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# Conservation Status of Virginia Populations of the Rare and Jeopardized Bridle Shiner, *Notropis bifrenatus*

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# Conservation Status of Virginia Populations of the Rare and Jeopardized Bridle Shiner, *Notropis bifrenatus*


## Abstract

The Bridle Shiner, *Notropis bifrenatus* (Cope, 1867), Family Leuciscidae, is a small minnow species that ranges in Atlantic coastal river drainages of eastern North America from the St. Lawrence in Canada southward to the Santee River basin of South Carolina. Over several decades notable declines throughout its range have been observed and multiple natural resource agencies note it to be jeopardized with probable extirpations evident in a number of drainages. The Virginia Department of Game and Inland Fisheries recognized the need to assess the status of the Bridle Shiner within its historical range in Virginia. Here we report the results of field surveys and preliminary molecular investigations undertaken to evaluate the species' current distribution and status. Currently, in Virginia, all remaining documented populations are confined to the greater James River basin in the Coastal Plain and lower Piedmont. Based on our findings, recommendations are made for targeted future sampling efforts to enhance detection of small, isolated populations of Bridle Shiner.



## Keywords

Bridle Shiner, Notropis bifrenatus, jeopardized, Virginia





## Cover Page Footnote

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## INTRODUCTION

The Bridle Shiner, *Notropis bifrenatus* (Cope, 1867), Family Leuciscidae (Figure 1), is a relatively small minnow, attaining a maximum total length of approximately 60 mm (Jenkins and Zorach 1970), though most specimens encountered are much smaller. Adults sport a black lateral stripe that continues forward around the snout, characteristically thinning at the upper lip, resulting in a bridle-like appearance that lends to the common name. The stripe may terminate in a darker, rectangular caudal spot. Small juveniles generally have a heavy black lateral stripe and distinct quadrangular caudal spot. Bridle Shiner in clearer waters generally reflect silvery blue iridescences over much of the body. Males in breeding condition develop a yellowish wash over much of the upper body and fins. The lateral line is usually incomplete, especially in juveniles, with pored scale counts increasing with growth. The Bridle Shiner is relatively short lived, attaining slightly over two years of age (Harrington 1948a, Jenkins and Burkhead 1994). This species is a midwater sight feeder with a diet consisting chiefly of various microcrustaceans and small aquatic insects (Harrington 1948b).

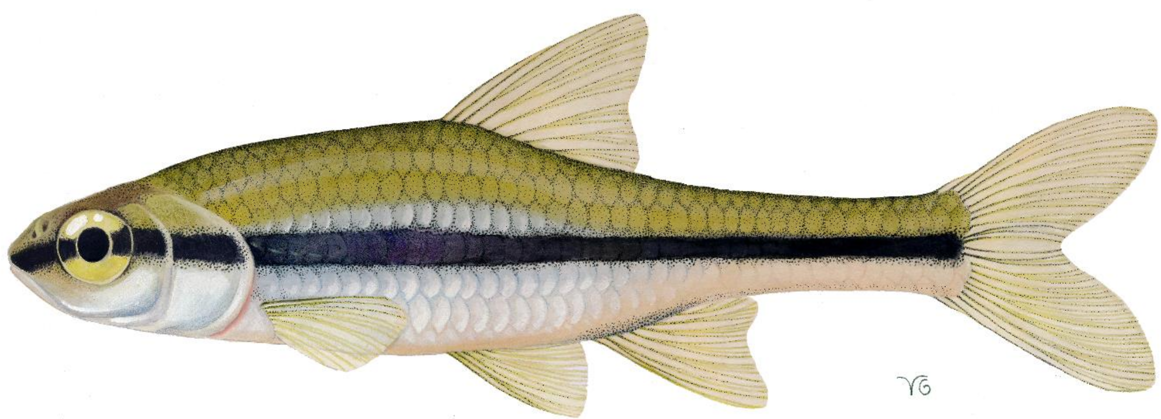

**Figure 1.** Bridle Shiner illustration by Virginia Greene.

The species' range extends from the St. Lawrence drainage, in and east of the Lake Ontario region in Quebec, New York, and New Hampshire, and in Atlantic coastal drainages from southern Maine to the Santee River basin of South Carolina (Arndt 2004, Carlson et al. 2016, Cooper 1983, Gilbert 1980, Hartel et al. 2002, Jenkins and Burkhead 1994, Jenkins and Zorach 1970, Langdon et al. 2006, McCabe 1948, Menhinick 1991, Murdy et al. 1997, 2013, Rohde et al. 2009, Scarola 1973, Tracy et al. 2020, Werner 2004, Whitworth 1996, Whitworth et al. 1968) (Figure 2 Inset). The distribution of Bridle Shiner is primarily on the Coastal Plain but does extend into the Piedmont in two Virginia river systems. Documented occurrences become increasingly disparate in the southern half of the range, with sporadic occurrences in Virginia (Jenkins and Burkhead 1994) and extremely isolated occurrences in North Carolina and South Carolina. In North Carolina, it had been considered extirpated since the 1960s until it was rediscovered in 2000 in the Neuse River basin and then in 2012 in the Chowan River basin (North Carolina Museum of Natural Sciences (NCSM) data; Menhinick 1991, Tracy et al. 2020). Subsequent attempts to verify the species' occurrence have yielded specimens in both the Chowan and Neuse basins. In South Carolina, Bridle Shiner was restricted to rare catches in two Santee River impoundments, lakes

Marion and Moultrie, during the 1950s and early 1980s (Rohde et al. 2009, NCSM data) but targeted efforts in these areas in 2009 by the authors in collaboration with the South Carolina Department of Natural Resources failed to detect its presence.

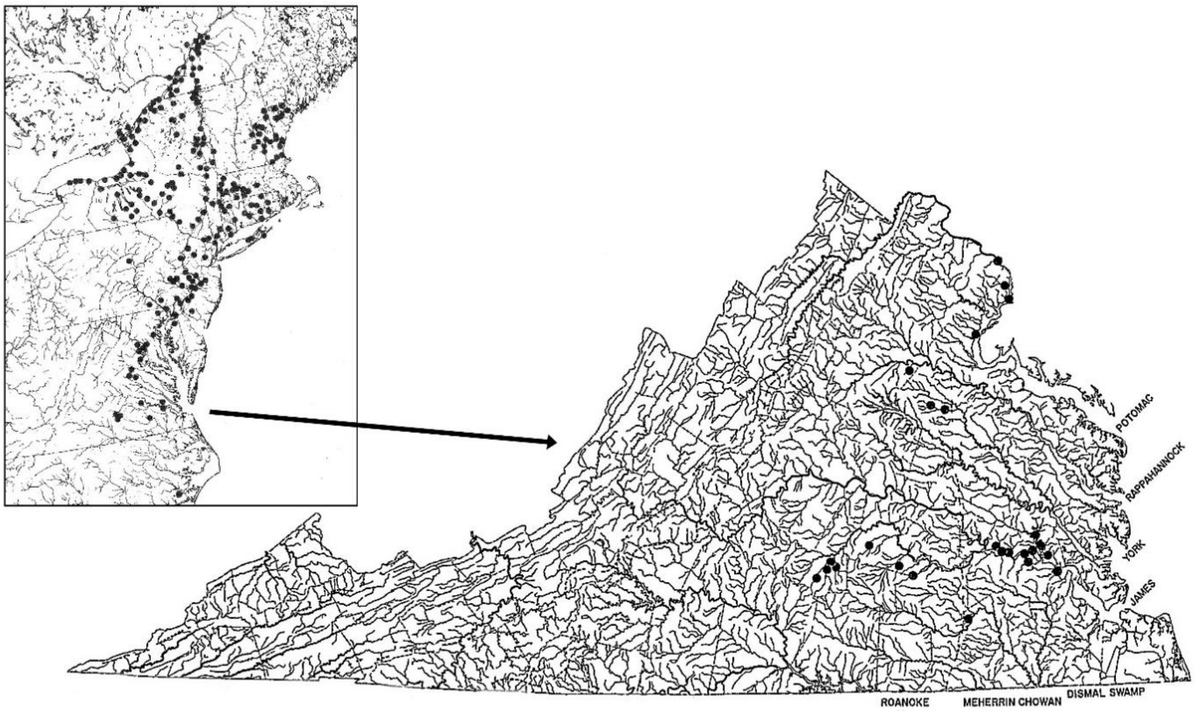


**Figure 2.** Historical distribution of Bridle Shiner in Virginia (taken from Jenkins and Burkhead 1994). Inset: Historical distribution of Bridle Shiner in North America (taken from Gilbert 1980). Distribution in South Carolina (Rohde et al. 2009) not shown.

The preferred habitats of the Bridle Shiner are generally backwaters or areas of sluggish current in streams, fresher portions of tidal streams, or in lakes and impoundments such as millponds. All suitable habitats possess considerable growth of submerged aquatic vegetation (SAV) that typically grows in soft bottoms, ranging from deep silt, detritus, and woody debris to firmer substrates of silt and sand. The association with SAV dictates to a degree that habitats are often in open canopy areas, in clearer waters, with good sunlight penetration. Depths may range from less than a meter to three meters plus. For those habitats where water quality data are available, pH is generally neutral or above (7.0+). Some tolerance of salinity in tidal habitats is indicated, up to 3 ppt (Jenkins and Burkhead 1994), though Hildebrand and Schroeder (1928) implied higher salinities were tolerated around Chesapeake Bay. In New England, this species most often occurred in association with an unspecified species of native Milfoil (*Myriophyllum* sp.), Stonewort (*Chara* sp.), and Coontail (*Ceratophyllum demersum*), also known as Hornwort, and was observed to spawn over this vegetation (Harrington, 1947). In the more southerly portions of its range, a close association with moderate to dense growths of native Coontail was usually found during our survey. In some areas, Coontail is often found in massive mixed beds with introduced and invasive Hydrilla (*Hydrilla verticulata*), particularly in large tidally influenced habitats. In one instance, it was also found in association with mixed Brazilian Elodea (*Egeria densa*), and native Slender Pondweed (*Potamogeton pusillus*).

Due to the types of habitats described above, which are often challenging to sample effectively, coupled with the tendency of Bridle Shiner to occur in very localized patchy aggregations, detection of this species can be notoriously difficult. For this reason, this species was chosen as a case study by Jensen and Vokoun (2013) who determined that a multi-state patch occupancy model approach to sampling was required to yield sufficient information to develop reliable quantitative habitat relationships and estimation of use of the habitat by the species. Regional faunal references, some going back three decades, noted perceived declines in Bridle Shiner populations in their respective areas of coverage. McCabe (1948) noted even earlier declines in Massachusetts lakes. Several reports have concluded it is in varied degrees of decline and imperilment from Pennsylvania northward through the northeastern states to Maine and in Canada (Chandler 1997, Criswell 1998, Fairchild et al. 1998, Holm et al. 2001, Horwitz 1985, Shervinskie 1998, Whittier et al. 1997, 2000, 2001). Collective consideration of state-based assessments deem it to be jeopardized throughout its entire distribution (Margolis 2003, Sabo 2000). This species is currently formally listed as: a species of Special Concern in Canada, Connecticut, Massachusetts, and South Carolina; Species of Greatest Conservation Need in Maine and New York; Vulnerable in Rhode Island; state Threatened in New Hampshire; state Endangered in Delaware, New Jersey, North Carolina, and Pennsylvania; and as Critically Endangered in Vermont. It is currently not listed in Maryland where it is considered to be extirpated (Kilian et al. 2011). In Virginia, this species was considered a Virginia Action Plan Tier Ia species by the Virginia Department of Game and Inland Fisheries. The description of this ranking indicates a critical need for conservation and that the species "faces an extremely high risk of extinction or extirpation. Populations of these species are at critically low levels, facing immediate threat(s), or occur within an extremely limited range. Intense and immediate management action is needed. Managers have identified 'on the ground' species or habitat management strategies expected to benefit the species; at least some of which can be implemented with existing resources and are expected to have a reasonable chance of improving the species' conservation status" (VDGIF 2015). This ranking allows the VDGIF to develop proactive programs to help species from becoming endangered. The ranking was reviewed and not changed in 2020 (M. Rollins, VANHP, personal communication), and is slated to retain the same status in the 2025 Virginia Action Plan (M.J. Pinder, VDGIF, personal communication).

## Distribution in Virginia

In Virginia, Bridle Shiner were formerly known from at least 25 localities extending from the lower Potomac River southward to the Nottoway River system of the Chowan River basin but was noted to be in major decline (Jenkins and Burkhead 1994) (Figure 2). Former occurrences of this species (Figure 2) were from mostly isolated populations in several river basins, extending from the Potomac through the James, and in the Chowan River basin. More contiguous populations were in the tidal lower James and Chickahominy rivers (Jenkins and Zorach 1970, Jenkins and Burkhead 1994). Distributional discussion in Jenkins and Burkhead (1994) gave indications that Bridle Shiner might be extirpated across most of eastern Virginia, except in small portions of the greater James River basin, including the Appomattox River system.

In the Potomac River basin documented historical occurrences of Bridle Shiner were few and restricted to the years 1898–1935 (Jenkins and Burkhead 1994, Starnes 2002, Starnes et al. 2011). Most records were distributed in the main river, or tributary embayments, from the vicinity of Chain Bridge in Arlington County downstream to the vicinity of Quantico in the Prince William-Stafford counties area (Figure 2). The last documented occurrence in 1935 was from a small tributary, Cameron Run, in Fairfax County (Jenkins and Burkhead 1994). Electrofishing and seine sampling of the main river and tributaries in the area between the Interstate 495 crossing west of the District of Columbia and the confluence of the Anacostia River, conducted by the authors in the mid-1990s and 2010 (Starnes 2002, Starnes et al. 2011), as well as extensive canvassing of resource agency collection data from this area, failed to reveal any reports of Bridle Shiner. Furthermore, Kilgore et al. (1989), in an electrofishing study targeting fishes inhabiting areas of SAV in much of the reach of the river formerly inhabited by Bridle Shiner, reported no catches.

The range of Bridle Shiner straddles the Rappahannock River basin, but only a single occurrence has been recorded based on material in the National Museum of Natural History (USNM 105319). It was collected in 1933 in Flat Run near Orange County. This is a tributary to the Rapidan River well up in the Piedmont portion of the basin. Despite its Piedmont context, Flat Run is a relatively low gradient stream and may have provided conditions favorable to habitation by Bridle Shiner and other lowland species. Attempts to duplicate this record by Starnes in 1979, plus attempts by others who have surveyed the Rappahannock (Maurakis et al. 1987, Jenkins and Burkhead 1994), failed to detect Bridle Shiner in the area.

Documented historical occurrences of Bridle Shiner in the greater York River basin are, surprisingly, limited to small rivers in the Piedmont headwaters portion of the drainage (Figure 2), with no known occurrences in the lower region akin to those known from the tidal Potomac and James rivers. For example, Raney and Massman (1953) reported abundant SAV in cove areas of a 67 km tidal reach of the Pamunkey River above West Point but recorded no Bridle Shiner from the 17 sites sampled over that reach. In the Piedmont, Jenkins and Burkhead (1994) indicated a population in the Matta River, Spotsylvania County, based on multiple records (CUMV 51933, NCSM 59054, and VIMS 0586), all taken from the vicinity of the highway US 1 crossing, in 1966, 1969, and 1983, respectively, and VIMS 12832, from further upriver at route CR 646. The 1983 collection was the last documented for the Matta River and the only vegetation present was some scattered growth of filamentous algae (*Spirogyra* sp.) on bedrock, with which the juvenile Bridle Shiner collected were taken in association. However, an additional collection from a sister system, the nearby Po River, was discovered during the present project in the collection of Mary Washington University, 38 specimens collected at the CR 612 crossing, Spotsylvania County, August 1988 (deposited as NCSM 66526). This is the last documented occurrence in the entire York River basin. These upland riverine habitats are decidedly different from those occupied by Bridle Shiner in the lower Coastal Plain, with very little SAV, rocky or sandy substrates, and considerable current in some areas.

Though the occurrence of Bridle Shiner was not known in the James River basin (Raney 1950), indications are that this basin has the most successful recent history of habitation by Bridle Shiner (Figure 2). There were several collections made in past decades from the lower estuarine area of the James River and its northern tributary, the Chickahominy River in Charles City James City, Prince George, and Surry counties (Figure 2). These collections were from in or near the large lower tidal reaches of tributary creeks and the main stem and spanned the years 1949–1975. Thus, the recent status of these populations was uncertain. More recently, Virginia Department of Game and Inland Fisheries (VDGIF) biologists (personal communication R. Greenlee) believed

Bridle Shiner had been encountered while sampling (e.g., in Upper Chippokes Creek) but these records were not vouchered. In addition to these large tidal water collections, Bridle Shiner were taken on multiple occasions between 1969 and 1995 from Jolly Pond and its outlet stream, located above the tidal portion of Gordon Creek, a tributary to the lower Chickahominy River, James County. In the Appomattox River (lower Piedmont), Bridle Shiner were formerly known from several tributary creeks in Amelia, Nottoway, and Prince Edward counties (Figure 2) based on a combination of museum records and collections made by Robert E. Jenkins in 1983.

Bridle Shiner have been reported from only one locality in the entire Albemarle Sound drainage, both within the Chowan River basin. This record is from Stony Creek, tributary to the Nottoway River, near the Interstate-95/US 301 highway crossing at the town of Stony Creek, Sussex County, collected on four occasions in the years 1967–1968 (CUMV 52945, VIMS 28500, 28562, 28993, 35131). Repeated attempts by subsequent workers to duplicate these finds have been without success, including by Robert E. Jenkins et al. over the years 1971–1984 (Jenkins and Burkhead 1994), and the authors for the present report in 2008. Extensive canvassing of the entire Chowan River Basin between 1967 and 1986, along with repeated attempts in Stony Creek, failed to reveal extant populations (Jenkins and Burkhead 1994).

Given the uncertainties surrounding the current status of Bridle Shiner populations in the state, the Virginia Department of Game & Inland Fisheries deemed it necessary to conduct a statewide survey to provide a status assessment for this species. Along with this survey, a preliminary genetic analysis was conducted, which allowed us to confirm the identifications of the Bridle Shiner collected and served as a primary assessment of variation among and within populations. The results of both the statewide survey and genetic analysis are provided here.

## SCOPE AND METHODS

In general, Bridle Shiner inhabit waters that are inherently challenging to sample effectively and a variety of methods were used in attempts to overcome this. The majority of habitats were too deep to permit the use of backpack electrofishing units thus this method was of limited use in the project. In wadeable streams, swamps, or shallow ponds and lakes, most sampling was conducted via 2 or 4 m seines with 5 mm mesh. Where possible, seines were dragged and beached or, where depth and distance did not permit this technique, hauls ended with a makeshift pursing of the seine. Another occasionally effective technique in waters too deep for wading was standing hip-deep along steep stream margins and casting the seines outward over the drop-off, dragging in vegetation and debris for inspection. Swimming of seines was generally not effective due to the force needed to overcome the drag of vegetation and constant snagging on woody debris. Dip nets were sometimes employed along the vegetated margins of deeper streams but failed to yield any catches of Bridle Shiner. In larger, deeper habitats, such as tidal rivers and streams, and impoundments, sampling was conducted using small mesh dip nets aboard electrofishing boats provided and piloted by VDGIF biologists. Electrofishing was mainly conducted above and along the margins of large SAV beds, or along banks with emergent aquatic vegetation and undercuts. Records of all species encountered were maintained and captured in the NCSM collection database. Voucher specimens were taken of any Bridle Shiner encountered. Preservation of these was either in 10% formalin (later transferred to 70% ethanol) or in 95% ethanol to provide voucher-linked tissues for DNA analyses that reside in ultracold storage at

NCSM. In addition to fish vouchers, submerged vegetation samples were occasionally preserved, particularly where the species were not readily recognized, and later identified with the aid of Aulbach-Smith and de Kozlowski (1996) or by personnel of the North Carolina Division of Water Resources.

The geographic scope of this project was defined by the overall range of Bridle Shiner in Virginia (Figure 2), as taken from taxonomic and regional literature (e.g., Jenkins and Zorach 1970, Jenkins and Burkhead 1994), and supplemental examination of museum collections. Those collections included The Academy of Natural Sciences of Drexel University, American Museum of Natural History, Cornell University (CUMV), National Museum of Natural History, Smithsonian Institution (USNM), North Carolina Museum of Natural Sciences (NCSM), Roanoke College, University of Mary Washington, University of Michigan Museum of Zoology, and the Virginia Institute of Marine Sciences (VIMS), plus a collective search on FishNet2.net in 2010.

The area surveyed ranged from the Potomac River basin southward through all the Chesapeake Bay tributaries and into the Chowan River subbasin of the Albemarle Sound basin. A total of 313 sites were chosen to be surveyed between 2010 and 2013 (Figure 3, see *Data Availability*). The breakdown of sites between river basins is as follows: Potomac–39, Great Wicomico–4, Rappahannock–25, Piankatank–2, York–58, James–128, and Chowan–57. Visual surveys were conducted at each site to evaluate the potential for Bridle Shiner habitat based on the previous collecting experience of the investigators and habitat descriptions in the literature. Sites visited were assessed as (1) water bodies with documented prior occurrences, (2) water bodies without prior occurrences but in proximity to known populations and sharing drainage connectivity and similar physiographic circumstances, and (3) sites that filled in gaps in the known distribution and offered some potential based on road reconnaissance and/or satellite imagery. Sites of prior occurrence were generally visited multiple times in an attempt to detect any persistence. In large tidal river or creek habitats coordinates for beds of SAV were located via satellite imagery and targeted for sampling. In the tidal Potomac River, further refinement of this technique was facilitated by correlating imagery with vegetation mapping provided in Hunter et al. (2009), which permitted pinpointing of SAV beds that contained a significant component of Coontail. Of the 313 sites visually assessed, 188 were deemed to have at least marginal potential for habitation by Bridle Shiner and were selected for further investigation (Figure 3). Also, nine sites that had been previously sampled by the investigators were considered as having marginal potential and were included. Because Bridle Shiner are known to occasionally inhabit some small impoundments such as millponds in the northern portion of their range, a number of these were also targeted for possible investigation.

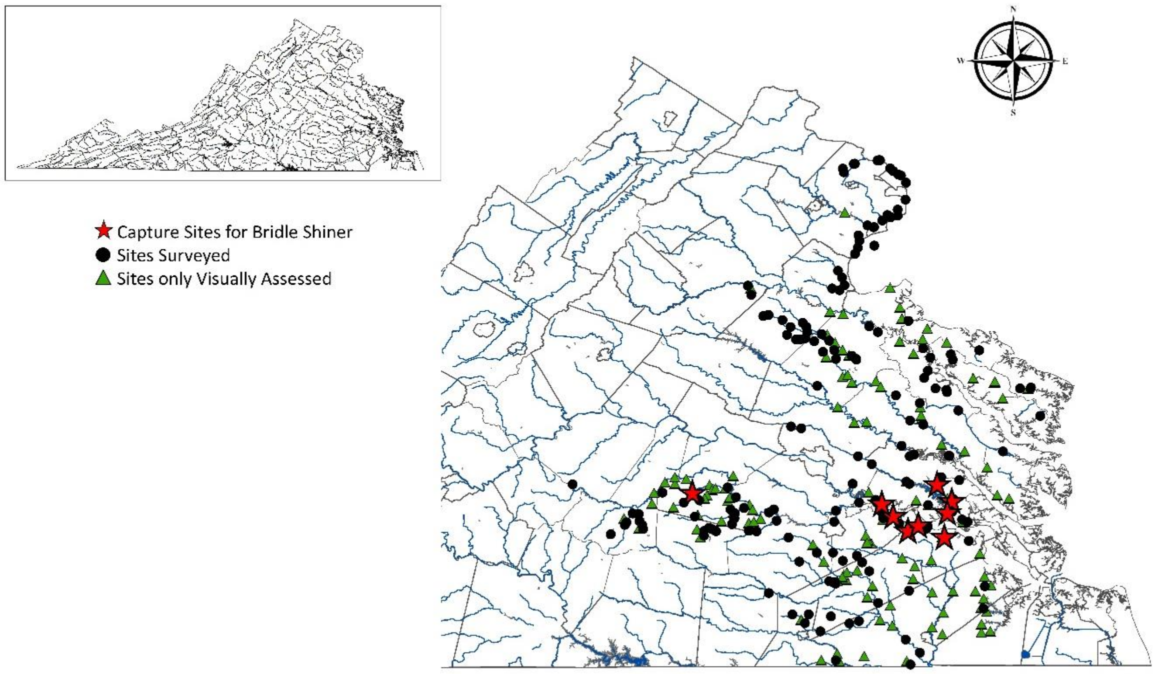


**Figure 3.** Map of Virginia detailing sites where populations of Bridle Shiner were documented, and sites surveyed versus only visually assessed.

A genetic analysis was also performed as a part of this study. DNA was sequenced (via DNA barcoding) from representative individuals vouchered at NCSM and the Yale Peabody Museum (Table 1) and sequences already deposited in GenBank. Comparisons were made against individuals in public databases (NCBI, etc.) and against those deposited in the International Barcode of Life Initiative (https://ibol.org/).

DNA was isolated from tissues (fin clips or muscle) from museum-vouchered specimens using either the ArchivePure DNA Tissue Purification Kit (5Prime Inc., Gaithersburg, MD, USA) or the Nucleospin Tissue Kit (Macherey-Nagel Inc., Bethlehem, PA, USA). DNA concentrations were standardized to no more than 50 ng/μL using a Nanodrop 1000 spectrophotometer. DNA barcodes (mitochondrial Cytochrome Oxidase I gene) were prepared for 99 individuals of Bridle Shiner and 2 individuals of Whitemouth Shiner, *Miniellus alborus*, using standardized protocols implemented by BOLD Systems (Ratnasingham and Hebert 2007). PCRs were assembled as 10 μL reactions containing 5 μL GoTaq MasterMix (Promega, Inc., Madison, WI, USA), 0.01 mM final concentration of each primer cocktail (protocols outlined in Ivanova et al. 2007) and using approximately 10–50 μg of genomic template. Successful PCR reactions were cleaned for sequencing using ExoSAP-IT (USB, Cleveland, OH, USA) following the manufacturer's recommendations. DNA sequencing was performed on an ABI 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) in the North Carolina Museum of Science's Genetics & Microbiology Research Lab following the manufacturer's recommended protocols for DNA sequencing and cleanup. Sequences were proofed and aligned using Sequencher ver. 5.1 (Gene Codes, Corp., Ann Arbor, MI, USA). Eleven additional Bridle Shiner sequences were downloaded from GenBank (Table 1). Whitemouth Shiner was included as an outgroup based on comments in Page and Burr (2011).

**Table 1.** Material examined for genetic analysis.

| Catalog Number | NCBI accession number | Species | Number of individuals sampled | Locality | Coordinates | Date collected |
|---|---|---|---|---|---|---|
| NCSM 30018 | PQ895383-PQ895384 | *Miniellus alborus* | 2 | NC; Union Co.; Rocky River; Bearskin Creek | 34.9969, -80.5258 | 02 Sep 2001 |
| NCSM 30364 | PQ895417-PQ895419 | *Notropis bifrenatus* | 3 | NC; Craven Co.; Neuse River; Bachelor Creek | 35.1785, -77.1319 | 10 Jul 2001 |
| NCSM 50873 | PQ895428-PQ895442 | *Notropis bifrenatus* | 15 | MA; Worcester Co.; East Branch Ware River | 42.4376, -71.9705 | 30 Jul 2008 |
| NCSM 50885 | PQ895443-PQ895447 | *Notropis bifrenatus* | 5 | MA; Bershire Co.; West Branch Farmington River | 42.2295, -73.1118 | 30 Jul 2008 |
| NCSM 50908 | PQ895448-PQ895452 | *Notropis bifrenatus* | 5 | MA; Bershire Co.; Housatonic River; Lily Pond Brook | 42.3358, -73.3102 | 30 Jul 2008 |
| NCSM 50913 | PQ895453-PQ895474 | *Notropis bifrenatus* | 22 | NJ; Morris Co.; Delaware River; Toomeys Pond (Weldon Brook) | 41.0084, -74.5864 | 30 Jul 2008 |
| NCSM 60673 | PQ895475 | *Notropis bifrenatus* | 1 | VA; Surry Co.; James River; Brandon Gut | 37.2255, -76.9821 | 16 Jul 2010 |
| NCSM 61895 | PQ895389-PQ895416 | *Notropis bifrenatus* | 28 | VA; Prince George-Surry counties; James River; Upper Chippokes Creek | 37.1988, -77.0315 | 18 Oct 2010 |
| NCSM 65252 | PQ895476-PQ895478 | *Notropis bifrenatus* | 3 | VA; Surry Co.; James River; Brandon Gut | 37.2255, -76.9820 | 28 Jun 2011 |
| NCSM 71642 | PQ895420-PQ895427 | *Notropis bifrenatus* | 8 | NC; Hertford Co.; Chowan River; Deep Swamp Branch | 36.3075, -76.7261 | 05 Jul 2012 |
| NCSM 74676 | PQ895479-PQ895483 | *Notropis bifrenatus* | 5 | VA; Amelia Co.; Appomattox River; Flat Creek | 37.3806, -78.0639 | 06 Sep 2013 |
| ROM 80219 | EU524967-EU524969; EU24172 | *Notropis bifrenatus* | 4 | Canada; Quebec; St. Lawrence River; Lake St. Paul | 46.292, -72.494 | 27 Sep 2005 |
| ROM 80224 | EU524963-EU524966; EU524173-EU524174 | *Notropis bifrenatus* | 6 | Canada; Quebec; St. Lawrence River | 46.30, -72.70 | 02 Sep 2005 |
| UAIC 12997 | JN027448 | *Notropis bifrenatus* | 1 | NJ; Somerset Co.; Millstone River; Pike Run | 40.4447, -74.6459 | 09 Feb 2001 |
| YPM ICH 020589 | PQ895385-PQ895388 | *Notropis bifrenatus* | 4 | MA; Berkshire Co.; West Branch Farmington River; Owl Lake | 42.1856, -73.0961 | 05 Jul 2008 |

Percent sequence divergence was calculated using MEGA ver. 5.2.2 (Tamura et al. 2011). Optimality of sequence evolution modeling was assessed with jModelTest 2.1.4 (Darriba et al. 2012). Bayesian analysis was performed in MrBayes 3.2.2 (Ronquist et al. 2003) as implemented on the CIPRES Science Gateway (Miller et al. 2010). Bayesian analysis was performed for 50 million generations with four chains sampled every 1000$^{th}$ generation. Posterior probabilities were generated after discarding a burn-in of 25% of the initially sampled trees. Data convergence among Bayesian MCMC chains in the analysis was assessed visually using the AWTY (Are We There Yet) Online Portal (Wilgenbusch et al. 2008). This product is no longer available but has been recreated in R as RWTY (Warren et al. 2017), though we did not use RWTY at the time.

## RESULTS

***Potomac River Basin*** – Considerable effort was made to ascertain if Bridle Shiner could be detected in some areas of the Potomac River basin. This effort, totaling 34 of the 39 sites considered for surveying, entailed boat electrofishing of the fresher portions of the main Potomac River, as well as in tidal embayments and lower courses of tributaries, plus seine sampling at road accesses of some wadeable tributaries (Figure 3). This coverage extended over an area of approximately 126 river km from the Chain Bridge area in Arlington County downstream on the Virginia side of the river and estuary to the Mattox Creek system in Westmoreland County. In addition, due to the greater percentage of Coontail reported by Hunter et al. (2009), the Mattawoman Creek embayment on the Maryland shore was included. These efforts yielded no discoveries of Bridle Shiner.

***Great Wicomico River Basin*** – This small Chesapeake Bay tributary has a limited drainage area and thus low potential for maintaining appreciable zones of fresh tidal waters that foster the growth of SAV. However, because of the history of occurrences of Bridle Shiner in some Chesapeake tidal tributaries, it was deemed worthwhile to explore this system for any possible expanses of habitat and, of four sites considered, three were chosen for sampling, including two large tidal creek arms and one wadeable site (Figure 3). SAV proved to be sparse at all of the sites and no Bridle Shiner were collected.

***Rappahannock River Basin*** – 25 sites were considered and 14 were chosen based on possible favorable characteristics. These sites extended from Orange County in the vicinity of Flat Run southeast over a linear distance of 133 km. They included wadeable streams, millponds, and large fresh tidal creek tributaries of the Rappahannock estuary as far south as the upper Corrotoman River system in Lancaster County (Figure 3). The main stem of the Rappahannock was excluded from sampling due to its propensity for turbidity and very poor growth of SAV (R. Greenlee and J. Odenkirk, VDGIF, personal communication). Particular emphasis focused on Flat Run and Russell Run. However, there were no captures of Bridle Shiner.

***Piankatank River Basin*** – Approximately 3.2 km of the lower tidal course were boat electrofished and two wadeable upstream sites, including one in 2008 prior to the project, were worked by seine and backpack electrofisher (Figure 3). SAV consisted of native Milfoil (*Myriophyllum* sp.) and Bladderwort (*Utricularia* sp.) at the most upstream site and was sparse in the relatively acidic and shaded lower reach. Assuming Bridle Shiner ever had historical access to the Piankatank River, despite the current good quality habitat in the system, conditions appear not conducive to supporting populations as no occurrences of Bridle Shiner were discovered.

***York River Basin*** – Despite the lack of records from the lower three-quarters of this basin, it was reasoned there could have been undiscovered populations in the lower portions that were the source of colonizers to the unusual upland populations of Bridle Shiner in the basin. Thus 58 stream, river, and tidal reach sites were assessed for possible habitat and 31 were selected for sampling. The sites were distributed from the lower Piedmont to near Chesapeake Bay, plus data from two other recent NCSM collections were included (Figure 3). Particular emphasis was placed on the rivers and streams of Spotsylvania County in the area of former occurrence, as well as on larger, heavily vegetated tidal reaches of the Mattaponi and Pamunkey rivers. These two rivers are the two major freshwater arms of the drainage above the euryhaline York River estuary. Despite these endeavors, no Bridle Shiner were discovered.

***James River Basin*** – In the lower James River area, extant populations of Bridle Shiner were documented in our study from five direct tidal tributaries to the James River: Grays Creek (Surry Co., NCSM 60753), Brandon Gut (Surry Co., NCSM 60673, 65252), Upper Chippokes Creek (Prince George-Surry cos., NCSM 61895), Flower Dew Hundred Creek (Prince George Co., NCSM 61883), and Herring Creek (Charles Co., NCSM 61847). Further, populations were verified in three eastern tidal creek tributaries, Gordon (James City Co., NCSM 67025), Yarmouth (James City Co., NCSM 67060), and Diascund (James City-New Kent cos., NCSM 67021) creeks, but no captures were recorded from the similar western tributary, Morris Creek, in Charles County. In these habitats, captures were generally from large beds of SAV with mixed growth of Coontail and Hydrilla (Figure 4). The sizable Upper Chippokes Creek and two Brandon Gut samples appeared to represent successful populations, reflected in the catch per unit effort for those sites (85.1, 7.1, and 8.6; calculated as number of individuals per hour). It is likely that all captures in the lower James River area represent semi-isolated subpopulations that are in occasional genetic communication with one another, thus operating essentially as metapopulations (e.g., Hanski and Gilpin 1997). Multiple efforts to confirm persistence of the formerly documented population in Jolly Pond on Gordon Creek met with no success, either in the impoundment or the outflow creek area. Likewise, none were discovered in any of the large or small impoundments sampled aside from Brandon Gut, despite the seemingly good habitat provided by abundant SAV, including Coontail, in some of these locations.

In the Appomattox subbasin of the James River basin, despite 30 collecting efforts, (Figure 3), Bridle Shiner were only detected in Flat Creek at the CR 644 crossing, Amelia County, in 2011 (NCSM 67046) and 2013 (NCSM 74676). This site was characterized by a large, easily seined, side water area about one meter in depth, fed by a tributary entering from an open canopy bottomland swamp (Figure 5). Captures were associated with small SAV growths of Brazilian Elodea and Slender Pondweed. Thorough sampling in the swamp tributary yielded no catches. Expectations of locating a population via boat electrofishing in the lower tidal reach near Petersburg, especially in backwaters associated with the Swift-Timsbury creeks complex, were not met.

**Figure 4.** Habitat typical for Bridle Shiner occurrences in large tidal creek reaches of the lower James River basin, including abundant SAV (Yarmouth Creek, James City County, Virginia).

**Figure 5.** Habitat in which Bridle Shiner were discovered in Flat Creek, Amelia County, Virginia.

***Chowan River Basin*** – Occurrences of Bridle Shiner in the Chowan River basin were extremely isolated. Much of the habitat in this basin consists of tannic, low pH waters with scarce SAV, atypical to most known habitats of the species. Due to the discovery of this species in the North Carolina portion of the basin, considerable effort was made to locate further populations. Fifty-seven sites, including the Nottoway and Blackwater rivers, tributary streams and swamps, and several impoundments, were evaluated across the area. Twenty-six sites were investigated, plus three prior collections were considered (Figure 3). Stony Creek was sampled at multiple localities in 2010 and 2013. However, these efforts did not reveal the presence of any Bridle Shiner populations in the Virginia portion of this basin.

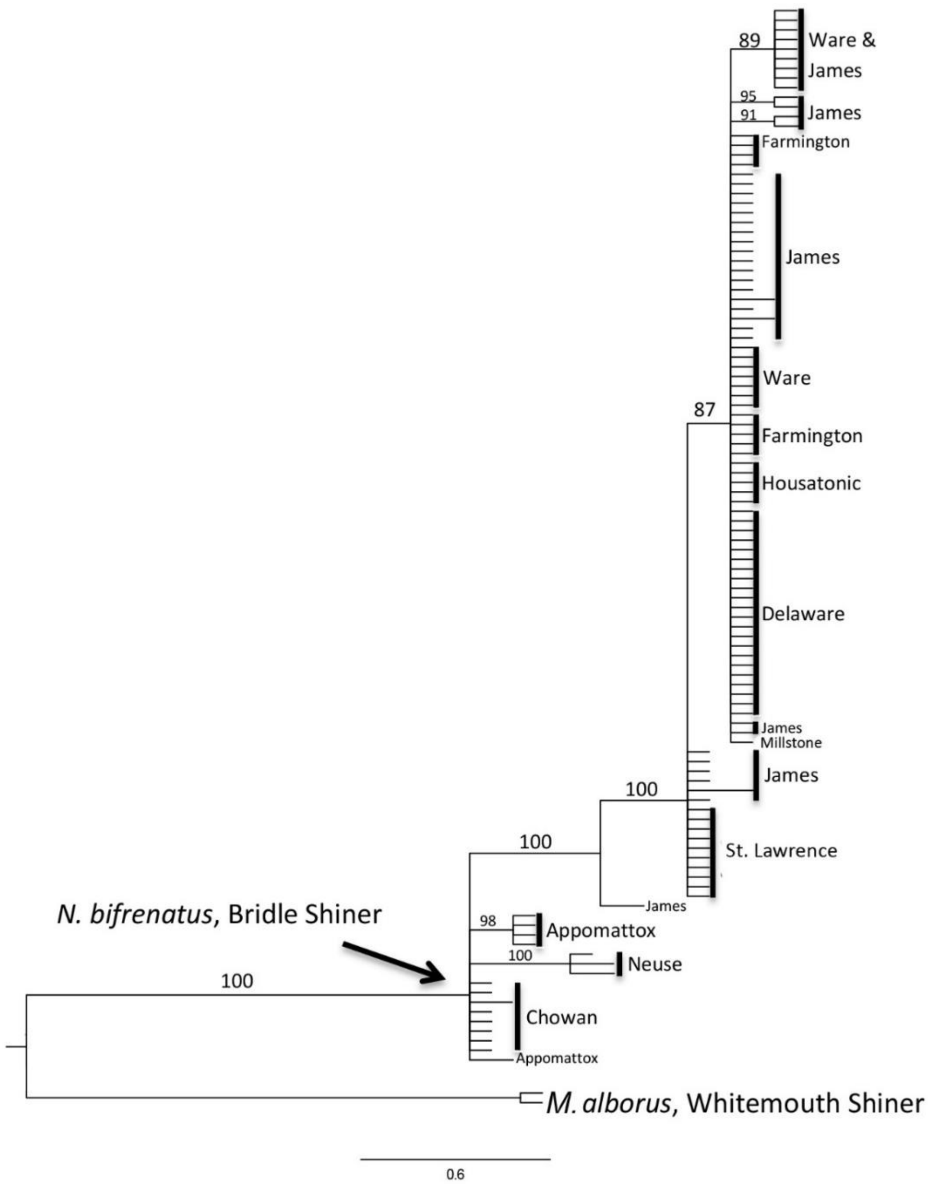


**Figure 6.** Phylogenetic relationships among Bridle Shiner individuals based on Cytochrome Oxidase I sequences. River basins correspond to those mentioned in the text and Table 1 and are indicated by thick vertical bars. Bayesian posterior probabilities are labeled on relevant nodes.

***Genetic Analysis*** – In general, percent sequence divergence was low. The largest divergence in the dataset, not inclusive of Whitemouth Shiner, was observed between individuals from the Neuse River, North Carolina and those from the Ware River, Massachusetts and certain James River individuals (approximately 2.5%). Most populations showed a divergence of less than 1.5% across the dataset. Bridle Shiner individuals from the Chowan and from the Appomattox rivers were slightly over 1.5% divergent from other Virginia and more northerly populations. Corrected Akaike Information Criterion (AICc) as implemented in jModeltest chose the HKY + I model as the best fit for the dataset. A monophyletic Bridle Shiner clade is recognized in the Bayesian tree (Figure 6) by analyzing all individuals in the dataset. In general, resolution within the tree was low, as would be expected with a DNA barcode dataset with a maximum of around 2.5% sequence divergence.

## SUMMARY AND DISCUSSION

Despite sampling efforts comprising almost 200 sites spanning seven river basins, collections of Bridle Shiner were few and restricted to the general areas from which prior occurrences were recorded. The persistence of populations in several of those areas could not be demonstrated. This species was not detected in the sites sampled in the Potomac, Great Wicomico, Rappahannock, Piankatank, York, and Chowan basins. While the deep and snag-ridden habitat of lowland populations is difficult to sample and may reduce detection probabilities, it is believed that some of this difficulty was likely overcome by the extent of sampling in these areas. In Piedmont habitats, however, it is believed that sampling methods were more efficient, and absences may represent possible extirpation, or years of extremely low population levels. In the Piedmont, both the Sandy River and Briery Creek have experienced reservoir construction, which has greatly altered those habitats since the captures of Bridle Shiner from those streams in the 1970s. Many sites examined in this area were shallow, moderate gradient streams with sandy – gravelly substrates, seemingly not appropriate for habitation by this species, though several were sampled. Several reaches of creeks sampled (e.g., Sweathouse Creek) have deep beaver impoundments with open canopy and some growth of SAV, though turbidity appears to curtail substantial growth of SAV. Field notes of Robert E. Jenkins indicated these beaver impoundments had been in place since at least the early 1980s. Such impoundments may have converted the character of these streams thus providing lowland-like habitat within the more upland context of the Piedmont and promoting colonization by typically lowland species. Considerable effort was made in the present study to ascertain if Bridle Shiner might have persisted in some areas of the Potomac at population levels that escaped detection over much of a century but might be resurging. This was an optimistic possibility considering the abundant regrowth of SAV some areas of the river have experienced in recent decades, following an earlier history of high sediment loads and turbidity (Starnes 2002).

Findings of this study only detected the presence of Bridle Shiner in the greater James River basin, in several tidal tributaries to the lower James and Chickahominy rivers, and in one tributary, Flat Creek, to the Appomattox subbasin of the James River in the Piedmont (Figure 3). A return to this site in 2016 failed to yield further specimens, despite vigorous collecting efforts in the precise areas of prior success. Further, a subsequent effort (Pinder and Wheaton 2020), extending over much of the length of Flat Creek, also failed to detect the presence of Bridle Shiner. The populations discovered in Grays and Flower Dew Hundred creeks in Surry and Prince George counties may represent previously undocumented ones. The former population in the Jolly Pond area, James County, may be extirpated. In 2016, biologists from the consulting firm EnviroScience, conducting surveys in the vicinity of the Surry Power Plant on the James River captured a single individual of Bridle Shiner (NCSM 112566) indicating that a population may persist in that reach or larval drift may be occurring from the populations upriver.

In this study, all habitats in which Bridle Shiner were collected were 1–3 or more meters in depth, with soft bottom, and SAV consisting most often of Coontail, or mixed Coontail and Hydrilla, and, in one instance, Brazilian Elodea and Slim Pondweed (Figures 4 and 5). The strong association between Bridle Shiner and Coontail and other submerged plants, including for reproductive purposes, was previously documented by Harrington (1947). All sites for which water quality data was available, pH readings were near neutral or greater, 6.5 up to 8.0, and salinities were 0 to 1.0 ppt. This included the several tidal creeks in the lower Chickahominy River area from which Bridle Shiner were collected (data provided by R. Greenlee, VDGIF), though they lie in a region of otherwise tannic and low pH water bodies. The tidal creeks where Bridle Shiner was found are likely influenced by major daily influxes of water derived from the large Piedmont portion of the James River watershed. This probably injects more neutral to basic conditions, including nitrates, into these systems, overwhelming the acidic effects of local input from small tannic streams. This tends to ameliorate salinity influences from downriver, thus providing the conditions necessary for good SAV growth, as well as freshening waters for species with low salinity tolerance. Goulder and Boatman (1971) have demonstrated the importance of nitrate content to the growth of Coontail in particular. These conditions may go far toward explaining the "island" of Bridle Shiner occurrences that is otherwise surrounded by a region of mostly tannic streams and swamps in southeastern Virginia that is seemingly devoid of the species (Figure 3).

The short lifespan of Bridle Shiner may render it vulnerable to local or broader scale extirpations during protracted periods of adverse conditions. Failure to recruit substantial numbers in two successive seasons could decimate a given population. Major threats and the causes of extirpations of Bridle Shiner likely involve habitat alterations that have occurred over the past two centuries. Chief among these is likely siltation and turbidity that retards growth of the SAV critical to Bridle Shiner's life history. A case in point, the Potomac River underwent a long period of increased turbidity, reduced water quality, and loss of SAV (Starnes 2002). Conditions have since improved and abundant SAV, though mostly Hydrilla, has returned but there may be no remnant population of Bridle Shiner to exploit this improvement. Aside from possible water quality impacts, the plethora of small (e.g., millponds) and larger impoundments that dot eastern Virginia may have taken a toll. It appears the former Briery Creek and Sandy River populations in the Appomattox subbasin found in the 1970s may have fallen victim to flow alterations and other factors brought on by the impoundment of these streams in the late 1980s–early 1990s. As for the many older and smaller millponds and small lakes, while these habitats often develop good growth of SAV, and offered some hope as potential habitats, it was observed that these semi-closed systems often become overly predator ridden making survival difficult for any potential forage species.

Bridle Shiner could possibly be extirpated from the great majority of its known former range in Virginia. The discovery of an extant population in the North Carolina portion of the Chowan basin lends some hope for an eventual rediscovery in Virginia. However, all of these statements must be couched in the context of known difficulties of detecting Bridle Shiner populations, occasionally even when they are known to be present and due to the effectiveness of the gear utilized (Jensen and Vokoun 2013, Pregler et al. 2015). There is a remote possibility that small, highly isolated, populations may exist in some of these areas that can eventually flourish and exploit any habitats that might experience improvements in the coming years.

The genetic analysis served two purposes: to confirm the identifications of the Bridle Shiner collected and served as a primary assessment of population-level variation for possible future study. While there isn't any widely accepted standard by which to measure diversity in a given species, IBOL employs BINs (Barcode Index Numbers) to help define operational taxonomic units, or OTUs (Ratnasingham and Hebert 2013) for barcoded taxa. These BINs aid researchers to identify errors in the database (incorrectly identified individuals) and to identify potential instances of currently unrecognized diversity within a species. BINs in fishes typically flag intraspecific instances of 2% divergence as worthy of further investigation, as seen at the base of the Bridle Shiner tree at 2.5% (Figure 6). Our genetic findings suggest further study is warranted with additional collection of DNA data. We also note that a finer-scaled analysis using, for example, microsatellite DNA might be warranted but was outside the scope of the project as defined in 2010–2012. Other studies such as Geneva et al. (2018) have documented basin-level population differentiation, and we would anticipate similar findings to be documented in Virginia. We also note that the use of eDNA as in Katz (2023) would likely help in documenting additional populations of this difficult to sample species.

Although the data presented here spans from 2010 to 2013, we believe it remains the most up-to-date and comprehensive dataset for comparison in any future status surveys conducted in Virginia. Our findings suggest that additional surveys in key areas would be valuable. Given the difficulties in effectively sampling this species, future survey designs should account for habitat preferences, gear effectiveness, and land-use changes (Pregler et al. 2015, 2019). Furthermore, future efforts should involve a more in-depth investigation of the genetic relationships among known populations, which would inform decisions regarding potential population augmentation. As noted by Pregler et al. (2019), a collaborative, range wide investigation of Bridle Shiner would provide the clearest picture of what is causing the decline of this species and how its resilience could be improved.

## Data Availability

The datasets that include all the site information (only visually assessed, surveyed, and capture sites for Bridle Shiner), and all of the other species encountered are available on Zenodo (https://doi.org/10.5281/zenodo.11164979).

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