DISCOVERY OF INTRODUCED AND CRYPTOGENIC COCHLIOPID GASTROPODS IN THE SAN FRANCISCO ESTUARY, CALIFORNIA

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ABSTRACT

We report the first discovery in the San Francisco Estuary ('Estuary') of two cochliopid gastropods, *Littoridinops monroensis* and *Tryonia porrecta*. These identifications were based on morphological criteria and supported by analysis of mitochondrial DNA (cytochrome *c* oxidase subunit I, COI) sequence data. We also report the first discovery of males in parthenogenetic *T. porrecta*. The new records represent large range extensions for both of these North American species, which were previously recorded from predominantly brackish habitats along the western Atlantic-Gulf Coast (*L. monroensis*) and thermal springs in the Great Basin and lower Colorado River region (*T. porrecta*). The COI haplotype observed in Estuary specimens of *L. monroensis* differed from those detected in two western Atlantic populations by only 1–3 bp, suggesting recent divergence which is not consistent with the separation of these two areas by imposing terrestrial barriers since at least the Pliocene. We suggest that *L. monroensis* was recently introduced to the Estuary by transoceanic shipping, adding to the large exotic biota that has invaded this highly disturbed ecosystem. The COI haplotype observed in Estuary specimens of *T. porrecta* is closely similar to haplotypes detected in Nevada and Utah populations and highly divergent relative to the single haplotype observed in other California populations. The implications of these findings for the status of *T. porrecta* in the Estuary are unclear, because the native range of this parthenogen has not been established and its scattered distribution in the West may be attributable to natural dispersal across land (on birds) and/or anthropogenic spread. Although we suggest treating *T. porrecta* as cryptogenic in the Estuary, a native status may be suggested by independent (subfossil) evidence that this snail was locally present prior to establishment of the area as a major centre of human population and commerce in the 1850s.

INTRODUCTION

The Cochliopidae (Caenogastropoda: Rissooidea) are a predominantly New World family of small gastropods that is found in brackish coastal waters and a wide variety of inland aquatic habitats (Hershler & Thompson, 1992; group elevated to separate family status by Wilke *et al.*, 2001). North America contains a diverse cochliopid fauna (23 genera, 65 species), which is largely concentrated in the Atlantic and Gulf Coastal plains and margins. Only three genera and 15 species live in western North America (Hershler, 1999, 2001) and only a single member of the family, *Tryonia imitator* (Pilsbry, 1899), has been found in the Pacific coastal waters that border this land mass. This species was once widely distributed in brackish coastal lagoons and estuaries of central and southern California and northernmost Baja California, but is thought to be now extinct over most of its historic range (USFWS, 1977; Kellogg, 1980, 1985; Taylor, 1981).

Unfamiliar cochliopid snails not conforming to *T. imitator* were recently discovered in the San Francisco Estuary (herein referred to as the Estuary), central California (Davis, 2004). Here, we detail these new records, which represent large range extensions for eastern Atlantic-Gulf Coastal *Littoridinops monroensis* (Frauenfeld, 1863) and western North American *Tryonia porrecta* (Mighels, 1845). We also analyse mitochondrial DNA

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sequence data to confirm our morphology-based identifications and assess divergence of the broadly disjunct Estuary representatives of these two species. Based on our findings and other pertinent information, we conclude that *L. monroensis* was recently introduced to the Estuary by transoceanic shipping. We are less certain of the status of *T. porrecta*, although our evidence suggests that this snail has been present in the Estuary for >150 years and may be a previously overlooked native.

MATERIAL AND METHODS

Littoridinops monroensis and *Tryonia porrecta* were first discovered in the Estuary in 1999 during the course of an ecological study of Point Edith Marsh, which is located on the southern shore of Suisun Bay (Fig. 1). Snails were collected within this wetland at three brackish (2.0–16.9 ppt salinity), *Typha*-dominated sites: a 200-m^2 pool at the western end of a 1-km long tidal channel along the north side of Waterfront Road ('Waterfront Marsh') (38°02'08"N, 122°04'14"W) (Fig. 2A); a 1,000-m^2 tidal pool *ca.* 1.25 km southeast of Point Edith ('Weir Marsh') (38°02'44"N, 122°03'41"W) (Fig. 2B); and a 5-m^2 nontidal pool ('Navy Pool') *ca.* 1.0 km north–northeast of the benchmark where Waterfront Road crosses Hastings Slough, Concord Detachment, (38°02'57"N, 122°02'59"W). Snails were found to persist and successfully recruit at these sites over a three-year period, suggesting that their populations are well established (Davis, 2004). In 2005, *L. monroensis* was coincidentally
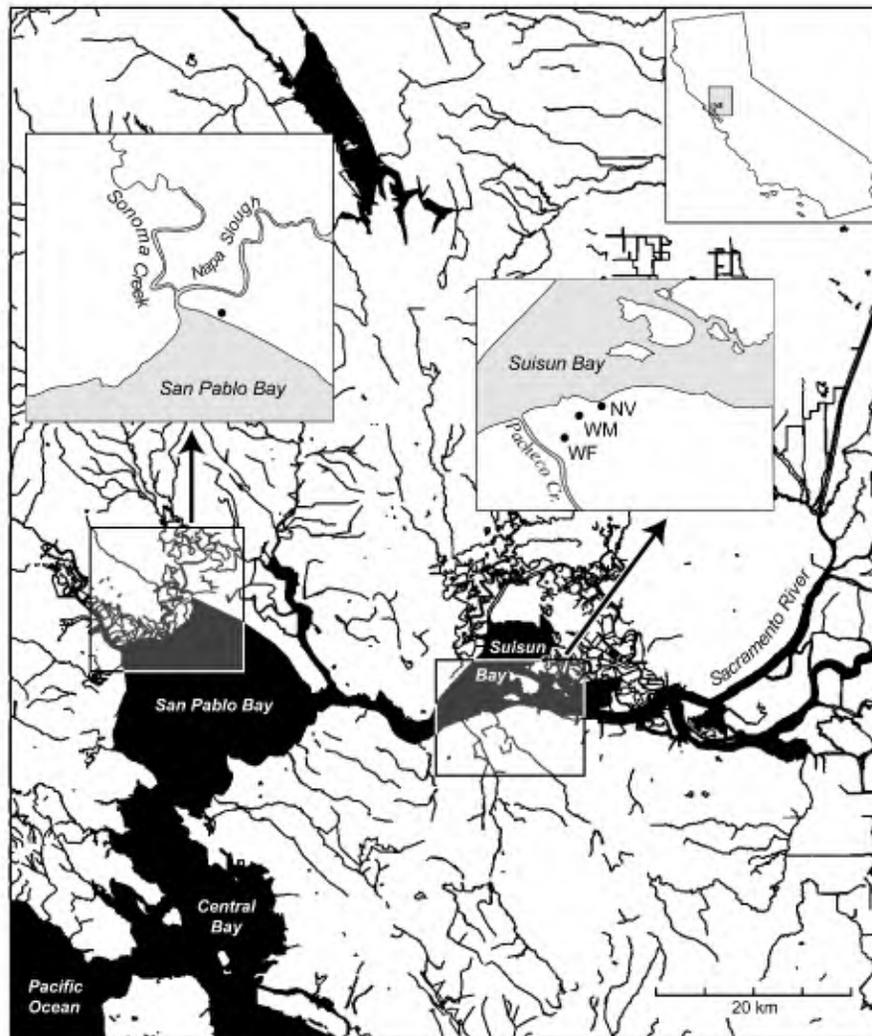
Figure 1. Drainage map of portion of San Francisco Estuary showing sampling localities. Abbreviations: NV, 'Navy Pool'; WF, 'Waterfront Marsh'; WM, 'Weir Marsh'.

discovered in a marsh along the northern shore of San Pablo Bay (Fig. 1), about 30 km distant from Point Edith Marsh. This locality is on Island No. 1 east of the confluence of Sonoma Creek and Napa Slough, alongside Skaggs Island Road just north of the intersection with CA Hwy 37, San Pablo National Wildlife Refuge (38°08′59″N, 122°22′49″W) (Fig. 2C). Salinity was not measured at this site, but is likely to be close to the ~15 ppt documented for nearby waters of San Pablo Bay (CLK, unpublished). All four collecting localities are in wetland areas that were reclaimed in the early part of the last century by construction of dikes and levees and later restored to tidal action by breaching of these structures.

Snails were collected by CLD using a small sieve or dip net and either directly preserved in 90% ethanol for molecular study; or relaxed with menthol crystals, fixed in dilute formalin and preserved in 70% ethanol for morphological study. Specimens were identified to species based on examination of shells (and opercula) using light microscopy and a scanning electron microscope and dissection of diagnostic aspects of soft part anatomy (e.g. penis and female oviduct). Identifications were also facilitated by comparison with relevant museum material and study of pertinent taxonomic literature. Both of these snails have been previously treated in detail (*L. monroensis,*

Thompson, 1968; *T. porrecta*, Hershler, 2001) and thus we provide only brief descriptions to help facilitate identification of the Estuary populations. Institutional abbreviations: Florida Museum of Natural History (UF); National Museum of Natural History, Smithsonian Institution (USNM).

Genomic DNA was isolated from individual snails using a CTAB protocol (Bucklin, 1992). The mitochondrial cytochrome *c* oxidase subunit I (COI) was amplified using primers COIL1490 and COIH2198 (Folmer *et al.*, 1994) via PCR. The amplification and cycle sequencing conditions were described in Liu, Hershler & Clift (2003). New sequences (only one sequence per haplotype per locality) were deposited in GenBank.

Two specimens from 'Waterfront Marsh' identified as *L. monroensis* were sequenced and compared to sequences from two Atlantic Coastal populations of this species; two of the other three described species in this genus, *L. palustris* Thompson, 1968 and *L. tenuipes* (Couper in Haldeman, 1844); undescribed *Littoridinops* from the Gulf Coast and six species that are closely related to *Littoridinops*: *Juturnia coahuilae* (Taylor, 1966), *J. kosteri* (Taylor, 1987), *J. tularosae* Hershler, Liu & Stockwell, 2002, *Pyrgophorus platyrachis* Thompson, 1968, *Spurwinkia salsa* (Pilsbry, 1905) and *Tryonia clathrata* Stimpson, 1865 (Hershler

Figure 2. Photographs of San Francisco Estuary sampling localities. **A.** 'Waterfront Marsh'. **B.** 'Weir Marsh'. **C.** Marsh, San Pablo Bay.

Table 1. Specimens used for molecular analysis of Estuary *Littoridinops monroensis* and relatives.

Species	Location	GenBank accession number(s)
L. monroensis[*]	Contra Costa Co., California	EF490565
L. monroensis	Dorchester Co, Maryland	AF367644
L. monroensis	Saint Johns Co., Florida	AF129323
L. palustris	Levy Co., Florida	AF129324
L. tenuipes[†]	Cumberland Co., Maine	EF490566, EF490567
L. sp.[‡]	Jackson Co., Mississippi	AF129328
L. sp.[§]	Tabasco, Mexico	EF490568, EF490569, EF490570
Juturnia coahuilae	Coahuila, Mexico	AF354761
J. kosteri	Chaves Co., New Mexico	AF129310
J. tularosae	Sierra Co., New Mexico	AF474371
Pyrgulopsis platyrachis[‡]	Hillsborough Co., Florida	AF129327, AF367632
Spurwinkia salsa	Cumberland Co., Maine	AF354765
Tryonia clathrata	Clark Co., Nevada	AF061767

[*]Sequenced for this study ('Waterfront Marsh', USNM 1087202). [†]Sequenced for this study (Harvey Brook, Freeport, USNM 1094132). [‡]Incorrectly identified as *Pyrgophorus* sp. in Hershler *et al.* (1999) and subsequent papers in which these sequences were utilized. [§]Sequenced for this study (Creek entering Laguna Michoacan 8.0 km southeast of Chiltepec, UF 155198).

et al., 2002). Localities and GenBank accessions numbers for these samples are in Table 1. (Additional locality details are available from the first author upon request.) *Tryonia clathrata* was used as the outgroup in the phylogenetic analysis of these data.

Specimens from 'Waterfront Marsh' (USNM 1089588) and 'Weir Marsh' (USNM 1093692) identified as *T. porrecta* were sequenced for the COI gene (three and four individuals from each locality, respectively). These sequences were added to a

previously generated nexus file (Hershler, Mulvey & Liu, 2005) which contained all available *T. porrecta* haplotypes; sequences from 13 other species of *Tryonia*; and a sequence of *Mexipyrgus carranzae* Taylor, 1996, which was used as the outgroup.

MrModeltest2.2 (Nylander, 2004) was used to obtain the most appropriate substitution model (using the Akaike Information Criterion) for Bayesian analyses of the COI datasets, which were performed using MrBayes 3.04 (Huelsenbeck & Ronquist, 2001). In the Bayesian approaches, three short runs were first conducted using the default random tree option to determine when the log likelihood sum reached a stable value (by plotting the log-likelihood scores of sample points against generation time). Metropolis-coupled Markov chain Monte Carlo simulations were then run with four chains using the model selected via MrModeltest2.2 for 1,000,000 generations and Markov chains were sampled at intervals of 10 generations to obtain 100,000 sample points. The sampled trees with branch lengths were used to generate a 50% majority rule consensus tree with the first 5,000 trees, equal to 50,000 generations, removed to ensure that the chain sampled on a stationary portion.

RESULTS

Taxonomic accounts

Littoridinops monroensis (Frauenfeld, 1863)
(Figs 3,4)

Hydrobia monroensis Frauenfeld, 1863: 1023 [type locality 'Lakeman Rok', Florida; per lectotype designation by Thompson, 1968; locality not identified, possible phonetic misinterpretation of Lake Monroe (Volusia County, Florida), which was also cited by Frauenfeld, 1863]. Full synonymy given by Thompson (1968).

Distribution: Coastal Georgia to Mississippi (Thompson, 1984); Cat Island, South Carolina (Wenner & Beatty, 1988); Chesapeake Bay, Maryland (Davis & McKee, 1989). Bahaman (Hutchinson, 1999) and Texas Gulf Coast (Odé, 1970, 1971; Heard, 1982) records require confirmation.

Estuary material: *Contra Costa County*: USNM 1087203, 'Navy Pool', 21.06.2005 – USNM 1087202, USNM 1087205, 'Waterfront Marsh', 06.07.2005, 08.06.2005 – USNM 1098719, 'Weir Marsh', 20.06.2006. *Solano County*: USNM 1087207, marsh alongside Skaggs Island Road near intersection with CA Hwy 37, 21.07.2005.

Description (of Estuary material): Shell (Fig. 3A–E) conical, apex pointed; maximum height 4.7 mm; whorls 5.5–6.0, slightly convex; sutures shallow. Aperture usually ovate and sharply angled above, sometimes sub-circular in large specimens (Fig. 3D). Inner lip complete; parietal lip usually a thin glaze, sometimes slightly thickened and narrowly disjunct; umbilical lip thin, slightly reflected; outer lip usually thin, sometimes slightly thickened, orthocline, sometimes slightly sinuate. Teleoconch sculptured with collabral growth lines and numerous fine spiral threads. Umbilicus narrow. Periostracum tan or brown. Shell measurements: height 3.6–4.3 mm; width 2.1–2.5 mm; body whorl height 2.5–2.9 mm; body whorl width 2.0–2.2 mm; aperture height 1.5–1.8 mm; aperture width 1.1–1.4 mm; shell height/width 1.68–1.85; aperture height/width 2.24–2.45; number of whorls/shell height 1.33–1.51 (USNM 1087202, $n = 14$).

Females oviparous. Penis (Fig. 4) large, narrow, ornamented with distal papilla on inner edge and numerous (21, 27, two specimens examined) papillae along outer edge that are arranged in three rows. Distal end of penis darkly pigmented, penial duct opening through large papilla.

Taxonomic remarks: This snail conforms to *Littoridinops* in having a relatively flat-whorled, conical shell with shallow sutures and weak spiral striations (Thompson, 1984). It also has the penial ornament (Thompson, 1968, 1984) and female reproductive groundplan (observed in dissection) (Hershler & Thompson, 1992) that are characteristic of this genus. *Littoridinops* includes well three described species that are difficult to distinguish by their closely similar and rather variable shells, but are readily identifiable by the arrangement of papillae on the penis (Thompson, 1968). (A fourth congener, *L. tampicoensis* [Pilsbry & Ferriss, 1907], is known only from river-drift shells.) Specimens from the Estuary populations agree with Thompson's (1968, 1984) description of *L. monroensis* in lacking a basal series of papillae near the inner edge of the penis, having relatively numerous papillae on the outer edge that are arranged in more than two rows and having a straight to slightly convex parietal margin of the operculum (Fig. 3F) (Thompson, 1968, 1984).

Comments: The Estuary populations conform to the 'brackish water variety' of *L. monroensis*, which has a relatively thick shell and a continuous inner lip across the parietal region (Thompson, 1968). *Littoridinops monroensis* is readily distinguished from *Tryonia porrecta* (discussed below), with which it co-occurs in the Estuary, by its broader, smoother shell; papillate shape of the distal end of the penis and different pattern of penial ornamentation.
Littoridinops monroensis was typically found in the Estuary on submerged vegetation in channels (rare specimens were collected from soft sediments) and was less abundant than *T. porrecta*. These brackish marsh habitats are typical of those occupied by this species elsewhere (Thompson, 1968; Davis & McKee, 1989; Wenner & Beatty, 1988).

Tryonia porrecta (Mighels, 1845)
(Figs 5,6)

Paludina porrecta Mighels, 1845: 22 (type locality, Oahu [Hawaii]). Full synonymy given by Hershler (2001).

Distribution: Lower Colorado River region (Salton Basin, California; Colorado River delta, Sonora, Mexico), Great Basin (Long Valley, California; eastern Lahontan Basin, Nevada; Bonneville Basin, Utah); Hawaii (Holocene only) (Hershler, 2001).

California material: *Contra Costa County*: USNM 1087388, 'Navy Pool', 21.06.2005 – USNM 1087206, USNM 1090038, USNM 1089588, 'Waterfront Marsh', 08.06.2005, 26.03.2006, 27.03.2006 – USNM 1087204, USNM 1093692, 'Weir Marsh', 21.06.2005, 20.06.2006 – USNM 1100180, 'Weir Marsh', from sediment core, 28.10.2003 (CLK coll.).

Description: Female shell (Fig. 5A–D) narrow conical or turriform; apex blunt, often eroded; maximum height 5.5 mm; whorls 5.5–6.5, slightly to medium convex, often having subsutural angulation; sutures shallow. Aperture narrowly ovate, slightly angled and sometimes thickened above. Inner lip complete; parietal lip usually slightly thickened, less frequently a thin glaze, broadly adnate or, in larger specimens, narrowly disjunct; umbilical lip thin, slightly reflected; outer lip thin, prosocline. Teleoconch sculptured with well-developed collabral growth lines and widely spaced spiral lirae or cords (about 7–10 on penultimate whorl), spiral sculpture usually weaker on body whorl. Umbilicus usually rimate, sometimes absent. Periostracum light tan, shell often covered with thick brown deposits. Shell measurements: height 4.3–5.5 mm; width 2.0–2.4 mm; body whorl height 2.5–3.0 mm; body whorl width 1.8–2.2 mm; aperture height 1.5–1.7 mm; aperture width 1.1–1.4 mm; shell height/width 2.08–2.47; aperture height/width 2.72–3.25; number of whorls/shell height 1.18–1.43 (USNM 1087204, $n = 20$).
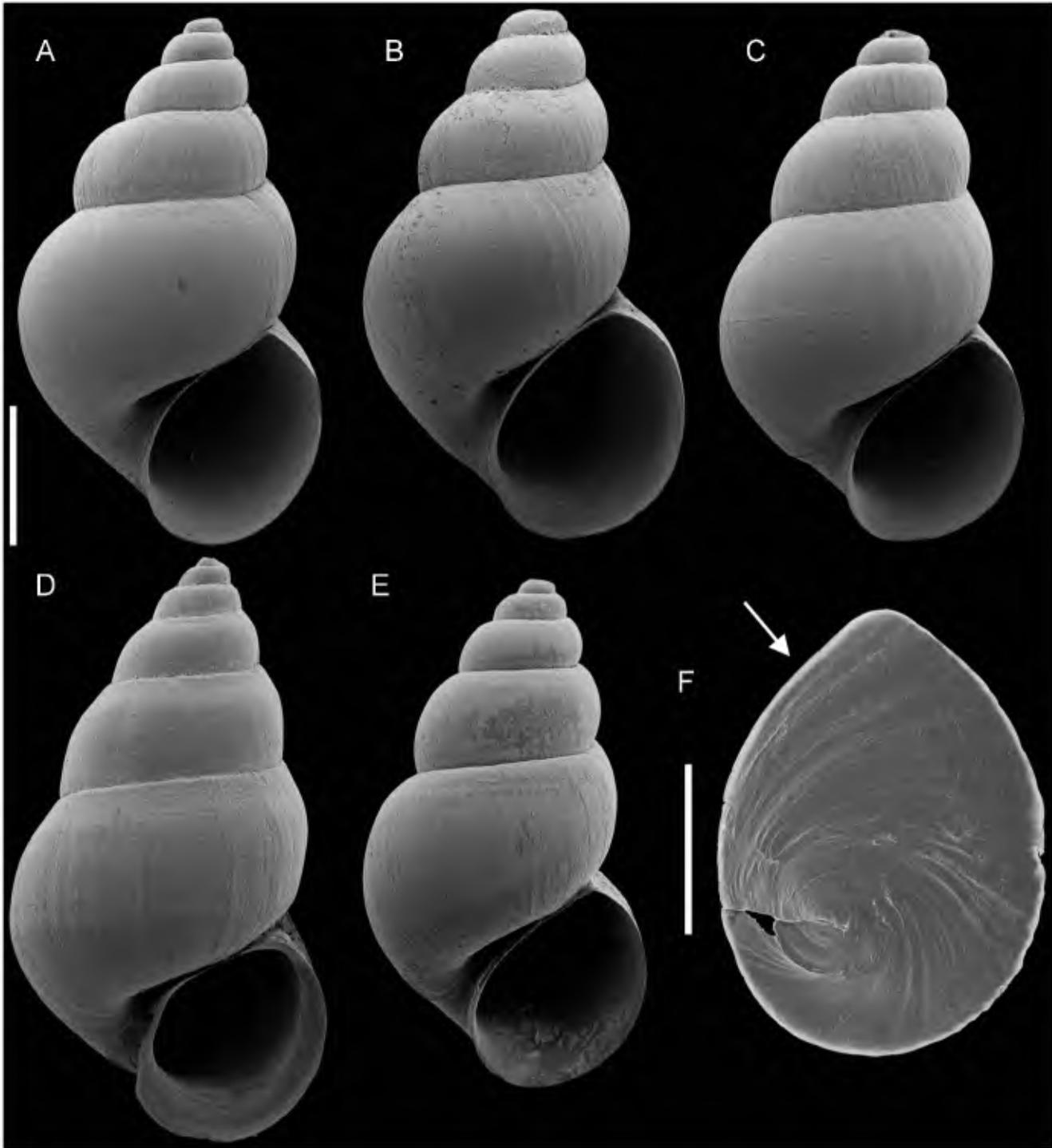
Figure 3. Scanning electron micrographs of shells and opercula of *Littoridinops monroensis*. **A–D.** Shells, 'Waterfront Marsh', USNM 1087202. **E.** Shell, marsh, San Pablo Bay, USNM 1087207. **F.** Opercula (outer side), 'Weir Marsh', USNM 1098719. Arrow points to parietal edge of opercula. Scale bars **A** = 1.0 mm; **F** = 300 μm. **B–E** printed to same scale as **A.**

Male shell (Fig. 5E, F) much smaller than that of females (maximum height about 2.8 mm; whorls 3.50–4.75) and further differentiated by its conical shape, typically more rounded whorls, weaker spiral sculpture and wider aperture. Shell measurements: height 1.7–2.8 mm; width 1.0–1.5 mm; body whorl height 1.2–1.9 mm; body whorl width 0.92–1.4 mm; aperture height 0.75–1.2 mm; aperture width 0.64–0.86 mm; shell height/width 1.46–1.85; aperture height/width 1.16–1.38; number of whorls/shell height 1.67–2.19 (USNM 1090038, $n = 10$).

Females ovoviviparous. Penis (Fig. 6) very large, elongate; inner edge ornamented with two distal papillae (a third, weak unit sometimes present centrally) and a nub-like papilla near base; outer edge having large papilla originating from upper
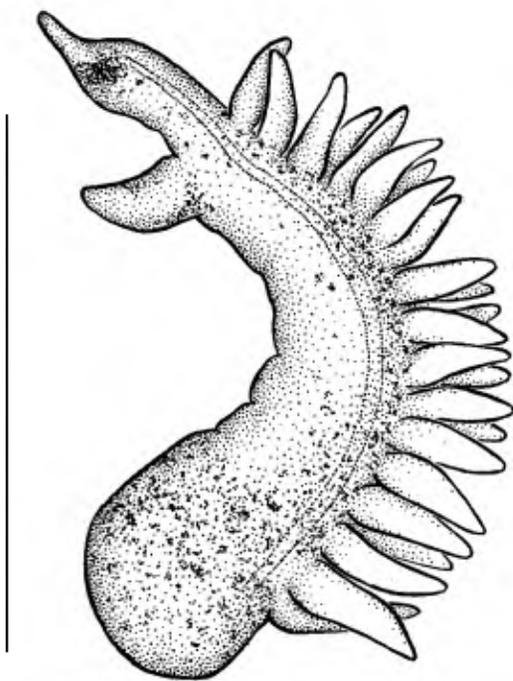
Figure 4. Penis of *Littoridinops monroensis*, 'Waterfront Marsh', USNM 1087205. Scale bar = 500 μm.

surface near base. Distal end of penis blunt, darkly pigmented, having well developed swelling along inner edge (Ds); penial duct opening through small stylet (St).

Taxonomic remarks: This snail is assignable to *Tryonia* based on the postero-dorsal insertion of the visceral vas deferens into the prostate gland (observed in dissection), a diagnostic feature of the genus (Hershler, 2001). It also conforms to *Tryonia* in having a narrow shell, glandular papillae on the penis, ovoviviparous mode of reproduction and a coiled sperm duct that opens to the bursal duct behind the posterior wall of the pallial cavity (observed in dissection) (Hershler, 2001). *Tryonia* contains 18 described species that are distributed in the Rio Grande basin and western North America (Hershler, 2001). The Estuary populations are referable to *T. porrecta* based on the dark pigmentation of the female seminal receptacle (observed in dissection), which is unique to this species within the genus (Hershler, 2001) and also closely conform to this species in their narrow, well-sculptured shells.

Comments: Males have not been previously reported for *T. porrecta* (Hershler, 2001), which genetically conforms to a parthenogen throughout most of its geographic range, although occasional sexual reproduction was hypothesized for several Utah populations based on allozyme evidence (Hershler *et al.*, 2005). Samples from only one of the Estuary populations contained males ('Waterfront Marsh'), which were somewhat less common than females.

Tryonia porrecta can be distinguished from *T. imitator* (Fig. 5G), which was originally described from the Estuary and may still live there (e.g. Petaluma Slough, Kellogg, 1985; Alviso Salt Ponds, Lonzarich & Smith, 1997), by its narrower, more strongly sculptured shell and less convex shell whorls. These species have a closely similar pattern of penial ornament (compare Fig. 6 with Hershler, 2001, Fig. 26E).

Tryonia porrecta was abundant at the Estuary localities and typically found on mud, with fewer numbers collected from vegetation (primarily *Enteromorpha*). In contrast with

the estuarine habitats of these snails, other populations of *T. porrecta* live in highly mineralized (1,760–9,500 μmho/cm), thermal (22–28°C) springs (Hershler, 1994; Hershler & Sada, 2000).

Molecular analyses

Littoridinops monroensis. MrModeltest2.2 selected the Hasegawa–Kishino–Yano (HKY) model (Hasegawa, Kishino & Yano, 1985), with some sites assumed to be invariable and with variable sites assumed to follow a discrete gamma distribution (e.g. HKY + I + G), as the best fit for the dataset using the Akaike Information Criterion. In the Bayesian tree (Fig. 7), Estuary specimens identified as *L. monroensis* (WF-1A, WF-1B) clustered with two other sequences of this species obtained from GenBank, forming a well-supported clade. The COI haplotype observed in the Estuary snails differs from those documented for Maryland (AF367644) and Florida (AF129323) specimens of *L. monroensis* by 1 and 3 bp, respectively.

Tryonia porrecta. MrModeltest2.2 selected the General Time Reversible (GTR) model (Tavaré, 1986), with variable sites assumed to follow a discrete gamma distribution (e.g. GTR + G), as the best fit for the dataset using the Akaike Information Criterion. In the Bayesian tree (Fig. 8), specimens from two Estuary samples (WF-A, WF-C, WF-D, WM-A, WM-B, WM-C, WM-D) were positioned in the *T. porrecta* clade as members of a well-supported subunit that also contained haplotypes from Utah (Horseshoe Springs, South Springs) and Nevada (Hualapai Flat) populations. The seven sequences obtained from Estuary samples of *T. porrecta* had an identical COI haplotype, which differs from all other haplotypes documented for this species by 2–17 bp. The Estuary haplotype differs from the only haplotype (I) found in other California populations (and that of the Colorado River delta) by 15 bp and is most closely similar to haplotypes observed in the Horseshoe Springs (Utah) (VI, 2 bp difference) and Hualapai Flat (Nevada) (VII, 3 bp difference) populations (Table 2).

DISCUSSION

The discovery of the broadly disjunct populations documented herein has interesting implications as *Littoridinops monroensis* has become the first cochliopid species to be found on both sides of the North American continental divide and *Tryonia porrecta* is now the first western American gastropod that has been recorded from thermal spring and cold estuarine habitats. However, our discoveries were made in a highly disturbed ecosystem (Nichols *et al.*, 1986) that has been heavily invaded by exotic biota (Cohen & Carlton, 1995), which suggests that these intriguing range extensions may be the product of recent introductions rather than natural biogeographic processes.

In the case of *L. monroensis*, the evidence for introduced status of its Estuary populations is very strong. This predominantly brackish water species has a well-established native range along the western Atlantic and eastern Gulf Coasts (Thompson, 1968) 3,000 km (airline distance) from the Estuary. Estuary specimens of this snail differ from those of Florida and Maryland by only 1–3 bp (<0.5%) for the COI gene, which suggests recent divergence. (Note that sampling of this species has been very limited and a more comprehensive genetic survey may reveal haplotype sharing between Estuary and eastern North American populations.) It is highly unlikely that the Estuary populations were founded by recent dispersal from eastern North America given the intervening barriers provided by the continental divide and other major geographic features. A vicariant origin of the Estuary populations is also highly improbable because severance of an ancestral coastal distribution extending
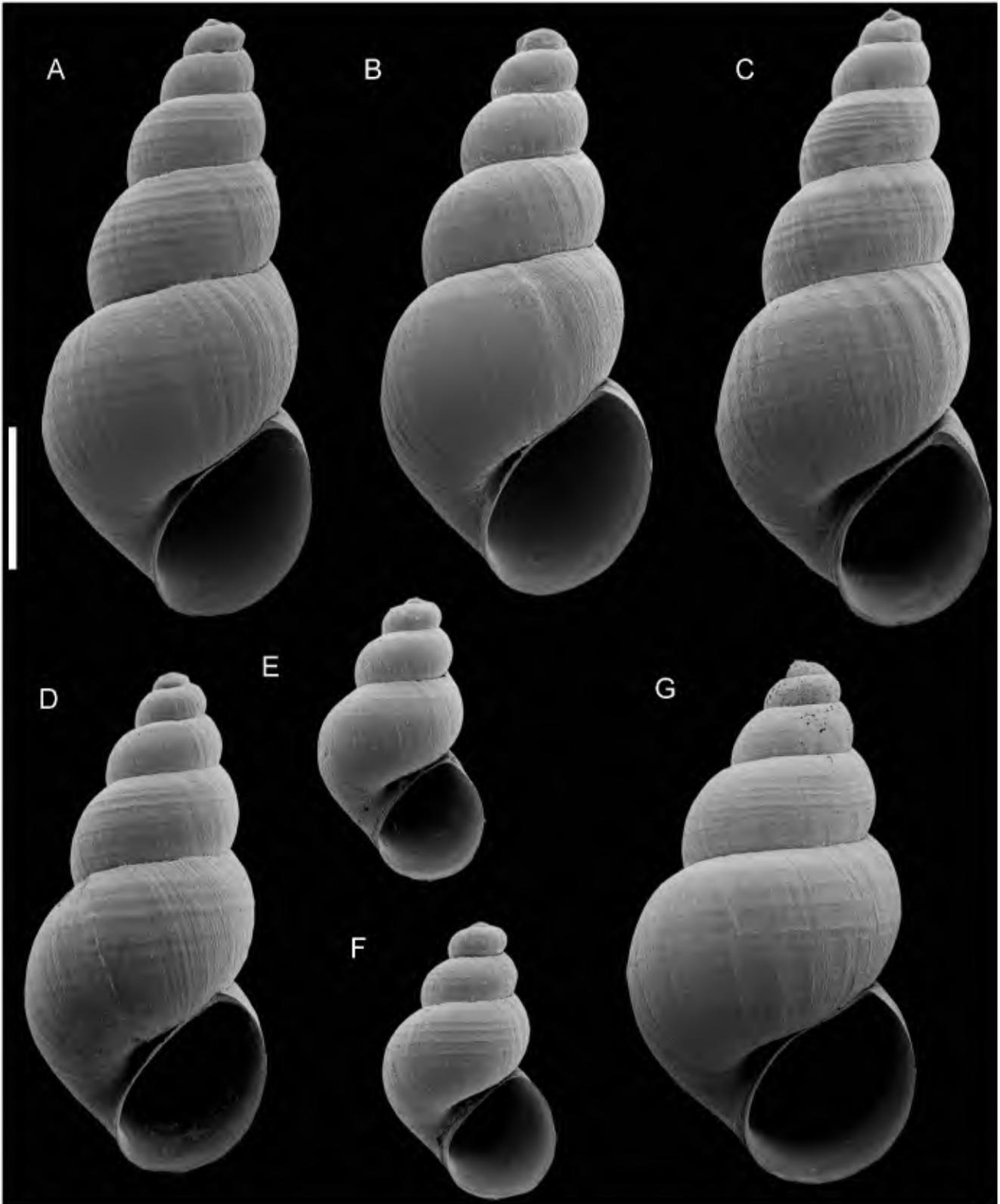
Figure 5. Shells of *Tryonia* species. **A, B.** *T. porrecta*, 'Weir Marsh', USNM 1087204. **C.** *T. porrecta*, 'Weir Marsh', from sediment core, USNM 1100180. **D–F.** *T. porrecta*, 'Waterfront Marsh', USNM 1090038 (**D**, female; **E**, **F**, males). **G.** *T. imitator*, Morro Bay, San Luis Obispo Co., CA, USNM 892057. Scale bar = 1.0 mm.

through the prior Central American Seaway would have occurred 3.1–2.8 Ma upon the rise of the Isthmus of Panama (Coates & Obando, 1996), implying much larger genetic divergence than we have observed. Furthermore, we consider it unlikely that *L. monroensis* formerly ranged in this manner given its broadly disjunct contemporary distribution and the
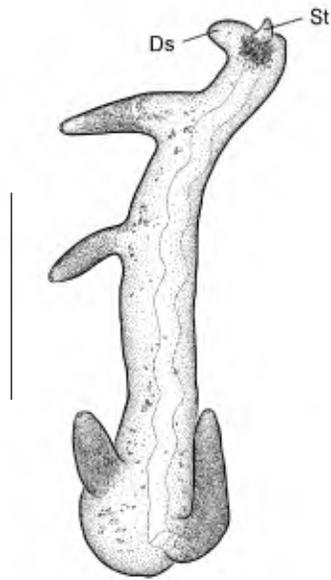
Figure 6. Penis of *Tryonia porrecta*, 'Waterfront Marsh', USNM 1090038. Abbreviations: Ds, distal swelling; St, stylet. Scale bar = 500 μm.
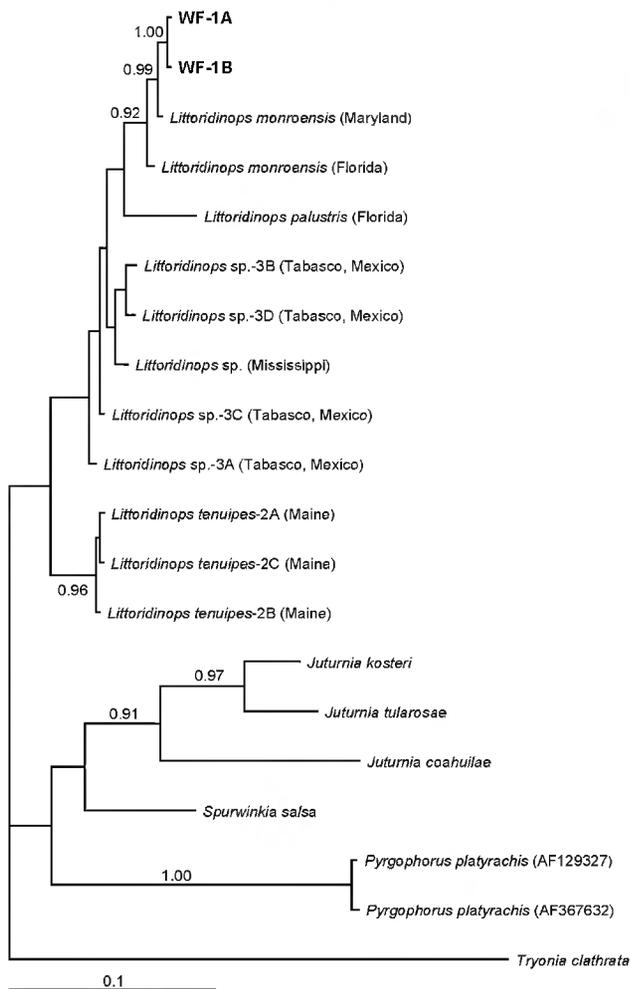

Figure 7. Bayesian tree based on COI sequences showing relationships among populations of *Littoridinops monroensis*, other congeners and closely related cochliopids. Estuary specimens of *L. monroensis* are highlighted by boldfaced type. Posterior probability values ≥90% are shown.
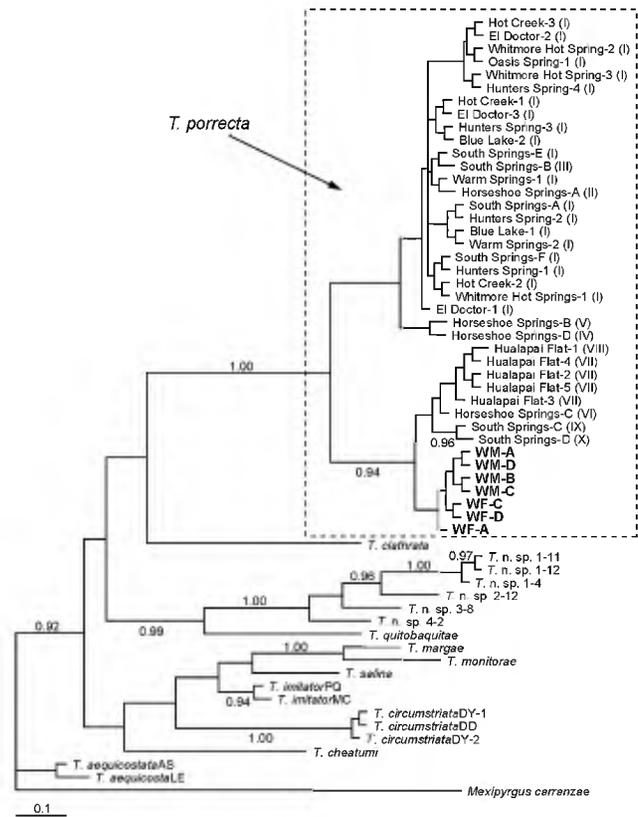

Figure 8. Bayesian tree based on COI sequences showing relationships among populations of *Tryonia porrecta* and closely related congeners. *Tryonia porrecta* haplotypes (I-X, Table 2) are indicated in parentheses following specimen codes. Estuary specimens of this species are highlighted by boldfaced type. Posterior probability values ≥90% are shown.

occurrence of other *Littoridinops* lineages in intervening Gulf Coastal waters (Fig. 7, Mississippi and Mexico specimens). In light of these details, we conclude that *L. monroensis* was recently introduced to the Estuary from the Atlantic or Gulf Coasts, joining a long list of local invasive species that originated from this region (Cohen & Carlton, 1995). Shipping would seem the most likely mechanism of introduction and given that that oviparous *L. monroensis* lacks a pelagic larval stage (Heard, Overstreet & Foster, 2002), we speculate that snails or egg capsules were transported in residual ballast sediment. (Note that a substantial portion of the exotic biota of the Estuary was apparently introduced by this mechanism; Cohen, 1998.) This hypothesis is supported by the close proximity of the Suisun Bay populations to a major naval shipping depot (Detachment Concord, formerly known as Port Chicago), which has been active since 1942 (NWSSB, 2007).

The status of *T. porrecta* in the Estuary is less clear because its native range has not been well established and its typically parthenogenetic mode of reproduction may facilitate a pattern of natural dispersal that cannot be readily distinguished from anthropogenic spread. This species has a scattered distribution in several parts of the Great Basin and lower Colorado River region (Hershler, 2001). The most closely proximal populations to the Estuary live 250 km distant in Owens Valley (western Great Basin), while the only other coastal colony lives in the Colorado River delta at the head of the Gulf of California. The California and lower Colorado River populations have been extensively surveyed using allozyme and mitochondrial DNA markers and were found to be

Table 2. COI haplotypes in *Tryonia porrecta* populations.*

Haplotype	9	20	47	56	63	110	167	176	183	206	207	248	299	306	335	342	348	358	364	375	423	437	555	569	653
I	T	G	G	A	G	T	G	G	G	G	C	G	G	A	T	C	A	C	C	A	T	T	G	C	T
II	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
III	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.
IV	.	.	A	G	.	.	.	.	.	.	.	A	.	.	.	.	G	.	.	.	.	.	.	.	.
V	.	.	.	G	.	.	.	.	.	.	.	A	.	.	.	.	G	.	G	.	.	.	.	.	.
VI	C	.	.	.	A	C	A	.	.	.	.	.	A	.	C	T	G	T	.	G	C	.	.	T	C
Estuary	C	A	.	.	A	C	A	.	.	A	.	.	A	.	C	T	G	T	.	G	C	.	.	T	C
VII	C	.	.	.	A	C	A	.	.	.	.	.	A	G	C	T	G	T	.	G	C	.	.	T	C
VIII	C	.	.	.	A	C	A	.	.	.	.	.	A	G	C	T	G	T	.	G	C	.	T	T	C
IX	C	.	.	.	A	C	A	A	.	.	.	.	A	.	C	T	G	T	.	G	C	C	.	T	C
X	C	.	.	.	.	C	A	A	.	.	.	.	A	.	C	T	G	T	.	G	C	C	.	T	C

*Haplotype I is found in California, Utah and Sonora (Mexico) populations; VII and VIII are found in Nevada populations; and II–VI, IX, X are found in Utah populations (Hershler *et al.*, 2005). Numbers designate positions along the 653 bp segment.

homogeneous members of a single clone (Hershler *et al.*, 2005). These populations are thought to have been recently founded by colonists from genetically variable Utah populations (whose diversity may be attributed to occasional or ancestral sexual reproduction), with transport occurring either on birds or through human mediated activities (Hershler *et al.*, 2005). The Estuary snails, which include sexual individuals based on the occurrence of males at one locality (haplotype variation was not observed in our small genetic samples and allozyme variation was not studied), are closely similar in COI sequence to haplotypes observed in Utah and Nevada populations (differing by 2–3 bp, 0.3–0.5%) and substantially divergent relative to the California-lower Colorado River haplotype (15 bp, 2.3%). Several alternative scenarios are suggested by these findings. The Estuary population could have been recently founded by colonists from Nevada or Utah, either 'naturally' (on birds) or through human activities, with the latter perhaps more likely given the large extent of intervening topography. It is also possible that spread occurred in the opposite direction, with sexual Estuary populations providing the founders of the genetically variable Nevada and Utah populations. The native status of Estuary *T. porrecta* implied by this scenario is supported by its unique haplotype (this snail has been well surveyed genetically across its range) and by shells of this species (Fig. 5C) from the base of a 140 cm deep core from the 'Weir Marsh' locality (Davis, 2004) that are estimated to be pre Gold Rush in age (before 1850s) based on the low mercury concentrations (<~0.05 μg/g) in surrounding sediments (J. Andrews, personal communication to CLK, June, 2001; see Hornberger *et al.*, 1999, for relevant background information). (This finding could alternatively be attributed to an older introduction of this species to Suisun Bay.) The possibility that *T. porrecta* is a previously overlooked native of the Estuary is also consistent with its discovery in difficult to access Suisun Bay marshlands that, to our knowledge, had not been sampled for benthic invertebrates prior to our studies. Nonetheless, for the time being we suggest that the Estuary populations of this species be treated as 'cryptogenic' (*fide* Carlton, 1996) pending a more confident assessment of their origin.

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