

Range extension for the region of sympatry between the nudibranchs *Hermisenda opalescens* and *Hermisenda crassicornis* in the northeastern Pacific

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Abstract

The mollusc nudibranch genus *Hermisenda* Bergh, 1879 was recently discovered to include three pseudocryptic species, dividing a single species *H. crassicornis* (sensu lato) into *H. crassicornis* Escholtz, 1831, *H. opalescens* J.G. Cooper, 1863, and *H. emurai* Baba, 1937. The species were distinguished by both genetic and morphological evidence, and the distribution of sampled animals suggested the three species had mostly distinct geographical ranges. Here, we report the presence of both *H. crassicornis* and *H. opalescens* in Barkley and Clayoquot Sounds, British Columbia, Canada, based on diagnostic characters and molecular data congruent with the differences described for these two species. This result extends the region of sympatry for the two species from northern California, USA, to, at least, Vancouver Island, British Columbia in 2016. Depending on how long this overlap has occurred, the possible northward expansion of *H. opalescens* would have implications for understanding the effects of short- or long-term environmental changes in ocean temperatures as well as complicating the interpretation of past neurobiological studies of *H. crassicornis* (sensu lato).

Key words: *Hermisenda*, distribution, pseudocryptic species, range shifts, Nudibranchia

Introduction

The heterobranch sea slug genus *Hermisenda* Bergh, 1879 was recently determined to include three pseudocryptic species: *H. crassicornis* Escholtz, 1831; *H. opalescens* J.G. Cooper, 1863; and *H. emurai* Baba, 1937 (Lindsay and Valdés 2016). The three species were distinguished by genetic analyses, morphological characters, and geographic ranges in the North Pacific (Table 1). This division has resurrected the three species names originally described in the genus that had previously been unified into a single trans-Pacific species, *H. crassicornis* (sensu lato) (O'Donoghue 1922). Beyond the biogeographic and evolutionary implications of recognizing the three species anew, the discovery that *H. crassicornis* (sensu lato) was a species complex may have important ramifications for interpreting more than 40 years of neurobiological study of the animals (e.g., Alkon 1973, 1980; Crow 2004;

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Table 1. Morphological differences among the three *Hermisenda* species as evaluated by Lindsay and Valdés (2016).

Character	<i>H. crassicornis</i>	<i>H. opalescens</i>	<i>H. emurai</i>
Range	Alaska–Northern California	Northern California–Sea of Cortez	Japan–Russian Far East
Body shape	Short	Short	Elongate
Body colour	Translucent to white	Translucent to white	Orange
Longitudinal stripe between rhinophores	Orange (light to bright in hue)	Orange (light to bright in hue)	Orange (dark to almost red)
Cerata background colour	Range from light to dark brown; may also be bright orange	Range from light to dark brown; may also be bright orange	Range from light to dark brown; may also be bright orange
Cerata tip colour	Not included in description	May or may not contain reddish to brown tipping	May or may not contain reddish to brown tipping
White cerata stripe	Present	Absent	Absent
Cerata group gaps	Small	Small	Large

Blackwell 2006; Cavallo et al. 2014; Gunaratne et al. 2014; Gunaratne and Katz 2016; Webber et al. 2017). It is possible that phenotypic heterogeneity amongst the species includes both neural and behavioural characters, and thus, care may be needed in integrating conclusions made in studies with animals from different locations. Gathering information on the respective species ranges is important for such retrospective work and would also be valuable in advance of further studies of *Hermisenda* (biogeographical, electrophysiological, or otherwise).

The evidence presented by Lindsay and Valdés (2016) indicated that both *H. crassicornis* and *H. opalescens* are present along the Pacific coast of North America. *H. opalescens* is a more southern species, found between Baja, California, Mexico, and Bodega Bay, California, USA, whereas *H. crassicornis* ranges from Alaska to central California. Based on the specimens examined, the species’ ranges should overlap along a relatively short stretch of coastline in central and northern California. However, unverified photographic records from the world wide web suggest *H. opalescens* may be present farther north (inaturalist.org/taxa/494603-Hermisenda-opalescens). The photos show specimens lacking white stripes on their cerata, matching the only morphological character distinguishing *H. opalescens* (stripe absent) from *H. crassicornis* (stripe present), as described by Lindsay and Valdés (2016). The northwestern Pacific species, *H. emurai*, also lacks the white stripe, but has a different body shape, colouration, and different spacing between groups of cerata, eliminating it from consideration. Furthermore, our initial observations in the summer of 2016 suggested that *H. crassicornis* (with a stripe) and *H. opalescens* (without a stripe) were both common on the west coast of Vancouver Island, British Columbia, Canada. Thus, our goals for this study were (i) to survey these locations on Vancouver Island to determine which species were present, and (ii) to confirm whether any morphological differences between species were consistent with those noted by Lindsay and Valdés (2016).

Materials and methods

Specimen collection

All animal collection and care followed the Canadian Council for Animal Care guidelines. Specimens were collected opportunistically from seven locations in Barkley Sound and one location in Clayoquot Sound, British Columbia (Fig. 1; Table S1). Collection sites included floating docks, intertidal zones, and subtidal habitats (sampled by SCUBA), spanning sites from 0 to 30 m in depth. Initial specimen collection occurred between 1–15 July 2016 at all sites, followed by further specimen collection at only a subset of sites in Barkley Sound between 25 August 2016 and 1 September 2016.

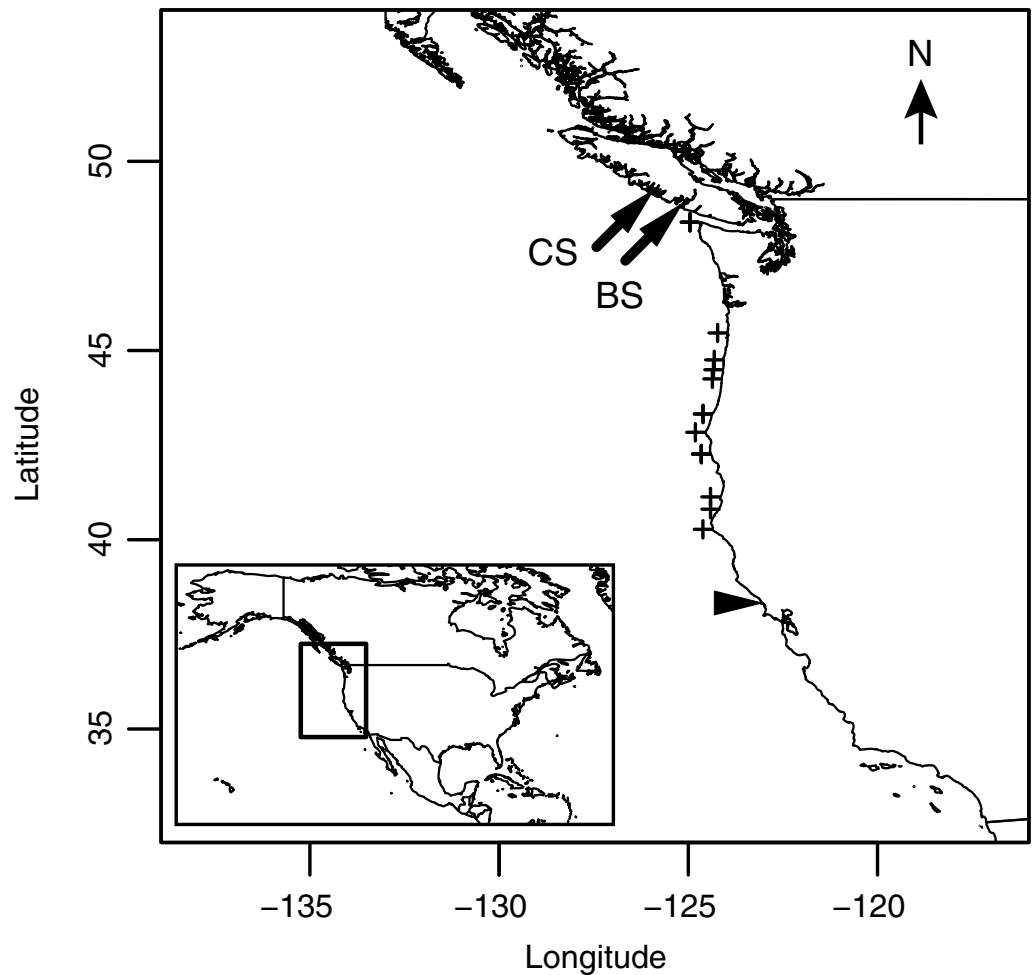


Fig. 1. Locations in Clayoquot Sound (CS) and Barkley Sound (BS), British Columbia where both *Hermisenda crassicornis* and *H. opalescens* were collected in 2016 (arrows). Previous northerly range limit for *H. opalescens* was Bodega Bay, California (arrowhead). Also shown are additional locations of anecdotal observations of *H. opalescens* (+) in Oregon and Washington in 2015 or 2016 (see Discussion). Inset shows location of map relative to North America. The map was produced in R v3.4.3 (R Core Team 2017) with the following packages: maps (Deckmyn et al. 2018), mapdata (Brownrigg et al. 2018), maptools (Bivand et al. 2017a), rgeos (Bivand et al. 2017b), scales (Wickham and RStudio 2017), raster (Hijmans et al. 2017), and shape (Soetaert 2018).

Measurements

Following collection, animals from different sites were individually segregated in mesh containers and kept in flow-through sea tables at Bamfield Marine Sciences Centre until measured. All individuals were photographed with either an Olympus STYLUS TG-3 Tough or a Pentax WG-3 camera. For morphometrics, photographs were taken of the dorsal, lateral, and ventral views (while specimens were crawling on a transparent surface). For each specimen, several morphological observations and measurements were tabulated, including ceratal and body colouration patterns, foot length (at the midline), foot width (at its widest point), foot area and perimeter (from a manual outline of the foot), and gap length between the first and second ceratal clusters (this was only measured in some specimens due to difficulties visualizing the gap). Specimens with broken tails were excluded from allometric analyses.

Software and statistical analyses

All measurements were made in ImageJ v1.50 and analyzed in R v3.3.0 with the ggplot2 package (Wickham 2009; R Core Team 2016). To assess whether foot width differed between the two species after adjusting for differences in foot length, we used analysis of covariance (ANCOVA) on \log_{10} -transformed data with species (identified based on the presence or absence of stripes on the cerata) and foot length as explanatory variables and foot width as the response variable (Jasiński and Bazzaz 1999).

DNA extraction, sequencing, and data analysis

Tissue samples preserved in 95% ethanol from five specimens matching the external colour pattern of both *H. crassicornis* ($n = 3$) and *H. opalescens* ($n = 2$) were obtained. One of each species was submitted to the Canadian Center for DNA Barcoding, University of Guelph, Canada (CCDB) for sequence analysis. DNA extraction, PCR amplification of the cytochrome *c* oxidase I (COI) barcode region, and DNA sequencing were performed at the CCDB following standard high-throughput protocols (Steinke et al. 2016). Complete voucher data, images, and GenBank accession numbers for these two specimens and all other *Hermisenda* records on BOLD (Ratnasingham and Hebert 2007) are available in the public dataset DS-HERMCOI under the DOI: [dx.doi.org/10.5883/DS-HERMCOI](https://doi.org/10.5883/DS-HERMCOI).

We processed the remaining three specimens ourselves. Genomic DNA was extracted using a DNeasy kit (Qiagen, Hilden, Germany) following the manufacturer protocols. Polymerase chain reaction (PCR) was used to amplify fragments of gene COI in a final volume of 50 μ L per sample. Each PCR sample included deionized water, 10 \times DreamTaq buffer, 10 μ mol/L dNTPs, 10 μ mol/L universal primers HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and LCOI490 (5'-GGTCAA CAAATCATAAAGATATTGG-3') (Folmer et al. 1994), and 5 mg/mL DreamTaq (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to amplify the regions of interest. The samples underwent an initial denaturation step of 95 $^{\circ}$ C for 3 min followed by an annealing period of 35 cycles of 94 $^{\circ}$ C for 45 s, 50 $^{\circ}$ C for 45 s, and 72 $^{\circ}$ C for 2 min, and a final extension period of 72 $^{\circ}$ C for 10 min. Agarose gel electrophoresis with ethidium bromide was used to detect the presence of DNA. Sequencing was outsourced to Source Bioscience Inc. (Santa Fe Springs, California, USA). Sequences were assembled and edited using Geneious Pro R8 (Kearse et al. 2012) and deposited in GenBank (accession numbers MH137939–MH137941). The geographic distribution and structure of the haplotypes was visualized by producing a haplotype network using the program PopArt v. 1.7 (Leigh et al. 2015) using the TCS option. Sequences were compared with data from both species reported by Lindsay and Valdés (2016) and obtained from GenBank (Table S2).

Results

Specimens of *Hermisenda* collected from both Barkley and Clayoquot Sounds included members of both *H. crassicornis* (sensu stricto) and *H. opalescens* (Fig. 2), based on the presence or absence of longitudinal white stripes on the anterior portion of the cerata (Tables 1 and 2). In July 2016, the proportion of individuals identified as *H. crassicornis* (with white ceratal stripes) was 81% (51 out of a total of 63) in Barkley Sound and 25% (8 out of a total of 32) in Clayoquot Sound were identified as *H. crassicornis* (Fig. S1). The remaining animals lacked white ceratal stripes and, thus, were identified as *H. opalescens*. Specimen collection in late August and early September 2016 in Barkley Sound recovered 96% (71 out of a total of 74) *H. crassicornis* and only 4% *H. opalescens*.

Our observations consistently showed one additional colouration difference between the species, apart from the diagnostic presence or absence of white ceratal stripes (Table 2). *H. crassicornis* had a tapered orange section at the distal ends of the cerata, with a very small translucent section at the apex. In contrast, in *H. opalescens*, the sections toward the distal ends of the cerata were distinctively



Fig. 2. Two *Hermissenda* congeners collected in Clayoquot Sound, British Columbia. Left: *H. opalescens* lacks longitudinal white stripes on its cerata. Right: *H. crassicornis* has distinctive anterior white stripes on its cerata. Scale = 5 mm.

Table 2. Distinguishing characters *Hermissenda crassicornis* and *H. opalescens* sampled on the west coast of Vancouver Island in the summer of 2016.

Character	<i>H. crassicornis</i>	<i>H. opalescens</i>
White stripe on cerata	Present	Absent
Colour on tip of cerata	Orange	White
Foot width relative to length	Narrower	Wider

white (again, with the small translucent section at the very tips). The two species otherwise shared patterns of colouration. The longitudinal stripe between the rhinophores was orange with a blue outline in all individuals of both species, whereas the background colour of the cerata varied from black to brown to orange among individuals of both species.

Measurements of various body dimensions also showed some consistent differences between the specimens we collected. Animals identified as *H. opalescens* were consistently larger than those identified as *H. crassicornis* (e.g., foot length with no stripe: 46 ± 2.3 mm, and with stripe: 29 ± 0.8 mm; mean \pm SE) with minimal overlap in size between the two species in our sample. Foot shape differed between species (Fig. 3); relative to a standard foot length, *H. crassicornis* had proportionally narrower feet than *H. opalescens* (ANCOVA species effect: $F_{1,112} = 677.99$, $P < 0.0001$; Fig. 4), and the slope of the relationship between foot length and foot width did not differ between the species (ANCOVA interaction term: $F_{1,112} = 1.42$, $P = 0.2364$; Fig. 4). No other morphometric differences were evident.

COI sequence data revealed the existence of five different haplotypes among the five specimens sequenced. In the haplotype network analysis (Fig. 5), two of the sequences (from animals without ceratal stripes) clustered with other specimens of *H. opalescens* obtained by Lindsay and Valdés (2016)

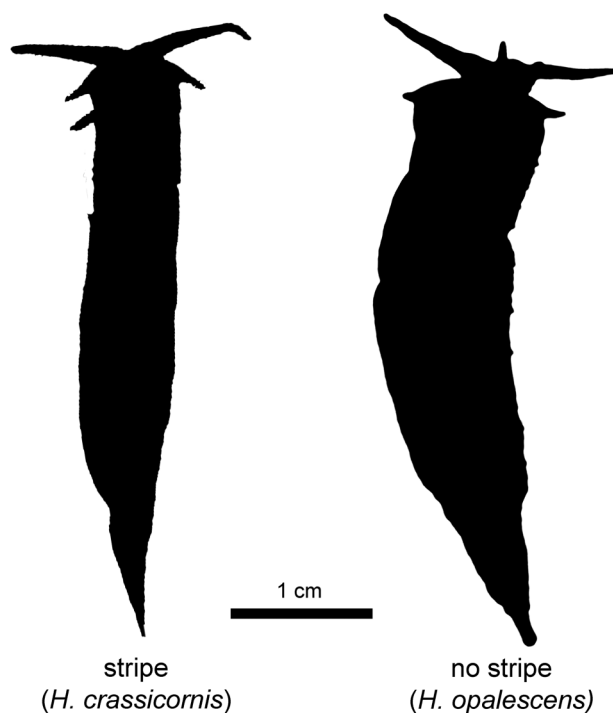


Fig. 3. Representative foot silhouettes show the differing foot length:width ratios for similarly sized individuals of *Hermissenda crassicornis* and *H. opalescens*.

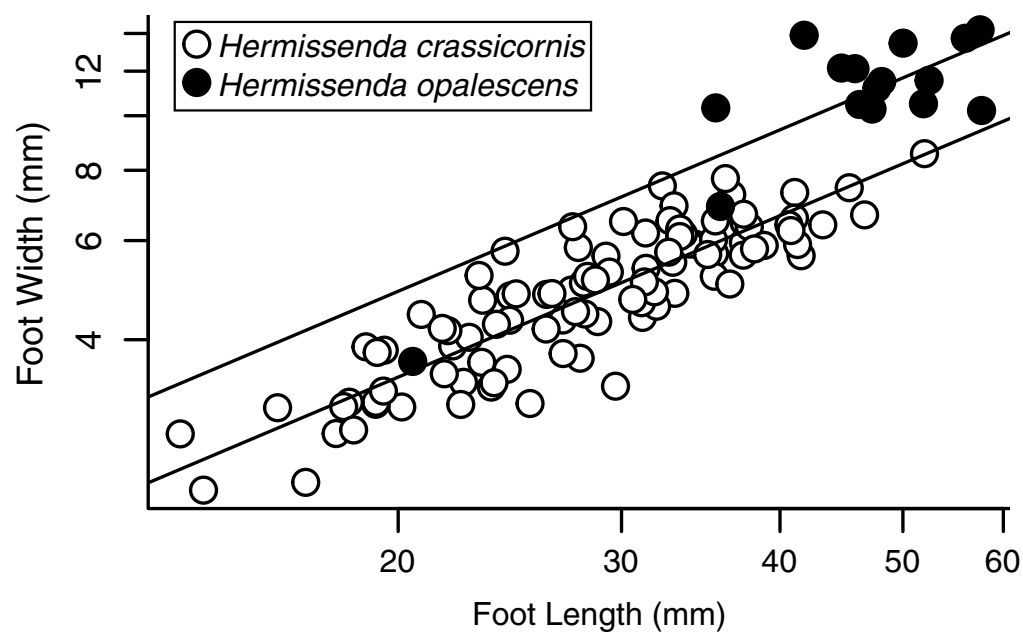


Fig. 4. Foot form of *Hermissenda crassicornis* (open circles) and *H. opalescens* (closed circles) sampled on the west coast of Vancouver Island in the summer of 2016. Lines show fit of ANCOVA after the non-significant interaction was removed from the model.

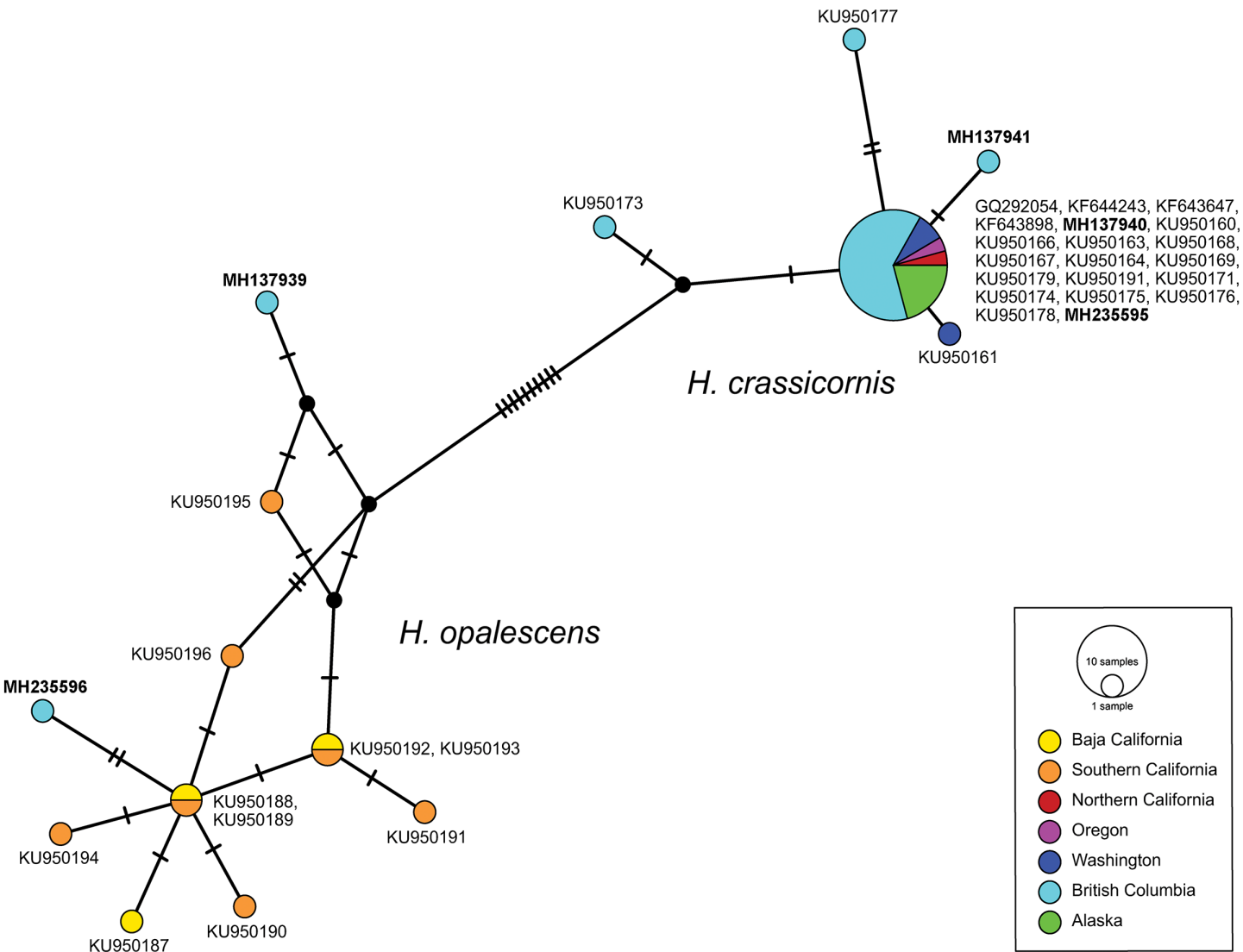


Fig. 5. Haplotype network with the geographic origin of the sequences colour coded for specimens from this study and specimens from Lindsay and Valdés (2016). Specimens from the current study are indicated in bold and group with their respective species as expected according to the presence (*H. crassicornis*) or absence (*H. opalescens*) of the white stripe. Circles are proportional to the number of haplotypes. Black circles correspond to hypothetical haplotypes not detected in the samples. Transverse lines indicate mutational substitutions.

from Mexico and southern California. The other three sequences (from animals with white ceratal stripes) clustered with samples of *H. crassicornis* ranging from northern California to Alaska.

Discussion and conclusions

We observed two species of *Hermisenda* on the west coast of Vancouver Island in the summer of 2016. The characteristics for each were consistent with those described for *H. crassicornis* (with white stripes on the cerata) and *H. opalescens* (without the stripes) (Lindsay and Valdés 2016) and field identification was confirmed with molecular data from five individuals belonging to the two species. Moreover, we identified two additional differences in morphology: colouration of the distal ends of

the cerata (excluding the very tips of the cerata, which were translucent in both species), and foot shape (at least for larger individuals). In the habitats we sampled, *H. crassicornis* had white ceratal stripes and an orange section on the tips of their cerata, and were smaller overall with proportionately narrower feet. *H. opalescens* had no white stripes on their cerata, the distal sections of the cerata were white, and the animals were consistently larger with proportionately wider feet. This difference in foot shape may or may not be a consequence of genuine differences between the species. Most of the *H. crassicornis* specimens we collected were smaller than the *H. opalescens* specimens we collected, so we cannot be certain that the difference in foot shape is maintained at comparable sizes. Instead, it may be that a change in shape arises during the growth of both species, but only the *H. opalescens* we collected had grown large enough to display the wider foot shape. It is, therefore, possible that the different *Hermisenda* foot shapes are a consequence of comparing different stages in parallel ontogenies (or some other form of shared phenotypic plasticity). However, no changes in foot shape have been reported in past studies of adult growth of *Hermisenda* spp. (Harrigan and Alkon 1978). Further investigation of the foot shape of small *H. opalescens* and large *H. crassicornis* will resolve whether the species consistently differ in foot shape at all sizes.

The presence of *H. opalescens* on the west coast of Vancouver Island constitutes a range extension for the species, which was previously described as only occurring as far north as Bodega Bay, California. A number of other anecdotal reports have also recorded *Hermisenda* individuals lacking ceratal stripes in both Oregon and Washington (J. Goddard, B. Green, K. Fletcher, D. Miller, T. Prestholdt, personal communication, 2017; Fig. 1). One possible reason for this apparent range extension is that *H. opalescens* may always have had a more northerly range. *Hermisenda* populations rapidly increase and decrease, and earlier sampling efforts may simply have missed the blooms of *H. opalescens* north of California. However, photos taken before 2016 by ourselves and contributors to The Sea Slug Forum (seaslugforum.net) show only animals with white ceratal stripes in Washington, Oregon, and British Columbia (Lindsay and Valdés 2016). This suggests that the presence of *H. opalescens* in British Columbia is a novel phenomenon. Northward range shifts in the northeast Pacific have also been documented over recent years for several other nudibranchs (Goddard et al. 2016), mole crabs (Wonham and Hart 2018), copepods (Peterson et al. 2017), several species of fish (Auth et al. 2018; Halpin et al. 2018a), and cetaceans (Halpin et al. 2018b). In the summer of 2016, we also recorded anecdotal observations of other southern species in the waters near the Bamfield Marine Sciences Centre on Vancouver Island, including the nudibranch, *Dirona picta* MacFarland, 1905 (C. Tamis, R. Wyeth, personal observation, 2016), striped shore crabs, *Pachygrapsus crassipes* Randall, 1840 (C. Neufeld, T. Eastham, personal observation, 2016), and brown pelicans *Pelecanus occidentalis* Linnaeus, 1766 (S. Gray, personal communication, 2016).

If the geographic range of *H. opalescens* is shifting northward, several possible mechanisms could explain this phenomenon. Given the broad diet of *Hermisenda* spp. (Avila and Kuzirian 1995), distributions are probably not restricted by prey availability, but rather driven by other factors such as survival and dispersal of the planktonic larvae (Harrigan and Alkon 1978), combined with adult tolerances for abiotic conditions. Optimal laboratory culture conditions have been explored for both larvae and adults (Harrigan and Alkon 1978; Rutowski 1983; Avila et al. 1997, 1998; Avila 1998). (The primary source of animals for these past studies was near Monterey Bay, California, approximately 200 km southward of the region of overlap for the two species (Lindsay and Valdés 2016), and therefore we presume they apply to *H. opalescens*.) However, these studies do not explore environmental tolerances that might set geographic limits for larvae or adults. Thus, we have limited information to infer range limits for either species without further experimentation.

An alternative approach could be to determine how conditions in northern regions are changing relative to historical conditions at southern locations with established records of *H. opalescens*.

Long-term warming trends in the Pacific, periodic warming as part of El Niño events, or the short-term North Pacific marine heat wave of 2014–2015 (Johnstone and Mantua 2014; Di Lorenzo and Mantua 2016; Jacox et al. 2016) all could have allowed *H. opalescens* to settle and grow successfully in the normally colder waters of Vancouver Island. Historically, the average annual sea surface temperatures are 1–3 °C warmer near Monterey Bay than Vancouver Island (NASA Worldview, worldview.earthdata.nasa.gov/), matching the approximately +2 °C temperature anomaly that occurred in 2015 (Di Lorenzo and Mantua 2016). Changes in ocean current patterns are also important to consider, given they have been shown to be a key driver in nudibranch recruitment, at least in California (Schultz et al. 2011). Either an increase in northward advection of the California Current (Kosro 2002; Schwing et al. 2003) or changes in upwelling (Jacox et al. 2015) and cross-shelf advection (causing longer pelagic transport of larvae) could lead to northern range expansions, as long as no thermal tolerance limits were encountered. Further monitoring is needed to determine if *H. opalescens* populations persist, and analysis of historical records and archival specimens are needed to establish more clearly when and where *H. opalescens* has been present north of California.

Our observations have important implications for interpreting other studies of *Hermisenda*. At least temporarily, the region of potential sympatry between *H. crassicornis* and *H. opalescens* extended from northern California to Vancouver Island in 2016. Depending on how long this range overlap has existed, earlier studies purportedly of *H. crassicornis* may have in fact been studying *H. opalescens* or a mixture of the two species. In particular, interpretations of the extensive history of neurobiological research with animals collected from various locations in California should now consider the potential effects of species-specific differences in neural circuits or behaviour (e.g., Alkon 1973, 1980; Cavallo et al. 2014; Gunaratne et al. 2014; Gunaratne and Katz 2016; Webber et al. 2017). In the future, careful species identification is required for all studies of *Hermisenda* along the northeast Pacific coast of North America, and further sampling is needed to verify both the northern range boundary for *H. opalescens* and the southern range boundary for *H. crassicornis*.

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Author contributions

EMM, KAM, NBS, CJN, TME, and RCW conceived and designed the study. EMM, KAM, NBS, ALKE-P, DS, and PDNH performed the experiments/collected the data. EMM, KAM, NBS, CJN, ALKE-P, AV, and RCW analyzed and interpreted the data. DS, PDNH, AV, and RCW contributed resources. EMM, KAM, NBS, CJN, TME, AV, and RCW drafted or revised the manuscript.

Competing interests

The authors have declared that no competing interests exist.

Data accessibility statement

All relevant data are within the paper, in GenBank (ncbi.nlm.nih.gov/genbank/; accession numbers MH137939–MH137941), and on BOLD (complete voucher data, images, and GenBank accession numbers for the two specimen samples from this study sent to the Canadian Center for DNA Barcoding (see Methods) and all other *Hermisenda* records on BOLD (Ratnasingham and Hebert 2007) are available in the public dataset DS-HERMCOI under the DOI: dx.doi.org/10.5883/DS-HERMCOI).

Supplementary Materials

The following Supplementary Material is available with the article through the journal website at doi:[10.1139/facets-2017-0060](https://doi.org/10.1139/facets-2017-0060).

Supplementary Material 1

Supplementary Material 2

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