Journal of Molluscan Studies

The Malacological Society of London

Journal of Molluscan Studies (2012) **78**: 321–329. doi:10.1093/mollus/eys017

MOLECULAR PHYLOGENY OF THE WESTERN NORTH AMERICAN PEBBLESNAILS, GENUS *FLUMINICOLA* (RISSOOIDEA: LITHOGLYPHIDAE), WITH DESCRIPTION OF A NEW SPECIES

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(Received 11 April 2012; accepted 28 June 2012)

ABSTRACT

The northwestern North American freshwater gastropod genus *Fluminicola* (Caenogastropoda, Lithoglyphidae) was previously hypothesized to be paraphyletic based on morphologic data, with *F. virens* positioned outside the clade containing other congeners. We further evaluated the phylogenetic relationships of *Fluminicola* using mitochondrial cytochrome *c* oxidase subunit I (COI) gene sequence data from 22 of 24 previously described congeners, a new congener (*F. gustafsoni*) that closely resembles *F. virens* morphologically, representatives of four other lithoglyphid genera, and several outgroups. A Bayesian analysis resolved two well supported *Fluminicola* clades, consistent with morphological heterogeneity. The clade composed of *F. virens* and *F. gustafsoni* was sister to the rest of the Lithoglyphidae; the clade containing the remaining congeners was sister to *Somatogyrus*. Our results confirm that *Fluminicola* is paraphyletic. However, this genus cannot be revised until the phylogenetic relationships of its possibly extinct type species are resolved. The new species described herein, *F. gustafsoni*, differs from *F. virens* in its smaller size, medium-convex and prominently shouldered teleoconch whorls, weakly angled shell aperture, presence of a well-developed rectangular wing on the outer marginal radular teeth, vertically oriented coiled oviduct, and in its COI sequences. *Fluminicola gustafsoni* is distributed in riverine habitats in the lower Snake River watershed.

INTRODUCTION

The freshwater caenogastropod family Lithoglyphidae (Rissooidea) is composed of small species (commonly known as pebblesnails) that usually have squat shells and simple penes lacking glands and lobes. The lithoglyphids are distributed primarily in the Holarctic region and typically live in fluvial or lacustrine habitats. This small group (containing about 13 genera and 116 species) was classified as a subfamily in the Hydrobiidae (Thompson, 1984) before it was recently elevated to a separate family (Wilke *et al.*, 2001; Bouchet & Rocroi, 2005). The scope and content of the Lithoglyphidae are still somewhat unsettled (e.g. Hershler & Thompson, 1990) and the phylogenetic relationships of the group are only beginning to be studied (e.g. Hausdorf, Röpstorf & Riedel, 2003; Hershler, Liu & Thompson, 2003; Hershler *et al.*, 2007).

Northwestern North America contains a relatively large lithoglyphid assemblage (24 species) that is currently classified as the genus *Fluminicola* (Hershler & Frest, 1996; Hershler, 1999; Hershler *et al.*, 2007). One of these species, *F. virens*, is well differentiated from its congeners by the following characters: short pedal commissure; anteriorly coiled vas deferens; broadly triangular, deeply creased penis having a terminal papilla; elongate duct of the bursa copulatrix; and several other morphological details (Hershler & Frest, 1996). Hershler & Frest's (1996) phylogenetic analysis of morphological characters positioned *F. virens* outside the clade containing the other members of the genus (those for which anatomical data were available). Although the phylogenetic relationships among *Fluminicola* species have been subsequently studied using molecular data (Hershler *et al.*, 2007), there have been no further investigations of the apparent paraphyly of the genus.

In this paper, we further investigate the phylogenetic relationships of *Fluminicola* and test for monophyly of the genus using mitochondrial DNA sequence data. This study was prompted in part by the discovery of a new species belonging to the *F. virens* lineage, which is described herein.

MATERIAL AND METHODS

Fresh material of the new species was collected by hand and by washing rocks. Subsamples were anaesthetized overnight with menthol crystals, fixed in dilute formalin and preserved in 70% ethanol for morphological study, and in 90% ethanol for molecular study. Shells, radula, opercula and soft anatomy

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were studied using methods employed in recent taxonomic studies of *Fluminicola* (Hershler & Frest, 1996; Hershler, 1999; Hershler *et al*., 2007).

The molecular phylogenetic analysis was based on partial sequences of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. We utilized a previously published *Fluminicola* dataset (Hershler *et al*., 2007) composed of 32 COI sequences from 22 congeners (and two taxonomically indeterminate lineages), and newly obtained sequences from five populations of the new congener described herein. Sequences are not available for the type species (*F. nuttallianus*) and *F. minutissimus*, both of which are known only from shells (Hershler & Frest, 1996). We also included sequences of lithoglyphids from eastern North America (*Somatogyrus*), Europe (*Lithoglyphus*) and Asia (*Benedictia*, *Kobeltocochlea*) to test the monophyly of *Fluminicola*. Two western North American rissooidean genera (of uncertain affinities) that are morphologically similar to lithoglyphids (*Pristinicola*, *Taylorconcha*; Hershler *et al*., 1994) were included as outgroups and *Phrantela marginata* (Hydrobiidae) was used to root the tree. Sample codes, locality details and GenBank accession numbers for the sequenced specimens are given in Table 1. Geographic coordinates are given in the Universal Transverse Mercator system (North American Datum 1983, zone 11). Collectors were as follows: Terry Frest (TF), Peter Hovingh (PH), Edward Johannes (EJ), Daniel Gustafson (DG), Jerry Landye (JL), Stewart Schell (SS) and Dwight Taylor (DT).

Genomic DNA was extracted from entire snails using a CTAB protocol (Bucklin, 1992). Amplifications were conducted in a 25 μl total volume, containing 5 μl of 5× buffer, 0.5 μl of dNTPs (10 mM), 2 μl of $MgCl_2$ (25 mM), 1.25 μl of each primer (10 μM), 1 unit Taq polymerase, 1 μl of template DNA (*c*. 100 ng double-stranded DNA) and 13.8 μl of sterile water. COIL1490 (Folmer *et al*., 1994) and COH654 were used to amplify a 634 bp fragment (excluding primers) of the COI gene. COH 654 (5′AAA TGC TGR TAT AAA ATT GG3′) was designed based on previously published *F. coloradensis* (AY962915) and *F. fuscus* (DQ372901) sequences. Thermal cycling was performed with an initial denaturation for 2 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 50°C, 2 min at 72°C, with a final extension of 10 min at 72°C. The amplified PCR product was cleaned using the Exonuclease I/Shrimp Alkaline Phosphatase method. Approximately 10–20 ng of cleaned PCR product was used as a template in a cycle-sequencing reaction using the BigDye Terminator v. 3.1 cycle-sequencing kit (Applied Biosystems). The following cycling conditions were used: 30 cycles of 96°C for 20 s, 50°C for 20 s and 60°C for 4 min. The cycle-sequenced product was cleaned using the ethanol-precipitation method and then run on an ABI 310 genetic analyser. Sequences were determined for both strands and then edited and aligned using Sequencher™ v. 4.8. Two to four specimens were sequenced for samples of the new species.

Base compositional differences were evaluated using the χ^2-test. Sequence divergences (uncorrected *p* distance) were calculated using MEGA5 (Tamura *et al*., 2011). Phylogenetic analysis was performed using Bayesian inference in MrBayes v. 3.2.1 (Ronquist *et al*., 2012). MrModeltest v. 2.3 (Nylander, 2004) selected the GTR + I + G model which best fitted these data under the Akaike Information Criterion. In the Bayesian analysis, the run was conducted using the best-fit model selected by MrModeltest v. 2.3 and the default random tree option. 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. At the end of the analysis, the average standard deviation of split frequencies was 0.0053 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% samples (equal to 125,000 sample points) removed to ensure that the chain sampled a stationary portion.

The posterior distribution of trees was used statistically to test *Fluminicola* monophyly using the methods outlined by Weisrock *et al*. (2006) and McMahon, Geheber & Piller (2010). A constraint (*Fluminicola* monophyly) created in PAUP* v. 4.0 (Swofford, 2002) was used to count the number of Bayesian trees that resolved *Fluminicola* as a monophyletic group. Monophyly was rejected under a Bayesian criterion if <5% of the total number of generated trees resolved a clade containing all of the *Fluminicola* sequences.

RESULTS

Fifteen specimens of the new species described herein were newly sequenced for this study. Five haplotypes were resolved and used in the phylogenetic analysis. The new sequences (one example of each haplotype per population) were deposited in GenBank under accession numbers JQ731609-JQ731615 (Table 1). The alignment of COI sequences yielded 658 bp, of which 263 sites were variable (39.97%) and 242 were parsimony informative (36.78%). Average base frequencies for COI were 27.3% A, 34.5% T, 19.2% C and 19.0% G. Base frequencies were homogeneous across all taxa (χ^2 =109.16, df= 141, $P = 0.98$).

The *Fluminicola* sequences were resolved as two evolutionarily distinct, well supported (≥95% posterior probability) clades in the Bayesian tree (Fig. 1). Clade A contained *F. virens* and the new species described herein and was positioned as sister to the rest of the lithoglyphids; clade B was composed of the rest of the *Fluminicola* sequences and was positioned distally as sister to *Somatogyrus* sp. Although the basal branches of the tree were generally poorly supported (<0.95 posterior probability), only 1,248 out of 500,001 trees (0.25%) delineated *Fluminicola* as a single clade, thus the hypothesized monophyly of this genus can be rejected.

DISCUSSION

Fluminicola was previously hypothesized to be paraphyletic based on morphological data (Hershler & Frest, 1996). Our findings, although based on a single gene fragment, provide independent, robust evidence that this genus is a composite of two separate lineages, one containing *F. virens* and the new species described below, and one containing 21 other congeners. The two lineages are genetically divergent (differing by 17.1 ± 1.3% COI sequence divergence) and do not appear to be closely related (Fig. 1), although additional studies will be needed to resolve further their relationships within the Lithoglyphidae. The average COI sequence divergence within clade A is 2.4 ± 0.3% (congeners ranging from 6.2 to 6.6%) and 8.0 ± 0.6% within clade B (congeners ranging from 1.5 to 14.3%), suggesting that these clades are either fairly old or have accelerated evolutionary rates.

Our results suggest that *Fluminicola* needs to be revised. However, nomenclatural changes cannot be made until the phylogenetic relationships of the type species, *F. nuttallianus*, are resolved. This species is known only from specimens that were collected in the lower Willamette River during the 1800s (Hershler & Frest, 1996). Although this watershed has been severely degraded by urbanization, pebblesnails of uncertain taxonomic status have been recently collected in many of its streams (Waite *et al*., 2008). We are currently reviewing this material to determine whether *F. nuttallianus* is extant.

Table 1. Samples of *Fluminicola* and rissooidean outgroups used for molecular analysis, with codes (used in Fig. 1, Table 2), locality details and Genbank accession numbers for COI.

Species	Code	Locality	GenBank
F. ahjumawi		Spring run north of Sam Wolfin Spring, Shasta Co., CA	AY962899
F. anserinus		Spring, Goose Valley, Shasta Co., CA	AY962908
F. caballensis		Davis Creek, Lassen Co., CA	AY962911
F. coloradensis		Green River above Warren Bridge, Sublette Co., WY	AY962915
F. dalli		Spring west of Thunderbolt Bay, Washoe Co., NV	AY962916
F. erosus	F10 202E	Spring southeast of Smokey Charley Spring, Modoc Co., CA	AY962917
F. erosus	F11 202A	Spring southeast of Smokey Charley Spring, Modoc Co., CA	AY962919
F. erosus	F12 202A	Spring southeast of Smokey Charley Spring, Modoc Co., CA	AY962921
F. favillaceus		Ash Creek, south culvert at Ash Valley Road crossing, Lassen Co., CA	AY962930
F. fremonti	D45aA	Hunters Spring, Lake Co., OR	AY962931
F. fremonti	D45aB	Hunters Spring, Lake Co., OR	AY962932
F. fuscus		Methow River, Okanogan Co., WA	DQ372901
F. gustafsoni	F67A	Clearwater River, Jim Ford Creek, Clearwater Co., ID	JQ731609
F. gustafsoni	F67B	Clearwater River, Jim Ford Creek, Clearwater Co., ID	JQ731610
F. gustafsoni	F68A	Clearwater River, Orofino, Clearwater Co., ID	JQ731611
F. gustafsoni	F69A	Salmon River, Pine Bar Rapids, Idaho Co., ID	JQ731612
F. gustafsoni	F70A	South Fork Clearwater River, Battlefield, Idaho Co., ID	JQ731613
F. gustafsoni	F73A	Snake River, below mouth of Couse Creek, Asotin Co., WA	JQ731614
F. gustafsoni	F73B	Snake River, below mouth of Couse Creek, Asotin Co., WA	JQ731615
F. insolitus		Page Springs, Harney Co., OR	AY962934
F. lunsfordensis	F359A	Lunsford Spring, Modoc Co., CA	AY962935
F. lunsfordensis	F359B	Lunsford Spring, Modoc Co., CA	AY962936
F. modoci		Spring at Three Springs Ranch, Modoc Co., CA	AY962938
F. multifarius		Big Springs (source), northwest of city of Mount Shasta	AY962977
F. neritoides		Willow Creek at lower end of Lower McBride Springs, Lassen Co., CA	AY962954
F. potemicus	F2 36A	Spring near Potem Creek, Shasta Co., CA	AY962956
F. potemicus	F2 36B	Spring near Potem Creek, Shasta Co., CA	AY962957
F. scopulinus		Northern-most spring southwest of Popcorn Spring, Shasta Co., CA	AY962958
F. seminalis		Pit River near confluence of Hat Creek, Shasta Co., CA	AY962969
F. turbiniformis		Roaring Springs, Harney Co., OR	AY962986
F. umbilicatus		Big Spring, tributary of Hat Creek, Shasta Co., CA	AY962989
F. virens		Willamette River at Canby Ferry, Clackamas Co., OR	AY962992
F. virginius		Hardscrabble Creek, Washoe Co., NV	AY962993
F. warnerensis		Parsnip Spring, Modoc Co., CA	AY962996
F. sp. (A)	SP113A	Springs west of Canby, Modoc Co., CA	AY962922
F. sp. (A)	F2SP373A	Spring at west end of Upper Rush Creek Campground, Modoc Co., CA	AY962925
F. sp. (A)	SP373B	Spring at west end of Upper Rush Creek Campground, Modoc Co., CA	AY962924
F. sp. (B)	SP373A	Spring at west end of Upper Rush Creek Campground, Modoc Co., CA	AY962923
F. sp. (B)	F2SP373E	Spring at west end of Upper Rush Creek Campground, Modoc Co., CA	AY962926
Benedictia baicalensis	A	Lake Baikal, Russia	Z92983
Benedictia baicalensis	B	Lake Baikal, Baikalsk, 15 m depth, Russia	AF445330
Benedictia maxima		Lake Baikal, Maloye More, 150 m depth, Russia	Y15380
Kobeltocochlea martensiana		Lake Baikal, Russia	Z92984
Lithoglyphus naticoides	A	Narew River near Drozdowo, Poland	AF367642
Lithoglyphus naticoides	B	River Ipel, Sahy, Slovakia	AF354770
Pristinicola hemphilli		Springs 1.8 km east of Lower Kalama Hatchery, Cowlitz Co., WA	AF520940
Somatogyrus sp.		Choctawatchee River, 3.2 km north of Geneva, Geneva Co., AL	AF520942
Taylorconcha insperata		Snake River, just below Davis Creek rapids, Wallowa Co., OR	DQ076027
Taylorconcha serpenticola		Banbury Springs outlets, Gooding Co., ID	DQ076022
Phrantela marginata		Tributary of Thirteen Mile Creek, Tasmania, Australia	AF129331

The discovery of these two divergent lineages underscores our limited knowledge of the diversity of the western North American component of the lithoglyphid radiation. More than half of the currently recognized species of *Fluminicola* (15/24) have been described since the genus was last reviewed in 1996 (Hershler, 1999; Hershler *et al*., 2007). The overwhelming majority of the pebblesnail populations in western North America are currently unassigned and the recognition of >40 putatively new *Fluminicola* species in recent contract reports (e.g. Frest & Johannes, 1993, 1995, 2000a, 2005) suggests a considerable

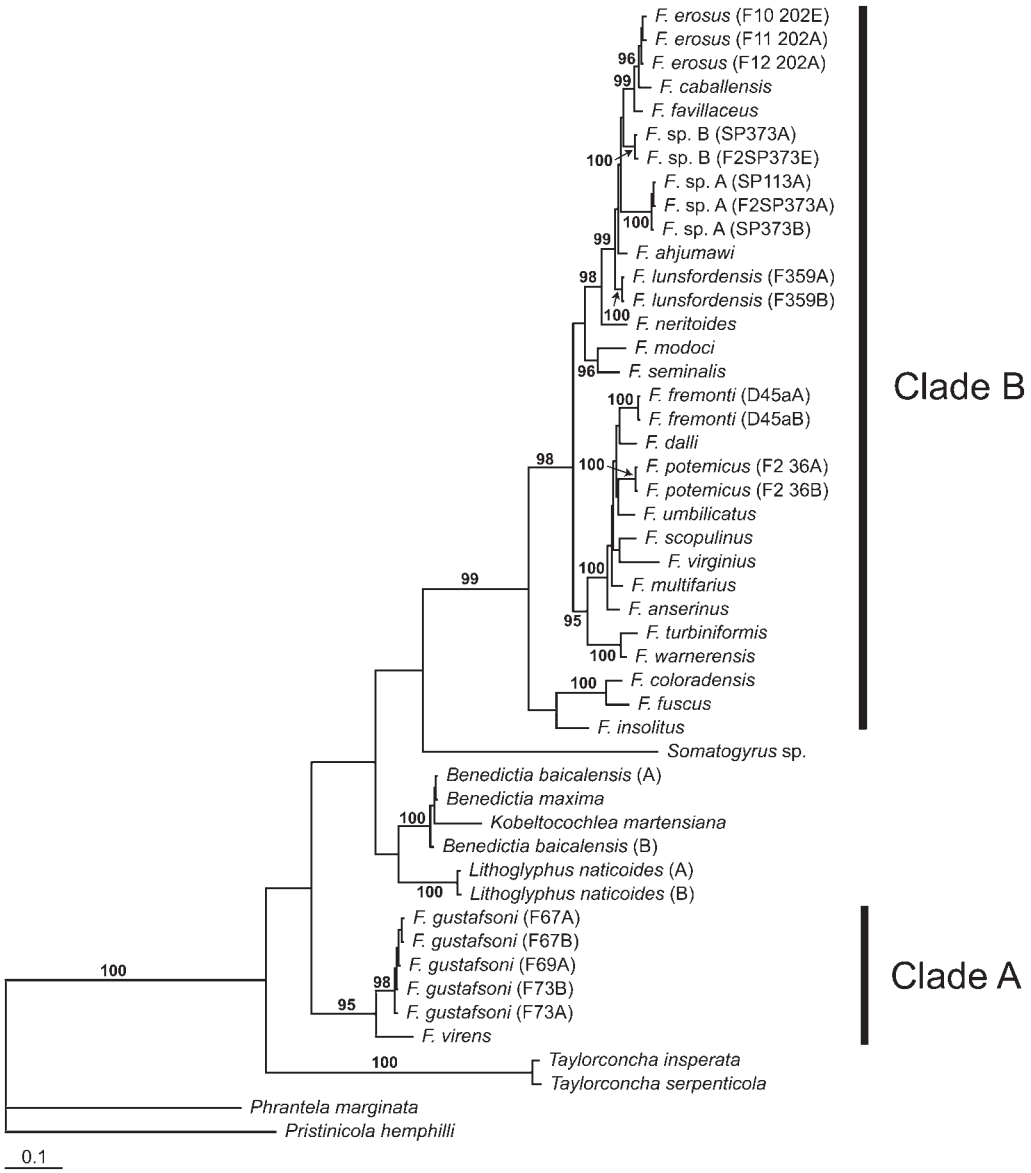

Figure 1. Phylogenetic analysis of *Fuminicola* species. Bayesian tree based on the COI dataset. Posterior probabilities are provided when >95%. Terminals and specimen codes are labelled as in Table 1.

amount of undescribed diversity that may possibly include additional divergent lineages.

SYSTEMATIC DESCRIPTION

FLUMINICOLA Carpenter, 1864

Fluminicola gustafsoni new species

Fluminicola fusca—Henderson, 1936: 139 (Clearwater River record).
Lithoglyphus nuttallianus—Taylor, 1966: fig. 14 (Clearwater River record).
Lithoglyphus virens—Schell, 1973: 463.
Fluminicola sp.—Hershler & Frest, 1996: 12 (Salmon River records).
Fluminicola undescribed sp. B—Frest & Johannes, 2000b:13.

Types: Holotype (Fig. 2A), USNM 905409, Salmon River at Pine Bar Rapids, Idaho County, Idaho (N 5082021, E 551897), coll. 23 April 2001, coll. DG. Paratypes (from same lot) USNM 1175400.

Etymology: Named after Daniel L. Gustafson (Montana State University), who collected much of the material used in this project, including the type material.

Referred material: IDAHO. *Clearwater County*: USNM 1082622, Clearwater R., Jim Ford Creek (N 5141362, E 560758), 31/7/2005, coll. DG; USNM 1082623, Clearwater R., Orofino (N 5148310, E 556435), 25/7/2005, coll. DG; USNM 883460, USNM 1144782, Clearwater R., US-12 km 54.4 (N 5149124,

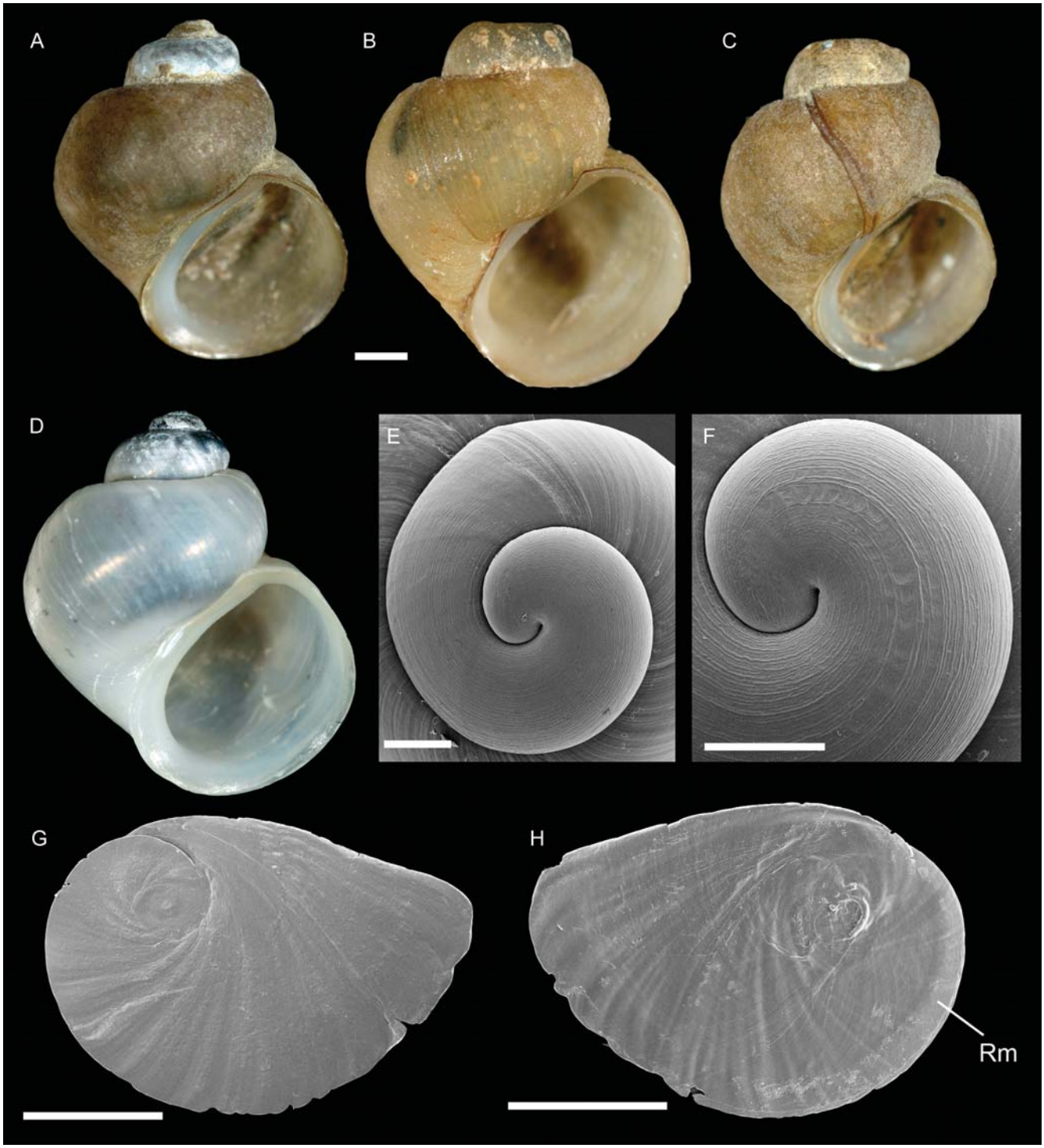

Figure 2. Scanning electron micrographs of shells and opercula of *Fluminicola gustafsoni* new species. **A**. Holotype, Salmon River at Pine Bar Rapids, Idaho County, Idaho (USNM 905409). **B**. Clearwater River at Spaulding Mission pull-off, Nez Perce County, Idaho (USNM 883458). **C**. Clearwater River, Jim Ford Creek, Clearwater County, Idaho (USNM 1082623). **D**. Salmon River at Pine Bar Rapids, Idaho County, Idaho (paratype, USNM 1175400) (shell cleaned with bleach). **E, F**. Shell apex, Clearwater River, Jim Ford Creek, Clearwater County, Idaho (USNM 1082623). **G, H**. Opercula (outer, inner sides), Clearwater River, Orofino, Clearwater County, Idaho (USNM 883460). Abbreviation: Rm, rim along outer margin. Scale bars **A–D** = 1.0 mm; **E, F** = 200 μm, **G, H** = 1.0 mm.

E 542294), 13/8/1989, coll. TF, EJ; USNM 883512, USNM 1144791, Clearwater R., US-12 km 78.8 (N 5141172, E 560782), 13/8/1989, coll. TF, EJ; USNM 883512; *Idaho County*: USNM 1082621, Salmon R., Pine Bar rapids (N 5082021, E 551897), 1/4/2005, coll. DG; USNM 1082629, South Fork Clearwater R., Battlefield (N 5098311, E 579134), 4/8/2005, coll. DG; USNM 1160580, Salmon R., Slate Creek access (N 5055498, E 555085), 2/10/2011, coll. RH, PH; USNM 1170785, Salmon R., km 93.2–93.8, Skookumchuck Creek access (N 5061045, E 553202), 10/8/1989, coll. TF, EJ; USNM

Table 2. Shell parameters for *Fluminicola gustafsoni* new species.

	WH	SH	SW	HBW	WBW	AH	AW
Holotype	*c.* 4.0	7.05	5.83	5.91	4.47	4.04	4.07
Paratypes (17)							
Mean	3.93*	7.07	6.10	6.09	4.57	4.30	4.26
Range	3.75–4.25	6.54–8.12	5.53–6.74	5.62–6.74	4.30–5.21	3.81–4.78	3.84–4.65
SD	0.21	0.50	0.36	0.34	0.25	0.26	0.22

Abbreviations: WH, total shell whorls; SH, shell height; SW, shell width; HBW, height of body whorl; WBW, width of body whorl; AH, aperture height; AW, aperture width;

*n=10.

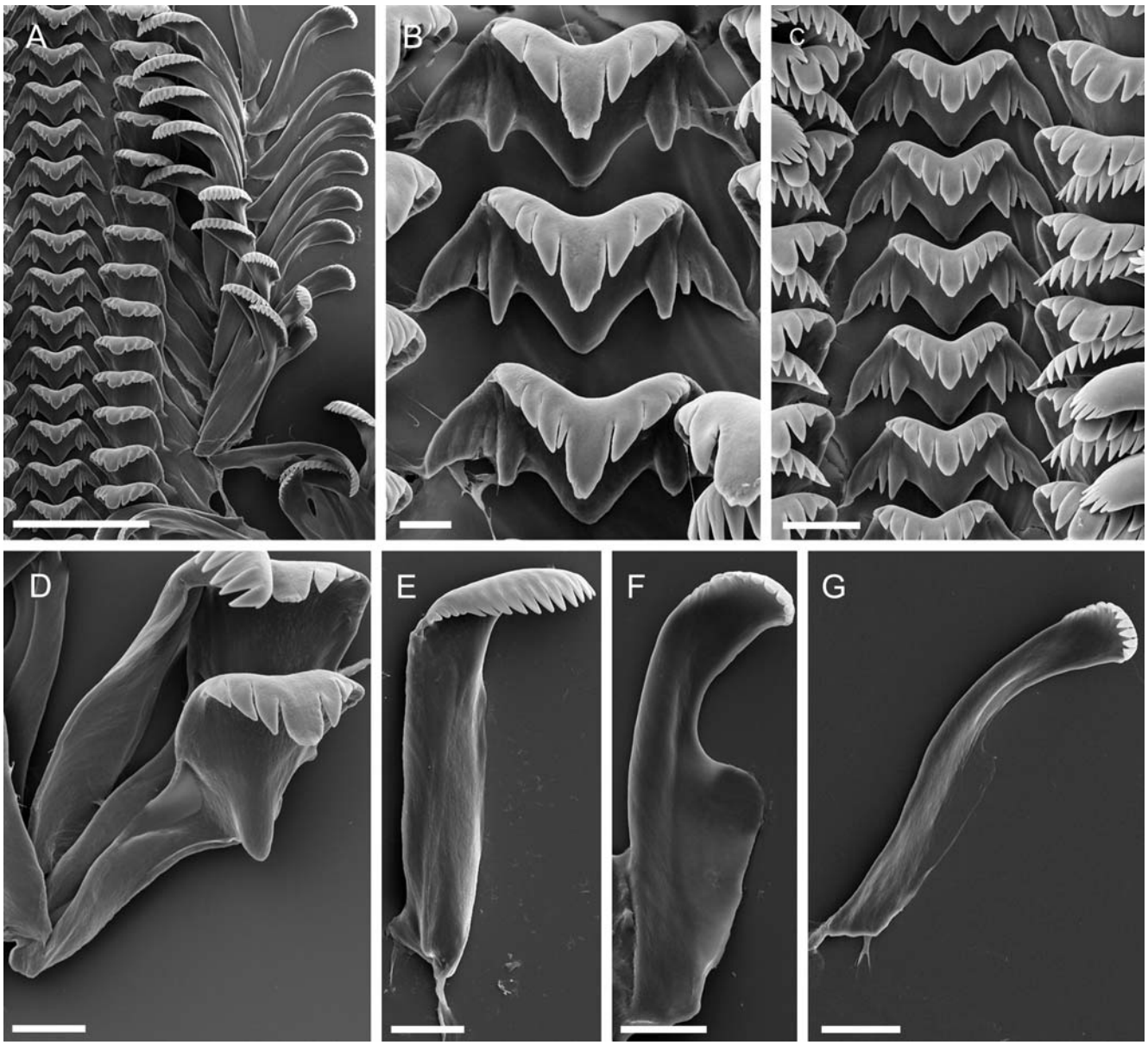

Figure 3. Scanning electron micrographs of radula of *Fluminicola gustafsoni* new species. **A**. Portion of radular ribbon, Salmon River at Pine Bar Rapids, Idaho County, Idaho (USNM 1175400). **B, C**. Central teeth, Salmon River at Pine Bar Rapids, Idaho County, Idaho (USNM 1175400); Clearwater River, Orofino, Clearwater County, Idaho (USNM 883460). **D**. Lateral and inner marginal teeth (USNM 1175400). **E**. Inner marginal tooth (USNM 1175400). **F, G**. Outer marginal tooth (USNM 1175400, USNM 883460). Scale bars **A** = 100 μm, **B** = 10 μm, **C–G** = 20 μm.

1170781, *ibid*., 10/8/1993, coll. TF, EJ; USNM 1170783, Salmon R. at Boles Bridge, to E of bridge for 0.4 km (N 5084361, E 545683), 29/9/1990, coll. TF, EJ; USNM 1170785, Salmon R., km 93.2–93.8, Skookumchuck Creek access (N 5061045, E 553202), 10/8/1989, coll. TF, EJ; USNM 1170808, Salmon R., river km 134.5–134.8, N of Riggins (N 5033541, E 554072), 9/8/1989, coll. TF, EJ; USNM 1170809, Salmon R. at S end of Lyons Bar (N 5072536, E 553287), 30/8/1990, coll. TF, EJ; *Lewis County*: USNM 1144788, Clearwater R., S Fork S of Stites (N 5104336, E 579137), 13/8/1989, coll. TF *et al*.; USNM 1144789, Clearwater R., US-12 km 113.3 (N 5115609, E 576571), 13/8/1989, coll. TF, EJ; USNM 883501, USNM 1144790, Clearwater R., US-12 km 87.2 (N 5134429, E 564112), 13/8/1989, coll. TF, EJ; *Nez Perce County*: USNM 883500, Hells Canyon, just inside mouth of Salmon R. (N 5078062, E 516048), 18/8/1993, coll. TF *et al*.; USNM 1144798, mouth of Salmon R., Hells Canyon (N 5078062, E 516048), 18/8/1989, coll. TF, EJ; UMMZ 219454, Clearwater R., Spaulding (N 5144246, E 514182), 12/8/1963, coll. DT; USNM 883458, USNM 1144724, Clearwater R. at Spaulding Mission pull-off, river km 13.4 (N 5144236, E 514173), 10/10/1993, coll. TF, EJ; USNM 1160583, Clearwater R., Gibbs Eddy access (N 5147409, E 518941), 2/10/2011, coll. RH, PH; USNM 758339, Clearwater R., 8/1969, coll. SS; USNM 758340, Clearwater R., 8/1969, coll. SS; USNM 874927, Clearwater R., 3.5 km W of Myrtle, on Hwy 9 (N 5147184, E 518947), 3/6/1968, coll. JL. WASHINGTON. *Asotin County*: USNM 1170810, Snake R., just below mouth of Couse Creek (N 5117061, E 502477), 2/10/2011, coll. RH, PH.

Diagnosis: A medium-sized *Fluminicola* having a trochoidal to ovate-conic shell and large, gently tapered penis. Differs from closely similar *F. virens* in its smaller size, medium-convex and prominently shouldered teleoconch whorls, weakly angled shell aperture, well developed rectangular wing on the outer marginal radular teeth, vertically oriented coiled oviduct, and in its mtDNA sequences (see Remarks below).

Description: Shell trochoidal to ovate-conic, spire usually eroded (Fig. 2A–D); height 6.5–7.2 mm; whorls about 4.0. Apex flat, slightly tilted. Protoconch of 1.5 whorls, diameter 0.95 mm; spiral striae numerous, well developed (Fig. 2E), apical region sometimes also having weak transverse elements (Fig. 2F). Teleoconch whorls medium convex, shouldered, sculptured with well developed collabral growth lines. Aperture ovate, weakly angled adapically. Parietal lip complete, adnate, thin and curved across body whorl, or thickened and nearly straight. Columellar lip usually somewhat thickened, straight, columellar shelf usually broad. Outer lip usually thin, prosocline. Umbilicus absent, umbilical region sometimes excavated. Shell clear-white (Fig. 2D), periostracum tan or light brown (Fig. 2A–C). Shell parameters are given in Table 2.

Operculum thin, light amber, ovate, multispiral, nucleus eccentric, last 0.25–0.50 whorl weakly frilled (Fig. 2G), outer margin sometimes having weak rim (Fig. 2H: Rm). Attachment scar margin smooth (Fig. 2H).

Radula having about 65 well-formed rows of teeth (Fig. 3A). Central tooth (Fig. 2B, C) 75 μm wide, cutting edge concave, lateral cusps 3–7; median cusp rounded or weakly pointed, usually parallel-sided proximally, broader and longer than laterals; basal cusps 2–3, innermost cusp largest; basal tongue rounded or V-shaped, approximately even with lateral margins. Lateral tooth (Fig. 3D) face rectangular, outer wing flexed, about 125% length of cutting edge, central cusp U-shaped, lateral cusps 2–6 (inner), 3–7 (outer). Inner marginal teeth (Fig. 3E) with 11–17 cusps. Outer marginal teeth (Fig. 3F, G) with 10–16 cusps; inner edge of tooth having rectangular wing. Radular data are from USNM 883460 and USNM 1175400.

Snout brown dorsally, ventral surface, small areas around eyes and distal tips pale. Foot generally pale, anterior portion brown, sole pale. Pallial roof and visceral coil black, pigment somewhat lighter on genital ducts. Ctenidium abutting pericardium posteriorly; ctenidial filaments 30–31 ($n = 5$), apices centrally positioned, lateral surfaces weakly ridged. Osphradium narrow, elongate, anteriorly hooked, positioned along posterior half of ctenidium. Renal organ with large pallial component. Hypobranchial gland thin, large, overlapping most of rectum and pallial roof. Pedal commissure short. Cephalopedal ganglia pale or very lightly pigmented.

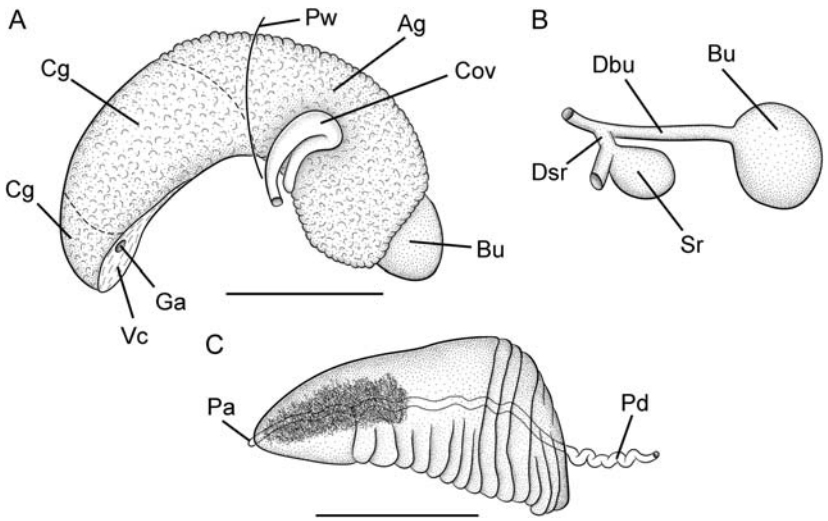

Figure 4. Reproductive anatomy of *Fluminicola gustafsoni* new species, Salmon River at Pine Bar Rapids, Idaho County, Idaho (USNM 1175400). **A**. Female glandular oviduct and associated structures (viewed from left side). **B**. Bursa copulatrix and seminal receptacle. **C**. Penis, dorsal surface (pigment darkly stippled). Abbreviations: Ag, albumen gland; Bu, bursa copulatrix; Cg, capsule gland; Cov, coiled oviduct; Dbu, bursa copulatrix duct; Dsr, seminal receptacle duct; Ga, genital aperture; Pa, penial papilla; Pd, penial duct; Pw, posterior wall of pallial cavity; Sr, seminal receptacle; Vc, ventral channel of capsule gland. Scale bars (**A–C**) = 500 μm.

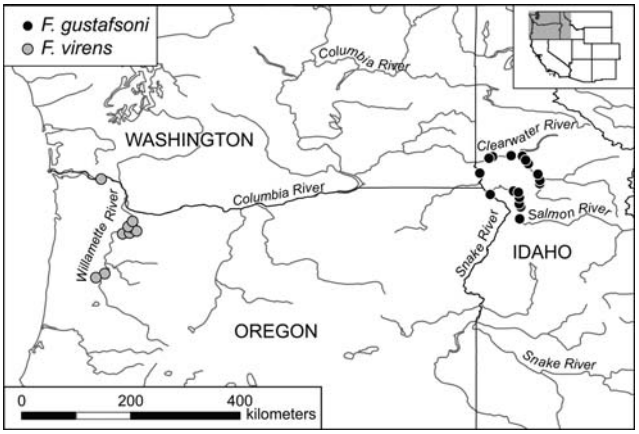

Figure 5. Map showing the geographic distributions of *Fluminicola gustafsoni* new species and *F. virens*. Data for the latter are from Hershler & Frest (1996).

Table 3. *Fluminicola gustafsoni* new species COI haplotypes and their distribution among samples.

Haplotype (specimen)	Base pair position					Sample code				
	143	200	259	359	376	F67	F68	F69	F70	F73
I (F67A)	A	A	T	T	C	1				
II (F67B)	A	A	T	C	C	2	4		2	
III (F69A)	G	A	C	C	C			3		
IV (F73A)	G	G	T	C	T					1
V (F73B)	G	G	T	C	C					2

Renal oviduct having connective tissues pigmented with black granules. Ovary composed of 3–4 compound lobes occupying about 0.5 whorl, slightly overlapping stomach anteriorly. Glandular oviduct and associated structures shown in Figure 4A, B. Coiled oviduct (Cov) narrow, nearly vertical, proximal arm kinked, posterior arm sometimes containing sperm. Bursa copulatrix (Bu) small, globose, partly overlapped by albumen gland (Ag). Bursal duct (Dbu) as long or slightly longer than bursa copulatrix, narrow, originating near anterior edge. Seminal receptacle (Sr) small, globose, duct (Dsr) short, positioned between coiled oviduct and bursa copulatrix, completely overlapped by albumen gland. Albumen gland (Ag) having short pallial component. Capsule gland (Cg) about as long as albumen gland, composed of two glandular zones, ovate in section. Rectum forming well-developed furrow on capsule gland (not shown in Fig. 4A). Ventral channel (Vc) narrow. Genital aperture (Ga) a small, subterminal pore.

Testis composed of compound lobes, occupying 1.25 whorls, overlapping stomach chambers. Seminal vesicle composed of thick coils occupying about 0.5 whorl, abutting posterior edge of stomach, opening little behind anterior edge of testis. Prostate gland large, pear-shaped, with about 25% of length in pallial roof. Visceral vas deferens opening to ventral edge of prostate gland just behind pallial wall. Pallial vas deferens opening from ventral edge of prostate gland in front of pallial wall, duct narrow, undulating. Penis (Fig. 4C) large, nearly straight, gently tapering. Base and median sections deeply folded along inner edge, distal tip weakly pointed. Penial duct (Pd) near centrally positioned, narrow, undulating, opening through small terminal papilla (Pa). Distal section of penis having dense core of internal black pigment.

Distribution and habitat: *Fluminicola gustafsoni* is distributed in the Clearwater River (and its south fork), lower Salmon River (downflow from Riggins), and the reach of the Snake River between the mouths of these two streams (Fig. 5). This species was collected in shallow water on rocks and cobbles. It occurs sympatrically with *F. fuscus* in both the Snake River and Salmon River.

Remarks: Five haplotypes were detected for *F. gustafsoni* which differed from each other by 1–4 base pairs (Table 3). One of the haplotypes (II) was observed in three samples, the others were either private alleles (III, V) or singletons (I, IV) (Table 3). The five samples of this species that were analysed differed from each other by 0.1–0.6% sequence divergence.

Fluminicola gustafsoni is distributed well upriver in the Columbia River basin from its sister species, *F. virens* (Fig. 5). These two species differed by $6.5 \pm 0.1\%$ COI sequence divergence. A close relationship between *F. virens* and Clearwater River pebblesnails was previously postulated by (former USNM curator) Joseph P.E. Morrison, who was asked to identify specimens of the former by parasitologist Stewart Schell (University of Idaho) in 1969. Morrison responded that 'The species you sent from the Clearwater River is close to, but not identical to *Lithoglyphus virens* (Lea) 1838 … It may be a new species; at the moment I'm not sure … List it as *Lithoglyphus* cf. *virens* (Lea), and you will be correct' (Morrison letter to Schell, April 9, 1970).

ACKNOWLEDGEMENTS

Karolyn Darrow (Smithsonian Institution) inked drawings; Yolanda Villacampa (Smithsonian Institution) prepared scanning electron micrographs, counted radula cusps and measured shells. Peter Hovingh assisted the first author in the field. This project was supported, in part, by an award from the Idaho Department of Fish and Game (Agreement no. T-3-7 0814). The manuscript was improved by comments provided by David Reid and an anonymous reviewer.

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