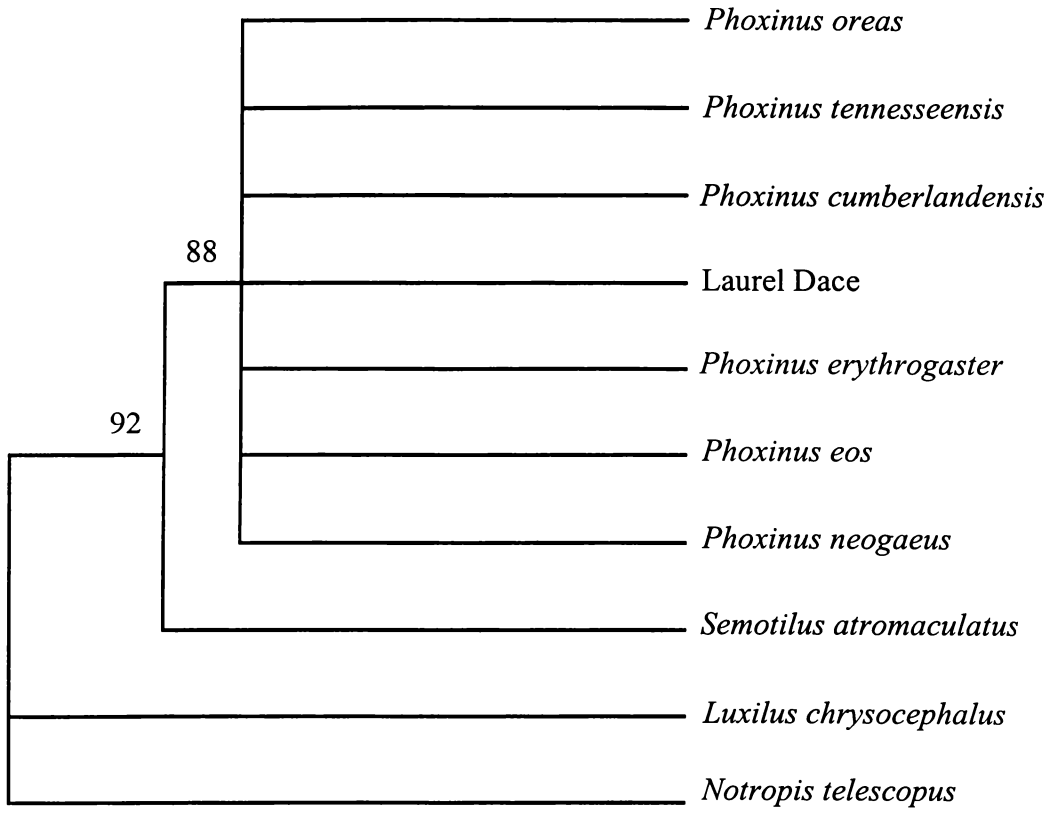


Figure 3-7. Bootstrap 50% majority rule consensus tree indicating relationships of the Nearctic *Phoxinus* and three outgroup taxa. Tree based on five most-parsimonious trees generated from PAUP analysis of 18 discretely coded allozyme characters.

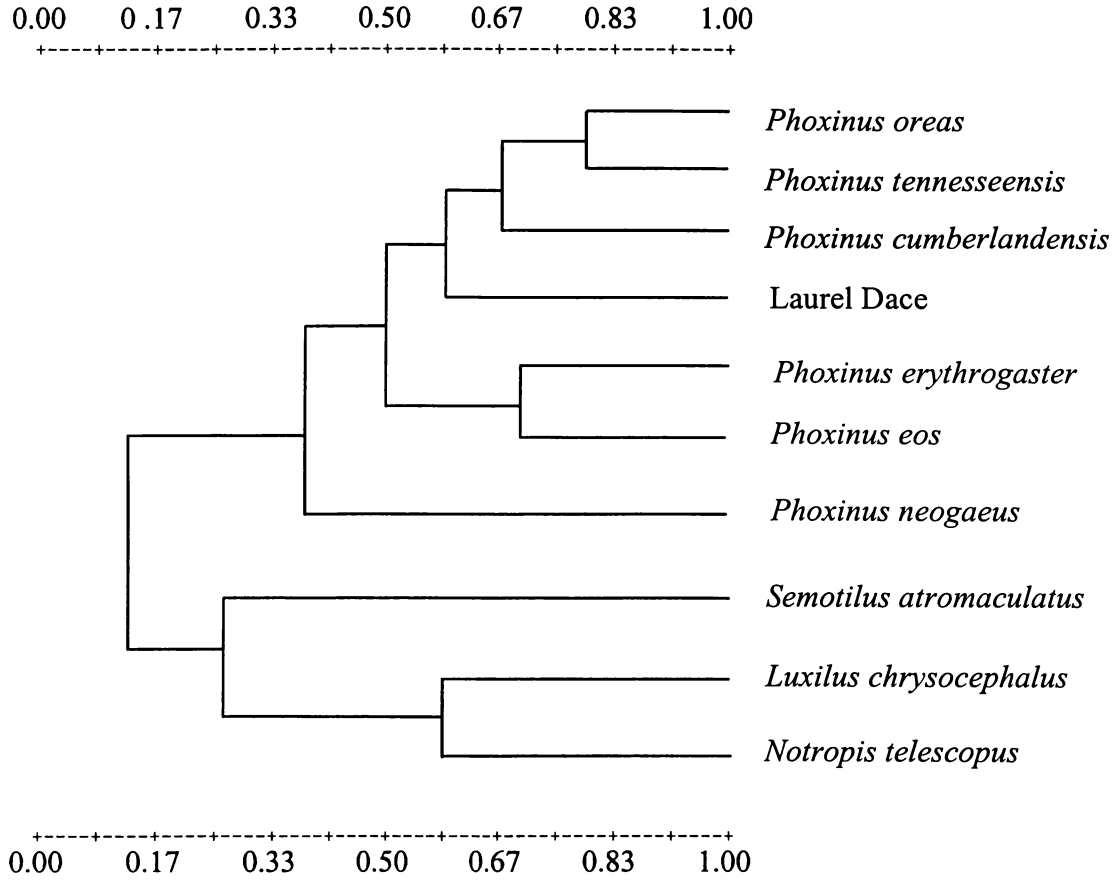


Figure 3-8. UPGMA phenogram of relationships of the Nearctic *Phoxinus* and three outgroup taxa based on Rogers (1972) genetic similarity.

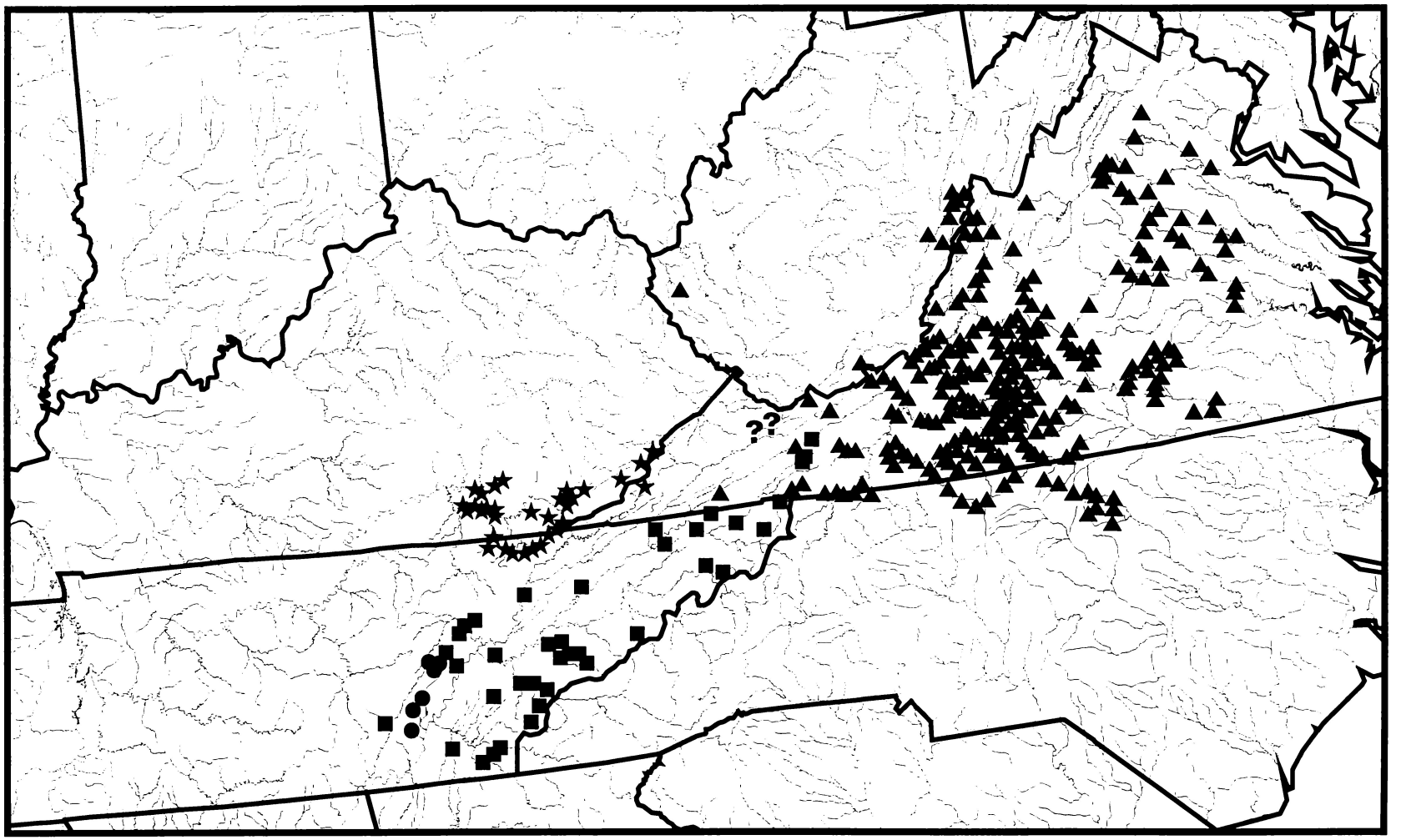


Figure 3-9. Distribution of Laurel Dace (circles), *Phoxinus* sp. cf. Laurel Dace (question marks), *P. tennesseensis* (squares), *P. oreas* (triangles), and *P. cumberlandensis* (stars).

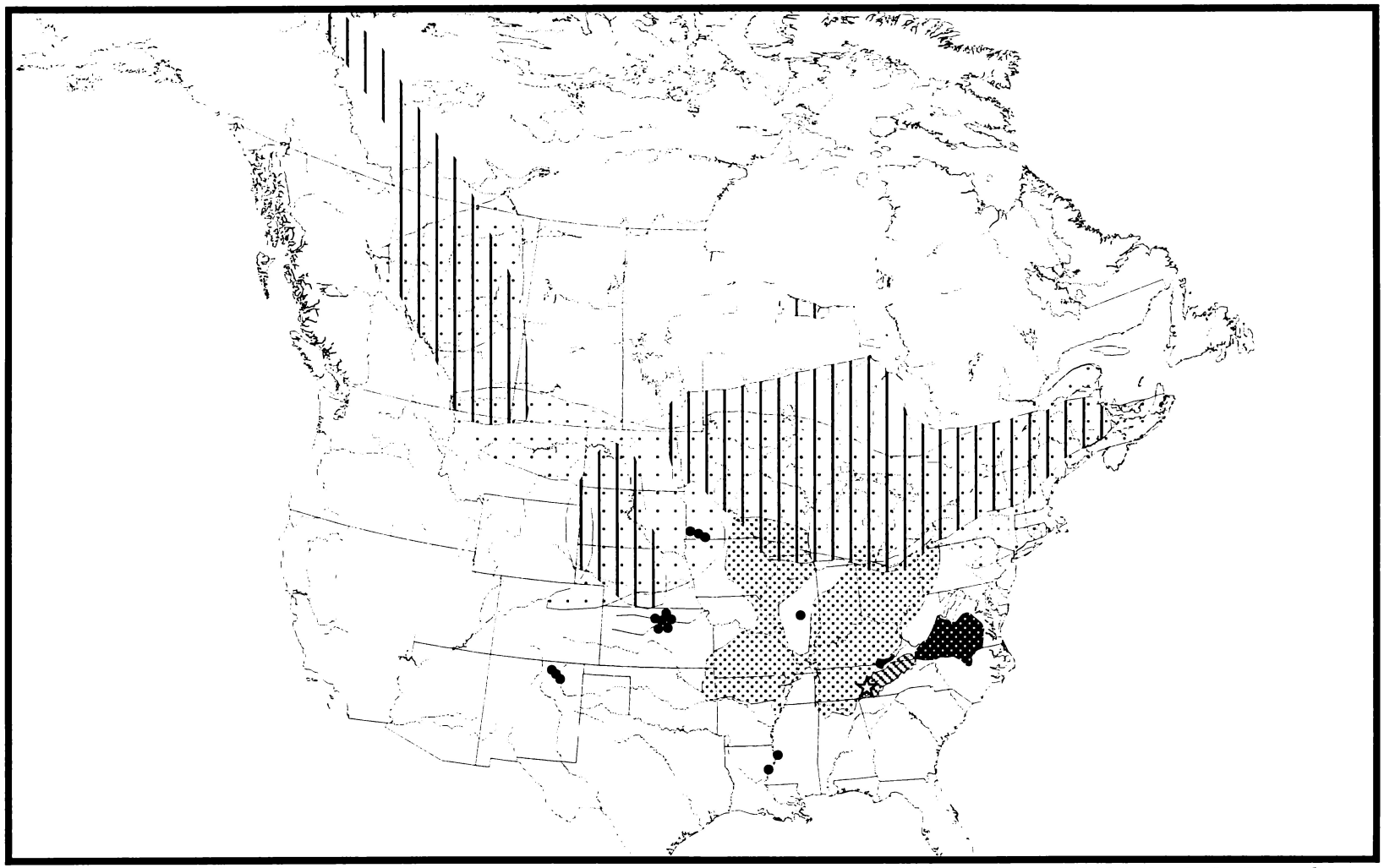


Figure 3-10. Ranges of the Nearctic members of the genus *Phoxinus*. *Phoxinus eos* (widely spaced dark stipples), *P. erythrogaster* (closed circles and dense dark stipples), *P. oreas* (white stipples), *P. tennesseensis* (slanted lines), *P. neogaeus* (vertical lines), *P. cumberlandensis* (oblong black shape on Kentucky-Tennessee border), and Laurel Dace (star).

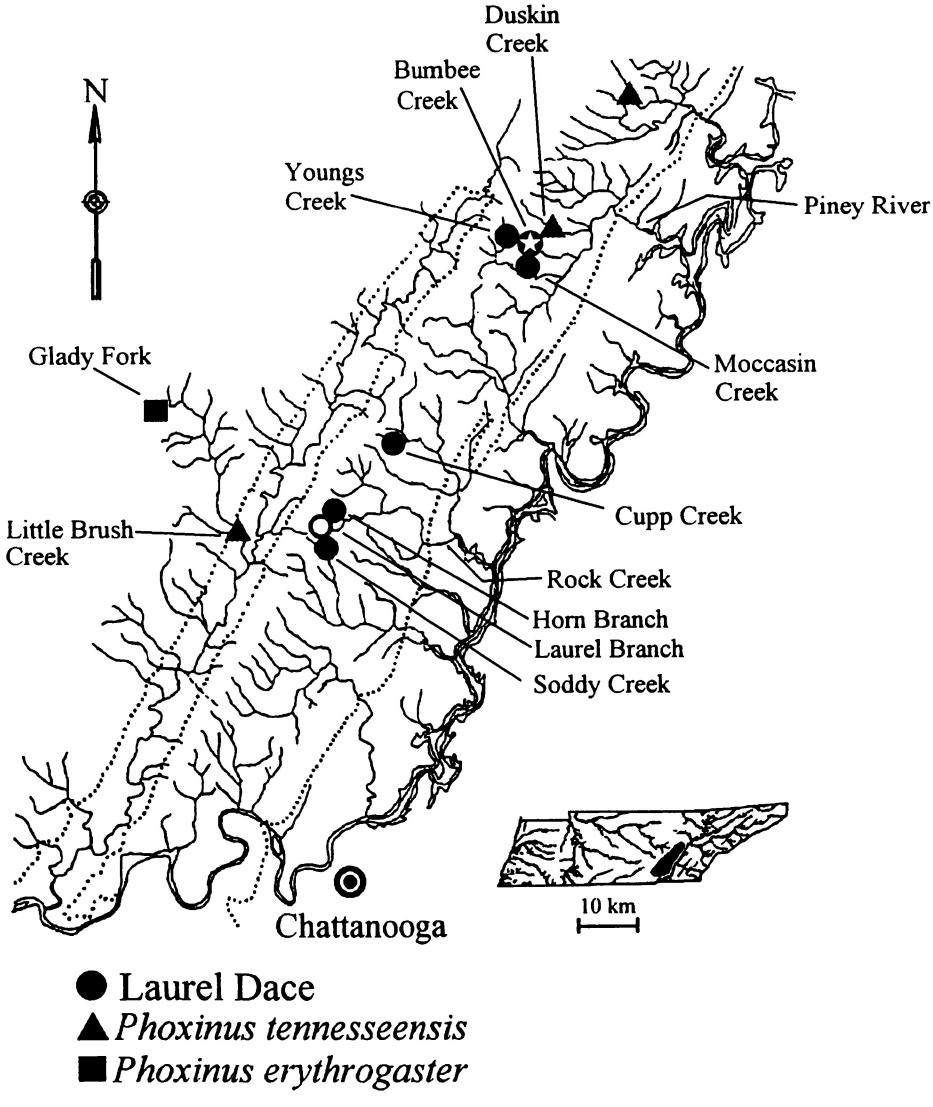


Figure 3-11. Known locations of Laurel Dace and proximate populations of *Phoxinus tennesseensis* and *Phoxinus erythrogaster*. The open circle represents an extirpated population of Laurel Dace, the star is the type locality of Laurel Dace, and the dotted line represents the Cumberland Escarpment

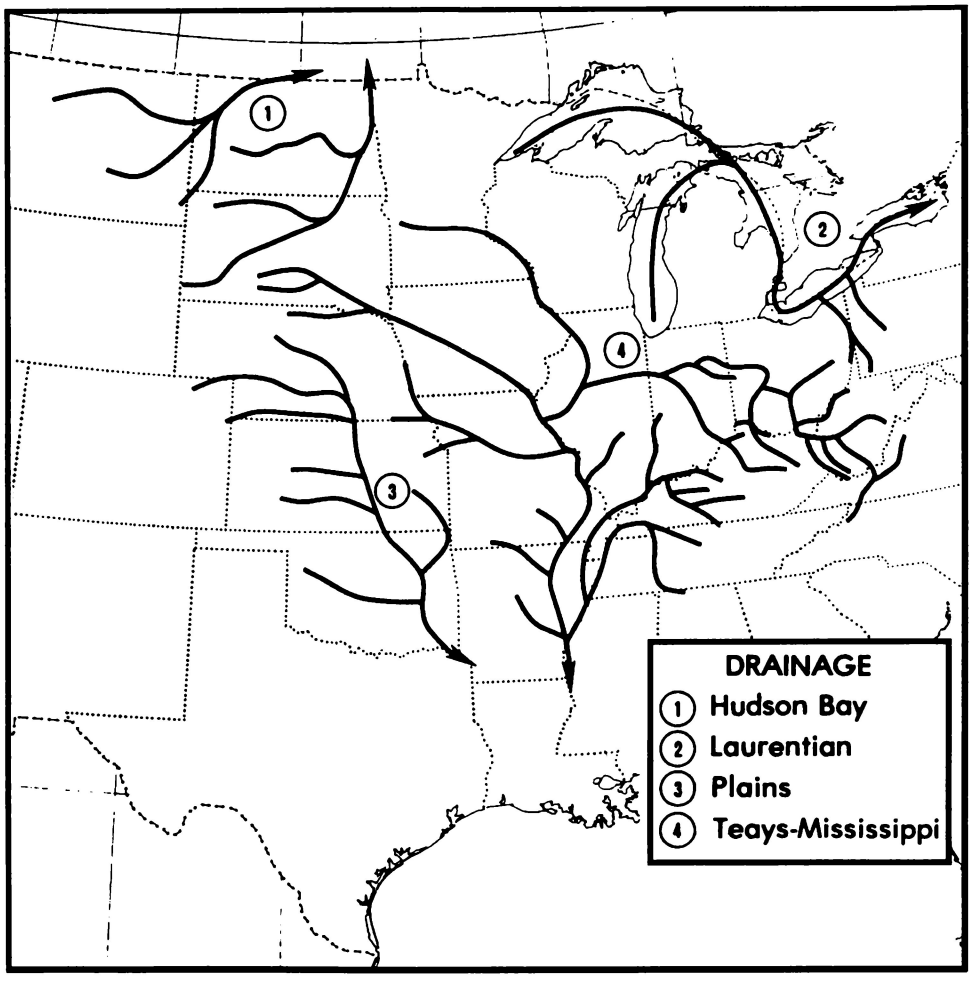


Figure 3-12. Hypothetical reconstruction of Pliocene drainages of the central United States (after Metcalf, 1966; Pflieger, 1971; Hocutt, 1979; as cited in Burr and Page, 1986).

Appendix 3-B. Collection localities of specimens used in genetic analyses.

Phoxinus cumberlandensis

Captive reared specimens from Conservation Fisheries, Incorporated, Knoxville, Tennessee.

Phoxinus oreas

Population 1: Tributary to Otter Creek, ca. 8 mi. ene of Bedford, Bedford County, Virginia, summer 1997.
Population 2: Wilson Creek adjacent to CR 723, ca. 5.8 air km ne Christiansburg, Montgomery County, Virginia, 22 June 1997.

Laurel Dace

Population 1: Bumbee Creek at only road crossing, 10.0 air km wsw of Spring City, Rhea County, Tennessee, 22 May 1996.
Population 2: Horn Branch at only road crossing, 21.9 air km ssw of Pikeville, Bledsoe County, Tennessee, 17 June 1997.
Population 3: Soddy Creek below Wolf Branch Road, ca. 25.7 air km s of Pikeville, Bledsoe County, Tennessee, 16 June 1997.

Phoxinus tennesseensis

Population 1: Pinhook Branch of E. Fork Poplar Creek at road 3.3 km e of jct. TN 95 and TN 58, ca 22.5 air km ne of Kingston center, Roane County, Tennessee.
Population 2: Brice Branch adjacent to Idumea Road, ca. 0.3 km from US 11W, and ca. 21.2 air km ne of Knoxville center, Knox County, Tennessee, 8 July 1997.

Phoxinus erythrogaster

Population 1: Glady Fork at SR8/SR111, ca. 18.5 air km nnw Dunlap center, Sequatchie Co., TN, 18 October 1996.
Population 2: Red Lick Creek at intersection of US 421 and KY 21, ca. 19.2 air km sse Richmond, Madison Co., KY. 6 May 1997.
Population 3: Unnamed trib. to Boone Fork at KY Hwy 1326, Rockcastle Co., KY. 6 May 1997.
Population 4: Possum Run at Possum Run Rd. ca. 4 mi. ne Irvine, Estill, Co., KY.

Population 5: Unnamed trib. to Knob Creek at Haywood Hollow Rd., ca 14.2 air km n of Columbia center, Maury Co., TN, 18 October 1996.

Phoxinus eos

Population 1: Little Rice Creek ca. 15 air km nw Bradley, Oneida County, WI, 24 August 1996.
Population 2: Tributary to Lake Saganagons, Ontario, Canada.

P. neogaeus

Population 1: Little Rice Creek ca. 15 air km nw Bradley, Oneida County, WI, 24 August 1996.
Population 2: Tributary to Lake Saganagons, Ontario, Canada.

Semotilus atromaculatus

Population 1: West Fork McCutcheon Creek, Spring Hill, Maury County, Tennessee, 18 October 1996
Population 2: Gravelly Creek Gateway Rd., ca 5.1 air km e of Maryville center, Blount County, Tennessee, 13 March 1997.

Notropis telescopus

Population 1: Hesse Creek at US 321, ca. 15.0 air km ese of Maryville center, Blount County, Tennessee. 13 March 1997.

Luxilus chrysocephalus

Population 1: Little River at US 411, ca 14.8 air km ene Maryville center, Blount County, Tennessee, 13 March 1997.
Population 2: Unnamed tributary to Bull Run Creek at intersection of US 441 and Pedigo Lane, ca. 11.4 air km n of intersection of I-75 and I-640 in Knoxville, Knox County, Tennessee. 20 March 1997.

Appendix 3-C. Morphological characters and character states. Asterisks denote feeding related characters.

*1. Shape of pharyngeal pad of basioccipital bone: 0 = winged; 1 = oval.

*2. Process on anterior edge of pharyngeal pad of basioccipital bone: 0 = not present; 1 = present.

*3. Pharyngeal tooth formula: 0 = 2,5-4,2; 1 = 2,4-4,2; 2 = 0,5-4,0; 3 = 0,5-5,0.

*4. Shape of anterior end of pharyngeal arch: 0 = pointed; 1 = spatulate.

5. Breast tuberculation of nuptial males exclusive of comb rows: 0 = absent; 1 = fine marginal tubercles; 2 = single tubercles with multiple points.

6. Comb scale rows anterior to pectoral fins on nuptial males: 0 = absent; 1 = present.

7. Dorsal head tuberculation of nuptial males: 0 = none; 1 = in rows; 2 = scattered large tubercles; 3 = scattered granular tubercles.

8. Marginal opercular tubercles on nuptial males: 0 = not present; 1 = present.

9. Body tuberculation of nuptial males: 0 = granular marginal tubercles on all scales; 1 = granular marginal dorsolateral tubercles; 2 = marginal tubercles concentrated posteriorly and midlaterally; 3 = large conical tubercles around anal fin; 4 = well developed tubercles on most of body; 5 = poorly developed and concentrated around anal fin.

10. Opercular tuberculation of nuptial males: 0 = none; 1 = scattered; 2 = dense triangular patch at posterior dorsal corner.

11. Dorsolateral striping of nuptial males: 0 = none; 1 = splotches; 2 = stripe present.

12. Lateral striping of nuptial males: 0 = none; 1 = wide stripe 4-5 scale rows deep; 2 = single narrow stripe 2-3 scale rows deep; 3 = interrupted and ventrally decurved stripe.

13. Lip color of nuptial males: 0 = ground color; 1 = black; 2 = red.

14. Gular color of nuptial males: 0 = Ground color; 1 = red; 2 = black.

15. Color at origin of paired fins of nuptial males: 0 = ground color; 1 = pearly patch at base of pectorals (size of eye) and base of pelvics (size of pupil); 2 = pearly patch at base of pectorals (size of eye) and base of pelvics (size of eye).

16. Cheek color of nuptial males: 0 = ground color; 1 = silver; 2 = gold.

17. Color of dorsal edge of operculum of nuptial males: 0= ground color; 1 = gold; 2 = silver.

18. Lower operculum color of nuptial males: 0 = ground color; 1 = gold; 2 = red.

19. Paired fin color of nuptial males: 0 = ground color; 1 = yellow.

20. Dorsal fin base color of nuptial males: 0 = ground color; 1 = red.

21. Pearl colored vertical bar at caudal fin base: 0 = absent; 1 = present.

22. Pearl colored spot at dorsal fin origin: 0 = absent; 1 = present.

23. Ventral and ventro-lateral coloration of nuptial males: 0 = ground color; 1 = red, yellow, or red and yellow completely covering belly; 2 = red, yellow, or red and yellow only on lower sides; 3 = red.

*24. Peritoneum color: 0 = silver; 1 = stippled brown; 2 = black.

*25. Gut shape: 0 = s-shaped; 1= moderately coiled (double looped); 2 = extremely coiled (multiple loops).

26. Number of lateral line scales: 0 = less than or equal to 40; 1 = between 40 and 70; 2 = greater than 70.

27. Lateral line completeness: 0 = complete; 1 = incomplete.

*28. Primary food items: 0 = animal; 1 = vegetable.

29. Pectoral rays of breeding males: 0 = not thickened; 1 = thickened.

30. Position of anterior anal pterigiophores: 0 = one or two forward of first hemal spine; 1 = three or four forward of first hemal spine (from Coburn and Cavender, 1992).

Appendix 3-D. Loci and allelic combinations used in phylogenetic analysis of the Nearctic *Phoxinus* and three outgroup taxa (Table 3-2).

1. Aat-A:
   0 = a; 1 = e; 2 = f; 3 = b; 4 = c; 5 = g; 6 = b
2. Aat-B:
   0 = b; 1 = a; 2 = ab
3. Ck-A:
   0 = d; 1 = c; 2 = b; 3 = a
4. Ck-B:
   0 = b; 1 = c; 2 = a
5. Est-1:
   0 = c; 1 = ac; 2 = bc;
6. Idh-B:
   0 = df; 1 = ab; 2 = c; 3 = e; 4 = cd
7. Gpi-A:
   0 = ab; 1 = b; 2 = d; 3 = e; 4 = beh; 5 = cfge
8. Gpi-B:
   0 = eg; 1 = g; 2 = c; 3 = d; 4 = af; 5 = ac; 6 = b
9. Ldh-A:
   0 = a; 1 = b; 2 = c
10. Ldh-B:
    0 = b; 1 = a; 2 = d; 3 = cd
11. Mdh-A:
    0 = d; 1 = a; 2 = e; 3 = b; 4 = c
12. Mdh-B
    0 = d; 1 = c; 2 = abc
13. Mdh-C:
    0 = cg; 1 = c; 2 = ef; 3 = ab; 4 = b; 5 = ad
14. Pep-A:
    0 = a; 1 = c; 2 = d; 3 = be; 4 = e; 5 = f; 6 = g; 7 = ad
15. Pgdh-A:
    0 = ce; 1 = c; 2 = b; 3 = ab; 4 = bc; 5 = bde; 6 = cd
16. Pgm-A:
    0 = ab; 1 = b; 2 = c; 3 = e; 4 = d; 5 = de
17. Gp-1:
    0 = b; 1 = a; 2 = d; 3 = ce; 4 = e
18. Sod-A:
    0 = b; 1 = a; 2 = c; 3 = e; 4 = d; 5 = g; 6 = f

Appendix 3-E. Computer code for phylogenetic analysis of allozyme characters using PAUP.

```
#NEXUS
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        Dimensions ntax=10 nchar=18;
        Format
                Symbols="01234567";
CHARLABELS
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        [2]     Aat-B           [8]     Gpi-B           [14]    Pep-A
        [3]     Ck-A            [9]     Ldh-A           [15]    Pgdh-A
        [4]     Ck-B            [10]    Ldh-B           [16]    Pgm-A
        [5]     Est-1           [11]    Mdh-A           [17]    Gp-1
        [6]     Idh-B           [12]    Mdh-B           [18]    Sod-A
        ;
MATRIX
        Oreas                   4120 1242 2241 4345 46
        Tenn                    4232 1435 2241 4424 45
        Cumberlandensis         4120 1332 2241 4524 43
        Laurel                  6130 0216 2342 4624 44
        Eryth                   5120 1414 2241 5254 43
        Eos                     5111 1253 2241 6764 33
        neogaeus                3111 2233 2231 3233 22
        Semotilus               2011 0122 1220 2122 01
        luxilus                 1010 1011 1110 1011 10
        telescopus              0000 0000 0000 0000 00
        ;
        end;

Begin Assumptions;
        usertype Aat-A = 7 0123456

                [0 1 2 3 4 5 6]
        [0]      0 2 2 2 2 2 2
        [1]      2 0 2 2 2 2 2
        [2]      2 2 0 2 2 2 2
        [3]      2 2 2 0 2 2 2
        [4]      2 2 2 2 0 2 2
        [5]      2 2 2 2 2 0 2
        [6]      2 2 2 2 2 2 0
        ;
```

```
usertype Aat-B = 3 012

        [0  1  2]
[0]      0  2  1
[1]      2  0  1
[2]      1  1  0
;
usertype Ck-A = 4 0123

        [0  1  2  3]
[0]      0  2  2  2
[1]      2  0  2  2
[2]      2  2  0  2
[3]      2  2  2  0
;
usertype Ck-B = 3 012

        [0  1  2]
[0]      0  1  2
[1]      2  0  2
[3]      2  2  0
;
usertype Est-1 = 3 012

        [0  1  2]
[0]      0  1  1
[1]      1  0  2
[2]      1  2  0
;
usertype Idh-B = 5 01234

        [0  1  2  3  4]
[0]      0  4  3  3  2
[1]      4  0  3  3  4
[2]      3  3  0  2  1
[3]      3  3  2  0  3
[4]      2  4  1  3  0
;
```

```
usertype Gpi-A = 6 012345

        [0  1  2  3  4  5]
[0]      0  1  3  3  3  6
[1]      1  0  2  2  2  5
[2]      3  2  0  2  4  5
[3]      3  2  2  0  2  3
[4]      3  2  4  2  0  5
[5]      6  5  5  3  5  0
;
usertype Gpi-B = 7 0123456

        [0  1  2  3  4 5  6]
[0]      0  1  3  4  4  4  3
[1]      1  0  2  3  3  3  2
[2]      3  2  0  3  3  1  2
[3]      4  3  3  0  2  2  3
[4]      4  3  3  2  0  2  3
[5]      4  3  1  2  2  0  3
[6]      3  2  2  3  3  3  0
;
usertype Ldh-A = 3 012

        [0  1  2]
[0]      0  2  2
[1]      2  0  2
[2]      2  2  0
;
usertype Ldh-B = 4 0123

        [0  1  2  3]
[0]      0  2  2  3
[1]      2  0  2  3
[2]      2  2  0  1
[3]      3  3  1  0
;
usertype Mdh-A = 5 01234

        [0  1  2  3  4]
[0]      0  2  2  2  2
[1]      2  0  2  2  2
[2]      2  2  0  2  2
[3]      2  2  2  0  2
[4]      2  2  2  2  0
```

;
usertype Mdh-B = 3 012

	[0	1	2]
[0]	0	2	4
[1]	2	0	2
[2]	4	2	0

;
usertype Mdh-C = 7 0123456

	[0	1	2	3	4	5	6]
[0]	0	1	4	4	3	3	3
[1]	1	0	3	3	2	2	2
[2]	4	3	0	4	3	3	5
[3]	4	3	4	0	1	3	3
[4]	3	2	3	1	0	2	4
[5]	3	2	3	3	2	0	2
[6]	3	2	5	3	4	2	0

;
usertype Pep-A = 8 01234567

	[0	1	2	3	4	5	6	7]
[0]	0	2	2	3	2	2	2	1
[1]	2	0	2	3	2	2	2	3
[2]	2	2	0	3	2	2	2	1
[3]	3	3	3	0	1	3	3	4
[4]	2	2	2	1	0	2	2	3
[5]	2	2	2	3	2	0	2	3
[6]	2	2	2	3	2	2	0	3
[7]	1	3	1	4	3	3	3	0

;
usertype Pgdh-A = 7 0123456

	[0	1	2	3	4	5	6]
[0]	0	1	3	4	2	4	2
[1]	1	0	2	3	1	3	1
[2]	3	2	0	1	1	1	3
[3]	4	3	1	0	2	2	4
[4]	2	1	1	2	0	2	2
[5]	4	3	1	2	2	0	2
[6]	2	1	3	4	2	2	0

;

```
        usertype Pgm-A = 6 012345

                [0  1  2  3  4  5 ]
        [0]      0  1  3  3  3  4
        [1]      1  0  2  2  2  3
        [2]      3  2  0  2  2  3
        [3]      3  2  2  0  2  1
        [4]      3  2  2  2  0  1
        [5]      4  3  3  1  1  0
        ;


        usertype Gp-1 = 5 01234

                [0  1  2  3  4]
        [0]      0  2  2  3  2
        [1]      2  0  2  3  2
        [2]      2  2  0  3  2
        [3]      3  3  3  0  1
        [4]      2  2  2  1  0
        ;
        usertype Sod-A = 7 0123456

                [0  1  2  3  4  5  6]
        [0]      0  2  2  2  2  2  2
        [1]      2  0  2  2  2  2  2
        [2]      2  2  0  2  2  2  2
        [3]      2  2  2  0  2  2  2
        [4]      2  2  2  2  0  2  2
        [5]      2  2  2  2  2  0  2
        [6]      2  2  2  2  2  2  0
        ;
typeset *stepmat=Aat-A:1, Aat-B:2, Ck-A:3, Ck-B:4, Est-1:5, Idh-B:6, Gpi-A:7,
        Gpi-B:8, Ldh-A:9, Ldhb:10, Mdh-A:11, Mdh-B:12, Mdh-C:13, Pep-A:14,
        Pgdh-A:15, Pgm-A:16, Gp-1:17, Sod-A:18;
end;
begin paup;
        set root=outgroup;
        outgroup telescopus luxilus;
end;
```

Appendix 3-F. Genotype Frequencies for the Nearctic *Phoxinus* and Three Outgroup Taxa. Number of specimens for each genotype in parentheses. Localities for each population listed in Appendix 3-A.

	Phoxinus oreas		*Phoxinus tennesseensis*		*P. cumb.*	Laurel Dace			*Phoxinus erythrogaster*		
Locus	Otter Creek	James River	Emory River	Holston River	No Bus. Creek	Bumbee Creek	Horn Branch	Soddy Creek	Glady Fork	Knob Creek	Redlick Creek
Aat-A	cc(07)	cc(07)	cc(06)	cc(05)	cc(10)	dd(05)	dd(06)	dd(03)	gg(07)	gg(07)	gg(10)
Aat-B	aa(07)	aa(07)	aa(06)	aa(03) ab(02)	aa(10)	aa(05)	aa(06)	aa(03)	aa(07)	aa(07)	aa(10)
Ck-A	bb(06)	bb(03)	aa(05)	aa(04)	bb(05)	aa(03)	aa(05)	aa(01)	bb(06)	--	bb(08)
Ck-B	bb(07)	bb(07)	aa(06)	aa(05)	bb(10)	bb(04)	bb(06)	bb(03)	bb(07)	--	bb(10)
Est-1	ac (03)	aa(04) ac(02)	ac(02) cc(04)	ac(01) cc(04)	ac(04) cc(05)	cc(03)	cc(04)	cc(03)	ac(01) cc(02)	--	aa(03) ac(03) cc(01)
Idh-A	aa(07)	aa(07)	aa(06)	aa(05)	aa(10)	aa(04)	aa(06)	aa(03)	aa(07)	--	aa(10)
Idh-B	cc(07)	cc(07)	cc(02) cd(02) dd(02)	cc(05)	ee(10)	cc(04)	cc(06)	cc(03)	ee(07)	--	cc(08) cd(01) dd(01)
Gapdh-A	aa(07)	aa(07)	aa(06)	aa(05)	aa(09)	aa(04)	aa(06)	a(03)	aa(06)	--	aa(10)
Gpi-A	ee(06) be(01)	ee(05) eh(02)	ee(06)	ee(05)	ee(10)	bb(05)	bb(06)	bb(03)	be(02) ce(02) ee(03)	ee(07)	bb(10)

Appendix 3-F, cont.

	Phoxinus oreas		*Phoxinus tennesseensis*		*P. cumb.*	Laurel Dace			*Phoxinus erythrogaster*		
Locus	Otter Creek	James River	Emory River	Holston River	No Bus. Creek	Bumbee Creek	Horn Branch	Soddy Creek	Glady Fork	Knob Creek	Redlick Creek
Gpi-B	cc(06)	cc(07)	aa(04) ac(02)	aa(03) ac(01) cc(01)	cc(10)	bb(05)	bb(06)	bb(03)	aa(05) ad(01) dd(01)	aa(07)	aa(07) af(03)
Lap-A	bb(07)	bb(07)	bb(06)	bb(05)	bb(10)	bb(05)	bb(06)	bb(03)	bb(07)	bb(07)	bb(10)
Ldh-A	cc(07)	cc(07)	cc(06)	cc(05)	cc(10)	cc(05)	cc(06)	cc(03)	cc(07)	cc(07)	cc(10)
Ldh-B	dd(07)	dd(07)	dd(06)	dd(05)	dd(10)	cc(05)	dd(06)	dd(03)	dd(07)	dd(07)	dd(10)
Mdh-A	cc(06)	cc(06)	cc(03)	cc(04)	cc(06)	cc(04)	cc(05)	cc(02)	cc(05)	cc(07)	cc(06)
Mdh-B	cc(06)	cc(05)	cc(03)	cc(04)	cc(06)	bb(01) ac(02) cc(01)	cc(05)	cc(02)	cc(05)	cc(06)	cc(06)
Mdh-C	bb(06)	bb(05)	bb(03)	bb(04)	bb(06)	bb(04)	bb(05)	bb(02)	dd(05)	aa(06) ad(01)	dd(06)
Pep-A	bb(04) be(02) ee(01)	bb(01) be(02) ee(04)	ee(06)	ee(05)	ff(10)	gg(05)	gg(06)	gg(03)	dd(07)	dd(07)	dd(10)

Appendix 3-F, cont.

	Phoxinus oreas		*Phoxinus tennesseensis*		*P. cumb.*	Laurel Dace			*Phoxinus erythrogaster*		
Locus	Otter Creek	James River	Emory River	Holston River	No Bus. Creek	Bumbee Creek	Horn Branch	Soddy Creek	Glady Fork	Knob Creek	Redlick Creek
Pgdh-A	bb(07)	bb(06) bc(01)	bb(06)	bb(05)	bb(10)	bb(05)	bb(06)	bb(03)	dd(07)	bb(01) be(01) dd(01) de(01) ee(03)	bd(06) dd(04)
Pgm-A	dd(07)	dd(07) de(02)	dd(06)	dd(05)	dd(10)	dd(05)	dd(06)	dd(03)	dd(07)	dd(07)	dd(10)
Gp-1	ee(07)	ee(07)	ee(06)	ee(05)	ee(10)	ee(05)	ee(06)	ee(03)	ee(07)	ee(07)	ee(10)
Sod-A	ff(06)	ff(06)	gg(06)	gg(05)	ee(10)	dd(05)	dd(06)	dd(03)	ee(03)		ee(09)

	Phoxinus erythrogaster		*Phoxinus eos*		*Phoxinus neogaeus*		*Semotilus atromaculatus*		*Notropis telescopus*	*Luxilus chrysocephalus*	
Locus	Boone Fork	Possum Run	Pine Creek	Lake Sag.	Pine Creek	Lake Sag.	McCutch. Creek	Gravelly Branch	Hesse Creek	Bull Run Creek	Little River
Aat-A	gg(05)	gg(08)	gg(07)	gg(06)	bb(03)	bb(07)	ff(06)	ff(04)	aa(08)	ee(03)	ee(08)
Aat-B	aa(05)	aa(08)	aa(07)	aa(06)	aa(03)	aa(07)	bb(06)	bb(04)	bb(08)	bb(03)	bb(08)
Ck-A	bb(03)	bb(07)	cc(05)	cc(03)	cc(02)	cc(05)	cc(01)	cc(03)	dd(08)	cc(03)	cc(07)

Appendix 3-F, cont.

	Phoxinus erythrogaster		*Phoxinus eos*		*Phoxinus neogaeus*		*Semotilus atromaculatus*		*Notropis telescopus*	*Luxilus chrysocephalus*	
Locus	Boone Fork	Possum Run	Pine Creek	Lake Sag.	Pine Creek	Lake Sag.	McCutch. Creek	Gravelly Branch	Hesse Creek	Bull Run Creek	Little River
Ck-B	bb(05)	bb(08)	cc(06)	cc(06)	cc(03)	cc(07)	cc(02)	cc(04)	bb(08)	bb(03)	bb(08)
Est-1	aa(01) ac(01)	aa(01) ac(02) cc(04)	ac(01) cc(03)	cc(04)	bb(01) cc(01)	cc(05)	cc(03)	cc(03)	cc(06)	aa(03)	ac(05) cc(01)
Idh-A	aa(05)	aa(08)	aa(06)	aa(06)	aa(03)	aa(07)	aa(02)	aa(04)	aa(08)	aa(03)	aa(08)
Idh-B	cc(05)	cc(04) cd(02) ee(01)	cc(06)	cc(06)	cc(03)	cc(07)	ab(02)	ab(04)	df(08)	df(03)	df(08)
Gapdh-A	aa(05)	aa(08)	aa(03)	aa(06)	aa(02)	aa(08)	aa(02)	aa(04)	aa(06)	aa(03)	aa(07)
Gpi-A	be(05)	bb(04) be(03) ee(01)	cc(03) cf(01) cg(02) ee(01)	ff(01) fg(03) gg(02)	ee(03)	ee(07)	dd(06)	dd(04)	ab(02) bb(06)	bb(03)	bb(08)
Gpi-B	aa(04) af(01)	aa(02) af(06)	aa(07)	ad(04) dd(01)	dd(03)	dd(07)	cc(06)	cc(04)	eg(03) gg(05)	gg(03)	gg(08)
Lap-A	bb(04)	bb(08)	bb(07)	bb(06)	bb(03)	bb(07)	bb(06)	bb(04)	bb(08)	aa(03)	aa(08)

Appendix 3-F, cont.

	Phoxinus erythrogaster		*Phoxinus eos*		*Phoxinus neogaeus*		*Semotilus atromaculatus*		*Notropis telescopus*	*Luxilus chrysocephalus*	
Locus	Boone Fork	Possum Run	Pine Creek	Lake Sag.	Pine Creek	Lake Sag.	McCutch. Creek	Gravelly Branch	Hesse Creek	Bull Run Creek	Little River
Ldh-A	cc(05)	cc(08)	cc(07)	cc(06)	cc(03)	cc(07)	bb(06)	bb(04)	aa(08)	bb(03)	bb(08)
Ldh-B	dd(05)	dd(08)	dd(07)	dd(06)	dd(03)	dd(07)	dd(06)	dd(04)	bb(08)	aa(03)	aa(08)
Mdh-A	cc(05)	cc(05)	cc(07)	cc(05)	bb(03)	bb(06)	ee(04)	ee(02)	dd(05)	aa(03)	aa(04)
Mdh-B	cc(04)	cc(04)	cc(06)	cc(03)	cc(02)	cc(06)	dd(03)	dd(02)	dd(04)	dd(03)	dd(04)
Mdh-C	dd(04)	dd(04)	cc(06)	ad(01) dd(02)	aa(02)	aa(05) bb(01)	ee(02) ff(01)	ee(02)	cg(04) gg(01)	cc(03)	cc(03)
Pep-A	dd(05)	dd(08)	aa(01) ad(05) dd(01)	dd(06)	dd(03)	dd(07)	cc(06)	cc(04)	aa(08)	aa(03)	aa(08)
Pgdh-A	bd(03) dd(02)	bd(04) dd(04)	dd(07)	cd(01) dd(04)	aa(01) ab(01) bb(01)	ab(03) bb(04)	bb(06)	bb(04)	cc(07) ce(01)	cc(03)	cc(08)
Pgm-A	dd(05)	dd(08)	dd(07)	dd(06)	ee(03)	ee(07)	cc(06)	cc(04)	ab(01) bb(07)	bb(03)	bb(08)

Appendix 3-F, cont.

	Phoxinus erythrogaster		*Phoxinus eos*		*Phoxinus neogaeus*		*Semotilus atromaculatus*		*Notropis telescopus*	*Luxilus chrysocephalus*	
Locus	Boone Fork	Possum Run	Pine Creek	Lake Sag.	Pine Creek	Lake Sag.	McCutch. Creek	Gravelly Branch	Hesse Creek	Bull Run Creek	Little River
Gp-1	ee(05)	ee(08)	cc(07)	cc(02) ce(03) ee(01)	dd(03)	dd(07)	bb(06)	bb(04)	bb(08)	aa(03)	aa(08)
Sod-A	ee(04)	ee(07)	ee(07)	ee(05)	cc(02)	cc(07)	aa(03)	aa(04)	bb(07)	bb(03)	bb(08)

PART 4: SUMMARY AND RECOMMENDATIONS

SUMMARY

The Laurel Dace was determined to be a new species of the genus *Phoxinus*. Its description brings the number of named Nearctic *Phoxinus* species to seven. It differs from its Nearctic congeners in aspects of osteology, feeding morphology, and nuptial coloration. The biology of the Laurel Dace appears to be similar to the remainder of the species in the genus. This species is endemic to the Tennessee River drainage and is currently known from only six localities on the Walden Ridge portion of the Cumberland Plateau. Based on this restricted distribution, I believe it needs protection at the federal level.

The relationships of the Nearctic *Phoxinus* were investigated using morphological and biochemical characters. There was little congruence between the morphological and the discretely coded allozyme data set. However, a UPGMA phenogram based on Rogers (1972) genetic similarity was highly congruent with a phylogenetic tree derived from one of the morphological data sets (Part 3, Figures 3-5A, 3-8). Because of their congruency, both hypotheses of relationships were considered viable.

Several other studies indicate that the Nearctic *Phoxinus* are derived from Eurasian stock and gained access to North America across the Beringia land bridge. There is also evidence that a form similar to *P. erythrogaster* was widespread in North America before the Pleistocene. A vicariant event (Nebraskan glaciation) probably split the *erythrogaster* stock into eastern and western populations in the Teays River drainage. Subsequent stream capture events between upper Teays tributaries and the Cumberland,

Tennessee, and Atlantic Slope drainages provided a scenario for evolution of the southeastern forms. The western population evolved into the present-day *P. erythrogaster*. Whether *P. eos* speciated from *P. erythrogaster* or vice versa can be argued based on current distributional patterns and the results of this study. The only difference between two preferred phylogenies was the relative placement of *P. eos* and *P. erythrogaster*. A UPGMA phenogram indicated that the two are sister species. In that event, *P. eos* probably evolved from *P. erythrogaster* during the Pleistocene. The hypothesis of relationships based on morphology indicated that *P. eos* is more primitive than *P. erythrogaster*. Under that scenario, *P. erythrogaster* would have evolved from *P. eos* prior to the Pleistocene. Some studies suggest that *Phoxinus neogaeus* is more closely related to its Eurasian congeners and likely entered North America during a second invasion.

RECOMMENDATIONS

There are many avenues for research regarding this group of fishes. Attempts to collect nuptial individuals of the recently discovered *Phoxinus* species from Virginia must continue in order to make a confident assessment of its specific status. Population genetic analysis should be completed with all known populations of Laurel Dace plus the unidentified populations. There is evidence that some population structuring (Part 3) exists within the species. This will be important as conservation management decisions are made concerning things such as artificial propagation and re-introductions.

Another possible project involves the newly discovered population of *P. cumberlandensis* from the upper Tennessee River drainage. It would be interesting to determine if this population is recently introduced, and if so, from what source population it was derived. Dr. Rex Strange at Southeastern Missouri State University has developed mtDNA primers for this species and may pursue the question.

This study and observations of other researchers suggest that *P. erythrogaster* may represent a species complex. Taxonomic evaluation across the range of *P. erythrogaster* is warranted.

VITA

Christopher E. Skelton was born in Augusta, GA in 1963. He graduated from Augusta College in 1987 with a B.S. in Computer Science. He was accepted into the Fisheries Department at The University of Tennessee in 1990 and completed his M.S. there in 1992. In August 1992 he began the Ph.D. program in the Department of Zoology at The University of Tennessee. Since October of 1998 he has been employed as the aquatic zoologist with the Georgia Department of Natural Resources, Georgia Natural Heritage Program.