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Phylogeography of Mesophotic Coral Ecosystems: Squirrelfish and Soldierfish (Holocentriformes: Holocentridae)

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Abstract: Mesophotic coral ecosystems (MCEs: ~30 to 100+ m depth) may be older and more stable than shallow coral ecosystems that are more prone to disturbances in both the long term (glacial sea level cycles) and short term (heavy weather and anthropogenic activities). Here, we assess the phylogeography of two MCE fishes, the soldierfish *Myripristis chryseres* (N = 85) and the squirrelfish *Neoniphon aurolineatus* (N = 74), with mtDNA cytochrome oxidase C subunit I. Our goal is to resolve population genetic diversity across the Central and West Pacific and compare these patterns to three shallow-reef species in the same taxonomic family (Holocentridae). Significant population structure (Φ_{ST} = 0.148, p = 0.01) was observed in *N. aurolineatus*, while no structure was detected in *M. chryseres* (Φ_{ST} = −0.031, p = 0.83), a finding that matches the shallow-water congener *M. berndti* (Φ_{ST} = −0.007, p = 0.63) across the same range. Nucleotide diversity in the MCE fishes was low (π = 0.0024–0.0028) compared to shallow counterparts (π = 0.003–0.006). Coalescence times calculated for *M. chryseres* (~272,000 years) and *N. aurolineatus* (~284,000 years) are more recent or comparable to the shallow-water holocentrids (~220,000–916,000 years). We conclude that the shallow genetic coalescence characteristic of shallow-water marine fishes cannot be attributed to frequent disturbance. We see no evidence from holocentrid species that MCEs are older or more stable habitats.

Keywords: mesophotic coral ecosystems; biodiversity; coalescent; coral reefs; deep reef refugia; fishes; mtDNA; population structure; vertebrates

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1. Introduction

Evaluating how marine biodiversity is shaped by geography, oceanography, climate, and natural history requires an understanding of the distribution of genetic diversity [1,2]. While every case is unique, documenting genetic partitions can reveal common drivers of biodiversity across various geographic scales. For example, Toonen et al. [3] documented concordant genetic breaks based on 27 invertebrate and vertebrate species in the Hawaiian Archipelago (see also [4]). Timm et al. [5], Barber et al. [6], Carpenter et al. [7], and Gaither & Rocha [8] discerned shared genetic breaks in the Coral Triangle and the wider Indo-Pacific, shedding light on factors that generate and maintain species diversity (see [9]).

Phylogeographic patterns of tropical marine organisms are extensively documented. However, most of this work is focused on the shallowest zones of the oceans, either in coastal waters or among the commercially valuable epipelagic fishes e.g., [10]. In particular, almost all genetic surveys of coral reef fishes are restricted to shallow habitats (<30 m). Yet, light-dependent coral ecosystems extend past 30 m, even down to 150 m. These mesophotic

coral ecosystems (MCEs) [11] share some fishes and invertebrates with the shallow zone, but also have a unique fauna restricted to the deeper reefs [12–17]. Very little is known about the phylogeography of organisms that inhabit MCEs, primarily due to the logistical and physiological constraints (time and depth limits) of sampling with scuba diving [18]. Indeed, those phylogeographic studies that have been accomplished below 30 m have been largely restricted to fishes that can be collected with hook and line, e.g., [19–21].

There are several reasons to suspect that MCE organisms may have phylogeographic patterns that are fundamentally different from their shallow-water counterparts. MCEs could provide refugia from the impacts of storms and anthropogenic stressors [22,23]—but see [24]—and MCEs may be more stable over both ecological and evolutionary timeframes [25,26]. In particular, MCEs reside largely below the zone extirpated by glacial cycles of sea level rise and fall, which encompass up to 120 m of the water column [27]. Stabler habitats would be expected to have older communities, relative to the shallow coalescence observed in most shallow-water organisms [28].

One group of fishes suited to comparing phylogeographic trends in shallow-water and mesophotic reef organisms are the Holocentridae (Beryciformes). This group of nocturnal fishes are characteristically red or silvery in color, with large eyes, large mouths, and coarse ctenoid scales [29]. Two circumtropical sub-families—Holocentrinae (squirrelfishes) and Myripristinae (soldierfishes)—comprise well-supported evolutionary lineages that diverged approximately 55 million years before the present day (BP) [30,31]. There are both shallow and mesophotic specialists in each sub-family, but as with most fish taxa, studies are restricted to shallow species. Phylogeographic studies of *Myripristis berndti* [32], a shallow water soldierfish, reveal a population structure on the broad scale of the Indian Ocean versus Central-West Pacific versus East Pacific. In the tropical Atlantic, the squirrelfish *Holocentrus adscensionis* has a low but significant population structure on the scale of ocean basins (Caribbean Sea versus Brazil, mid-Atlantic ridge, and Gulf of Guinea (East Atlantic)), while the soldierfish *Myripristis jacobus* has no population structure across the same range [33]. The three holocentrids surveyed to date show little population structure, indicating extensive larval dispersal, possibly facilitated by a post-larval stage that remains in the water column [34]. The mtDNA networks for all three species reveal many closely related haplotypes and low nucleotide diversity ($\pi = 0.003–0.006$), indicating late Pleistocene coalescence of mtDNA lineages.

In the present study, we compare phylogeographic patterns between mesophotic and shallow-water fishes. We collected two species of MCE holocentrids, the Yellowfin Soldierfish *Myripristis chryseres* (Jordan and Evermann 1903) and the Yellowstriped Squirrelfish *Neoniphon aurolineatus* (Liénard 1839), across the Hawaiian Archipelago and elsewhere in the Pacific. The nocturnal Yellowfin Soldierfish is occasionally found in shallow water, but typically inhabits depths of 30–235 m in tropical waters from Africa to Hawai'i [35]. This has been observed with coelacanths in caves of the western Indian Ocean at depths > 180 m [36]. The Yellowstriped Squirrelfish is also nocturnal, with a similar distribution across the Indo-Pacific at depths of 30–190 m [37]. Both species sampled in this study were found only in the lowest mesophotic zone (>90–150 m), while the three species we compare them to (*M. berndti*, *M. jacobus*, and *H. adscensionis*) were found exclusively in shallower strata, based on 317 transects conducted from 0–150 m in the Pacific and Western Atlantic [24]. By comparing phylogeographic patterns among these species, we hope to provide the first steps towards a true comparative assessment of mesophotic phylogeography.

2. Materials and Methods

2.1. Specimen Collection and DNA Extraction

Tissue samples were collected throughout the Hawaiian Archipelago, Johnston Atoll, Pohnpei, and the Philippines (Figure 1) for *M. chryseres* (N = 85) and *N. aurolineatus* (N = 74). Fish were collected with a pole spear on a closed-circuit rebreather from 2012 to 2018 at 50–80 m depth. Tissue samples were stored in saturated salt-dimethylsufoxide (DMSO)

buffer [38]. Total genomic DNA was extracted from all specimens using the "HotSHOT" protocol [39] and stored at −20 °C for later amplification.

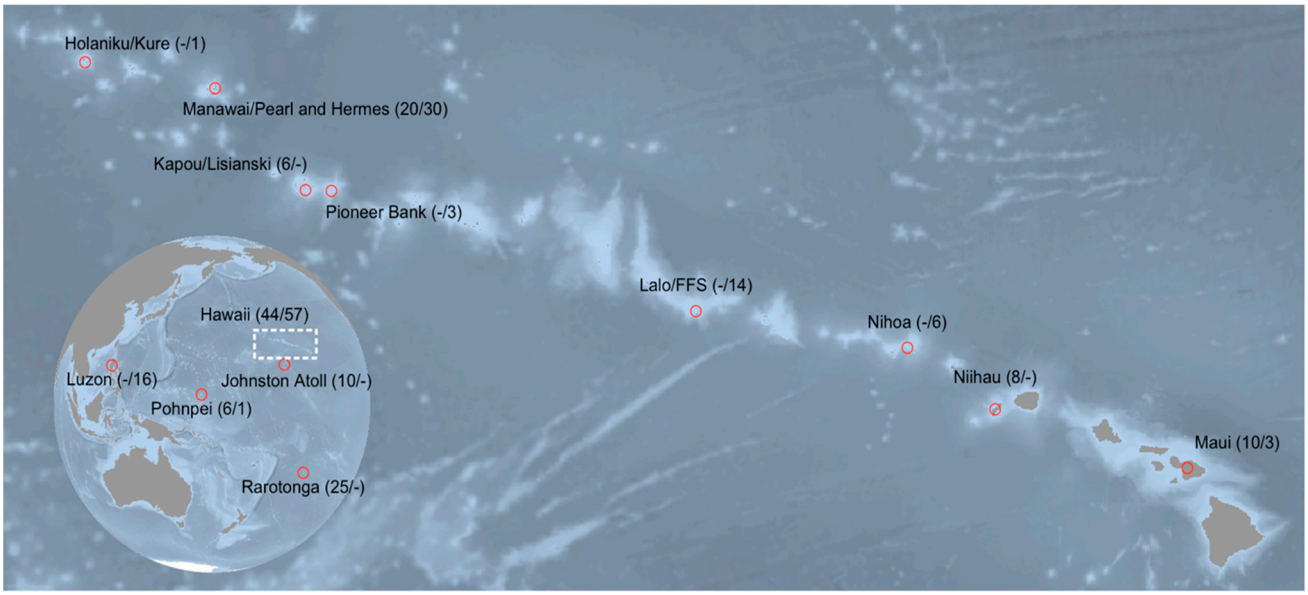

Figure 1. Bathymetric map of sampling locations and number of specimens taken per site for *Myripristis chryseres* and *Neoniphon aurolineatus* (*M. chryseres*/*N. aurolineatus*). Main map indicates sampling sites in the Hawaiian Archipelago, inset indicates additional sample sites in the Central and West Pacific.

2.2. PCR and Sequencing

A segment of the mitochondrial DNA (mtDNA) cytochrome oxidase C subunit 1 (COI) was resolved using fish-specific primers, FishF2 and FishR2 [40]. When these primers did not amplify, overlapping FISH BCL and FISH BCH primers [41] were used instead.

Polymerase chain reaction (PCR) was performed in a 15 µL reaction containing 7.5 µL BioMix^{TM} Red (Bioline Inc., Springfield, NJ, USA), 0.2 µM of each primer, 5–50 ng template DNA, and nanopure water (Thermo Scientific, Barnstead, Dubuque, IA, USA) to volume. PCR cycling parameters were as follows: initial 95 °C denaturation for 10 min, followed by 35 cycles of 94 °C for 30 s, annealing temperature 55 °C in all cases for 30 s, and 72 °C for 30 s, followed by a final extension of 72 °C for 10 min. PCR products were visualized using a 1.5% agarose gel with GelRed^{TM} (Biotium®, Freemont, CA, USA) and then cleaned by incubating with 0.75 units of Exonuclease and 0.5 units of Shrimp Alkaline Phosphatase (ExoSAP; USB®, Cleveland, OH, USA) per 7.5 µL of PCR product for 30 min. at 37 °C, followed by 85 °C for 15 min. Sequencing was conducted in the forward and reverse direction using a genetic analyzer (ABI 3730XL, Applied Biosystems, Foster City, CA, USA) at the ASGPB Genomics Sequencing Facility at the University of Hawai'i at Mānoa. The sequences were aligned, edited, and trimmed to a common length using Geneious Pro v.10.2.6 DNA analysis software (Biomatters Ltd., Auckland, New Zealand).

2.3. Data Analysis

Sequence alignment data were imported into the R [42] environment using the 'Biostrings' [43] and 'ape' [44] packages. The number of segregating sites and unique haplotypes, as well as haplotype diversity (h), nucleotide diversity (π) and Tajima's D, were calculated at all sampling locations for each species using the 'ape' and 'pegas' [45] packages, while Fu's F was calculated using the 'hfufs' package [46]. To assess the level of geographic structure across our sampling locations, analyses of molecular variance (AMOVA) were performed using 'pegas'. If the AMOVA determined there was significant structure among sampling locations, pairwise Φ_{ST} values were calculated for populations

with a minimum of five samples using the 'haplotypes' package [47]. The *p*-values for pairwise Φ_{ST} comparisons were adjusted using the Benjamini and Yekutieli [48] modified false discovery rate (B-Y FDR), as discussed by Narum [49], using the function p.adjust in R.

To infer the population histories and coalescence times of each species, we performed Bayesian skyline analyses in BEAST v2.6.0 [50]. We allowed BEAST to iterate over all possible molecular evolution models and choose the model with the highest likelihood given the data, as implemented in the bModelTest package [51]. We performed 120–170 million simulations using a strict clock model for each species, discarding the first 10% of results as burn-in. Our present data are mtDNA COI sequences, and the rate of 2% per million years estimated in previous studies was based on the mtDNA cytochrome b (cytb) gene. Comparisons between the two loci indicate similar mutation rates, albeit slightly higher (but not significantly different) in cytb [52,53]. Both are non-recombining protein-coding regions in the same metabolic pathway that are linked and inherited as a unit. Thus, we used the same molecular clock rate (2%/Ma) as that applied in previous studies of holocentrids [32,33], and, in addition, we ran analyses using a clock rate of 1.6% per million years, an estimate for COI derived from geminate species pairs separated by the Isthmus of Panama [54]. As those previous studies based divergence time estimates on values of τ and θ from Arlequin analyses, we reanalyzed the respective datasets in BEAST to ensure equivalence when making comparisons. We confirmed convergence of MCMC runs and model parameters in BEAST analyses using TRACER v1.7.2 [55].

Haplotype networks were constructed for our two study species to visualize the relationship between sampled haplotypes and their prevalence by location. These were also plotted on a composite plot containing reproduced haplotype networks from the three previously studied species to compare the topologies by depth zone and sub-family. Data from previous studies [32,33] were extracted from the original Arlequin [56] files, converted into FASTA format using PGDSpider [57], and imported into R as described above. All haplotype networks were created using 'pegas' and plotted using the 'ggplot2' [58], 'colorRamps' [59], 'patchwork' [60], and 'magick' [61] R packages.

New sequences generated for this study were deposited in GenBank (accession numbers ON998515–ON998673). GenBank accession numbers for previously published sequencing data are available in the original publications.

3. Results

3.1. Myripristis Chryseres

We resolved 530 bp of mtDNA COI containing 20 polymorphic sites and 23 unique haplotypes (Table 1). The most common haplotype was found at every location (Figure 2). Overall haplotype diversity (*h*) was 0.712 and nucleotide diversity (π) was 0.0024. Haplotype diversity was greatest in Pohnpei, nucleotide diversity was greatest in Maui, and both measures of diversity were lowest at Kapou/Lisianski. Tajima's D and Fu's F were negative at each individual location and overall. There was no geographic structure detected across sampling locations (AMOVA: global $\Phi_{ST} = -0.031$, $p = 0.830$). A continuous expansion was inferred for this species based on Bayesian skyline analysis (Supplemental Figure S1), and the lineages sampled in this study coalesced at ~272,000 years BP (95% HPD: 87,000–537,000 years) (Figure 3). BEAST runs were fully converged and all model parameters had ESS values > 200. Here and below, we report coalescence results based on the 1.6%/Ma mutation rate, but they do not differ substantially from those based on 2.0%/Ma. For complete coalescence results, see Supplemental Table S1.

Table 1. Population genetic diversity summary statistics for *Myripristis chryseres* across all sampling locations (n = number of samples sequenced, S = number of segregating sites, H = number of unique haplotypes, *h* = haplotype diversity, π = nucleotide diversity, D = Tajima's D, F = Fu's F). Bold numbers indicate summary values.

Location	n	S	H	*h*	π	D	F
Manawai/Pearl and Hermes	20	9	9	0.705	0.0020	−1.969	−6.279
Kapou/Lisianski	6	1	2	0.333	0.0006	−0.933	−0.023
Ni'ihau	8	4	4	0.643	0.0022	−1.030	−0.914
Maui	10	8	6	0.778	0.0039	−1.161	−1.756
Johnston Atoll	10	5	5	0.756	0.0025	−1.035	−1.596
Pohnpei	6	5	5	0.933	0.0031	−1.337	−2.526
Rarotonga	25	11	10	0.757	0.0026	−1.781	−5.732
Overall	**85**	**20**	**23**	**0.712**	**0.0024**	**−2.002**	**−22.942**

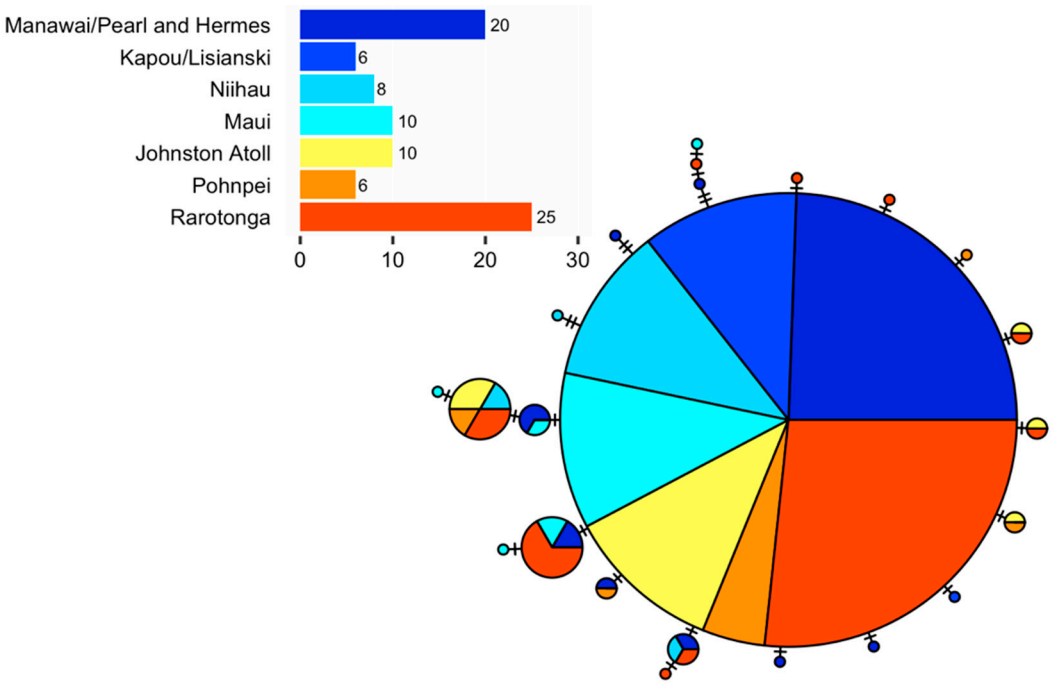

Figure 2. *Myripristis chryseres* haplotype network. Circle sizes correspond to the frequency of each sampled haplotype. Cool colors represent samples from locations in the Hawaiian Archipelago, while warm colors represent samples from locations across the wider Indo-Pacific. Sample sizes indicated by bar graph.

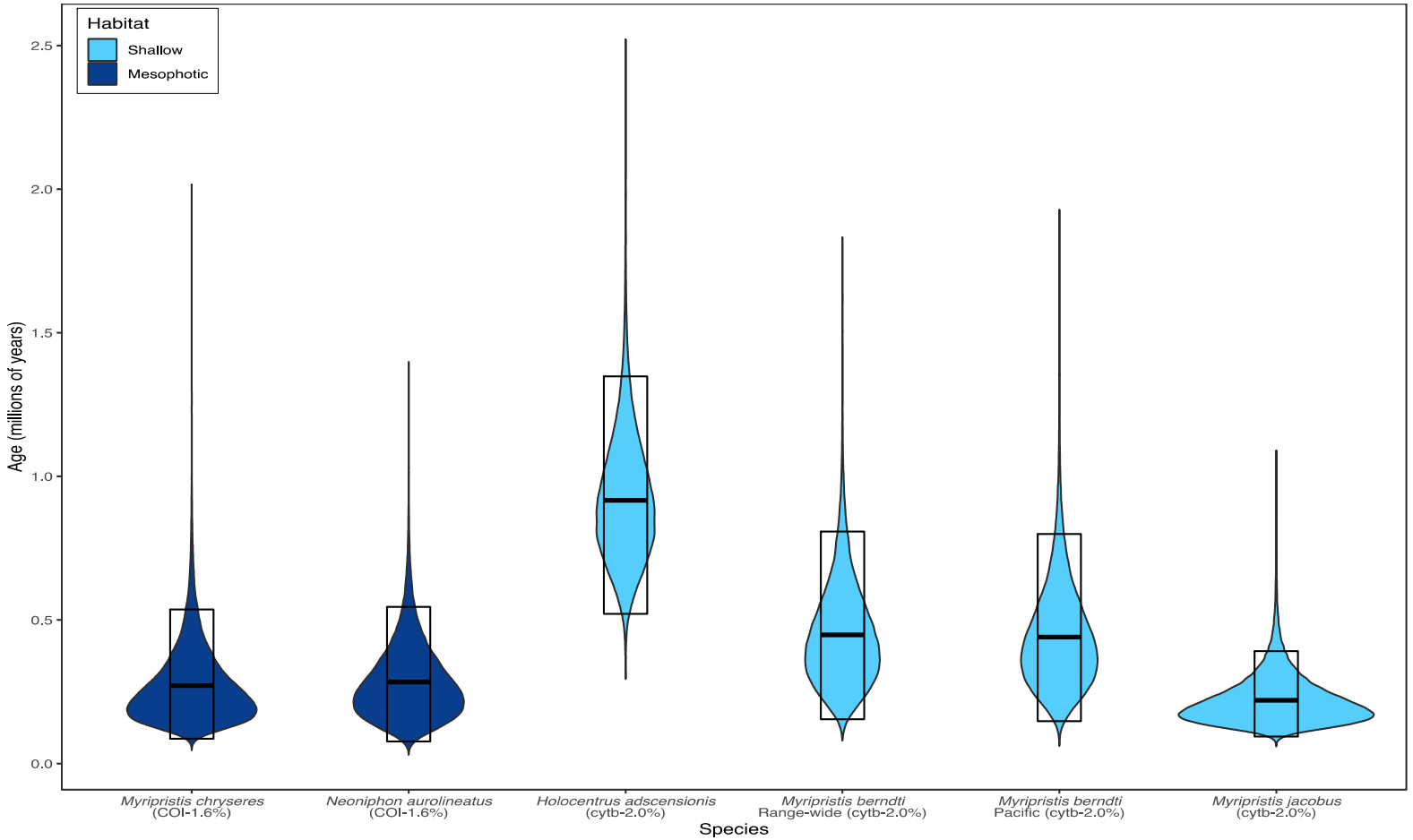

Figure 3. Violin plot comparing coalescence ages of species examined in this study, including *Myripristis berndti*, *M. jacobus*, *Holocentrus adscensionis* (shallow reefs), and *M. chryseres* and *Neoniphon aurolineatus* (deep reefs). Boxes indicate mean age (central bar) and 95% highest posterior density (upper and lower box borders). These include two plots for *M. berndti*: one for the entire Indo-Pacific range of the species, and one for the Pacific range that is comparable to the surveys for the deep-reef species, *M. chryseres* and *N. aurolineatus*.

3.2. *Neoniphon Aurolineatus*

We resolved 539 bp of COI for *Neoniphon aurolineatus.* The 77 sequences contained 12 polymorphic sites and 14 haplotypes (Table 2). The most common haplotype was observed at every location where at least two individuals were sampled (Figure 4). Overall haplotype diversity (*h*) was 0.793 and nucleotide diversity (π) was 0.0028. All three specimens in Maui had different haplotypes, while Luzon had the next highest haplotype and highest nucleotide diversity. Tajima's D and Fu's F were negative overall and at every location where more than four individuals were sampled.

Table 2. Population genetic diversity summary statistics for *Neoniphon aurolineatus* across all sampling locations (n = number of samples sequenced, *S* = number of segregating sites, H = number of unique haplotypes, *h* = haplotype diversity, π = nucleotide diversity, D = Tajima's D, F = Fu's F). Bold numbers indicate summary values.

Location	n	*S*	H	*h*	π	D	F
Hōlanikū/Kure	1	-	1	-	-	-	-
Manawai/Pearl and Hermes	30	7	8	0.752	0.0025	−0.687	−2.708
Pioneer Bank	3	2	2	0.667	0.0025	0.000	1.032
Lalo/French Frigate Shoals	14	6	6	0.736	0.0026	−0.893	−1.921
Nihoa	6	3	4	0.800	0.0022	−0.447	−1.466
Maui	3	2	3	1.000	0.0025	0.000	−1.216
Pohnpei	1	-	1	-	-	-	-
Luzon	16	7	9	0.850	0.0030	−0.865	−5.256
Overall	**74**	**12**	**14**	**0.793**	**0.0028**	**−1.088**	**−6.681**

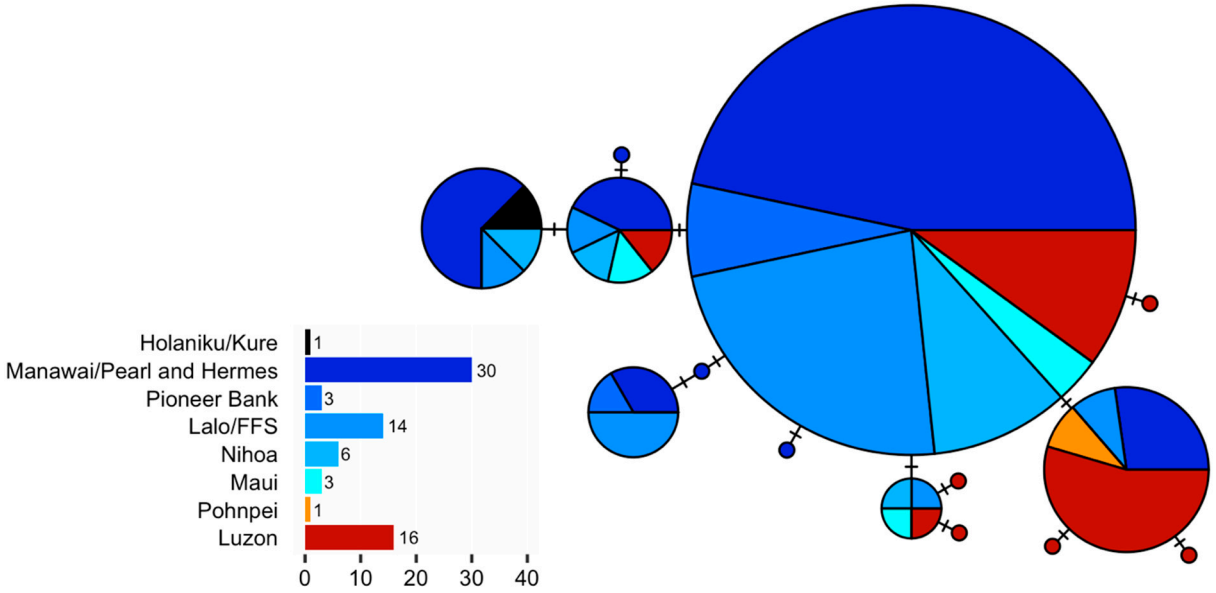

Figure 4. *Neoniphon aurolineatus* haplotype network. Circle sizes correspond to the frequency of each sampled haplotype. Cool colors represent samples from locations in the Hawaiian Archipelago, while warm colors represent samples from locations across the wider Indo-Pacific and correspond with those in Figure 2. Sample sizes indicated by bar graph.

Significant overall geographic population structure was detected (AMOVA: global Φ_{ST} = 0.148, p = 0.009). Pairwise Φ_{ST} was only significant between Luzon and both Lalo/French Frigate Shoals (Φ_{ST} = 0.131, p = 0.005) and Manawai/Pearl and Hermes (Φ_{ST} = 0.151, p = 0.001) after B-Y FDR correction (6 comparisons: $p < 0.008$). Φ_{ST} was similarly high between Luzon and less-sampled (n = 6) Nihoa (Φ_{ST} = 0.123), and was nearly significant (p = 0.05). Values of Φ_{ST} were less than 0.005 between Hawaiian locations. A continuous expansion was inferred for this species based on Bayesian skyline analysis

(Supplemental Figure S1), and the lineages sampled in this study coalesce at ~284,000 years BP (95% HPD: 77,000–546,000 years) (Figure 3). BEAST runs were fully converged and all model parameters had ESS values > 200.

3.3. Multi-Species Haplotype Network Comparison

Both mesophotic species have similar haplotype network topologies, haplotype diversity, and nucleotide diversity (Figure 5). Two of three Myripristinae species (*Myripristis chryseres* and *M. jacobus*) also have similar network topologies: one central haplotype comprising most individuals and sampling areas, with many closely related, less frequent haplotypes. *Myripristis berndti* had two haplotypes found at almost all locations (but this network was built with nearly three times more samples than collected for any species in the present study). *Holocentrus adscensionis* has at least twice as much nucleotide diversity and considerably greater haplotype diversity than *Neoniphon aurolineatus* or the Myripristinae species.

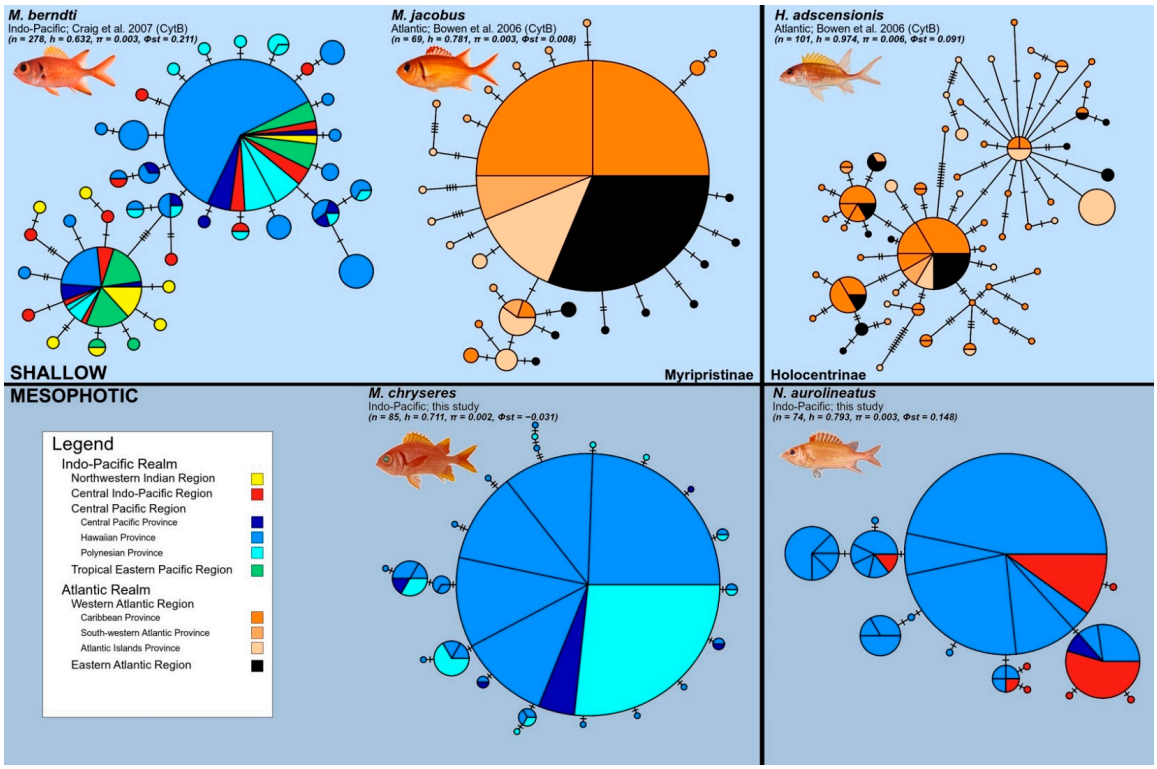

Figure 5. Haplotype networks for five reef-associated holocentrids organized by sub-family and depth strata, including *Myripristis berndti*, *Myripristis jacobus*, *Neoniphon adscensionis* (shallow), and *M. chryseres* and *N. aurolineatus* (mesophotic). Circle sizes correspond to the frequency of each sampled haplotype. Plots include ocean basin, data source, sample size, and global population genetic statistics for each sampled species. Colors correspond to biogeographic regions and provinces, as discussed in Kulbicki et al. [62], while circle segments correspond to individual sampling locations. For the *M. berndti* network (adapted from data in Craig et al. [32]), all Hawaiian sampling locations are grouped into the same segment and all circle sizes are square root-standardized (due to the higher sample size). Photo credits: Jeffrey Williams (*M. berndti, M. jacobus, M. adscensionis*), Barry Hutchins (*M. chryseres*), John Randall (*N. aurolineatus*).

4. Discussion

Specimens of the deep-reef holocentrids *M. chryseres* and *N. aurolineatus* were collected across the Hawaiian Archipelago and elsewhere in the Pacific to resolve phylogeographic patterns in mesophotic fishes and to compare genetic architecture between deep- and shallow-water holocentrids. Data from congeneric surrogates indicate that these two species may have similar dispersal patterns. Tyler et al. [34] estimated a pelagic

larval duration (PLD) of 40–58 d for the Atlantic species *M. jacobus*. Lefèvre and Lecomte-Finiger [63] estimated a PLD of 38–59 d for the Pacific species *Neoniphon sammara*. Despite putative similarity in larval duration, the two species have distinctly different population structures across the Central and West Pacific: Φ_{ST} = 0.151 (p = 0.009) for *N. aurolineatus*, while no structure was detected in *M. chryseres* (Φ_{ST} = 0.000, p = 0.830). In both this study and previous studies, species in the Myripristinae sub-family appear to be more dispersive than members of the Holocentrinae sub-family.

The comparative phylogeography of MCE fishes can address several issues pertinent to these understudied ecosystems. Reefs of the Indo-Pacific are subject to glacio-eustatic sea level changes up to 120 m below contemporary levels, so that shallow reefs may be displaced on a cycle of 10^5–10^6 years [64–66]. Fauvelot et al. [67] were among the first to suggest that low mtDNA diversity in marine fishes might be due to these sea level fluctuations. Ludt et al. [68] hypothesized that lagoon species have shallower genetic histories than fore-reef species for similar reasons, although they were unable to show such differences between *Halichoeres* wrasses. Delrieu-Trottin et al. [69] reported that shallow coalescences dominated the endemic reef fish fauna of Rapa Nui (Easter Island). Ludt and Rocha [27] documented that up to 92% of shallow habitat is lost in some regions of the world during sea level lows. Based on these observations, Pyle and Copus [25] and Copus et al. [26] suggested that populations in MCEs may be older and more stable than those of shallow-water congenerics. If correct, the stability of MCE populations should be apparent in genetic signatures such as coalescence times, as older, more stable populations would have higher levels of genetic diversity [70]. There is also a tendency for deep-water organisms to be highly dispersive, having little to no population structure across ocean basins [20,21,71,72]. Here, we tested both predictions (older and more dispersive populations) against the existing body of population genetic literature on the Holocentridae family.

The only other genetic survey of MCE fishes is Tenggardjaja et al. [73]. They examined the phylogeographic patterns of *Chromis verater* across the Hawaiian Archipelago, a species that occupies upper MCEs, as well as shallow reefs. No genetic structure was observed between deep and shallow populations, or among locations across the archipelago.

4.1. Comparisons to Shallow Holocentrids

Prior to comparing our results with those of the shallow holocentrids, three caveats must be addressed. First, we are comparing conclusions based on COI sequences to previous studies using cytb sequences. We ran our analyses using both the same mutation rate as previous studies, as well as one estimated for COI based on transisthmian geminate species pairs, with no substantial difference in results. Thus, we feel that qualitative comparisons are justified. A second concern is that range sizes vary among the five holocentrids, and this could affect estimates of genetic diversity and coalescence times. Both are expected to increase with range size if that equates to increased population size. We partially correct for this by reporting values for *M. berndti* over both the entire range and the restricted Pacific range we surveyed for the mesophotic holocentrids in this study. Third, we lacked the resources to survey *M. chryseres* and *N. aurolineatus* through the Indian Ocean, so these are not range-wide surveys. However, the comparison of coalescence values for the entire range of *M. berndti* (448,000 years) versus the restricted Pacific range (441,000 years) indicates that conclusions about recent coalescence are robust.

Members of the Holocentridae family comprise about 83 species, most with depth ranges in the upper 100 m [74]. Pelagic larval duration is generally 40–60 days, however PLD can be a poor predictor of dispersal [4,75]. Forecasts of dispersal capability are further confounded because this family may have a post-larval pelagic phase, a unique 'rhynchichthys' juvenile stage that is characterized by extreme cranial supination [34]. This post-larval stage has potential to considerably extend the pelagic duration and dispersal capability of holocentrid fishes. Furthermore, holocentrid larvae are much larger than those of other reef fishes, and size may be a good predictor of swimming ability [76]. Overall, squirrelfishes and soldierfishes seem well equipped for extensive dispersal, but we caution

that the two sub-families, Myripristinae and Holocentrinae, diverged early in the history of the family, at about 55 million years BP [31,77,78]. Certainly, sufficient time has passed to evolve alternative life histories and dispersal strategies, although if the larvae of both shallow and deep-specialist species (within their respective sub-families) behave similarly, they may be found in the same parts of the water column and therefore experience similar dispersal outcomes. Here, we show that the deep soldierfish *M. chryseres* and the deep squirrelfish *N. aurolineatus* have phylogeographic patterns that are fundamentally the same as shallow members of their sub-families.

Myripristis berndti occurs throughout the tropical Indo-Pacific basin from Africa to the Americas [79]. While this species may occur to at least 160 m depth, it is primarily a shallow-water reef fish [80]. Preliminary examination of *M. berndti* otoliths indicates a PLD of about 55 days (B. Victor unpubl. data). A range-wide mtDNA survey revealed population structure at the largest scale, between the Indian Ocean and the West Pacific (Φ_{ST} = 0.583, $p < 0.001$) and between the Central and East Pacific (Φ_{ST} = 0.278, $p < 0.001$) [32]. Each of these ocean basins is separated by substantial but semi-permeable biogeographic barriers [54,81].

Myripristis jacobus inhabits all major biogeographic provinces of the tropical Atlantic, including the Caribbean, Brazil, mid-Atlantic ridge, and the Gulf of Guinea. The PLD for this species is estimated at 40–58 days [34]. This species may occur to depths of 210 m, but is primarily found at 2–35 m [82]. A range-wide survey of mtDNA cytb revealed no detectable population structure across the tropical Atlantic (Φ_{ST} = 0.008, p = 0.228) [33].

Holocentrus adscensionis inhabits a tropical Atlantic range similar to *M. jacobus*, with a reported PLD of 43–56 days, approximately the same as the other squirrelfish [34]. However, members of the *Holocentrus* genus have an additional "meeki" stage after the rhynchichthys stage, extending the pelagic duration to about 71 days, the longest of the holocentrids examined here. It is reported to depths of 180 m, but primarily occurs in the shallow range of 8–30 m [83]. Despite a pelagic duration about 22% longer than the co-distributed *M. jacobus*, this species had significant population partitions between the eastern, central (mid-Atlantic ridge), and western Atlantic (Φ_{ST} = 0.091, $p < 0.001$) based on cytb sequences [33].

How do these five species compare in mtDNA diversity and coalescence times (Table 3)?

Table 3. Overview of phylogeographic comparisons among holocentrid species (h = haplotype diversity, π = nucleotide diversity).

Species	Habitat	Φ_{ST}	h	π	Age (Ma)	Locus	Ref.
M. chryseres	deep	−0.031	0.712	0.0024	0.272	COI	-
N. aurolineatus	deep	0.148 [a]	0.793	0.0028	0.284	COI	-
M. berndti	shallow	−0.007 [b]	0.632 [c]	0.003 [c]	0.448	cytb	[32]
M. jacobus	shallow	0.008	0.781	0.003	0.220	cytb	[33]
H. adscensionis	shallow	0.091a	0.974	0.006	0.917	cytb	[33]

[a] Statistically significant. [b] Value for Central-West Pacific range only. [c] Values for the entire range including the East Pacific.

- *M. berndti* in the Indo-Pacific has haplotype diversity h = 0.632, nucleotide diversity π = 0.003, a range-wide coalescence at ~448,000 years (95% HPD: 154,000–807,000 years), and a restricted Pacific coalescence of ~441,000 years (95% HPD: 144,000–799,000 years), based on cytb sequences [32].
- *M. jacobus* in the Atlantic has h = 0.781, π = 0.003, and a coalescence at ~220,000 years (95% HPD: 94,000–391,000 years), based on cytb sequences [33].
- *M. chryseres* in the mesophotic Pacific has h = 0.712, π = 0.0024, and a coalescence at 272,000 years (95% HPD: 87,000–537,000 years), based on COI sequences (present study)

- *H. adscensionis* in the Atlantic has $h = 0.974$, $\pi = 0.006$, and a coalescence at ~917,000 years (95% HPD: 521,000–1.3 M years), based on cytb sequences [33].
- *N. aurolineatus* in the mesophotic Pacific has $h = 0.793$, $\pi = 0.0028$, and a coalescence at 284,000 years (95% HPD: 77,000–546,000 years), based on COI sequences (present study).

In four out of five cases, the mtDNA haplotype networks included one or two abundant haplotypes that occur at most or all locations, centrally located in a network of low-frequency haplotypes that differ by one or two mutations from the common haplotype (the exception is *H. adscensionis*, see below). The commonality of this outcome across deep and shallow lineages contradicts predictions of older, stabler habitats in the mesophotic realm.

The squirrelfish *H. adscensionis* is the only species in our study to have a more complex mtDNA network, with more than two high-frequency haplotypes (Figure 5). While explanations could include differences in mutation rate or effective population size (N_e), we note that this fish had the highest population structure observed in the Atlantic. Less dispersive fishes usually have more complex networks, a trend that reaches an apex in freshwater fishes, e.g., [84,85]. Coalescence analysis also recovered an older origin for this species in comparison to the other four (~917,000 vs. 219,000–434,000 years), which could lead to greater haplotype diversity over time. Thus, we propose that despite longer PLD, lower dispersal and greater age may be at least part of the explanation for the more complex network in *H. adscensionis*.

Across the other shallow species we compared, population structure is low, and, in one case (*M. jacobus* in the tropical Atlantic), non-existent across their surveyed ranges. *Myripristis berndti* has population structure on the scale of the Indian versus the Pacific Ocean, but not on the scale of the Central-West Pacific (as for the mesophotic *M. chryseres* of this study). We provisionally conclude that mesophotic fishes are neither more nor less dispersive than shallow-water species, a finding that requires additional studies to verify or reject.

4.2. Age and Stability of Mesophotic Populations

The mesophotic squirrelfish and soldierfish we surveyed have population characteristics that are similar to their shallow-water counterparts. Copus et al. [26] formally proposed the *habitat persistence hypothesis*, which posits that reef communities below the depths affected by glacio-eustatic sea level fluctuations, and perhaps beyond the reach of storm surges, may have older, more stable populations. Our data do not support the prediction of older and more stable populations on mesophotic reefs. To the contrary, coalescence times in the MCE fishes are shorter than or comparable to their shallow-water counterparts. Part of the explanation may be that mesophotic reefs are still within the grasp of shallow-water perturbations. Rocha et al. [24] observed evidence of storm disturbance down to 135 m on mesophotic reefs in the Bahamas (see also [86]). However, we discount the 'not deep enough' explanation for shallow coalescence, because the same shallow coalescence was observed in four snappers (Lutjanidae family) that dwell below the mesophotic zone > 200 m; [20,21]. Therefore, recent coalescence seems to be a nearly ubiquitous feature of marine fishes. Previous explanations focused on the possibility of unstable and bottlenecked populations through time [28]. However, shallow coalescence and unstable N_e through time can result from several different processes that may not be easily distinguishable (or mutually exclusive) using single locus markers (e.g., mtDNA) over evolutionary time. Thus, the finding that shallow and MCE squirrelfish species have similar population histories prompts us to search for alternate explanations.

Strong selection can cause a decrease in genetic diversity that may erase the signature of previous demographic stability, but this process operates at the population genetic level [87] and is therefore unlikely to be the primary cause of shallow coalescence across diverse groups of marine fishes and invertebrates [88] on its own. On the other hand, reproductive strategies that are shared across diverse taxa tend to influence levels of N_e

and genetic diversity in similar ways. The r-selected strategy of most marine organisms (fishes included), wherein hundreds or thousands of eggs and larvae are produced, but very few survive to reproductive maturity, yields a small N_e relative to the actual number of spawning adults. N_e is based on the harmonic means of reproducing individuals [89], and is strongly affected by occasional 'drought' periods of low reproductive success [90,91]. The r-selected strategy, combined with high variance in reproductive success, in which a few individuals produce most of the next generation [92], could explain the shallow mtDNA coalescence in marine fishes, without having to invoke demographic changes (bottlenecks) and selective forces caused by glacial sea level changes.

5. Conclusions

Based on one of the first phylogeographic surveys of fish species in MCE habitats, these communities have population histories that are concordant with shallow-water species in the same taxonomic family. Results do not support the habitat persistence hypothesis for MCEs. However, this finding must be regarded as preliminary until more species and more loci are surveyed. Every holocentrid species surveyed to date shows extensive dispersal capacity (with population structure being relevant at the scale of ocean basins) and coalescence in recent evolutionary history. The data from fishes in coastal habitats [28], pelagic denizens [10], mesophotic fishes (current study), and submesophotic snappers [20,21] all indicate that shallow population histories are a ubiquitous feature of marine fishes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14080691/s1. Table S1. Coalescence estimates for all species examined in this study, including *Myripristis berndti*, *M. jacobus*, *Neoniphon adscensionis* (shallow reefs), and *M. chryseres* and *N. aurolineatus* (deep reefs). For specimens where COI was sequenced, results are presented for both 1.6% and 2.0% mutation rates. Figure S1. Baysian skyline plots for five species of holocentrid fishes, including *Myripristis berndti*, *M. jacobus*, *Neoniphon adscensionis* (shallow reefs), and *M. chryseres* and *N. aurolineatus* (deep reefs).

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Data Availability Statement: Sequence data for the mesophotic species, Yellowfin Soldierfish *Myripristis chryseres* and the Yellowstriped Squirrelfish *Neoniphon aurolineatus*, are available in GenBank under accession numbers ON998515–ON998673. GenBank accession numbers for previously published sequence data are available in the original papers.

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