

Research Article

Introduced *Hemigrapsus oregonensis* (Dana, 1851) formerly colonized an inland Texas salt spring, as now underpinned by COI barcode sequence analysisDarryl L. Felder¹ and Amanda M. Windsor^{1,2,*}¹Department of Biology, University of Louisiana – Lafayette, Louisiana 70504, USA²National Museum of Natural History, Smithsonian Institution, Washington, DC 20013, USAAuthor e-mails: amwindsor@gmail.com (AMW), darryl.felder1@louisiana.edu (DLF)

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Abstract

Described as a grapsoid crab endemic to the Estelline Salt Spring near Estelline, Texas, *Hemigrapsus estellinensis* Creel, 1964 has long been regarded as an enigmatic taxon representing a small, now extinct, population of unexplained origins. At the time of its description from the panhandle region of Texas, the species was acknowledged to be of close relationship to the North American Pacific coast species, *H. oregonensis* (Dana, 1851), from which it was reported to differ in relatively few morphological characters. No hypotheses were offered to explain a potential common lineage, and none seemed plausible given the long historical separation of waterways that might have joined this isolated population with Pacific congeners. We have examined morphology of archived type specimens of *H. estellinensis* and successfully sequenced the DNA barcode region of COI from one of the paratypes. Morphological and molecular genetic comparisons were made to selected congeners. We conclude that the Estelline Salt Spring population represented an introduction of *H. oregonensis*, and that *H. estellinensis* should hereafter be regarded as its junior synonym. Barnacles found in Estelline Salt Spring at the time that the grapsoid crabs were collected are also now known to represent two North American Pacific coast species. Recollections by an elderly ranch hand from Estelline, Texas of the area in the early 1900s lead us to postulate that railway cargos of Pacific coast timber may have been the pathway for these introductions.

Key words: Brachyura, invasion pathway, Pacific North West, taxonomy, synonymy**Introduction**

In the course of a biotic survey of the Texas Panhandle in the early 1960s, Dr. Gordon Creel made the remarkable discovery of living brachyuran crabs inhabiting near-surface perimeter waters of the Estelline Salt Spring on the property of the Mill Iron Ranch in Hall County. In February 1962, 16 specimens were collected, becoming the holotype and paratypes for *Hemigrapsus estellinensis* Creel, 1964, as they will remain cataloged in the Smithsonian Institution, National Museum of Natural History (USNM). The salt spring habitat was at the time evident as a surface pool of about 20 m diameter, with depths reaching to at least 37 m, where the diameter narrowed to an opening from which water flowed at an estimated rate of

11,350 liters per minute, at nearly constant salinity of 43 PSU, and temperature of 18–22 °C. The pool overflow emptied into the Prairie Dog Town Fork of the Red River, though the crabs were not observed to populate this outflow.

Especially intriguing was a brief mention by Creel (1964) that many other invertebrate species occurred in the spring and that these included barnacles. This mention recalls a similar rare case of marine barnacles found in saline waters of an African desert lake and the apparently unresolved debate over whether these animals represented relicts or introductions (Nilsson-Cantell 1948). The barnacles found in north Texas were not alive at the time, but their scars and shells were abundantly distributed over the surfaces of rocks recovered from the spring by SCUBA divers on the same day that the crabs were collected (G. Creel *pers. comm.*). While archived communications from the late Dr. Creel indicated that most of the other invertebrate specimens were provided to (and apparently lost by) intended collaborators, he did fortunately retain one rock bearing barnacle tests, and it was subsequently loaned to DLF for study.

Unfortunately, the crabs and likely most other invertebrates of Estelline Salt Spring were extinct by the time they were reported, as noted by Creel (1964) and as recorded in a more comprehensive but unpublished 1963 Final Report to the U.S. National Science Foundation that he generously shared with DLF. Severe disturbance of the habitat, whether by SCUBA divers massively suspending sediments on the day the crabs were collected, or by U.S. Army Corps of Engineers salt control projects (Creel 1964), has made all subsequent studies of the populations, including the present one, a postmortem review.

Following a visit to Estelline Salt Spring in December 1980, preliminary studies of the provided barnacle shells were conducted along with examinations of the *H. estellinensis* type series and comparisons to congeners in the USNM. This built a case for regarding the Estelline Salt Spring crab and barnacle occurrences as introductions, as was presented publicly and alluded to in an abstract (Felder and Spivey 1981) but never definitively published.

Herewith, previously available evidence for origins of these crustacean populations is reviewed and augmented with new observations, including comparisons of the sequences of the DNA barcode region of COI to the North American Pacific coastal species *Hemigrapsus nudus* (Dana, 1851) and *H. oregonensis* (Dana, 1851), as well to *H. penicillatus* (De Haan, 1835), *H. sanguineus* (De Haan, 1835), and *H. takanoi* Asakura & Watanabe, 2005 from the western Pacific. Our findings, placed in context of recollections by a life-long resident of Estelline, Texas, lead us to postulate a pathway for colonization of Estelline Salt Spring by an introduced marine crab and barnacle species common to the environment from which it likely originated.

Materials and methods

Existing records and resources

Records and material collections were reviewed from a short expedition to Estelline Salt Spring, made by DLF and colleagues December 14–16, 1980. The periphery of the now enclosed pond at the spring's surface was thoroughly searched for macroinvertebrates, and shallow tows were made with a zooplankton net in search of larvae or other evidence of marine invertebrates. SCUBA was used in dives to approximately 25 m depth, searching pocketed walls of the spring for any evidence of crabs, barnacles, or other macrofauna. Near-surface rubble and pilings of a small pier extending into the pond were closely inspected.

A barnacle-fouled rock provided by Dr. Gordon Creel was sectioned to determine its composition. Barnacle tests were closely inspected to recover opercular plates, where present. Semi-intact tests, available opercular plates, and plate micrographs were furnished to Dr. Henry Spivey (Florida State University) for identifications. Following studies, the rock with attached barnacles was returned to Dr. Creel at the Department of Biology, Angelo State University, San Angelo, Texas.

Hemigrapsus morphological studies

The holotype (USNM 107855) and 15 paratypes (USNM 107856) of *H. estellinensis* were used in synoptic studies of selected morphological features alongside representatives of *H. oregonensis* (USNM 18295, 20163, 140774), *H. nudus* (2056, 14802, 14804, 207634, 44500), and *H. penicillatus* (De Haan, 1835) (USNM 62896). Along with sex, the carapace width (cw) of examined and photographed specimens was determined with dial calipers to the nearest 0.1 mm. Color characters for distinction from *H. oregonensis*, while proposed by Creel (1964), were not deemed applicable to materials with varied preservation histories, especially for intertidal populations that are known to vary strikingly in natural pigment patterns (Lamb and Hanby 2005; Jensen 2014; Jensen and Egnotovitch 2015). However, color photographs of preserved specimens were obtained for selected specimens of *H. estellinensis* (Figure 1A–C) and examples of *H. oregonensis* (Figure 1D–F) to show similarities in general habitus and those elements of pigmentation patterns that persisted after long preservation. Comparisons excluded relative separation of the third maxillipeds, relative size of male chelipeds, and length of the fifth pereopod dactyli previously used as characters by Creel (1964), as preliminary examinations determined them to be excessively prone to maturational and ecomorphic variation. While not previously reported in *H. estellinensis*, though commonly used as an apomorphic character for species-level distinctions in other grapsoids (for example Abele 1992), terminal sculpture of the first gonopod (G1) tip was thoroughly examined in paratype males of *H. estellinensis* (Figure 2). For two

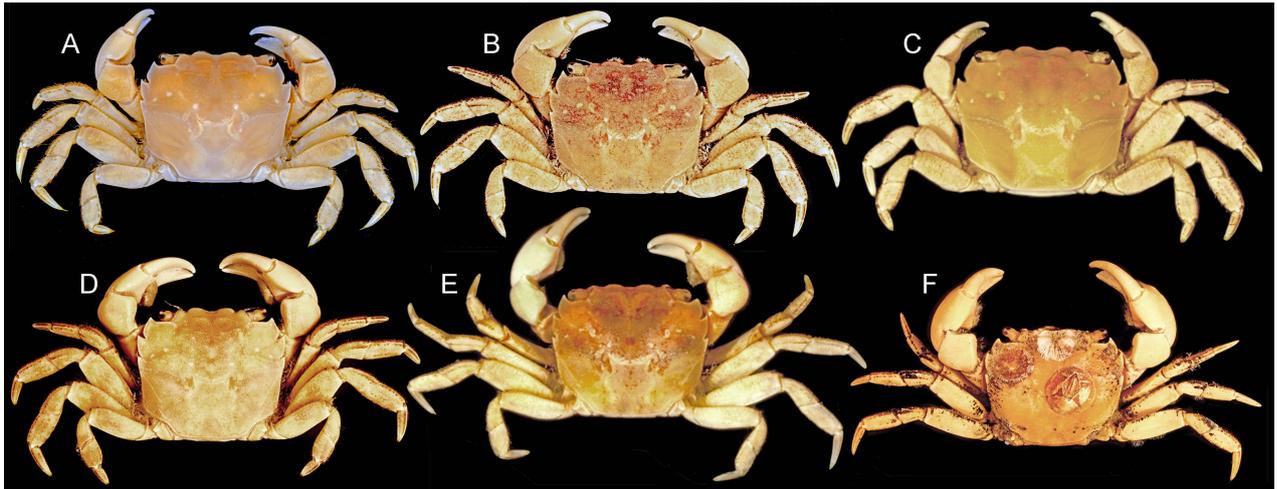


Figure 1. Color photographs of selected alcohol-preserved specimens representing *Hemigrapsus estellinensis* (A–C) and *Hemigrapsus oregonensis* (D–F). A, male holotype, cw 19.9 mm (USNM 107855); B, male paratype, cw 18.0 mm (USNM 107856); C, female paratype, cw 22.0 mm (USNM 107856); D, male, cw 20.4 mm (USNM 20163, Angel Island, California); E, male, cw 16.1 mm (USNM 140774, Baja California, Mexico); F, male with barnacle shells and scars, cw 24.0 mm (USNM 55263, San Francisco Bay, California). Photos by DLF.

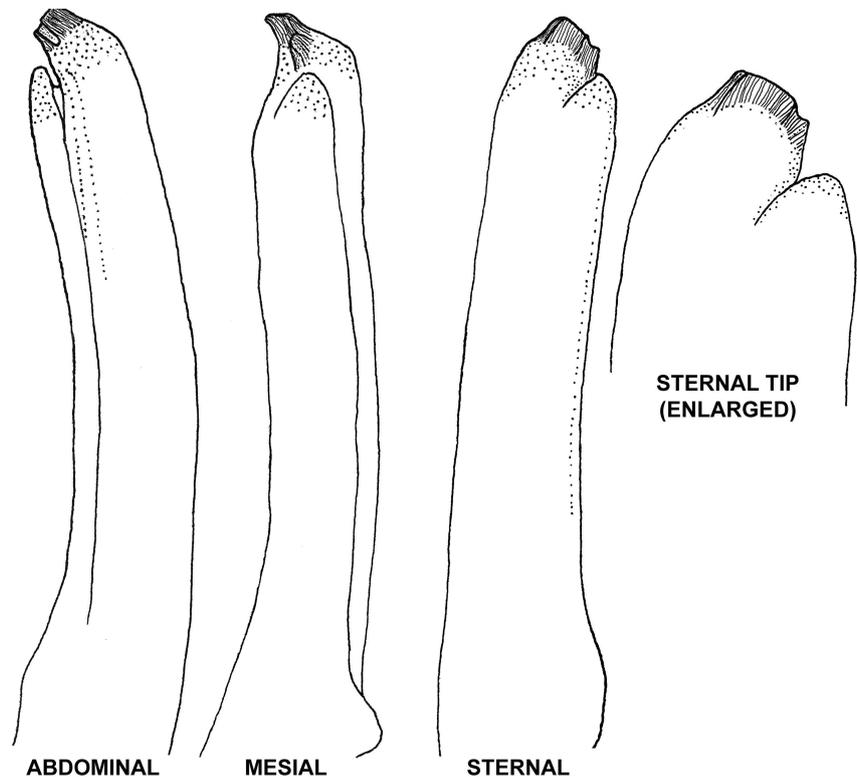


Figure 2. First gonopod tips of *Hemigrapsus estellinensis* male paratype (USNM 107856), cw 18.0 mm, showing abdominal, mesial, and sternal surfaces (setae removed, setal pits shown).

specimens of the paratype series (cw 18.0 and 19.1 mm), the left gonopod was removed and setae of the terminus carefully broken free with a dissecting pin to fully expose the sculptured corneous tip (Figures 2, 3A). For comparisons of this sculpture, the same protocol was repeated on gonopods of four male *H. oregonensis*, four male *H. nudus*, and two male *H. penicillatus* (Figure 3B–D). Typical examples were illustrated under a dissecting microscope equipped with a camera lucida.

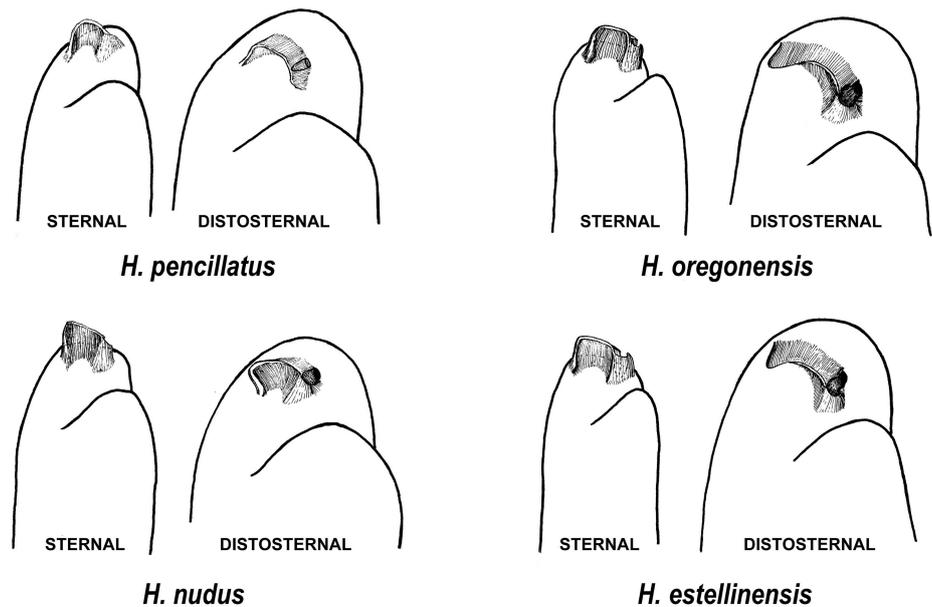


Figure 3. Representative first gonopod tips for A) *H. estellinensis* (USNM 107856, cw 18.0 mm, paratype); B) *H. nudus* (USNM 207834, cw 24.5 mm); C) *H. oregonensis* (USNM 20163, cw 20.4 mm); and D) *Hemigrapsus pencillatus* (USNM 26896, cw 17.7 mm). For each species: left perspective is sternal surface and right is slightly enlarged distosternal surface (setae removed, setal pits not shown).

Molecular genetics

Genomic DNA was extracted from pereopod muscle of one paratype specimen of *H. estellinensis* (USNM 107856) using a phenol chloroform extraction protocol on an Autogen Geneprep 965 automated extractor. The 658 bp barcode region of COI (Hebert et al. 2003) was amplified with the primers JgLCO1490 and JgHCO2189 (Geller et al. 2013). PCR was carried out using Promega GoTaq G2 hot start master mix (Promega M7432) for 45 cycles with an annealing temperature of 42 °C. PCR product was visualized by agarose gel electrophoresis (1.5% agarose) and purified with ExoSAP-IT (Affymetrix) prior to sequencing. Sequencing reactions were performed using 1 µL of purified PCR product in a 10 µL reaction containing 0.5 µL primer, 1.75 µL Big Dye buffer and 0.5 µL Big Dye (Life Technologies) and sequenced on an ABI 3730XL automated sequencer.

Geneious 9.1.8 (Biomatters) was used to visualize, trim, edit, and assemble a contig from forward and reverse sequences. This sequence was aligned to previously archived GenBank sequences of comparable length for a selection of the most divergent COI sequences available for North American Pacific specimens of *H. oregonensis* and four congeners *H. nudus*, *H. penicillatus*, *H. sanguineus*, and *H. takanoi* (Table 1). An unrooted neighbor joining dendrogram with 1000 bootstrap pseudoreplicates was constructed in Geneious to visualize this alignment (Figure 4). All laboratory work was carried out in the Laboratories of Analytical Biology at the Smithsonian Institution.

Table 1. GenBank accession numbers for COI sequences used in analysis.

| Taxon Name | GenBank Accession | Specimen Origin |
|----------------------------------|-----------------------|---------------------------|
| <i>Hemigrapsus estellinensis</i> | MN711692* | Estelline Spring, TX, USA |
| <i>Hemigrapsus oregonensis</i> | KX039688 ^a | BC, Canada |
| <i>Hemigrapsus oregonensis</i> | KX039710 ^a | BC, Canada |
| <i>Hemigrapsus oregonensis</i> | KX039691 ^a | BC, Canada |
| <i>Hemigrapsus oregonensis</i> | KX039718 ^a | BC, Canada |
| <i>Hemigrapsus oregonensis</i> | KX039700 ^a | BC, Canada |
| <i>Hemigrapsus oregonensis</i> | MG936338 [†] | BC, Canada |
| <i>Hemigrapsus oregonensis</i> | MG936586 [†] | BC, Canada |
| <i>Hemigrapsus nudus</i> | KM611510 [†] | BC, Canada |
| <i>Hemigrapsus nudus</i> | KM611726 [†] | BC, Canada |
| <i>Hemigrapsus nudus</i> | KM611732 [†] | BC, Canada |
| <i>Hemigrapsus nudus</i> | MG318135 [†] | BC, Canada |
| <i>Hemigrapsus penicillatus</i> | KC771068 ^c | Aomori, Japan |
| <i>Hemigrapsus penicillatus</i> | KC771069 ^c | Aomori, Japan |
| <i>Hemigrapsus penicillatus</i> | KC771070 ^c | Aomori, Japan |
| <i>Hemigrapsus takanoi</i> | KC771062 ^c | Aomori, Japan |
| <i>Hemigrapsus takanoi</i> | KC771064 ^c | Aomori, Japan |
| <i>Hemigrapsus takanoi</i> | KC771065 ^c | Aomori, Japan |
| <i>Hemigrapsus takanoi</i> | KT208924 ^b | North Sea, Germany |
| <i>Hemigrapsus sanguineus</i> | KT208564 ^b | North Sea, Germany |
| <i>Hemigrapsus sanguineus</i> | KT208665 ^b | North Sea, Germany |
| <i>Hemigrapsus sanguineus</i> | KT307116 ^b | ME, USA |

* This study, ^a Castelin et al. 2016, ^b Raupach et al. 2015, ^c Markert et al. 2014, [†] unpublished submissions

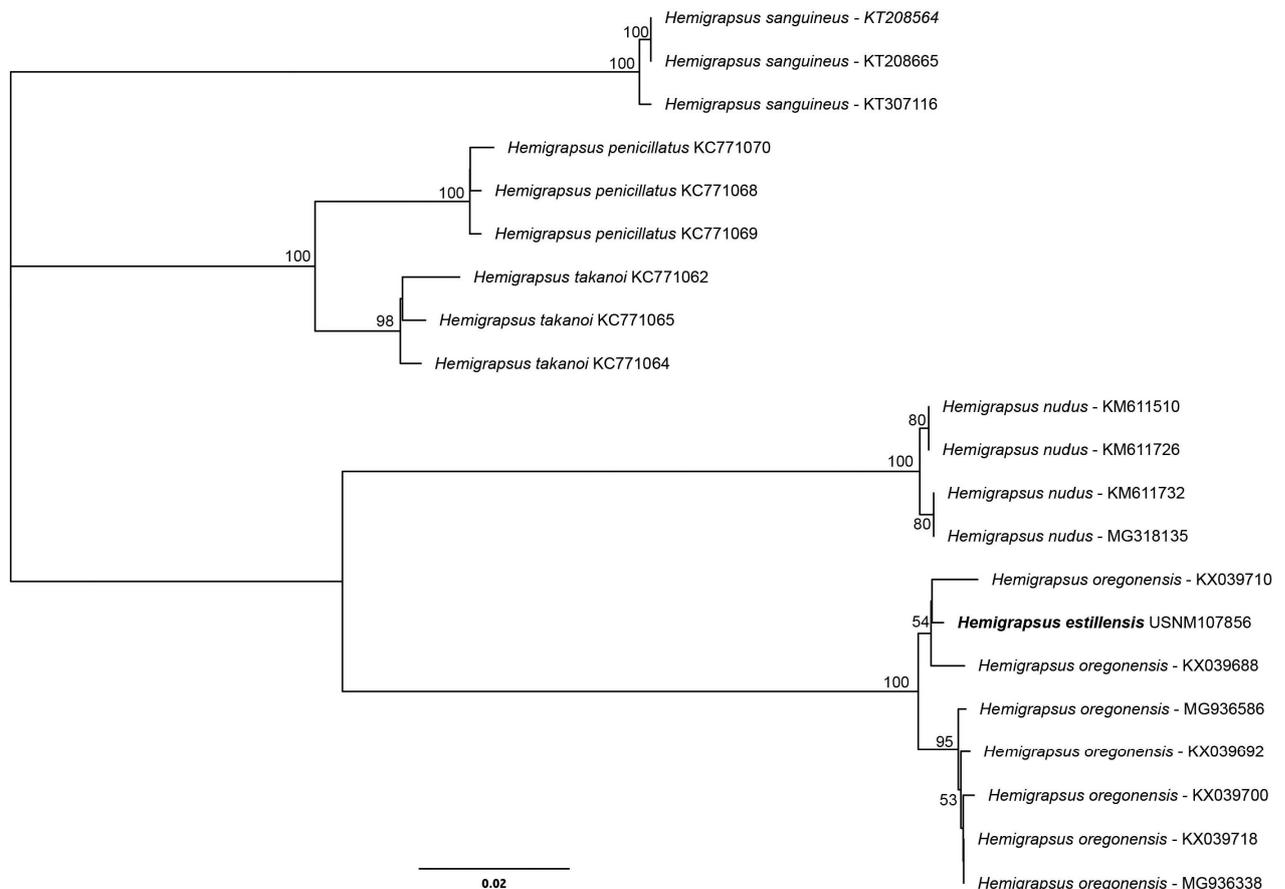


Figure 4. Neighbor joining dendrogram with bootstrap support values illustrating an alignment of five species of *Hemigrapsus*, including a selection of the most divergent COI sequences available in GenBank for North American Pacific specimens of *H. oregonensis*, and the sequence generated herein from a paratype of *H. estellinensis* (USNM107856).

Results

Previously unreported field and laboratory observations

In December 1980, Estelline Salt Spring was found by DLF and colleagues to be largely confined within a circular elevated dike that had been constructed by the U.S. Army Corps of Engineers to create a pond forming a pressure head curtailing the outflow of saline water. No flow or circulation of water was detected during SCUBA dives to a maximum of about 25 m, these dives being terminated by safety concerns due to instability of wall sediments as the chamber narrowed with pond depth. The cord of a conductivity probe was extended to a maximum depth of about 40 m, and salinity was found to range from 59 psu at depth to 61 psu near the surface. Shallow margins near the pond surface were sparsely populated with salt-tolerant submerged widgeon grass, *Ruppia maritima* L., common in regional inland waters. No evidence of live or dead crabs or barnacles was found in the course of SCUBA dives, searches of the pond periphery, or examinations of hard substrates. Terrestrial isopods, *Porcellio* sp., were common under concrete fragments surrounding the pond margin. Zooplankton tows captured no larvae of crabs or barnacles, though the cosmopolitan harpacticoid copepod *Cletocamptus deitersi* (Richard, 1897) was abundant. Remarkably, the catch of one plankton tow that briefly touched bottom was later found to include a single small (< 3 mm) snail (provisionally identified as *Tryonia?* sp.), slightly misshapen but apparently alive at the time of collection, now archived in the University of Florida Museum of Natural History (UF 546998).

The single rock that Dr. Creel obtained in 1962 was cut with a rock saw by technicians of the UL Lafayette Geology Department and found to be composed of concrete aggregate, consistent with scattered concrete debris that remained at the site in 1980. On the rock surface were found 11 shells of *Chthamalus dalli* Pilsbry, 1916, one with the opercular valves intact, along with 32 shells of *Balanus glandula* Darwin, 1854, five with the opercular valves intact, and 55 additional barnacle scars mostly representing *B. glandula* (Figure 5A, B). The mean basal diameter for those barnacles with valves intact was about 10 mm.

Comparisons of morphological characters

Strikingly similar in general habitus (Figure 1), specimens assigned to *Hemigrapsus estellinensis* and *H. oregonensis* could not be consistently distinguished by morphological features suggested by Creel (1964: 238–239) when comparisons included large series and broad size ranges of *H. oregonensis* in the USNM collections. Just as ranges of carapace measurements and relative male cheliped size overlap in Creel (1964: table 1), relative separation of the third maxillipeds and relative lengths of the fifth pereopod dactyls were not found to be of diagnostic value for separations,

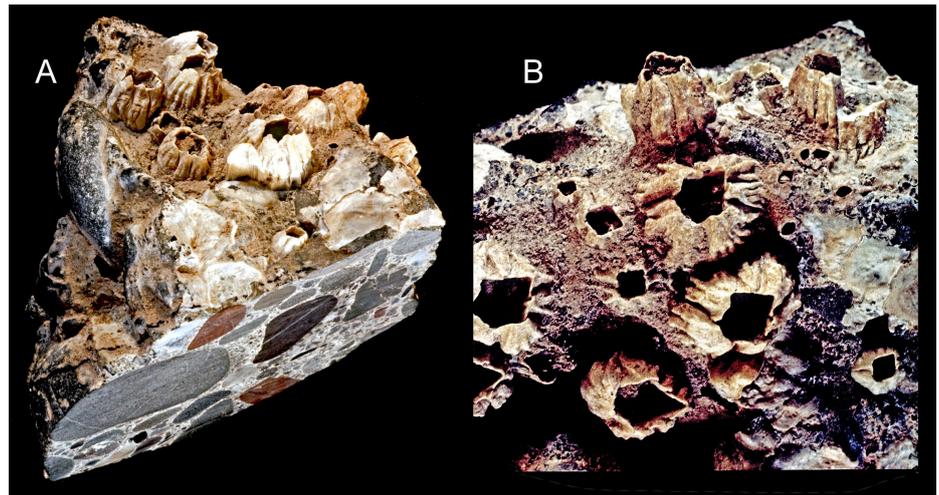


Figure 5. Barnacle shells and scars representing *Chthamalus dalli* and *Balanus glandula* on surfaces of aggregate concrete rock collected by G. Creel from Estelline Salt Spring in 1962. A, barnacles above cut rock surface; B, barnacles on adjacent surface. Photos by DLF.

contrary to suggestions in the original text. Morphology of the first gonopod tips was also strikingly similar in the two ostensibly separate species (Figure 3A, C), both being readily distinguishable in these features from *H. nudus* and *H. penicillatus* (Figure 3B, D).

Mitochondrial gene sequences

The resulting sequence was accessioned to GenBank (MN711692). A BLAST® search of nucleotide records in GenBank revealed our sequence for *H. estellinensis* to be a 99% match to COI sequences of *H. oregonensis* currently deposited in GenBank. Our sequence varied from between 1 and 3 base pairs when compared to the first 30 GenBank search results. Placed in a neighbor joining dendrogram among a selection of the most divergent COI sequences (i.e., those with the highest pairwise distances) available for North American Pacific specimens of *H. oregonensis* (Figure 4), the sequence for *H. estellinensis* fell within the range of population genetic structure evident among Pacific coast populations.

Discussion

For varied species of *Hemigrapsus* in which sequencing of COI presently allows insights into population divergence, all show evidence of population structure (Figure 4). In one case, the western Pacific species, *Hemigrapsus takanoi* was separated from and named as a sibling species of *H. penicillatus* on the basis of color, minor morphological differences, and genetic measures that initially supported limited divergence (Takano et al. 1997; Asakura and Watanabe 2005; Yamasaki et al. 2011; Mingkid et al. 2006). Molecular genetic separation of these populations as species was shown to be well-supported by Markert et al. (2014), a conclusion supported herein (Figure 4). Were a similar level of morphological and genetic divergence to be observed

between subpopulations of *H. oregonensis*, a species widely distributed from Alaska to Baja California, separate taxonomic designations might also be considered for its subclades. Our representation of the most divergent presently available COI sequences (Figure 4) suggests that genetics might infer subpopulation separation, but there is no present evidence that genetic isolation, geography, ecology, reproduction, or other agents are operating to conserve divergent eastern Pacific lineages of *H. oregonensis* at a level worthy of taxonomic status. For the present, our sequence for *H. estellinensis* clearly falls among others for *H. oregonensis*, as presently recognized. We interpret it to represent a founder lineage, introduced to an inland salt spring in northern Texas from the North American Pacific coast.

Genetics and morphology rule out continued recognition of *H. estellinensis* as a relict endemic crab that once populated a north Texas salt spring and require that we relegate this species to the synonymy of *H. oregonensis*. The alternative of *H. estellinensis* being a relict sibling species of *H. oregonensis* would seem untenable for a variety of reasons, including that the genus *Hemigrapsus* Dana, 1851 is not evident in the fossil record prior to the Pliocene and that the earliest records of *H. oregonensis* and *H. nudus* are Pleistocene (Rathbun 1926). Thus, fossil records are from long after closure of seaways that might have provided contiguous habitats for bridging of lineages to anywhere near north Texas salt springs, not to mention that a host of geological and climatic effects following the Pliocene would almost certainly have catastrophically disrupted habitat stability in the Texas Panhandle (Stanley 1999). Finally, even if a common lineage of *Hemigrapsus* were to have existed and been separated by the closure of Central American seaways, sequence divergence at least four times greater than observed (no less than 4%) should be expected, as has been measured for other geminate crustacean species of such origins (Lessios 2008).

The conclusion that *H. estellinensis* represents an introduction from the Pacific is further supported by the apparent co-occurrence in Estelline Salt Spring of two additional crustaceans known to be from the North American Pacific coast, the barnacles *Chthamalus dalli* and *Balanus glandula*. Both are endemic to northeastern Pacific coastal waters, with *C. dalli* the only member of that genus populating the U.S. coastlines north of San Francisco (Chan et al. 2016). While opercular valves vary across the broad latitudinal range of *B. glandula*, morphology of those from Estelline Salt Spring most closely match others near the center of its North American Pacific range in and around Puget Sound, Washington, further narrowing their probable origins (H. Spivey *pers. comm.*). Long after its collection from Estelline Salt Spring, *B. glandula* has become well known for its invasive potential along marine coastlines that presently include at least Belgium, South Africa, Argentina, and Japan (Rashidul Alam et al. 2014; Kerckhof et al. 2018), underscoring its ability to adapt to varied ecological settings when introduced. Among its adaptations, this species is known to

tolerate and reproduce in very high salinity waters (Bergen 1968). Thus, we submit that three west coast marine species were at some point introduced into a small, isolated salt spring in north Texas, and we suggest that a common pathway of introduction could apply to all. While it is also possible that the small snail collected in 1980 could represent a fourth species similarly introduced, we cannot for now definitively affiliate that single and likely immature specimen with a source species or population. Furthermore, it may represent a littoridinine species that is not of marine origins. Just as is known for the widgeon grass, *Ruppia*, that was found in Estelline Salt Spring and is dispersed into other brackish to alkaline inland waters (Phillips 1960), it could have been introduced by coastal waterfowl during migratory stopovers (Miura et al. 2011).

Shortly following field investigations in 1980, one of us (DLF) conducted a lengthy telephone interview with the late R.A. Eddleman, in his mid-80s at the time, regarding his locally reputed knowledge of Estelline Spring over his long history of work as a cowboy and manager on the Mill Iron Ranch. He recollected the site being used heavily in stopovers by migratory waterfowl, including geese, ducks, and swans. He also noted that at one point there were on its margins a small bath house and improvised diving board, and that at least one drowning of a prominent citizen had occurred there. The spring was also rumored to have been used briefly for dropping of dummy bombs in target practice by military aircraft, as well as by a SCUBA diving club based at a nearby Air Force facility in Childress, Texas. However, most provocative, he recollected an old sawmill adjacent to the salt spring, where logs were held in the surface pond, all in close proximity to the Fort Worth and Denver Railway line. Near the turn of the century, this railway provided the first efficient connections for freight transports from the western states and the Pacific U.S. coast into north Texas (Overton 1953, 1976).

While Mr. Eddleman's recollections suggested several possibilities for introductions, none is more compelling than a sawmill on Estelline Salt Spring being fed logs by a railway connected to the U.S. Pacific Northwest. Among the primary rail cargos shipped eastward by the Northern Pacific Railroad from Pacific Northwest coastlines in the early 1900's were timber and lumber, some of these likely untrimmed trunks rafted together for loading along coastal Pacific shorelines, like those of Puget Sound (Renz 1980). By 1920, over 30% of U.S. timber production was from the Pacific northwest (Marchak 1995). Barnacles could have colonized surfaces of rafted logs or crab carapaces, as commonly occurs (e.g., Figure 1F), and the desiccation-tolerant species found in Estelline Salt Spring (Newman 1967; Kozloff 1996), especially under high humidity. They are also well adapted to salinity extremes found in intertidal zones. Likewise, *Hemigrapsus oregonensis* ranges into the intertidal zone and can survive extended periods above water when tucked into cavities, beneath intertidal rocks, or in other damp

confines (MacGinitie 1935; Garth and Abbott 1980; Kozloff 1996; Lamb and Hanby 2005). Such could easily be provided between water-soaked logs or beneath loosened bark and other saturated debris potentially associated with transported timber.

The days to weeks required for rail transport could likely be tolerated by a number of intertidal marine organisms in those settings, especially if the journey were to end in a salty sawmill pond. For *Hemigrapsus*, successful introduction of both males and females would of course be required for the population to reproduce and survive over multiple decades between the early 1900's and 1962. However, this is also plausible as multiple individuals of *H. oregonensis* are known to group in damp cavities or substrate interstices, both adults and juveniles, during tidal exposure (MacGinitie 1935; Garth and Abbott 1980; Lamb and Hanby 2005; Jensen 2014). As for survival and establishment of *H. oregonensis* in a hypersaline spring, the species is known to tolerate varied salinities (Dehnel 1962), with selected populations sometimes adapting to extreme hypersalinity (Garth and Abbott 1980). It is also apparent that the salt spring could have provided both nutrition and salinities of a range adequate for metamorphosis of its larvae (Sulkin et al. 1998). Furthermore, the report by Creel (1964), augmented by his 1963 Final Report to the National Science Foundation, contends that a once diverse marine community existed in the spring, including varied invertebrates and an array of diatoms and other benthic microalgae and phytoplankton. These organisms, larvae of once abundant barnacles, and other opportunistic colonizers such as the aforementioned cosmopolitan harpacticoids, could have provided essential food substrates for larvae of *Hemigrapsus* to survive and metamorphose there.

As noted by Creel (1964), the Mill Iron Ranch foreman first mentioned his observation of several crabs at Estelline Salt Spring in September 1960, and he "did not think it unusual" at the time. One can only guess that their presence there might have been noticed by others and also considered unremarkable over decades preceding the formal report of their discovery. However, it does appear that the crab population was no longer extant by December 1962, 10 months following the only collections made there, and our survey in December 1980 also found no evidence of crabs nor further evidence of live or dead barnacles. Creel (1964) indicated that the normally clear waters of the salt spring were greatly disturbed by SCUBA divers charting the spring on the date he made his collections, perhaps causing hypoxia. However, the U.S. Army Corps of Engineers was also at this time actively working to stop flow of the spring by varied means, with the intent of improving water quality of adjacent river drainages into which the spring flowed. Their efforts included construction of an encircling dike in 1963 that was regarded to have destroyed "a unique habitat, its fauna and flora, ...the Estelline Salt Spring" (G. Creel, Final Report to US National Science Foundation, Grant GB 497, December 31, 1963). The unfortunate

destruction of this biota before it could be more fully studied indeed represents a lost opportunity, regardless of present conclusions that must draw upon the limited remaining evidence of what once lived there.

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