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Complex interplay of ancient vicariance and recent patterns of geographical speciation in north-western North American temperate rainforests explains the phylogeny of jumping slugs (*Hemphillia* spp.)

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The history of the currently disjunct temperate rainforests of the Pacific Northwest of North America has shaped the evolution and diversity of endemics. This study focuses on how geological and climatic perturbations have driven speciation in the area by isolating lineages. We investigated the phylogenetic relationships and historical biogeography of the endemic jumping slugs (genus *Hemphillia*) using a multi-locus phylogeny. We evaluated the spatial distribution and divergence times of major lineages, generated ancestral area probabilities and inferred the biogeographical history of the genus. Our study revealed eight genetic lineages that formed three clades: one clade consisting of two Coast/Cascade lineages, and two reciprocally monophyletic clades that each contain a Coast/Cascade and two Rocky Mountains taxa. The results of the biogeographical analysis suggest that the ancestral range of the genus occupied Coast/Cascade habitats and then spread across into Northern Rocky Mountain interior habitats with subsequent fragmentations isolating coastal and inland lineages. Finally, there have been more recent speciation events among three lineage pairs that have shaped shallow structures of all clades. We add to our knowledge of the biogeographical history of the region in that we discovered diversification and speciation events that have occurred in ways more complex than previously thought.

ADDITIONAL KEYWORDS: endemism – *Hemphillia* – Pacific Northwest – refugia – temperate rainforest.

INTRODUCTION

North-western North America supports temperate rainforests in both its Pacific coast and northern Rocky Mountain interior regions. These forests are dominated by western hemlock (*Tsuga heterophylla*) and western red cedar (*Thuja plicata*) and contain many other endemic plant and animal taxa (e.g. DellaSala, 2011). These two disjunct rainforests are currently separated by xeric habitats of the Columbia Plateau (Graham, 1993, 1999), a flood basalt, shrub-steppe grassland spanning 300 km between the Coast/Cascade ranges and northern Rockies. This plateau

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formed from successive flows of basalt between 6 and 17 Mya (Tolan *et al.*, 2009), and the Cascades Range uplift between 4 and 7 Mya (Priest, 1990). The latter generated the current rain shadow that further aridified the Columbia Plateau, which reached near-modern conditions by 4 Mya (Ashwill, 1983), and the transformation of the Plateau into a xeric sage-shrub habitat was complete by ~2 Mya (Brunsfeld *et al.*, 2001). The currently disjunct mesic forests of the Coast/Cascade Mountains and northern Rocky Mountains have thus been spatially isolated since xerification became complete. Subsequently, throughout the Pleistocene (2.6–0.012 Mya), the region was heavily affected by glacial cycles, and the Plateau experienced repeated flooding from proglacial Lake Missoula (Waitt, 1985; O'Connor & Baker, 1992; Booth *et al.*, 2003). Most of the northern portions of the current range of the rainforest were covered by glaciers and smaller alpine glaciers formed in mountains during the Pleistocene.

These dramatic changes in the landscape have strongly affected the diversification patterns of rainforest endemics. Indeed, such taxa exhibit a phylogeographical break (*sensu* Swenson & Howard, 2005) between the Coast/Cascade and the northern Rockies (reviewed by Brunsfeld *et al.*, 2001). This division has been explained by ancient vicariance caused by the aridification of the Columbia Plateau ecoregion, which has probably been unsuitable for dispersal between mountain ranges throughout the Pleistocene (Brunsfeld *et al.*, 2001; Carstens & Richards, 2007) and into the Holocene. Phylogeographical studies of some regional endemics show Pacific coast and interior populations as being genetically differentiated (Nielson *et al.*, 2001; Carstens *et al.*, 2005; Steele *et al.*, 2005), suggesting a pre-Pleistocene vicariance. Likewise, research on other non-endemic plant (Li & Adams, 1989; Albach *et al.*, 2006) and animal (Demboski & Cook, 2001; Barrowclough *et al.*, 2004; Galbreath *et al.*, 2009; Kerhoulas *et al.*, 2015) species from the region have uncovered phylogeographical divisions between the Coast/Cascade and northern Rockies phylogroups. Conversely, the phylogeography of other taxa suggests the presence of gene flow across the Columbia Basin (e.g. Ruffley *et al.*, 2018), with the disjunction in some species having occurred via post-Pleistocene dispersal (e.g. Carstens *et al.*, 2005; Smith *et al.*, 2017).

For taxa with a long history (pre-Pleistocene) in inland mesic forests, the Pleistocene glacial cycles may have resulted in compartmentalized refugia in the northern Rockies (Brunsfeld *et al.*, 2001). Specifically, isolation would have been promoted by montane glaciers, which were extensive in the Rocky Mountains north-west of the Wyoming Basin during the Last Glacial Maximum (LGM; Porter *et al.*, 1983; Brouillet & Whetstone, 1993). In this scenario, populations of the northern Rockies would be expected to exhibit strong phylogeographical substructure and the presence of divergent lineages associated with different mountain systems. Such patterns have been identified in the inland endemics Constance's bittercress (*Cardamine constancei*; Brunsfeld & Sullivan, 2005), and Rocky Mountain tailed frogs (*Ascaphus montanus*; Nielson *et al.*, 2006, Metzger *et al.*, 2015), where phylogroups are consistent with the existence of a complex glacial refugium with multiple compartments.

To date, there has been considerable biogeographical research in the area, but the taxa previously examined typically have a high dispersal capacity. In contrast, there are many small, endemic animals that live hidden and isolated, such as terrestrial invertebrates. Many of these organisms have a very low dispersal capacity, and therefore are good systems to provide additional insight into the biogeographical history of the Pacific Northwest (PNW). Among these invertebrates, molluscs exhibit substantial diversity and endemism within the PNW (Pilsbry, 1948; Frest & Johannes, 1995; Burke & Leonard, 2013). In particular, many endemic gastropod taxa occur in the disjunct rainforest ecosystems of the PNW (Burke & Leonard, 2013). Here, we conduct phylogenetic and biogeographical analyses on jumping slugs of the genus *Hemphillia*, a terrestrial gastropod genus that is endemic to temperate rainforests in the PNW. *Hemphillia* slugs are appropriate species for understanding the influence of the landscape on the diversification of species associated with the PNW disjunct rainforests because species are spatially structured and restricted to the region. We aim to unravel the complex interplay between environmental change and geographical speciation in PNW rainforests by using *Hemphillia* as model taxa. To do this, we investigate the origin of the major *Hemphillia* clades in space and time, test whether the timing of lineage diversification occurred in concert with the progression of the PNW ecosystem, and consider phylogeographical concordance with other co-distributed, endemic taxa.

MATERIAL AND METHODS

Study system

Species of *Hemphillia* (jumping slugs; Fig. 1) have traditionally been separated into two species-groups. One group, including *H. burringtoni* (Pilsbry, 1948) and *H. glandulosa* (Bland & Binney, 1872), is formed by smaller-bodied taxa known from western Washington and adjacent parts of western Oregon and western British Columbia (Burke & Leonard, 2013). A third species within this group, *H. pantherina* (Branson, 1975), is of uncertain status as it was described from a

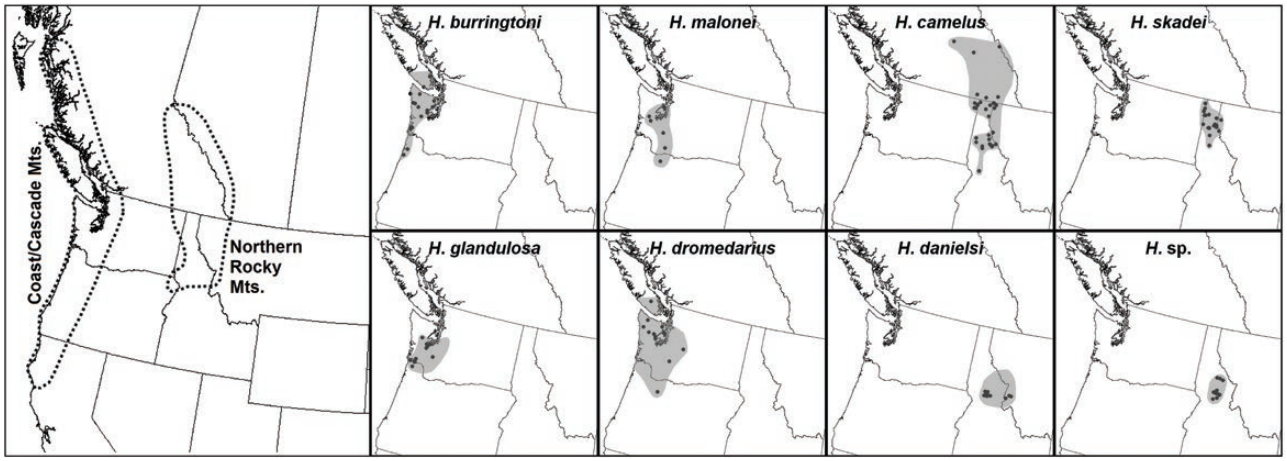

Figure 1. Area of study and *Hemphillia* sampling used in this study. Shaded areas represent distributions for *H. burringtoni*, *H. glandulosa*, *H. camelus*, *H. danielsi*, *H. dromedarius* and *H. malonei*, adapted from Burke & Leonard (2013), and tentative distributions for *H. skadei* and *H.* sp.

single specimen and is now viewed as not warranting specific recognition (Burke 2015, T. E. Burke, personal communication, 2017). The second group is composed of larger-bodied taxa and includes *H. camelus* (Pilsbry & Vanatta, 1898), *H. danielsi* (Vanatta, 1914), *H. dromedarius* (Branson, 1972), *H. malonei* (Pilsbry, 1917), the newly described *H. skadei* (Lucid *et al.*, 2018) and a suspected new species that closely resembles *H. danielsi* (Kelley *et al.*, 1999; Burke & Leonard, 2013), hereafter referred to as *H.* sp. Both *H. dromedarius* and *H. malonei* have Coast/Cascades Mountain distributions whereas the other species occur in the interior Rocky Mountain forests of south-eastern British Columbia, north-eastern Washington, western Montana and northern Idaho.

Taxon sampling and molecular data

We obtained data from 200 *Hemphillia* specimens gathered from field or museum collections (Fig. 1; *H. burringtoni* = 20, *H. glandulosa* = 13, *H. camelus* = 43, *H. danielsi* = 18, *H. dromedarius* = 14, *H. malonei* = 14, *H. skadei* = 32 and *H.* sp. = 46; (Supporting Information, Appendix S1). Field personnel preserved specimens in 70% ethanol and identified all ethanol-preserved specimens based on external morphological characters and geographical range following Burke & Leonard (2013). Museum specimens were identified in a similar manner by their respective collectors. For details of specimen IDs, localities, museum catalogue numbers and GenBank accession numbers, see Supporting Information (Appendix S1). Total DNA was extracted from the foot of each specimen (N = 156; 10–15 mg) using the DNeasy Blood and Tissue Kit (Qiagen) per the manufacturer's protocols. Partial sequences of the mitochondrial cytochrome *c* subunit I (*COI*) gene, mitochondrial 16S rRNA gene, nuclear internal transcribed spacer 1 (ITS1) marker and nuclear *actin* gene were amplified by PCR using the primers listed in Table 1. All PCRs were carried out in 50-μL reactions containing 3 μL DNA, 37.75 μL water, 5 μL buffer, 1 μL of 25 mM $MgCl_2$, 1 μL of 10 mM dNTPs (Thermo Fisher Scientific), 1 μL of 10 mM forward and reverse primer, and 0.25 μL of 5 U/μL of Taq polymerase (New England Biolabs). PCR amplification consisted of an initial denaturation step at 95 °C for 2 min, followed by 30 cycles of a denaturation step at 95 °C for 35 s, an annealing step (Table 1) for 60 s and an elongation step at 72 °C for 45 s, and a final extension step at 72 °C for 5 min. Amplicons were electrophoresed in a 1% agarose gel to verify the amplifications and cleaned using the Qiaquick PCR cleanup kit (Qiagen). Bi-directional DNA sequencing was carried out by Eurofins (eurofinsgenomics.com) MWG Operon using the ABI Big Dye Terminator kit (v.3.1) and an automated DNA sequencer (model ABI 3730 XL). Sequences produced by Eurofins were visually examined and edited with Chromas v.2.6.2 (Technelysium, http://www.technelysium. com.au/chromas.html). Consensus sequences were then produced from both forward and reverse strands. To these data we added a set of mitochondrial (mtDNA) sequences from an additional 44 *Hemphillia* specimens generated according to the methods described by Wilke & Duncan (2004) (Supporting Information, Appendix S1). Multiple sequence alignments were constructed for each locus separately using MAFFT online (http://www.ebi.ac.uk/Tools/msa/mafft/). In the 16S and ITS1 data sets, many regions were too divergent to be aligned across lineages, and therefore we used the Gblocks algorithm (Castresana, 2000; http://molevol.cmima.csic.es/castresana/Gblocks_server.

Table 1. Oligonucleotide sequences and annealing temperatures used for amplification of genetic markers for this study

Locus	Primer	Sequence (5′→3′)	Annealing temperature (°C)	Reference
COI	LCO1490	TAAACTTCAGGGTGACCAAAAAATCA	52	Folmer *et al.* (1994)
	HCO2198	GGTCAACAAATCATAAAGATATTGG		
16S	16Sbr-H	CCGGTCTGAACTCAGATCACGT	47.5	Lucid *et al.* (2018)
	16Sar-L	CGCCTGTTTATCAAAAACAT		
ITS1	ITS1F	GCTGCGTTCTTCATCGATGC	52	Armbruster *et al.* (2000); Mumladze *et al.* (2013)
	ITS1R	TAACAAGGTTTCCGTAGGTGAA		
actin	ActinA_S	ATGACATGGAGAAGATCTGGC	51.5	Rowson *et al.* (2011)
	ActinBAS	TCCATACCAAGGAAAGATGGC		

html) to eliminate ambiguous regions and extract the conserved regions for subsequent analysis. No indels or premature stop codons were found in the *COI* and *actin* protein coding genes.

Phylogenetic inference

We conducted preliminary analyses to reconstruct gene trees for the four markers to assess potential incongruence. Each of the four separate data alignments was subjected to maximum-likelihood (ML) phylogenetic estimation. We used the automodel command in PAUP* v.4.0a152 (preview release; Swofford, 2003) to select a model of nucleotide sequence evolution using the Bayesian Information Criterion and decision theory (Minin *et al.*, 2003). The GTR+I+Γ model was specified for *COI*, GTR+Γ for 16S, JC+I for ITS1 and K2P+I+Γ for *actin*. ML analyses were performed in Garli (Zwickl, 2006) using default parameters, and each ML tree was first determined by conducting ten replicate runs with random starting trees. Node support was assessed using 100 bootstrap replicates with two tree searches per bootstrap. We used the resulting ML phylogenies to test the assumption that each data set has evolved in a clock-like fashion by testing for a global molecular clock in PAUP* v.4.0a152 using the likelihood-ratio test (LRT) of Felsenstein (1988). As the strict clock model was rejected, the relaxed clock model was used for subsequent analyses (Drummond *et al.*, 2006).

Moreover, because nuclear data could not be obtained for specimens from all sites, we identified the major mitochondrial clades in the genus *Hemphillia*. We concatenated the mitochondrial *COI* and mitochondrial 16S data sets (*COI*+16S) and used ML inference to identify the diversification branching pattern among *Hemphillia* species. Eight specimens representing the genus *Prophysaon*, another endemic slug of the PNW, were used as outgroups (*P. andersoni*: AY357610/AY357657, *P. coeruleum*: AY357617/AY357664, *P. dubium*: AY357611/AY357658, *P. foliolatum*: AY357612/AY357659, *P. humile*: AY357613/AY357660, *P. obscurum*: AY357614/AY357661, *P.* sp.: AY357616/AY357663 and *P. vanattae*: AY357615/AY357662) (Wilke & Duncan, 2004). ML analysis was conducted in Garli using the methods described above but with the data set partitioned by gene.

Species-tree inference

We performed species-tree inference under a multi-species coalescent model with *BEAST (Heled & Drummond, 2009) using the *COI*, 16S, *actin* and ITS1 data sets (*COI*+16S+*actin*+ITS1), implemented in BEAST 2.4.4 (Bouckaert *et al.*, 2014). The data matrix was partitioned by gene with unlinked substitution and clock models, unlinked ITS1 and *actin* trees, but linked *COI* and 16S trees. We used a relaxed lognormal molecular clock, a birth–death speciation tree prior, and a linear and constant root model for population size prior. One specimen each from the PNW endemic slug species *Zacoleus idahoensis* (XXX) and *Magnipelta mycophaga* (XXX) were used as outgroups. Because there are several published substitution rate estimates for both the *COI* and the 16S gene in terrestrial gastropods and given a lack of time calibration for the taxa, we applied a range of mtDNA substitution rates to estimate divergence times. The rates for the *COI* gene in terrestrial gastropods are reported to vary between 2.8×10^{-8} and 1.3×10^{-7} substitutions/site/year (Van Riel *et al.*, 2005), and rates for 16S are reported to vary between 1.6×10^{-8} and 1.29×10^{-7} substitutions/site/year (Thomaz *et al.*, 1996; Chiba, 1999; Van Riel *et al.*, 2005). Therefore, we estimated the timing of cladogenetic events by applying a normally distributed rate prior truncated to 0 and 0.2 for both *COI* and 16S, with a mean *COI* site substitution rate of 0.08 per million years (SD: 0.03), and a mean 16S

site substitution rate of 0.07 per million years (SD: 0.04). The 95% interval of these distributions included all the values reported above. The analysis consisted of 500 million generations with a sampling interval of 50 000 and a burn-in of 25%. The BEAST output was analysed using Tracer v.1.4 (Rambaut & Drummond, 2007) to verify an effective sample size exceeding 200 for all parameters being estimated. The BEAST tool TreeAnnotator was used to produce a median branch length maximum clade credibility tree from the post-burn-in trees.

Simultaneous divergence test

We tested a null hypothesis of simultaneous divergence under a hierarchical Approximate Bayesian Computation (hABC) approach as implemented by the PyMsBayes package (Oaks, 2014). Specifically, we tested whether divergence happened synchronously in four species pairs that represent phylogenetically related disjunct taxa (*H. danielsi*/*H.dromedarius*, *H.* sp./*H. dromedarius*, *H. camelus*/*H. malonei* and *H. skadei*/*H. malonei*). The PyMsBayes program implements a modified version of *msbayes* (Huang *et al.*, 2011) that specifies a Dirichlet-process prior (*dpp-msbayes*) over the hyperprior specifying the number of divergence events (Oaks *et al.*, 2013). The *dpp-msbayes* model can use multiple loci to infer the temporal pattern of divergence across species pairs by comparing summary statistics among empirical and simulated data sets. We used both mitochondrial (*COI* and 16S) and nuclear (*actin* and ITS1) loci together in the *dpp-msbayes* model to examine temporal congruence of divergence times between the four aforementioned species pairs. Our *BEAST divergence time estimates guided prior selection for *dpp-msbayes* as follows: concentration parameter of the Dirichlet process hyperprior~gamma [1000, 0.00055] such that there was an equal prior probability of one ($Tdiv_A = Tdiv_B$) or two ($Tdiv_A > Tdiv_B$ or $Tdiv_A < Tdiv_B$) divergence events, population-scaled mutation rate (θ)~gamma [1, 0.0082], divergence times (τ)~gamma [1, 0.036], and the transition-to-transversion rate ratio (kappa) of the HKY substitution model, implemented in *dpp-msbayes*, was estimated for each alignment separately using PAUP*. We performed 1 000 000 simulations and retained the 1000 simulations with the best fit to the empirical data to estimate posterior parameter values.

Historical biogeographical analyses

We used *BioGeoBEARS* (Matzke, 2013) to conduct a historical biogeographical analysis in R (R Core Team, 2013). This package estimates ancestral geographical ranges using a time-calibrated phylogeny and current ranges, under an ML framework. Model testing was then performed to determine the fit of alternative biogeographical models. We used and compared two biogeographical models implemented in *BioGeoBEARS* to determine their fit to our data: (1) dispersal–extinction–cladogenesis (DEC; Ree *et al.*, 2005; Ree & Smith, 2008) and (2) DEC+j. The DEC model focuses on vicariance, or allopatric speciation due to separation of the geographical range (when an ancestor with distribution ABC splits into two distributions A and BC) and allows for sympatric speciation (when an ancestor with a distribution ABC splits into two distributions A and ABC; Ronquist & Sanmartín, 2011). The DEC model has two free parameters that specify the rate of range expansion (d = dispersal) and range contraction (e = extinction), whereas the DEC+j model adds a third free parameter (j = jump dispersal) that corresponds to founder-event speciation (Matzke, 2014). The j parameter was initially implemented for island systems, in which new lineages may be established by colonization of a new island without a continuous ancestor (Clark *et al.*, 2008). To infer ancestral ranges at internal nodes of the *Hemphillia* phylogeny, we used a pruned version of the multilocus species tree from *BEAST containing only in-group taxa. We coded each *Hemphillia* species as being present or absent in the PNW coastal and PNW interior region. The maximum range size, which limits the number of areas by tips and nodes, was set to two.

Species distribution modelling

To obtain an independent perspective on the distribution and divergences for *Hemphillia* species, we developed species distribution models (SDMs) for each species based on georeferenced locality data (Supplementary Information, Appendix S1) and current climate data. This allowed us to assess how closely the predicted habitat suitability of individual species reflected actual occurrence and to estimate the range of each species. Projections were based on a subset of seven uncorrelated (Pearson's correlation coefficient < |0.70|) standard bioclimatic parameters to include in models to describe the suitable climate of each species and to allow for comparisons across species. Variables included the following: BIO3 = Isothermality, BIO5 = Maximum Temperature of Warmest Month, BIO6 = Minimum Temperature of Coldest Month, BIO7 = Temperature Annual Range, BIO13 = Precipitation of Wettest Month, BIO14 = Precipitation of Driest Month and BIO15 = Precipitation Seasonality. We obtained these seven bioclimatic data layers for current (1950–2000) conditions from the WorldClim database at a resolution of 30″ (Hijmans *et al.*, 2005) and cropped the

study area to be between 100° and 180°W and 32° and 72°N. Setting a working area polygon to include only western North America was to avoid sampling habitat greatly outside the species' known occurrences for the selection of background points, which are meant to be compared with the presence data and differentiate the environmental conditions which the species can potentially occur. Nine modelling methods were used to calculate separate SDMs: Generalized Linear Model (GLM), Boosted Regression Trees (GBM), Generalized Additive Model (GAM), Classification Tree Analysis (CTA), Artificial Neural Network (ANN), Surface Range Envelop (SRE), Flexible Discriminant Analysis (FDA), Multiple Adaptive Regression Splines (MARS) and Random Forest (RF). The relative contributions of these alternative models to the final combined model were weighted using the area under the receiver operating characteristic curve (AUC). All analyses were run using the R (R Core Team, 2013) package biomod2 (Thuiller *et al.* 2009), and for each method we used the default settings.

RESULTS

Phylogenetic and species-tree estimation

Inference of individual gene trees indicated no conflict between mitochondrial gene trees and no strongly supported incongruence between mitochondrial and nuclear gene trees. Each genealogy revealed eight genetic clusters that correspond to species assignments (Supporting Information, Figs S1–S4). For ML analysis of the concatenated mitochondrial data set (Fig. 2), the deepest split within *Hemphillia* was inferred to be between the smaller bodied *H. burringtoni* (bootstrap support of 70) and *H. glandulosa* (97) (Clade I; 72) and the remaining larger bodied *Hemphillia* species (80). Within the larger bodied species-group, there were two clades (Clades II and III), each with three species. Within Clade II (93), the coastal species *H. dromedarius* (100) is sister to the reciprocally monophyletic *H. danielsi* and *H.* sp. (56), both inland species (100 and 100, respectively). Clade II is sister to Clade III (72), which contains the coastal species *H. malonei* (100) as sister to the reciprocally monophyletic *H. camelus* and *H. skadei* (88), both inland species (99 and 100, respectively). Relative to *H. burringtoni* and *H. glandulosa*, the six large-bodied species show similar, short intraspecific branch lengths. In contrast, branch lengths within *H. glandulosa* and *H. burringtoni* are half the total tree depth within the genus.

The *BEAST species-tree based on both mitochondrial and nuclear markers (Fig. 3) recovered branching patterns that were identical to those of

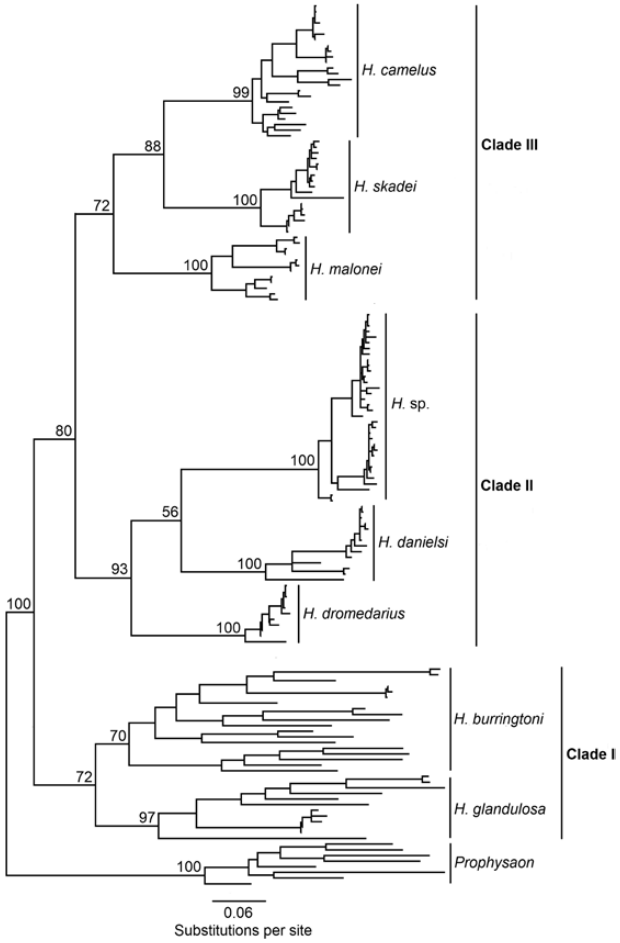

Figure 2. Best maximum-likelihood phylogeny for *Hemphillia* based on mtDNA haplotypes. The scale is given in substitutions per site, and node labels indicate maximum-likelihood bootstrap values for major groups.

the ML mtDNA analysis, although Bayesian nodal support was higher. One notable observation is that both reconstructions group *H. dromedarius*, *H. danielsi* and *H.* sp. together with high support (i.e. Clade II is well supported), but there is low support for the *H. danielsi*/*H.* sp. clade (bootstrap support of 56 and posterior probability of 0.71). Based on assumed substitution rates for *COI* and 16S (see above), the analysis converged on a mean rate of 0.13 [95% highest posterior density (HPD): 0.084–0.17] substitutions/site/Myr for the *COI* locus, 0.037 (95% HPD: 0.023–0.052) for 16S, 0.014 (95% HPD: 0.0081–0.02) for *actin* and 0.012 (95% HPD: 0.007–0.017) for ITS1. The analysis suggests that *Hemphillia* is a relatively ancient lineage, with the deepest split between the large-bodied *Hemphillia* species (Clades II and III) and the small-bodied *H. burringtoni*/*H. glandulosa* (Clade I) placed at about 4.54 Mya (95% HPD: 3.05–6.19 Mya), and the split between *H. burringtoni* and

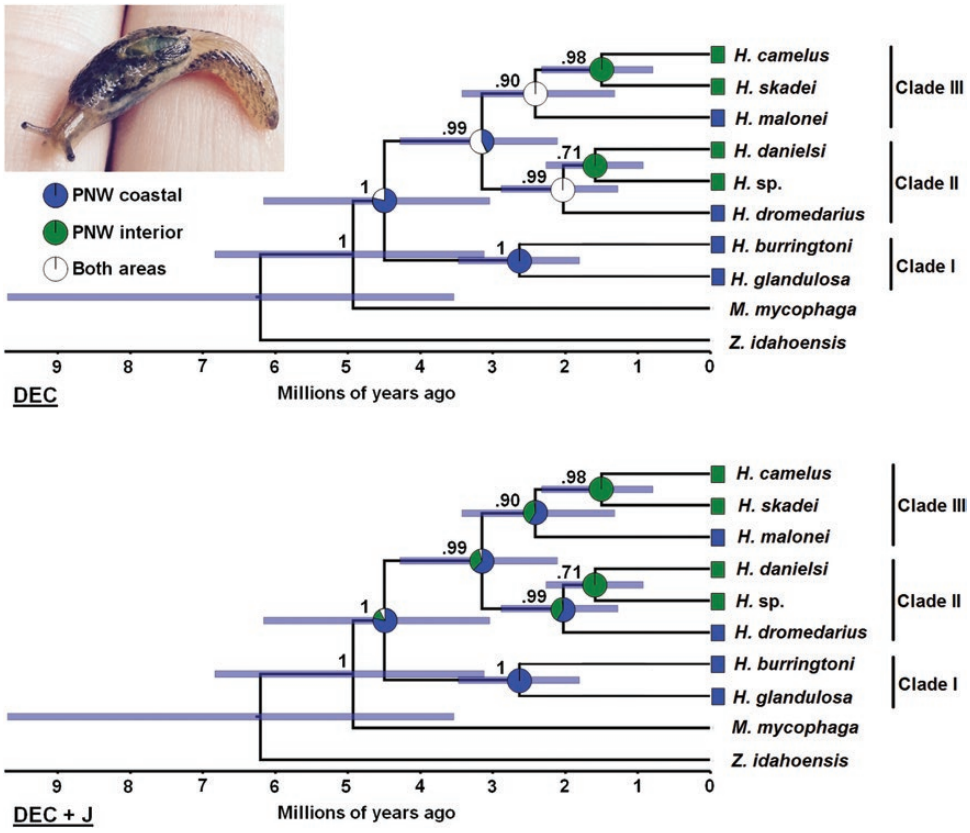

Figure 3. Maximum clade credibility species tree from the *BEAST analysis estimated from both mitochondrial and nuclear markers. Branch lengths are shown in millions of years and represent the median values of those present in the sampled trees. Node labels indicate Bayesian posterior probabilities for major groups and horizontal bars are the 95% highest posterior density (HPD) for the node estimates. Pie charts at nodes indicate the most probable ancestral range location(s) inferred from *BioGeoBEARS*, for both the DEC (top) and the DEC+j (bottom) models. Photo is of *Hemphillia camelus* (photo credit: Jack M. Sullivan).

H. glandulosa (Clade I) placed at 2.6 Mya (95% HPD: 1.79–3.56 Mya). Within the large-bodied species group, Clades II and III display a split dated at 3.2 Mya (95% HPD: 2.14–4.36 Mya). *Hemphillia dromedarius* split from *H. danielsi*/*H.* sp. 2.09 Mya (95% HPD: 1.27–3 Mya), and *H. danielsi*/*H.* sp. split 1.58 Mya (95% HPD: 0.9–2.33 Mya). *Hemphillia malonei* split from *H. camelus*/*H. skadei* around 2.44 Mya (95% HPD: 1.49–3.49 Mya), while the latter two split 1.5 Mya (95% HPD: 0.75–2.22 Mya).

Simultaneous divergence test

We used *dpp-msbayes* to test for simultaneous divergence across the Columbia Plateau by estimating divergence times for four species pairs (*H. danielsi*/*H.dromedarius*, *H.* sp./*H. dromedarius*, *H. camelus*/*H. malonei* and *H. skadei*/*H. malonei*) and estimating the posterior probability of the number of divergence episodes. Results from analysis with *dpp-msbayes* support synchronous diversification (Fig.

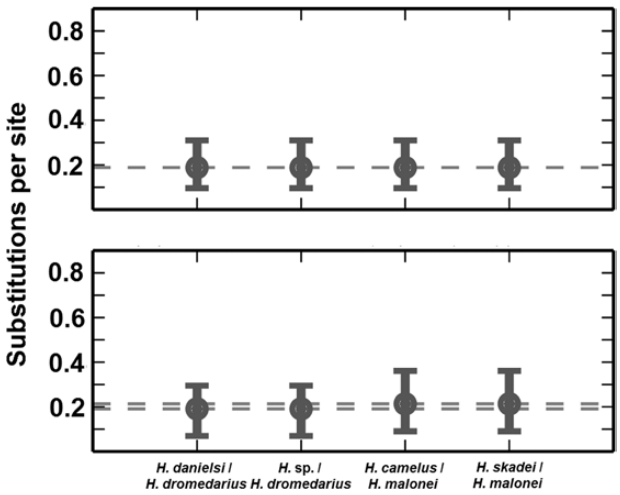

Figure 4. Results from analysis with *dpp-msbayes*. Top: posterior probability was highest for a single divergence event (0.59), whereas the next best supported scenario (two divergence events; bottom) received a posterior probability of 0.068.

4). The posterior probability for a single divergence was 0.59, whereas the next best supported scenario (two divergence events) received a posterior probability of 0.068, suggesting that Clades II and III began diversifying at approximately the same time. This inference is also supported by the broadly overlapping HPD intervals in the topology of the species tree (Fig. 3).

Historical biogeography

The species-tree inference from *BEAST yielded the overall highest likelihood for the DEC+j model in our *BioGeoBEARS* analysis [weighted corrected Akaike Information Criteria (AICc): 0.928; Table 2], with much less weight given to the DEC model (AICc: 0.0725; Table 2). However, Ree & Sanmartin (2018) stressed that the likelihoods of the DEC and DEC+j models are not statistically comparable, and because the latter model favours direct dispersal over widespread ranges owing to the assumption of extremely low extinction rates, the DEC+j model may not be adequate for reconstructing the history of older lineages (Sanmartin & Meseguer, 2016). We therefore consider the results from both the DEC and DEC+j models.

The branching pattern of the species-tree identifies one of the two primary branches leading exclusively to coastal species, whereas the second primary branch leads to both coastal and inland species. Given this, the area of the common ancestor of the genus *Hemphillia* is estimated as primarily in the coast for both DEC and DEC+j (Fig. 3) inferences. The coastal region is also the most likely range for the ancestor of Clades II and III when considering the DEC+j model; however, DEC inference slightly favours a widespread range for this node, with the ancestral lineage having a distribution spanning the coast and inland (CI). Furthermore, in the DEC model, the ancestor of Clade II and the ancestor of Clade III show high support for a widespread range, which posits that the split between *H. dromedarius* and *H. danielsi*/*H.* sp. and the split between *H. malonei* and *H. camelus*/*H. skadei* occurred when an ancestral lineage with a distribution spanning the coast and inland (CI) split into two lineages with distribution C and I (widespread vicariance). However, the added jump-dispersal parameter emphasized by the DEC+j model suggests that long-distance dispersal/founder-event speciation may have had some important effects in obtaining the current disjunct distribution without the presence of widespread ancestors. That is, conditional on this model, there were two eastward colonization and founder effect speciation events driving the initial diversification of Clades II and III.

Species distribution modelling

The projection of SDMs onto current climatic conditions shows that areas of high occurrence probability include moist forest stands, riparian and other wet, cool areas in the Cascade/Coastal Mountains and northern Rocky Mountain wet-belt ecosystem (Supporting Information, Fig. S5). Sister species *H. burringtoni* and *H. glandulosa* exhibit a parapatric distribution with contact zones (Fig. 1) but more overlapping SDMs. For species of Clade II, the SDM for *H. dromedarius* closely matches its known distribution (Fig. 1), but sister species *H. danielsi* and *H.* sp. show SDMs that are broader than their current distributions (Fig. 1). The two species are known from the Clearwater and Salmon River watersheds, Idaho, and part of the Bitterroot Range, but show high occurrence probability across the Blue-Wallowa Mountains and Central Oregon Highland Mountains. In addition, the predicted SDM of *H.* sp. appears nested within that of *H. danielsi*. For species of Clade III, the SDM for *H. malonei* corresponds well with its known distribution (Fig. 1), whereas the SDMs of *H. camelus* and *H. skadei* show areas of high probability corresponding to their distribution as well as coastal/Cascade habitat. Lastly, the SDM of *H. skadei* is nested within that of *H. camelus*, similar to the observation between *H. danielsi* and *H.* sp.; however, evidence suggests that *H. camelus*/*H. skadei* are largely allopatric while *H. danielsi* and *H.* sp are largely sympatric (Fig. 1).

DISCUSSION

Regional biogeography

The temperate rainforests of the Pacific coast and northern Rocky Mountain interior regions offer a compelling opportunity to study the impact of a

Table 2. *BioGeoBEARS* results for each model implemented in the analysis: log-likelihood (LnL), number of parameters, dispersal (*d*), extinction (*e*), founder (*j*), corrected Akaike Information Criteria (AICc) and AICc weight.

Model	LnL	Number of parameters	*d*	*e*	*j*	AICc	AICc weight
DEC	−10.12	2	0.074	1.00×10^{-12}	0	24.24	0.019
DEC+j	−5.16	3	1.00×10^{-12}	1.00×10^{-12}	0.25	16.32	0.98

disjunct ecosystem on structuring biotic diversity. An ancient vicariance hypothesis has been posited to explain the existence of extant, disjunct taxa in both the Coast/Cascade and northern Rocky Mountains components (Brunsfeld *et al.*, 2001). Specifically, under this hypothesis, disjunct taxa are the result of formerly contiguous distributions that were split by the xerification of the Columbia Plateau associated with orogenesis of the Cascades. Here, we investigated how separation of the coastal and inland rainforests has shaped phylogenetic diversity for a genus of terrestrial slugs with putatively limited dispersal ability and a wide distribution in the PNW, to gain insights into the biogeographical history of the ecosystem.

Palaeontological data suggest that coniferous forests have been present in the northern Rocky Mountains since the mid-Eocene (Graham, 1993, 1999), or since the formation of the northern Rocky Mountains 45–36 Mya (English & Johnson, 2004). Thus, prior to uplift of the Cascades, a presumably continuous coniferous forest habitat stretched across the PNW. Establishment of the Columbia Plateau xeric habitat following uplift of the Cascades is thought to have fragmented this habitat, leading to the current disjunct range of the rainforest. Within this general historical framework, our results support the ancient vicariance hypothesis for the general coastal/inland diversification of the group in the sense that the divergences between coastal and inland types are older, substantially pre-dating possible post-Pleistocene dispersal (Brunsfeld *et al.*, 2001). However, in contrast to other animals (e.g. *Ascaphus montanus*/*A. truei*, Nielson *et al.*, 2001; *Plethodon idahoensis*/*P. vandykei*, Carstens *et al.*, 2004), the structure in *Hemphillia* is more complex in that there are replicated ancient vicariance events in different lineages with subsequent inland speciation events within each lineage. The deep pre-Pleistocene structure characterized by the three major phylogenetic groups (Clades I, II and III), as well as shallow structure shaping those individual clades, indicates that there were multiple occurrences of range expansion/fragmentation or perhaps multiple long-distance dispersal/founder-event speciation events that structured diversity.

The two deepest phylogenetic breaks (between Clades I, II and III) are older than 2.14 Mya, and probably reflect Pliocene events. The most recent common ancestor of *Hemphillia*, which probably existed from 3 to 6 Mya, probably had a Pacific coast distribution (Fig. 3), and after the initial diversification of the group, the DEC model suggests that the ancestor of the larger-bodied species group spread across to the Northern Rocky mountains (NRM) interior region such that there were contiguous populations from the Pacific coast to the interior during the Pliocene. The succeeding divergence of Clades II and III – the two reciprocally monophyletic large-bodied *Hemphillia* species groups – is interesting because to our knowledge a similar phylogeographical pattern this old has not been observed in previously studied taxa from the region. However, a latitudinal split between northern populations still connected by the Okanogan Highlands (in north-central Washington) and southern populations connected via the Central Oregon Highlands may explain this pattern. Incidentally, the SDMs for both *H. danielsi* and *H.* sp. show areas of high suitability in the Central Oregon Highlands (Supporting Information, Fig. S5), although they are not currently known to occur in the region. The climatic influences of the early Cascades and associated loss of suitable habitat connecting the coastal and inland rainforest ecosystems probably split contiguous populations of Clades II and III into eastern and western groups, which would then have retracted to coastal and inland distributions.

The model with the highest likelihood in our *BioGeoBEARS* analysis was the DEC+j model, which received nearly all the weighted model support (as measured using AICc weights). Taken at face value, the resulting inference would suggest that long-distance dispersal/founder-event speciation played an important role in the invasions of the interior region, although this inference potentially contrasts with characteristics of *Hemphillia*, such as their sedentary nature and low vagility. However, in spite of their low vagility, there are reports of mollusc specimens or their eggs being dispersed by birds (e.g. Pearce *et al.*, 2012; Shikov & Vinogradov, 2013), and rare colonization events such as being moved across the Columbia Basin by birds to the NRM interior forests may be possible, especially given the hermaphroditic nature of these organisms. Notably, other terrestrial gastropods (e.g. members of the tail-dropper genus *Prophysaon*) lack deep genetic structure across the Columbia basin (e.g. Wilke & Duncan, 2004; Smith *et al.*, 2017, 2018), suggesting that there have been opportunities for gene flow across the disjunction. One possibility is that individuals dispersed along river corridors such as the Columbia River drainage basin, perhaps during large-scale flooding episodes that followed deglaciation, although such dispersal would have to have run against flood currents (generally NW to SE). Both river valleys and other events such as bird dispersal provide possible mechanisms for jump-dispersal/founder event speciation to have played an important role in colonization of the NRM interior forests by Clades II and III. However, given the broadly overlapping HPD intervals in the topology of the species tree (Fig. 3) and high posterior probability support for a single divergence event from the *dpp-msbayes* analysis (Fig. 4), two independent and simultaneous long-distance dispersal events seem an unlikely mechanism of diversification, and

it is more plausible that simultaneous divergence was driven by an environmental mechanism, such as xerification of the Columbia Plateau. Thus, we favour the inferences derived under the DEC model over those from the DEC+j model, following the arguments of Ree & Sanmartin (2018). A similar reasoning has been invoked to explain comparable disjunction patterns in clades of amphibians (e.g. *Ascaphus montanus*/*A. truei*, Nielson *et al.*, 2001; *Plethodon idahoensis*/*P. vandykei*, Carstens *et al.*, 2004; and *Dicamptodon copei*/*D. aterrimus*, Carstens *et al.*, 2005). Carstens *et al.* (2005; using a coalescent-based method) estimated the mean time of divergence between the *Ascaphus*, *Plethodon* and *Dicamptodon* lineages to be 3.1, 4.1 and 1.2 Mya, respectively. Likewise, here (using a tree-based approach) we estimated the splits between coastal and interior sister groups of *Hemphillia* (*H. dromedarius* vs. *H. danielsi*/*H.* sp. and *H. malonei* vs. *H. camelus*/*H. skadei*) to be 2.09 Mya (95% HPD: 1.27–3 Mya) and 2.44 Mya (95% HPD: 1.49–3.49 Mya). These lineages may well have responded to Pliocene (5.33–2.49 Mya) drought in a similar manner. The xerification of the Columbian Plateau was probaby not a discrete event but instead a long-term gradual drying, and therefore sister-groups may not have been strictly allopatric but perhaps paraptric with potential areas of contact. Ongoing hybrid zones may have slowed the process of lineage sorting to post-date the ecological separation of the Coast–Cascade mountains and Northern Rocky Mountains.

A corollary of the ancient vicariance hypothesis is that populations became isolated in both the Cascades and northern Rocky Mountains after uplift of the Cascades. This suggests that isolates persisted in coastal and inland refugia throughout the Pleistocene (2.6–0.012 Mya) glaciations, and until the present (Brunsfeld *et al.*, 2001) (i.e. the most recent speciation events in Clades II and III probably reflect Pleistocene glacial events shaping the structure of those individual clades). During the Pleistocene glacial cycles, most of the PNW was subjected to repeated glaciation. Only the northernmost portions of the Cascades Range were affected, with montane glaciers in the Cascades probably pushing forest habitats to lower altitudes, and ice sheets covering a significant portion of the northern Rocky Mountains (Pielou, 1991; Delcourt & Delcourt, 1993). Nevertheless, the inland rainforest ecosystem contains a collection of pre-Pleistocene endemic species (i.e. old endemics), including Constance's bittercress (*Cardamine constancei*; Brunsfeld & Sullivan, 2005), Coeur d'Alene salamanders (*Plethodon idahoensis*; Carstens *et al.*, 2004), Rocky Mountain tailed frogs (*Ascaphus montanus*; Nielson *et al.*, 2006) and Idaho giant salamanders (*Dicamtodon aterrimus*; Steele *et al.*, 2005), which are thought to have persisted through the Pleistocene glacial cycles in one or more inland refugia. In *Hemphillia*, the depth of phylogenetic divergence between the sister species *H. camelus* and *H. skadei*, as well as the divergence between *H. danielsi* and *H.* sp., suggests that inland *Hemphillia* species have also persisted in the region throughout the Pleistocene climatic fluctuations. *Hemphillia danielsi* and *H.* sp. appear to be deeply divergent sister species; however, they do not appear to have differentiated spatially (Supporting Information, Fig. S5) with their distributions centred around the Clearwater River drainage, a historically non-glaciated part of the inland ecosystem that has been a suspected refugium for many mesic forest endemics (Daubenmire, 1975; Carstens *et al.*, 2004; Brunsfeld & Sullivan, 2005). Similarly, *H. camelus* and *H. skadei* are also well-separated sister species that nevertheless are predicted to have overlapping but not equal spatial distributions (Supporting Information, Fig. S5). However, the distribution of the two species is predominately allopatric, suggesting some form of ecological exclusion. *Hemphillia skadei* has been found in the Coeur d'Alene, Saint Joe and Selkirk mountains in northern Idaho, and its range appears to be nested within that of *H. camelus* (Lucid *et al.*, 2018). The latter occurs directly south of populations of *H. skadei*, but also in previously glaciated areas of the Selkirk and Purcell mountains of northern Idaho and the surrounding regions. Lucid *et al.* (2018) provide additional information on the geographical association among the subclades of *H. camelus* and *H. skadei*. It is likely that these northern areas were colonized through western Montana, surrounding the range of *H. skadei*, a pattern like that found for the Rocky Mountain tailed frogs (*Ascaphus montanus*; Metzger *et al.*, 2015). The presence of more northerly, largely allopatric populations (Fig. 1) suggests the possibility of northern refugia in these species. It is possible that more northerly refugia occurred along other river canyons, such as the St. Joe or Coeur d'Alene rivers of northern Idaho, a hypothesis also supported by the phylogeographical structure identified in Constance's bittercress (*Cardamine constancei*; Brunsfeld & Sullivan, 2005). Our results thus provide more support for the presence of multiple, compartmentalized refugia within the northern Rockies (Brunsfeld *et al.*, 2001), and further demonstrate the complexity of the biogeographical patterns and structure observed in northern Rocky Mountain endemics (reviewed by Shafer *et al.*, 2010). Future work on *Hemphillia* and other inland endemics should focus on the presence of these potential northern refugia in the region.

Within the Coast/Cascade mountains, there was a much greater extent of unglaciated habitat during the Pleistocene (Brunsfeld *et al.*, 2001). However, *H. glandulosa* and *H. burringtoni* separate into two phylogroups that parse according to geography and

appear to have separated during the late Pliocene/early Pleistocene. The current distribution of the two species is parapatric (Fig. 1), although they show very similar SDM projections (Supporting Information, Fig. S5). It is possible that these two groups were isolated in separate Coast Mountain refugia and Cascades Mountain refugia during the Pleistocene and have only recently come into secondary contact. Individuals of the *H. burringtoni* clade occur on Vancouver Island, south throughout the Olympic Peninsula and western Washington, and along the north-eastern Oregon coast while individuals of the *H. glandulosa* clade occur from the central coast of Washington to the south-western Washington Cascades and north-western Oregon, and the two species come into contact in some areas of Washington and Oregon (Wilke & Ziegltrum, 2004; Burke *et al.*, 2005). Further information on spatial population structure of *H. glandulosa* and *H. burringtoni* is discussed in Wilke & Ziegltrum (2004).

Lastly, members of the genus *Hemphillia* are often recognized as species of conservation importance in the states and provinces in which they occur (e.g. IDFG, 2017). This is due to their endemism and limited distribution, and a lack of taxonomic and natural history knowledge (IDFG, 2017). Our phylogenetic analyses help to clear some previous taxonomic difficulties of the group. For example, our large-scale sampling shows that *H. glandulosa* and *H. burringtoni* separate into two phylogroups (see also Wilke & Ziegltrum, 2004), and therefore represent two genetically distinct species that parse according to geography. *Hemphillia camelus* and *H. skadei* appear to be deeply divergent sister species, even though *H. skadei* has long been treated as *H. camelus* (Burke & Leonard, 2013) due to their highly similar external morphologies, and has only recently been described (Lucid *et al.*, 2018). Our results also confirm that the suspected new taxon (*H.* sp.) is genetically distinct from the morphologically similar, sympatric species *H. danielsi* (Burke & Leonard, 2013), and appears to be endemic to Idaho's Clearwater River drainage. The biogeographical information we have detailed in this study will help guide managers to appropriately allocate resources for species conservation, and the support we document for previously suggested refugia will help guide land management to conserve potential evolutionary, and thus biodiversity, hotspots.

CONCLUSIONS

Many studies of phylogenetic concordance involving taxa endemic to the PNW show clear genetic breaks between coastal and inland populations, as well as evidence of multiple refugia in both coastal and inland regions throughout the Pleistocene glaciations. Our molecular data assembled for individuals of the endemic slug genus *Hemphillia* show elements of both ancient and shallow biogeographical patterns, suggesting that the biogeographical structure of taxa such as this one is more complex than seen in others from the region. For example, recent studies on other PNW invertebrates with limited dispersal abilities and wide distributions have shown either shallow divergence between coastal and inland populations (*Prophysaon* slugs; Wilke & Duncan, 2004; Smith *et al.*, 2018), or genetic division between – but not within – coastal and inland populations (*Chonaphe* millipedes; Espíndola *et al.*, 2016). Our data suggest that *Hemphillia* experienced replicated ancient vicariance events in two separate lineages, as well as more recent speciation events in both the coast and inland regions. We posit that late Pliocene and Pleistocene climatic oscillations, in conjunction with the geological and physiographic heterogeneity of the coastal and inland mesic forests, promoted allopatric diversification between the sister species pairs *H. burringtoni*/*H. glandulosa*, *H. camelus*/*H. skadei* and *H. danielsi*/*H.* sp. Today, two of the lineage pairs (*H. burringtoni*/*H. glandulosa* and *H. camelus*/*H. skadei*) are mostly non-overlapping, while *H. danielsi*/*H.* sp. are largely sympatric (Fig. 1). Our study demonstrates that a complex interplay of ancient vicariance and more recent speciation events has shaped the biogeography of *Hemphillia* in north-western North American temperate rainforests.

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REFERENCES

Albach DC, **Schoenswetter P**, **Tribsch A. 2006.** Comparative phylogeography of the *Veronica alpina* complex in Europe and North America. *Molecular Ecology* **15:** 3269–3286.

Armbruster GF, **van Moorsel CH**, **Gittenberger E. 2000.** Conserved sequence patterns in the non-coding ribosomal ITS-1 of distantly related snail taxa. *Journal of Molluscan Studies* **66:** 570–573.

Ashwill M. 1983. Seven fossil floras in the rain shadow of the Cascade Mountains, Oregon. *Oregon Geology* **45:** 107–111.

Barrowclough GF, **Groth JG**, **Mertz LA**, **Gutiérrez RJ. 2004.** Phylogeographic structure, gene flow and species status in blue grouse (*Dendragapus obscurus*). *Molecular Ecology* **13:** 1911–1922.

Bland T, **Binney WG. 1874.** XVI.—Description of *Hemphillia*, a new genus of terrestrial mollusks. *Annals of the New York Academy of Sciences* **10:** 208–211.

Booth DB, **Troost KG**, **Clague JJ**, **Waitt RB. 2003.** The Cordilleran ice sheet. *Developments in Quaternary Sciences* **1:** 17–43.

Bouckaert R, **Heled J**, **Kühnert D**, **Vaughan T**, **Wu CH**, **Xie D**, **Drummond AJ. 2014.** BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* **10:** e1003537.

Branson BA. 1972. *Hemphillia dromedarius*, a new Arionid slug from Washington. *Nautilus* **85:** 100–106.

Branson BA. 1975. *Hemphillia pantherina*, a new Arionid slug from Washington. *The Veliger* **18:** 93–94.

Brouillet L, **Whetstone RD. 1993.** Climate and physiography. In: FONAE Committee, ed. *Flora of North America North of Mexico.* New York: Oxford University Press, 15–46.

Brunsfeld SJ, **Sullivan J. 2005.** A multi-compartmented glacial refugium in the northern Rocky Mountains: evidence from the phylogeography of *Cardamine constancei* (Brassicaceae). *Conservation Genetics* **6:** 895–904.

Brunsfeld SJ, **Sullivan J**, **Soltis DE**, **Soltis PS. 2001.** Comparative phylogeography of northwestern North America: a synthesis. In: Silvertown J, Antonovics J, eds. *Integrating ecological and evolutionary processes in a spatial context*, *Vol.* **14**. Oxford: Blackwell Science, 319–339.

Burke T. 2015. *Examination of* Hemphillia pantherina *type specimen photographs*. Portland: US Forest Service Interagency Special Status/Sensitive Species Program.

Burke TE, **Leonard WP. 2013.** *Land snails and slugs of the Pacific Northwest*. Portland: Oregon State University Press.

Burke TE, **Wainwright M**, **Duncan N. 2005.** *Conservation assessment for four species of the genus* Hemphillia. Oregon and Washington: USDA Forest Service Region 6 and USDI Bureau of Land Management.

Carstens BC, **Brunsfeld SJ**, **Demboski JR**, **Good JM**, **Sullivan J. 2005.** Investigating the evolutionary history of the Pacific Northwest mesic forest ecosystem: hypothesis testing within a comparative phylogeographic framework. *Evolution* **59:** 1639–1652.

Carstens BC, **Richards CL. 2007.** Integrating coalescent and ecological niche modeling in comparative phylogeography. *Evolution* **61:** 1439–1454.

Carstens BC, **Stevenson AL**, **Degenhardt JD**, **Sullivan J. 2004.** Testing nested phylogenetic and phylogeographic hypotheses in the *Plethodon vandykei* species group. *Systematic Biology* **53:** 781–792.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17:** 540–552.

Chiba S. 1999. Accelerated evolution of land snails *Mandarina* in the oceanic Bonin Islands: evidence from mitochondrial DNA sequences. *Evolution* **53:** 460–471.

Clark JR, **Ree RH**, **Alfaro ME**, **King MG**, **Wagner WL**, **Roalson EH. 2008.** A comparative study in ancestral range reconstruction methods: retracing the uncertain histories of insular lineages. *Systematic Biology* **57:** 693–707.

Daubenmire R. 1975. *Floristic plant geography of eastern Washington and northern Idaho*. Provo: Brigham Young University Press.

Delcourt PA, **Delcourt HR. 1993.** Paleoclimates, paleovegetation, and paleofloras during the late Quaternary. In: Flora North America Editorial Committee, ed. *Flora of North America*, **Vol. 1**. New York: Oxford University Press, 71–94.

DellaSala D, **Alaback P**, **Craighead L**, **Goward T**, **Pacquet P**, **Spribille P. 2011.** Temperate and boreal rainforests of inland northwestern North America. In: DellaSalla DA, ed. *Temperate and boreal rainforests of the world: ecology and conservation*. Washington: Island Press, 82–110.

Demboski JR, **Cook JA. 2001.** Phylogeography of the dusky shrew, *Sorex monticolus* (Insectivora, Soricidae): insight into deep and shallow history in northwestern North America. *Molecular Ecology* **10:** 1227–1240.

Drummond AJ, **Ho SY**, **Phillips MJ**, **Rambaut A. 2006.** Relaxed phylogenetics and dating with confidence. *PLoS Biology* **4:** e88.

English JM, **Johnston ST. 2004.** The Laramide orogeny: what were the driving forces? *International Geology Review* **46:** 833–883.

Espíndola A, **Ruffley M**, **Smith ML**, **Carstens BC**, **Tank DC**, **Sullivan J. 2016.** Identifying cryptic diversity with predictive phylogeography. *Proceedings of the Royal Society B: Biological Sciences* **283:** 20161529.

Felsenstein J. 1988. Phylogenies from molecular sequences: inference and reliability. *Annual Review of Genetics* **22:** 521–565.

Folmer O, **Black M**, **Hoeh W**, **Lutz R**, **Vrijenhoek R. 1994.** DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3:** 294–299.

Frest TJ, **Johannes EJ. 1995.** *Interior Columbia Basin mollusk species of special concern*. Final Report Contract #43-0E00-4-9112. Washington: Interior Columbia Basin Ecosystem Management Project.

Galbreath KE, **Hafner DJ**, **Zamudio KR. 2009.** When cold is better: climate-driven elevation shifts yield complex patterns of diversification and demography in an alpine specialist (American pika, *Ochotona princeps*). *Evolution* **63:** 2848–2863.

Gavin DG, **Hu FS**, **Walker IR**, **Westover K. 2009.** The northern inland temperate rainforest of British Columbia: old forests with a young history? *Northwest Science* **83:** 70–78.

Graham A. 1993. History of the vegetation: Cretaceous–Tertiary. In: Flora North America Editorial Committee, eds. *Flora of North America North of Mexico*, **Vol. 1**. New York: Oxford University Press, 57–70.

Graham A. 1999. *Late Cretaceous and Cenozoic history of North American vegetation*. New York: Oxford University Press.

Heled J, **Drummond AJ. 2009.** Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution* **27:** 570–580.

Hijmans RJ, **Cameron SE**, **Parra JL**, **Jones PG**, **Jarvis A. 2005.** Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25:** 1965–1978.

Huang W, **Takebayashi N**, **Qi Y**, **Hickerson MJ. 2011.** MTML-msBayes: approximate Bayesian comparative phylogeographic inference from multiple taxa and multiple loci with rate heterogeneity. *BMC bioinformatics* **12:** 1.

Idaho Department of Fish and Game. **2017.** *Idaho State Wildlife Action Plan, 2015*. Boise: Idaho Department of Fish and Game. Grant No.: F14AF01068 Amendment #1. Available at: http://fishandgame.idaho.gov/. Sponsored by the US Fish and Wildlife Service, Wildlife and Sport Fish Restoration Program.

Kelley R, **Dowlan S**, **Duncan N**, **Burke T. 1999.** *Field guide to survey and manage terrestrial mollusk species from the Northwest Forest Plan*. Portland: USDI Bureau of Land Management.

Kerhoulas NJ, **Gunderson AM**, **Olson LE. 2015.** Complex history of isolation and gene flow in hoary, Olympic, and endangered Vancouver Island marmots. *Journal of Mammalogy* **96:** 810–826.

Li P, **Adams WT. 1989.** Range-wide patterns of allozyme variation in Douglas-fir (*Pseudotsuga menziesii*). *Canadian Journal of Forest Research* **19:** 149–161.

Lucid MK, **Rankin A**, **Espindola A**, **Chichester L**, **Ehlers S**, **Robinson L**, **Sullivan J. 2018.** Taxonomy and biogeography of *Hemphillia* (Gastropoda: Pulmonata: Arionidae) in North American rainforests, with description of a new species (Skade's jumping-slug, *Hemphillia skadei* sp. nov.). *Canadian Journal of Zoology* **96:** 305–316.

Matzke NJ. 2013. *BioGeoBEARS: BioGeography with Bayesian (and likelihood) evolutionary analysis in R Scripts*. R package, version 0.2.1, 2013. http://CRAN.R-project.org/package=BioGeoBEARS. Accessed 5 November 2017.

Matzke NJ. 2014. Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Systematic Biology* **63:** 951–970.

Metzger G, **Espíndola A**, **Waits LP**, **Sullivan J. 2015.** Genetic structure across broad spatial and temporal scales: Rocky Mountain tailed frogs (*Ascaphus montanus*; Anura: Ascaphidae) in the inland temperate rainforest. *Journal of Heredity* **106:** 700–710.

Minin V, **Abdo Z**, **Joyce P**, **Sullivan J. 2003.** Performance-based selection of likelihood models for phylogeny estimation. *Systematic Biology* **52:** 674–683.

Mumladze L, **Tarkhnishvili D**, **Murtskhvaladze M. 2013.** Systematics and evolutionary history of large endemic snails from the Caucasus (Helix buchii and H. goderdziana) (Helicidae). *American Malacological Bulletin* **31:** 225–235.

Nielson M, **Lohman K**, **Sullivan J. 2001.** Phylogeography of the tailed frog (*Ascaphus truei*): implications for the biogeography of the Pacific Northwest. *Evolution* **55:** 147–160.

Nielson M, **Lohman K**, **Daugherty CH**, **Allendorf FW**, **Knudsen KL**, **Sullivan J. 2006.** Allozyme and mitochondrial DNA variation in the tailed frog (Anura: *Ascaphus*): the influence of geography and gene flow. *Herpetologica* **62:** 235–258.

O'Connor JE, **Baker VR. 1992.** Magnitudes and implications of peak discharges from glacial Lake Missoula. *Geological Society of America Bulletin* **104:** 267–279.

Oaks JR. 2014. An improved approximate-Bayesian model-choice method for estimating shared evolutionary history. *BMC Evolutionary Biology* **14:** 150.

Oaks JR, **Sukumaran J**, **Esselstyn JA**, **Linkem CW**, **Siler CD**, **Holder MT**, **Brown RM. 2013.** Evidence for climate-driven diversification? A caution for interpreting ABC inferences of simultaneous historical events. *Evolution: International Journal of Organic Evolution* **67:** 991–1010.

Pearce TA, **Mulvihill RS**, **Porter KA. 2012.** Land slugs (Gastropoda: Pulmonata) on birds demonstrate dispersal potential. *Nautilus* **126:** 38–40.

Pielou EC. 1991. *After the ice age: the return of life to glaciated North America*. Chicago: University of Chicago Press.

Pilsbry HA. 1917. A new Hemphillia and other snails from near Mt. Hood, Oregon. *The Nautilus* **30:** 117–119.

Pilsbry H. 1948. *Land Mollusca of North America (north of Mexico)*, *Vol.* **2**. Philadelphia: The Academy of Natural Sciences of Philadelphia Monographs No. 3.

Pilsbry HA, **Vanatta EG. 1898.** Revision of the North American slugs: *Binneya*, *Hemphillia*, *Hesperarion*, *Prophysaon* and *Anadenulus*. *Proceedings of the Academy of Natural Sciences of Philadelphia* **50:** 219–261.

Porter SC, **Pierce KL**, **Hamilton TD. 1983.** Late Wisconsin mountain glaciation in the western United States. Late Quaternary environments of the United States. In: Perter SC, ed. *The late Pleistocene*, **Vol. 1**. Minneapolis: University of Minnesota Press, 71–111.

Priest GR. 1990. Volcanic and tectonic evolution of the Cascade Volcanic arc, central Oregon. *Journal of Geophysical Research* **95:** 19583–19600.

R Core Team. 2013. *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: http://www.R-project.org/.

Rambaut A, Drummond AJ. 2007. *Tracer v1.4*. Available at: http://beast.bio.ed.ac.uk/Tracer.

Ree RH, Sanmartín I. 2018. Conceptual and statistical problems with the DEC+ J model of founder-event speciation and its comparison with DEC via model selection. *Journal of Biogeography* **45:** 741–749.

Ree RH, Smith SA. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology* **57:** 4–14.

Ree RH, Moore BR, Webb CO, Donoghue MJ. 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution* **59:** 2299–2311.

Ronquist F, Sanmartín I. 2011. Phylogenetic methods in biogeography. *Annual Review of Ecology, Evolution, and Systematics* **42:** 441–464.

Rowson B, Tattersfield P, Symondson WO. 2011. Phylogeny and biogeography of tropical carnivorous land-snails (Pulmonata: Streptaxoidea) with particular reference to East Africa and the Indian Ocean. *Zoologica Scripta* **40:** 85–98.

Ruffley M, Smith ML, Espíndola **A, Carstens BC, Sullivan J, Tank DC. 2018.** Combining allele frequency and tree-based approaches improves phylogeographic inference from natural history collections. *Molecular Ecology* **27:** 1012–1024.

Sanmartín I, Meseguer AS. 2016. Extinction in phylogenetics and biogeography: from timetrees to patterns of biotic assemblage. *Frontiers in Genetics* **7:** 35.

Shafer A, Cullingham CI, Cote SD, Coltman DW. 2010. Of glaciers and refugia: a decade of study sheds new light on the phylogeography of northwestern North America. *Molecular Ecology* **19:** 4589–4621.

Shikov EV, Vinogradov AA. 2013. Dispersal of terrestrial gastropods by birds during the nesting period. *Folia Malacologica* **21:** 105–110.

Smith ML, Ruffley M, Espíndola A, Tank DC, Sullivan J, Carstens BC. 2017. Demographic model selection using random forests and the site frequency spectrum. *Molecular Ecology* **26:** 4562–4573.

Smith ML, Ruffley M, Rankin AM, Espíndola **A, Tank DC, Sullivan J, Carstens BC. 2018.** Testing for the presence of cryptic diversity in tail-dropper slugs (Prophysaon) using molecular data. *Biological Journal of the Linnean Society* **124:** 518–532.

Steele CA, Carstens BC, Storfer A, Sullivan J. 2005. Testing hypotheses of speciation timing in *Dicamptodon copei* and *Dicamptodon aterrimus* (Caudata: Dicamptodontidae). *Molecular Phylogenetics and Evolution* **36:** 90–100.

Swenson NG, Howard DJ. 2005. Clustering of contact zones, hybrid zones, and phylogeographic breaks in North America. *The American Naturalist* **166:** 581–591.

Swofford DL. 2003. *PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4*. Sunderland: Sinauer Associates.

Thomaz D, Guiller A, Clarke B. 1996. Extreme divergence of mitochondrial DNA within species of pulmonate land snails. *Proceedings of the Royal Society of London B* **263:** 363–368.

Thuiller W, Lafourcade B, Engler R, Araújo MB. 2009. BIOMOD–a platform for ensemble forecasting of species distributions. *Ecography* **32:** 369–373.

Tolan TL, Martin BS, Reidel SP, Anderson JL, Lindsey KA, Burt W. 2009. An introduction to stratigraphy, structural geology, and hydrogeology of the Columbia River Flood-Basalt Province—A primer for the GSA Columbia River Basalt Group field trips. In: O'Connor JE, Dorsey RJ, Madin IP, eds. *Volcanoes to vineyards—Geologic field trips through the dynamic landscape of the Pacific Northwest: Geological Society of America Field Guide*, **Vol. 15**, 599–643.

Vanatta EG. 1914. Montana shells. *Proceedings of the Academy of Natural Sciences of Philadelphia* **66:** 367–371.

Van Riel P, Jordaens K, Van Houtte N, Martins AMF, Verhagen R, Backeljau T. 2005. Molecular systematics of the endemic Leptaxini (Gastropoda: Pulmonata) on the Azores islands. *Molecular Phylogenetics and Evolution* **37:** 132–143.

Waitt RB Jr. 1985. Case for periodic, colossal jökulhlaups from Pleistocene glacial Lake Missoula. *Geological Society of America Bulletin* **96:** 1271–1286.

Walsh HE, Kidd MG, Moum T, Friesen VL. 1999. Polytomies and the power of phylogenetic inference. *Evolution* **53:** 932–937.

Wilke T, Duncan N. 2004. Phylogeographical patterns in the American Pacific Northwest: lessons from the arionid slug *Prophysaon coeruleum*. *Molecular Ecology* **13:** 2303–2315.

Wilke T, Ziegltrum J. 2004. *Genetic and anatomical analysis of the jumping slugs*. USFS Contract Report #43-05g2-1-10086 Final Report. 2003

Zwickl DJ. 2006. *GARLI: genetic algorithm for rapid likelihood inference*. Available at: http://www.bio.utexas.edu/faculty/antisense/garli/Garli.html.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Figure S1. *COI* maximum-likelihood gene tree.
Figure S2. 16S maximum-likelihood gene tree.
Figure S3. ITS1 maximum-likelihood gene tree.
Figure S4. *Actin* maximum-likelihood gene tree.
Figure S5. Species distribution models (SDMs) for each *Hemphillia* species. Scales indicate suitability values.
Appendix S1. Data from 200 *Hemphillia* specimens.