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Cryptic Genetic Diversity in the Coastal Isopod *Alloniscus oahuensis* from the Pacific Ocean

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The isopod sub-order Oniscidea includes over 3,700 species and is known to occur in all terrestrial environments, except those at extreme elevations and polar latitudes. Current estimates of the biodiversity of the Oniscidea may be underestimates, as recent molecular studies have uncovered high levels of cryptic diversity in several taxa in the sub-order. High levels of cryptic diversity have been found in coastal species, species from remote and isolated regions, and species with complex taxonomic histories. *Alloniscus oahuensis* is a good candidate to harbor cryptic diversity, as it is a coastal isopod species with a geographic range that spans several remote and isolated archipelagos in the Pacific Ocean and has a complex taxonomic history. In this study, we used sequences for three mitochondrial genes and one nuclear gene to determine whether *A. oahuensis* harbors highly divergent lineages that may represent cryptic species. By characterizing 60+ *A. oahuensis* individuals from 17 localities from various Pacific Ocean archipelagos, we uncovered two deeply divergent lineages with disjunct distributions. The levels of genetic divergence observed amongst the two lineages match or exceed those reported across other cryptic species in the Oniscidea, suggesting that *A. oahuensis* may represent a cryptic species complex in need of a taxonomic revision. The extremely low lineage diversities within *A. oahuensis* indicate that the lineages may have spread across the Pacific Ocean recently, potentially due to anthropogenic activity.

Key words: Oniscidea, Cryptic species, Hawaiian Islands, *Alloniscus brevis*, Mitochondrial.

BACKGROUND

The isopod sub-order Oniscidea includes over 3,700 terrestrial species found in all terrestrial habitats, with the exception of those found in the poles and at extreme elevation (Schmalfuss 2003). Recent molecular studies have uncovered high levels of cryptic diversity within several different oniscidean taxa, indicating that our understanding of the biodiversity of the sub-order Oniscidea is likely an underestimate (*e.g.*, Hurtado et al. 2010; Hurtado et al. 2014; Raupach et al. 2014; Greenan et al. 2018; Delhoumi et al. 2019; Guzik et al. 2019; Santamaria 2019; Yoshino and Kubota 2022; Wang et al. 2022). Amongst these studies, striking reports of cryptic diversity have been made for coastal oniscideans (*e.g.*, Hurtado et al. 2010 2013 2014; Eberl et al. 2013; Greenan et al. 2018), species found in remote and isolated regions (*e.g.*, Santamaria et al. 2014 2017; Santamaria 2019; Wang et al. 2022), and those with complex taxonomic histories (*e.g.*, Santamaria et al. 2014; Hurtado et al. 2018). To wit, molecular studies of *Ligia hawaiiensis*, a species previously thought to be the sole coastal *Ligia* species endemic to the remote Hawaiian Islands, found this species to represent a cryptic species complex comprised of at least 8 species (Taiti et al. 2003; Santamaria et al. 2013; Santamaria 2019). Additional studies of coastal oniscidean taxa thus hold the potential to uncover previously unknown

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cryptic diversity and thus further our understanding of oniscidean biodiversity.

The coastal isopod *Alloniscus oahuensis* Budde-Lund, 1885 holds the potential to harbor cryptic diversity, as it has a complex taxonomic history and geographic range that includes several remote archipelagos throughout the Pacific Ocean. This species was first described in 1885 by Budde-Lund from specimens collected on the Hawaiian island of O'ahu, and afterwards reported in several archipelagos of the Pacific including Samoa (Jackson 1927), the Marquesas (Jackson 1933) and southern Polynesia (Jackson 1941 1938). The validity of *A. oahuensis*, however, was doubted by Jackson (1927) who determined *A. oahuensis* to be a junior synonym of *Alloniscus brevis* Budde-Lund 1885, a separate species described from specimens collected in the East Indies by Budde-Lund in the same study where he described *A. oahuensis* (no further type-locality information was provided by Budde-Lund). Jackson (1933) later determined *A. oahuensis* to be the senior synonym of *A. brevis*, as the former was included as a nomen nudum in Budde-Lund's previous work (Budde-Lund 1879). Arcangeli (1960) later revived *A. brevis* and placed it in an *Alloniscus* subgenus separate from *A. oahuensis*; however, the taxonomic confusion between these two species persisted. In 1984, Schultz re-described *A. oahuensis* based on specimens from the Hawaiian Archipelago but did not determine the validity of *A. brevis*. Similarly, Ferrara and Taiti (1985) left unanswered whether *A. brevis* represented a synonym of *A. oahuensis* or a valid species. More recently, Green et al. (1990) inspected the holotype of *A. brevis* and considered this species likely to be a junior synonym of *A. oahuensis*. Since then, *A. oahuensis* has been reported from other habitats in the Indo-Pacific including the Krakatau islands (Green et al. 1990), the Togian Islands (Taiti et al. 1992), and Henderson Island (Benton and Lehtinen 2008), while *A. brevis* is not considered a valid species (Schmalfuss 2003).

The complex taxonomic history of *A. oahuensis* as well as its broad geographic range suggests this species may harbor highly divergent and cryptic genetic lineages. In this study, we use molecular approaches to characterize *A. oahuensis* individuals collected in several Pacific Ocean localities. Our aim is to determine whether this taxon harbors highly divergent genetic lineages that may represent cryptic species, and if so the geographic distribution of said genetic lineages. Our findings may not only be informative for future taxonomic revisions of *A. oahuensis* but also help further our understanding of the biodiversity of oniscidean isopods and intertidal coastal habitats, especially the highly biodiverse Pacific Ocean.

MATERIALS AND METHODS

We collected *Alloniscus* specimens in 17 localities across the Pacific Ocean (Fig. 1), including sites in Yap (M01), The Philippines (M02), the Hawaiian Islands (P01–09), Vanuatu (P10-12), and Fiji (P15). In the field, individuals were hand-caught and preserved in 70% ethanol. We used Schultz's (1984) key to identify to species 2–10 individuals per locality. All individuals inspected were identified as *A. oahuensis*. Two more specimens were obtained on loan from Collection Crustacea at the Senckenberg Research Institute and Natural History Museum Frankfurt: an *A. oahuensis* specimen from Samoa (P14; Crustacea – SMF Catalogue # 41234) and an *Alloniscus* sp. specimen from Tahiti (P12; Crustacea – SMF Catalogue # 40898). Detailed locality and specimen information is provided in table 1.

Total genomic DNA was extracted from pereopods using the Quick g-DNA MiniPrep Kit (Zymo Research) for a subset of 1–5 individuals per locality prior to PCR amplification of three mitochondrial and one nuclear gene fragments using previously published primers and conditions: (a) a 658-bp segment of the Cytochrome Oxidase I gene (hereafter *COI*, primers LCO1490 and HCO2198; Folmer et al. 1994), (b) a ~459-bp segment of the 12S rDNA gene (primers crust-12Sf/crust-12Sr; Podsiadlowski and Bartolomaeus 2005), (c) a ~490-bp segment of the 16S rRNA gene (primers 16Sar/16Sbr; Palumbi 1996), and (d) a ~280-bp segment of the Histone H3 gene (primers H3AF/H3AR; Colgan et al. 1998). All PCR products were visualized on a 1% agarose gel stained using SYBR-Safe (Invitrogen) prior to sequencing at the University of Arizona Genetics Core (UAGC).

Sequences were assembled, checked for quality, and trimmed to remove read ends in Geneious Prime v2022.0.2. Protein coding genes were aligned independently using the online MAFFT server (Katoh et al. 2019) with default settings. In these alignments we did not observe early stop codons, nonsynonymous mutations, or internal gaps. The two ribosomal genes included in our analyses (16S rDNA, 12S rDNA) were aligned independently in the GUIDANCE2 server (Sela et al. 2015) using the MAFFT algorithm (Katoh and Standley 2013) with default settings. Positions in the alignment with an alignment score < 0.93 were considered poorly aligned and excluded from analyses (see GUIDANCE Manual).

We sought to identify an appropriate outgroup to root analyses of *A. oahuensis* by adding publicly available sequences for other *Alloniscus* species to our dataset; however, only five *COI* sequences for *A. balssi* were available at the time of analysis. We aligned these

sequences (GenBank Accession Numbers: LC655938-39, LC655942-43, and LC655949) to our *COI* dataset and estimated a neighbor-joining (hereafter NJ) phylogenetic tree in Geneious Prime v2022.0.2 under the Tamura-Nei model of nucleotide evolution (Tamura and Nei 1993), assuming *A. balssi* to be the outgroup. Support for relationships was estimated by calculating one thousand bootstrap replicates. This analysis supported the existence of two separate and divergent clades within *A. oahuensis* (see Results; Fig. 2 Panel A). Given the monophyly of each lineage observed within *A. oahuensis* and that all specimens in the ingroup were identified as *A. oahuensis* on the basis of morphology, we used each of these two clades to root the other in our analyses that contained all genes and specimens of *A. oahuensis*, an approach similar to that used by Mateos et al. (2012) to root phylogenetic analyses of Bythograeid crabs.

To reconstruct phylogenetic relationships amongst *A. oahuensis*, we concatenated individual gene alignments in Geneious Prime v2022.0.2 after removing poorly aligned sites in the 12S and 16S rDNA datasets. The concatenated alignment was used to estimate a NJ phylogenetic tree in Geneious Prime v2022.0.2. One thousand bootstrap replicates were carried out under the Tamura-Nei model of nucleotide evolution (Tamura and Nei 1993) with all other settings as default. We calculated a majority-rule consensus tree with all bootstrap replicates using the SumTrees command of DendroPy v3.10.1 (Sukumaran and Holder 2010). We also visualized relationships between haplotypes for each dataset by reconstructing branch

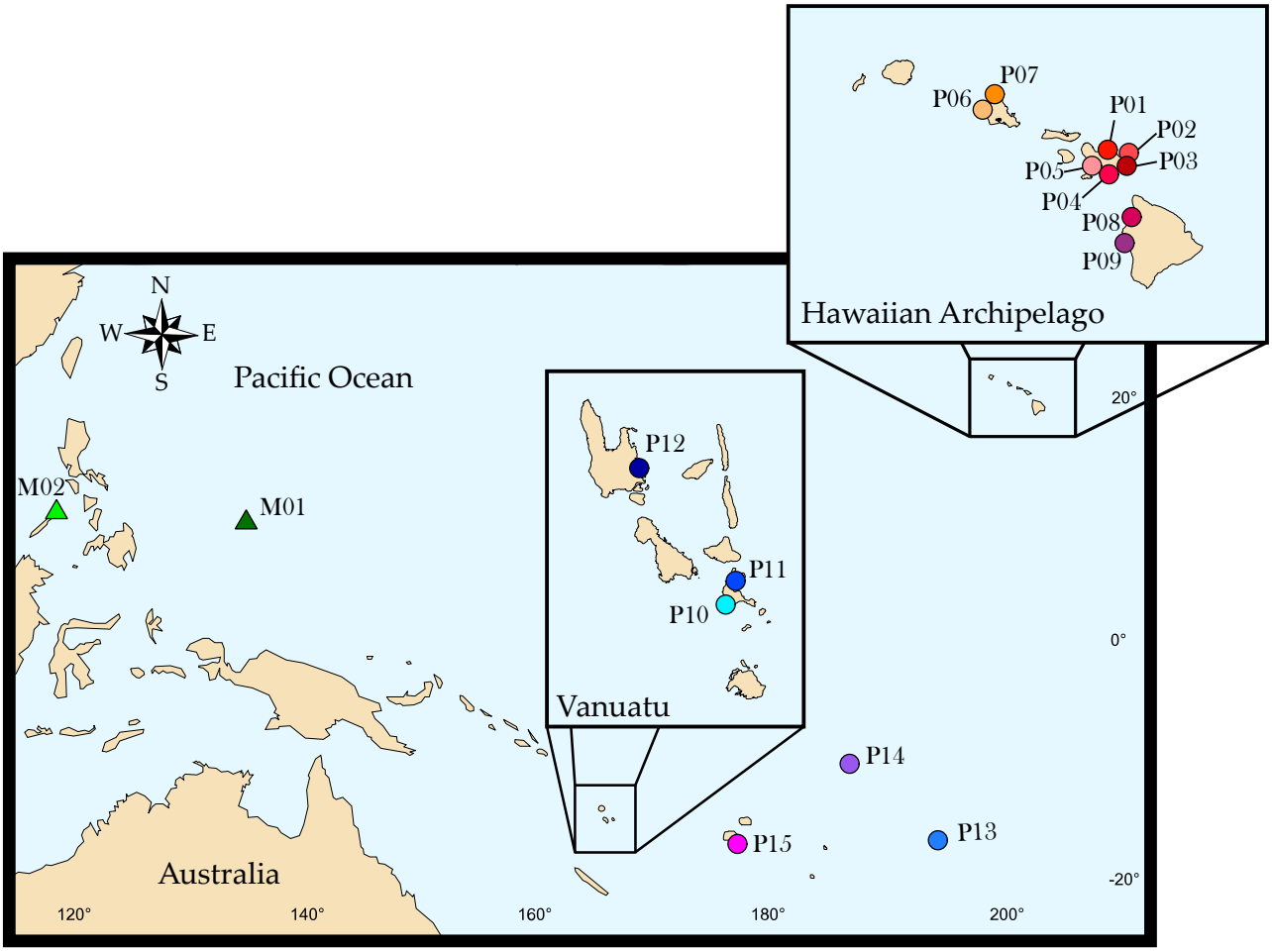

Fig. 1. Map of localities included in this study. M01-Wanead, Yap, Federated States of Micronesia; M02-Las Cabanas Beach, Palawan, Philippines; P01-Baby Beach, Maui, HI, USA; P02-Waiʻānapanapa, Maui, HI, USA; P03-Koki Beach Park, Maui, HI, USA; P04-Hanakaoʻo Park, Maui, HI, USA; P05-Kihei, Maui, HI USA; P06-Kapalaoa Beach, Oʻahu, HI, USA; P07-Pūpūkea Beach Park, Oʻahu, HI, USA; P08-Spencer Beach Park, Hawaiʻi, HI, USA; P09-Nāpoʻopoʻo Park, Hawaiʻi, HI, USA; P10-Blue Pango, Efate, Vanuatu; P11-Pele Island, Vanuatu; P12-Champagne Beach, Espiritu Santo, Vanuatu; P13-Opunohu bay, Moorea, Tahiti, French Polynesia; P14-Upolu, Samoa; P15-The Beachhouse, Viti Levu, Fiji. Colors, shapes, and labels for each locality correspond with other figures and tables. Detailed information for each locality is presented in table 1. Map is edited from a map in the public domain in Wikimedia Commons at https://commons.wikimedia.org/wiki/File:Pacific_Theater_Areas;map1.svg.

Table 1. Geographic and haplotype information for localities included in this study. Latitude and longitude are provided when available. The GenBank accession number for each unique haplotype is listed in the localities where individuals harbored the haplotype, with number in parenthesis indicating how many individuals harbored the haplotype

Locality	Map Label	Lat	Long	12S rDNA
Wanead, Yap, Federated States of Micronesia	M01	09°36'22.50"N	138°10'41.60"E	ON980549 (2)
				ON980550 (2)
Las Cabanas Beach, Palawan, Philippines	M02	11°08'41.90"N	119°23'28.90"E	ON980547 (2)
				ON980548 (3)
Baby Beach, Maui, HI, USA	P01	20°54'45.22"N	156°24'15.78"W	ON980551 (5)
Waiʻānapanapa, Maui, HI, USA	P02	20°47'20.68"N	156°00'11.76"W	ON980551 (5)
Koki Beach Park, Maui, HI, USA	P03	20°43'41.60"N	155°59'06.10"W	ON980551 (5)
Hanakaoʻo Park, Maui, HI, USA	P04	20°54'34.10"N	156°41'18.90"W	ON980551 (5)
Kihei, Maui, HI USA	P05	20°43'12.80"N	156°26'51.10"W	ON980551 (4)
				ON980552 (1)
Kapalaoa Beach, Oʻahu, HI, USA	P06	21°36'33.40"N	157°54'33.60"W	ON980551 (3)
				ON980555 (2)
Pūpūkea Beach Park, Oʻahu, HI, USA	P07	21°39'07.40"N	158°03'42.90"W	ON980551 (4)
				ON980554 (1)
Spencer Beach Park, Hawaiʻi, HI, USA	P08	20°01'23.50"N	155°49'20.50"W	ON980551 (1)
Nāpoʻopoʻo Park, Hawaiʻi, HI, USA	P09	19°28'16.30"N	155°55'15.60"W	ON980553 (2)
Blue Pango, Efate, Vanuatu	P10	17°46'32.20"S	168°18'00.90"E	ON980556 (2)
Pele Island, Vanuatu	P11	17°29'22.00"S	168°23'43.80"E	ON980554 (5)
Champagne Beach, Espiritu Santo, Vanuatu	P12	15°08'36.30"S	167°07'17.20"E	ON980554 (5)
Opunohu bay, Moorea, Tahiti, French Polynesia	P13	N/A	N/A	ON980551 (1)
Upolu, Samoa	P14	N/A	N/A	ON980554 (1)
The Beachhouse, Viti Levu, Fiji	P15	18°13'45.87"S	177°46'33.45"E	ON980554 (4)
				ON980556 (1)

Locality	16SrDNA	*COI*	H3A
Wanead, Yap, Federated States of Micronesia	ON980544 (4)	ON959209 (3)	ON980649 (2)
	ON980545 (1)		
Las Cabanas Beach, Palawan, Philippines	ON980542 (5)	N/A	ON980649 (5)
Baby Beach, Maui, HI, USA	ON980546 (5)	ON959207 (2)	ON980648 (5)
		ON959208 (3)	
Waiʻānapanapa, Maui, HI, USA	ON980546 (5)	ON959207 (4)	ON980648 (5)
		ON959208 (1)	
Koki Beach Park, Maui, HI, USA	ON980546 (5)	ON959208 (5)	ON980648 (5)
Hanakaoʻo Park, Maui, HI, USA	ON980546 (5)	ON959207 (1)	ON980648 (5)
		ON959208 (3)	
Kihei, Maui, HI USA	ON980546 (5)	ON959207 (1)	ON980648 (5)
		ON959208 (4)	
Kapalaoa Beach, Oʻahu, HI, USA	N/A	ON959208 (1)	N/A
Pūpūkea Beach Park, Oʻahu, HI, USA	ON980546 (5)	ON959207 (1)	ON980648 (5)
		ON959208 (4)	
Spencer Beach Park, Hawaiʻi, HI, USA	ON980546 (1)	ON959208 (1)	ON980648 (1)
Nāpoʻopoʻo Park, Hawaiʻi, HI, USA	ON980546 (2)	ON959207 (2)	ON980648 (2)
Blue Pango, Efate, Vanuatu	ON980546 (2)	ON959208 (5)	ON980648 (2)
Pele Island, Vanuatu	ON980546 (5)	ON959207 (1)	ON980648 (5)
		ON959208 (1)	
Champagne Beach, Espiritu Santo, Vanuatu	ON980546 (5)	ON959208 (5)	ON980648 (5)
Opunohu bay, Moorea, Tahiti, French Polynesia	ON980546 (1)	N/A	ON980648 (1)
Upolu, Samoa	ON980546 (1)	N/A	ON980648 (1)
The Beachhouse, Viti Levu, Fiji	ON980543 (3)	ON959208 (3)	ON980648 (4)
	ON980546 (2)		

connections using the TCS network option (Clement et al. 2002) of PopArt v1.7 (Leigh and Bryant 2015). We used ASAP (Puillandre et al. 2021), a distance-based molecular species delimitation approach, to identify the putative number of species present in *A. oahuensis*. ASAP analyses were conducted on the ASAP webserver (https://bioinfo.mnhn.fr/abi/cgi-bin/web_asap.cgi) on the *A. oahuanesis* concatenated mitochondrial dataset after all individuals with 2 or more missing genes were removed from the dataset. Analyses were

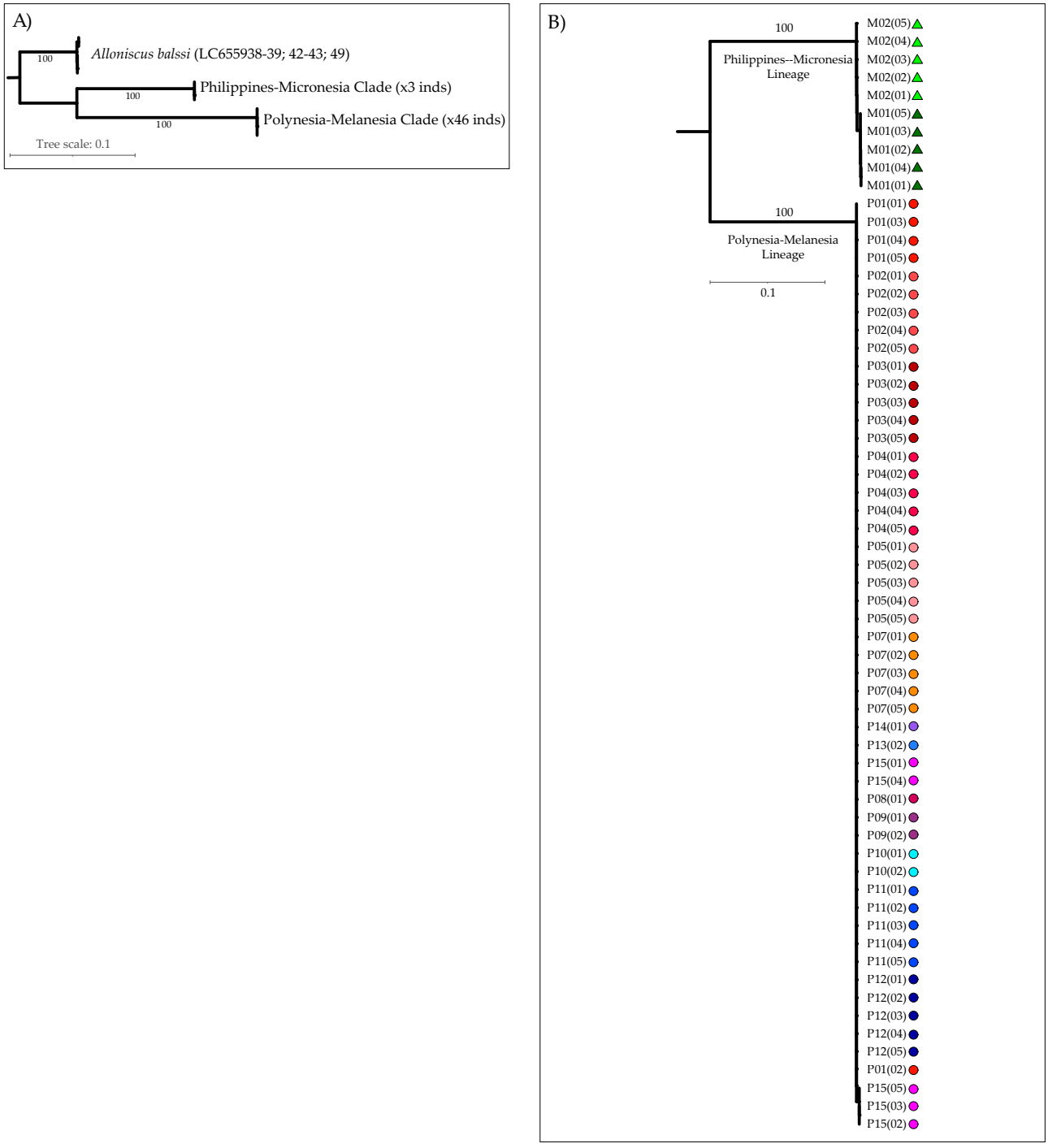

Fig. 2. Neighbor-Joining trees produced in this study. Panel A represents a Neighbor-Joining tree of *A. oahuensis* and *A. balssi* based on *COI* mitochondrial sequences, while Panel B shows the Neighbor-Joining tree of *A. oahuensis* based on concatenated dataset including the four genes used in this study. Locality IDs, colors, and shapes correspond with those used in all other Figures, Tables, and Datasets. Bootstrap support values are provided for each major lineage recovered in analyses.

carried out under the Kimura 2-Parameter (K2P) nucleotide evolution model, assuming a Pmin value of 0.01, a Pmax of 0.20, and a relative width of 2 with all ambiguous sites masked. All other settings were as default. Lastly, we estimated genetic distances between all individuals sequenced for each gene dataset using the Kimura-2-Parameter correction (hereafter K2P; Kimura 1980) in MEGA v11.0.10 (Tamura et al. 2021).

RESULTS

We successfully sequenced at least one mitochondrial gene for 66 specimens identified as *A. oahuensis* using Schultz's (1984) dichotomous key: 66 individuals for the 12S rDNA gene, 62 for the 16S rDNA gene, and 51 for the *COI* gene fragment. We also sequenced the nuclear H3A gene for 58 individuals. We were able to produce at least one sequence of each gene for each locality with the exception of H3A (no sequences from locality P06 in in O'ahu, Hawai'i) and *COI* (no sequences from localities in the Philippines [M02], Tahiti [P13], and Samoa [P14]). All unique haplotypes produced in this study were deposited into GenBank under Accession Numbers ON980547-ON980556, ON980542-ON980546, ON959207-ON959209, ON980648-ON980649. A detailed breakdown of the number of individuals and haplotypes identified in each location is provided in table 1.

The final concatenated alignment consisted of 1,739 positions after the removal of poorly aligned sites. The final alignment included 452-bp of the 12S rDNA gene (7 sites removed), 419-bp of the 16S rDNA gene (39 sites removed), 588-bp of the COI gene, and 280-bp of the H3A gene. Of the 1,739 positions included in the final alignment, 345 were parsimony informative (12S rDNA: 130; 16S rDNA: 89; *COI*: 118; H3A: 10).

The consensus tree produced from NJ bootstrap replicates based on this alignment identified two well-supported clades (Bootstrap Support = 100; Fig. 2 Panel B): a "Philippines-Micronesia" clade consisting of all *A. oahuensis* individuals collected in Yap (M01) and The Philippines (M02), and a "Polynesia-Melanesia" clade that included all individuals collected in the Hawaiian Archipelago (P01–09), Vanuatu (P10–12), Tahiti (P13), Samoa (P14), and Fiji (P15). K2P genetic distances amongst these clades were high regardless of the gene under consideration, with all amongst-clade pairwise comparisons exceeding 23.0% for the mitochondrial genes and 3.0% for the nuclear H3A gene (Table 2). Average K2P distances amongst the clades were 34.87%, 25.53%, 23.74%, and 3.67% for the 12S rDNA, 16S rDNA, *COI*, and H3A genes respectively (Table 2).

Haplotype network reconstructions produced results consistent with the above findings regardless of the gene under analysis (Fig. 3). For the 12S rDNA gene we recovered eleven haplotypes: four corresponding to the "Philippines-Micronesia" lineage with the other seven belonging to the "Polynesia-Melanesia" lineage (Fig. 3, Panel A). Haplotypes from the two lineages differed by 134–140 mutational steps. Within the "Philippines-Micronesia," two haplotypes were recovered from individuals collected in Yap (M01) and another two recovered from specimens collected in the Philippines (M02). The Yap haplotypes differed from each other in a single nucleotide and to those found in the Philippines by 2–4 steps. The two haplotypes found in the Philippines differed by a single nucleotide position. The seven "Polynesia-Melanesia" lineage haplotypes were separated by 1–5 differences, with most individuals exhibiting one of two haplotypes that differed from each other by a single position. Of these two haplotypes, one was recovered from 32 individuals collected in localities from the Hawaiian Archipelago (P01–09) and Tahiti (P13), while the other was recovered in 16 individuals from the Hawaiian Archipelago (P01–09), Fiji (P15), Samoa (P14), and Vanuatu (P10–12). The remaining "Polynesia-Melanesia" haplotypes were observed in 1–3 individuals (Fig. 3, Panel A) and differed by 1–3 steps from the two more frequent haplotypes and each other.

We recovered four 16S rDNA haplotypes, two each in "Philippines-Micronesia" and "Polynesia-

Table 2. K2P genetic distances within and amongst lineages. For each gene we provide the minimum, maximum, and mean K2P genetic pairwise genetic distance

	"Polynesia-Melanesia"			"Philippines-Micronesia"			Amongst Lineages		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
12S	0	0.67	0.17	0	1.81	0.92	34.41	36.02	34.87
16S	0	0.24	0.03	0	0.49	0.18	25.31	26.35	25.53
COI	0	0.17	0.0652	0	0	0	23.67	23.93	23.74
H3A	0	0	0	0	0	0	3.67	3.67	3.67

Melanesia" localities (Fig. 3, Panel B). Haplotypes in the two groups differed by 87–90 steps. Within the "Philippines-Micronesia" group, we found two haplotypes from individuals from Yap (M01) and one from individuals from the Philippines (M02). These haplotypes differed by 1–2 nucleotides. The two haplotypes recovered in "Polynesia-Melanesia" localities also differed by a single position from each other, with one haplotype recovered from 3 individuals collected in Fiji (P15) and the other found in all other individuals from this group.

For the *COI* gene, we recovered two haplotypes from individuals in the "Polynesian-Melanesia" localities and a single haplotype from individuals from the "Philippines-Micronesia" locality, Yap (Fig. 3, Panel C). In the "Polynesia-Melanesia" group, both haplotypes were of about equal frequency with both present in all regions of the Pacific where this group was observed.

For the nuclear H3A gene, we recovered two haplotypes that differed by 10 mutational steps (Fig. 3, Panel D). One haplotype was shared by all specimens from the "Polynesia-Melanesia" localities, and the other was shared by all individuals from the "Philippines-Micronesia" localities.

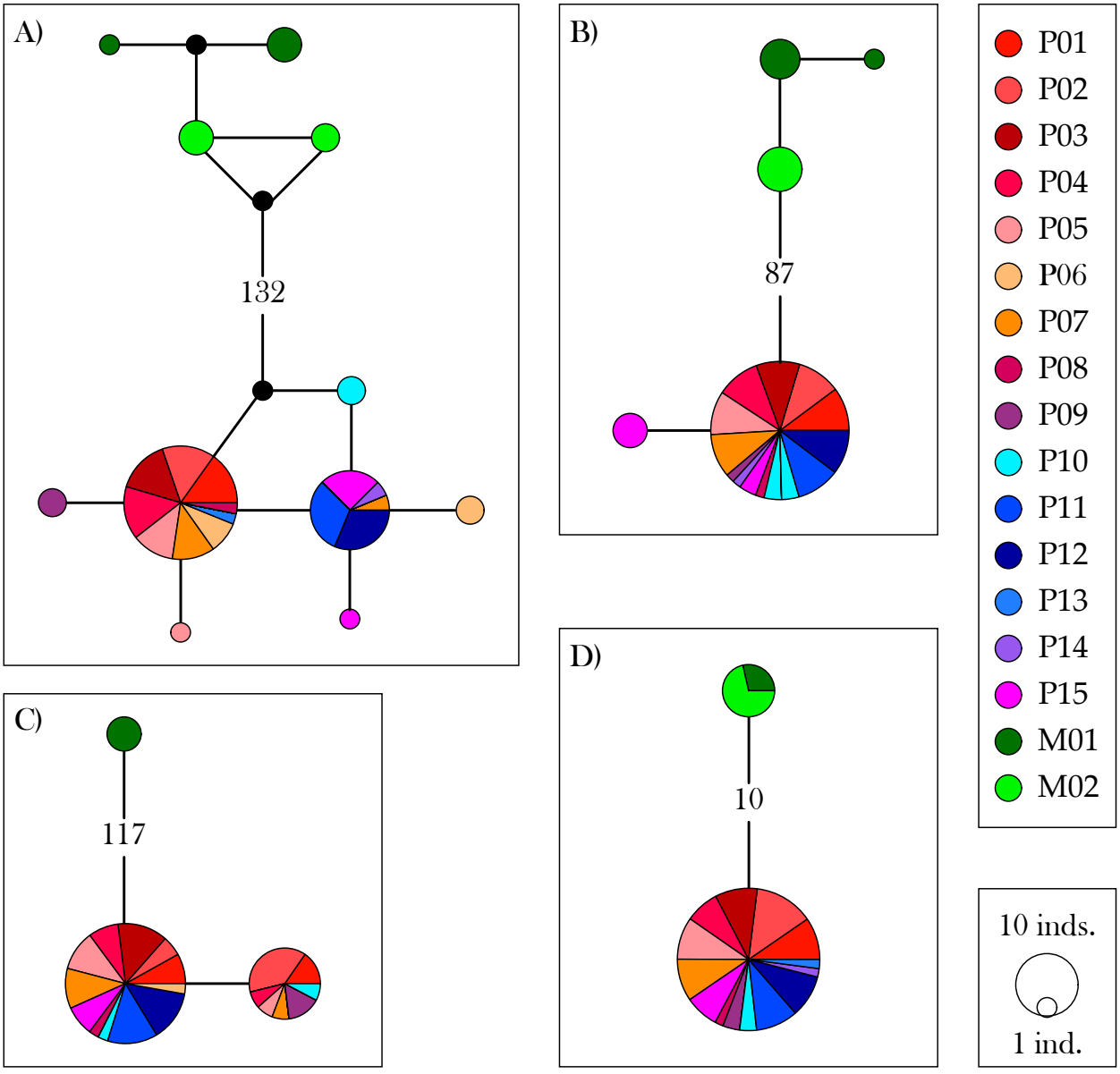

Fig. 3. Haplotype networks for *A. oahuensis*. Panels correspond with the 12S rDNA gene (Panel A), 16S rDNA gene (Panel B), *COI* (Panel C), and H3A (Panel D). Colors and locality IDs correspond with those used in all other Figures. Black circles represent unsampled (*i.e.*, missing) haplotypes, with the size of circles proportional to the frequency at which each haplotype was recovered.

ASAP analyses identified a two species solution as the most likely primary species hypothesis for our *A. oahuensis* dataset [ASAP score = 1, *p*-value = 1×10^{-5} (rank = 1), W value = 3.06×10^{-3} (rank 1), threshold = 0.041296]. The two putative species identified by ASAP corresponded with the two major clades recovered in phylogenetic reconstructions: all individuals belonging to the "Polynesia-Melanesia" were placed in one putative species, with and the other putative species consisting of all "Philippines-Micronesia" individuals.

DISCUSSION

The purpose of this study was to determine whether *A. oahuensis* harbors any divergent genetic lineages possibly representing cryptic species and to describe the geographic distribution of these lineages. To this end, we used both mitochondrial and nuclear markers to characterize specimens identified as *A. oahuensis* based on morphological traits from locations in the Pacific Ocean. Our results indicate that *A. oahuensis* is likely a cryptic species complex comprised of at least two highly divergent genetic and geographically disjunct lineages that may have recently dispersed throughout the region.

We identified two highly divergent genetic lineages within *A. oahuensis*: a "Philippines-Micronesia" lineage, identified from specimens collected in The Philippines and Yap (Federated States of Micronesia), and a "Polynesian-Melanesia" lineage, from specimens collected in Vanuatu, Fiji, Samoa, Tahiti, and Hawai'i. Divergences amongst these lineages were high across all genes studied, exceeding 23.0% K2P for all mitochondrial genes and 3.0% for the nuclear H3A. These divergences are equal to or higher than amongst-species divergences reported for other coastal oniscidean isopods (Jung et al. 2008; Hurtado et al. 2010 2014; Raupach et al. 2014; Hurtado et al. 2018; Greenan et al. 2018), including species from islands in the Pacific Ocean (Taiti et al. 2003; Santamaria et al. 2013; Santamaria 2019). For instance, the *COI* K2P divergences amongst *A. oahuensis* lineages herein reported greatly exceed those reported amongst cryptic *Ligia* species from the Hawaiian Islands (*COI* K2P 4.0–16.60%; Santamaria et al. 2013; Santamaria 2019). Indeed, ASAP analyses identified the two lineages recovered in phylogenetic reconstructions as separate putative species. Thus, our findings suggest that *A. oahuensis* may represent a cryptic species complex of at least two species in need of taxonomic revision.

Our findings of cryptic diversity in *A. oahuensis*, a coastal crustacean species considered to be widely distributed across islands in the Pacific and Indo-Pacific, are in accordance with recent reports of such diversity for other coastal crustaceans in the region. Shahdadi et al. (2019) used morphological and molecular evidence to describe *Parasesarma austrawati*, a cryptic mangrove crab species found in northern Australia. Shih and Poupin (2020) used similar approaches to describe *Austruca citrus*, a novel species of fiddler crab whose distribution is thought to be limited to Fiji, Wallis & Futuna, and Samoa. As for isopods, Santamaria (2019) used molecular, morphological, and distributional evidence to redescribe *Ligia hawaiensis* and propose seven new cryptic species of *Ligia* isopods from the Hawaiian Islands. Interestingly, our findings contrast with those of Santamaria (2019) as we did not observe the high levels of genetic diversity reported for *Ligia* amongst populations in the Hawaiian Islands.

Divergences within the lineages reported herein are of note, as they appear to be very low. Within the "Philippines-Micronesia" group, K2P divergences did not exceed 2.0% for any mitochondrial gene. Furthermore, 12S and 16S rDNA haplotypes found in the two locations of this gene were only 1–3 differences apart. Divergences within the "Polynesian-Melanesia" were even lower, with all pairwise comparisons amongst members of the lineage resulting in K2P distances under 1.0% K2P regardless of the gene under consideration. Furthermore, haplotype sharing amongst sites in this clade was common for all genes. These patterns appear at odds with the biology of *Alloniscus* (*e.g.*, direct development, no planktonic larval stages), the large geographic distances across sites included in this study, and the high levels of genetic divergence reported amongst populations of other poorly dispersing coastal isopods (Taiti et al. 2003; Jung et al. 2008; Hurtado et al. 2010 2014 2018; Santamaria et al. 2013; Raupach et al. 2014; Greenan et al. 2018; Santamaria 2019). For example, the lack of genetic divergence and high degree of haplotype sharing observed within *A. oahuensis* populations in the Hawaiian Islands severely contrasts with recent findings of population substructure of poorly dispersing coastal organisms in the archipelago, including the coastal isopod genus *Ligia* (Taiti et al. 2003; Santamaria et al. 2013; Santamaria 2019), the shrimp *Halocaridinia rubra* (Craft et al. 2008), and *Cellana* limpets (Bird et al. 2011). These findings either suggest that *A. oahuensis* dispersed recently across the Polynesian and Micronesian islands or that gene flow amongst these populations is higher than for other coastal organisms in the region with similar biology.

Despite lacking planktonic larvae, coastal isopods are known to disperse by rafting on sea wrack (*e.g.*, wood, kelp) and by forming air bubbles when curling into a ball (Kensley 1974). Genetic signatures of rafting having been reported for *Limnoria* isopods in Chile

(Haye et al. 2012) and laboratory experiments suggest *Idotea metallica* exhibits adaptations to long-term over-sea dispersal on rafts (Gutow 2006). Thus, the widespread distribution of the "Polynesian-Melanesian" *A. oahuensis* lineage across the Pacific Ocean may be explained by rafting. On the other hand, the genetic similarity amongst localities in this area and the low levels of divergence amongst individuals in this lineage would suggest high levels of gene flow and would appear more in line with anthropogenic dispersal. Coastal isopods have been introduced to Pacific islands by human activities including in highly remote islands of the Pacific such as Midway Atoll (Santamaria et al. 2022), indicating the possibility that *A. oahuensis* has been dispersed by human activity. Distinguishing between these possibilities requires additional research.

CONCLUSIONS

We uncovered two deeply divergent genetic lineages within *A. oahuensis* by using mitochondrial and nuclear gene sequences to characterize 60+ individuals from 17 localities across the Pacific Ocean: a "Philippines-Micronesia" lineage comprising individuals collected in The Philippines and Yap, and a "Polynesia-Melanesia" lineage comprising individuals from the Hawaiian Islands, Vanuatu, Fiji, Tahiti, and Samoa. The genetic divergences amongst these two lineages match or exceed those reported across other cryptic species in Oniscidea, suggesting that *A. oahuensis* may represent a cryptic species complex in need of a taxonomic revision.

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Competing interests: CAS and MRK declare that they have no conflict of interests.

Availability of data and materials: All unique haplotypes produced in this study can be found in GenBank under Accession Numbers ON980547-ON980556, ON980542-ON980546, ON959207-ON959209, ON980648-ON980649. A concatenated dataset including all sequences used in this study is provided as supplementary file 1.

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Supplementary materials

Supplementary file 1. (download)