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9 | APPENDIX

THREE NEW SPECIES OF *FONTIGENS* (CAENOGASTROPODA: FONTIGENTIDAE) FROM CAVES IN THE APPALACHIAN RIDGE AND VALLEY PROVINCE, VIRGINIA

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Abstract

We describe three new species of the stygophilic hydrobiid genus *Fontigens* from extensive collections made by the Virginia Department of Conservation and Recreation, Division of Natural Heritage Karst Program 2008 – 2019: *F. hershleri* n. sp. from Scott County and *F. benfieldi* n. sp. and *F. davisii* n. sp. from Giles County, VA. *Fontigens hershleri* is a member of the *nickliniana* group, bearing a tripartite penis with two tubular accessory glands, known only from Lane Cave near Copper Creek. *Fontigens hershleri* is distinguished by unique shell morphology and a minimum COI sequence divergence of 13.1% from all previously sequenced *Fontigens*. *Fontigens benfieldi*

and *F. davisii* are both members of the *orolibas* group, bearing a tripartite penis with one tubular and one bulbous gland, and occur in the two largest known caves within the Wabash Spring Basin: the Sugar Run Cave System (*F. benfieldi*) and Dulaneys Cave (*F. benfieldi* and *F. davisii*). They are also distinguished by shell morphology, a 9.1% COI sequence divergence from each other, and an 8.5% sequence divergence from the closest previously sequenced *Fontigens* population. **Key words:** Gastropoda, Truncatelloidea, springsnails, shell morphology, reproductive anatomy, COI, mtDNA sequence divergence, molecular phylogeny, karst springs.

Introduction

Truncatelloid gastropods of the family Hydrobiidae (s.s.) are widespread and locally common in cool, well-oxygenated waters throughout North America. Their high levels of endemism, especially in the American West, have led to substantial conservation concerns (Hershler *et al.* 2014). Important systematic studies have been contributed for *Pyrgulopsis* by Hershler (1994; 1998) and Liu & Hershler (2005), for *Tryonia* by Hershler (2001) and Hershler *et al.* (2011), and for *Fluminicola* by Hershler & Frest (1996) and Hershler & Liu (2012).

Until recently, hydrobiids of the eastern and midwestern genus *Fontigens* have been less well-studied than groups inhabiting the west. The genus is distinguished by shell morphology, basal cusps on the central radular tooth, and a tripartite penis. Taylor (1966) suggested that *Fontigens* comprised a distinct hydrobiid subfamily, the Fontigentinae, which Hershler *et al.* (1990) synonymized under the Emmericiinae of Brusina (1870). Wilke *et al.* (2013) did not confirm a close association between *Fontigens* (represented by a single sample) and the European genus *Emmericia*, however, returning the Fontigentinae to subfamilial status. Gladstone & Whelan (2022) have recently elevated the Fontigentidae to full family rank.

Hershler *et al.* (1990) recognized nine species of *Fontigens*, with a tenth of uncertain status, five stygobionts restricted to subsurface

waters and nearly endemic, the other five stygophilic, inhabiting spring runs and more widespread. Recently *Liu et al.* (2021) have published a survey of COI mtDNA sequence variation conducted across seven *Fontigens* species, 14 populations and 88 individuals. The authors have reported strikingly high sequence divergence among conspecific populations, ranging up to 14.3%. Nevertheless, the seven species remained phylogenetically distinct, demonstrating 8.7 – 21.0% sequence divergence.

The center of *Fontigens* diversity is the Valley and Ridge physiographic province of Virginia and West Virginia, inhabited by six of the ten known species. The Virginia Department of Conservation and Recreation Division of Natural Heritage (DCR-DNH) Karst Program and legally permitted collaborators regularly perform biological collections in caves, karst springs, and karst spring runs throughout this section of the Commonwealth, not infrequently collecting samples of gastropods. Here we report the discovery of three new species of cave dwelling *Fontigens* in these extensive collections.

Materials and Methods

From 2008 to 2019 DCR-DNH survey teams collected gastropod specimens from a total of 87 sites across 19 counties down the length of western Virginia from Frederick County to Lee County: 19 collections were from drainages of the Shenandoah, 9 from the James, 1 from the Roanoke, 33 from the New, and 25 from the Tennessee. These 87 collections were sent to the FWGNA Laboratory in Charleston, SC, for dissection and identification. Three well-characterized species of *Fontigens* were identified, together with a variety of other freshwater and terrestrial gastropods: *Fontigens orolibas* Hubricht (25), *Fontigens morrisoni* Hershler *et al.* (4), and *Fontigens bottimeri* (Walker) (4).

In addition, our observations suggested that DCR-DNH teams had gathered three *Fontigens* species not previously described: one inhabiting Lane Cave in Scott County, VA, and two inhabiting a pair of hydrologically connected caves in Giles County, VA. Subsets of

these especially interesting samples were selected for sequencing, together with a control *F. orolibas* population sampled from Tawneys Cave in Giles County (Virginia Speleological Survey #2526, approx. coords. 37.31, -80.51).

Our DNA sequencing methodology has been described by Liu *et al.* (2021). Ultimately, we sequenced 3 individuals from Lane Cave, 5 individuals from the main stream of the Sugar Run Cave System, 3 individuals from Dulaneys Cave, and 3 individuals from Tawneys Cave.

Genomic DNA was extracted from entire snails using a CTAB protocol (Bucklin 1992). A partial (709 bp) segment of mitochondrial cytochrome c oxidase subunit I (mtCOI) corresponding to “Folmer’s fragment” (Folmer *et al.* 1994) was amplified and sequenced with primers LCO1490 and HCOI2198 following the protocols of Liu *et al.* (2003). Sequences were determined for both strands and then edited and aligned using Sequencher™ version 5.4.1.

Identification	Codes	Locality	GenBank Accession
<i>Fontigens hershleri n.sp.</i>	LC21A, LC23A, LC23B	Lane Cave, Scott Co, VA	
<i>Fontigens benfieldi n.sp.</i>	SRC20A, SRC20B, SRC25A, SRC25B, SRC25C	Birthday entrance to the Sugar Run Cave System, Giles Co, VA	
<i>Fontigens benfieldi n.sp.</i>	DC24B	Dulaneys Cave, Giles Co, VA	
<i>Fontigens davisii n.sp.</i>	DC24A, DC24C	Dulaneys Cave, Giles Co, VA	
<i>Fontigens antroecetes</i>		St. Clair Co, IL	MT425002
<i>Fontigens bottimeri</i>		Washington, DC	MT425003
<i>Fontigens bottimeri</i>		Frederick Co, VA	MT425004
<i>Fontigens cryptica</i>		Bullitt Co, KY	MT425005
<i>Fontigens morrisoni</i>		Highland Co, VA	MT425006
<i>Fontigens nickliniana</i>		LaGrange Co, IN	MT425007
<i>Fontigens nickliniana</i>		Bath Co, VA	MT425008-14
<i>Fontigens nickliniana</i>		Shenandoah Co, VA	MT425015-19
<i>Fontigens nickliniana</i>		Washington Co, VA	MT425020-27
<i>Fontigens nickliniana</i>		Kalamazoo, MI	JX970609
<i>Fontigens orolibas</i>	oro27A, oro27B, oro27C	Tawneys Cave, Giles Co, VA	
<i>Fontigens orolibas</i>		Page Co, VA	MT425028
<i>Fontigens orolibas</i>		Augusta Co, VA	MT425029
<i>Fontigens orolibas</i>		Tazewell Co, VA	MT425030-31
<i>Fontigens tartarea</i>		Greenbrier Co, WV	MT425032-33
<i>Bythinella tentaculata</i>			MK308073
<i>Bythinella austriaca</i>			FJ028979
<i>Bythinella pannonica</i>			HQ149623
<i>Bythinella viridis</i>			FJ029102
<i>Emmericia expansilabris</i>	-		KC810061

Table 9.1. Specimen codes, localities, and GenBank accession numbers.

Sequence divergences (uncorrected p distance) within and between phylogenetic lineages were calculated using MEGA7 (Kumar *et al.* 2016) with pairwise deletion for missing data.

Phylogenetic analyses were formed using Bayesian inference. Our analysis included the 14 newly sequenced specimens and 38 sequences retrieved from GenBank - 32 sequences contributed by Liu *et al.* (2021) and 6 others, including 5 hydrobioid outgroups, as listed in Table 9.1.

Trees were rooted with *Bythinella viridis* (Poiret), following Wilke *et al.* (2013). MrModeltest2 (Nylander 2004) was used to obtain an appropriate substitution model (using the Akaike Information Criterion) and parameter values for the analyses. Bayesian inference was performed using MrBayes v. 3.2.7a (Ronquist *et al.* 2012). In the Bayesian analysis Metropolis-coupled Markov chain Monte Carlo simulations were performed with four chains for 5,000,000 generations and Markov chains were sampled at intervals of 10 generations to obtain 500,000 sample points. We used the default settings for the priors on topologies and the GTR + I + G model parameters selected by MrModeltest2 as the best fit model. At the end of the analysis, the average standard deviation of split frequencies was 0.001 and the potential scale reduction factor was 1, indicating that the runs had reached convergence. The sampled trees with branch lengths were used to generate a 50% majority rule consensus tree, with the first 25% of the samples removed to ensure that the chain sampled a stationary portion.

Results

Upon initial examination, the small sample of *Fontigens* collected from Lane Cave (N = 5) demonstrated a unique ovate-conic shell morphology not readily matched to any known species of *Fontigens*. Anatomically they were indistinguishable from *Fontigens nickliniana*

(Lea), demonstrating pigmented bodies and a tripartite penis with two tubular accessory lobes.

	<i>F. morrisoni</i>	<i>F. orolibas</i>	<i>F. tartarea</i>	<i>F. benfieldi</i>	<i>F. davisii</i>
<i>F. morrisoni</i> (1)	N/C				
<i>F. orolibas</i> (7)	0.189	0.089			
<i>F. tartarea</i> (2)	0.090	0.189	0.003		
<i>F. benfieldi</i> (6)	0.108	0.183	0.092	0.001	
<i>F. davisii</i> (2)	0.094	0.175	0.085	0.091	0.000

Table 9.2. Mean (uncorrected) COI sequence divergence in the *orolibas* group.

Similarly, the DCR-DNH sample of *Fontigens* from the main stream in the Sugar Run Cave System (N = 25) demonstrated a unique shell morphology, best described as ovoid. Anatomically that sample was placed in the *orolibas* group, characterized by unpigmented bodies and a tripartite penis with one accessory lobe tubular and the other accessory lobe bulbous. The group also includes *F. morrisoni* and *F. tartarea* Hubricht, as well as *F. orolibas* (Hershler *et al.* 1990).

The DCR-DNH sample from Dulaneys Cave (N = 39) was mixed. That sample included N = 31 individuals matching the population from the Sugar Run Cave System, adults bearing entirely white, ovoid shells approximately 1.6 – 1.9 mm in standard length. Also present in the same sample were N = 8 individuals of a third apparently unknown species bearing a distinctive elongate-conic shell, generally stained with mineral deposit, adults approximately 2.4 – 2.7 mm in standard length. Subsamples from both sets anatomically matched the *orolibas* group. The three individuals we selected to sequence from this mixed sample included 2 Dulaneys-elongate and 1 Dulaneys-ovoid.

A phylogenetic analysis placing samples from the Lane, Sugar Run, Dulaneys and Tawneys (*F. orolibas* - control) populations into the context of the larger analysis of Liu *et al.* (2021) is shown in Figure 1. Table 2 shows the values of percent sequence divergence within and among eight *Fontigens* populations (18 individuals) in

the *orolibas* group, and Table 3 reports percent sequence divergence within and among six *Fontigens* populations (25 individuals) in the *nickliniana* group.

	<i>nickliniana</i> Indiana	<i>nickliniana</i> Bath Co	<i>nickliniana</i> Shenandoah Co	<i>nickliniana</i> Washington Co	<i>nickliniana</i> Michigan	<i>hershleri</i> Lane Cave
<i>F. nickliniana</i> Indiana (1)	N/C					
<i>F. nickliniana</i> Bath Co, VA (7)	0.109	0.003				
<i>F. nickliniana</i> Shenandoah Co, VA (5)	0.010	0.110	0.009			
<i>F. nickliniana</i> Washington Co, VA (8)	0.101	0.108	0.104	0.009		
<i>F. nickliniana</i> Michigan (1)	0.003	0.113	0.010	0.107	N/C	
<i>F. hershleri</i> n. sp. Lane Cave (3)	0.131	0.144	0.133	0.156	0.136	0.000

Table 9.3. Mean (uncorrected) COI sequence divergence in the *nickliniana* group.

The Tawneys Cave sequences (oro27A, oro27B, oro27C) and the three previously analyzed *F. orolibas* sequences from Virginia were resolved as a strongly supported monophyletic clade. The mean sequence divergence between the Tawneys sample and the three other *F. orolibas* populations ranged from 10.5-12.0%, which is within previously recorded values (8.7-14.3%).

Our sample of N = 5 sequences from the *Fontigens* population at the main stream in the Sugar Run Cave System (SRC20A, SRC20B, SRC25A, SRC25B, SRC25C) clustered with two other species of the *orolibas* group, *F. morrisoni* and *F. tartarea*, although not closely related to our (now four) populations of *F. orolibas* itself. And our sample of N = 3 sequences from Dulaneys Cave was heterogeneous. The single sequence from the Dulaneys-ovoid specimen (DC24B) clustered with the five Sugar Run sequences, at a maximum of only 0.2% sequence divergence. And the pair of Dulaneys-elongate sequences were distinct. Although not differing from each other, the two Dulaneys-elongate specimens (DC24A and DC24C) differed from the Dulaneys-ovoid sequence by 9.3%.

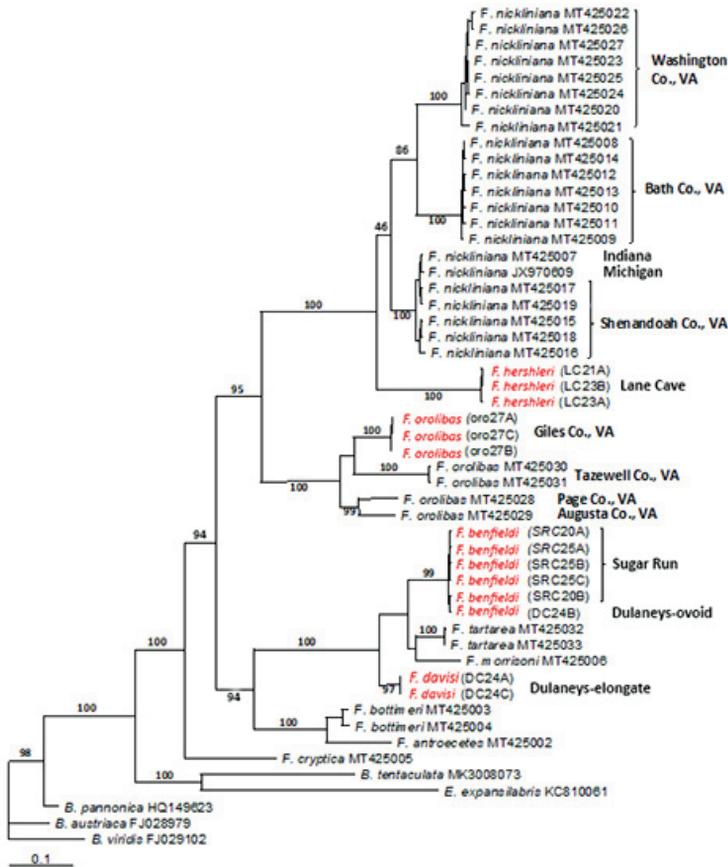


Fig 9.1. Bayesian tree based on COI sequence data for specimens listed in Table 1, posterior probabilities at the nodes. New data highlighted in red.

Consistent with the anatomical evidence, the three sequences obtained from Lane Cave (LC21A, LC23A, and LC23B) clustered with the five populations of *F. nickliniana* previously analyzed (3 Virginia, 1 Indiana, 1 Michigan), although distinct from them. The percent sequence divergences among the five previously analyzed *nickliniana* populations ranged from 0.3 to 11.0%. The percent sequence

divergence between our Lane Cave population and the five *nickliniana* ranged from 13.1 – 15.6%.

Discussion

The Appalachian Mountains are a geologically complex landform, the result of an ancient orogenic belt sculpted during the Mesozoic and Cenozoic eras by processes of uplift and erosion that continue through the present. Stratigraphic and structural bedrock relationships were established during the Paleozoic Era by deposition of sedimentary rocks (including extensive carbonate bedrock) punctuated by a series of orogenic events that included extensive folding and faulting and resulted in the formation of Pangaea approximately 270 million years ago (Hatcher 1987; van der Voo 1988; Clark 2008). Uplift and erosion of the roots of this ancient mountain range has occurred intermittently since then, beginning with the rifting of Pangaea at the start of the Mesozoic Era 250 million year ago, forming the modern Atlantic Ocean (Olsen *et al.* 1991). Significant uplift has been documented at least as recently as 5 million years ago near the end of the Miocene Epoch, Neogene Period, Cenozoic Era (Gallen *et al.* 2013).

The Valley and Ridge Physiographic Province, an approximately 1,000 km long band between the Blue Ridge and the Appalachian Plateau provinces, is underlain by Paleozoic strata that include thick sequences of limestone and dolomite bedrock. Most of the caves and other karst structures known in this province probably developed from the Pliocene (5 Ma) through the middle Pleistocene (300 ka) (White 2007; Anthony & Granger 2004; Schwartz *et al.* 2009), with older caves and passages at higher elevations. Extensive sedimentation of caves occurred during the Pleistocene, especially in the late Calabrian stage (White 2007; Granger *et al.* 2001). These Pleistocene sediments are nearly ubiquitous in the caves of the Valley and Ridge Province and are slowly being eroded away by modern karst hydrological systems.

The diverse and often endemic subterranean invertebrate fauna inhabiting this region has been reviewed by Culver *et al.* (2003), Holsinger *et al.* (2013) and Niemiller & Zigler (2013). Some quantitative investigations have suggested that the cave fauna may be as old or older than many of the caves themselves (Hutchins *et al.* 2010).

Lane Cave is developed in Cambrian Marysville Limestone (Brent 1963) where it is exposed in a narrow, east to northeast belt atop a 60 m bluff parallel to and just south of Copper Creek, a tributary to the Clinch River in the upper Tennessee River Basin. At nearly 0.8 km long and just over 60 m deep, Lane Cave is among the larger of the 10 – 12 caves known from this belt (Holsinger 1975). The large stream in the cave is probably fed by multiple, small surface streams flowing from the southwest that sink into the Marysville limestone. It flows from northeast to southwest, likely resurging at a spring tributary to Copper Creek, west of the cave, and on through the Clinch/Tennessee River system.

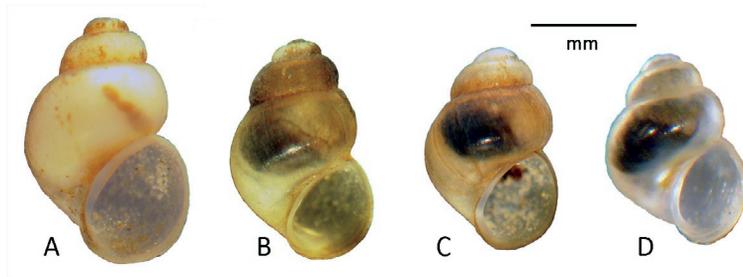


Fig 9.2. *Fontigens hershleri* n. sp. (A) Holotype, (B – D) paratypes destroyed for analysis.

Anatomically, the population of *Fontigens* inhabiting Lane Cave is most similar to *F. nickliniana*, widespread and locally common in above-ground springs and spring runs from Virginia to Michigan, occasionally discovered in caves. The ovate-conic shell morphology demonstrated by the Lane Cave population is distinctive, however. Genetically, our Lane Cave sample demonstrated 13.1 – 15.6% mtDNA sequence divergence from five *F. nickliniana* populations surveyed

across three states by Liu et al (2021). The Bayesian analysis shown in Figure 1 returned weak support for some of the interior branches of the larger *nickliniana*-group cluster, but nevertheless depicted the Lane Cave sample as most distinct.

Approximately 175 km NE of Lane Cave lie Dulaneys Cave and the Sugar Run Cave System, developed approximately 2 km apart in limestones of Middle Ordovician age exposed along the northeast flank of Sugar Run Mountain (Schultz et al, 1986). Both are deep, multi-pitch vertical cave systems with a combined total of nearly 45 km of passage and a depth of over 200 m (Virginia Speleological Survey, personal communication). They are hydrologically connected, although not connected by human passage.

The main stream in the Sugar Run Cave System is formed where Sugar Run (proper) sinks into its bed, about 6.4 km NW of Wabash Spring (Savko 2001). The main stream in Dulaneys Cave is fed by numerous small streams draining the northeast slope of Sugar Run Mountain, which sink at the limestone contact and run underground approximately 1 km E to join subterranean Sugar Run. Waters from both caves then flow together to the southeast and discharge at Wabash Spring, then to Walker Creek and through the New/Kanawha River system.

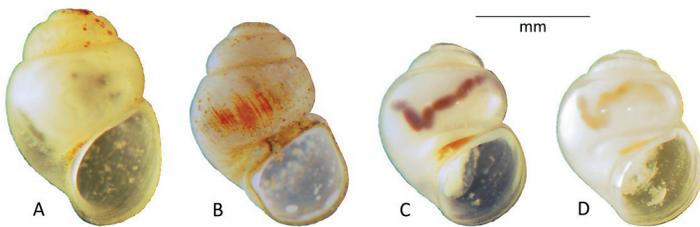


Fig 9.3. *Fontigens benfieldi* n. sp. (A) Holotype, (B – D) paratypes destroyed for analysis.

Our anatomical observations suggested that the *Fontigens* population inhabiting the main stream in the Sugar Run Cave System

are most similar to *F. orolibas*, widespread from Pennsylvania to Tennessee, both in underground waters and at above-ground spring-heads (Gladstone *et al.* 2021). The Sugar Run population bears a distinctive shell, however, best described as ovoid.

Genetically, however, the Sugar Run population proved distinct from the three *F. orolibas* populations previously sequenced by Liu *et al.* (2021), and from a fourth (control) *F. orolibas* population newly sequenced from Tawneys Cave nearby (ranged from 17.9% - 19.0% and averaged 18.3%). Our mtDNA sequence data suggested a closer relationship between the Sugar Run population and two other previously described species from the *orolibas* group, *F. tartarea* (9.2%) and *F. morrisoni* (10.8%).

The *Fontigens* collected by DCR-DNH teams from the waters of Dulaneys Cave were a mixture. The population inhabiting the main stream seemed to match the population in the main stream at the Sugar Run Cave system, both in their ovoid shell morphology and in mtDNA sequence (0.2% sequence divergence.) A population inhabiting an intermittent stream at the Dulaneys Cave entrance, however, were larger in adult size and bore shells of a distinctive elongate-conic morphology. Our mtDNA analysis suggested that the Dulaneys-elongate population was genetically sister to the Dulaneys-ovoid population, although differing by 9.1% mtDNA sequence divergence.

These observations combine to suggest that DCR-DNH Karst Program teams have gathered samples of three previously undescribed species of *Fontigens*, as detailed in the systematic account that follows. The Lane Cave species we propose to name *Fontigens hershleri*, the ovoid species from the main stream of the Sugar Run Cave System *Fontigens benfieldi*, and the elongate-conic species from the entrance series stream at Dulaneys Cave *Fontigens davisii*.

It seems likely to us that *Fontigens hershleri* may prove endemic or near endemic to Lane Cave. No other populations of *nickliniana*-group *Fontigens* have been documented from Scott County, or indeed, anywhere further downstream in Tennessee. The nearest population of *F. nickliniana* has been reported from a surface spring

near Dickensonville, in Russell County about 40 km east (Hershler *et al.* 1990). Our plans include exploration of any sinking streams that may be found to recharge Lane Cave, as well as its discharge spring. Other caves in this outcrop belt with potentially suitable aquatic habitat that should be sampled for hydrobiid snail fauna include Coley Cave No. 2, Blair-Collins Cave, Mill Springs Cave No.1.

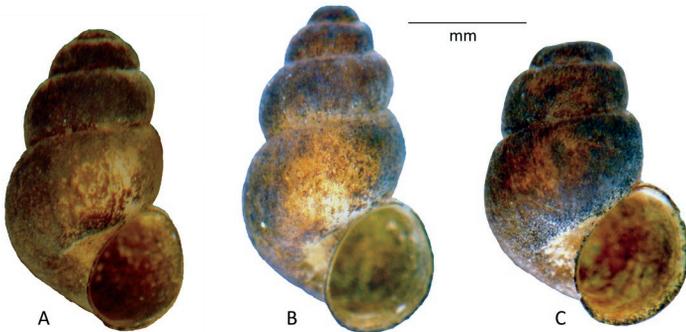


Fig 9.4. *Fontigens davisii* n. sp. (A) Holotype, (B – C) paratypes destroyed for analysis.

It also seems likely that *Fontigens benfieldi* and *F. davisii* may prove endemic or near-endemic to caves in the Wabash Spring basin. In contrast to the situation with *F. hershleri*, Hershler *et al.* (1990) have documented four populations of cave-dwelling *orolibas*-group *Fontigens* in the immediate Giles County vicinity. Our mtDNA sequence data suggest, however, that neither *F. benfieldi* nor *F. davisii* is especially similar to Giles County *F. orolibas*. It should be noted that Tawneys Cave, where our control *F. orolibas* were collected, is located on the opposite (East) side of the New River from the Sugar Run / Dulaneys / Wabash Spring basin. The New River appears to be a significant barrier for several groups of stygofauna, as shown by the truncation of several species distributions displayed in Holsinger *et al.* (2013).

The Sugar Run Cave System is the second longest cave in Virginia, but together with Dulaneys Cave spans only about one half of the

underground Wabash Spring basin. The complex cave systems within the Wabash Spring basin provide ample habitat variation and isolation to promote speciation. We suggest that the ancestor of *Fontigens benfieldi* and *F. davisii* may have evolved from an *orolibas*-group ancestor, and that the two species we describe may here have subsequently diverged, entirely within the single, large, complex Wabash Spring basin system.

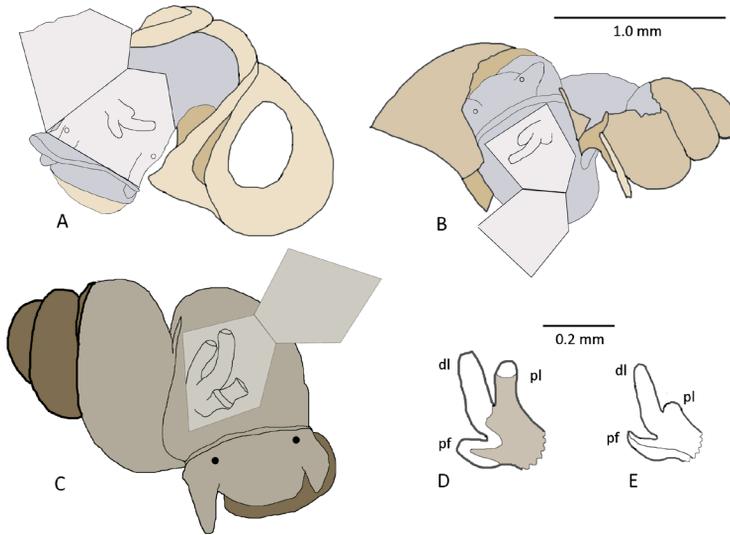


Fig 9.5. *Fontigens* opened to demonstrate dorsal penial morphology.

(A) *F. benfieldi* n. sp., (B) *F. davisii* n. sp., (C) *F. hershleri* n. sp., (D) ventral penial morphology of *F. hershleri*, (E) ventral penial morphology of *F. benfieldi* and *F. davisii*. pf = penial filament, dl = distal lobe, pl = proximal lobe.

The results we present here confirm and reinforce those of Liu *et al.* (2021) with respect to the unusually high levels of genetic divergence observed among populations of the hydrobiid genus *Fontigens*. Western hydrobioid groups typically demonstrate less than 1% mtDNA sequence divergence among conspecific populations and 2% minima among species, while we here report no less than 8.5% between any of our three new species and any of the seven *Fontigens*

species previously analyzed. Such observations would seem to suggest that the *Fontigens* populations of eastern North America are older than hydrobioid populations of the west.

Molecular clock hypotheses are fraught with assumptions (Wilke *et al.* 2009), but the Wilson & Sarich (1969) estimate of 2% sequence divergence per million years might still be accepted as a rule of thumb for protein-encoding mitochondrial genes. Then if, as we speculate, *Fontigens benfieldi* and *F. davisii* speciated and diverged entirely within the Sugar Run / Dulaneys / Wabash Spring basin, the 9.1% sequence divergence we report between them would imply an age of approximately 4.5 my for the cave system, consistent with a Pliocene origin.

Systematic Account

Caenogastropoda

Superfamily Truncatelloidea Gray, 1840

Family Fontigentidae Taylor, 1966

Genus *Fontigens* Pilsbry, 1933

Fontigens hershleri, new species

(Figures 9.2, 9.5C)

Description. Adult shell 1.8 – 2.4 mm standard length and 1.2 – 1.6 mm standard width, ovate-conic, light gray. Adult whorls 3 – 4. Apex moderately acute; sutures lightly impressed; body whorl 75 – 80% of adult shell length. Umbilicus slightly open. Operculum transparent. Body and eyespots pigmented. Penis bearing two accessory lobes, both tubular.

DNA Sequences. Accession numbers xxxxxxxx, deposited in GenBank. (LC21A, LC23A, LC23B)

Etymology. Named in honor of Dr. Robert (Bob) Hershler, recently retired from his position as curator of mollusks at the U.S. National Museum (Smithsonian). Dr. Hershler became the foremost

authority on the systematics and evolution of American hydrobioid gastropods, publishing almost 100 papers and monographs in his productive career. He remains a good friend.

Type Locality. Lane Cave. VA: Scott County, 11 km southwest of Snowflake. Virginia Speleological Survey (VSS) Identification Number: 1395. Approximate coordinates 36.66, -82.61. Clinch River drainage.

Material. Holotype ANSPA480684. Paratype (N=1) ANSPA480685.

Distribution and habitat. Stygophilic, possibly stygobiontic. Found grazing on and under rocks and cobbles in flowing waters in the main Lane Cave stream. The population is mixed with juvenile above-ground-dwelling *Pleurocera simplex* (Say) at this point, suggesting that the stream in which they live communicates with the outside. This observation, together with the fact that the bodies of *F. hershleri* bear some pigment, suggests that *F. hershleri* might be capable of living in surface waters.

Remarks. Both penial morphology and phylogenetic analysis suggest that *Fontigens hershleri* is a member of the larger “*nickliniana* group” of Hershler *et al.* (1990), widespread throughout the Eastern and Midwestern United States. Three species formerly comprised the group: *F. nickliniana*, *F. turritella* Hubricht, and *F. aldrichi* (Call & Beacher), to which we here add a fourth. Both *F. nickliniana* and *F. turritella* bear elongate-conic shells, readily distinguishable from the ovate-conic *F. hershleri*. Morphologically our new *F. hershleri* is most similar to *F. aldrichi* of Missouri, but the previously described *aldrichi* is distinguished by a shell bearing an entirely open umbilicus and an “almost flat” apex.

***Fontigens benfieldi*, new species**

(Figures 9.3, 9.5A)

Description. Adult shell 1.6 – 1.9 mm standard length and 1.2 – 1.4 mm standard width, colorless, ovoid. Adult whorls approximately three. Apex depressed; body whorl exceptionally convex; sutures

lightly impressed; body whorl about 80 – 85% of adult shell length, umbilicus variable. Operculum transparent. Body and eyespots unpigmented. Penis bearing two accessory lobes: a tubular distal lobe and a bulbous proximal lobe.

DNA Sequences. Accession numbers xxxxxxxx, deposited in GenBank. (SRC20A, SRC20B, SRC25A, SRC 25B, SRC 25C, and DC24B)

Etymology. Named in honor of Dr. Ernest F. (Fred) Benfield, Emeritus Professor of Biology at Virginia Tech, an influential undergraduate mentor of the senior author, and friend. He saw to it that RTD had the equipment and facilities to follow his passion, occasionally offered gentle advice, and otherwise got out of his way.

Type Locality. Sugar Run Cave System, main stream. VA: Giles County, 6 km west of Staffordsville. Virginia Speleological Survey (VSS) Identification Number: 3113. Approximate coordinates 37.24, -80.78. New River drainage.

Material. Holotype ANSP A480681. Paratypes ANSP A480682 (N = 14, locality above). Paratypes ANSP A480683 (N=30) Dulaneys Cave. VA: Giles County, 8 km west of Staffordsville. Virginia Speleological Survey (VSS) Identification Number: 790. Approximate coordinates 37.25, -80.81. New River drainage.

Distribution and habitat. Apparently stygobiontic. Found grazing on and under rocks and cobbles in flowing waters in the main stream of the Sugar Run Cave System and in the main stream of Dulaneys Cave (a tributary of Sugar Run).

Remarks. Both penial morphology and phylogenetic analysis suggest that *Fontigens benfieldi* is a member of the larger “*orolibas* group” of Hershler *et al.* (1990), widely distributed in caves and springs of Valley and Ridge Province from southern Pennsylvania into East Tennessee (Dillon 2019). Three species formerly comprised the group: *F. orolibas*, *F. tartarea*, and *F. morrisoni*, to which two new species are added here, *benfieldi* and *davisi*. *Fontigens orolibas*, *F. tartarea*, and *F. davisi* bear elongate-conic shells, strikingly different from the ovoid or fusoid appearance of *F. benfieldi*. Our new *F. benfieldi* is most morphologically similar to *F. morrisoni*, but the latter is

a typically pigmented species, bearing a more conic shell, with apex less depressed.

***Fontigens davisi*, new species**

(Figures 9.4, 9.5B)

Description. Adult shell 2.4 – 2.7 mm standard length and 1.6 – 1.7 mm standard width, elongate-conic, convex, colorless but often stained with mineral deposit. Adult whorls approximately four. Apex variably depressed; sutures lightly impressed; body whorl about 70% of adult shell length. Umbilicus slightly open. Operculum transparent. Body and eyespots unpigmented. Penis bearing two accessory lobes: a tubular distal lobe and a bulbous proximal lobe.

DNA Sequences. Accession numbers xxxxxxxx, deposited in GenBank. (DC24A, DC24C)

Etymology. Named in honor of Dr. George M. Davis, retired from his position as Chairman of the Department of Malacology at the Academy of Natural Sciences of Philadelphia. Dr. Davis published approximately 100 papers and monographs in his productive career, most famously on the hydrobioid faunas of Asia, and served as Editor-in Chief of the journal *Malacologia* for many years. He was an influential graduate mentor of the senior author and remains a good friend.

Type Locality. Dulaneys Cave, entrance stream. VA: Giles County, 8 km west of Staffordville. Virginia Speleological Survey (VSS) Identification Number: 790. Approximate coordinates 37.25, -80.71. New River drainage.

Material. Holotype ANSPA480679. Paratypes (N=5) ANSPA480680.

Distribution and habitat. Apparently stygobiontic. Found grazing on and under rocks in an intermittent stream near the entrance of Dulaneys Cave.

Remarks. Both penial morphology and phylogenetic analysis suggest that *Fontigens davisi* is a member of the larger “*orolibas* group” of

Hershler *et al.* (1990), widely distributed in caves and springs of Valley and Ridge Province from southern Pennsylvania into East Tennessee (Dillon 2019). Three species formerly comprised the group: *F. orolibas*, *F. tartarea*, and *F. morrisoni*, to which two new species are added here, *benfieldi* and *davisi*. *Fontigens morrisoni* and *F. benfieldi* bear ovate or ovoid shells, strikingly different from the elongate-conic *orolibas*, *tartarea*, and *davisi*. Of the elongate-conic subset, *F. orolibas* is distinguished by a more slender shell, body whorl no more than approximately 65% of shell length. Morphologically our new *F. davisi* is most similar to *F. tartarea*, but the shell apex of the latter is more depressed, and the sutures more impressed.

The close proximity of *F. benfieldi* and *F. davisi* within the same spring basin and cave suggests these species may occur sympatrically, and hence may be reproductively isolated, but collections to date have been from distinct, separate locations.

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