

Description of three species of *Salvelinus* (Teleostei: Salmonidae) from the Great Smoky Mountains National Park, Tennessee, U.S.A.

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Three new species of *Salvelinus* are described from the Great Smoky Mountains National Park, Tennessee. The light spots on a dark background clearly place all three species in the genus *Salvelinus*. All three species are in subgenus *Baione*, which to date contains only one extant species, *Salvelinus fontinalis*. Synapomorphies of *Baione* include mottled vermiculated markings on the dorsal portion of the body, on caudal and dorsal fins; the black edge and white border of the pelvic and anal fins; and the lack of teeth on the basibranchials. The three new species all possess these synapomorphies of *Baione*. The higher number of vertebrae and retrognathous jaws, which form a slightly inferior mouth distinguish all three species from *Salvelinus fontinalis*. *Salvelinus kingi*, new species, is distinguished from *Salvelinus multidentatus*, new species, by the fewer number of teeth on the basihyal (5–7 vs. 8–9) and from *Salvelinus angustus*, new species, by fewer lateral-line scales (109–115 vs. 118–130). The number of lateral-line scales of *S. angustus* (118–130) differentiates it from *S. multidentatus* (88–111).

Introduction

Salmonid fishes inhabiting lower latitudes in North America present significant challenges to conservation biologists that attempt to identify and maintain ecological and evolutionary processes within and among populations. To date, Brook Trout (*Salvelinus fontinalis* (Mitchill, 1814)) functions as a keystone species in some headwater streams. Great Smoky Mountains National Park (GRSM) is committed to monitoring ecological and evolutionary functions and processes of park ecosystems. Since 1900, Brook Trout have lost 75 % of their range in GRSM due to fragmentation and compression (Moore et al., 2005). The presence

of the introduced Rainbow Trout (*Oncorhynchus mykiss* (Walbaum, 1792)) historically has been the major cause of continued decline in the distribution of *Salvelinus* spp. in the GRSM (Kelly, 1980; Moore et al., 1986). Geographic constraints of the region limit migration to higher elevations in the same basin, which are already occupied, blocked by natural barriers, or unsuitable due to low pH, lack of reliable flows, or stream habitat in small headwater streams. Functionally, these geographic populations are veritable “islands” (Flebbe et al., 2006: 1372) as there is little or no opportunity for gene flow among populations. Demographically, these processes reduce effective population size and accelerate extinction (Gilpin

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Fig. 1. *Salvelinus fontinalis*, PSU 11387, neotype, 170.8 mm SL; U.S.A.: New York State: Long Island: Connetquot River.

& Soulé, 1986). Moreover, the historic use of hatchery-reared *S. fontinalis* for supplemental and restorative stocking in GRSM and other southern Appalachian streams underscores the need to recognize the evolutionary relationships among stream populations.

Brook Trout is the only native charr recorded from Tennessee (Etnier & Starnes, 1993). Crête-Lafrenière et al. (2012) completed a phylogenetic portrait of Salmonidae and supported the monophyly of the genus *Salvelinus*, with *S. fontinalis* being a sister species to the other members of this genus. They further acknowledged that unresolved issues within the salmonids may be attributed to parallel/convergent evolution, similarity of niches, rapid radiation, frequent hybridization, and local adaptations. The taxonomic status of *S. fontinalis* has been problematic, since its original description by Mitchill (1814). Stauffer & King (2014) recently designated an individual (PSU 11387) from the Connetquot River, Long Island, New York as the neotype of *S. fontinalis* (Fig. 1) and provided evidence for six distinct lineages within this species. They further presented data showing that Brook Trout genotyped at 13 microsatellite loci from the North Atlantic Slope, as represented from samples from Long Island, were phylogeographically distinct from (1) those that were sea run, (2) occur in the St. Lawrence River and Great Lakes drainages, (3) occur in the Upper Interior Basin (Ohio River), (4) occur on the southern Atlantic Slope, and (5) occur in the Lower Interior Basin (Ohio River via Tennessee River). The collections from the locality of the neotype are morphologically distinct from

populations in the Great Smoky Mountains National Park (Stauffer & King, 2014). Certainly, both migratory and resident populations co-occur in some of these systems.

The existence of sibling species of salmonids is well documented (e.g. Behnke, 1972). These populations are fragile, and every effort must be made to protect their genetic diversity (Behnke, 1972). The independent evolution of adaptively divergent phenotypes among closely related lineages is most likely the consequence of natural selection (King & Wilson, 1975), but may be accelerated by sexual selection (Seehausen et al., 1999; Kellogg et al., 2000; Stauffer et al., 2013). The purpose of this paper is to describe three new species of *Salvelinus* (subgenus *Baione*) from the Pigeon/French Broad Drainage in Great Smoky Mountains National Park.

Material and methods

Fishes were collected from Indian Camp Creek (35°44.2'N 83°16.6'W) on 3 August 2009; Cosby Creek (35°44.8'N 83°12.0'W) on 3 August 2009; and Little Greenbrier Creek (35°45.9'N 83°15.2'W) on 5 August 2009 of the Pigeon/French Broad drainage (Fig. 2) with the aid of a backpack electro-shocking unit. All sites were generally above 680 m altitude. I had permission to preserve 10 specimens at each site. 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 transferred to 70 % ethanol for permanent storage

at The Pennsylvania State University (PSU) Fish Museum (IACUC Protocol# 37480). Pigmentation patterns and colour were recorded in the field. Counts and measurements follow Stauffer (1991). Fin clips were not taken on the type series, since fin clips used for genetic analysis were collected simultaneously from fishes that were caught and released (see Stauffer & King, 2014); thus, I assumed that the type series were the same genetically as those fish from which fin clips were removed. All counts and measurements were taken from the left side of the body except for gill-raker counts, which were taken on the right side.

The head of the holotype and all the paratypes of the three new species and the neotype of *Salvelinus fontinalis* were scanned on the high-resolution x-ray computed tomography (HRCT) system in the Center for Quantitative X-Ray Imaging (CQI) at Penn State University. The mandibles were scanned with target pixel and slice resolutions of approximately 30 μm and the data stored on Morphosource. Scan data were reconstructed as 16-bit TIFF images with a 1024 \times 1024 pixel grid. The volumetric image datasets for each fish were used to create a three-dimensional isosurface reconstruction using the visualization software Avizo 6.1 (VSG, Burlington, MA). Because all the fishes were HRCT scanned with the same energy settings and voxel resolutions, a global threshold was used for all datasets to separate bone from non-bone for the three-dimensional reconstructions. The whole body of each of the above specimens and nine specimens collected concurrently with the neotype of *S. fontinalis* were x-rayed using the HRCT system to obtain counts of vertebrae.

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 species were illustrated by plotting the sheared second principal component (SPC2) of the morphometric data against the first principal component (PC1) of the meristic data (Stauffer & Hert, 1992). An ANOVA was used to determine whether the minimum polygon clusters of different populations were significantly different along one axis ($p < 0.05$), independent of the other axis. If the clusters were significantly different a Duncan's Multiple Range Test was

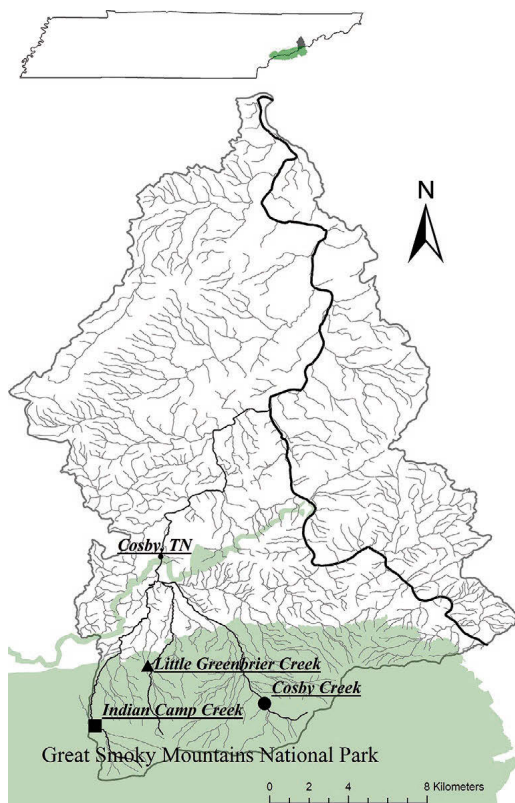


Fig. 2. Map of the upper French Broad drainage, with main channel Pigeon River in bold, in the Great Smoky Mountains National Park (■), Tennessee State (inset), U.S.A., showing the collection sites of the holotypes of *Salvelinus angustus* (▲), *S. kingi* (●), and *S. multidentatus* (■).

used to determine which clusters were different from each other (Stauffer & Konings, 2006).

Results

The species described herein all possess light spots on a dark background, which is a synapomorphy of the genus *Salvelinus* (Kendall & Behnke, 1984). Furthermore, mottled vermiculated markings on the dorsal portion of the body, caudal fins, and dorsal fins; the black edge and white border of the pelvic and anal fins; and the lack of teeth on the basibranchial places all these species in the subgenus *Baione*. *Salvelinus fontinalis* is endemic to northeastern North America and until now is the only extant species in the subgenus *Baione*, although Behnke (1972) recognized *Salvelinus*

agassizii (Garman, 1885), which is probably extinct, as a member of *Baione* and regarded it as a valid species distinct from the sympatric *S. fontinalis* in Dublin Pond, New Hampshire. Henn & Rickenbach (1925) differentiated between *Salvelinus timagamiensis* Henn & Rickenbach, 1925 (Aurora Char) and sympatric *S. fontinalis* in White Pine Lake, Ontario; however, Sale (1967) and Qadri (1968) regarded it as a subspecies of *S. fontinalis* because intergrades were found between it and the

introduced *S. fontinalis*. Behnke (1980) diagnosed *S. fontinalis* by the presence of 57–62 vertebrae, 13–22 gill rakers, and 25–50 pyloric caeca. Additionally, *S. fontinalis* lacks basibranchial teeth, except for one population from Canada (Behnke, 1980).

Observations revealed the fishes described were sexually mature at the sizes reported herein. Eggs were stripped from fishes of similar size by Kulp et al. (2017: 69, fig. 2).

Table 1. Morphometric and meristic values of the holotype (PSU 13088) and paratypes (PSU 11392) of *Salvelinus kingi* from Cosby Creek, Great Smoky Mountains National Park. SD, standard deviation.

	Holotype	range	mean	SD
Standard length, mm	118.3	91.4–118.3	102.2	8.4
Head length, mm	31.5	24.2–34.5	27.7	3.1
In percents of standard length				
Head Length	26.6	26.1–30.5	27.1	1.3
Body depth	26.2	21.5–30.6	24.9	2.5
Snout to dorsal-fin origin	48.5	47.7–50.8	49.0	1.1
Snout to pelvic-fin origin	52.6	51.1–53.7	52.5	0.8
Dorsal-fin base length	14.7	14.4–17.5	15.5	1.0
Anterior dorsal to anterior anal	36.2	33.2–38.8	35.4	1.6
Anterior dorsal to posterior anal	44.0	41.2–45.1	42.9	1.4
Posterior dorsal to anterior anal	23.8	20.3–24.6	22.4	1.3
Posterior dorsal to posterior anal	29.8	24.1–30.0	27.6	1.8
Posterior dorsal to ventral caudal	41.1	37.4–42.8	39.8	1.7
Posterior adipose to posterior anal	11.1	11.1–13.7	11.9	0.8
Posterior anal to dorsal caudal	20.0	18.1–21.1	19.5	1.0
Anterior dorsal to pectoral-fin origin	30.9	29.9–35.8	32.1	1.8
Posterior dorsal to pectoral-fin origin	40.8	40.1–43.6	41.5	1.0
Caudal-peduncle length	15.6	15.2–18.3	16.7	1.1
Least caudal-peduncle depth	11.2	9.8–11.7	10.7	0.6
In percents of head length				
Snout length	22.9	21.4–26.1	24.1	1.6
Postorbital head length	47.2	47.0–49.4	48.0	0.9
Horizontal eye diameter	30.4	28.2–36.5	31.6	2.3
Vertical eye diameter	27.0	26.4–31.5	28.5	1.7
Lower-jaw length	73.3	65.1–73.3	68.4	2.6
Head depth	77.3	62.9–77.3	70.9	4.0
Counts				
			mode	frequency %
Dorsal-fin rays	11	10–11	11	90
Anal-fin rays	9	9–10	9	70
Pectoral-fin rays	12	12–14	14	70
Pelvic-fin rays	6	7–9	7	40
Lateral-line scales	110	109–115	114	40
Pored scales post. to hypural plate	3	2–7	4	40
Gill rakers on first ceratobranchial	7	7–10	10	40
Gill rakers on first epibranchial	6	5–8	6	60
Teeth in outer row of left lower jaw	14	12–17	14	60
Teeth on basihyal	7	5–7	7	50
Parr marks	6	5–7	6	50
Number of vertebrae	62	57–64	61	40

Salvelinus kingi, new species
(Fig. 3, Table 1)

Holotype. PSU 13088, male, 118.3 mm SL; U.S.A.: Tennessee State: Haywood County: Pigeon/French Broad Drainage: Cosby Creek, Great Smoky Mountains National Park, 35°44'53"N 83°12'01"W; T. C. Weathers, T. L. King, J. L. Fischer and J. R. Stauffer Jr., 3 Aug 2009.

Paratypes. PSU 11392, 9, 91.4–108.2 mm SL, data as for holotype.

Diagnosis. A member of the subgenus *Baione* distinguished from all other members of this subgenus in the following manner. The retrognathous jaws, which form a slightly inferior mouth, distinguish it from *S. fontinalis*, which has isognathous jaws and a terminal mouth (Stauffer & King, 2014). Additionally, there are generally more vertebrae (57–64) present in *S. kingi* than

Table 2. Morphometric and meristic values of the holotype (PSU 13087) and paratypes (PSU 11393) of *Salvelinus angustus* from Indian Camp Creek, Great Smoky Mountains National Park. SD, standard deviation.

	Holotype	range	mean	SD
Standard length, mm	97.2	77.0–107.7	92.1	9.6
Head length, mm	25.6	19.1–28.3	23.6	2.8
In percents of standard length				
Head Length	26.3	24.0–26.3	25.6	0.8
Body depth	23.0	21.4–26.9	24.4	1.7
Snout to dorsal-fin origin	48.6	44.7–48.6	47.4	1.2
Snout to pelvic-fin origin	52.9	47.7–53.5	50.5	1.9
Dorsal-fin base length	13.0	13.0–15.6	14.4	0.9
Anterior dorsal to anterior anal	33.9	33.9–35.8	35.0	0.6
Anterior dorsal to posterior anal	44.4	41.6–44.4	43.0	0.9
Posterior dorsal to anterior anal	22.8	20.9–28.5	22.9	2.2
Posterior dorsal to posterior anal	28.7	26.5–29.7	28.6	1.0
Posterior dorsal to ventral caudal	44.6	39.7–44.6	41.3	1.6
Posterior adipose to posterior anal	11.6	11.1–12.6	11.9	0.5
Posterior anal to dorsal caudal	20.7	18.7–22.7	20.2	1.2
Anterior dorsal to pectoral-fin origin	28.2	28.2–32.4	30.9	1.2
Posterior dorsal to pectoral-fin origin	37.7	37.7–43.8	41.0	1.7
Caudal-peduncle length	17.5	16.2–20.1	17.5	1.3
Least caudal-peduncle depth	9.6	9.3–10.9	10.1	0.5
In percents of head length				
Snout length	23.4	19.4–28.3	21.6	1.7
Postorbital head length	47.3	45.4–51.1	48.3	1.5
Horizontal eye diameter	31.0	28.7–35.3	32.2	2.1
Vertical eye diameter	27.5	26.3–30.4	28.2	1.3
Lower-jaw length	70.5	55.4–70.5	61.8	4.1
Head depth	71.8	67.1–78.2	72.6	3.4
Counts				
			mode	frequency %
Dorsal-fin rays	10	9–11	10/11	40
Anal-fin rays	10	9–10	10	70
Pectoral-fin rays	13	13–15	14	60
Pelvic-fin rays	8	8–10	10	40
Lateral-line scales	119	118–130	118/126	20
Pored scales post. to hypural plate	2	2–7	2/3	30
Gill rakers on first ceratobranchial	11	8–11	9/10/11	30
Gill rakers on first epibranchial	6	6–8	7	50
Teeth in outer row of left lower jaw	14	14–17	15	50
Teeth on basihyal	6	6–8	6	70
Parr marks	6	9–11	10	50
Number of vertebrae	63	63–66	63	70



Fig. 3. *Salvelinus kingi*, PSU 13088, holotype, 118.3 mm SL; U.S.A.: Tennessee State: Cosby Creek.

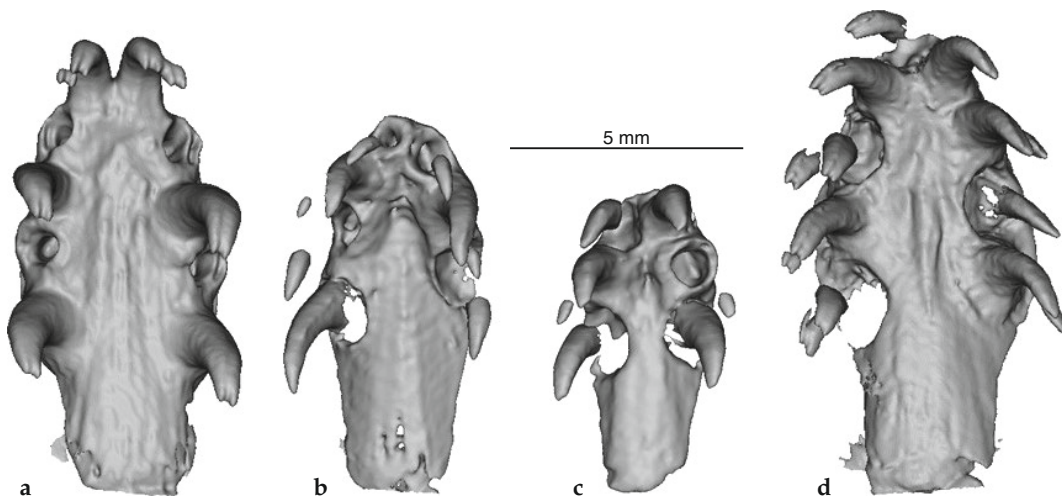


Fig. 4. Teeth on the basihyal (in dorsal view) of members of subgenus *Baione*: a, *Salvelinus fontinalis*, PSU 11387, neotype, 170.8 mm SL; b, *Salvelinus kingi*, PSU 13088, holotype, 118.3 mm SL; c, *Salvelinus angustus*, PSU 13087, holotype, 97.2 mm SL; d, *Salvelinus multidentatus*, PSU 13089, holotype, 135.4 mm SL.

found in *S. fontinalis* (55–59) from the Connetquot River, Long Island. The number of teeth on the basihyal (5–8, Fig. 4) further distinguishes *S. kingi* from *S. fontinalis* (8–9) and from *S. multidentatus*, new species (8–9). The number of lateral-line scales of *S. kingi* (109–115) distinguishes it from *S. angustus*, new species (118–130).

Description. Morphometric ratios and meristic data in Table 1. Body fusiform and compressed, deepest at origin of dorsal fin. Head elongate (26.1–30.5 % SL). Large eyes (HED 28.2–36.5 % HL; VED 26.4–31.5 % HL). Jaws large (LJL 65.1–73.3 % HL) and retrognathous. Caudal fin trun-

cate. Caudal peduncle length (15.2–18.3 % SL) greater than least caudal peduncle depth (9.8–11.7 % SL). Head acute and pointed with straight predorsal profile. Mature adults with 5–7 parr marks. Lateral-line scales 109–115, holotype with 110; scales posterior to hypural plate 2–7. Gill rakers on first ceratobranchial 7–10, holotype 7.

Pectoral-fin insertion low on body and almost adjacent to posterior edge of opercular. Anterior insertion of anal fin below 6–7th anterior rays of dorsal fin.

Live specimens with olive green head with pale yellow vermiculations; white gular; interior of mouth black; cheek and opercle pale yellow.



Fig. 5. *Salvelinus angustus*, PSU 13087, holotype, 97.2 mm SL; U.S.A.: Tennessee State: Little Greenbrier Creek.

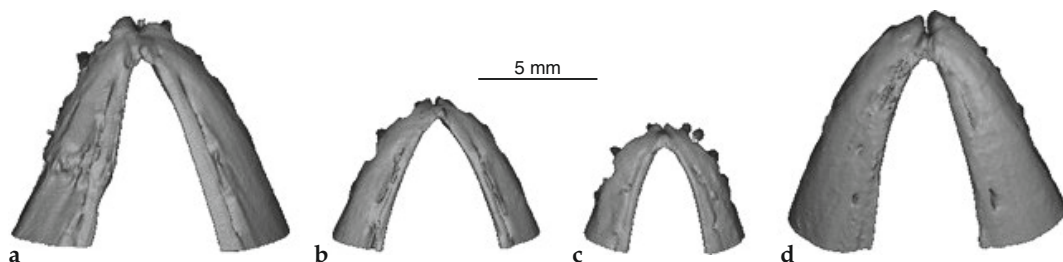


Fig. 6. Lower jaw (in ventral view) in members of the subgenus *Baione*: **a**, *Salvelinus fontinalis*, PSU 11387, neotype, 170.8 mm SL; **b**, *Salvelinus kingi*, PSU 13088, holotype, 118.3 mm SL; **c**, *Salvelinus angustus*, PSU 13087, holotype, 97.2 mm SL; **d**, *Salvelinus multidentatus*, PSU 13089, holotype, 135.4 mm SL.

Above lateral line dorsally, body dark olive green and yellow/grey ventrally; dark grey parr marks, ventral portion and belly posterior to pectoral fin orange; anterior to pectoral fin white; pale yellow spots usually in parallel rows above and below lateral line, some with red centers along sides. Dorsal fin pale yellow with black vermiculations; caudal fin pale orange with black markings; pectoral fins, anal fins, and pelvic fins with black submarginal and white marginal bands.

Distribution. To date only known from Cosby Creek in Great Smoky Mountains National Park and LeConte Creek, where it has been introduced.

Etymology. Named after Timothy L. King, who devoted his professional life to studying fishes, gathered extensive information on species of Brook Trout throughout their range, and was a great friend and mentor to me. A noun in the genitive case.

Salvelinus angustus, new species (Fig. 5, Table 2)

Holotype. PSU 13087, 97.2 mm SL; U.S.A.: Tennessee State: Haywood County: Pigeon/French Broad Drainage: Little Greenbrier Creek, Great Smoky Mountains National Park, 35°44'16"N 83°16'40"W; S. E. Moore, J. P. Predel, J. Henry, T. L. King and J. R. Stauffer Jr., 5 Aug 2009.

Paratypes. PSU 11393, 9, 77.0–107.7 mm SL, data as for holotype.

Diagnosis. A member of the subgenus *Baione* distinguished from all other members of this subgenus in the following manner. The retrognathous jaws, which form a slightly inferior mouth distinguishes it from *S. fontinalis*, which has isognathous jaws and a terminal mouth (Stauffer & King, 2014). *Salvelinus angustus* has more vertebrae (63–66) than *S. kingi* (57–64) and *S. fontinalis* (55–59) from the Connetquot River, Long Island. The presence

of 6–8 teeth on the basihyal (Fig. 4) separates *S. angustus* from *S. multidentatus*, new species, which has 8–9. The number of lateral-line scales of *S. angustus* (118–130) distinguishes it from *S. multidentatus* (88–111) and *S. kingi* (109–115).

Description. Morphometric ratios and meristic data in Table 2. Body fusiform and compressed, deepest at origin of dorsal fin. Head elongate (24.0–26.3 % SL). Large eyes (HED 28.7–35.3 % HL; VED 26.3–30.4 % HL). Jaws large (LJL 55.4–70.5 % HL) and retrognathous. Caudal fin truncate. Caudal peduncle length (16.2–20.1 % SL) greater than least caudal peduncle depth (9.3–10.9 % SL). Head acute and pointed with straight predorsal profile. Mature adults with 9–11 parr marks. Lateral-line scales 118–130, holotype with 119; scales posterior to hypural plate 2–7. Gill rakers on first ceratobranchial 8–11, holotype 11.

Pectoral-fin insertion low on body and almost adjacent to posterior edge of opercular. Anterior insertion of anal fin below 7–8th anterior rays of dorsal fin.

Live specimens with olive green head with pale yellow vermiculations; white gular; inter-orbital olive green; interior portion of mouth black; cheek and opercle pale yellow. Above lateral line dorsally, body dark olive green with light vermiculations anteriorly and light spots posteriorly; dark grey parr marks, lateral sides olive green light spots usually in parallel rows above and below lateral line; some light spots with red centers; ventral posterior $\frac{1}{4}$ red/orange with light bars to lateral line. Ventral portion of belly with black stripe. Dorsal fin black with pale yellow vermiculations. Adipose fin olive green with light yellow vermiculations. Caudal fin orange/red with black vermiculations; pectoral fin, anal fin and pelvic fin with black submarginal bars and white marginal bars.

Distribution. To date, only known from Little Greenbrier Creek in Great Smoky Mountains National Park and LeConte Creek, where it has been introduced.

Etymology. *Angustus*, -a, -um, referring to the relative narrow width of the lower jaw when compared to *S. kingi* and *S. multidentatus* (Fig. 6). A Latin adjective, meaning narrow, strait, contracted.

Salvelinus multidentatus, new species (Fig. 7, Table 3)

Holotype. PSU 13089, male, 135.4 mm SL; U.S.A.: Tennessee State: Haywood County: Pigeon/French Broad Drainage: Indian Camp Creek, Great Smoky Mountains National Park, 35°45' 56" N 83°15'14" W; T. C. Weathers, T. L. King, J. L. Fischer and J. R. Stauffer, Jr., 5 Aug 2009.

Paratypes. PSU 11391, 9, 96.5–153.1 mm SL, data as for holotype.

Diagnosis. A member of the subgenus *Baione* distinguished from all other members of this subgenus in the following manner. The retrognathous jaws, which form a slightly inferior mouth distinguishes it from *S. fontinalis*, which has isognathous jaws and a terminal mouth (Stauffer & King, 2014). The number of vertebrae of *S. multidentatus* (60–64) is greater than those found in *S. fontinalis* from the Connetquot River, Long Island (55–59). *Salvelinus multidentatus* generally has more teeth (8–9) on the basihyal (Fig. 5) than *S. kingi* (5–8) and fewer lateral-line scales (88–111 vs. 109–115). The number of lateral-line scales of *S. multidentatus* (88–111) distinguishes it from *S. angustus* (118–130). The lower jaw of *S. multidentatus* is wider when compared with *S. angustus* (Fig. 6).

Description. Morphometric ratios and meristic data in Table 3. Body fusiform and compressed, deepest at origin of dorsal fin. Head acute and pointed with straight predorsal profile. Head elongate (25.3–30.2 % SL). Large eyes (HED 26.2–32.1 % HL; VED 23.8–28.8 % HL). Jaws large (LJL 61.5–79.6 % HL) and retrognathous. Caudal fin truncate. Caudal peduncle length (15.1–17.6 % SL) greater than least caudal peduncle depth (10.1–11.3 % SL). Mature adults with 7–9 parr marks. Lateral-line scales 88–111, holotype with 90. Gill rakers on first ceratobranchial 8–10, holotype 9.

Pectoral-fin insertion low on body and almost adjacent to posterior edge of opercular. Anterior insertion of anal fin below 7–8th anterior rays of dorsal fin.

Live specimens with olive green head dorsally; white gular; interior portion of mouth white; cheek and opercle pale yellow. Above lateral line, body dark olive green. Numerous small light spots throughout; dark grey parr marks, ventral portion olive; some light spots with red centers; Ventral portion of belly with pale orange stripe. Dorsal fin

pale yellow with black vermiculations. Adipose fin olive green with light yellow vermiculations. Caudal fin orange/red with black vermiculations; pectoral fin, anal fin, and pelvic fin with black submarginal bars and white marginal bars.

Distribution. To date only known from Indian Camp Creek in Great Smoky Mountains National Park and LeConte Creek, where it has been introduced.

Etymology. *Multidentatus*, -a, -um, referring to the many teeth (8–9) found on the basihyal. A Latin adjective, meaning many teeth.

Remarks. Stauffer & King (2014: 565, fig. 3) differentiated the population from which the neotype was designated from those of the Great Smoky Mountains National Park. A plot of the sheared second principal component (SPC2) of the morphometric data against the first principal component (PC1) of the meristic data (Fig. 8) il-

Table 3. Morphometric and meristic values of the holotype (PSU 13089) and paratypes (PSU 11391) of *Salvelinus multidentatus* from Little Greenbrier Creek, Great Smoky Mountains National Park. SD, standard deviation.

	Holotype	range	mean	SD
Standard length, mm	135.4	96.5–153.1	115.5	1.7
Head length, mm	40.9	26.7–43.2	32.0	0.6
In percents of standard length				
Head Length	30.2	25.3–30.2	27.6	1.5
Body depth	26.5	23.9–26.8	25.3	1.1
Snout to dorsal-fin origin	50.8	46.5–50.8	48.9	1.3
Snout to pelvic-fin origin	52.6	49.6–53.4	51.7	1.2
Dorsal-fin base length	14.9	12.9–15.3	14.6	0.7
Anterior dorsal to anterior anal	35.8	34.4–36.3	35.5	0.6
Anterior dorsal to posterior anal	43.3	30.3–44.3	42.0	4.1
Posterior dorsal to anterior anal	23.7	22.2–23.8	23.0	0.6
Posterior dorsal to posterior anal	28.1	27.4–29.9	28.6	0.9
Posterior dorsal to ventral caudal	40.6	39.2–42.8	40.8	1.3
Posterior adipose to posterior anal	12.3	11.0–12.6	11.9	0.5
Posterior anal to dorsal caudal	19.5	17.6–20.5	19.0	1.0
Anterior dorsal to pectoral-fin origin	31.5	30.3–32.7	31.5	0.7
Posterior dorsal to pectoral-fin origin	43.0	39.6–43.2	41.4	1.1
Caudal-peduncle length	17.2	15.1–17.6	16.5	0.9
Least caudal-peduncle depth	11.3	10.1–11.3	10.8	0.4
In percents of head length				
Snout length	26.7	19.7–26.7	23.0	1.7
Postorbital head length	47.8	47.6–50.9	49.2	1.1
Horizontal eye diameter	26.7	26.2–32.1	29.7	2.0
Vertical eye diameter	23.8	23.8–28.8	26.3	1.8
Lower-jaw length	74.5	61.5–79.6	69.2	5.4
Head depth	62.2	62.2–81.1	71.8	5.0
Counts				
			mode	frequency %
Dorsal-fin rays	10	10–11	10	60
Anal-fin rays	9	9–10	9/10	50
Pectoral-fin rays	14	14	14	100
Pelvic-fin rays	8	8–10	9	60
Lateral-line scales	90	88–111	90/99/100	20
Pored scales post. to hypural plate	3	3–4	3	90
Gill rakers on first ceratobranchial	9	8–10	9	60
Gill rakers on first epibranchial	7	5–8	7	50
Teeth in outer row of left lower jaw	14	14–16	14	50
Teeth on basihyal	9	8–9	8	80
Parr marks	8	7–9	8	80
Number of vertebrae	60	60–64	61	30



Fig. 7. *Salvelinus multidentatus*, PSU 13089, holotype, 135.4 mm SL; U.S.A.: Tennessee State: Indian Camp Creek.

illustrated that the three species described herein form distinct clusters. The first principal component (size variable) of the morphometric data explained 91.5 % of the observed variance; the sheared second principal component explained 21.2 % of the remaining variance. Variables that had the highest loadings on the sheared second principal components of the morphometric data were distance between the posterior insertion of the dorsal fin to the anterior insertion of the anal fin (0.36), body depth (0.30), and lower jaw length (−0.28). The first principal component of the meristic data explained 39.8 % of the variance. Variables with the highest loadings on the first principal components of the meristic data were the number of parr marks (−0.51), number of teeth on the basihyal (−0.46), and number of lateral-line scales (0.47). The three species were significantly different ($p < 0.05$) along the x-axis (PC1 of the meristic data); thus supporting that these three populations represent unique taxa.

The neotype and eight conspecifics of *S. fontinalis* collected in Connetquot River, Long Island, New York (Stauffer & King, 2014) had 55–57 vertebrae, with one specimen having 59. The insertion of the anal fin of all specimens from GRSM is posterior to the insertion of the anal fin of *S. fontinalis* (below 4–5th anterior ray of dorsal fin vs. 7–8th anterior ray of dorsal fin) collected in Connetquot River, Long Island. One specimen from GRSM possessed 57 vertebrae and the remainder between 62–67.

The neotype of *Salvelinus fontinalis* and the holotype of *S. multidentatus* (Fig. 4) possess bicuspid teeth on the basihyal, while the holotypes of

S. kingi and *S. angustus* possess unicuspid teeth. This character state was not consistent among all specimens examined. Paratypes of all three described species consisted of individuals with bicuspid and unicuspid teeth, while the two specimens collected with the neotype of *S. fontinalis* possessed unicuspid teeth.

Discussion

Analysis of tissue samples of fishes from the three species described herein formed three distinct clusters when genotyped at 13 microsatellite loci (Stauffer & King, 2014: 561, fig. 1). As part of an effort to re-establish native fishes, GRSM fishery managers removed introduced trout (i.e. *Salmo trutta* Linnaeus, 1758, *O. mykiss*) from LeConte Creek, which flows through Gatlinburg, Tennessee. They subsequently introduced “Brook Trout” from Cosby (*S. kingi*), Little Greenbrier (*S. angustus*), and Indian Camp (*S. multidentatus*) creeks into LeConte Creek. At least 76 % of the fish sampled seven years post-stocking (only 35 % of the founding fish were sampled) from LeConte Creek resulted from parents originating from the same source stream. Sampling of an additional 90 fish, 11 years post-stocking, showed at least 82 % of sampled fish originated from same-stream matings (Kulp et al., 2017). Thus, three discernible populations of *Salvelinus* spp. co-exist in LeConte Creek.

In a series of artificial crosses in the laboratory, Kulp et al. (2017) successfully produced within-stream and between-stream crosses of

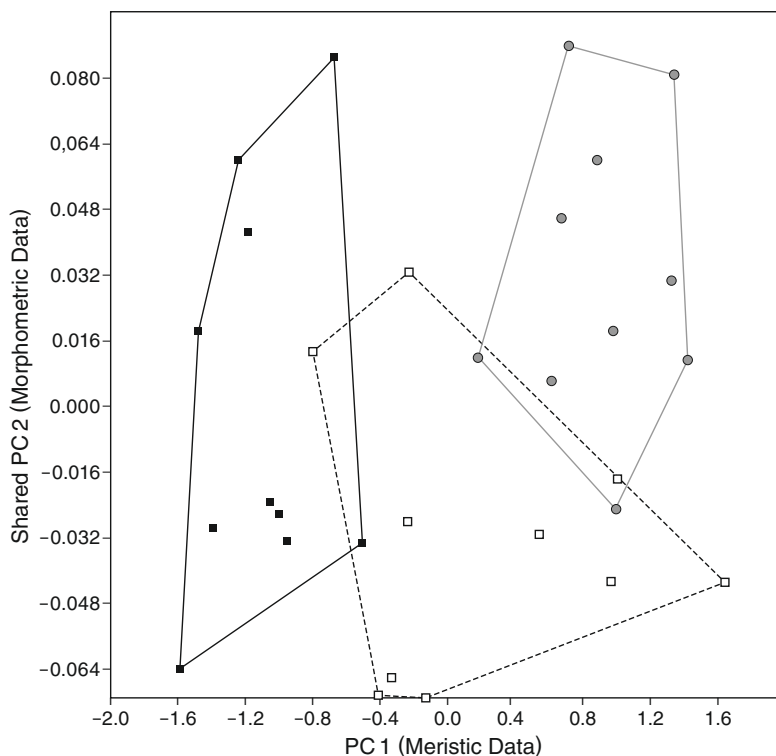


Fig. 8. Sheared second principal component (morphometric data) plotted against the first principal component of the meristic data of *Salvelinus angustus* (●), *S. kingi* (□), and *S. multidentatus* (■).

the species described herein. They explained the low genetic admixture in LeConte Creek as a physiological response to spawning influenced by environmental conditions (i.e. stream temperature). They further postulated that long term-term isolation and fragmentation may be limiting spawning among parents from different sources. They ignored the fact that these populations are different species that evolved allopatrically and that there are either premating isolating mechanisms or post-mating depression in place. The fact that different species produce offspring in the laboratory does not negate the fact that they are heterospecific. Laboratory studies that provided both males and females from allopatric populations and used genetic paternity testing demonstrated a high level of assortative mating among allopatric populations of cichlids from Lake Malawi, which supported incipient species status (Knight & Turner, 2004).

In describing the species herein, I used morphological characters to diagnose them. Although, advancements in the field of molecular genetics

since the mid-1960s have provided fisheries scientists with powerful investigative tools that can be used to answer questions related to the evolutionary relationships among fish populations, it is still the existence of unique phenotypes that is used to delineate most species. In effect, if allopatric populations have evolved one or more derived characters, then the evolutionary modification of primitive characters has been satisfied and the population should be given species status (Cracraft, 1983). From a cladist's perspective, it is the presence of uniquely derived conditions (autapomorphies) that determine specific status, not gene trees. The presence of isolated species of *Salvelinus* that historically were known as *Salvelinus fontinalis* is similar to isolated populations of species historically identified as *S. trutta* in Europe. Several species regarded as subspecies of *S. trutta* were elevated to species rank by Kottelat (1997). *Salmo trutta* from North Africa (Delling & Doadrio, 2005) and the Balkan (Delling, 2003) were evaluated. A total of 29 species in this complex was postulated by Kottelat & Freyhof (2007).

Additionally, Turan et al. (2009) elevated subspecies of *S. trutta* to species from streams along the Black Sea coast.

Weathers et al. (2019) reported high levels of neutral genetic differentiation and allelic fixation among populations of *Salvelinus* cf. *fontinalis* throughout the Great Smoky Mountains National Park suggesting there is little gene flow among these populations. The absence of gene flow, however, can be a function of either geographic or biological separation. The genetic data for the introduced populations in LeConte Creek supported my contention that they are distinct species. The three distinct populations that were introduced into LeConte Creek could be delimited 11 years after introductions created sympatry. Among the introduced fishes, there is either positive (selective) assortative mating (i.e. premating isolating mechanism) and/or some form of post-reproductive isolating mechanism; thus, these populations are in fact separate species sensu the biological species concept. As with the coregonine fishes, a more complete study of the systematics of the genus *Salvelinus* would be a significant contribution to the management of these stocks (Behnke, 1972). A typological approach must be avoided, if the inherent diversity of these fishes is to be conserved. Species are ontological individuals, sensu Ghiselin (2002) and as such cannot be defined but are recognized, diagnosed, and discovered (Wiley, 2002).

The initial step in preserving the adaptive potential of a trust resource is to delineate the fundamental unit of management (e.g. species, metapopulation, population). The approach espoused by Kulp et al. (2017) to capture as much genetic variance of source stocks and to enhance population connectivity via translocation may, in fact, drive local allopatric species to extinction. Although changes in land use practices and habitat fragmentation are a threat to populations of Brook Trout, the presence of introduced fishes can overshadow these threats (Hudy et al., 2008). Competing species, notably *O. mykiss* were considered to be the most important threat to native Brook Trout (Kelly et al., 1980). The introduction of northern strains of Brook Trout in an attempt to bolster populations of native trout met with limited success, perhaps because of the unsuitability of these strains (Lennon & Parker, 1959). As noted by Behnke (1972), every effort should be made to protect the genetic diversity of these postglacial salmonid populations, which are

fragile and susceptible to extirpation by introductions. In effect, resource managers must plan for an evolutionary future for trust species, as such ecological and evolutionary processes – those that maintain genetic diversity and provide the raw material for evolution and adaptation of populations – must be explicitly identified (Stockwell et al., 2003). The current paradigm for conservation of species and associated habitats emphasizes the recognition and protection of irreplaceable evolutionarily distinct lineages; in effect, we must be able to recognize and identify distinct species.

Comparative material. *Salvelinus fontinalis*, PSU 11387, neotype, 170.8 mm SL; Connetquot River, Long Island, New York, U.S.A. – PSU 11388, 9, 103.6–166.1 mm SL; collected with the neotype.

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