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Genome skimming supports two new crayfish species from the genus *Pacifastacus* Bott, 1950 (Decapoda: Astacidae)

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Abstract

Recent phylogenetic analyses have suggested that the Signal Crayfish, *Pacifastacus leniusculus* (Dana, 1852), contains two highly distinct lineages that merit recognition as species. We further investigate these lineages here using genome skimming to conduct phylogenetic analyses on mitogenomes and highly repetitive 18S, 28S, and H3 nuclear markers. We also analyze morphological characters of these putative species to identify traits that may facilitate their identification in the field. Phylogenetic trees of mitogenomes support these lineages as species in the family Astacidae, and phylogenetic trees based on concatenated nuclear markers return comparable topologies. We describe these crayfishes as the Misfortunate Crayfish, *Pacifastacus malheurensis* **sp. nov.**, which occurs in central and eastern Oregon, United States, and the Okanagan Crayfish, *Pacifastacus okanaganensis* **sp. nov.**, which occurs in south central British Columbia, Canada and north central Washington, United States. Both of these species of *Pacifastacus* face conservation risks from displacement by non-native invasive crayfishes, but *P. malheurensis* **sp. nov.** is especially vulnerable to the rapidly spreading Rusty Crayfish, *Faxonius rusticus* (Girard, 1852), in central Oregon.

Key words: mitogenome, Misfortunate Crayfish, Okanagan Crayfish, *Pacifastacus leniusculus, Pacifastacus malheurensis* sp. nov., *Pacifastacus okanaganensis* sp. nov.

Introduction

The Signal Crayfish, *Pacifastacus leniusculus* (Dana, 1852), is an important decapod crustacean globally, well-known for its negative impacts as an invasive species in Asia (e.g., Nakata & Goshima 2006; Usio *et al.* 2009), Europe (e.g., Galib *et al.* 2021; Robinson *et al.* 2018), and North America (e.g., Light *et al.* 1995; Scordo *et al.* 2023). However, *P. leniusculus* also has cultural and commercial value within its native range in the Pacific Northwest region of the United States (US) and Canada, including substantial harvest for human consumption (Larson & Olden 2011; Meyer-Arendt *et al.* 2020). A complicated taxonomic history likely affects conservation and management of *P. leniusculus* and associated congeners in the Pacific Northwest (Larson & Williams 2015). Miller (1960) recommended recognition of *Pacifastacus klamathensis* (Stimpson, 1857) and *Pacifastacus trowbridgii* (Stimpson,

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1857) as subspecies of *P. leniusculus* based on intermediate morphologies interpreted as hybrids, which was adopted by subsequent taxonomic keys for North America (Hobbs 1972). These three *P. leniusculus* subspecies—*P. l. klamathensis*, *P. l. leniusculus*, and *P. l. trowbridgii*—have been de-emphasized by researchers over the past several decades, likely because well-studied invasive populations in Asia and Europe include limited phylogenetic diversity relative to *P. leniusculus* in its native range (Petrusek *et al.* 2017, Usio *et al.* 2016). However, a tendency to interpret *P. leniusculus* as a uniform, globally cosmopolitan invasive species may leave subspecies, unique lineages, or cryptic species in the native range at risk of population declines or extinctions consistent with other members of the genus *Pacifastacus* (Bouchard 1977; Egly & Larson 2018; Light *et al.* 1995).

Recent work has identified highly distinct lineages of *P. leniusculus* that seemingly merit elevation to species status. Larson *et al.* (2012) used 16S mitochondrial DNA (mtDNA) to identify two highly divergent lineages of *P. leniusculus* that were more distinct from the rest of this species than a within-genus outgroup, the Snake River Pilose Crayfish *Pacifastacus connectens* (Faxon, 1914). Both of these lineages resembled *P. l. klamathensis* by morphology but were geographically isolated from the type locality of *P. l. klamathensis* at Klamath Lake, Oregon (Stimpson 1857). Further, Larson *et al.* (2012) recovered a moderately divergent but monophyletic lineage of *P. leniusculus* from the Klamath River watershed and adjacent coastal rivers of southern Oregon (i.e., the Umpqua River) with the morphology of *P. l. klamathensis*. Accordingly, Larson *et al.* (2012) termed the two non-monophyletic lineages of *P. leniusculus* as the "Central Oregon" and "Okanagan" crayfishes in reference to their geographic ranges. Larson *et al.* (2016) revisited this phylogenetic work by expanding taxonomic coverage across the extant species of *Pacifastacus* and including an additional mtDNA marker (COI) and one nuclear marker (GAPDH). Although GAPDH was relatively uninformative for distinguishing species of *Pacifastacus*, phylogenetic species delimitation on well-supported COI trees continued to identify the Central Oregon and Okanagan crayfishes as distinct species (Larson *et al.* 2016). Conservation concern for these putative species of *Pacifastacus* includes risk of competitive displacement by invasive species including the Rusty Crayfish, *Faxonius rusticus* (Girard, 1852), in central Oregon (Messager & Olden 2018; Olden *et al.* 2009) and the Virile Crayfish, *Faxonius virilis* (Hagen, 1870), in the vicinity of the Okanagan and Thompson plateaus of British Columbia and Washington (Larson *et al.* 2010; Phillips 2024). Further, *P. leniusculus* itself is a threat to congeners where introduced over their populations, as evidenced by the role of this species in the extinction of the Sooty Crayfish, *Pacifastacus nigrescens* (Stimpson, 1857), and the US Endangered Species Act (ESA) listing of the Shasta Crayfish, *Pacifastacus fortis* (Faxon, 1914), in California (Bouchard 1977; Light *et al.* 1995; U.S. Fish and Wildlife Service 1988).

Here, we augment existing phylogenetic information on *Pacifastacus* using genome skimming (Hoban *et al.* 2022), with an emphasis on species status for the Central Oregon and Okanagan lineages identified by Larson *et al.* (2012) and Larson *et al.* (2016). We conduct phylogenetic analyses on whole mitogenomes and highly repetitive 18S, 28S, and H3 nuclear markers recovered from genome skimming (Grandjean *et al.* 2017). We then identify morphological characters that may be useful in the field identification of these crayfishes relative to populations of *P. leniusculus* used in our phylogenetic analyses. Lastly, we describe the Central Oregon lineage as the Misfortunate Crayfish, *Pacifastacus malheurensis* **sp. nov**., and the Okanagan lineage as the Okanagan Crayfish, *Pacifastacus okanaganensis* **sp. nov.**, from specimens vouchered at the Royal British Columbia Museum, Victoria, British Columbia, Canada (RBCM). We conclude with a summary of needs for management and conservation of these newly described Pacific Northwest crayfishes.

Materials and methods

Specimens and DNA extraction. We dissected abdominal muscle tissue from specimens of *Pacifastacus* vouchered at the RBCM. These vouchers originated from Larson *et al.* (2012) and include two geographically distinct populations of both *P. malheurensis* **sp. nov.** and *P. okanaganensis* **sp. nov.**, as well as the broader geographic extent and phylogenetic diversity of *P. leniusculus* (Fig. 1, Table 1). Populations of *P. malheurensis* **sp. nov.** included the Columbia River-draining South Fork of the John Day River, as well as the upper Silvies River, isolated over a watershed divide and draining into the endorheic or closed Harney Basin of southeastern Oregon. Populations of *P. okanaganensis* **sp. nov.** included Jewel Lake in south central British Columbia, which drains to the Columbia River by an intermittent tributary stream to the Kettle River, and the southernmost known population of this lineage at Deep Lake in Washington, which lacks contemporary surface water connections to the Columbia River.

TABLE 1. Crayfish specimens (Population) used in genome skimming analyses for descriptions of *P. malheurensis* **sp. nov.** and *P. okanaganensis* **sp. nov.** with collection location (Location) including abbreviated state or province names as British Columbia (BC), Idaho (ID), Nevada, (NV), Oregon (OR), and Washington (WA), geographic coordinates (Latitude, Longitude) in WGS84, lot numbers (Lot) at either the Royal British Columbia Museum (RBCM) or Illinois Natural History Survey (INHS) where available (N/A otherwise), the number of individuals used in either genome skimming (first) or morphological analyses (second) with the count of male (M) and female (F) crayfish used in morphology measurements, and GenBank accession numbers when available (N/A otherwise) for new sequence data (Mitogenome, 18S, 28S, H3).

Population	Location	Latitude, Longitude	Lot	Genome, Morphology	Mitogenome	18S	28S	H3
P. malheurensis **sp. nov.**	Camas Creek, OR	45.0460°, -118.9805°	RBCM 012-00093-001	0, 5 (3 M, 2 F)	N/A	N/A	N/A	N/A
P. malheurensis **sp. nov.**	N. Fork John Day River, OR	44.9979°, -118.9358°	RBCM 012-00094-001	0, 6 (4 M, 2 F)	N/A	N/A	N/A	N/A
P. malheurensis **sp. nov.**	S. Fork John Day River OR	44.3981°, -119.5472°	RBCM 012-00086-001, RBCM 012-00086-003, RBCM 012-00086-004	1, 5 (3 M, 2 F)	PV231284	PQ166728.1	PQ186832.1	PQ217109.1
P. malheurensis **sp. nov.**	Upper Silvies River	44.1941°, -119.1844°	RBCM 012-00095-001	1, 6 (3 M, 3 F)	N/A	PQ166729.1	PQ186833.1	PQ217110.1
P. okanaganensis **sp. nov.**	Blueberry Creek, BC	49.2593°, -117.9389°	RBCM 012-00122-011	0, 16 (9 M, 7 F)	N/A	N/A	N/A	N/A
P. okanaganensis **sp. nov.**	Deep Lake, WA	47.5878°, -119.3385°	RBCM 012-00100-001	1, 4 (3 M, 1 F)	N/A	PQ166727.1	PQ186831.1	PQ217108.1
P. okanaganensis **sp. nov.**	Idabel Lake, BC	49.7404°, -119.1794°	RBCM 012-00124-001	0, 7 (4 M, 3 F)	N/A	N/A	N/A	N/A
P. okanaganensis **sp. nov.**	Jewel Lake, BC	49.1827°, -118.6000°	RBCM 012-00121-001, RBCM 012-00121-003, RBCM 012-00121-004	2, 4 (2 M, 2 F)	PV231282, PV231283	PQ166718.1, PQ166719.1	PQ186822.1, PQ186823.1	PQ217099.1, PQ217100.1
P. okanaganensis **sp. nov.**	Kettle River, BC	49.1097°, -118.9792°	RBCM 012-00123-001	0, 2 (2 M, 0 F)	N/A	N/A	N/A	N/A
P. okanaganensis **sp. nov.**	Okanagan Lake, BC	50.1802°, -119.4412°	RBCM 012-00125-001	0, 2 (1 M, 1 F)	N/A	N/A	N/A	N/A
P. okanaganensis **sp. nov.**	Park Lake, WA	47.5879°, -119.3964°	RBCM 012-00304-001	0, 6 (3 M, 3 F)	N/A	N/A	N/A	N/A
P. connectens	Box Canyon Springs, ID	42.7071°, -114.8101°	INHS 16455	1, 0	PV231278	PQ166717.1	PQ186821.1	PQ217098.1

.....continued on the next page

TABLE 1. (Continued)

Population	Location	Latitude, Longitude	Lot	Genome, Morphology	Mitogenome	18S	28S	H3
P. fortis	Crystal Lake, CA	40.9349°, -121.5534°	N/A	2, 0	PV231279	PQ166738.1, PQ166739.1	PQ186842.1, PQ186843.1	PQ217119.1, PQ217120.1
P. gambelii	Salt Creek, WY	42.3911°, -110.9729°	INHS 16477	2, 0	PV231280, PV231281	PQ166725.1, PQ166726.1	PQ186829.1, PQ186830.1	PQ217106.1, PQ217107.1
P. leniusculus	John Day River, OR	44.4214°, -118.9519°	RBCM 012-00089-001	1, 8 (5 M, 3 F)	PV231289	PQ166734.1	PQ186838.1	PQ217115.1
P. leniusculus	Lajoie Lake, BC	50.839°, -122.8999°	RBCM 012-00112-001	1, 27 (8 M, 19 F)	PV231292	PQ166721.1	PQ186825.1	PQ217102.1
P. leniusculus	Loon Lake, WA	48.0460°, -117.6239°	RBCM 012-00104-001	1, 2 (2 M, 0 F)	PV231293	Q166724.1	PQ186828.1	PQ217105.1
P. leniusculus	Malheur River, OR	43.7853°, -118.3320°	RBCM 012-00097-001	1, 7 (2 M, 5 F)	PV231288	PQ166733.1	PQ186837.1	PQ217114.1
P. leniusculus	Muriel Lake, BC	49.1268°, -125.6194°	RBCM 12-00116-001	1, 19 (12 M, 7 F)	PV231291	PQ166722.1	PQ186826.1	PQ217103.1
P. leniusculus	Owhyee River, NV	41.8887°, -116.054°	RBCM 012-00128-001	1, 1 (1 M, 0 F)	N/A	PQ166732.1	PQ186836.1	PQ217113.1
P. leniusculus	Paulina Lake, OR	43.7135°, -121.2738°	RBCM 012-00099-001	1, 5 (3 M, 2 F)	PV231290	PQ166723.1	PQ186827.1	PQ217104.1
P. leniusculus	Pitt River, BC	49.3485°, -122.6184°	RBCM 012-00108-001	1, 13 (5 M, 8 F)	N/A	PQ166735.1	PQ186839.1	PQ217116.1
P. leniusculus	Ririe Reservoir, ID	43.5758°, -111.7375°	RBCM 012-00129-001	1, 1 (1 M, 0 F)	PV231287	PQ166731.1	PQ186835.1	PQ217112.1
P. leniusculus	Steamboat Creek, OR	43.3453°, -122.7362°	RBCM 012-00098-001	1, 10 (4 M, 6 F)	PV231285	PQ166730.1	PQ186834.1	PQ217111.1
P. leniusculus	Umtanum Creek, WA	46.8555°, -120.4879°	RBCM 012-00103-001	1, 0	PV231286	PQ166720.1	PQ186824.1	PQ217101.1
P. *leniusculus*	Wynoochee Lake, WA	47.4063°, -123.589°	RBCM 012-00074-001	2, 3 (2 M, 1 F)	PV231294, PV231295	PQ166736.1, PQ166737.1	PQ186840.1, PQ186841.1	PQ217117.1, PQ217118.1

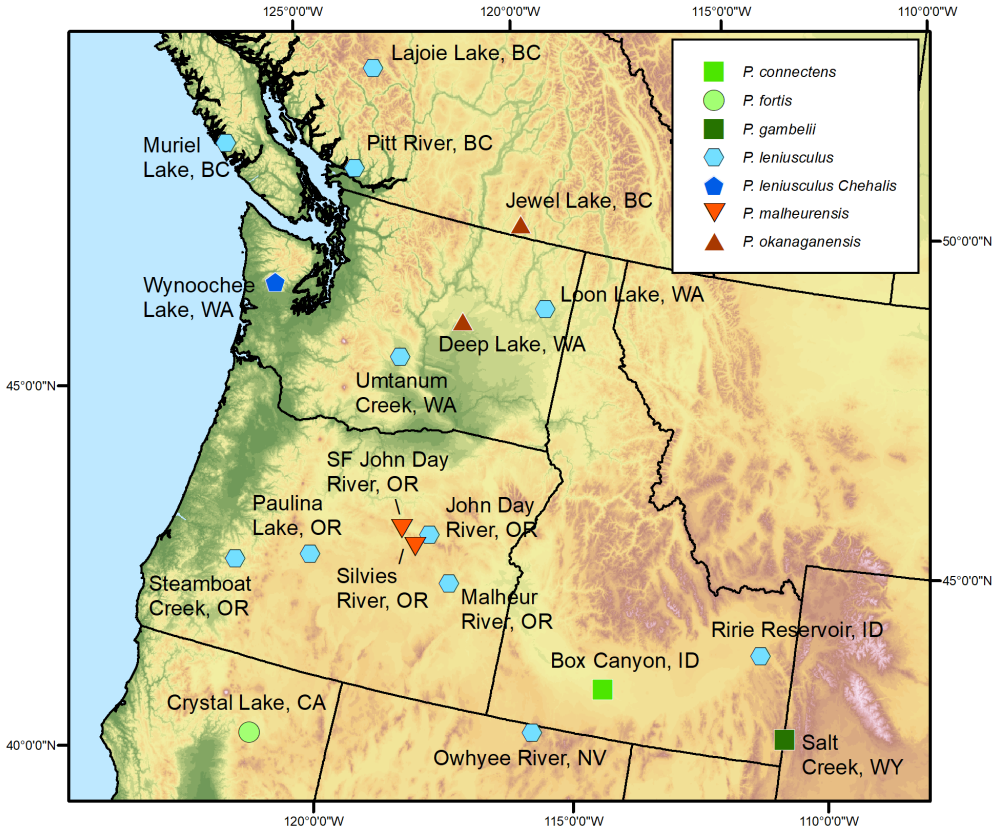

FIGURE 1. Populations used in genome skimming of *Pacifastacus* for phylogenetic analyses (Table 1).

Populations of *P. leniusculus* included geographic coverage from Steamboat Creek of the Umpqua River watershed in southwestern Oregon, which included individuals with the morphology of *P. l. klamathensis* according to Larson *et al.* (2012), north to three locations in western British Columbia (Muriel Lake, Lajoie Lake, and Pitt River) and east to populations in northern Nevada (Owhyee River) and southeastern Idaho (Ririe Reservoir). Some of these populations are hypothesized as anthropogenic introductions (Larson *et al.* 2012). Notable phylogenetic diversity in *P. leniusculus* was included from both Umtanum Creek in central Washington and Wynoochee Lake on the southern Olympic Peninsula in western Washington, which was identified as a distinct "Chehalis" lineage by Larson *et al.* (2012). This Chehalis lineage was not supported as meriting species status by phylogenetic species delimitation in Larson *et al.* (2016).

For complete taxonomic coverage of the extant species of *Pacifastacus*, we also included one population of *P. connectens* from Box Canyon, Idaho and one population of the Pilose Crayfish *Pacifastacus gambelii* (Girard, 1852) from Salt Creek, Wyoming, as well as one population of *P. fortis* from Crystal Lake, California (Fig. 1, Table 1). Abdominal muscle tissue from *P. connectens* and *P. gambelii* was dissected from specimens vouchered at the Illinois Natural History Survey (INHS) crustacean collection from Principe *et al.* (2021). Gill tissue salvaged from fresh *P. fortis* mortalities collected under ESA permit was provided by Koen Breedveld of Spring Rivers Ecological Sciences (https://springrivers.com).

Total genomic DNA was extracted from crayfish tissue using the Qiagen DNeasy blood and tissue kit (Qiagen, Inc., Redwood City, California, US). DNA extractions from *P. malheurensis* **sp. nov.**, *P. okanaganensis* **sp. nov.**, and *P. leniusculus* were performed at the Pacific Biological Station in Nanaimo, British Columbia, Canada, with the following two changes to the manufacturer's protocol: elution buffer was heated to 56°C and two final DNA elutions of 100 µl were done and subsequently combined. DNA extractions from *P. connectens*, *P. gambelii*, and *P. fortis* were performed at the University of Victoria, British Columbia, Canada, following the manufacturer's protocol.

Library preparation and sequencing. DNA extracts with concentrations ranging from 15.1–133.3 ng/µL were sent to the Michael Smith Genome Sciences Centre at BC Cancer, British Columbia, Canada for library preparation

and whole genome shotgun sequencing. To reduce library bias and coverage gaps caused by PCR amplification of high GC or AT-rich regions, the TruSeq DNA PCR-free library prep kit (Illumina Inc., San Diego, California, US) was used, which was automated on a Microlab NIMBUS liquid handling robot (Hamilton Robotics, Reno, Nevada, US). A total of 500 ng genomic DNA was arrayed in a 96-well microtiter plate and sheared by sonication using Covaris LE220 (Covaris, Woburn, Massachusetts, US). The sheared DNA was then end-repaired and size selected using PCRClean DX paramagnetic beads (Aline Biosciences, Boston, Massachusetts, US) targeting 300–400 bp fragments. After 3' A-tailing, full-length TruSeq adapters (Illumina Inc., San Diego, California, US) were ligated and purified using PCRClean DX paramagnetic beads. The concentrations of the PCR-free genome libraries were quantified using a qPCR Library Quantification kit (KAPA, Wilmington, Massachusetts, US) prior to sequencing. Libraries were pooled into equimolar quantities and sequenced in a single run on an Illumina NovaSeq 6000 (Illumina Inc., San Diego, California, US) using S4 reagents with a sequencing run length of 150 bp (paired end).

Mitogenome and nuclear marker assembly. Mitogenomes were all assembled, circularized, and standardized by running mtGrasp (Mitochondrial Reference-Grade Genome Assembly and Standardization Pipeline, v. 1.1.0, https://github.com/bcgsc/mtGrasp) with default custom k-mer size and k-mer coverage cutoff. All mitogenomes were standardized by orienting the sequences from 5' to 3' end and having tRNA-Phe as the starting sequence (Lopez *et al.* 2025). Mitochondrial genome annotations were generated using MITOS (Bernt *et al.* 2013). We manually reviewed all 18 annotations and corrected them as needed using the reference mitochondrial genome (NC_033509.1). Highly repetitive nuclear 18S RNA, 28S RNA, and Histone H3 genes were assembled from our focal crayfish (Table 1) using Geneious Prime 2023.2.1 consistent with Hoban *et al.* (2022). We used the "Map to Reference" function to relate our sequence data to 18S, 28S, and H3 reference sequences from *P. leniusculus* in the United Kingdom generated by Grandjean *et al.* (2017; Table 2), with sensitivity set to "Medium – Low" and iterated up to five times. We then inspected and trimmed ends of resulting assemblies where coverage was low.

TABLE 2. Additional crayfish sequences from Grandjean *et al.* (2017) used here in phylogenetic analyses with NCBI GenBank accession numbers for mitogenomes and highly repetitive 18S rRNA, 28S rRNA, and histone H3 nuclear genes.

Species	Geographic origin	Mitogenome	18S	28S	H3
A. astacus	France	KX279347.1	N/A	N/A	N/A
A. astacus	France	KX279348.1	N/A	N/A	N/A
A. astacus	France	N/A	KX444559.1	KX444580.1	KX444601.1
A. pallipes	France	KP205430.1	KX444562.1	KX444583.1	KX444604.1
A. torrentium	Germany	KX268734.1	KX444563.1	KX444584.1	KX444605.1
P. leniusculus	United Kingdom	KX268740.1	KX444579.1	KX444600.1	KX444621.1
P. leptodactylus	France	KX279349.1	N/A	N/A	N/A
P. leptodactylus	France	KX279350.1	N/A	N/A	N/A
P. leptodactylus	Turkey	N/A	KX444561.1	KX444582.1	KX444603.1
P. acutus	United States	KX268741.1	KX444577.1	KX444598.1	KX444619.1

Phylogenetic analyses. We built both mitochondrial and nuclear phylogenetic trees using mitogenomes and highly repetitive nuclear genes recovered from genome skimming. We specifically sought to compare phylogenetic trees from the mitogenome to those using concatenated 18S, 28S, and H3 sequences to evaluate whether the nuclear genome supported previous mitochondrial phylogenies of *Pacifastacus* and species status for *P. malheurensis* **sp. nov.** and *P. okanaganensis* **sp. nov.** (Larson *et al.* 2012, 2016). Both sets of trees used additional crayfish sequence data from Grandjean *et al.* (2017), including White River Crayfish, *Procambarus acutus* (Girard, 1852), as an outgroup from the family Cambaridae and Noble Crayfish (*Astacus astacus* (Linnaeus, 1758)), White-Clawed Crayfish (*Austropotamobious pallipes* (Lereboullet, 1858)), Stone Crayfish (*Austropotamobius torrentium* (von Paula Schrank, 1803)) and Narrow-Clawed Crayfish (*Pontastacus leptodactylus* (Eschscholtz, 1823)) for taxon coverage within the family Astacidae from Europe (Table 2). Lastly, we included the same *P. leniusculus* sequences from Grandjean *et al.* (2017) used in assembly of 18S, 28S, and H3 markers from genome skimming.

Mitogenomes were aligned with MAFFT v7.490 (Katoh & Standley 2013) in Geneious Prime 2023.2.1 with the automatic algorithm, a scoring matrix of 200PAM / k = 2, and a 1.53 gap open penalty with 0.123 offset value. We built a Bayesian phylogenetic tree on mitogenomes using MrBayes 3.2.6 (Huelsenbeck & Rondquist 2001) in Geneious Prime 2023.2.1 using the HKY85 substitution model, gamma rate variation with four categories, a chain length of 1,100,000, four heated chains with a temperature of 0.2, a sampling frequency of 200, burn-in length of 100,000, a random seed of 20,399, unconstrained branch lengths of 1, 0.1, 1 and 1, and an exponential (10) shape parameter. *Procambarus acutus* was defined as the outgroup. We built a maximum likelihood phylogenetic tree on mitogenomes using PhyML (Guindon *et al.* 2010) in Geneious Prime 2023.2.1 with the HKY85 substitution model, bootstrap branch support with 100 bootstraps, an estimated transition / transversion ratio, invariable sites fixed as zero, four substitution rate categories, and an estimated gamma distribution parameter. Concatenated sequences (18S, 18S, H3 together) were aligned with MUSCLE5.1 (Edgar 2021) using the PPP algorithm in Geneious Prime 2023.2.1. Bayesian and maximum likelihood phylogenetic tree building for concatenated nuclear sequences followed the above methods for mitogenomes, with a random seed of 13,393 in MrBayes. Where trees with shared topologies were returned between MrBayes and PhylML, Bayesian posterior probabilities and bootstrap support are reported together on nodes.

Morphological analyses. Larson *et al.* (2012) conducted a discriminant function analysis (DFA) on *P. leniusculus* and the lineages described here as *P. malheurensis* **sp. nov.** and *P. okanaganensis* **sp. nov.** using 16 morphological ratios calculated for each individual crayfish from specimens vouchered at the RBCM. This DFA classified 90% of individuals to their correct lineage by mtDNA. Here, we sought to simplify recommendations for field identification of *P. malheurensis* **sp. nov.** and *P. okanaganensis* **sp. nov.** to fewer morphological characters than Larson *et al.*'s (2012) DFA. We used all vouchered lots of *P. malheurensis* **sp. nov.** and *P. okanaganensis* **sp. nov.** at the RBCM that had been sequence confirmed by mtDNA in Larson *et al.* (2012) and Larson *et al.* (2016), as well as populations of *P. leniusculus* at the RBCM used in the current genome skimming and phylogenetic analyses (Table 1). For *P. malheurensis* **sp. nov.**, we used 22 total individuals as 13 males and nine females from four populations, with a size range of 19.7–37.6 mm total carapace length (TCL). For *P. okanaganensis* **sp. nov.**, we used 41 individuals as 24 males and 17 females from seven populations, with a size range of 19.6–47.0 mm TCL. For *P. leniusculus*, we used 96 individuals as 45 males and 51 females from 11 populations, with a size range of 19.4–65.8 mm TCL.

Morphological measurements followed Miller (1960) and Larson *et al.* (2012) to the nearest 0.1 mm using digital Vernier calipers. We calculated the same 16 morphological ratios as Larson *et al.* (2012) and converted to percentages. We then used a classification tree in *rpart* 4.1.11 (Therneau *et al.* 2015) in R 3.4.2 (R Core Team 2017) to classify individual crayfish to *P. leniusculus*, *P. malheurensis* **sp. nov.**, and *P. okanaganensis* **sp. nov.** The classification tree was built using default settings and then pruned with a complexity parameter of 0.1. Results were visualized as both the classification tree and a biplot of discriminating morphological ratios. Lastly, we summarized geographic distributions of confirmed populations of *P. malheurensis* **sp. nov.** and *P. okanaganensis* **sp. nov.** from the RBCM vouchers (Table 1) and mtDNA sequence results from Larson *et al.* (2012) and Larson *et al.* (2016) not vouchered at museums.

Results

Sequence reads and genome skimming. We recovered 1.98 to 2.4 million reads (2.1 ± 0.3 million) per library. From this, we were able to assemble 18 nearly complete or complete-circularized mitogenomes ranging from 15,006 to 16,852 bp in length. We also assembled 23 sequences for each of 18S, 28S, and H3 genes across our focal crayfishes. GenBank accession numbers for sequences are reported in Table 1.

Phylogenetic analyses. Equivalent, well-supported phylogenetic trees were returned by MrBayes and PhyML on mitogenomes, which recovered *P. malheurensis* **sp. nov.** and *P. okanaganensis* **sp. nov.** as highly diverged lineages relative to *P. leniusculus* (Fig. 2). *Pacifastacus leniusculus*, *P. malheurensis* **sp. nov.**, and *P. okanaganensis* **sp. nov.** were a monophyletic lineage, but *P. malheurensis* **sp. nov.** and *P. okanagaensis* **sp. nov.** were as distinct from each other and *P. leniusculus* as other species-level distinctions within the family Astacidae. MrBayes and PhyML returned comparable topologies for phylogenetic trees on concatenated nuclear genes, although these were less supported than mitogenome trees (Fig. 2).

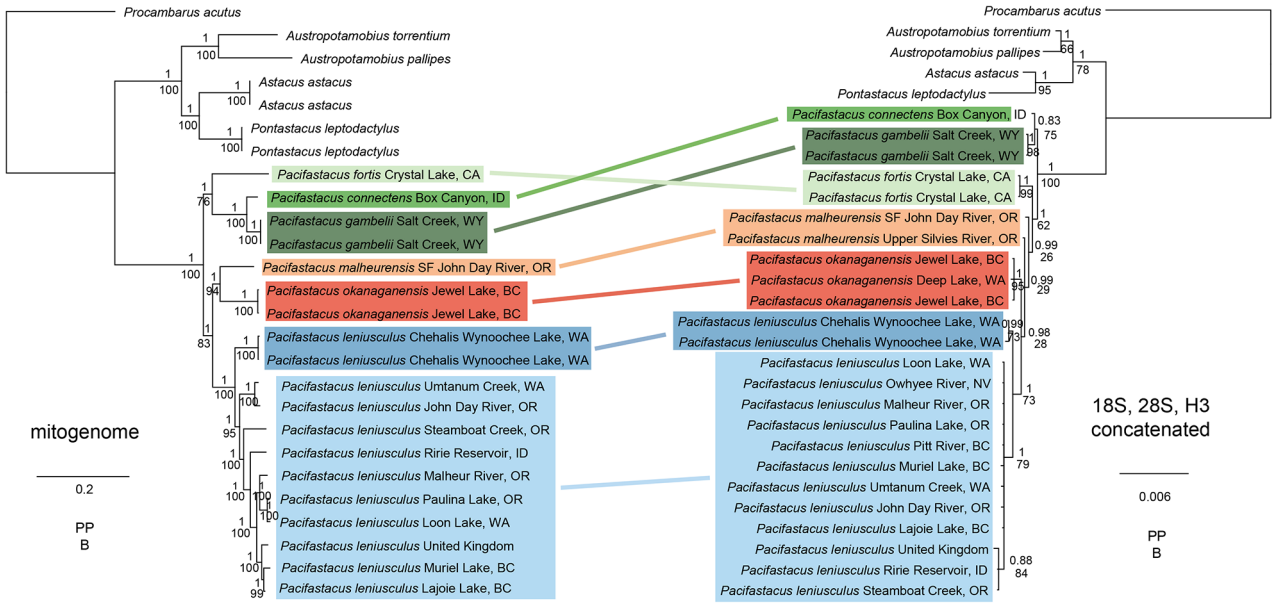

FIGURE 2. Phylogenetic trees from mitogenomes (left) and concatenated 18S, 28S, and H3 nuclear genes (right) for *Pacifastacus* with Bayesian posterior probabilities (PP) and bootstrap support (B) on nodes.

Morphological analyses. Our pruned classification tree used two splits to identify individual crayfish as *P. leniusculus*, *P. malheurensis* **sp. nov.**, or *P. okanaganensis* **sp. nov.** (Fig. 3). The first split classified crayfish with acumen lengths more than 79.4% of anterior rostrum width as *P. leniusculus.* This decision rule correctly classified 86 of 96 of *P. leniusculus* individuals (89.6%), but four *P. malheurensis* **sp. nov.** (19.0%) and two *P. okanaganensis* **sp. nov.** (4.9%) individuals were misclassified as *P. leniusculus*. The second split classified crayfish with rostrum lengths more than 18.85% of TCL as *P. okanaganensis* **sp. nov.**, and crayfish with rostrum lengths below this decision rule as *P. malheurensis* **sp. nov.** This decision rule correctly classified 38 of 41 *P. okanaganensis* **sp. nov.** individuals (92.6%) but misclassified 10 *P. leniusculus* (10.4%) and five *P. malheurensis* **sp. nov.** (22.7%) as *P. okanaganensis* **sp. nov.** This decision rule also correctly classified 13 of 22 *P. malheurensis* **sp. nov.** individuals (59.0%), misclassified no *P. leniusculus*, and only one *P. okanaganensis* **sp. nov.** (2.4%) as *P. malheurensis.* The one *P. okanaganensis* **sp. nov.** misclassified as *P. malheurensis* **sp. nov.** was our holotypic male (Table 3). Cumulatively, our simple, two-split classification tree assigned 137 of 159 crayfish individuals to their correct species (86.1%). *Pacifastacus malheurensis* **sp. nov.** was the species most likely to be misclassified as a congener, whereas *P. okanaganensis* **sp. nov.** was the least likely.

Taxonomy

Family Astacidae Latreille, 1802
Genus *Pacifastacus* Bott, 1950

Pacifastacus malheurensis Larson sp. nov.

urn:lsid:zoobank.org:act:D296A360-6997-43CD-9FCB-C0C58CEEA7F5
(Fig. 4, Table 3)

Pacifastacus klamathensis.—Miller, 1960:130, 132, 197, 198 pl. VIII fig. 39 [all in part].
Pacifastacus leniusculus klamathensis (Stimpson).—Miller, 1960: 133, 146, 160, 180, 181 [all in part].
Pacifastacus leniusculus.—Hobbs, 1972: 21 [by implication].—Larson & Olden, 2011: 64 [in part].
Pacifastacus leniusculus leniusculus.—Hobbs, 1972: 21 [in part].—Hobbs, 1974: 6 [in part; neither fig. 5 nor 6, p. 81 = *P. okanaganensis* **sp. nov.**].
Pacifastacus leniusculus klamathensis.—Hobbs, 1974: 22 [in part].—Larson *et al.* 2012: 3, 6, 12, fig. 1 [all in part].—Larson & Williams, 2015: 413, 419, 424, fig. 17.2 [all in part].

Pacifastacus (*Pacifastacus*) *leniusculus klamathensis*.—Bouchard, 1978: 431 [by implication, in part].—Hobbs, 1989: 82 [in part].
Pacifastacus (*Pacifastacus*) *leniusculus leniusculus*.—Hobbs, 1989: 82 [in part].
Pacifastacus lenisculus lenisculus.—Fitzpatrick, 1983: 155 [erroneous spelling].
Pacifastacus leniusculus (Dana, 1852).—Larson *et al.*, 2012: 2–6 [all in part].—Williams & Weaver, 2019: 286, 287, 291, 296, 297.
Pacifastacus klamathensis (Stimpson, 1857).—Larson *et al.*, 2012: 2, 3 [all in part]
Central Oregon Group.—Larson *et al.*, 2012: 7, 8, 10, 12 [by implication], 13, fig. 2, table 1, 2.—Larson & Williams, 2015: 419, 426, fig. 17.2.— Larson *et al.*, 2016: 10, 12, fig. 3.
Pacifatacus leniusculus klamathensis.—Larson & Williams, 2015: 413 [erroneous spelling].
P. l. klamathensis.—Hart & Hart, 1974: 129.
Pacifatacus leniusculus klamathensis.—Larson & Williams, 2015: 413 [erroneous spelling].
Pacifastacus l. klamathensis.—Larson & Williams, 2015: 413, 419, 424, fig. 17.2 [all in part].
Central Oregon Group.—Larson & Williams, 2015: 419, 426, 427, fig. 17.2.—Larson *et al.*, 2016: 10, 12, fig. 3.

Type material. Holotype (RBCM 012-00086-003) male, South Fork of the John Day River, Oregon (44.3981°, -119.5472°). Allotype (RBCM 012-00086-004) female, South Fork of the John Day River, Oregon (44.3981°, -119.5472°).

Other material. RBCM 012-00086-001, South Fork of the John Day River, Oregon (44.3981°, -119.5472°); RBCM 012-00093-001, Camas Creek, Oregon (45.0460°, -118.9805°); RBCM 012-00094-001, North Fork of the John Day River, Oregon (44.9979°, -118.9358°); RBCM 012-00095-001, Upper Silvies River, Oregon (44.1941°, -119.1844°). Number of specimens by sex in Table 1.

Type locality. South Fork of the John Day River, Oregon (44.3981°, -119.5472°).

Diagnosis. *Pacifastacus* with rostrum bearing only single pair of marginal tubercles or spines; acumen length less than 79.4% of anterior rostrum width; rostrum length less than 18.85% of TCL (Fig. 3).

Description. Body and eyes pigmented. Eyes not reduced. Rostrum deflected ventrally, base and anterior broad, margins parallel to sub-parallel, non-serrate; median carina subtle; acumen strongly converging, not separated from remainder of rostrum by spines; acumen length 68% of anterior rostrum width (11% sd). Rostrum length including acumen 145% of basal rostrum width (15% sd) and 19% of TCL (2% sd); anterior rostrum width 82% of posterior rostrum width (6% sd). Cephalothorax subcylindrical; postorbital ridge not terminating in spine, occasionally terminating in tubercle; TCL 203% of carapace width (11% sd); areola length 273% of areola width (44% SD), 34% of TCL (1% sd), width 26% of TCL (5% sd). Third pereopods without hook on ischium. Chelae without tubercles; palm length 82% of maximum chela width at palm (5% sd); palm length 35% of propodus length (2% sd); chela height 63% of maximum chela width at palm (2% sd). First pleopod (gonopod) of males nondescript, typical for genus. Annulus ventralis lacking, typical for genus.

Holotypic male. Body compressed dorsoventrally (Fig. 4A). Carapace slightly wider (102%) than abdomen. Rostrum broad, margins parallel to sub-parallel, anterior width 85% of posterior width, without spines or tubercles, deflected ventrally; with weak median carina (Fig. 4B). Rostrum length 139% of posterior width, 18% of TCL; acumen 30% of rostrum length. Carapace maximum depth less (85%) than carapace width; TCL 25.1 mm; areola 200% longer than wide, 32% of TCL (Fig. 4B); short postorbital ridges terminating in small tubercle; surface otherwise lacking tubercles or spines. Abdomen slightly shorter than carapace (97%). Palm length 87% of palm width; palm depth 64% of palm width (all measurements and counts from right chela; Fig. 4C). Gonopod nondescript, typical for genus (Fig. 4D, E). Epistome with semi-circular anterior lobe, lacking setae (Fig. 4F). Right antennal scale 4.1 mm long and 2.0 mm wide (Fig. H). Third pereopods without hook on ischium.

Allotypic female. Differing from holotype in following respects: TCL 33.9 mm; areola 286% longer than wide, length 35% of TCL; anterior rostrum width 69% of posterior rostrum width; rostrum length 133% of posterior width and 17% of TCL; acumen 34% of rostrum length; palm length 81% of palm width, depth 59% of palm width (all measurements and counts based on right chela). Antennal scale 5.0 mm long, 2.2 mm wide. Annulus ventralis absent (Fig. 4G).

Size. The largest individual measured was 37.6 mm TCL.

Color. Olive brown (Fig. 5) to yellow or orange. The white mark at the joint of the dactyl and propodus in *P. leniusculus* is generally absent or reduced.

Etymology. For the Malheur region of eastern Oregon, including the Malheur National Forest and Malheur Lake, from the French "malheur" meaning misfortune. We propose the common name the "Misfortunate Crayfish"

due to its discovery while studying spread of invasive *F. rusticus* throughout the John Day River watershed in Oregon, which has displaced *P. malheurensis* **sp. nov.** from a substantial proportion of its former distribution.

Geographic distribution and habitat. *Pacifastacus malheurensis* **sp. nov.** occurs in the John Day River watershed of central Oregon and its tributary streams, as well as over a watershed divide into the endorheic Harney Basin of southeastern Oregon (Fig. 6). Populations visualized in Fig. 6 are vouchered at the RBCM except the most downstream location in the John Day River and the East Canal at Page Springs Dam population reported in Larson *et al.* (2016). Harney Basin tributaries include the upper Silvies River and East Canal at Page Springs Dam, an irrigation-modified tributary of the Donner und Blitzen River. Both the Silvies and Donner und Blitzen rivers drain to the saline Malheur Lake. Whether *P. malheurensis* **sp. nov.** occurs in other rivers of the Blue Mountains in northeast Oregon, or other endorheic watersheds of the Great Basin of California, Idaho, Oregon, or Nevada, is unknown.

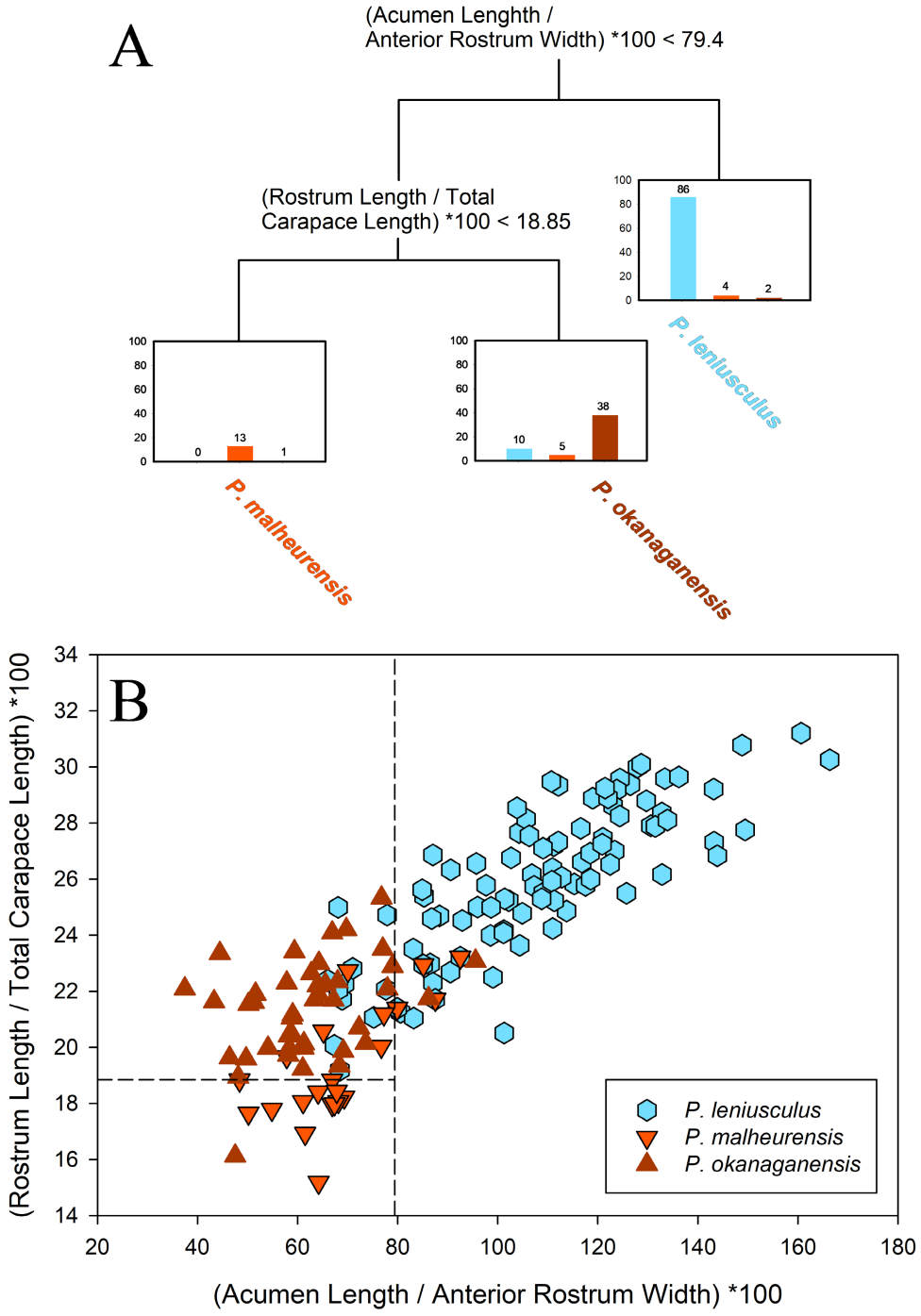

FIGURE 3. Classification tree for *P. leniusculus*, *P. malheurensis* **sp. nov.**, and *P. okanaganensis* **sp. nov.** (A) using morphological ratios, with biplot (B) of the two morphological characters contributing to the best discriminating splits and associated thresholds (dashed lines).

TABLE 3. Measurements of holotypic male (M; 012-00086-003) and allotypic female (F; 012-00086-004) for *Pacifastacus malheurensis* **sp. nov.** and holotypic male (M; (012-00121-003) and allotypic female (F; 012-00121-004) for *Pacifastacus okanaganensis* **sp. nov.**

Character	*P. malheurensis* M	*P. malheurensis* F	*P. okanaganensis* M	*P. okanaganensis* F
Carapace				
Total Length	25.1	33.9	27.4	21.7
Maximum Width	12.5	16.9	13.9	10.5
Depth	10.5	13.1	11.5	8.7
Rostrum				
Length	4.6	5.6	4.4	4.1
Anterior Width	2.8	2.9	3.1	2.4
Posterior Width	3.3	4.2	4.0	3.1
Acumen Length	1.4	1.9	1.5	1.3
Antennal Scale				
Length	4.1	5.0	4.9	3.7
Width	2.0	2.2	1.7	1.4
Areola				
Length	8.0	12.0	9.3	7.3
Width	4.0	4.2	4.1	3.2
Chelae				
Propodus Length	20.7	27.8	23.6	13.8
Palm Length	7.7	9.5	8.9	5.1
Palm Width	8.9	11.7	9.9	5.9
Palm Depth	5.7	6.9	6.1	3.6
Dactyl Length	12.0	15.5	11.5	7.7
Abdomen				
Length	24.4	34.5	29.6	21.9
Width	12.2	18.4	13.5	10.4
Gonopod				
Length	7.0	N/A	7.0	N/A

Pacifastacus malheurensis **sp. nov.** has never been detected west of the Cascade Mountains in coastal California, Oregon, or Washington. *Pacifastacus malheurensis* **sp. nov.** has not been collected from lentic ecosystems, but natural lakes are scarce within its distribution excluding saline lakes of endorheic basins that are unlikely to be viable for these crayfish.

Life history. Life history of *P. malheurensis* **sp. nov.** has not been studied, and berried or ovigerous individuals are not included among the RBCM vouchers. *Pacifastacus malheurensis* **sp. nov.** life history might be expected to broadly resemble other crayfishes of the genus *Pacifastacus*, as slower-growing and with lower fecundity proportional to carapace length relative to members of the family Cambaridae native to eastern North America (Momot 1984).

Conservation status. *Pacifastacus malheurensis* **sp. nov.** has likely been displaced from much of its native range in the John Day River of Oregon by ongoing spread of invasive *F. rusticus* (Messager & Olden 2018; Olden *et al.* 2009). Urgent conservation attention is needed, with an emphasis on preventing invasive crayfish introductions to isolated *P. malheurensis* **sp. nov.** populations in the Silvies River and Donner und Blitzen River. The rapid pace of the spread of *F. rusticus*, and associated displacement of *P. malheurensis* **sp. nov.** from the John Day River, suggests an International Union for the Conservation of Nature (IUCN) status of globally Endangered due to likely range declines of ≥70% over 10 years or three generations (IUCN 2012). As only six occurrences of *P. malheurensis* **sp. nov.** are known (Fig. 6), an Oregon natural heritage ranking of Imperiled is recommended (https://inr.oregonstate.edu/orbic/rare-species/ranking-definitions).

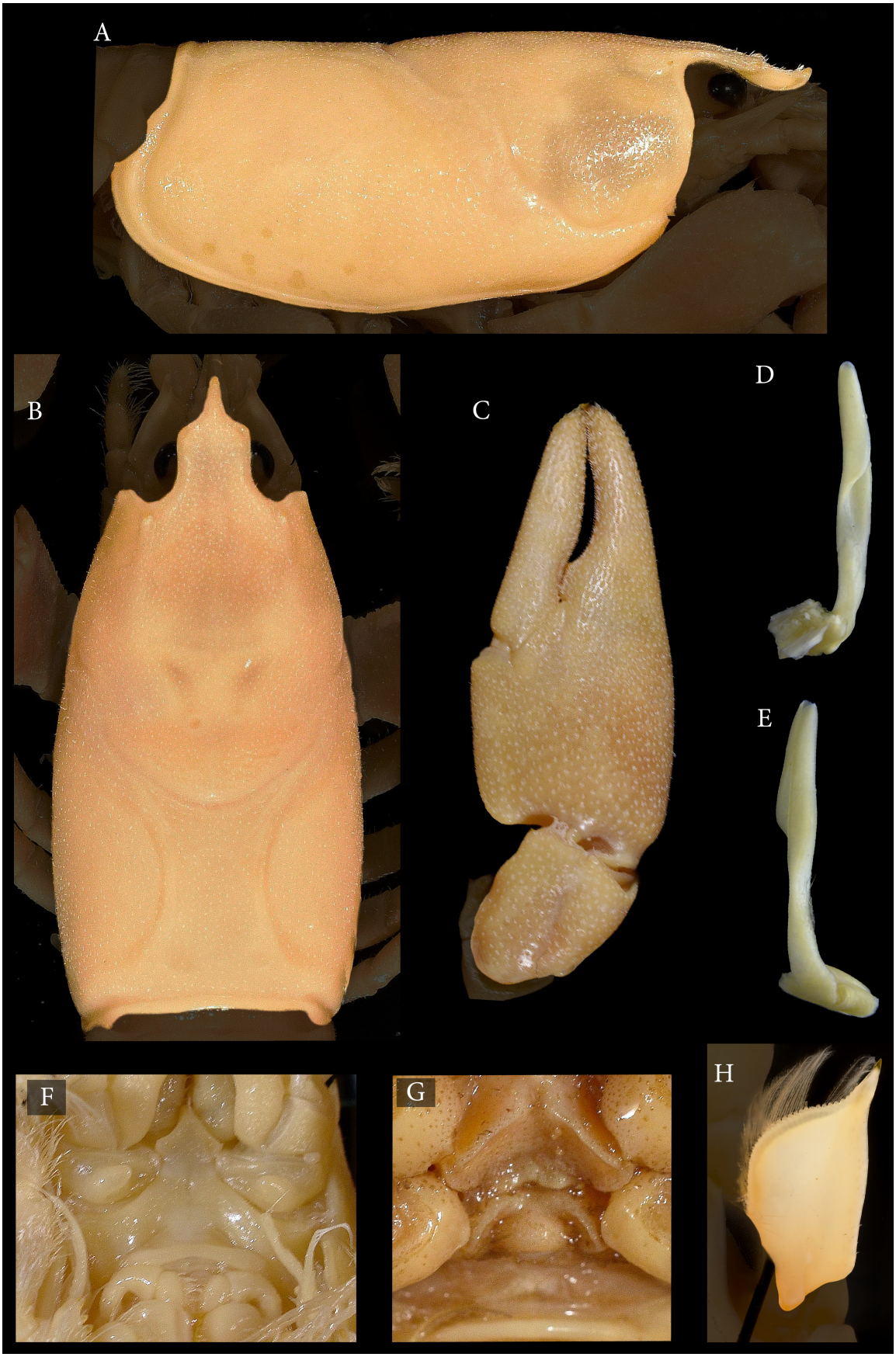

FIGURE 4. *Pacifastacus malheurensis* **sp. nov.**, A–F, H: holotype, RBCM 012-00086-003; G; female allotype, RBCM 012-00086-004. Lateral carapace view (A); dorsal carapace view (B); right chelae (C); gonopods (D, E); epistome (F); annulus ventralis (G); and antennal scale (H).

FIGURE 5. *Pacifastacus malheurensis* **sp. nov.** individual from the North Fork of the John Day River, Oregon showing olive brown color of habitus.

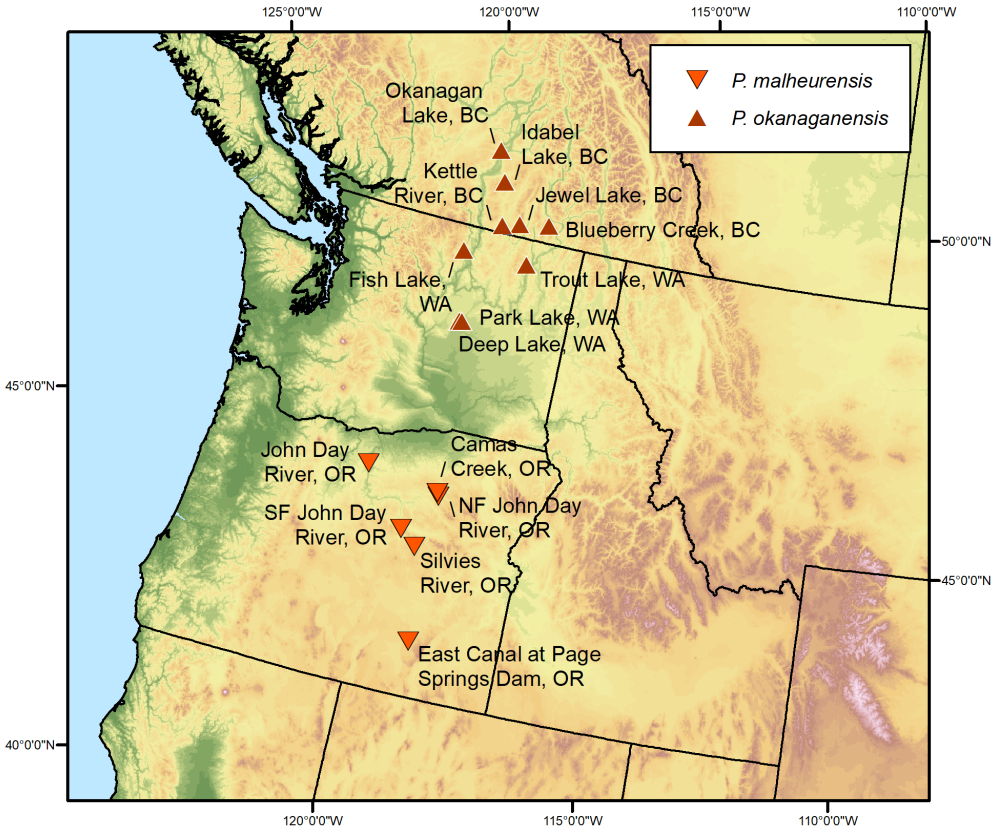

FIGURE 6. Map of known populations for *P. malheurensis* **sp. nov.** and *P. okanaganensis* **sp. nov.** from RBCM vouchers (Table 1) and past papers (Larson *et al.* 2012, 2016).

Crayfish associates. *Faxonius rusticus* is now the dominant crayfish throughout much of the John Day River and its tributaries in Oregon (Messager & Olden 2018; Olden *et al.* 2009). *Pacifastacus malheurensis* **sp. nov.** was not collected in sympatry with *F. rusticus* by Larson *et al.* (2012) or Larson *et al.* (2016). *Pacifastacus leniusculus* was collected from the mainstem John Day River in the town of John Day by Larson *et al.* (2012) and included in phylogenetic and morphological analyses here (Table 1). These *P. leniusculus* did not co-occur with *P. malheurensis* **sp. nov.** and mtDNA haplotypes of the two lineages have never been recovered from the same location. *Pacifastacus*

connectens is also known from some locations in the Harney Basin of eastern Oregon, which constitutes its western range boundary, but *P. connectens* has not been collected with *P. malheurensis* **sp. nov.** and is known instead from isolated springs in the vicinity of Harney Lake (Egly & Larson 2018; Principe *et al.* 2021).

Relationships and comparisons. *Pacifastacus malheurensis* **sp. nov.** is morphologically similar to *P. okanaganensis* **sp. nov.** and *P. l. klamathensis* crayfishes of coastal, southwestern Oregon and northwestern California. *Pacifastacus malheurensis* **sp. nov.** generally has an acumen length less than 79.4% of anterior rostrum width, whereas *P. leniusculus* generally has an acumen length greater than 79.4% of anterior rostrum width (Fig. 3). *Pacifastacus malherensis* **sp. nov.** generally has a rostrum length less than 18.85% of TCL, whereas *P. okanaganensis* **sp. nov.** has a rostrum length greater than 18.85% of TCL (Fig. 3). *Pacifastacus l. leniusculus* in particular are easy to differentiate from *P. malheurensis* **sp. nov.** by the long, spiny rostrum and chelae with short, convex palms (Hobbs 1972). *Pacifastacus malheurensis* **sp. nov.** has not been collected in sympatry with *P. okanaganensis* **sp. nov.** or coastally distributed *P. l. klamathensis* and may be differentiated by geographic range. *Pacifastacus connectens* can be differentiated from *P. malheurensis* **sp. nov.** by a serrated rostrum with many pairs of marginal tubercles or spines and the presence of patches of setae on the dorsal margins of the chelae. *Pacifastacus connectens* in the Harney Basin often has a distinctive color pattern of orange or red mottling on a yellow background (Principe *et al.* 2021).

Pacifastacus okanaganensis **Larson sp. nov.**

urn:lsid:zoobank.org:act:F9687AA1-D8A4-485D-A0BE-78F56253E559
(Fig. 7, Table 3)

Astacus klamathensis.—Lord, 1866: 278.
Astacus Klamathensis.—Hagen, 1870: 93, 94, 98, 102, pl. III fig. 169a, b, c [all in part].
C. Klamathensis.—Hagen, 1870: 102 [erroneous combination].
A. klaymathensis.—Huxley, 1880: 223 [in part; erroneous spelling].
Astacus Klamathensis.—Faxon, 1884: 151 [in part].
Astacus klamathensis.—Faxon, 1885: 130, 131, 132 [all in part].
Astacus klamathensis.—Faxon, 1890: 634 [in part].
Astacus klamathensis.—Faxon, 1898: 665 [in part].
Potamobius (*Potamobius*) *klamathensis* (Stps.).—Ortmann, 1902: 286 [in part].
Pacifastacus klamathensis.—Bott, 1950: 24 [by implication, in part].—Miller, 1960: 130, 132, 197, 198, pl. VIII fig. 39 [all in part].
Pacifastacus leniusculus klamathensis.—Miller, 1960: 133, 146, 160, 180, 181 [all in part].
Pacifastacus leniusculus.—Hobbs, 1972: 21 [by implication].—Larson & Olden, 2011:64 [in part].
Pacifastacus leniusculus leniusculus.—Hobbs, 1972: 21 [in part].—Hobbs, 1974: 6 [in part; neither fig. 5 nor 6, p. 81 = *P. okanaganensis* **sp. nov.**].
Pacifastacus leniusculus klamathensis.—Hobbs, 1974: 22 [in part].—Larson *et al.*, 2012: 3, 6, 12, fig. 1 [all in part].—Larson & Williams, 2015: 413, 419, 424 [in part], fig. 17.2.
Pacifastacus (*Pacifastacus*) *leniusculus klamathensis*.—Bouchard, 1978: 431 [by implication; in part].—Hobbs, 1989: 82 [in part].
Pacifastacus (*Pacifastacus*) *leniusculus leniusculus*.—Hobbs, 1989: 82 [in part].
Pacifastacus lenisculus lenisculus.—Fitzpatrick, 1983: 155 [erroneous spelling].
Pacifastacus leniusculus.—Larson *et al.*, 2012: 2, 3, 4, 5, 6 [all in part].
Pacifastacus klamathensis.—Larson *et al.*, 2012: 2, 3 [in part]
Okanagan group.—Larson *et al.*, 2012: 7, 8, 10, 12 [by implication], 13, fig. 2, table 1, 2. —Larson & Williams, 2015: 419, 426, fig. 17.2.—Larson *et al.*, 2016: 10, 12, fig. 3.
Pacifatacus leniusculus klamathensis.—Larson & Williams, 2015: 413 [erroneous spelling].

Type material. Holotype (RBCM 012-00121-003), male, Jewel Lake, British Columbia (49.1827°, -118.6000°). Allotype (RBCM-012-00121-004), female, Jewel Lake, British Columbia (49.1827°, -118.6000°).

Other material. RBCM 012-00100-001, Deep Lake, Washington (47.5878°, -119.3385°); RBCM 012-00121-001, Jewel Lake, British Columbia (49.1827°, -118.6000°); RBCM 012-00122-011, Blueberry Creek, British Columbia (49.2593°, -117.9389°); RBCM 012-00123-001, Kettle River, British Columbia (49.1097°, -118.9792°); RBCM 012-00124-001, Idabel Lake, British Columbia (49.7404°, -119.1794°); RBCM 012-00125-001, Okanagan Lake, British Columbia (50.1802°, -119.4412°); RBCM 012-00304-001, Park Lake, Washington (47.5879°, -119.3964°). Number of specimens by sex in Table 1.

Type locality. Jewel Lake, Jewel Lake Provincial Park, British Columbia (49.1827°, -118.6000°).

Diagnosis. *Pacifastacus* with rostrum bearing single pair of marginal tubercles or spines; acumen length less than 79.4% of anterior rostrum width; rostrum length more than 18.85% of TCL (Fig. 3).

Description. Body and eyes pigmented. Eyes not reduced. Rostrum deflected ventrally, base and anterior broad, margins sub-parallel, non-serrate; median carina subtle; acumen strongly converging, separated from remainder of rostrum by weak spines or tubercles; length 62% of anterior rostrum width (12% sd). Rostrum length including acumen 150% of base rostrum width (14% sd) and 21% of TCL (2% sd); anterior rostrum width 75% of posterior rostrum width (5% sd). Cephalothorax subcylindrical; postorbital ridge not terminating in spine, occasionally terminating in tubercle; TCL 203% of carapace width (9% sd); areola length 33% of TCL (1% sd), 233% of areola width (29% SD); areola width 29% of total carapace width (4% sd). Third pereopods without hook on ischium. Chelae without tubercles; palm length 87% of maximum chelae width at palm (12% sd); 36% of propodus length (10% sd); chelae height 63% of maximum chelae width at palm (3% sd). First pleopod (gonopod) of males nondescript, typical for genus. Annulus ventralis lacking, typical for genus.

Holotypic male. Body compressed dorsoventrally (Fig. 7A). Rostrum broad, deflected ventrally; margins sub-parallel with anterior width 78% of posterior width, without spines or tubercles; median carina weak (Fig. 7B). Rostrum length 110% of posterior width and 16% of TCL; acumen 34% of rostrum length. Carapace slightly wider (103%) than abdomen, maximum depth less (83%) than carapace width; TCL 27.4 mm; areola 227% longer than wide, 34% of TCL (Fig. 7B); short postorbital ridges terminating in small tubercle, carapace otherwise lacking tubercles or spines. Abdomen slightly longer than carapace (108%). Palm length 90% of palm width, palm depth 62% of palm width (all measurements and counts based on right chela; Fig. 7C). Gonopod nondescript, typical for genus (Fig. 7D, E). Epistome with semi-circular anterior lobe, lacking setae (Fig. 7F). Right antennal scale 4.9 mm long and 1.7 mm wide (Fig. 7H). Third pereopods without hook on ischium.

Allotypic female. Differing from holotype in following respects: TCL 21.7 mm; areola length 34% of TCL, 228% longer than wide; anterior rostrum width 77% of posterior rostrum width; rostrum length 132% of posterior width, 19% of TCL, acumen 32% of rostrum length; palm length 86% of palm width, palm depth 61% of palm width (all measurements and counts based on right chela). Antennal scale 3.7 mm long and 1.4 mm wide. Annulus ventralis absent (Fig. 7G).

Size. The largest individual measured at RBCM was 47.0 mm TCL.

Color. Olive brown to brick red (Fig. 8). The white mark at the joint of the dactyl and propodus in *P. leniusculus* is generally absent or reduced.

Etymology. From an Okanagan-Salish language place name. We propose the common name of the "Okanagan Crayfish" due to the distribution of *P. okanaganensis* **sp. nov.** throughout the Okanagan and Thompson plateaus and Okanagan Lake, British Columbia, as well as Okanogan County, Washington, and due to the Okanagan lineage terminology of Larson *et al.* (2012) and Larson *et al.* (2016).

Geographic distribution and habitat. *Pacifastacus okanaganensis* **sp. nov.** has been most often collected from relatively isolated, mid-elevation lakes in the Okanagan and Thompson plateaus of British Columbia and Washington (Fig. 6). The species has also been collected from large Okanagan Lake in British Columbia, and lakes below Dry Falls in central Washington at Sun Lakes State Park (Deep and Park lakes). The only lotic records for the species are from the Kettle River and Blueberry Creek in British Columbia. Blueberry Creek was sampled as the outlet stream immediately below Nancy Greene Lake in Nancy Greene Provincial Park. Whether the species was more prevalent in the mainstem Columbia River before invasion of *F. virilis* is unknown (Larson *et al.* 2010). Fish and Trout lakes in Washington, documented by mtDNA sequencing in Larson *et al.* (2012), are the only locations from past sequencing work not vouchered at RBCM (Fig. 6). The eastern range extent of the species into the upper Columbia River watershed of British Columbia, Idaho, and Montana is unknown. *Pacifastacus okanaganensis* **sp. nov.** has never been detected west of the Cascade Mountains in coastal British Columbia or Washington.

Life history notes. Life history of *P. okanaganensis* **sp. nov.** has not been studied, and berried or ovigerous individuals are not included among the RBCM vouchers. *Pacifastacus okanaganensis* **sp. nov.** life history might be anticipated to broadly resemble other congeners, as slower-growing and with lower fecundity proportional to carapace length relative to members of the family Cambaridae native to eastern North America (Momot 1984).

Conservation status. *Pacifastacus okanaganensis* **sp. nov.** could be vulnerable to displacement by invasive *F. virilis,* common in the mainstem Columbia River of Washington (Larson *et al.* 2010) and recently discovered in this watershed in British Columbia (Phillips 2024). *Pacifastacus leniusculus* could also threaten *P. okanaganensis* **sp. nov.** with displacement, as it has impacted congeners in California (Bouchard 1977; Light *et al.* 1995; U.S. Fish and Wildlife Service 1988). *Pacifastacus okanaganensis* **sp. nov.** is known from more locations than *P. malheurensis* **sp.**

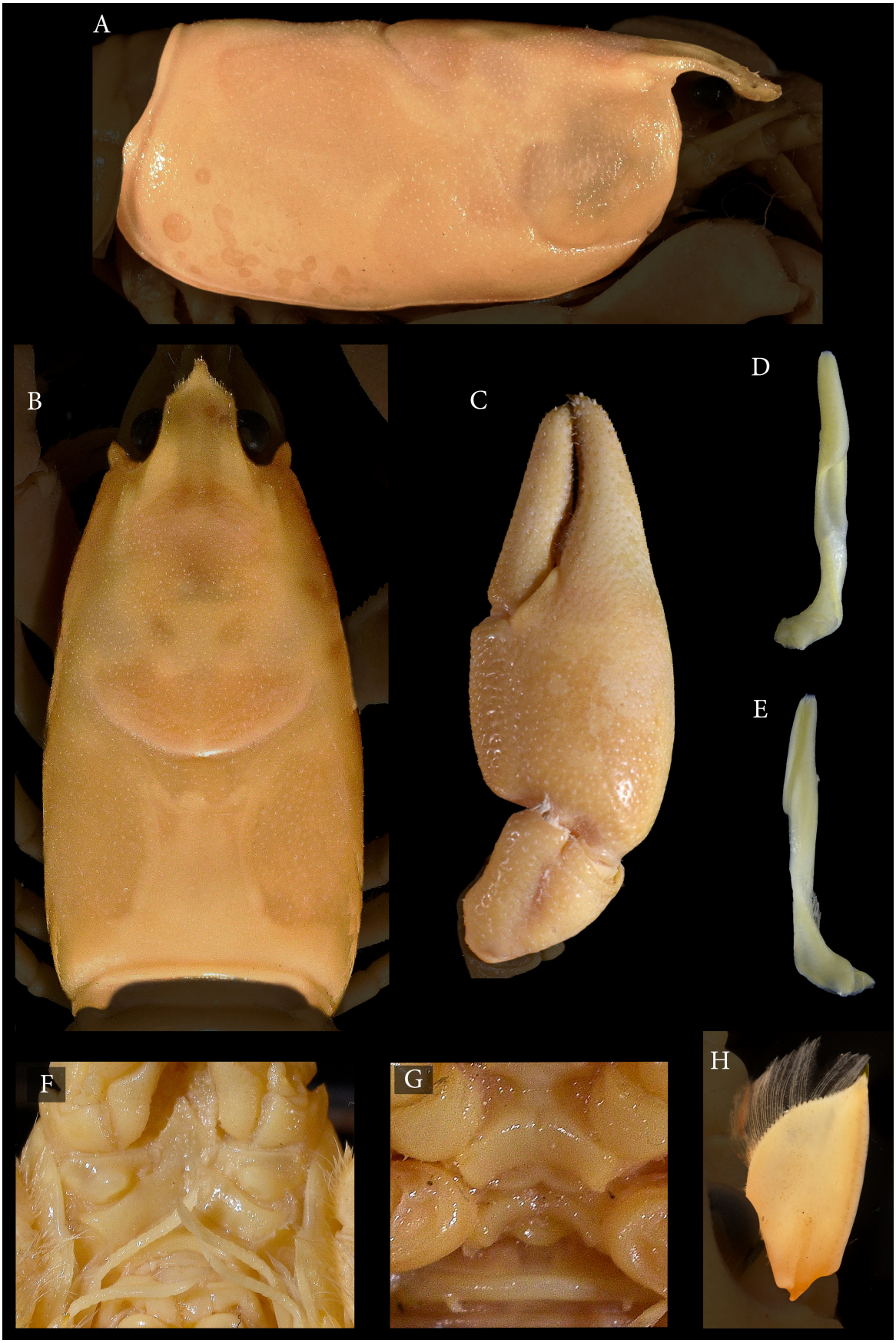

FIGURE 7. *Pacifastacus okanaganensis* **sp. nov.**, A–F, H: holotype, RBCM 012-00121-003; G; female allotype, RBCM 012-00121-004. Lateral carapace view (A); dorsal carapace view (B); right chelae (C); gonopods (D, E); epistome (F); annulus ventralis (G); and antennal scale (H).

nov., and many of these locations are relatively isolated, mid-elevation lakes that may be difficult for other crayfish species to spread into without the assistance of human introductions. As the magnitude and timing of displacement of *P. okanaganensis* **sp. nov.** by either *F. virilis* or *P. leniusculus* in the Columbia River watershed is unknown, we recommend a global IUCN conservation status of data deficient (IUCN 2012). We recommend rankings of Imperiled in both British Columbia and Washington because only four or five occurrences of *P. okanaganensis* **sp. nov.** are known from each of these jurisdictions (Fig. 6).

Crayfish associates. *Pacifastacus okanaganensis* **sp. nov.** was not collected in sympatry with other crayfish species by Larson *et al.* (2012) or Larson *et al.* (2016). Both *F. virilis* and *P. leniusculus* occur in the vicinity of *P. okanaganensis* **sp. nov.** populations, including in the mainstem Columbia River.

Relationships and comparisons. *Pacifastacus okanaganensis* **sp. nov.** is morphologically similar to *P. malheurensis* **sp. nov.** and *P. l. klamathensis* from coastal, southwestern Oregon and northwestern California. *Pacifastacus okanaganensis* **sp. nov.** generally has an acumen length less than 79.4% of anterior rostrum width, whereas *P. leniusculus* generally has an acumen length greater than 79.4% of anterior rostrum width (Fig. 3). *Pacifastacus okanaganensis* **sp. nov.** generally has a rostrum length greater than 18.85% of TCL, whereas *P. malheurensis* **sp. nov.** generally has a rostrum length less than 18.85% of TCL (Fig. 3). *Pacifastacus l. leniusculus* in particular are easy to differentiate from *P. okanaganensis* **sp. nov.** by the long, spiny rostrum and chelae with short, convex palms (Hobbs 1972). *Pacifastacus okanaganensis* **sp. nov.** has not been collected in sympatry with *P. malheurensis* **sp. nov.** or coastally distributed *P. l. klamathensis* and may be also differentiated by geographic range.

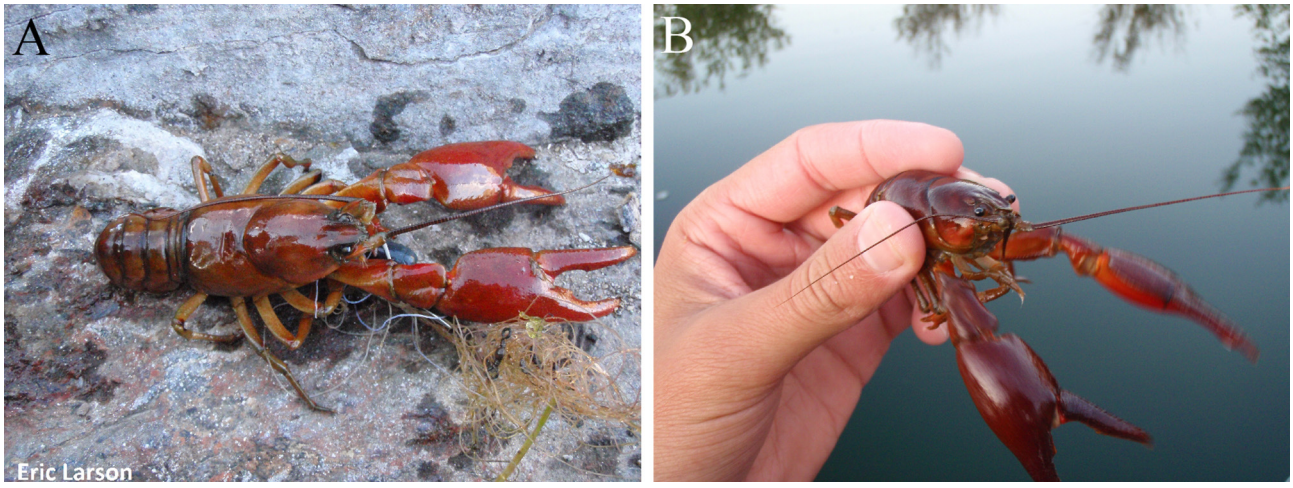

FIGURE 8. *Pacifastacus okanaganensis* **sp. nov.** from Fish Lake, Washington (A) and Park Lake, Washington (B) showing brick red color of habitus.

Discussion

New phylogenetic information derived here from genome skimming supports previous phylogenetic work (Larson *et al.* 2012, 2016) related to recognizing two highly distinct lineages of *P. leniusculus* as separate species, herein named *P. malheurensis* **sp. nov.** and *P. okanaganensis* **sp. nov**. In particular, the phylogenetic trees built on concatenated, highly repetitive nuclear RNA sequences largely corroborated the mitochondrial phylogeny of these crayfishes. Recognition of *P. malheurensis* **sp. nov.** and *P. okanaganensis* **sp. nov.** is supported by consistent results across a decade of phylogenetic analyses and motivated by urgent threats to both species from invasive crayfishes in the Pacific Northwest region (Larson *et al.* 2010; Messager & Olden 2018; Pearl *et al.* 2013).

We described *P. malheurensis* **sp. nov.** and *P. okanaganensis* **sp. nov.** as new species despite their similar morphology to *P. l. klamathensis.* Both new species were among those populations identified as *P. l. klamathensis* by Miller (1960), occurring from the Pacific Ocean-draining Klamath River in northern California and southwestern Oregon into the interior Pacific Northwest east of the Cascade Mountains north to Okanagan Lake in British Columbia (Larson *et al.* 2012). We declined to ascribe either *P. malheurensis* **sp. nov.** or *P. okanaganensis* **sp. nov.** to *P. klamathensis* because Larson *et al.* (2012) recovered a unique lineage of *P. leniusculus* from the Klamath River

and adjacent watersheds with *P. l. klamathensis* morphology, including in Steamboat Creek of the Umpqua River watershed included in this analysis. These crayfish, especially in the watershed of the Klamath Lake type locality, have precedent as the subspecies, *P. l. klamathensis*. *Pacifastacus leniusculus* in the Klamath River watershed and adjacent rivers should be investigated in more detail, particularly as they are likely affected by multiple invasive crayfishes in this region (Pearl *et al.* 2013). Similarly, distinct crayfishes from the Olympic Peninsula and Chehalis River of Washington, termed the Chehalis lineage by Larson *et al.* (2012) and represented by Wynoochee Lake in this paper, might be investigated as an appropriate match by geography and morphology to *P. l. trowbridgii*. As our analyses here demonstrate, morphological distinctions between these crayfishes are subtle, likely reflecting similar environmental pressures overlaid on a relatively recent pattern of divergence within the genus.

Research is needed at the interface of geography and ecology for both *P. malheurensis* **sp. nov.** and *P. okanaganensis* **sp. nov.** The northern range extent of *P. malheurensis* **sp. nov.** and southern range extent of *P. okanaganensis* **sp. nov.** are unknown. It is a common pattern among crayfishes that some species tolerate larger freshwater habitats with more predation pressure, whereas other species specialize on isolated, headwater, or disturbance-prone ecosystems (Creed 2006; Dorn & Trexler 2007; Flinders & Magoulick 2003). As such, the lower Columbia River may function as a dispersal barrier to both *P. malheurensis* **sp. nov.** and *P. okanaganensis* **sp. nov.**, especially if *P. leniusculus* is better able to exploit this large river habitat consistent with the historic distribution of *P. l. leniusculus* (Larson *et al.* 2012; Miller 1960). Other range boundaries for *P. malheurensis* **sp. nov.** and *P. okanaganensis* **sp. nov.** are also poorly known. *Pacifastacus malheurensis* **sp. nov.** could occur beyond the John Day River in other Columbia River tributaries of northeastern Oregon or southeastern Washington, like rivers draining the Blue Mountains. This species might also occur beyond the Harney Basin in other endorheic watersheds of the Great Basin. *Pacifastacus okanaganensis* **sp. nov.** may occur farther east into Columbia River tributaries of British Columbia, Idaho, and Montana than documented here, or alternatively, may have been excluded from these regions by glaciation, the Lake Missoula floods, or other factors (Larson *et al.* 2012; Larson & Williams 2015). Neither species has been documented from any locations west of the Cascade Mountains. Whether human introductions have affected contemporary distributions of these species is also unknown, although these species have not been documented among invasive *P. leniusculus* populations in Europe or Japan (Petrusek *et al.* 2017; Usio *et al.* 2016).

The genus *Pacifastacus* has experienced extinction of *P. nigrescens* (Bouchard 1977), ESA listing of *P. fortis* (Light *et al.* 1995; U.S. Fish and Wildlife Service 1988), and substantial range declines of both *P. connectens* and *P. gambelii* (Egly & Larson 2018). Displacement by invasive crayfishes has been implicated in each of these events. We are similarly concerned about extinction risk to *P. okanaganensis* **sp. nov.** and especially *P. malheurensis* **sp. nov.** from invasive crayfishes in western North America. Since its discovery in 2005 (Olden *et al.* 2009), *F. rusticus* has spread rapidly throughout the John Day River in Oregon (Messager & Olden 2018). Long-term persistence of *P. malheurensis* **sp. nov.** will require protecting isolated populations from further introduction and spread of *F. rusticus*, including those populations over drainage divides in the Harney Basin or potentially above crayfish dispersal barriers like waterfalls or road culverts within upstream tributaries of the John Day River (Foster & Keller 2011). Barrier construction or maintenance might be used to protect *P. malheurensis* **sp. nov.** from *F. rusticus* (Cowart *et al.* 2018; Manfrin *et al.* 2019). *Pacifastacus okanaganensis* **sp. nov.** may be threatened by populations of *F. virilis* or *P. leniusculus* that are common in the mainstem Columbia River (Larson *et al.* 2010; Phillips 2024). However, populations of *P. okanaganensis* **sp. nov.** in mid-elevation lakes of the Okanagan and Thompson plateaus may be easier to protect from spread of invasive crayfishes by connectivity management (e.g., Fausch *et al.* 2009) than *P. malheurensis* **sp. nov.** in free-flowing rivers of central Oregon. Lake outlet streams may be amenable to crayfish barrier construction, and fish-passable but crayfish-impassable barrier designs are possible (Frings *et al.* 2013; Cowart *et al.* 2018). Long-term persistence of both species will benefit from regulations and their enforcement to discourage further non-native crayfish invasions into their native ranges (DiStefano *et al.* 2023; Dresser & Swanson 2013; Ricciardi & MacIsaac 2022).

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