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Unraveling cryptic morphological diversity in a marine snail species complex using nuclear genomic data

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Abstract: *Conus lividus* (Hwass in Bruguière, 1792) and *Conus sanguinolentus* (Quoy and Gaimard, 1834) are closely related Indo-West Pacific cone snails that have largely overlapping distributions. Previous population genetic analyses of these species found that some individuals that were identified as *C. lividus* possessed mitochondrial gene sequences that were similar or in some cases identical to those of *C. sanguinolentus*. While these species tend to be easily distinguished based on shell color patterns, it is possible that some individuals of *C. sanguinolentus* were misidentified as *C. lividus*. The result though could also be due to introgression of the mitochondrial genome of *C. sanguinolentus* into *C. lividus*. We used a ddRAD approach to obtain sequences of short fragments of more than 7,000 nuclear genomic loci to examine patterns of variation and evaluate these explanations. Results showed that the two parental species are genetically differentiated at nuclear loci and all putative hybrids were unambiguously assigned to *C. sanguinolentus* based on shared patterns of variation. These results demonstrate that variation in shell color patterns of *C. sanguinolentus* overlaps with that of *C. lividus*, and extend the distribution of *C. sanguinolentus* into the Hawaiian Archipelago. Additional analyses of patterns of genetic variation among populations of the two species revealed that while *C. lividus* shows no genetic population structure, the population of *C. sanguinolentus* from Hawaii is genetically differentiated from populations elsewhere as found in prior analyses based on mitochondrial sequence data.

Key words: Conidae, shell variation, population structure

The family Conidae (i.e., ‘cone snails’) is a species rich group of predatory marine gastropods that is largely distributed throughout tropical and subtropical regions of the world’s oceans. Although many cone snail species are well differentiated in shell color pattern, some species are not easily distinguished from each other and several cases of cryptic species are known (Duda *et al.* 2008, 2009, Lawler and Duda 2017). *Conus lividus* (Hwass in Bruguière, 1792) and *Conus sanguinolentus* (Quoy and Gaimard, 1834) are closely related Conidae species that are largely sympatric throughout much of their ranges in the Indo-West Pacific, although *C. sanguinolentus* is apparently absent in the central Indian Ocean and Hawaiian Archipelago (Duda and Kohn 2005, Röckel *et al.* 1995) (Fig. 1). The two species occupy similar microhabitats (Röckel *et al.* 1995), but show differences in feeding ecology (Kohn 1959, 1960, 1968, 1981, Kohn and Nybakken 1975, Marsh 1971, Reichelt and Kohn 1985) and are also generally distinguishable by their shell color patterns. In particular, *C. lividus* exhibits a prominent light-colored band around the shoulder area and center of the last whorl of the shell whereas *C. sanguinolentus* does not (Fig. 2). Previous population genetic investigation of these species based on analyses of an approximately 650 base pair region of the cytochrome oxidase c subunit I (COI) gene revealed a conundrum: while most individuals of the two species exhibited fixed differences at 57 or more positions of this gene region, some of the individuals that were identified as *C. lividus* (based on shell color patterns) possessed haplotypes that were more similar or, in some cases, identical to sequences from individuals that were identified as *C. sanguinolentus* (Duda *et al.* 2012).

Several factors might contribute to the pattern observed, including ambiguity with regards to taxonomic identity (*i.e.*, ‘imperfect taxonomy’), interspecific hybridization, incomplete lineage sorting, and presence of NUMTs (*i.e.*, nuclear mitochondrial DNA sequences or mitochondrial sequences that have been incorporated into the nuclear genome; Funk and Omland 2003). Given the levels of divergence and patterns of segregation of COI sequences, incomplete sorting of ancestral polymorphism and the presence of NUMTs seem inadequate explanations for these observations. It is possible though that morphological variation of *C. sanguinolentus* overlaps with that of *C. lividus* and some individuals of *C. sanguinolentus* may not be easily distinguished from individuals of *C. lividus*. Another explanation is that mitochondrial genomes of *C. sanguinolentus* introgressed into *C. lividus* via past or recent hybridization. If introgression is responsible for the morphological and mitochondrial discordance, individuals with the morphology of *C. lividus* and mitochondrial gene sequences of *C. sanguinolentus* represent hybrid or backcrossed individuals (hereafter referred to as ‘putative hybrids’). Nuclear gene regions of these individuals may show evidence of mixed ancestry or, given the many examples of cases in which mitochondrial genomes have introgressed with little to no introgression of nuclear gene loci (Ballard and Whitlock 2004, Good *et al.* 2015, Toews and Brelsford 2012), they may be only derived from *C. lividus*.

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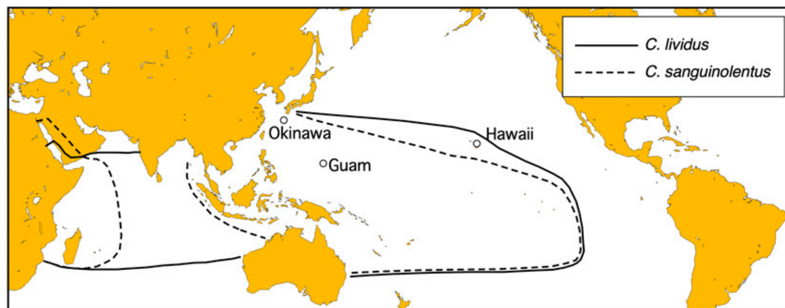

Figure 1. Map showing previously recognized ranges of *C. lividus* and *C. sanguinolentus* in the Indo-West Pacific. As described in the text, *C. sanguinolentus* is present in the Hawaiian Archipelago. Locations from which specimens used in the study were collected are also indicated.

Because individuals with the morphology of *C. sanguinolentus* and mitochondrial gene sequences of *C. lividus* have not yet been detected, mitochondrial genome introgression, if it has occurred, may be unidirectional (*i.e.*, predominantly occurring from *C. sanguinolentus* to *C. lividus*). Otherwise, if *C. sanguinolentus* are in some cases misidentified as *C. lividus*, nuclear gene regions of putative hybrids will share identity with those of *C. sanguinolentus* individuals.

To evaluate these possible explanations, we obtained nuclear genomic data from putative hybrid individuals and representatives of the two parental species using the double digest restriction site associated DNA (ddRAD) sequencing method (Peterson *et al.* 2012). We utilized this approach instead of sequencing small regions of a few nuclear genes to increase our power of detecting alleles of nuclear loci that may have introgressed among the two species. Although sequencing ddRAD libraries requires use of next generation sequencing approaches, which can be expensive, the costs of directly sequencing multiple gene regions from multiple individuals using traditional sequencing approaches is comparable to the cost of next generation sequencing, especially when multiple libraries are combined in a single sequencing run (see Peterson *et al.* 2012).

We sought to identify ddRAD sequences that are unique to each parental species and then evaluate whether or not putative hybrid individuals show evidence of mixed ancestry (*i.e.*, contain sequences from both parental species) or possess sequences from only one of the two parental species. We included putative hybrid individuals from different parts of their ranges in the Indo-West Pacific (Okinawa, Guam, and Hawaii) to gauge the potential extent of hybridization where parental species are apparently sympatric (Okinawa and Guam) and allopatric (Hawaii) if hybridization is confirmed or to contrast patterns of genetic variation determined from nuclear DNA to those previously determined from mitochondrial data (Duda *et al.* 2012) if it is not.

MATERIALS AND METHODS

Specimens

We utilized genomic DNA that had already been extracted from 18 specimens of *C. lividus*, *C. sanguinolentus*, and putative hybrid individuals from collections of the Museum of Comparative Zoology (MCZ 318157) and the University of Michigan Museum of Zoology (UMMZ 301862, 305870, 305875, 305876, 305878, 305908, 305909, and 305910). These included seven individuals of *C. lividus* from Okinawa (*N*=2), Guam (*N*=2), and Hawaii (*N*=3); six putative hybrid individuals from Okinawa (*N*=1), Guam (*N*=2), and Hawaii (*N*=3); and five individuals of *C. sanguinolentus* from Okinawa (*N*=2) and Guam (*N*=3). The DNA extractions were previously performed for phylogeographic analyses of these species (Duda *et al.* 2012) and stored at -80°C since then. The specimens were originally identified as *C. lividus* or *C. sanguinolentus* based on shell color patterns (*i.e.*, the presence (*C. lividus*) or absence (*C. sanguinolentus*) of a prominent light colored band around the center of the last whorl of the shell); putative hybrid individuals resembled *C. lividus* (*i.e.*, exhibited a band) but possessed COI gene sequences that were most similar or identical to sequences of this gene region from individuals identified as *C. sanguinolentus* (Duda *et al.* 2012).

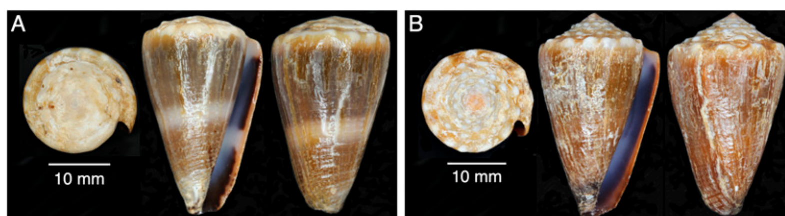

Figure 2. Comparison of shell color patterns of *C. lividus* and *C. sanguinolentus*. A. Specimen of *C. lividus* (MCZ 318156) from Guam that illustrates the well-defined white transverse bands at the center and shoulder area of shell and the entirely white spire. B. Specimen of *C. sanguinolentus* (UMMZ 305886) from Guam that illustrates the characteristic absence of white bands around the shoulder area and center of shell as well as yellowish-brown color between coronations on the spire and shoulder.

Preparation and sequencing of the ddRAD library

We measured concentrations of the DNA extractions with a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA) and utilized

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approximately 200 ng of DNA for ddRAD library preparation following established protocols (Peterson *et al.* 2012). We digested genomic DNA samples with restriction enzymes EcoRI-HF and MseI (New England Biolabs, Ipswich, MA) at recommended conditions. We targeted 294-394 basepair (bp) fragments from the digested DNA with a Pippen Prep device (Sage Science, Beverly, MA) following recommended protocols. We then submitted size-selected libraries to the University of Michigan DNA Sequencing Core facilities (now known as the Advanced Genomics Core). The samples were indexed and run together on a single lane with other samples on the Illumina HiSeq 4000 platform with 150 bp paired-end sequencing.

Processing of ddRAD data

We utilized Fastqc v.0.11.5 (Andrews 2010) to evaluate the quality of the multiplexed sequences. We used ipyrad v.0.7.29 (Eaton 2014, Eaton and Overcast 2018) to process, identify and align putative orthologous sequences. In particular, we sorted sequences by barcode to demultiplex them and then trimmed barcodes, adapters and remaining restriction site regions from the sequences. These analyses did not permit any barcode mis-matches and allowed for only five low quality bases; we treated low quality base reads as ambiguous characters, defined as those with Phred-scores of less than 20, and replaced these with Ns. We aligned sequences for each individual using a similarity threshold of 85% and removed those with a depth of coverage of less than six. We then clustered and aligned sequences from all individuals at 85% similarity. For the results presented herein, we examined datasets that included putative loci that occurred in at least 13 of the 18 individuals examined (*i.e.*, present in more than 70% of individuals). We also evaluated datasets with loci that were represented in all individuals and as few as five individuals, but because results utilizing datasets derived from different cut-offs were similar, we only report results from analyses of the 70% cut-off dataset.

Analyses of ddRAD data

We utilized MEGA v.7.0.26 (Kumar *et al.* 2016) to build a phylogeny based on analyses of Kimura 2-parameter distances with neighbor-joining methods. We performed 1,000 bootstrap replicates to estimate support for branches and midpoint rooted the tree with MEGA. We also utilized MEGA to calculate average Kimura 2-parameter distances among and within the major clades of the tree. These distances were calculated while deleting missing data for only the affected pairwise comparisons that included them (as opposed to deleting them for all pairwise comparisons).

We utilized STRUCTURE v.2.3.4 (Hubisz *et al.* 2009, Pritchard *et al.* 2000) to test for introgression and evaluate whether or not putative hybrid individuals (*i.e.*, those that contained mitochondrial gene sequences of *C. sanguinolentus* but morphologically resemble *C. lividus*) show evidence of mixed ancestry as well as to examine patterns of genetic population structure. We used PGDspider v2.1.1.5 (Lischer and Excoffier 2012) to produce a datafile that included only one SNP from each polymorphic locus. We then used STRUCTURE to perform iterative analyses of the dataset 10 times per *K* with default parameters for 500,000 generations after 150,000 generations of burn-in. The *K* parameter ranged from one to nine; the maximum value used reflects the total number of distinct sets of individuals (*i.e.*, populations)—*C. lividus* from Hawaii, Guam, and Okinawa; *C. sanguinolentus* from Guam and Okinawa; and putative hybrids from Hawaii, Guam, and Okinawa—plus one. We utilized STRUCTURE HARVESTER (Earl and vonHoldt 2012) to assess stationarity and evaluate the optimal *K* using the ΔK method (Evanno *et al.* 2005). We also used Clumpak v1.1 (Kopelman *et al.* 2015) to assess posterior probabilities and visualize the results of the STRUCTURE analyses.

To evaluate patterns of population genetic structure of *C. lividus* and *C. sanguinolentus* from Hawaii, Guam, and Okinawa, we used ipyrad to filter the dataset to retain individuals from each species. We analyzed these datasets with STRUCTURE as described above, except K-values ranged from one to four (*i.e.*, the total number of populations plus one). We also utilized Arlequin v3.5.2.2 (Excoffier and Lischer 2010) to estimate Φ_{ST} values among population pairs of each species using Kimura 2-parameter distances; these estimates were based on analyses of concatenated sequences of variable sites only due to limitations on sequence input length in Arlequin. We also used Arlequin to determine if estimated values of Φ_{ST} deviated significantly from the null hypothesis of no differences between populations based on the proportion of 10,100 resampled data that produced Φ_{ST} values greater to or equal the observed Φ_{ST} values. Nonetheless, P-values are not very informative for inferring the strength of population differentiation when sample sizes are low as is the case with some of the comparisons.

RESULTS

ddRAD data

We recovered 660,805 to 8,268,728 raw sequence reads from each individual (mean=3,027,915). The average depth of coverage was 22.6 and ranged between 12.2 and 41.1. Filtering and clustering procedures ultimately recognized 7,636 putative orthologous loci that occurred in at least 13 of 18 individuals. Sequence data was accessioned into NCBI's Sequence Reads Archive (BioProject PRJNA591397: accession numbers SRR10522747-SRR10522762).

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Analyses of ddRAD data

We examined concatenated sequences in MEGA to reconstruct a phylogeny and evaluate levels of divergence among major clades in the tree. The entire concatenated dataset included 2,061,988 nucleotides that contained 90,090 variable sites. Given that we did not have sequences from an outgroup species, we midpoint rooted the tree. Individuals of *C. lividus* and *C. sanguinolentus* occur in one of two well separated main clades and all putative hybrids cluster with individuals of *C. sanguinolentus* with high bootstrap support (Fig. 3). Within the main clade containing putative hybrid and *C. sanguinolentus* individuals, putative hybrids from Guam and Okinawa cluster indiscriminately with high support with individuals identified as *C. sanguinolentus*, while putative hybrids from Hawaii are paraphyletic with respect to these individuals (Fig. 3). The average genetic distance among the two main clades based on Kimura 2-parameter distances is 0.017 (range: 0.0157-0.0174). The mean distances within each main clade are 0.004 for the group including specimens of *C. lividus* (range: 0.0033-0.0046) and 0.006 for the group including specimens of *C. sanguinolentus* plus putative hybrid individuals (range: 0.0054-0.0064).

Based on examination of ΔK values determined from STRUCTURE analyses of data from all 18 individuals, the optimal K is two (i.e., two genetic groups: ΔK =3538.9; next highest ΔK-value was 199.8 for K=3). The two groups defined at K=2 are clearly separated by species, with all putative hybrid individuals assigned unambiguously to *C. sanguinolentus* (Fig. 4A).

We further analyzed subsets of data from individuals of each of these groups to determine if they exhibit genetic population structure: one containing seven individuals of *C. lividus* from Okinawa (N=2), Guam (N=2), and Hawaii (N=3) and one containing a total of 11 putative hybrid and *C. sanguinolentus* individuals from Okinawa (N=3), Guam (N=5), and Hawaii (N=3). Analyses of the *C. lividus* data with STRUCTURE did not reveal any evidence of structure, with low ΔK values recovered for K=2 and K=3 (1.2 and 3.1, respectively) and no clear separation of individuals based on geographic sampling locality. Estimates of pairwise Φ_{ST} values with Arlequin range between -0.019 and 0.054 (P-values were all greater than 0.1) and hence not strongly indicative of any genetic differentiation among these populations. STRUCTURE analyses of the data for *C. sanguinolentus*, on the other hand, revealed strong evidence of population structure, with clear support for separation and unambiguous assignment of individuals into two geographically defined groups: one for individuals from Hawaii and the other for individuals from Guam and Okinawa (Fig. 4B). The ΔK for K=2 was 9.3 while the next highest ΔK was 1.1 for K=3. Estimates of pairwise Φ_{ST} values reveal the same pattern with a low value of 0.005 estimated for the comparison involving populations from Okinawa and Guam (P-value=0.407) and larger values among comparisons involving population pairs Okinawa-Hawaii and Guam-Hawaii (0.204 and 0.191; with P-values of 0.101 and 0.017, respectively).

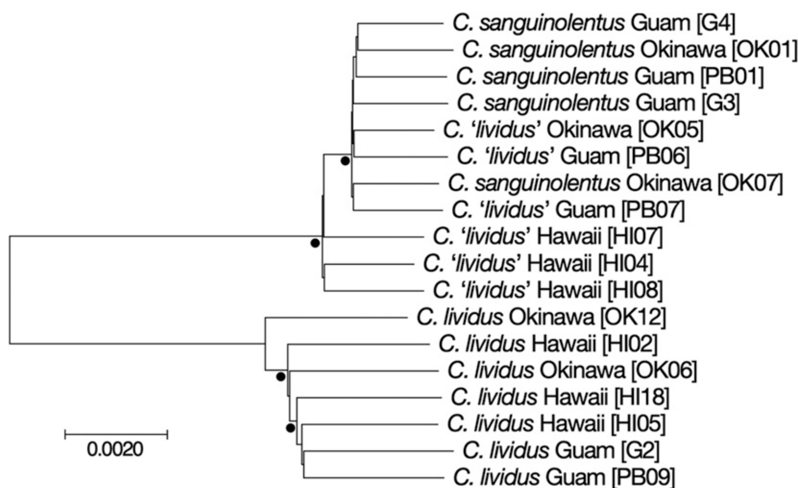

Figure 3. Midpoint-rooted neighbor-joining tree constructed from analyses of concatenated sequences of 7,636 ddRAD loci. 100% support for branches as estimated from 500 bootstrap replicates indicated with filled circles. Putative hybrids (*i.e.*, individuals identified as *C. lividus* but that possessed mitochondrial COI sequences that were more similar to those from *C. sanguinolentus*) are indicated with specific epithet of *C. lividus* in single quotation marks (*i.e.*, *C.* '*lividus*'). Specimen codes in brackets.

DISCUSSION

Analyses of 7,636 ddRAD loci reveal that *C. lividus* and *C. sanguinolentus* are differentiated at nuclear loci and that some individuals that were identified as *C. lividus* are indistinguishable genetically from *C. sanguinolentus* at mitochondrial *and* nuclear gene regions. Furthermore, patterns of genetic differentiation of populations of *C. sanguinolentus* at nuclear loci corroborate those detected previously from mitochondrial COI sequences. On the other hand, population genetic analyses of data from *C. lividus* revealed no evidence of structure. Implications of these results are discussed below.

Differentiation at nuclear loci

As revealed in prior analyses of mitochondrial COI sequences of *C. lividus* and *C. sanguinolentus* (Duda *et al.* 2012), individuals of each species occur in one of two reciprocally monophyletic

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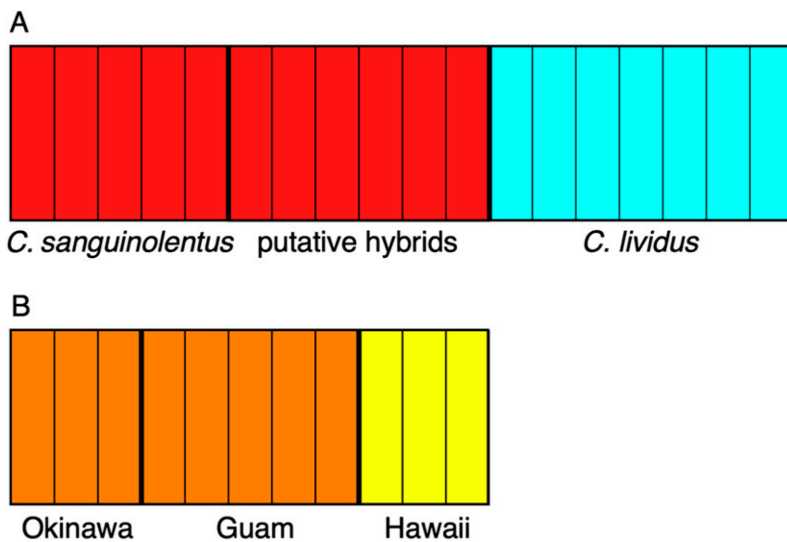

Figure 4. Barplots illustrating results of population assignment of individuals based on Bayesian clustering analysis. A. Assignment of individuals of *C. sanguinolentus*, *C. lividus*, and putative hybrid individuals with *K*=2. B. Assignment of putative hybrids and *C. sanguinolentus* individuals from Okinawa, Guam, and Hawaii with *K*=2.

groups in phylogenetic reconstructions of the concatenated ddRAD dataset (Fig. 3). Hence, both mitochondrial and nuclear sequences provide clear genealogical support for recognition of *C. lividus* and *C. sanguinolentus* as separate species.

Individuals of *C. lividus* and *C. sanguinolentus* exhibit an overall average genetic distance of 1.7% at the 7,636 nuclear loci that were analyzed. This level of differentiation is clearly much less than that observed for COI sequences which exhibited genetic distances of more than 8% (Duda *et al.* 2012). Nonetheless, rates of nucleotide substitution of nuclear DNA are often less than those of mitochondrial gene sequences and so this result is not unexpected (Brown *et al*. 1979, Moritz *et al*. 1987). For example, while some cephalaspidean sea slug species differ at COI sequences at as little as 8% of nucleotide sites for some species pairs and as many as 22% for other species pairs, levels of divergence at a nuclear gene, histone H3, range from 0% to nearly 5% (Ornelas-Gatdula *et al.* 2011). In addition, examination of patterns of variation in mitochondrial and nuclear mutation rates of animals based on levels of divergence at neutral sites reports a fourfold difference in these rates in most molluscs (Allio *et al.* 2017). Thus, the difference in levels of divergence of mitochondrial and nuclear sequences of *C. lividus* and *C. sanguinolentus* is not without precedent.

STRUCTURE analyses assign all putative hybrid individuals unambiguously with *C. sanguinolentus* (Fig. 4A). Hence, given these results and the corroborating evidence from mitochondrial COI sequences (Duda *et al*. 2012), these individuals represent specimens of *C. sanguinolentus*. Hereafter we refer to these putative hybrids as *C. sanguinolentus*.

Morphological boundaries

Conus lividus and *C. sanguinolentus* differ in aspects of shell color patterns in that pale whitish transverse bands that occur around the shoulder and center of shells of *C. lividus* are absent in *C. sanguinolentus* (Cernohorsky 1964) (Fig. 2). The presence of these bands also affects color patterns on the interior of the shell: while the aperture of *C. sanguinolentus* is purple in its entirety, the color of the aperture of *C. lividus* is purple that is interrupted by pale bands at the shoulder and center (Cernohorsky 1964) (Fig. 2). Nonetheless, the strength and abruptness of edges of these bands tend to vary among individuals with some individuals having more robust bands than others (Figs. 2B, 5). We examined shells of specimens used in this and previous studies (Duda *et al.* 2012) and observed that individuals of *C. sanguinolentus* that were shown to have been misidentified (as *C. lividus*) have bands that are fainter and less well-defined in outline than individuals of *C. lividus* (Fig. 5). Although several taxonomic treatments have noted that the characteristic bands of *C. lividus* may be faint in individuals of *C. sanguinolentus* (Cernohorsky 1964, Röckel *et al.* 1995, Walls 1979), it is not readily apparent from these works how faint the bands need to be for the individual to be considered *C. sanguinolentus*. Moreover, given that *C. sanguinolentus* was reported to be absent from the Hawaiian Archipelago, the variation in shell color exhibited by the specimens that were determined to represent *C. lividus* in Hawaii was assumed (by us) to reflect the variation in shell color pattern that is exhibited by this species at other locations. This is clearly not the case as our results show that *C. sanguinolentus* occurs in Hawaii. Based on examination of shells of individuals that were misidentified as *C. lividus*, we infer that individuals with well-defined bands are *C. lividus,* while those with faint, less well-defined bands (or no bands at all) are *C. sanguinolentus* (see Fig. 5), although more work is needed to support the notion that individuals with faint bands are *C. sanguinolentus*.

Another morphological character that appears to differentiate *C. lividus* from *C. sanguinolentus* well is spire color (Cernohorsky 1964). While the spire and coronations on the shoulder of *C. lividus* are white, the interspaces between the white colored coronations on the shoulder and spire are a yellowish-brown color (Fig. 2). Based on examination of shells of specimens of these two species, this character appears to be reliable in differentiating specimens of *C. sanguinolentus* from those of *C. lividus* (Fig. 5).

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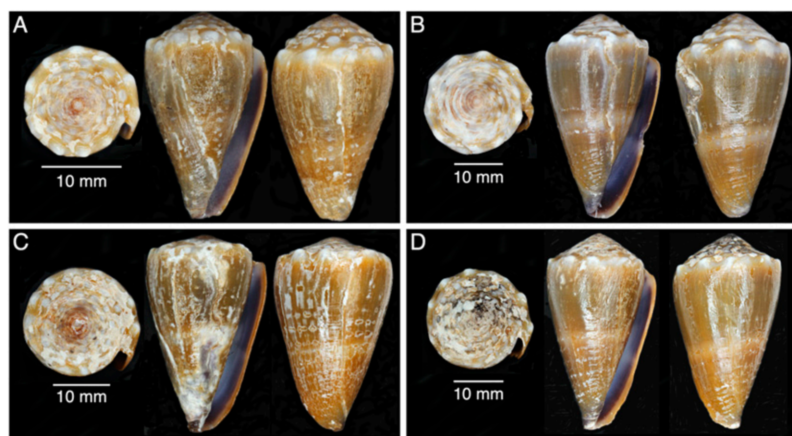

Figure 5. A-D. Shells of specimens of *C. sanguinolentus* from Hawaii (MCZ 318158) that were assumed to represent *C. lividus* based on the reported absence of *C. sanguinolentus* at this location and the presence of pale transverse bands at the centers of shells.

Distribution of *C. sanguinolentus*

Conus lividus occurs throughout most shallow water locations in tropical parts of the Indo-West Pacific, from approximately the Red Sea to the Hawaiian Islands and Tuamotu Archipelago in French Polynesia (Fig. 1). The distribution of *C. sanguinolentus* has heretofore been considered to be more restricted as this species was thought not to occur in the central Indian Ocean and in the Hawaiian Archipelago (Röckel *et al.* 1995) (Fig. 1). Our results show that *C. sanguinolentus does* in fact occur in Hawaii and, as correctly surmised by Walls (1979), can be confused with *C. lividus* at this location. In her treatment of marine molluscs of Hawaii, Alison Kay (1979) synonymized *C. sanguinolentus* with *C. lividus*, although it is not clear if she made this decision based on her observations of shell color phenotypes of *C. lividus* in Hawaii or for some other reason.

We have not detected nor are we aware of any reports of individuals of *C. sanguinolentus* in the Hawaiian Archipelago with shells that are completely lacking of bands as is typical of shells of some individuals of this species elsewhere (Fig. 2B). The absence of individuals with this color pattern may result from the genetic differentiation of the population at Hawaii (see below) or because of unique biotic or abiotic characteristics at Hawaii that preclude development of this particular phenotype. In addition, because we did not include specimens from the central Indian Ocean in our study, we do not know if *C. sanguinolentus* is also confused for *C. lividus* here as well, but suspect that this is the case.

Population differentiation of *C. sanguinolentus* in the Indo-West Pacific

During previous phylogeographic analyses of *C. lividus* and *C. sanguinolentus* based on analyses of mtDNA COI sequences, Duda *et al.* (2012) assumed that individuals that resembled *C. lividus* but possessed sequences that were more similar or identical to those from *C. sanguinolentus* were misidentified and actually represented *C. sanguinolentus*. Hence, they combined sequences from these individuals with sequences from *C. sanguinolentus* in population genetic analyses. Their results showed that the population at Hawaii is genetically differentiated from populations at Guam and Okinawa (Duda *et al.* 2012). Indeed, most individuals at Hawaii possessed a haplotype that was relatively rare at other locations (*i.e.*, only detected in a few individuals from Okinawa) and one substitutional step away from two haplotypes that were common elsewhere (Fig. 2C from Duda *et al.* 2012). Otherwise, populations elsewhere exhibit no evidence of population structure (Duda *et al.* 2012). Although the larval development of *C. sanguinolentus* has not previously been investigated, given the lack of structure in parts of it range, we suspect that it exhibits indirect development with a feeding, free-living veliger larval stage that lasts up to several weeks, as do many other cone snail species (Kohn and Perron 1994), including *C. lividus* (see below).

Individuals of *C. sanguinolentus* from Guam and Okinawa cluster together separately from those from Hawaii in phylogenetic reconstruction based on analyses of concatenated ddRAD loci (Fig. 2). STRUCTURE analyses also support the lack of admixture among populations of *C. sanguinolentus* from Hawaii and Guam and Okinawa (Fig. 4B) as do estimates of Φ_{ST} values (see Results). The Φ_{ST} values calculated from the ddRAD data (i.e., 0.005 for Okinawa-Guam, 0.205 for Okinawa-Hawaii, and 0.191 for Guam-Hawaii population pairs) are similar to those reported previously for COI sequences (i.e., -0.011, 0.322, and 0.235, respectively; Duda *et al*. 2012). As previously discussed by Duda and coauthors (2012), *C. sanguinolentus* is the only Conidae species that is known to exhibit a strong signal of a genetic 'break' between populations at Hawaii and populations elsewhere, although other cone snail species are known or appear to be endemic to the Hawaiian Archipelago, including *Conus abbreviatus* (Reeve, 1843); *Conus leviteni* (Tucker, Tenorio and Chaney, 2011); *Conus paukstisi* (Tucker, Tenorio and Chaney, 2011); and *Conus peasei* (Brazier, 1877) (Lawler and Duda 2017, Tucker *et al.* 2011). Given that the Hawaiian Archipelago is rather isolated from other landmasses and islands in the Pacific and a number of other marine species are endemic to this region (Bird *et al*. 2007, 2011, Bowen *et al.* 2013, Kay and Palumbi 1987), the genetic isolation of the population of *C. sanguinolentus* at Hawaii is not surprising, but this pattern is not shared by other cone snails, including *Conus ebraeus*

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(Linnaeus, 1758) and *Conus chaldaeus* (Röding, 1798) (Duda *et al*. 2012) as well as *C. lividus* given its apparent lack of population structure (see below). Genetic isolation of the population of *C. sanguinolentus* at Hawaii might explain the absence of individuals that exhibit the customary phenotype of this species here that completely lacks a light-colored band at the center of the shell (*i.e.*, Fig. 5D).

We did not detect any significant population genetic structure for *C. lividus* and, unlike *C. sanguinolentus*, the population of *C. lividus* at Hawaii is not genetically differentiated from populations elsewhere. Because sample sizes of mitochondrial sequence data from this species were small, Duda *et al.* (2012) did not use these data to analyze the genetic structure of this species and so we cannot directly compare patterns of variation exhibited by this species at nuclear and mitochondrial loci. Nonetheless, our results complement and reiterate studies that have shown that the genetic structure and location of genetic breaks among populations of widespread cone snail species in the Indo-West Pacific often differ, even among species with similar traits like dispersal potential (see treatment of this theme in Duda *et al.* 2012). While *C. lividus* is known to exhibit indirect development with a minimum larval period of four weeks (Kohn and Perron 1994), the life history of *C. sanguinolentus* has not been described. Nonetheless, because it is possible that past studies of the larval biology of *C. lividus* may have inadvertently included specimens of *C. sanguinolentus* in their analyses, we suspect that *C. sanguinolentus* has a similar life history as *C. lividus.* Hence, differences in the population genetic structure of these species may not reflect differences in dispersal abilities, but instead are due to differences in other species level traits or reflect the effects of stochasticity on realized dispersal.

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