Limnol. Oceanogr., 53(2), 2008, 675–689

Islands under islands: The phylogeography and evolution of *Halocaridina rubra* Holthuis, 1963 (Crustacean: Decapoda: Atyidae) in the Hawaiian archipelago

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Abstract

The genetic structure and evolutionary history of an endemic anchialine species, the shrimp *Halocaridina rubra* Holthuis, 1963 (Crustacean: Decapoda: Atyidae), was investigated across its range in the Hawaiian archipelago using mitochondrial (e.g., cytochrome oxidase subunit I and large subunit ribosomal) gene sequences. A survey of 573 individuals collected from 34 sites on the islands of Hawai'i, Maui, and Oahu revealed 13 distinct genetic groups belonging to eight divergent lineages. In general, a *Halocaridina* genetic group or lineage was restricted to a particular region of a single Hawaiian Island, with no individuals being exchanged between them. This pattern

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stems from a combination of intrinsic organismal properties such as large egg size, abbreviated development, restricted larval habitat and larval feeding mode, and extrinsic obstacles to gene flow in the form of a marine barrier and geologic features that compartmentalize the islands' aquifers. The phylogeographic structuring on and between islands suggests that evolutionary diversification in *Halocaridina* is driven by population fragmentation, isolation, and subsequent diversification in the aquifers of the Hawaiian Islands. Calibration of cytochrome oxidase subunit I sequence divergence between sister *Halocaridina* lineages to the geologic age of Kilauea volcano on Hawai'i implies that diversification in the genus is proceeding at a short-term rate of 20% per million years. The examined mitochondrial genes were generally inadequate for inferring phylogenetic relationships between the *Halocaridina* lineages.

Anchialine environments are characterized as "bodies of haline waters, usually with restricted exposure to air, always with extensive subterranean connections to the sea, and showing noticeable marine and terrestrial influences" (Stock 1986). Habitats fitting this definition can be found around the world (Maciolek 1983) and occur as coastal, land-locked open pools, pools in caves, or submerged cave passages (Holthuis 1973; Iliffe 2000). The tidal rhythms in these habitats are the result of water movement between them and the sea via subterranean connections that perforate the basin of the pool or cave. Typically, the infiltrating oceanic water mixes with terrestrial groundwater, resulting in a varying salinity (i.e., 0.5 to 30) over the tidal cycle. Thus, anchialine habitats are unique from an ecological and evolutionary perspective since their waters represent an extension of the underlying freshwater aquifer while simultaneously having a persistent connection to the sea.

Although a variety of organisms exploit anchialine habitats (Maciolek and Brock 1974; Iliffe et al. 1984), the genetic structure and population connectivity of just two species from this habitat have been examined to date (Kano and Kase 2004; Santos 2006). For this reason, little is known regarding the evolutionary processes operating on endemic anchialine organisms, including the factors promoting population differentiation in these habitats. Differentiation results from a reduction or cessation of gene flow among populations and is often considered an important initial step in the process of speciation (reviewed by Coyne and Orr 2004). Given that anchialine habitats represent an interface between the marine and subterranean groundwater environments, alternative hypotheses can be proposed regarding how populations differentiate in these habitats. One possibility is that anchialine organisms are comparable with those occupying the marine environment. If this were the case, populations would typically be considered to be demographically "open" because of larval dispersal and differentiation would only occur in the presence of strong barriers (reviewed by Caley et al. 1996; but *see* Warner and Cowen 2002). The anchialine gastropod *Neritilia cavernicola* exhibits just such a system, with no genetic differentiation being observed between two islands in the Philippines separated by ~200 km (Kano and Kase 2004). On the other hand, anchialine habitats are also an extension of the subterranean freshwater environment, and it can be hypothesized that diversification results from processes similar to those that act on groundwater organisms. Under this scenario, fragmentation events are the prevailing mechanism promoting divergence (reviewed by Sbordoni et al. 2000), with support for this coming from patterns of genetic diversity in subterranean organisms such as crustaceans (e.g., Buhay and Crandall 2005; Finston et al. 2007) and the fauna of Cape Range peninsula in Western Australia (Humphreys and Adams 1991). Along with extrinsic factors such as barriers, intrinsic properties of an organism, specifically dispersal ability, can also significantly influence population differentiation (e.g., Sale and Kritzer 2003; Havel and Shurin 2004). For example, a large-scale survey of organisms from varying environments found a significant negative correlation between dispersal ability and population divergence (Bohonak 1999). Thus, the factors contributing to population differentiation can be complex. A powerful approach in elucidating these factors and their relative contribution to the process of differentiation is to combine knowledge on life history with information on the geographic distribution of genetic variation (i.e., phylogeography) for the organism in question (Avise et al. 1987). Analyses like these on organisms from environments that have received little attention will not only contribute to a better understanding of the factors promoting differentiation in that context (in this case, anchialine habitats), but may also provide insight into the mode and tempo of evolution in general.

Having the largest concentration of anchialine environments in the world (~520; Brock et al. 1987), the Hawaiian archipelago is an ideal location for studying evolutionary processes in these habitats. Hawaiian anchialine habitats are home to diverse assemblages of microorganisms (Bailey-Brock and Brock 1993) as well as a macrofauna dominated by endemic gastropod mollusks and crustaceans (Maciolek and Brock 1974; Maciolek 1983). The shrimp *Halocaridina rubra* Holthuis, 1963 (Crustacea: Decapoda: Atyidae), commonly referred to as '*ōpae* '*ula* (*lit.* tiny red shrimp), is particularly characteristic of the endemic

Acknowledgments

We are extremely grateful to Ann Fielding of Island Explorations for logistical support on the island of Maui and the following individuals and entities for allowing access to anchialine ponds on lands that they administer: V. Yamanaka (Pōhue Bay, Ka'ū), M. Ikeda (Queen Liliuokalani Children's Center, Kona-Kohala), D. Chai (Hualālai, Kona-Kohala), the U.S. National Park Service (Hawai'i Volcano National Park; permit HAVO-2005-SCI-0030), and the U.S. Fish and Wildlife Service (Kalaeloa Unit of the Pearl Harbor National Wildlife Refuge; permit SUP ORC-PK4-07). We also thank the two anonymous reviewers whose comments improved this manuscript.

This is contribution 29 to the Auburn University (AU) Marine Biology Program.

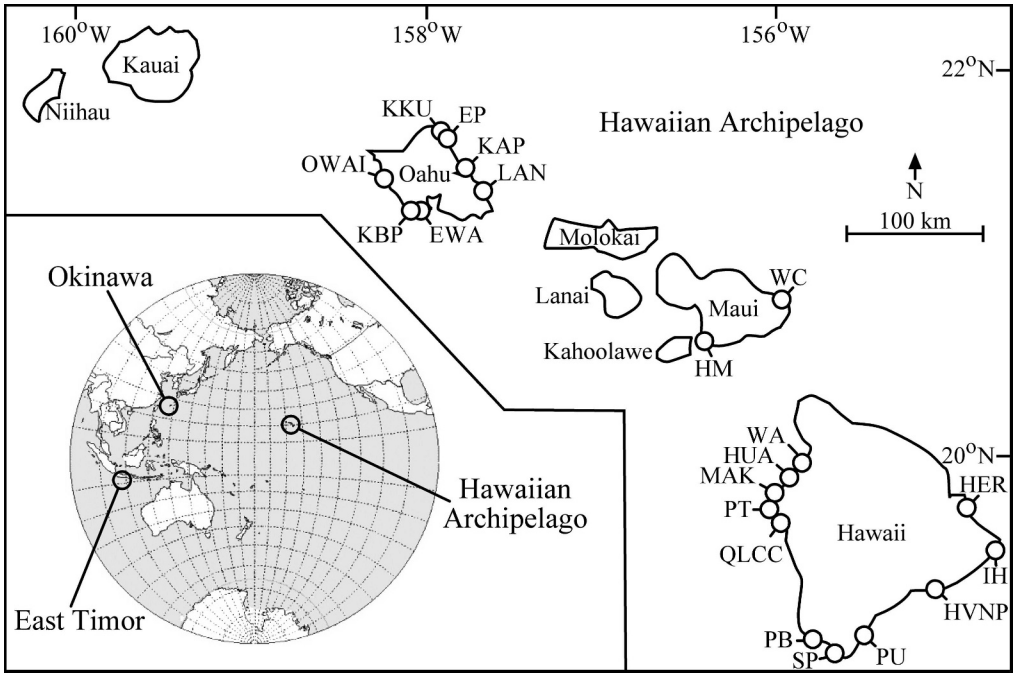

Fig. 1. Map of the Pacific Ocean and Hawaiian archipelago depicting locations where *Halocaridina*, *Halocaridinides trigonophthalma*, and the *Antecaridina* sp. were sampled for this study. *Halocaridinides trigonophthalma* and the *Antecaridina* sp. were collected from Irabu Island, Okinawa, Japan and the Lautem land district of East Timor, respectively. *Halocaridina rubra* was collected from 20 primary localities on three islands: Hawai'i, Maui, and Oahu. Site labels are listed in Table 1. Geographical coordinates of sampling sites are available from the corresponding author upon request.

Hawaiian anchialine fauna. These small (~10 mm in length), microphagous grazers have a distribution spanning Hawai'i, Kahoolawe, Maui, Molokai, and Oahu (reviewed by Santos 2006). This distribution, combined with the fact that each of these islands had to be colonized de novo following its creation, implies dispersal through the marine environment at some frequency. Additionally, *H. rubra* appears to have an intimate association with the subterranean (i.e., hypogeal) environment since (1) berried (i.e., egg-carrying) females and larvae have never been observed in the epigeal (i.e., well-lighted) portion of these environments (Maciolek 1983; Bailey-Brock and Brock 1993; pers. obs.), implying that reproduction and larval development are restricted to the hypogeal, and (2) rapid colonization of man-made anchialine habitats on Kahoolawe (Brock and Bailey-Brock 1998) and Oahu (Maciolek 1983; pers. obs.) that have no or few natural anchialine habitats suggest the mobilization of *H. rubra* populations already residing within the aquifer.

For *H. rubra*, a prior study on the island of Hawai'i demonstrated strong subdivision and little to no gene flow between populations separated by >30 km, with limited oceanic dispersal and regional hydrology influencing this pattern (Santos 2006). This led us to hypothesize that population differentiation and evolutionary diversification in *H. rubra* of the Hawaiian archipelago result from population fragmentation or isolation events (or both), similar to what has been observed for subterranean groundwater organisms. To test this hypothesis, sequence variation at two mitochondrial (i.e., cytochrome oxidase subunit I [COI] and large subunit ribosomal [16S-rDNA]) genes was examined from an extensive survey of *H. rubra* populations across its range in the archipelago. A further goal was to combine the available phylogeographic and biological data for *H. rubra* with geologic information from the islands to identify the particular life history traits and barriers contributing to population differentiation in this species.

Methods

Biological materials and habitat characteristics—Sampling sites were chosen to exhaustively survey areas of each island in the Hawaiian archipelago with recorded anchialine habitats and *H. rubra* populations. Although *Halocaridina* has been reported from Kahoolawe, Molokai, and Lua o Palahemo lava tube in the South Point region of Hawai'i in the past (Maciolek 1983; Kensley and Williams 1986; Brock and Bailey-Brock 1998), recent trips to these sites were unable to identify extant populations (pers. obs.). For Maui and Oahu, specimens of *H. rubra* were collected from 17 sites (Fig. 1; Table 1) between February 2005 and July 2006. Anchialine habitats on Maui occupied basalt basins, whereas those on Oahu occurred in fossil coral (e.g., calcium carbonate) reefs; all habitats except Waianapanapa Cave (site WC) on Maui exhibited daily tidal fluctuations. Temperature and salinity ranged from ~17°C to 30°C and 1 to 16, respectively, across sites. Between 6 and 33 *H. rubra*

Table 1. Sampling sites for *Halocaridina, Halocaridinides trigonophthalma*, and the *Antecaridina* sp. in this study. † = lower-case letters designate discrete anchialine ponds located within 100 m of each other at a site.

Genus/species	Geographic location	Island or land district	Site name	Site code(s)†
Halocaridina rubra	Hawaiian archipelago	Hawai'i	Hawaii Volcano National Park	HVNPa, HVNPb
			Herman's House	HERa, HERb
			Hualālai	HUAa, HUAb
			Isaac Hale	IH
			Makalawena	MAK
			Pine Trees	PT
			Pōhue Bay	PBa, PBb
			Puhi Ula Cave	PU
			Queen Liliokalani Children's Center	QLCCa, QLCCb
			Wai'ahukini	SP
			Waikoloa	WAa, WAc
		Maui	Cape Hanamanioa	HM
			Joe's Pond	JOE
			Kanahena Pond	MLOM
			Kauhinalakini Pond	KAUP
			Kealia Well Shaft	KWS
			Skippy's Pond	SKIP
			Waianapanapa Cave	WC
		Oahu	Eric's Pond	EP
			Ewa Beach	EWA
			Kalaeloa Unit	KBP
			Kapapa Island	KAP
			Kahuku Well Shaft	KKU
			Popoia Island	LAN
			Waianae Boat Harbor	OWAI
			White Plains Holes	WP6, WP7, WP8
Halocaridinides trigonophthalma	Okinawa	Irabu Jima		IJ
Antecaridina sp.	East Timor	Lautem District		ET

were sampled from each site (a sample size of 245 individuals) using a small net and preserved either in 95% ethanol or 100% acetone for subsequent molecular analyses.

The Maui and Oahu sampling was supplemented with published data from Santos (2006), who examined 305 individuals of *H. rubra* from 16 sites on the island of Hawai'i (GenBank accession numbers DQ399124–DQ399258). Additionally, 23 *H. rubra* from Puhi Ula cave on Hawai'i, which were collected after the publication of Santos (2006), are presented here (Table 1). Individuals of two anchialine atyid species from outside the Hawaiian archipelago are also included for comparison with *H. rubra* and for use in phylogenetic analyses. Specifically, these were 15 specimens of *Halocaridinides trigonophthalma* (Fujino and Shokita 1975) from Irabu Jima, Okinawa, Japan and six individuals of an *Antecaridina* sp. (on the basis of morphological and genetic data, T. J. Page and J. W. Short pers. comm. 2007) from the Lautem land district of East Timor (Fig. 1; Table 1). The *H. trigonophthalma* and the *Antecaridina* sp. were collected in September 2005 and November 2003, respectively, and preserved as described above. In total, 594 individuals from 36 discrete anchialine habitats and three distinct geographic locations in the world are examined in this study (Fig. 1; Table 1).

Laboratory techniques—Total nucleic acids were extracted from each specimen according to the methods in Santos (2006). From all individuals, ~10–30 ng of nucleic acids were utilized as template to amplify a ~670 base-pair (bp) fragment of the mitochondrial (mtDNA) COI gene via the polymerase chain reaction (PCR). Reactions were conducted in 25-μL volumes containing 10 mmol L^{-1} tris hydrocloride (pH 8.3), 50 mmol L^{-1} potassium chloride, 0.001% gelatin, 2.0 mmol L^{-1} magnesium chloride, 200 μmol L^{-1} of each deoxynucleotide triphosphate (dATP, dCTP, dGTP, dTTP), 1 U of *Taq* polymerase, and 0.4 μmol L^{-1} each of primers LCO1490 and HCO2198 (Folmer et al. 1994) in a PTC-100 thermocycler (MJ Research) under the following profile: initial denaturing step of 94°C for 5 min, 15 cycles of 94°C for 45 s, 40°C for 45 s, 72°C for 60 s; 25 cycles of 94°C for 45 s, 55°C for 45 s, 72°C for 60 s; and a final extension of 72°C for 5 min.

In addition to COI, sequence data from the mtDNA large subunit ribosomal (16S-rDNA) gene were obtained from two to three individuals per genetic group (*see* below; Table 2) for phylogenetic analyses (*see* below). For 16S-rDNA, PCRs were performed in 25-μL volumes with 0.4 μmol L^{-1} each of primers CRUST16SF and CRUST16SR (Ivey and Santos 2007) and the reaction constituents described above. A "touchdown" thermocycler profile was utilized for 16S-rDNA amplifications: initial denaturing step of 94°C for 4.5 min; 11 cycles of 94°C for 45 s, 60°C for 45 s (−1°C per cycle), 72°C for 60 s; 26 cycles of 94°C for 45 s, 50°C for 45 s, 72°C for 60 s; and a final extension of 72°C for 3 min.

Table 2. Genetic diversity measures and results of neutrality tests for *Halocaridina, Halocaridinides trigonophthalma*, and the *Antecaridina* sp. in this study. Values in parentheses in *n* and *nh* columns represent the actual number of individuals or recovered haplotypes, respectively, from that location. Differences in values represent individuals within a population belonging to a distinct *Halocaridina* lineage or genetic group from what is endemic to that geographic area. These outliers were excluded from the diversity measures and neutrality tests of the particular genetic group. See text for additional details. *n* = number of sampled individuals; *nh* = number of recovered haplotypes; *h* = haplotype diversity. † = lower-case letters designate discrete anchialine ponds located within 100 m of each other at a site. * = $p < 0.05$.

Genus/species	Geographic location	Island or land district	Lineage	Genetic group	Site code(s)†	*n*	Diversity measures		Neutrality tests	
							nh	*h*±SD	Tajima *D*	Fu F_s
Halocaridina rubra	Hawaiian archipelago	Hawai'i	East Hawai'i	Puna	IH, HVNPa, HVNPb	60 (61)	33 (34)	0.949±0.016	−2.01*	−26.2*
				South Hilo	HERa, HERb	32	11	0.786±0.065	−0.95	−2.96
			Ka'ū	Na'alehu	PU	23	5	0.629±0.072	−1.07	−1.32
			West Hawai'i	Kona	WAa, WAc, HUAa, HUAb, MAK, PT, QLCCa, QLCCb	162	67	0.924±0.014	−2.02*	−26.3*
				Ka'ū	PBa, PBb, SP	50	26	0.902±0.031	−2.15*	−24.8*
		Maui	South Maui	Kinau	HM, MLOM, JOE, SKIP, KWS, KAUP	56	24	0.884±0.033	−1.91*	−16.6*
			East Maui	Hana	WC	29	13	0.702±0.096	−1.64*	−5.03*
		Oahu	South Oahu	Ewa	EWA, WP6, WP7, WP8	33	12	0.782±0.061	−0.69	−3.76
			West Oahu	Kalaeloa	KBP	28 (33)	4 (6)	0.458±0.096	−0.87	−1.3
				Waianae	OWAI	28	7	0.389±0.116	−2.09*	−6.42*
			Windward Oahu	Kahuku	KKU, EP	30	8	0.659±0.087	−1.31	−2.83*
				Lanikai	LAN	6	2	0.333±0.215	−0.93	−0.003
			West Hawai'i	Kapapa	KAP	30	7	0.637±0.082	−1.41	−3.76*
	Okinawa	Irabu		Okinawa	IJ	15	5	0.476±0.155	−1.75*	−1.73*
Halocaridinides trigonophthalma	East Timor	Lautem		East Timor	ET	6	5	0.933±0.122	−0.11	−0.81
Antecaridina sp.										

Amplified products were purified with Montage PCR filter units (Millipore) according to the supplier's recommendations, cycle-sequenced in both directions using BigDye Terminators v.3.1, and read on a PRISM 3100 genetic analyzer (Applied Biosystems). Ambiguities in the chromatograms were corrected by comparison with the complementary DNA strand in Sequencher version 4.6 (Gene Codes). Representatives of novel COI and 16S-rDNA sequences generated in this study were deposited into GenBank under accession numbers EF173755–EF173847.

Population genetic analyses—Sequence data from COI were utilized for population-level analyses. Tests of genetic differentiation between populations of *Halocaridina rubra*, *Halocaridinides trigonophthalma*, and the *Antecaridina* sp. were conducted with pairwise Φ_{ST} statistics, which incorporated information from both haplotype frequencies and molecular divergence. The Tamura and Nei (1993) model of DNA evolution with rate variation among sites (i.e., TN + G [G = 1.25]), selected by the Akaike information criterion (AIC) in Modeltest version 3.6 (Posada and Crandall 1998), was utilized in the pairwise Φ_{ST}. In cases where differentiation between populations was not significant, these were consolidated into "genetic groups." Estimates of haplotype (*h*) diversity (Nei 1987) and tests of neutrality (i.e., Tajima's *D*, Tajima [1989]; Fu's F_s, Fu [1997]) were calculated on the basis of these groups. Statistical significance in the pairwise Φ_{ST} and neutrality tests was assessed by 10,000 permutations. The above analyses were performed with Arlequin version 3.11 (Excoffier et al. 2005).

The relationships among haplotypes were visualized via networks constructed with the program TCS version 1.21 (Clement et al. 2000). The analysis was conducted using the default settings, which provides the 95% parsimoniously plausible branch connections between haplotypes. Loops or reticulations between haplotypes, which represent ambiguous connections in a network, were resolved using the methods of Crandall et al. (1994).

Phylogenetic analyses—Phylogenetic analyses were utilized to infer the evolutionary relationships among *Halocaridina rubra* in the Hawaiian archipelago. Both *Halocaridinides trigonophthalma* and the *Antecaridina* sp. served as outgroups in the analyses. Sequences were aligned manually with SE-AL v2.0a11 (available at http://evolve.zoo.ox.ac.uk/). For COI, the third position of each codon was excluded from the data set to avoid potential issues of phylogenetic noise due to mutational saturation between ingroup and outgroup taxa while insertion-deletions (indels) in the 16S-rDNA alignment were treated as missing data. Phylogenetic signal of the individual genes was assessed by the skewness of tree length distributions (g_1) (Hillis and Huelsenbeck 1992), calculated on the basis of 10^6 randomly sampled parsimony trees. To determine if the two genes had significantly different phylogenetic signals, a partition homogeneity (incongruence length difference) test (Farris et al. 1995) was conducted. Replicates were analyzed using the parsimony criterion, and branch

swapping using tree–bisection–reconnection (TBR) was performed with one tree held at each step during the stepwise addition. Following Cunningham (1997), a p value of 0.01 was taken as a significance criterion for the test. The g_1- and partition homogeneity test were conducted with PAUP* version 4.0 b10 (Swofford).

For the maximum parsimony (MP) analysis, character optimization with accelerated transformation (ACCTRAN), 10 repetitions of random sequence additions, starting trees obtained by stepwise addition, and branch swapping by TBR was utilized. The maximum likelihood (ML) analysis was conducted under the model of DNA evolution and parameters chosen by the AIC in ModelTest v3.6 and used the full heuristic search option, 10 repetitions of random sequence additions, starting trees obtained by stepwise addition, and branch swapping by TBR. Branch supports in the MP and ML trees were estimated by bootstrap analysis of 1,000 and 100 replicates, respectively, in PAUP*.

Molecular clock analyses and divergence rate estimation—To test the hypothesis of a molecular clock (i.e., lineages evolve at the same rate), likelihood scores from ML trees constructed with (L_1) and without (L_2) enforcing a clock were obtained with PAUP*. Likelihood ratio tests (LRTs) were then conducted on these scores according to Felsenstein (1981). The LRTs were done separately for COI (inclusion of all nucleotide positions) and 16S-rDNA, with gene-specific models of DNA evolution (each selected by the AIC in Modeltest) being utilized in the construction of the ML trees.

To estimate a rate of divergence specific to these atyids, the COI sequence divergence between *Halocaridina rubra* genetic groups, *Halocaridinides trigonophthalma*, and the *Antecaridina* sp. were first calculated as "net between-group means," which corrects for within-group polymorphism, using MEGA version 3.1 (Kumar et al. 2004). Values were expressed as uncorrected (p) genetic distances. Sequence divergence between monophyletic sister lineages of *Halocaridina rubra*, as identified from the phylogenetic analyses, was then calibrated to the geological age of the region from which those lineages were collected. The resulting evolutionary rate estimate was then applied to the remaining pairwise comparisons.

Results

Population genetic analyses—On the basis of 630 bp of COI, 228 haplotypes were identified from the 594 specimens included in the study. One hundred thirty-five of these haplotypes were previously described from 305 *H. rubra* on the island of Hawai'i (Santos 2006). The 23 samples from Puhi Ula Cave, Hawai'i, belonged to five haplotypes that had not been encountered on the island in the earlier survey of Santos (2006). The 245 *H. rubra* sampled from Maui and Oahu yielded 78 haplotypes, all of which were distinct from those found on Hawai'i. The 15 *Halocaridinides trigonophthalma* from Okinawa were represented by five haplotypes, whereas five of the six *Antecaridina* sp. from East Timor possessed unique haplotypes. Across the entire data set, 59 haplotypes were sampled more than once; the remaining 169 occurred as singletons (Santos 2006; *see* Web Appendix 1: www.aslo.org/lo/toc/vol_53/issue_2/0675a1.pdf). In all, 232 (36.8%) sites were variable, with the difference between any two haplotypes ranging from 1 (0.16%) to 150 (23.8%) substitutions. Whereas most (i.e., 251) substitutions were "silent," 20 of the 271 were nonsynonymous in nature. These nonsynonymous changes were typically to amino acids with similar biochemical properties and represented fixed differences between *Halocaridina rubra*, *Halocaridinides trigonophthalma*, or the *Antecaridina* sp. (data not shown).

Significant genetic differentiation was observed between most *H. rubra* populations. Only those in relative proximity (~0.1–5 km, but up to 30 km in the Kona-Kohala land district of Hawai'i [Santos 2006]) exhibited no statistically significant structure between them. Following consolidation of these populations, 13 genetic groups of *Halocaridina rubra* were identified, with *Halocaridinides trigonophthalma* and the *Antecaridina* sp. forming two additional groups (Table 2). Pairwise Φ_{ST} values between *Halocaridina rubra* genetic groups were highly significant, and, in most cases, approached the maximum limit of 1.0 (Table 3). Thus, few, if any, individuals are being exchanged between these genetic groups (but *see* below). Haplotype (h) diversity values for the genetic groups were variable and ranged from 0.333 (±0.215) to 0.949 (±0.016), indicating modest to high diversity within each (Table 2). Corrected average genetic distances between genetic groups were 0.71–38.61 (within *H. rubra*) and 143.46–184.46 (between *H. rubra*, *Halocaridinides trigonophthalma*, and the *Antecaridina* sp.) (Table 3). Six *Halocaridina rubra* genetic groups possessed significant negative Tajima's D and Fu's F_S values, whereas two additional groups had significant negative values for Fu's F_S only (Table 2). Of these, five of the eight genetic groups were from the geologically younger islands of Maui and Hawai'i (Table 2). Significant negative values of Tajima's D and Fu's F_s reflect an excess of rare polymorphisms in these genetic groups and is often obtained from populations that have experienced recent expansion (Tajima 1989; Fu 1997).

Ten discrete networks were recovered from the statistical parsimony (TCS) analysis of the COI data set. *Halocaridina rubra* haplotypes from Oahu, Maui, or Hawai'i belonged to one of eight networks (referred to hereafter as lineages), whereas *Halocaridinides trigonophthalma* and the *Antecaridina* sp. haplotypes each formed their own networks (Fig. 2A). A *Halocaridina* lineage was typically comprised of one to three genetic groups, and in general, a lineage was confined to a particular region of a single Hawaiian Island, with each island harboring at least two lineages (Fig. 2A; Table 2). Notably, two exceptions were found to this pattern. In the first case, the population at Kalaeloa, Oahu (site KBP, Fig. 2A) was the only instance where haplotypes belonging to two distinct lineages were found to co-occur at the same site. This was observed at a frequency of seven Kalaeloa haplotypes to one Ewa haplotype (Table 2). Of the five Ewa haplotypes recovered at the KBP site, four were identical to the most common haplotype in the Ewa

Table 3. Pairwise Φ_{ST} statistics (below diagonal) and corrected average genetic differences (above diagonal) between *Halocaridina* genetic groups, *Halocaridinides trigonophthalma*, and the *Antecaridina* sp. All calculations are under the Tamura and Nei (1993) model of DNA evolution. Corrected average pairwise differences are reported as differences in base pairs. All Φ_{ST} values are significant at $p < 0.05$.

	Genetic group														
	Puna	South Hilo	Na‘alehu	Kona	Ka‘ū	Kinau	Hana	Kahuku	Kapapa	Lanikai	Ewa	Kalaeloa	Waianae	Okinawa	East Timor
Puna	-	7.45	15.35	23.67	23.52	35.70	27.05	22.49	25.28	23.03	29.83	27.92	27.74	181.28	162.64
South Hilo	0.698	-	13.24	21.16	21.15	34.74	27.19	23.77	22.61	21.04	30.28	27.51	25.28	180.90	162.96
Na‘alehu	0.843	0.879	-	25.50	26.29	38.61	32.79	25.57	28.15	28.58	30.30	31.38	29.44	184.46	168.09
Kona	0.879	0.875	0.899	-	0.71	27.57	25.59	20.33	6.02	20.23	17.74	18.79	21.02	166.49	157.36
Ka‘ū	0.886	0.899	0.934	0.193	-	28.51	27.02	22.26	6.39	21.62	18.39	19.73	22.33	165.57	157.92
Kinau	0.918	0.929	0.946	0.901	0.918	-	27.41	28.06	27.68	30.21	27.47	27.35	28.29	179.67	149.47
Hana	0.882	0.905	0.945	0.890	0.913	0.904	-	22.71	26.26	22.22	25.77	28.84	28.42	170.54	158.80
Kahuku	0.866	0.909	0.958	0.868	0.908	0.914	0.878	-	21.61	6.11	22.19	21.29	20.49	177.25	156.17
Kapapa	0.882	0.910	0.972	0.656	0.745	0.915	0.902	0.950	-	20.88	16.33	16.87	20.58	170.57	155.20
Lanikai	0.872	0.905	0.974	0.868	0.910	0.922	0.889	0.859	0.978	-	24.58	23.40	20.43	174.65	152.47
Ewa	0.902	0.922	0.941	0.854	0.884	0.911	0.898	0.899	0.874	0.914	-	16.97	20.21	174.07	154.36
Kalaeloa	0.854	0.851	0.874	0.833	0.825	0.864	0.824	0.768	0.727	0.789	0.778	-	6.72	174.69	143.46
Waianae	0.914	0.943	0.979	0.885	0.932	0.935	0.952	0.971	0.978	0.980	0.926	0.625	-	181.45	149.12
Okinawa	0.983	0.989	0.995	0.983	0.988	0.987	0.987	0.994	0.995	0.996	0.988	0.971	1.00	-	157.46
East Timor	0.978	0.984	0.992	0.980	0.985	0.981	0.977	0.982	0.986	0.986	0.982	0.956	0.99	0.989	-

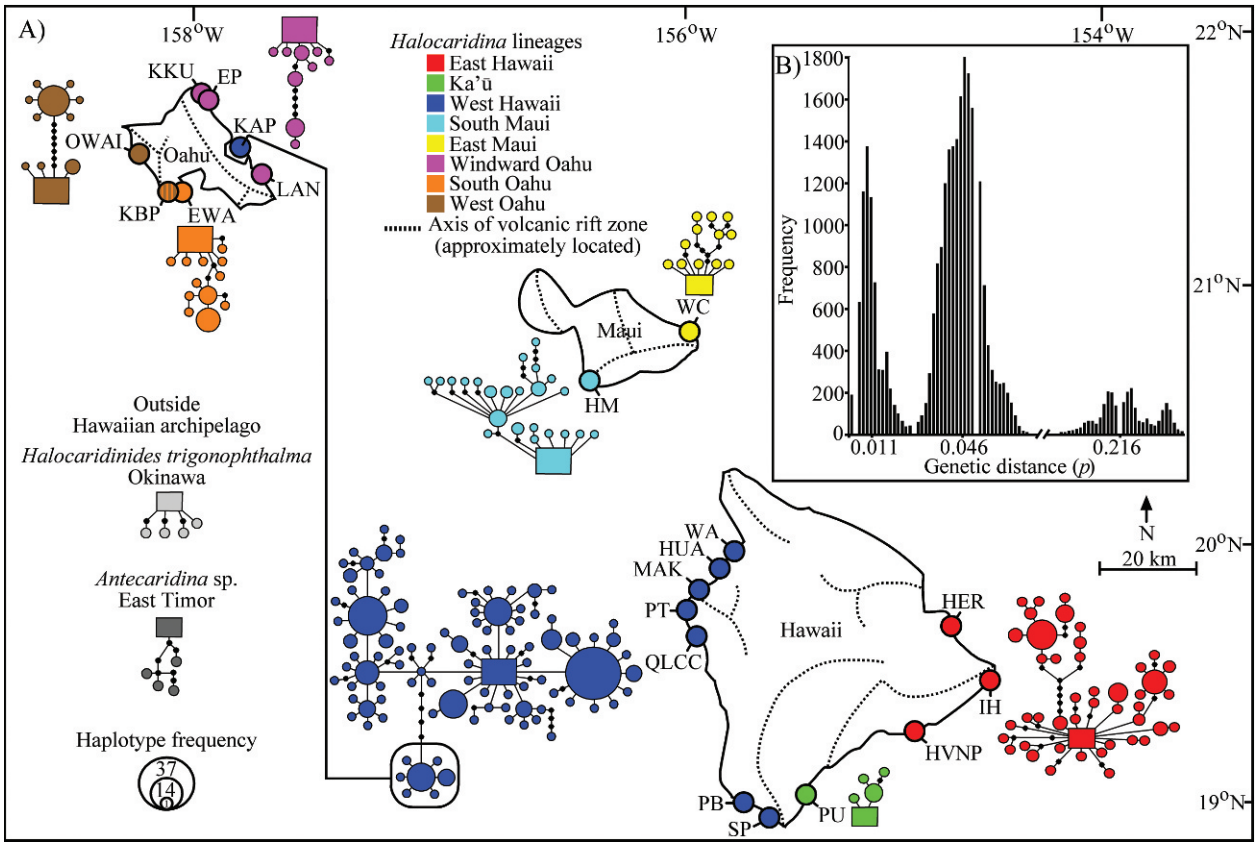

Fig. 2. Genetic diversity of *Halocaridina* in the Hawaiian archipelago. (A) Geographic distribution of the eight identified *Halocaridina* lineages. Networks depict relationships between cytochrome oxidase subunit I (COI) haplotypes within each lineage. Sampled haplotypes are indicated by a shape of solid color, whereas small black dots represent unsampled (i.e., missing) haplotypes within a network. Rectangles represent the haplotype with the highest outgroup probability in each network. Size of circles and rectangles is proportional to the frequency at which a haplotype was recovered (see Web Appendix 1 and Santos [2006] for exact frequencies). Color codes for each lineage are presented in the legend. Note that in spite of variable lengths, each branch in a network implies a single mutational difference between haplotypes. Networks for *Halocaridinides trigonophthalma* and the *Antecaridina* sp. are presented for comparison. (B) Histogram of pairwise genetic distances among all unique *Halocaridina*, *Halocaridinides trigonophthalma*, and *Antecaridina* sp. COI haplotypes. Genetic distances were calculated as uncorrected (*p*) values.

genetic group, suggesting a recent introduction of haplotypes from the Ewa genetic group into the KBP site. In the second case, *Halocaridina* from Kapapa Island, Oahu (site KAP) and the western coast of Hawai'i (sites WA, HUA, MAK, PT, QLCC, PB, and SP) formed the sole lineage spanning two islands (depicted by connecting line in Fig. 2A).

A histogram of pairwise *p* genetic distances between all 228 unique haplotypes (a total of 25,878 pairwise comparisons) revealed a trimodal distribution (Fig. 2B). The first mode (~0.2–2.4%) was mainly comprised of genetic distances between haplotypes in the same *Halocaridina* lineage, or within *Halocaridinides trigonophthalma* or the *Antecaridina* sp. The exception to this involved some pairwise genetic distances (e.g., values in the range of ~1.9–2.4%) between haplotypes of two *Halocaridina* lineages (i.e., East Hawai'i and Ka'ū) from the island of Hawai'i. On the other hand, the second (~2.6–6.9%) mode encompassed genetic distances between haplotypes in different *Halocaridina* lineages (excluding those comparisons noted above). Last, the third (~19.8–24%) mode in the histogram corresponded to genetic distances between *Halocaridina*, *H. trigonophthalma*, and *Antecaridina* sp. haplotypes (Fig. 2B).

Phylogenetic analyses—The partition homogeneity test yielded a probability score greater than 0.01 ($p = 0.974$), indicating low incongruence between trees generated from the COI (420 bp) and 16S-rDNA (839 bp) sequences (*see* Web Appendix 2: www.aslo.org/lo/toc/vol_53/issue_2/0675a2.pdf). Given this, the two genes were concatenated for phylogenetic analyses. The g_1-test detected significant phylogenetic signal (mean = 1,706.11, SD = 85.21, $g_1 = -2.71$, $p < 0.01$) in the resulting 1,259 bp alignment. A total of 410 characters in the alignment was parsimony-informative and the TVM + I + G model of evolution was chosen by the AIC (I = 0.36, G = 0.47; support values of −lnL = 4,285.43; K = 9; AIC = 8588.86) for the ML analysis.

Phylogenetic trees constructed under MP and ML gave identical topologies (Fig. 3). For both methods, *Halocaridina* was monophyletic with strong (100%) support. Relationships between *Halocaridina* genetic groups of the same lineage were also strongly supported, consistent with their clustering into distinct networks via the statistical parsimony analysis. For example, the Kapapa genetic group on Oahu and the Kona and Ka'ū genetic groups of Hawai'i formed a clade with strong (97–99%) support (Fig. 3). However, relationships between the eight *Halocaridina* lineages were largely unresolved (Fig. 3). The exception to this was moderate (84–87%) support for a sister relationship between the East Hawai'i and Ka'ū lineages of *Halocaridina* on the island of Hawai'i (Fig. 3). Inclusion of third codon position from COI, or having either *Halocaridinides trigonophthalma* or the *Antecaridina* sp. as a sole outgroup, did not alter the tree topology or overall bootstrap values in the MP and ML analyses (data not shown).

Molecular clock analyses and divergence rate estimation—The LRTs could not reject the molecular clock hypothesis for either COI (L_1 [−Ln likelihood] = 2,539.4304, L_2 = 2,523.8917, $\chi^2 = 31.08$, df = 29, $p = 0.362$) or 16S-rDNA (L_1 = 3,275.1189, L_2 = 3,259.1012, $\chi^2 = 32.04$, df = 29, $p = 0.318$). Thus, the rate of divergence across the examined *Halocaridina* lineages appears to be relatively homogeneous. Following correction for within-group variation, *p* distances within *Halocaridina* were 0.1–5.7%, whereas those for *Halocaridina* compared with *Halocaridinides trigonophthalma* and *Antecaridina* sp. were 19.3–23.6% (Table 4).

Sequence divergence in COI between the sister *Halocaridina* lineages of East Hawai'i and Ka'ū ranged from 2.0% (Na'alehu–South Hilo genetic groups) to 2.3% (Na'alehu–Puna genetic groups) (Table 4). The three genetic groups in the two lineages are confined to the eastern coast of Hawai'i (Fig. 2A; Santos 2006) and occur along the flank of Kilauea volcano. This volcano is the youngest of five on the island (Carson and Clague 1995) and the estimated age of its earliest subaerial (i.e., atmospheric) eruption is 50,000–100,000 yr ago (http://hvo.wr.usgs.gov/kilauea/). Assuming the ancestor of these two lineages colonized the hypogeal water system of the area soon after the emergence of Kilauea, this implies that a minimum of 2.0% of sequence divergence has transpired in a maximum of 100,000 yr, or a COI molecular clock of 20% per million years (Myr^{-1}). Application of this rate to the other lineages within *Halocaridina* suggests that all have diverged from each other within the last 0.3 Myr, whereas the split between *Halocaridina, Halocaridinides trigonophthalma*, and the *Antecaridina* sp. is estimated to have occurred ~1.0 Myr (Table 4).

Discussion

Ecological isolation and evolutionary diversification in Halocaridina via marine and geological barriers and the role of life history—This study clearly demonstrates substantial genetic diversity and population structure on an island, as well as between islands, for *Halocaridina* in the Hawaiian archipelago. In spite of possessing a number of traits thought to be conducive to dispersal between anchialine habitats (reviewed by Santos 2006), the extensive sampling of populations on Oahu and Maui conducted here and previously for Hawai'i reveal only sporadic cases of *Halocaridina* haplotypes outside the regions in which they are typically found. These cases are limited to the western and eastern coasts of Hawai'i (Santos 2006; Table 2) and the southern coast of Oahu (Fig. 2A; Table 2), and in all instances involve one to five haplotypes from a *Halocaridina* genetic group or lineage from the same region of that island. This phylogeographic pattern, particularly across multiple islands, further supports the idea that the open ocean represents a significant isolating barrier for *Halocaridina* (Santos 2006), similar to what has been observed for other atyid genera that exhibit some level of salinity tolerance (e.g., *Paratya*, Page et al. 2005; *Caridina*, Page and Hughes 2007*a*).

Along with the ocean, the geologic features that define the subterranean hydrology of a landmass can also significantly influence the population structure and diversification of anchialine organisms by acting as barriers to dispersal via the hypogeal water system. For example, volcanic rift zones appear to dictate the distribution of

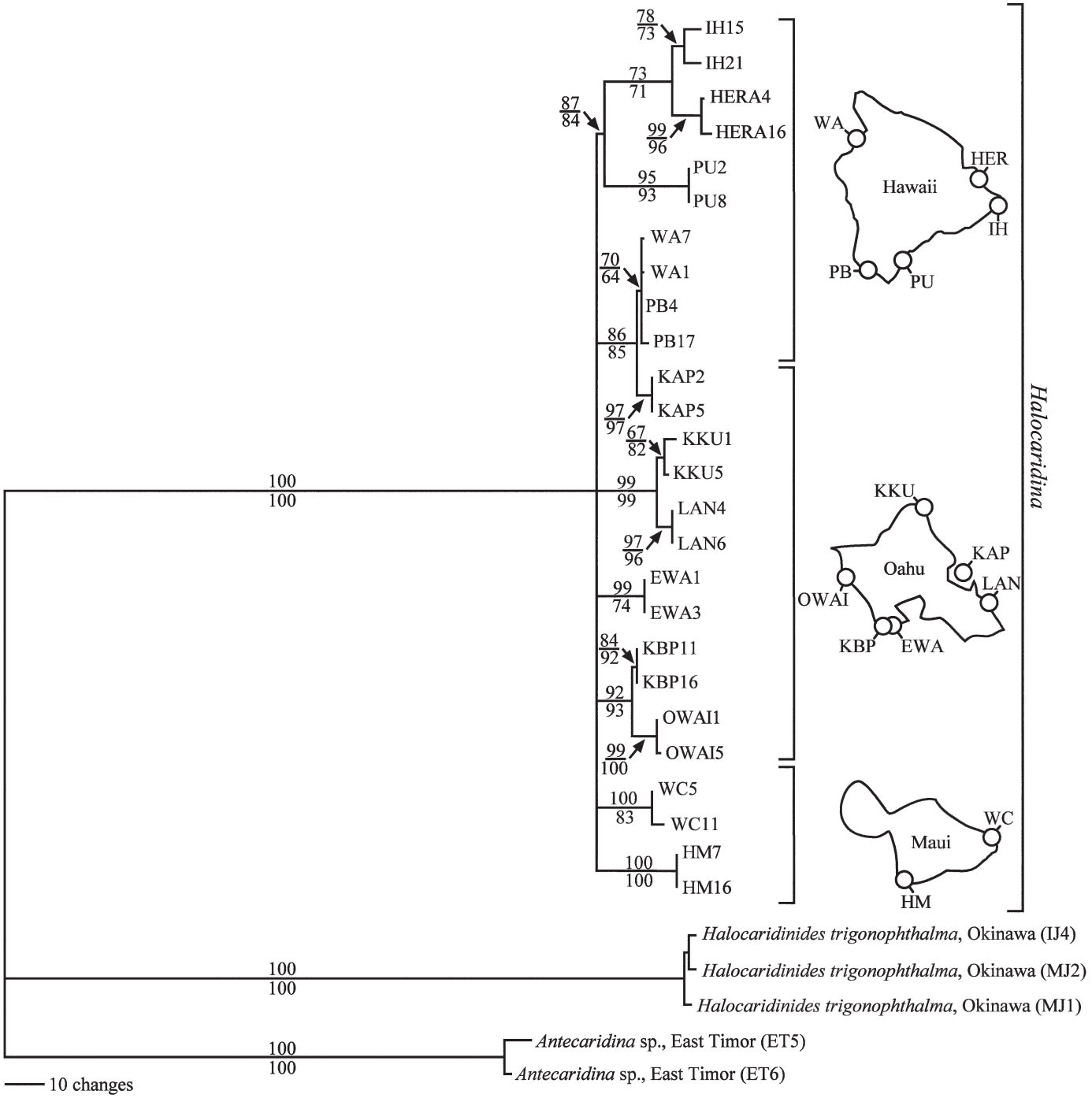

Fig. 3. Phylogenetic relationships inferred for *Halocaridina* in the Hawaiian archipelago. Branches belonging to *Halocaridina* genetic groups or lineages of a particular island are bracketed at right. Site labels are as listed in Table 1. The length of the most parsimonious tree was 604 steps with a consistency index (CI) of 0.85, homoplasy index (HI) of 0.15, and retention index (RI) of 0.93. Maximum likelihood (ML) tree ($-\ln L = 4{,}290.41$). Values above and below vertical lines represent bootstrap support as percentages of 1,000 resamplings for maximum parsimony (MP) and 100 resamplings for maximum likelihood (ML) analyses, respectively.

Halocaridina genetic groups and lineages in the Hawaiian Islands (Fig. 2A). Specifically, these rift zones are geohydrologic boundaries that compartmentalize the hypogeal water system of an island into distinct aquifers (e.g., Hunt 1996; Scholl et al. 1996). The correlation between distributions of *Halocaridina* genetic groups or lineages and aquifer compartmentalization is clearly demonstrated with hydrological data for Oahu (Hunt 1996). For example, the Windward Oahu lineage of *Halocaridina* exhibits genetic and geographic substructuring that follows the distribution of the aquifers, with the Kahuku and Lanikai genetic groups confined to the Kahuku and Koolau Rift Zone

Table 4. Cytochrome oxidase subunit I (COI) divergence time estimates for *Halocaridina* genetic groups, *Halocaridinides trigonophthalma*, and the *Antecaridina* sp. Estimated times of divergence (above diagonal) assume a COI mutation rate of 20% per million years (2.0% per 100,000 years); genetic distances (below diagonal) are "net between group" averages (i.e., corrected for within-group polymorphisms) and expressed as uncorrected *p* values.

	Genetic group														
	Puna	South Hilo	Na'alehu	Kona	Ka'ū	Kinau	Hana	Kahuku	Kapapa	Lanikai	Ewa	Kalaeloa	Waianae	Okinawa	East Timor
Puna	-	0.06	0.12	0.18	0.18	0.27	0.2	0.17	0.2	0.18	0.23	0.24	0.21	1.16	1.06
South Hilo	0.012	-	0.1	0.16	0.16	0.26	0.21	0.18	0.17	0.16	0.23	0.23	0.19	1.16	1.06
Na'alehu	0.023	0.02	-	0.2	0.2	0.29	0.25	0.2	0.22	0.22	0.23	0.27	0.23	1.18	1.1
Kona	0.036	0.032	0.039	-	0.005	0.21	0.2	0.16	0.05	0.16	0.14	0.18	0.16	1.08	1.03
Ka'ū	0.036	0.032	0.04	0.001	-	0.22	0.2	0.17	0.05	0.17	0.14	0.18	0.17	1.08	1.04
Kinau	0.053	0.051	0.057	0.042	0.043	-	0.21	0.22	0.21	0.23	0.21	0.24	0.22	1.15	0.99
Hana	0.04	0.041	0.049	0.04	0.04	0.041	-	0.18	0.2	0.17	0.2	0.25	0.22	1.11	1.04
Kahuku	0.034	0.036	0.039	0.031	0.034	0.043	0.035	-	0.17	0.05	0.17	0.19	0.16	1.15	1.03
Kapapa	0.039	0.034	0.043	0.009	0.01	0.042	0.04	0.033	-	0.16	0.13	0.16	0.16	1.11	1.02
Lanikai	0.035	0.032	0.043	0.031	0.033	0.046	0.034	0.01	0.032	-	0.19	0.21	0.16	1.13	1.01
Ewa	0.045	0.045	0.045	0.027	0.028	0.042	0.039	0.034	0.025	0.037	-	0.18	0.16	1.12	1.02
Kalaeloa	0.047	0.046	0.053	0.035	0.036	0.047	0.049	0.038	0.032	0.041	0.036	-	0.07	1.15	0.97
Waianae	0.042	0.038	0.045	0.032	0.034	0.043	0.043	0.032	0.032	0.032	0.031	0.013	-	1.16	0.98
Okinawa	0.231	0.232	0.236	0.216	0.215	0.23	0.222	0.229	0.221	0.226	0.224	0.229	0.232	-	1.04
East Timor	0.212	0.212	0.219	0.206	0.207	0.198	0.208	0.205	0.204	0.201	0.203	0.193	0.196	0.208	-

aquifers, respectively (Fig. 4). A similar situation is observed for the West Oahu lineage; the Waianae genetic group is localized to the Waianae Rift Zone aquifer, whereas the Kalaeloa genetic group is restricted to the adjacent Southern Oahu aquifer along with the Ewa genetic group. Last, although the *Halocaridina* population from Kapapa Island (Kapapa genetic group) is not compartmentalized by an aquifer, this small (i.e., 0.012 km^2) islet is located ~3.2 km off the east coast of Oahu and is effectively isolated by the marine environment of Kaneohe Bay (Fig. 4). Analogous scenarios of aquifer compartmentalization to those on Oahu are also apparent for *Halocaridina* genetic groups and lineages from Maui and Hawai'i (Fig. 2A; Santos 2006), suggesting that this pattern is not unique to a single island.

Life history and dispersal potential may also play a significant role in the population differentiation of marine (e.g., Sale and Kritzer 2003) and freshwater (e.g., Havel and Shurin 2004) organisms. Likewise, life history characteristics appear to also influence the phylogeography and evolution of anchialine organisms. Shokita (1979) distinguished types of reproduction among atyid and palaemonid shrimps on the basis of egg size, which dictates the number of larval stages, and larval habitat. On the basis of this scheme, *Halocaridinides trigonophthalma* was classified as being medium egg (~1.0 mm and abbreviated development) and landlocked (Shokita 1979). With its similar egg size (Couret and Wong 1978) and larval habitat, Maciolek (1983) subsequently placed *Halocaridina* in the same category of medium egg–landlocked atyids. Support for the conclusions of Maciolek (1983) (and by extension, Shokita [1979]) is not only found in the data presented here for *Halocaridina* but also from studies of the freshwater stream atyid genus *Caridina*, where dispersal ability and population structure significantly correlate with variation in egg size (Page and Hughes 2007*b*). In this context, *Caridina* species with the largest geographic range and lower levels of genetic structure have small eggs (~0.4 mm), whereas those with large eggs (~1.6 mm) have more restricted distributions and higher levels of genetic structure (Page and Hughes 2007*b*). Notably, *Caridina* with an egg size of ~1.0 mm exhibit extreme genetic structure (i.e., Φ_{ST} values approaching the maximum limit of 1.0, Page and Hughes 2007*b*), identical to what is observed between most genetic groups of *Halocaridina* (Table 3). This trend strongly implies that egg size is an effective predictor of dispersal ability and population structure for the Atyidae and it will be interesting to see if this pattern extends to other caridean shrimp as well.

Comparing the larval biology of *Halocaridina* to that of the anchialine gastropod *N. cavernicola* also suggests that larval feeding mode may serve as a good predictor of dispersal potential and population structure for anchialine organisms in general. Under laboratory conditions, *Halocaridina* larvae hatch as free-swimming lecithotrophic zoeae (Couret and Wong 1978; pers. obs.). This is in contrast to the larvae of *N. cavernicola*, which are planktotrophic (Kano and Kase 2004). Generally, differences in larval feeding mode can have significant effects on population connectivity since lecithotrophic larvae are thought to be energy constrained, with long periods in the plankton leaving little reserves for metamorphosis and successful colonization (more structured populations); planktotrophic larvae, on the other hand, may not have such limits because of the ability to feed during this developmental stage (less structured populations) (reviewed by Palumbi 1994). This suggests that the energy reserves carried by *Halocaridina* larvae are only sufficient for long-distance colonization under rare and exceptional circumstances, contributing to the highly structured nature of populations on, as well as between, the Hawaiian Islands.

Here, a model of evolutionary diversification is proposed for *Halocaridina* that incorporates the identified extrinsic obstacles to gene flow as well as the intrinsic organismal

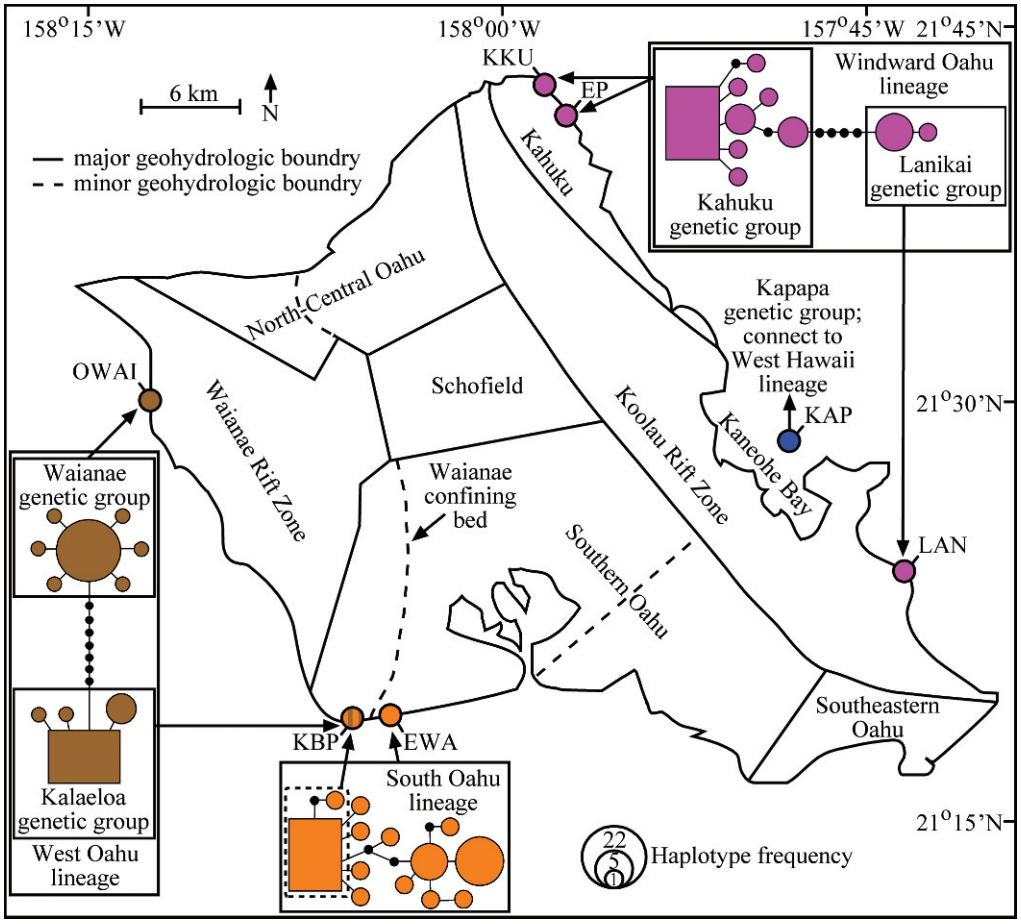

Fig. 4. Geographic correlation between *Halocaridina* lineages and genetic groups and aquifers for the island of Oahu. Major and minor geohydrological boundaries are represented by solid and dashed lines, respectively. Networks depict relationships between cytochrome oxidase subunit I (COI) haplotypes within each lineage. Sampled haplotypes are indicated by a shape of solid color, whereas small black dots represent unsampled (i.e., missing) haplotypes within a network. Rectangle represents the haplotype with the highest outgroup probability in each network. Size of circles and rectangles is proportional to the frequency at which a haplotype was recovered. Color codes for each lineage are as in Fig. 2. Note that in spite of variable lengths, each branch in a network implies a single mutational difference between haplotypes. Map modified after Hunt (1996).

properties of these atyids. Although the model is based on *Halocaridina* in the Hawai'i archipelago, it likely extends to other anchialine species (e.g., *Halocaridinides trigonophthalma*) possessing life history traits of large egg size, abbreviated larval development, lecithotrophic larvae, or a restricted larval habitat as well. Because of these traits, *Halocaridina* has low long-distance dispersal ability via oceanic routes and populations are landlocked in ecological timescales. Following a rare, but successful, colonization event, population expansion (implied by significant negative values of Tajima's D and Fu's F_S; Table 2) and range extension occurs through the hypogeal water system of an island and continues until strong physical barriers, such as the volcanic rift zones that divide aquifers, are encountered. During this phase, peripheral populations may undergo genetic differentiation due to restricted gene flow and isolation by distance, which has been observed at the extremes of the Kona-Kohala land district on the island of Hawai'i (Santos 2006). On occasion, a small number of individuals from populations adjacent to a barrier may colonize the neighboring aquifer, most likely by a short-distance coastal route around the barrier. This idea is supported by the apparently recent one-way migration of *Halocaridina* haplotypes from the Ewa genetic group into the Kalaeloa population across the geohydrological boundary of the Waianae confining bed (Fig. 4). Colonization of the adjacent aquifer, however, results in migrants becoming isolated from their parental population because of the factors outlined previously and, over time, divergence in allopatry transpires between the two. Evidence for this scenario comes from the larger-than-average number of steps connecting haplotypes in divergent *Halocaridina* genetic groups of the same lineage but from neighboring aquifers (e.g., genetic groups in the Windward

Oahu, West Oahu, and East Hawai'i lineages; Fig. 4, Santos 2006), which is indicative of such allopatric fragmentation events (Templeton 2004). Ultimately, sufficient divergence (in this case, ~2.0% *p* distance) is accrued between sister genetic groups in adjacent aquifers, resulting in a split and the creation of independent lineages (e.g., the East Hawai'i and Ka'ū lineages of *Halocaridina* on the island of Hawai'i [Fig. 3]). Thus, the evolution of *Halocaridina* in the Hawaiian archipelago is driven by population fragmentation, isolation, and subsequent diversification in "islands" (e.g., isolated aquifers) under the islands. Given this, the diversity reported here is likely a gross underestimate of the total diversity within the species or genus (*see* below), since numerous aquifers (and the *Halocaridina* populations that may reside within them) remain to be sampled throughout the islands (Figs. 2, 4).

Halocaridina "species"—Two morphospecies are currently recognized in *Halocaridina*: *Halocaridina rubra* Holthuis (Holthuis 1963), described from specimens collected on the eastern and western coasts of Hawai'i and later recorded from the other islands (Holthuis 1973, Maciolek 1983, Brock and Bailey-Brock 1998), and *Halocaridina palahemo* Kensley and Williams (Kensley and Williams 1986), described from a single location, Lua o Palahemo lava tube at South Point on Hawai'i. Notably, a reexamination of individuals from Hawai'i (including *H. palahemo* from Lua o Palahemo) and Oahu revealed overlapping morphological characters within, as well as between, populations of the two species (Bailey-Brock and Brock 1993), which questions the validity of *H. palahemo* as well as the use of morphology for species separation in *Halocaridina*. Unfortunately, attempts to obtain *H. palahemo* for this study were unsuccessful (*see* Methods); thus, the genetic identity of this "species" and its relationship to *H. rubra* remain unknown. However, in light of Bailey-Brock and Brock (1993), *H. palahemo* and *H. rubra* should be considered members of the same morphospecies (i.e., *H. rubra*, by historical precedent).

What do the eight lineages described here represent in the context of *H. rubra*? Two lines of evidence suggest that these lineages correspond to "cryptic species" and that *Halocaridina* represents a "species complex." First, modal distributions of pairwise genetic distances have been used to infer the existence of cryptic species in marine crustaceans (e.g., Held 2003). Similarly for *Halocaridina*, by utilizing the third mode (~19.8–24%, mean = 21.8%) in the distribution of pairwise genetic difference between unique COI haplotypes (Fig. 2B) as a calibration point of genetic distances among genera (e.g., *Halocaridina*, *Halocaridinides*, and the *Antecaridina* sp.), backtracking in a taxonomic framework implies that the second (~2.6–6.9%, mean = 4.3%) and first (~0.2–2.4%, mean = 0.8%) modes represent genetic variation between species of the same genus (e.g., interspecific divergence between *Halocaridina* species) and genetic variation within a species (e.g., intraspecific divergence in a single *Halocaridina* species), respectively. Inter- and intraspecific COI divergences comparable with these *Halocaridina* species have been reported from a variety of invertebrates (Hebert et al. 2003), including crustaceans (e.g., Witt et al. 2006). Second, the lineages fulfill the criteria of multiple species concepts, further corroborating their potential species status. For example, each is a well-supported monophyletic assemblage (Fig. 3), consistent with the principles of the phylogenetic species concept (de Queiroz and Donoghue 1990). Likewise, the lineages meet the definition of "cohesion species" on the basis of the interchangeability criteria of the cohesion species concept (Templeton 2001). However, further work is needed to satisfactorily resolve the taxonomic status of *Halocaridina* and its constituent lineages. In this regard, a combination of morphological and molecular taxonomy, which has been successfully applied to the genus *Caridina* (Page et al. 2005), may prove fruitful. Nevertheless, it is important in the interim to recognize the potential species status of these lineages as well as the regional endemism of genetic groups within them. This is particularly relevant when establishing conservation and management strategies for *Halocaridina* and anchialine habitats in the Hawaiian archipelago (Santos 2006).

The molecular clock—Diversification in *Halocaridina* is proceeding at an exceptional rate, 20% Myr^{-1}. This molecular clock estimate for COI is in sharp contrast to those previously reported and commonly utilized for arthropods, which range from 1.7% Myr^{-1} (Williams and Knowlton 2001) to 2.3% Myr^{-1} (Brower 1994). One hypothesis to account for this discrepancy is that divergence between the sister *Halocaridina* lineages of East Hawai'i and Ka'ū occurred on an older island, with subsequent dispersal of each to Hawai'i. Although the remaining high islands in the archipelago are >1 Myr in age (Carson and Clague 1995), which would allow sufficient time for 2% of divergence to be achieved at the published rates, it is highly improbable that sister lineages would independently colonize adjacent aquifers on Hawai'i given the limited dispersal and colonization success of *Halocaridina* in general. Alternatively, these lineages might be evolving at an accelerated rate. The LRTs, however, could not reject a clock hypothesis for either COI or 16S-rDNA, suggesting that the rate of divergence is relatively homogeneous across lineages. Thus, this molecular clock estimate appears to be justified.

Although exceptional, mitochondrial molecular clocks on par with that of *Halocaridina* have been reported from other organisms. For instance, estimates of 14% and 10% Myr^{-1} have been proposed for Hawaiian tree snails in the subfamily Achatinellinae (Thacker and Hadfield 2000) and the land snail genus *Mandarina* (Chiba 1999), respectively. Notably, high sequence divergence rates were previously hypothesized for land snails because of their strong genetic structure (Thomaz et al. 1996), a trait that is also characteristic of *Halocaridina* and consistent with theoretical and empirical data that small effective sizes of founding populations can lead to faster rates of molecular evolution (DeSalle and Templeton 1988 and references therein). Nevertheless, it should be noted that this molecular clock (1) is specific to *Halocaridina* and not necessarily applicable to other atyids or arthropods in general, and (2) represents an estimate at the population–species boundary, rather than a long-term

evolutionary rate, for these atyids. This latter caveat stems from evidence that molecular clocks can exhibit a measurable transition from a high, short-term (<1–2 Myr) mutation rate to a low, long-term substitution rate over time (reviewed by Ho and Larson 2006). Currently, the approximate value of this long-term rate is unknown because of a lack of appropriate calibration points (Ho and Larson 2006) or a well-resolved phylogeny for the genus (Fig. 4). It is predicted, however, that the long-term rate will be appreciably lower then the presented short-term rate, similar to what has been observed in other organisms (e.g., Ho et al. 2005). This being the case, the estimated times since divergence at the generic level (e.g., *Halocaridina, Halocaridinides trigonophthalma* or *Antecaridina* sp., Table 4) should be considered underestimates of their actual values (i.e., splits occurred >1 Myr in the past).

Phylogenetics of Halocaridina—Phylogenetic analyses on the basis of a concatenation of COI and 16S-rDNA found relationships among most *Halocaridina* lineages to be unresolved, whereas those of genetic groups within lineages were strongly supported (Fig. 4). An analogous situation of well-supported tip clades, but a lack of resolution for deeper relationships, has also been observed for *Caridina* (Page et al. 2007). These examples are in contrast to the atyids *Paratya* and *Troglocaris*, where deeper branches in phylogenies of the genera were well supported when using the same genes (Page et al. 2005; Zakšek et al. 2007). Thus, the phylogenetic signal and subsequent ability to resolve relationships with these mitochondrial genes varies among atyid genera.

Although a robust phylogeny for *Halocaridina* remains to be elucidated, its general topology can be hypothesized from studies of other endemic Hawaiian organisms. In this context, phylogenies typically possess a topology where the orders of clades correlate with the physical and temporal sequence of the islands (reviewed by Fleischer et al. 1998). This pattern results from dispersal and colonization being generally from older to younger islands in the archipelago and is known as the ''progression rule'' (Funk and Wagner 1995). The finding that *Halocaridina* from Kapapa Island, Oahu (i.e., an older island) shares an evolutionary history with populations on the western coast of Hawai'i (i.e., a younger island) suggests that these atyids also follow a form of this rule. However, *Halocaridina* does not seem to adhere strictly to the progression rule since the founders of the western coast of Hawai'i appear to have originated from Oahu rather than the geographically closer and geologically younger island of Maui (Fig. 2A). If a well-supported phylogeny for *Halocaridina* is found to significantly deviate from the serial area cladograms usually recovered from other Hawaiian organisms, the degree and manner of its departure may offer new perspectives on dispersal and colonization in the archipelago.

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Received: 26 June 2007
Accepted: 1 October 2007
Amended: 17 October 2007