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Designation of a neotype for brook trout, *Salvelinus fontinalis*

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Abstract.—The taxonomic status of *Salvelinus fontinalis* (Mitchill) is problematic. Difficulties in comparison of populations are exacerbated by the lack of type material. Here we designate a neotype from Connetquot River, Long Island, New York. We provide genetic and morphological data for the neotype, conspecifics, and other populations (Swan Creek, Nissequogue Creek) from Long Island, New York. We demonstrate, using molecular markers, that the population from Connetquot River most likely has not been influenced by the major broodstock strains utilized in the Northeast for supplemental and restorative stocking programs. We distinguish the above populations morphologically from lower interior basin populations, represented by fishes from the Pigeon-French Broad drainage, North Carolina and Tennessee. Finally, we position populations from Long Island, New York, within six distinct lineages of *S. fontinalis*.

Keywords: brook trout, neotype, New York, *Salvelinus fontinalis*

The brook trout, *Salvelinus fontinalis* (Mitchill), evolved a great variety of life history, developmental and physiological traits in response to a broad range of habitats. Populations of brook trout are native to headwater streams and cold-water lakes of the Mississippi River drainage east to the Atlantic Slope drainages; they are found from northeastern Canada south through the Great Lakes and into the southern Appalachian mountains (Power 1980). The geological history of this region reveals numerous events (Hocutt et al. 1986) that would serve to isolate fish populations, including extensive glacial impoundment and stream captures in the northern parts of its range, and montane glaciations and stream captures affecting southern populations.

Brook trout, along with most other salmonids, show exceptional levels of life-

history variation (e.g., resident and migratory types often co-occur). Upon reaching sexual maturity individuals undergo dramatic morphological (e.g., male kype formation), physiological, and behavioral adaptations. This life-history variation appears to be influenced by complex interactions between genetic and environmental factors (Hendry et al. 2004). Anthropogenic disturbances and climate change have resulted in the proliferation of demographically small isolated populations, thus, providing the opportunity to study the accumulation of phenetic and genetic traits in allopatric populations. Additionally, the overlapping translocation of isolated populations into new areas permits the study of reproductive isolation of introduced populations in natural environments.

Salvelinus fontinalis was described from Long Island, New York (Mitchill 1814). Unfortunately, neither type material nor a

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type locality were designated. Mitchill (1815:438) further described the brook trout as a "most dainty fish" that lives in running waters; however, he also reported fish that weighed in excess of two kg. Certainly, there were both resident and sea-run individuals present on Long Island during this time. Behnke (1980) posed the question of whether there were distinct northern and southern groups of brook trout, or a single homogenous stock established since the last glacial retreat during the Pleistocene. Morgan & Danzmann (1997, 2001) and Hall et al. (2002) suggested high mtDNA RFLP diversity within, and differentiation among, brook trout populations in the mid-Atlantic when compared to northern populations analyzed previously (Jones et al. 1997, Danzmann et al. 1998). By contrast, brook trout from the mid-Atlantic region belong to five of the six established mtDNA assemblages (Morgan & Danzmann 1997, 2001; Danzmann et al. 1998, Hall et al. 2002). Additionally, most allozyme-based studies of populations from the southern Appalachians have produced evidence that genetic diversity is relatively high in this region of the native range of brook trout (McCracken et al. 1993, Hayes et al. 1996, Krieglner et al. 1995). Surveys of microsatellite DNA, however, suggest less allelic diversity in the southeast portion of the species' range (Richards et al. 2008). In order to determine species status within this complex lineage, it is necessary to have a reference point to which these other native populations can be compared. It is the purpose of this paper to designate a neotype for *S. fontinalis* from Long Island and redescribe the species.

Materials and Methods

Fishes were collected on Long Island with the aid of a New York Department of Environmental Conservation crew led by

Charles Guthrie at the following localities: Nissequogue Creek 40°49.61039'N, 073°13.593'W; Swan Creek 40°46.551'N, 072°59.6238'W; and Connetquot River 40°47.1714'N, 073°10.1334'W. We used brook trout from the Pigeon-French Broad system, located in Great Smoky Mountains National Park (GRSM), Tennessee to use as representatives of the Lower Interior Basin for comparative purposes. Specifically, we collected fishes from Indian Camp Creek 35°44.265'N, 083°16.674'W; Cosby Creek 35°44.881'N, 083°12.020'W; and Greenbrier Creek 35°45.9247'N, 083°15.2272'W. All fish were collected by backpack electro-shocking. All sites in the Lower Interior Basin were above 680 m altitude. All fish were anesthetized with clove oil, euthanized in 1% formalin, pinned in trays so that the bodies were flat and the fins erect, preserved in 10% formalin, and placed in permanent storage in 70% ethanol. Pigmentation patterns and color were recorded in the field via direct observation. Counts and measurements follow Stauffer (1991). All counts and measurements were taken from non-spawning specimens from the left side of the body with the exception of gill-raker counts, which were taken on the right side. Fin-clips were preserved in 99% ethanol.

Morphometric data were analyzed using a sheared principal component analysis, which factors the covariance matrix and restricts size variation to the first principal component (Humphries et al. 1981, Bookstein et al. 1985). Meristic data were analyzed using a principal component analysis in which the correlation matrix was factored. Differences among populations were illustrated by plotting the sheared second principal components (SPC2) of the morphometric data against the first principal components (PC1) of the meristic data (Stauffer & Hert 1992).

We brought fin tissue samples to the United States Geological Survey (USGS)

Table 1.—Hatchery brook trout stocks utilized for comparisons to putative wild collections.

Hatchery strain	State	Year sampled	Sample size
Phillips Hatchery	Maine	2005	60
Sandwich Hatchery	Massachusetts	2003	37
Hyde Pond Strain	New York	2005	35
Mountain Pond Strain	New York	2005	31
Big Hill Pond Strain	New York	2005	55
Rome Hatchery	New York	2005	50
Bellefonte Hatchery	Pennsylvania	2004	31

Leetown Science Center, Kearneysville, West Virginia, for molecular analyses. Genomic DNA was extracted from tissue using the Puregene Kit (Gentra Systems, Minneapolis, Minnesota). All samples were screened for 13 microsatellite loci designed specifically for brook trout (*SfoB52*, *SfoC24*, *SfoC28*, *SfoC38*, *SfoC79*, *SfoC86*, *SfoC88*, *SfoC113*, *SfoC115*, *SfoC129*, *SfoD75*, *SfoD91*, *SfoD100*; King et al. 2012). Details of the master mix composition, thermal cycling parameters, and multiplexing are provided in King et al. (2012). PCR amplifications were performed on either PTC-200 or PTC-225 thermal cyclers (Bio-Rad Laboratories, Hercules, California), and microsatellite allele sizes were determined on an Applied Biosystems (Foster City, California) ABI 3130. Genetic Analyzer GeneScan 3.7 and GeneMapper Fragment Analysis software (Applied Biosystems) were used to score, bin, and output allelic data.

No records indicate fish being stocked into the Long Island, New York streams. We used GeneClass (Cornuet et al. 1999) to determine the probability of each individual collected having genotypes found among seven potential hatchery source populations (Table 1) used for supplementation in the northern Atlantic Slope region. Because detailed records of supplementation were not available for the Long Island, New York strains, we assumed any hatchery source could have been stocked in any drainage and, therefore, tested for the presence of the most

commonly stocked hatchery strains. Population allele frequencies were estimated in GeneClass using the Bayesian option (Rannala & Mountain 1997). The probability that an individual belonged to one of the hatchery populations was calculated by simulating 10,000 genotypes and calculating the probability of the individuals genotyped being observed in that simulated hatchery population. While no fish was determined to be of stocked origin based on assignment testing, a principal coordinates analysis, PAST (Hammer et al. 2001), was utilized to compare the proportion of shared alleles distance among all individuals.

The evolutionary relationships among brook trout collections from throughout its range (Table 2) were visualized through the construction of a Neighbor-Joining tree (Saitou & Nei 1987). Genetic distances between each pair of collections were summarized with genetic distance matrices calculated using the Cavalli-Sforza & Edwards (1967) chord distance in MEGA5 (Tamura et al. 2011). The strength of support for each node in the phylogenetic tree was tested by bootstrapping over loci using njbpop (J.-M. Cornuet, INRA, Montpellier, France).

Results

Populations inhabiting the North Atlantic Slope (represented by fish from Long Island, New York) were distinguished from those populations that 1) sea run, 2) inhabit the North Atlantic slope, 3) inhabit the St. Lawrence River and the Great Lakes drainages, 4) inhabit the Upper Interior Basin (Ohio River), 5) inhabit the southern Atlantic Slope, and 6) inhabit the lower interior basin (Ohio River) (Fig. 1). Ordination of the inter-individual genetic distance suggested that some degree of relatedness (overlap) existed between some putative wild individuals from Nissequogue Creek and fish from the Bellefonte (Penn-

Table 2.—List of collections included in a range-wide genetic analysis at 13 microsatellite DNA loci in brook trout (*Salvelinus fontinalis*). The survey consists of fish sampled from 18 locations representing at least six of the major phylogeographic groupings within the species' range. Results of the comparison are presented in a neighbor-joining tree (Fig. 1).

Major drainage	Primary drainage	General location	Year sampled	# fin clips sampled
Atlantic Ocean	Freshwater R.	Freshwater River	2000	50
Atlantic Ocean	Waterm Cove	Waterm Cove	2000	36
Hudson Bay	Lake Mistassini	Pepeshquasati River	2000	50
Hudson Bay	Lake Mistassini	Cheno River	2000	43
Gulf of Maine	Bass Harbor/Eastern Passage	Marshall Brook	2002	56
Gulf of Maine	Bracy Cove/Eastern Way	Jordan Stream	2002	50
Atlantic Ocean	Great South Bay	Nissequogue Creek	2010	30
Atlantic Ocean	Great South Bay	Swan Creek	2010	30
Atlantic Ocean	Great South Bay	Connetquot River	2010	30
St. Lawrence R.	Lake Superior	Grace Creek	1994	29
St. Lawrence R.	Lake Superior	Tobin Harbor	1996	56
Pee Dee R.	Yadkin R./Pee Dee R.	Harris Creek	2006	20
Pee Dee R.	Yadkin R./Pee Dee R.	Mitchell River	2004	20
Mississippi R.	Youghiogheny R./Monongahela R./Ohio R.	Puzzley Run	1998	49
Mississippi R.	Youghiogheny R./Monongahela R./Ohio R.	Little Bear Creek	1999	49
Mississippi R.	Pigeon R./French Broad R./Holston R./Tennessee R./Ohio R.	Cosby Creek	2004	48
Mississippi R.	Pigeon R./French Broad R./Holston R./Tennessee R./Ohio R.	Greenbrier Creek	2004	27
Mississippi R.	Pigeon R./French Broad R./Holston R./Tennessee R./Ohio R.	Indian Camp Creek	2000	33

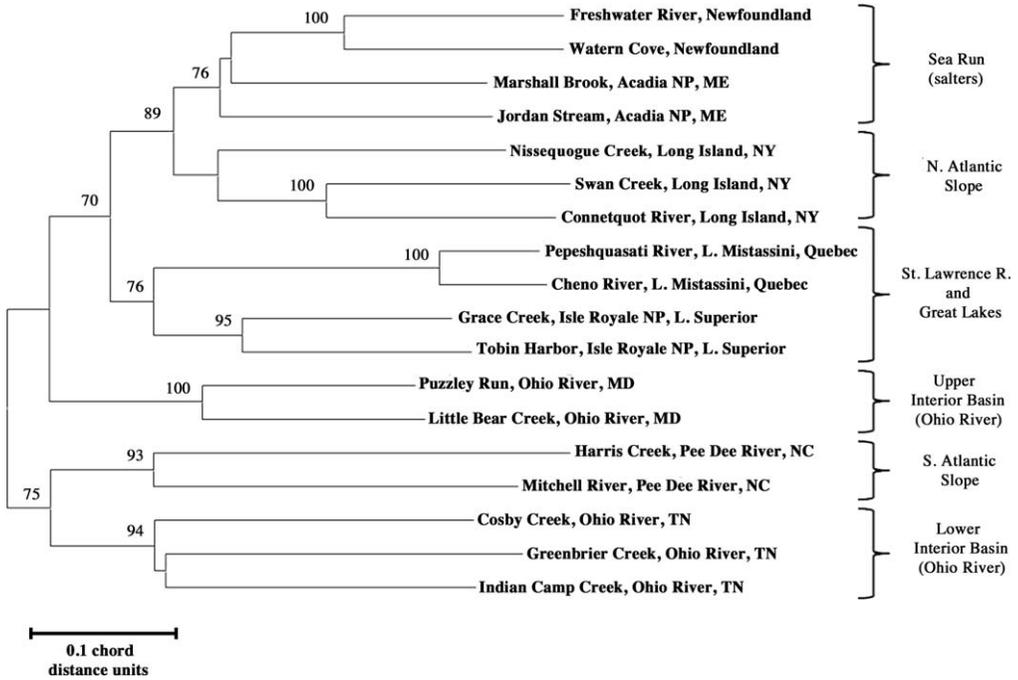


Fig. 1. Evolutionary relationships of brook trout *Salvelinus fontinalis* genotyped at 13 microsatellite loci inferred using the neighbor-joining algorithm (Saitou & Nei 1987) applied to the Cavalli-Sforza & Edwards (1967) chord distance for 18 collections representing six phylogeographically distinct assemblages (sea run, northern Atlantic slope, St. Lawrence-Great Lakes, upper interior basin/Ohio River, southern Atlantic slope, and lower interior basin/Ohio River). The phylogenetic tree was generated using njbpop (J.-M. Cornuet, INRA, Montpellier, France). Numbers along branches represent bootstrap support for nodes generated from 5000 randomizations. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Abbreviations: L. = Lake, MD = Maryland, ME = Maine, NC = North Carolina, NP = National Park, NY = New York, TN = Tennessee.

sylvania) and Rome (New York) hatchery strains. No overlap of populations from Swan Creek and Connetquot River with hatchery stocks existed (Fig. 2). Thus, we chose the neotype from Connetquot River, Long Island, New York.

Salvelinus fontinalis (Mitchill)

Neotype.—PSU 11387, 178.8 mm SL, collected by Charles Guthrie and Rachel Yoder, Connetquot River, Connetquot River State Park, 40°47.1714'N, 073°10.1334'W, Long Island, New York, 13 Jul 2010.

Material examined.—Nine specimens, PSU 11388, 103.6–166.1 mm SL; collection data as for neotype. Ten specimens, PSU

11389, 130.1–206.3 mm SL, collected by Charles Guthrie and Rachel Yoder, Nissequogue Creek at Blydenburgh County Park Office, Long Island, New York, 14 Jul 2010. Ten specimens, PSU 11390, 123.9–170.1 mm SL, collected by Charles Guthrie and Rachel Yoder, Swan Creek at end of Roberts Street, Long Island, New York, 13 Jul 2010.

Description of neotype.—The brook trout is a member of the subgenus *Baione*; as such it possesses minute teeth on the maxillaries and intermaxillaries, a patch of minute teeth on the vomer, and a series of teeth on the outer edges of the tongue (De Kay 1842). Populations from Long Island have isognathous jaws, which form a terminal mouth that differentiates it from

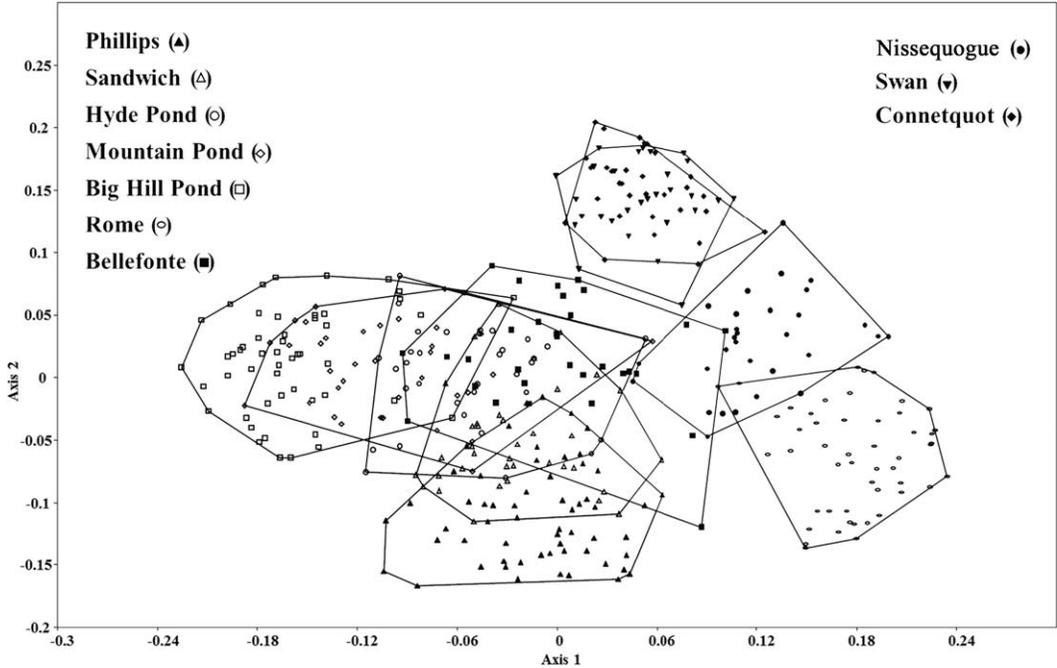


Fig. 2. Principal coordinates analysis depicting the relationship of the pairwise proportion of shared alleles from a survey of 13 microsatellite DNA markers among selected regional brook trout *Salvelinus fontinalis* hatchery strains and three wild collections (Nissequoque Creek, Swan Creek, and Connetquot River) sampled from Long Island, New York.

southern populations represented by fishes from the Lower Interior Basin, which have retrognathous jaws that form a slightly inferior mouth.

Jaws isognathous; teeth on upper and lower jaws and on vomer. Lateral line scales 109–138, neotype with 119; pored scales posterior to lateral-line terminus at hypural plate 5–10. Gill rakers on first ceratobranchial 8–10, neotype 10. Principal morphometric data and meristic data are shown in Tables 3 and 4, respectively.

Differentiation.—We present data that *Salvelinus fontinalis*, genotyped at 13 microsatellite loci (Table 2), demonstrates at least six phylogeographically distinct assemblages (sea run, northern Atlantic slope, St. Lawrence-Great Lakes, upper interior basin/Ohio River, southern Atlantic slope, and lower interior basin/Ohio River).

We further show morphological distinction of populations from Long Island from

the lower interior basin populations from Great Smoky Mountains National Park (Tables 5, 6). The plot of the sheared second principal component of the morphometric data versus the first principal component of the meristic data shows that the minimum polygon cluster formed by fishes from Long Island, New York, does not overlap with that formed by those collected in the Pigeon-French Broad Basin in Great Smoky Mountains National Park (Fig. 3). Variables with the highest standardized scoring coefficients for the meristic data were pored scales posterior to the lateral line (0.27), teeth on the lower jaw (0.26), and lateral-line scales (0.26). Size accounted for 95% of the observed variance and the second principal component for 31% of the remaining variation. Variables with the highest loadings on the sheared second principal component were dorsal-fin base length (–0.37), lower jaw

Table 3.—Morphometric data of brook trout, *Salvelinus fontinalis*, from Long Island, New York streams.

	Connetquot River			Nissequoque Creek			Swan Creek			
	Neotype	\bar{x}	SD	Range	\bar{x}	SD	Range	\bar{x}	SD	Range
Standard length, mm	170.8	152.8	19.7	103.6–170.8	160.0	24.9	130.1–206.3	149.7	15.3	123.9–170.1
Head length, mm	45.2	39.0	5.3	27.4–46.0	42.1	6.0	32.8–51.9	38.8	4.2	32.9–45.6
Percent standard length										
Body depth	25.4	25.1	1.3	23.5–28.1	23.3	1.2	21.1–25.2	25.1	1.2	23.7–27.3
Snout to dorsal-fin origin	47.0	46.0	0.8	44.7–47.0	47.5	1.2	45.2–49.7	46.7	1.3	44.5–48.5
Snout to pelvic-fin origin	52.6	50.8	1.6	48.4–53.6	53.6	1.4	51.8–56.0	51.7	1.7	48.2–53.9
Dorsal-fin base length	17.6	17.3	1.4	15.7–20.1	14.6	0.8	12.7–15.5	16.6	1.2	14.7–19.0
Anterior dorsal to anterior anal	37.7	37.2	1.0	35.7–39.3	36.0	1.0	34.3–38.1	36.6	0.7	35.6–37.7
Anterior dorsal to posterior anal	46.5	44.4	1.0	43.1–46.5	42.5	1.2	41.0–44.3	43.4	0.9	41.8–44.7
Posterior dorsal to anterior anal	24.9	24.2	1.0	22.6–25.9	24.1	0.8	22.8–25.4	23.9	0.8	22.5–25.3
Posterior dorsal to posterior anal	29.9	29.4	0.8	28.1–31.0	29.4	1.0	28.3–31.0	28.6	0.8	27.3–29.6
Posterior dorsal to ventral caudal	43.1	42.6	1.1	41.1–44.0	41.8	1.1	39.3–43.3	42.4	0.9	41.1–44.0
Anterior adipose to posterior anal	15.6	14.4	1.1	13.3–16.1	13.2	0.4	12.4–13.8	13.4	0.6	12.7–14.4
Posterior anal to dorsal caudal	20.6	20.3	0.9	18.6–21.5	18.8	1.1	17.9–21.4	19.6	0.6	18.4–20.6
Anterior dorsal to pelvic-fin origin	27.1	25.6	1.5	23.4–28.4	23.7	0.9	22.5–25.0	25.2	1.1	23.8–27.4
Posterior dorsal to pelvic-fin origin	25.9	24.4	1.3	22.5–26.4	22.2	1.0	20.7–23.7	24.0	0.9	22.7–25.3
Caudal-peduncle length	16.3	16.7	0.8	15.3–18.1	15.2	1.1	13.2–17.2	16.3	0.9	14.9–17.6
Least caudal-peduncle depth	11.6	10.6	0.7	10.0–11.8	10.6	0.5	10.0–11.3	10.6	0.7	9.3–11.8
Percent head length										
Snout length	23.3	22.8	1.1	21.4–25.2	23.9	1.6	21.4–26.8	22.9	1.6	20.0–25.2
Postorbital head length	54.1	51.2	1.5	49.4–54.1	49.7	2.0	45.5–52.8	51.3	2.1	47.1–53.3
Horizontal eye diameter	25.6	27.5	1.5	25.6–29.6	28.4	2.0	24.0–31.1	28.5	1.6	26.9–31.6
Vertical eye diameter	22.3	24.6	1.8	21.7–28.1	25.7	1.6	22.8–27.8	24.8	1.9	22.9–29.2
Lower-jaw length	68.3	68.9	2.9	64.8–74.2	70.3	4.0	65.1–75.4	65.1	3.4	60.0–71.1
Head depth	63.7	66.5	3.2	62.8–72.4	66.7	2.7	63.6–71.0	64.8	3.6	57.5–69.5

Table 4.—Meristic data of brook trout, *Salvelinus fontinalis*, from Long Island, New York streams.

Counts	Connetquot River				Nissequoque Creek			Swan Creek		
	Neotype	Mode	% Freq.	Range	Mode	% Freq.	Range	Mode	% Freq.	Range
Dorsal-fin rays	12	12/13	40	11–14	13	50	9–13	13	40	11–13
Anal-fin rays	10	10	100	10	10	90	10–11	11	50	10–12
Pectoral-fin rays	14	13	60	12–14	14	60	12–14	14	60	13–14
Pelvic-fin rays	10	9	70	9–10	9	70	9–10	10	70	8–10
Lateral-line scales	119	127	20	111–138	117	30	112–130	116	20	109–130
Pored scales posterior to lateral line	6	6	50	5–8	5	60	5–6	6	40	5–10
Gill rakers on first ceratobranchial	10	9	50	8–10	9	60	8–9	8	100	8
Gill rakers on first epibranchial	6	5/6	50	5–6	6	60	6–7	5	60	5–6
Teeth in outer row of left lower jaw	15	16	50	15–17	17	50	17–19	18	40	16–19
Parr marks	7	7	60	7–8	8	80	8–10	8	50	6–9

length (0.36), and vertical eye diameter (0.28).

Discussion

In Article 75.3 of the International Code of Zoological Nomenclature (<http://www.nhm.ac.uk/hosted-sites/iczn/code/index>.

jsp?article=75&nfv=true) it states that a neotype is validly designated when the express purpose is to 1) clarify the taxonomic status or the type locality of a nominal taxon (75.3.1); 2) a statement of the characters differentiating the neotype from other taxa (75.3.2); 3) present data that is sufficient to ensure recognition of the neotype (75.3.3); 4) statement of

Table 5.—Morphometric data of brook trout, *Salvelinus fontinalis*, from the French Broad Drainage in Great Smoky Mountains National Park.

	\bar{x}	SD	Range
Standard length, mm	104.3	15.6	78.5–156.6
Head length, mm	27.7	5.2	19.7–43.8
Percent standard length			
Body depth	24.3	2.2	19.9–30.8
Snout to dorsal-fin origin	47.8	1.2	44.9–49.8
Snout to pelvic-fin origin	51.3	1.0	49.5–54.1
Dorsal-fin base length	15.4	1.2	13.5–17.0
Anterior dorsal to anterior anal	35.8	1.4	32.8–39.7
Anterior dorsal to posterior anal	42.7	1.1	40.7–45.0
Posterior dorsal to anterior anal	22.4	1.1	20.1–24.5
Posterior dorsal to posterior anal	27.8	107	21.8–30.2
Posterior dorsal to ventral caudal	40.4	1.7	37.1–43.8
Anterior adipose to posterior anal	18.0	1.2	15.6–20.6
Posterior anal to dorsal caudal	19.1	1.1	17.0–21.9
Anterior dorsal to pelvic-fin origin	24.0	1.7	21.2–29.9
Posterior dorsal to pelvic-fin origin	21.5	1.5	19.1–24.5
Caudal-peduncle length	16.3	1.3	13.9–19.8
Least caudal-peduncle depth	10.7	5.7	10.0–11.9
Percent head length			
Snout length	21.6	1.8	18.6–25.2
Postorbital head length	48.5	2.5	43.9–53.1
Horizontal eye diameter	32.0	2.3	28.0–36.5
Vertical eye diameter	28.5	2.5	24.8–33.9
Lower-jaw length	63.2	5.8	53.1–76.0
Head depth	67.5	4.1	60.2–75.3

Table 6.—Meristic data of brook trout, *Salvelinus fontinalis*, from the French Broad Drainage in Great Smoky Mountains National Park.

Counts	Mode	Range
Dorsal-fin rays	11	10–12
Anal-fin rays	9	9–11
Pectoral-fin rays	13	12–14
Pelvic-fin rays	9	9–11
Lateral-line scales	94/105	78–128
Pored scales posterior to lateral line	2	0–6
Gill rakers on first ceratobranchial	8	7–9
Gill rakers on first epibranchial	7	4–8
Teeth in outer row of left lower jaw	13	8–15
Parr marks	9	6–11

reasons for believing the name-bearing type specimen was lost or destroyed (75.3.4); 5) provide evidence that the neotype is consistent with the known name-bearing type from the original description (75.3.5); and 6) provide evidence that the neotype came from as near as possible to the type locality.

Certainly the taxonomic status of populations of *S. fontinalis* must be examined further. To facilitate these studies, we provide genetic and morphological data for the neotype and associated populations from Long Island, New York. We further show allelic diversity throughout the range and have differentiated the Long Island populations morphologically from Interior Basin populations. We supply both genetic and morphological characters to describe and ensure recognition of the neotype. A type specimen of this taxon was never preserved or catalogued into a museum. Finally, we collected the neotype from localities where the collections were made upon which the original description was based and demonstrated that there was no discernable evidence that hatchery stocks had altered the genetic composition of these populations.

Acknowledgments

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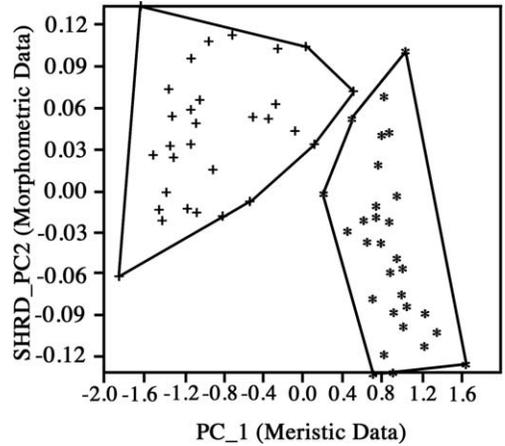


Fig. 3. Second sheared principal components (morphometric data) plotted against the first principal components (meristic data) of *Salvelinus fontinalis* populations from Long Island, New York (*) and Great Smoky Mountains National Park (+).

ronmental Conservation, who organized a crew and arranged for the collecting of fish on Long Island, New York. We thank Rachel Yoder for aiding in the collection of fish. Fish were collected under the approved IACUC 40122 research program.

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