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Molecular and Ecological Characterization of Extralimital Populations of Red-Legged Frogs from Western North America

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ABSTRACT.—**Extralimital populations of red-legged frogs have recently been found on Graham Island (Queen Charlotte Islands), British Columbia, and on Chichagof Island, Alaska. Both islands are well north of the traditionally understood (or core) range of red-legged frogs in western North America. The Chichagof Island frogs are known to be introduced, and the Graham Island frogs are suspected to be introduced. However, species-level identification of these populations remains uncertain. Recent phylogeographic analyses have demonstrated that there are two species of red-legged frogs, *Rana aurora* and *Rana draytonii*, and *R. aurora* is more closely related to the Cascades Frog, *Rana cascadae* (i.e., [*aurora* + *draytonii*] is not monophyletic). Here, we compare new mtDNA sequence data from these extralimital populations to available sequences from 50 populations from the core range of red-legged frogs. These results demonstrate that both extralimital populations are the Northern Red-Legged Frog, *R. aurora*, and are most closely related to haplotypes found in the most northern clade of *R. aurora*. Further, we conduct ecological niche modeling under current conditions and future conditions that assume a global warming scenario to assess habitat suitability in southeastern Alaska and the Queen Charlotte Islands and the potential for the persistence and expansion of the extralimital populations. These analyses suggest that the extralimital populations occur in the most suitable habitat on Graham and Chichagof Islands and that suitability will increase on Graham and decrease on Chichagof Island in the future. These results are used to discuss several management options for the extralimital *R. aurora*.**

The red-legged frogs of western North America (*Rana aurora* and *Rana draytonii*) comprise two distinct species whose ranges narrowly overlap in coastal southern Mendocino County, California (Fig. 1; Shaffer et al., 2004). The California Red-Legged Frog, *R. draytonii*, occurs from northern Baja California, Mexico to Mendocino County, California, along the coast and in a few isolated localities in the Sierra Nevada, although it formerly occurred throughout the Sierra Nevada foothills (Fig. 1; Stebbins, 2003; Fellers, 2005). The Northern Red-Legged Frog, *R. aurora*, occurs west of the Cascade Crest from Mendocino County, California, north to Vancouver Island and the adjacent mainland up to Sullivan Bay, British Columbia (Fig. 1; Stebbins, 2003; Pearl, 2005). Although once considered conspecifics, *R. aurora* and *R. draytonii* are now recognized as distinct species. Moreover, these two species are not each other's closest relatives, as *Rana cascadae*, a long-recognized species of the western United States, is more closely related to *R. aurora* than it is to *R. draytonii* (Shaffer et al., 2004).

Recently, extralimital populations of red-legged frogs have also been reported from Graham Island, British Columbia (Ovaska et al., 2002) and Chichagof Island, Alaska (Fig. 1; Hodge, 2004; J. C. Sargent, A. Hutton, and J. Waatti, Discovery of the red-legged frog (*Rana aurora*) in northeast Chichagof Island: an introduced species, Tongass National Forest, Hoonah Ranger District, Hoonah, Alaska, unpubl. data, 2003). Graham Island is the larger of the two main islands in the Queen Charlotte (Haida Gwaii) Archipelago, and Chichagof Island is in the Alexander Archipelago of southeastern Alaska. The Graham and Chichagof populations are approximately 400 km and 850 km, respectively, north of the nearest recognized native populations in Sullivan Bay, British Columbia (Fig. 1). Although both of these populations likely result from introductions, we refer to them as extralimital because only the Chichagof population is unquestionably an introduction; the situation is less clear for the Graham Island frogs which were considered to be of uncertain origin by Ovaska et al. (2002) and to be introduced by Matsuda et al. (2006). Through-

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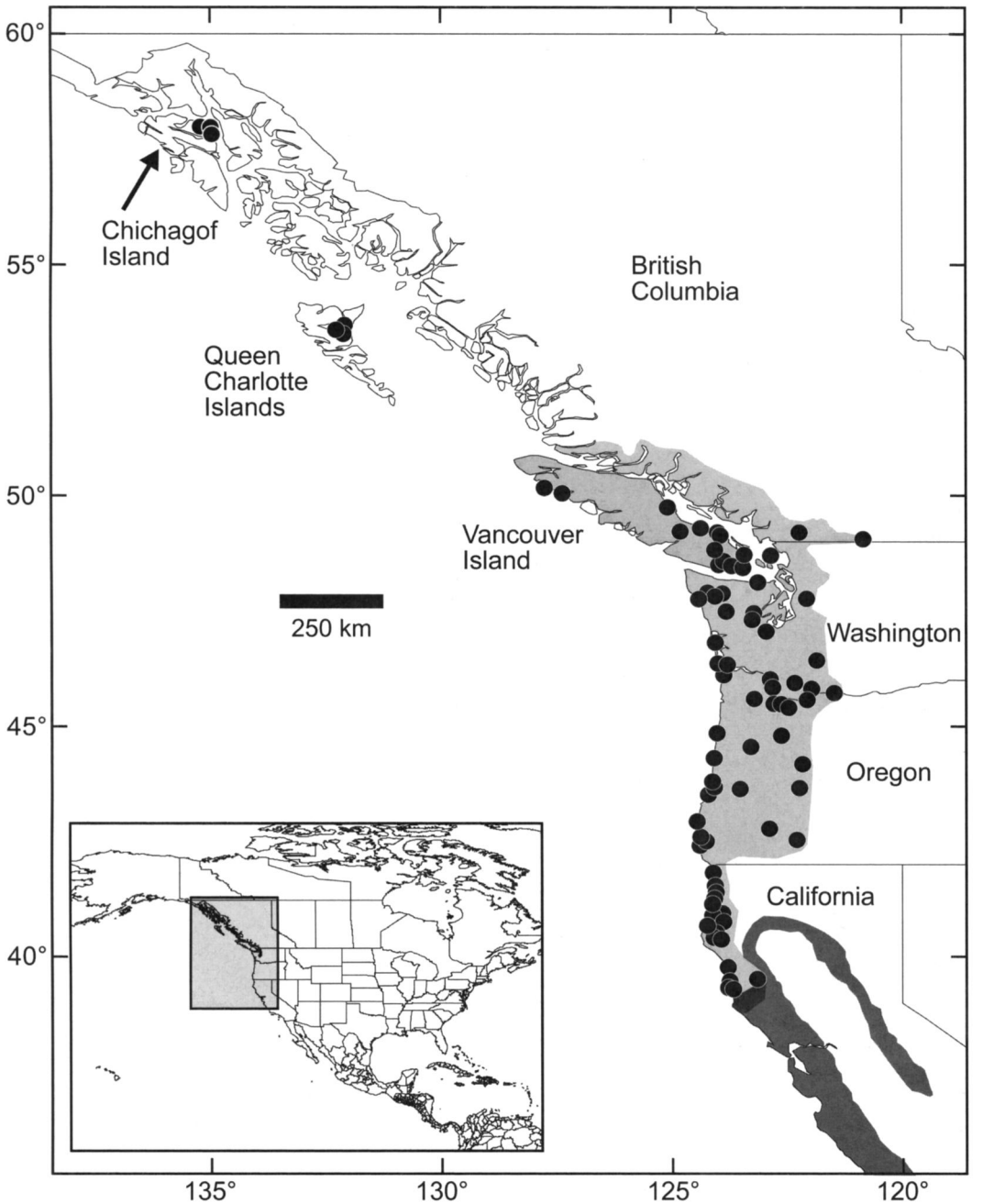

FIG. 1. Map showing the core range of *Rana aurora* (light shading) and portions of the historical range of *Rana draytonii* (medium shading). *Rana draytonii* has been extirpated from much of its range in coastal southern California and the Sierra Nevada. The range of *R. aurora* is depicted as far south as the Big River, Mendocino County, California; the dark shading just south of the Big River depicts the intergrade populations as recovered in Shaffer et al. (2004). Black circles depict all 72 localities used for ecological niche modeling as well as three localities on Graham Island, British Columbia and three localities on Chichagof Island, Alaska. These six extralimital localities were used in comparisons of habitat suitability.

out this work, we will refer to the range in which red-legged frogs are unquestionably native as the "core range."

The frog fauna of the Queen Charlottes consists primarily of a single unquestionably native species, the Western Toad (*Bufo boreas*) and the introduced Pacific Treefrog (*Pseudacris regilla*). In 1961 or 1962, *P. regilla* was introduced in the vicinity of Port Clements, Graham Island and has become increasingly common and widespread on the Queen Charlottes (Reimchen, 1991). In 2002, a third anuran species, a red-legged frog, was also discovered on Graham Island (Ovaska et al., 2002). As with *P. regilla*, the first populations of red-legged frogs were found in the vicinity of Port Clements. Further, red-legged frogs were not encountered during repeated amphibian surveys of Graham Island (see Reimchen, 1991) until the 2002 surveys by Ovaska et al. Thus, it seems probable that red-legged frogs are recent additions to the island's fauna.

The Chichagof Island red-legged frogs are believed to be the result of an introduction in about 1982 by a schoolteacher from the northern Chichagof Island town of Hoonah. This teacher purchased one or two red-legged frog egg masses from Powell Laboratories, Gladstone, Oregon (now Carolina Biological Supply Company). Powell Labs sold wild collected specimens from the northwestern United States (Nace et al., 1971), and available documentation indicates Powell Labs collected *R. aurora* egg masses from Oregon's Columbia River Gorge during this time (Hodge, 2004). The schoolteacher later released the surviving metamorphs into a pond near Kennel Creek, approximately 30 km southeast of Hoonah (Hodge, 2004). The existence of these frogs went undocumented until 2000, when U.S. Forest Service biologists obtained a single specimen (L. Lerum and R. Piehl, Southeast Alaska, Chichagof Island red-legged frog population status, Admiralty Island National Monument, USDA Forest Service, Juneau, Alaska, unpubl. data, 2007). Surveys in 2002 confirmed the presence of red-legged frogs at several ponds in the Kennel Creek watershed (J. C. Sargent, A. Hutton, and J. Waatti, Discovery of the red-legged frog (*Rana aurora*) in northeast Chichagof Island: an introduced species, Tongass National Forest, Hoonah Ranger District, Hoonah, Alaska, unpubl. data, 2003). Broader scale field surveys in 2006 documented red-legged frogs over a 30-km long corridor of contiguous wetland habitat (approximately 6,000 ha) that includes the Kennel Creek and other watersheds (L. Lerum and R. Piehl, Southeast Alaska, Chichagof Island red-legged frog population status, Admiralty Island National Monument, USDA Forest Service, Juneau, Alaska, unpubl. data, 2007).

In contrast to the population expansion on Chichagof Island, Northern and California Red-Legged Frogs are declining throughout much of their native range. As a result of these declines, *R. draytonii* is listed as Threatened under the U.S. Endangered Species Act (United States Fish and Wildlife Service, 1996), and *R. aurora* is considered a Species of Special Concern in British Columbia (COSEWIC, 2002) and California (California Department of Fish and Game, 1994) and as Sensitive-Vulnerable in the Willamette Valley of Oregon and Sensitive-Undetermined in the rest of the state (Oregon Department of Fish and Wildlife, unpubl. data, 1997).

Both the Chichagof and Graham Island populations were described as red-legged frogs prior to the recognition of two different red-legged frog species. Unfortunately, these two species are not easily differentiated based solely on morphological characters. Therefore, a molecular diagnosis is a better alternative for a conclusive identification of these extralimital populations. We conduct molecular sequencing analyses to identify whether these frogs are *R. aurora* or *R. draytonii* and to identify the potential source region for any introduced populations. We also use ecological niche modeling to assess the potential for the persistence and expansion of the extralimital populations and to predict future changes in habitat suitability under a global warming scenario. Finally, we combine the genetic and ecological analyses to assess the conservation and management implications of these extralimital populations.

Materials and Methods

Molecular Analysis.—Our general strategy for assessing the genetic relationships among red-legged frogs from the extralimital and core ranges (both *R. aurora* and *R. draytonii*) was to sequence a large (approximately 1,050 bp) fragment of the mitochondrial cytochrome *b* gene of the Chichagof and Graham Islands frogs and compare these new sequences to the dataset of Shaffer et al. (2004). This dataset consists of a smaller fragment of cytochrome *b* (approximately 400 bp and nested within the larger fragment) from 50 populations of red-legged frogs (77 individuals of *R. aurora* and *R. draytonii*) as well as additional samples from three congeners, *Rana cascadae*, *Rana muscosa*, and *Rana boylii*. Once we identified the haplotypes from the core range (i.e., from the Shaffer et al. dataset) that are most similar to the extralimital haplotypes, we acquired these individuals from

the personal collection of H. Bradley Shaffer (HBS). These individuals were then sequenced for the larger cytochrome *b* fragment in hopes that the larger fragment would provide increased resolution in assessing the relationships among these similar haplotypes.

In total, we extracted DNA and sequenced approximately 1,050 bp of cytochrome *b* from 12 specimens from Chichagof Island, the only available specimen from the Queen Charlotte Islands, British Columbia, and three specimens from the Shaffer et al. (2004) dataset (Appendix 1). The 12 Chichagof samples were collected in June and August 2006 from eight localities. The samples include six tadpoles, one metamorph (25.5 mm SVL), three juveniles (33.7–41.2 mm), and two adult males. All animals were collected live in the field and later sacrificed in the lab. Whole animals or liver samples (for the two adults) were preserved in 95% ethanol for the molecular analyses. Tissue samples used for DNA extraction included fin clips from the tadpoles, leg muscle from the metamorph and juveniles, and liver from the two adults.

DNA was extracted using the Viogene DNA/RNA Extraction Kit. The primers MVZ15-L (Moritz et al., 1992) and CytbAR-H (Goebel et al., 1999) were then used to amplify approximately 1,050 bp of cytochrome *b* using the following thermal cycle profile: 2 min at 94°C, followed by 35 cycles of 94°C for 30 sec, 48°C for 40 sec, and 72°C for 90 sec, and a final extension phase at 72°C for 7 min. This fragment was chosen because it completely overlaps the fragment used by Shaffer et al. (2004) and has been used effectively in other inter- and intraspecific studies of ranids (e.g., Austin et al., 2003). Purified PCR products were sequenced in both directions and analyzed on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Sequences were edited and assembled using Sequencher 4.1 (Gene Codes Corp.) and manually aligned to the Shaffer et al. (2004) dataset in MacClade 4.08 (Sinauer Associates, Sunderland, MA). Variable sites were verified by examining the original chromatograms.

To compare the extralimital individuals to the haplotypes from the core range, we reduced the length of this dataset to 293 bp so that all individuals were represented by complete sequences. Further, we only included one representative of each unique haplotype for the ingroup sample (with one exception) and three unique *R. boylii* haplotypes as the outgroup. As a result, 10 *aurora*, 14 *draytonii*, 5 *muscosa*, and 4 *cascadae* haplotypes were included in the ingroup. Multiple representatives of the most geographically widespread *R. aurora* haplotype were also included. Outgroup choice was based on Shaffer et al. (2004) and Hillis and Wilcox (2005). The most appropriate model of evolution was assessed using the Akaike Information Criterion (AIC) and hierarchical likelihood ratio tests (hLRT) as implemented in MrModeltest (vers. 2.2; J. A. A. Nylander, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden, 2004). Phylogenetic relationships among these haplotypes were assessed under maximum likelihood using GARLI (Zwickl, 2006). Five stochastic likelihood searches from different starting trees were conducted to ensure recovery of the best tree. Nodal support was assessed by conducting a likelihood bootstrap analysis in GARLI using 200 replicates and through a maximum parsimony nonparametric bootstrap in PAUP* (vers. 4.0b10; Sinauer Associates 2003). This bootstrap included 1,000 replicates with 100 random addition sequence replicates per bootstrap replicate and TBR branch swapping.

The phylogenetic analyses of the smaller (293 bp) cytochrome *b* fragment indicated several individuals in the core range that shared sequence identity with the extralimital individuals. To better resolve relationships among these individuals and thereby narrow down the geographic region of any potential source populations, three individuals from the Shaffer et al. (2004) study were then sequenced for the larger (1,050 bp) fragment. Uncorrected sequence divergence among these samples was then assessed using PAUP*.

Niche Modeling.—Niche models are based on environmental values at localities of known occurrence of the target species, which then are used to identify geographic regions that have similar combinations of values. The input for model building consists of a set of localities of known occurrence of the target species and environmental data from digital maps (e.g., annual temperature, annual precipitation, altitude) for the target region.

Rana aurora localities were obtained from Shaffer et al. (2004) and from the following natural history collections: California Academy of Sciences, Museum of Vertebrate Zoology at the University of California Berkeley, Museum of the High Plains at Fort Hays State University, Royal British Columbia Museum, and Royal Ontario Museum. Most records were accessed through the HerpNET data portal (http://www.herpnet.org). Localities without coordinates were georeferenced using the gazetteers from the Alexandria Digital Library Project (http://middleware.alexandria.ucsb.edu) and the Atlas of Canada (http://atlas.nrcan.gc.ca). To reduce spatial autocorrelation, we only included localities separated by at least 10 km.

Because the genetic results demonstrated that the extralimital populations are *R. aurora*, we also wanted to be certain that we were only including native *R. aurora* in the niche modeling. The mtDNA study of Shaffer et al. (2004) suggests that pure *R. aurora* occur north of the Big River, Mendocino County, California, but that both *aurora* and *draytonii* haplotypes can be found in a narrow zone of overlap south of the Big River (Fig. 1). A larger mtDNA dataset of over 600 individuals and additional sampling of the nuclear gene Tropomyosin support this finding (H. B. Shaffer, unpubl. data). Therefore, we only included *R. aurora* localities north of the Big River in our analysis. In total, 72 known localities within the core range of *R. aurora* were included (Fig. 1).

The environmental data for niche modeling consisted of 12 raster maps (11 bioclimatic variables and altitude; resolution = 10 km × 10 km per cell) obtained from WorldClim (Hijmans et al., 2004). We originally examined 19 bioclimatic rasters, some of which were highly correlated. We removed redundant rasters from the analysis by estimating Spearman's Rho pairwise correlations (using the software JMP 5.1; SAS Institute, Cary, NC, 2003). Pairs of highly correlated rasters (rho > 0.9) were identified, and one raster was then removed from the analysis. The raster removed was the one we considered less biologically meaningful (e.g., precipitation of the coldest quarter was removed in favor of precipitation of the wettest month; rho = 0.996). We retained the following bioclimatic rasters: (1) annual mean temperature; (2) mean temperature diurnal range; (3) isothermality; (4) temperature seasonality; (5) maximum temperature of warmest month; (6) mean temperature of driest quarter; (7) mean temperature of coldest quarter; (8) annual precipitation; (9) precipitation of wettest month; (10) precipitation seasonality; and (11) precipitation of driest quarter.

To estimate the future distribution of suitable habitat for *R. aurora*, we projected the environmental niche model to future climate conditions. The prediction is based on the CCM3 global climate model that assumes a doubled concentration of atmospheric CO_2 relative to the preindustrial concentration. Raster assembly for future conditions is described in Hijmans and Graham (2006).

To predict the current and future geographic distribution of suitable habitat we used Maxent, a maximum entropy algorithm that generates a probability distribution of habitat suitability across the target region (Phillips et al., 2006). We chose Maxent among several modeling options because of its high efficiency and predictive performance (Elith et al., 2006; Hijmans and Graham, 2006; Phillips et al., 2006). Maxent operates under the maximum-entropy principle, which seeks to generate a probability distribution by avoiding assumptions beyond those imposed by a set of constraints. In its application to niche modeling, the constraints are defined by the environmental values at the localities of known occurrence of the species (for a description of its mathematical definition and its use in environmental niche modeling, see Phillips et al., 2006). We ran the analyses using Maxent version 2.3 (http://www.cs.princeton.edu/~schapire/maxent) under the default modeling parameters: convergence threshold = 10^{-5}, maximum iterations = 500, regularization multiplier = 1.0. The output from Maxent is a raster map with pixel values ranging from zero to 100, which give a relative index of habitat suitability (higher values for higher suitability). Hereafter, we refer to the suitability values as the suitability index (SI).

Niche models were built with all 72 available localities (i.e., 72 for training; zero for testing) except in tests of model performance, which were conducted to assess model appropriateness (i.e., whether the niche models were accurately predicting the distribution of suitable habitat). For the tests of model performance, localities were partitioned into two halves with random assignment: a training and a testing set. The training set was used for model building and the testing set for model evaluation. By setting an arbitrary threshold for the SI, the continuous model was transformed into a binary map (suitable vs. unsuitable conditions). Then, we applied a binomial test to compare the proportion of localities from the testing set correctly predicted within the suitable habitat versus the expected proportion for a random model with the same amount of suitable habitat (e.g., a random model with suitable habitat occurring on 50% of the total area is expected to include 50% of the localities just by chance). This procedure was repeated 10 times, each for a random partition of localities. In each replicate, the binomial test was applied to 10 commonly used thresholds for the SI.

To compare habitat suitability between the core range (modeled from unquestionably native localities) and the two extralimital ranges, we generated 100 random localities within each range with a visual basic macro for ArcMap 9.1 (ESRI, Redlands, CA; http://arcscripts.esri.com). We determined the SI for each random locality and compared SIs between ranges using a Student's *t*-test. Within the core range, we only included areas with SIs equal or higher than the LPT or "lowest presence threshold" (LPT is the minimum SI assigned to any of the localities used to build the model). For the

comparisons, we generated 100 localities within (1) the core range, (2) the Queen Charlotte Islands (all points were on Graham and Moresby Islands, which are the two main islands of the Queen Charlottes), and (3) Chichagof Island. We included both Graham and Moresby Islands in the sample because they are separated by a narrow channel (only 75 m wide in some areas), and natural or human-mediated colonization of Moresby Island by *R. aurora* seems probable (as has occurred with *P. regilla* [Reimchen, 1991]). We used these same randomly generated localities to compare habitat suitability (SI values) between current and future conditions, within each range, with paired *t*-tests. We also compared habitat suitability between the extralimital localities and the random localities within the core range. For the extralimital sites, we considered the eight collection sites from Chichagof Island (Appendix 1) and the ten published localities on Graham Island (Ovaska et al., 2002). After excluding sites within 10 km of each other, this list was reduced to six localities.

Results

Molecular Analysis.—The Shaffer et al. (2004) dataset included 37 individuals representing 33 unique haplotypes. The HKY + G model was selected as the most appropriate using both the Akaike Information Criterion and likelihood ratio tests. This model was used in all maximum likelihood (GARLI) analyses, and all five searches recovered the same tree ($-\ln L = 1149.50486$). For the 293 bp fragment, the 12 Chichagof frogs and the single Graham Island sample were identical to the northernmost *R. aurora* haplotype in the Shaffer et al. (2004) dataset (this clade was represented by three geographically disparate individuals in our dataset; Fig. 2). This haplotype was shared by all 10 individuals from the five northernmost populations sampled in Shaffer et al. (2004). This northern clade ranges from Oregon to Vancouver Island (Fig. 2; Shaffer et al., 2004: fig. 1). As with Shaffer et al. (2004), we recovered *R. cascadae* as the sister taxon to *R. aurora*, and the relationship among *R. draytonii*, *R. muscosa*, and the *R. aurora/cascadae* clade was equivocal. Although the maximum likelihood topology suggested that *R. muscosa* is the sister taxon to (*aurora* + *cascadae*), this relationship was only weakly supported (ML bootstrap = 45%; MP bootstrap = 62%; Fig. 2). The next most-favored reconstruction places *R. muscosa* and *R. draytonii* as sister taxa (ML bootstrap = 29%; MP bootstrap = 35%).

We then sequenced the larger cytochrome *b* fragment for three representatives of the northern *R. aurora* clade: HBS 26019 from Vancouver Island, British Columbia; HBS 30292 from Thurston County, Washington; and HBS 19824 from Coos County, Oregon. Therefore, in total we sequenced 16 individuals for the larger cytochrome *b* fragment; sequences are available from GenBank (Accession Numbers EU552211–EU552226). The final alignment of this fragment yielded 928 bases shared by all individuals. Maximum uncorrected sequence divergence for these 16 samples was 0.32% (Table 1). All 12 Chichagof samples remained identical for this larger fragment and were most similar to the Oregon sample (Table 1), a result consistent with Hodge's (2004) suggestion that the source population is from northern Oregon. The specimens from Graham Island, Vancouver Island, and Washington remained identical and differed by only three bases from the Chichagof Island frogs (Table 1).

Niche Modeling.—To determine whether the niche models were predicting the occurrence of suitable habitat better than random models, we conducted threshold-dependent binomial tests. Predictions were better than random in all 10 replicates and at all threshold values ($P < 0.001$ in 100 tests).

The minimum suitability value assigned to any of the training localities (LPT) was 3.205. The LPT identifies regions predicted to be at least as suitable as the localities where the species has been recorded. According to this threshold, the distribution of suitable habitat predicted by the model closely approximates the known range of *R. aurora* (Fig. 3). Continuous suitable habitat is predicted to occur up to Margaret Bay, British Columbia, which is only about 25 km north of the northernmost *R. aurora* locality from the vicinity of Sullivan Bay. Close matching between the predicted suitable habitat (areas with SI > 3.205) and the known range continues along the eastern range boundary through British Columbia, Washington, and Oregon. Of particular note are the high suitability scores recovered for the Fraser River Valley in southwestern British Columbia and the Columbia River Valley on the Oregon-Washington border (Fig. 3); in both of these areas, *R. aurora* occurs further inland (Pearl, 2005; Matsuda et al., 2006). In California, however, the known distribution is largely restricted to the Coast Mountain Range (Fig. 1), where the highest suitability index (SI 25–100) closely matches the known localities (compare Figs. 1 and 3); suitable habitat with slightly lower scores (i.e., SI = 3.205–25) is found in the Klamath, Shasta-Trinity, and extreme southern Cascade Ranges, which are all further inland in areas where *R. aurora* has not been found. The model also predicts

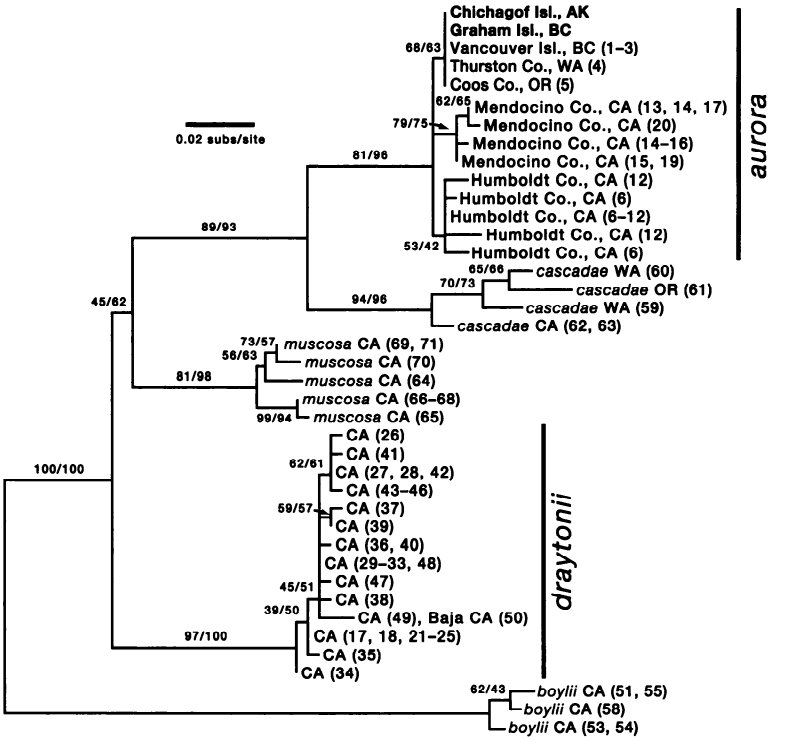

FIG. 2. Maximum likelihood tree for five species of western North American ranids for the 293 bp fragment of cytochrome *b*. Only unique haplotypes were analyzed except in the northern *Rana aurora* clade for which multiple localities were included for comparison to the extralimital (bolded) populations. Numbers along key branches are ML bootstrap values/MP bootstrap values. Localities for *R. aurora* are given as the county and state or province of occurrence: California (CA), Oregon (OR), Washington (WA), British Columbia (BC), and Alaska (AK). Localities for the other species are given as the province or state only. Population numbers as described in Shaffer et al. (2004) are given in parentheses. For haplotypes found in multiple localities, all localities and population numbers are listed.

suitable habitat along the coast south of Mendocino County and along the foothills of the Sierra Nevada as far south as Tulare County, California. Although *R. aurora* has never been found in these regions, this area is, or was historically, occupied by its close congener *R. draytonii*.

The LPT was used to differentiate between "suitable" and "unsuitable" regions. According to this threshold, all *R. aurora* localities on Graham and Chichagof Islands are suitable (SI range 3–21; $N = 6$). However, only 23% of the random localities on Chichagof are greater than or equal to the LPT suggesting that the potential for population expansion is limited on this island (but see Discussion). In contrast, on the Queen Charlotte Islands, 90% of the random localities had a habitat suitability score greater than or equal to the LPT. Nevertheless, habitat suitability on Chichagof and the Queen Charlotte Islands, as measured at the 100 randomly selected localities, is significantly lower than in

TABLE 1. Distance matrix showing the number of bases that differ among 16 individuals for 928 bases of cytochrome *b*.

Number of bases between	1	2	3	4	5
Chichagof Isl., AK ($N = 12$)	–				
Graham Isl., BC (RBCM 1945)	3	–			
Vancouver Isl., BC (HBS 26019)	3	0	–		
Washington (HBS 30292)	3	0	0	–	
Oregon (HBS 19824)	2	1	1	1	–

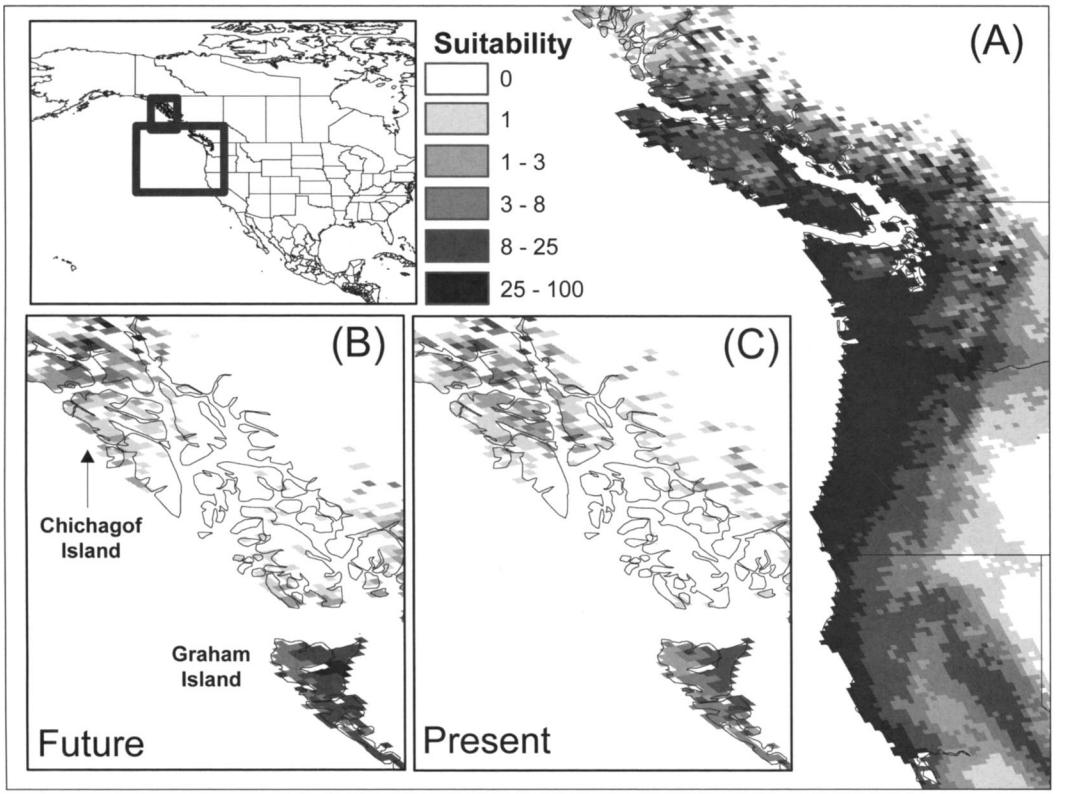

FIG. 3. Results for ecological niche modeling depicting predicted habitat suitability for *Rana aurora* in western North America. (A) Suitability under current conditions in the core (i.e., unquestionably native) range; (B) suitability under future conditions (global warming) in the extralimital range; (C) suitability under current conditions in the extralimital range. Higher index values indicate higher habitat suitability. Regions with suitability scores below three have conditions that are less suitable than conditions at the 72 recorded localities used to build the niche model.

the native range ($t = 6.44$, $P < 0.001$ for the Queen Charlotte's; $t = 9.34$, $P < 0.001$ for Chichagof). Suitability is also lower at the six extralimital localities compared to the random localities within the native range ($t = 3.26$, $P = 0.008$).

Predictions for the future under a global warming model show opposite effects for the extralimital populations (Fig. 3). On the Queen Charlotte Islands, habitat suitability is expected to improve at the three localities of known occurrence on Graham Island and also at all 100 random localities (paired $t = 26.59$, $P < 0.001$). In the global warming model, all random localities on the Queen Charlotte Islands have SI values above the LPT. Thus, expansion of this population is expected based on niche modeling. In contrast, on Chichagof Island, the habitat will be unsuitable (SI < LPT) at the three localities of known occurrence and at 98 of the 100 random localities. Conditions at 86% of the random localities on Chichagof are expected to remain equally suitable or become less suitable (recall that scores at 77% of the random localities were less than the LPT under the present conditions model). Future suitability is significantly lower than present suitability among the random localities on Chichagof (paired $t = -7.36$, $P < 0.001$).

DISCUSSION

Extralimital Populations.—Red-legged frogs have a surprisingly long history of introductions. The earliest documented introduction occurred in 1857 when frogs were released on Oahu, Hawaii, but disappeared after several months (McKeown, 1996). An introduced population of *R. draytonii* was also documented on Santa Cruz Island off the coast of southern California in 1919, but it is also believed to have disappeared shortly thereafter (Jennings, 1988; Fellers, 2005). More recently, red-legged frogs were introduced to multiple localities in Nevada as part of frog farming ventures in the 1930s and 1940s, and several populations

became established (Linsdale, 1940; Reaser, 2003). The current status of these populations is unknown, although following observations made in 1983 Green (1985) described the Duckwater population as "substantial." Green (1985) used allozyme data to demonstrate that the Duckwater frogs are *R. draytonii*, and the other Nevada populations have subsequently been assumed to also be the California Red-Legged Frog (Reaser, 2003).

Here we used DNA sequence data to examine two extralimital populations of red-legged frogs that occur far north of the core range of *R. aurora*. The Graham Island, British Columbia and Chichagof Island, Alaska frogs are clearly nested within the geographically widespread northern clade of *R. aurora* (Fig. 2). Moreover, the available data suggests that the Graham Island frogs are most closely related to frogs from Vancouver Island and Washington (Table 1). The lack of available samples from this region and the lack of sequence variation prevent more specific identification of any possible source population if in fact the Graham Island frogs are the result of an introduction. Similarly, the Chichagof frogs are most closely related to the only available sample from Oregon, a result consistent with Hodge's (2004) claim that the source population is from the Columbia River Gorge. The complete lack of variation among the 12 Chichagof Island samples is also consistent with a recent introduction and range expansion from a small source population, as would be expected if the founding population only included metamorphs of one or two clutches.

Are the Graham Island Frogs Introduced?.—Since their discovery in 2002, whether or not the Graham Island frogs are introduced has been debated. Although Matsuda et al. (2006) consider this island population to be introduced, Ovaska et al. (2002) suggest that they could be native. The expectation for an introduced population is the lack of variation across multiple samples (i.e. a genetic bottleneck), as we found in the Chichagof samples. Unfortunately, multiple samples from Graham Island are not currently available; hence, a genetic diagnosis is not possible. However, the niche modeling and the genetic relationships among populations in the core range provide some insight. The habitat suitability index rapidly decreases below the lowest presence threshold (LPT = 3.25) northwest of Sullivan Bay, British Columbia (Fig. 3). Therefore, the niche modeling and the known northernmost localities are in close agreement that the northern range limit of *R. aurora* on the mainland is near Sullivan Bay. This congruence indicates that we can be confident in the current interpretation of the northern range limit of this species on the mainland and, therefore, that source populations for natural colonization of the Queen Charlotte Islands are at least 400 km south of the populations currently found on Graham Island. Further, Shaffer et al. (2004) demonstrate that the majority of the genetic variation in *R. aurora* occurs in the southernmost portion of the species range. For example, in the 293 bp fragment, nine of the 10 haplotypes recovered for *R. aurora* occur between Big River, Mendocino County and Redwood Creek, Humboldt County, a region spanning only 225 km in northern California, while only a single haplotype was found among the five populations from central Oregon to southern Vancouver Island, a region spanning approximately 630 km. These results suggest a recent, postglacial, range expansion northward along the coast, a phylogeographic pattern found in other similarly distributed amphibians (e.g., the Rough-Skinned Newt, *Taricha granulosa*; Kuchta and Tan, 2005). Therefore, *R. aurora* has likely only relatively recently expanded into these northern areas and historically has been even more distant from Graham Island, further decreasing opportunities for nonhuman mediated colonization.

Additional evidence supporting a recent introduction comes from previous faunal surveys of the Queen Charlotte Islands. General surveys indicate that the islands have a depauperate vertebrate fauna (Foster, 1965). These surveys and more focused anuran surveys centered in the area where *R. aurora* is now found failed to find this species (Reimchen, 1991), even though Ovaska et al. (2002) noted that *R. aurora* is now "widespread" in portions of the area that were previously surveyed. Introductions of frogs to the Queen Charlotte Islands have also been previously documented; the Pacific Treefrog, *Pseudacris regilla*, was introduced to Port Clements on Graham Island in the early 1960s from individuals collected near Comox Lake on Vancouver Island (Reimchen, 1991; Matsuda et al., 2006). Interestingly, *R. aurora* is also known from Comox Lake (e.g., RBCM 0918). All of this evidence suggests that *R. aurora* is introduced to the islands. Further confirmation of an introduction would require information from residents who may have personal knowledge of introductions, as was reported for *P. regilla* (Reimchen, 1991), or additional genetic data (e.g., microsatellites) from multiple individuals to test for a recent population bottleneck.

Habitat Suitability on Chichagof and Graham Island.—The extralimital populations occur in the most suitable habitat on their respective islands. The eastern half of Chichagof Island,

including the peninsula between Freshwater Bay and Tenakee Inlet where *R. aurora* occurs, has the most suitable habitat (Fig. 3). Nevertheless, the suitability scores of much of this region are close to the LPT recovered for the localities in the core range suggesting that the habitat is only marginally suitable. The prediction under global warming suggests that habitat suitability will decrease below the LPT almost everywhere across Chichagof Island. Overall, niche modeling suggests that the Chichagof population will expand over a relatively restricted area and may even decline in the future. However, these predictions could be biased if either of two critical assumptions is not met: (1) that the niche model accurately predicts ecological tolerances and requirements; and (2) that the niche of *R. aurora* will be conserved in the extralimital populations and these frogs will not adapt to the novel and changing future conditions. Our model does seem to accurately predict the ecological requirements as evidenced by the close match between habitat suitability and the known range. However, biotic factors not considered by the models (e.g., ecological release in the exotic range) could lead to sustained population increase and range expansion. The same outcome could result from adaptive responses to environmental conditions in the exotic range. A putative example of this response is given by *Bufo marinus* (classification follows Hillis [2007] and G. B. Pauly, D. M. Hillis, and D. C. Cannatella [unpubl. data] who reject recent arbitrary proposed changes to the generic names of many North and South American ranids and bufonids), which has expanded its niche in its exotic range in Australia, even surpassing the breadth of environmental conditions that this species occupies in its native range in the Neotropics (Urban et al., 2007).

On Graham Island, habitat suitability is highest around Port Clements and along the Massett Peninsula to the northeast of Port Clements. Future conditions appear to be even more suitable, suggesting that these frogs are likely to expand to other areas of the Queen Charlotte Islands. The spread of these frogs may be especially likely if the population is in fact the result of an introduction and their current range on Graham Island is already the result of expansion from the point of introduction.

Management and Conservation of Rana aurora.—The obvious question is what to do about introduced populations of *R. aurora*. Here, we will only consider management issues for the Chichagof population for which introduced status is unquestioned. If the status of the Graham Island population can be unquestionably established as being introduced, then the management concerns and options discussed below can be similarly applied on Graham Island. Here, we discuss two management options and suggest a specific strategy for the Chichagof population.

One option is to attempt to remove all *R. aurora* from the introduced range. Removal would thwart a variety of potential problems associated with the introduction of nonnative wildlife including predation on native wildlife and introduction of pathogens and disease. The diet of *R. aurora* largely consists of invertebrate prey (Licht, 1986), but they can also consume vertebrates including amphibians (Rabinowe et al., 2002). *Bufo boreas* is native to Chichagof (MacDonald and Cook, 2007) and, therefore, has the potential to be adversely affected. The possible introduction of disease is particularly worrisome as chytrid fungus, *Batrachochytrium dendrobatidis*, is known to be a central factor in global amphibian declines (Lips et al., 2006; Pounds et al., 2006), and chytrid is known to occur in multiple species of ranids including *R. aurora* (Pearl et al., 2007). Further, the possibility of disease transmission to the native *B. boreas* is particularly troubling because chytrid fungus has been implicated in the decline of this species in Colorado (Scherer et al., 2005). However, complete eradication is likely to have a high financial cost and a low likelihood of success given the remote and unpopulated landscape of northeastern Chichagof Island.

Another option is to allow the frogs to persist, at least while potential effects on native species are evaluated. Here the goal should be to keep the frogs isolated to Chichagof. Given the number of intentional human transplants and introductions of other amphibians to southeastern Alaska (e.g., *P. regilla* to Revillagigedo Island, *Rana sylvatica* to Douglas Island, and *Taricha granulosa* to Baranof Island and on the mainland north of Juneau; MacDonald and Cook, 2007), this may not be a simple task and likely will require efforts to increase the public's knowledge of the hazards of amphibian translocations. Niche modeling suggests that regions north of Chichagof on the mainland in Glacier Bay National Park have suitable habitat and may be especially at risk for future introductions (Fig. 3). The *R. aurora* on Chichagof should be studied for potential impacts on native wildlife and monitored for disease occurrence. Continued study of range changes on Chichagof should also be undertaken (including estimates of the rate of range expansion); if *R. aurora* continues to expand, then the additional localities could be used in further niche modeling to assess changes in ecological tolerances and requirements in the introduced range (sensu Urban et al., 2007). As this information is

gathered, management decisions should be reevaluated.

We believe this latter approach may be best for the Chichagof population. Given limited resources for amphibian conservation in Alaska, and the likely expense associated with eradication, we advocate increased monitoring of this population to assess changes in range size and threats to native species. As long as there is no evidence of significant ecological impacts or high likelihood of subsequent introductions, we suggest allowing this population to persist. This strategy may benefit future conservation of *R. aurora*. Given the global decline of amphibians and declines of all western North American ranid species, it may be useful to allow these disjunct populations to persist as an insurance against future extinctions. Similar justifications have been used for maintaining an introduced colony of Bolson's tortoise, *Gopherus flavomarginatus*, in New Mexico (H. W. Greene, pers. comm.; Donlan et al., 2006), which is far north of the only other extant population in a single small valley in central Mexico.

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Literature Cited

Austin, J. D., S. C. Lougheed, P. E. Moler, and P. T. Boag. 2003. Phylogenetics, zoogeography, and the role of dispersal and vicariance in the evolution of the *Rana catesbeiana* (Anura: Ranidae) species group. Biological Journal of the Linnean Society 80:601–624.

California Department of Fish and Game. 1994. Amphibian and Reptile Species of Special Concern in California, Northern Red-legged Frog. California Department of Fish and Game. Rancho Cordova, CA.

COSEWIC. 2002. COSEWIC Assessment and Status Report on the Red-Legged Frog, *Rana aurora*. Committee on the Status of Endangered Wildlife in Canada. Ottawa, Ontario, Canada.

Donlan, C. J., J. Berger, C. E. Bock, J. H. Bock, D. A. Burney, J. A. Estes, D. Foreman, P. S. Martin, G. W. Roemer, F. A. Smith, M. E. Soulé, and H. W. Greene. 2006. Pleistocene rewilding: an optimistic agenda for twenty-first century conservation. American Naturalist 168:660–681.

Elith, J., C. H. Graham, R. P. Anderson, M. Dudik, S. Ferrier, A. Guisan, R. J. Hijmans, F. Huettmann, J. R. Leathwick, A. Lehmann, J. Li, L. G. Lohmann, B. A. Loiselle, G. Manion, C. Moritz, M. Nakamura, Y. Nakazawa, J. M. Overton, A. T. Peterson, S. J. Phillips, K. Richardson, R. Scachetti-Pereira, R. E. Schapire, J. Soberon, S. Williams, M. S. Wisz, and N. E. Zimmermann. 2006. Novel methods improve prediction of species' distributions from occurrence data. Ecography 29:129–151.

Fellers, G. M. 2005. *Rana draytonii*, California Red-Legged Frog. *In* M. J. Lannoo (ed.), Amphibian Declines: The Conservation Status of United States Species, pp. 552–554. University of California Press, Berkeley.

Foster, J. B. 1965. The evolution of the mammals of the Queen Charlotte Islands, British Columbia. Occasional Papers of the British Columbia Provincial Museum 14:1–130.

Goebel, A. M., J. M. Donnelly, and M. E. Atz. 1999. PCR primers and amplification methods for 12S ribosomal DNA, the control region, cytochrome oxidase I, and cytochrome *b* in bufonids and other frogs, and an overview of PCR primers which have amplified DNA in amphibians successfully. Molecular Phylogenetics and Evolution 11:163–199.

Green, D. M. 1985. Biochemical identification of Red-Legged Frogs, *Rana aurora draytonii* (Ranidae) at Duckwater, Nevada. Southwestern Naturalist 30:614–616.

Hillis, D. M. 2007. Constraints in naming parts of the Tree of Life. Molecular Phylogenetics and Evolution 42:331–338.

Hillis, D. M., and T. P. Wilcox. 2005. Phylogeny of the New World true frogs (*Rana*). Molecular Phylogenetics and Evolution 34:299–314.

Hijmans, R. J., and C. H. Graham. 2006. The ability of climate envelope models to predict the effect of climate change on species distributions. Global Change Biology 12:2272–2281.

Hijmans, R. J., S. Cameron, and J. Parra. 2004. WORLDCLIM. Museum of Vertebrate Zoology, University of California, Berkeley.

Hodge, R. P. 2004. Geographic distribution. *Rana aurora*. Herpetological Review 35:79.

Jennings, M. R. 1988. Origin of the population of *Rana aurora draytonii* on Santa Cruz Island, California. Herpetological Review 19:76.

Kuchta, S. R., and A. M. Tan. 2005. Isolation by distance and post-glacial range expansion in the Rough-Skinned Newt, *Taricha granulosa*. Molecular Ecology 14:225–244.

Licht, L. E. 1986. Food and feeding behavior of sympatric Red-Legged Frogs, *Rana aurora*, and Spotted Frogs, *Rana pretiosa*, in southwestern British Columbia. Canadian Field-Naturalist 100:22–31.

Linsdale, J. M. 1940. Amphibians and reptiles in Nevada. Proceedings of the American Academy of Arts and Sciences 73:197–257.

LIPS, K. R., F. BREM, R. BRENES, J. D. REEVE, R. A. ALFORD, J. VOYLES, C. CAREY, L. LIVO, A. P. PESSIER, AND J. P. COLLINS. 2006. Emerging infectious disease and the loss of biodiversity In a Neotropical amphibian community. Proceedings of the National Academy of Sciences of the United States of America 103:3165–3170.

MACDONALD, S. O., AND J. A. COOK. 2007. Mammals and Amphibians of Southeast Alaska. Special Publication Number 8, Museum of Southwestern Biology, University of New Mexico, Albuquerque.

MATSUDA, B., D. M. GREEN, AND P. T. GREGORY. 2006. The Amphibians and Reptiles of British Columbia. Royal British Columbia Museum, Victoria, BC, Canada.

MCKEOWN, S. 1996. A Field Guide to Reptiles and Amphibians in the Hawaiian Islands. Diamond Head Publishing, Los Osos, CA.

MORITZ, C., C. J. SCHNEIDER, AND D. B. WAKE. 1992. Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. Systematic Biology 41:273–291.

NACE, G. W., J. K. WAAGE, AND C. M. RICHARDS. 1971. Sources of amphibians for research. BioScience 21:768–773.

OVASKA, K., L. HYATT, AND L. SOPUCK. 2002. Geographic distribution. *Rana aurora*. Herpetological Review 33:318.

PEARL, C. A. 2005. *Rana aurora*, Northern Red-Legged Frog. *In* M. J. Lannoo (ed.), Amphibian Declines: The Conservation Status of United States Species, pp. 528–530. University of California Press, Berkeley.

PEARL, C. A., E. L. BULL, D. E. GREEN, J. BOWERMAN, M. J. ADAMS, A. HYATT, AND W. H. WENTE. 2007. Occurrence of the amphibian pathogen *Batrachochytrium dendrobatidis* in the Pacific Northwest. Journal of Herpetology 41:145–149.

PHILLIPS, S. J., R. P. ANDERSON, AND R. E. SCHAPIRE. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling 190:231–259.

POUNDS, J. A., M. R. BUSTAMANTE, L. A. COLOMA, J. A. CONSUEGRA, M. P. L. FOGDEN, P. N. FOSTER, E. LA MARCA, K. L. MASTERS, A. MERINO-VITERI, R. PUSCHENDORF, S. R. RON, G. A. SÁNCHEZ-AZOFEIFA, C. J. STILL, AND B. E. YOUNG. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature 439:161–167.

RABINOWE, J. H., J. T. SERRA, M. P. HAYES, AND T. QUINN. 2002. *Rana aurora aurora* (Northern Red-Legged Frog). Diet. Herpetological Review 33:128.

REASER, J. K. 2003. Occurrence of the California Red-Legged Frog (*Rana aurora draytonii*) in Nevada, USA. Western North American Naturalist 63:400–401.

REIMCHEN, T. E. 1991. Introduction and dispersal of the Pacific Treefrog, *Hyla regilla*, on the Queen Charlotte Islands, British Columbia. Canadian Field-Naturalist 105:288–290.

SCHERER, R. D., E. MUTHS, B. R. NOON, AND P. S. CORN. 2005. An evaluation of weather and disease as causes of decline in two populations of Boreal Toads. Ecological Applications 15:2150–2160.

SHAFFER, H. B., G. M. FELLERS, S. R. VOSS, J. C. OLIVER, AND G. B. PAULY. 2004. Species boundaries, phylogeography and conservation genetics of the Red-Legged Frog (*Rana aurora/draytonii*) complex. Molecular Ecology 13:2667–2677.

STEBBINS, R. C. 2003. A Field Guide to Western Reptiles and Amphibians. 3rd ed. Houghton Mifflin, Boston, MA.

UNITED STATES FISH AND WILDLIFE SERVICE (USFWS). 1996. Endangered and Threatened Wildlife and Plants: Determination of Threatened Status for the California Red-Legged Frog. Federal Register 25813–25833. USFWS, Portland, OR.

URBAN, M. C., B. L. PHILLIPS, D. K. SKELLY, AND R. SHINE. 2007. The Cane Toad's (*Chaunus* [Bufo] *marinus*) increasing ability to invade Australia is revealed by a dynamically updated range model. Proceedings of the Royal Society of London B 274:1413–1419.

ZWICKL, D. J. 2006. Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets under the Maximum Likelihood Criterion. Ph.D. diss., University of Texas, Austin.

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APPENDIX 1

Locality data for the 16 specimens sequenced for the molecular analysis. Voucher numbers and the sample size examined from each site are provided in parentheses. AB = National Marine Fisheries Service, Auke Bay Laboratory, Juneau, Alaska. TNHC = Texas Natural History Collection, University of Texas, Austin. RBCM = Royal British Columbia Museum. HBS = the personal collection of H. Bradley Shaffer.

Chichagof Island, Alaska. 57.88659°N, 135.16895°W. (N = 3, AB 07-0001–0002, TNHC 67059).

Chichagof Island, Alaska. 57.85360°N, 135.10093°W. (N = 1, TNHC 67051).

Chichagof Island, Alaska. 57.80419°N, 135.08469°W. (N = 3, TNHC 67052–67054).

Chichagof Island, Alaska. 57.94577°N, 135.26190°W. (N = 1, TNHC 67055).

Chichagof Island, Alaska. 57.83732°N, 135.07881°W. (N = 1, TNHC 67056).

Chichagof Island, Alaska. 57.80321°N, 134.99011°W. (N = 1, TNHC 67057).

Chichagof Island, Alaska. 57.92670°N, 135.24064°W. (N = 1, TNHC 67058).

Chichagof Island, Alaska. 57.92978°N, 135.21234°W. (N = 1, TNHC 67060).

Graham Island, Queen Charlotte Islands, British Columbia, Canada. 53.63889°N, 132.21111°W. (N = 1, RBCM 1945.00).

Prospect Lake Road, Vancouver Island, British Columbia, Canada. 48.5014°N, 123.4428°W. (N = 1, HBS 26019).

Grass Lake, Olympia, Thurston County, Washington. 47.0543°N, 122.9496°W. (N = 1, HBS 30292).

Saunders Lake off Hwy 101, Coos County, Oregon. 43.5358°,N 124.2167°W. (N = 1, HBS 19824).