Genetic Drivers of Diversification in the *Conus* Adaptive Radiation

by

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ABSTRACT

Adaptive radiations offer striking examples of biodiversity. Their rapid diversification is facilitated by adaptive traits, which allow co-occurring species to partition niche space. My research is motivated by a desire to understand how these adaptive phenotypes evolve and diversify to produce evolutionary novelty. Specifically, I focus my efforts on characterizing the genetic basis of adaptive traits and detecting patterns of molecular evolution. Predatory marine gastropods in the family Conidae – "cone snails" – are a particularly compelling radiation in which to explore these themes. Since their emergence in the fossil record some 55 million years ago, they have rapidly diversified to ~800 extant species, most of which belong to the genus *Conus*. Cone snails often occur in sympatry with many conspecifics, including close relatives, frequently occupying the same microhabitats. These attributes point to several factors that may contribute to the exceptional rate of diversification of *Conus* which I explore in my dissertation research.

First, hybridization among recently diverged species is thought to contribute to ecological diversification by assembling novel combinations of genetic variation. In my first chapter, I ask whether hybridization and introgression are viable mechanisms for genetic exchange in *Conus*. I generated a phylogenomic dataset from venom duct transcriptomes of species belonging to the *Virroconus* subgenus and use it to clarify phylogeny of the group and detect evidence of hybridization and introgression. I find strong evidence for

mitochondrial and nuclear introgression among several species. These results demonstrate the viability of hybridization as a mechanism for driving diversification in Conidae.

A second factor that likely contributes to the rapid diversification of Conidae is the evolution of adaptive phenotypes that facilitate resource partitioning. Cone snails exhibit extreme trophic specialization, and most research to date focuses on the role of venom in facilitating that specialization. In my second two chapters, I expand our understanding of the predatory phenotype of cone snails beyond venom to include olfaction, the primary sensory modality of gastropods. In my second chapter, I characterize the olfactory receptor (OR) gene repertoire of *Conus ebraeus* using a transcriptomic approach. I identify 88 candidate OR genes and produce a phylogeny that illustrates the high diversity of this gene family relative to other gastropods. I examine patterns of evolution throughout the tree and estimate levels of gene expression. Results from these analyses suggest that one particular clade of OR genes has undergone rapid diversification and that these loci.

In my final chapter, I compare the OR repertoire of *Conus ebraeus* to its sister species *Conus judaeus*, which exhibits a highly distinct dietary specialization. I then examine evolutionary mechanisms driving divergence of gene family composition and function. I recover a more diverse repertoire of OR genes from *Conus judaeus*, and detect several differentially expressed loci. I identify eight orthologous loci that exhibit evidence of positive selection. These results suggest that divergence of a small number of orthologs and differences in gene expression contribute to the distinct prey specializations of these

species. Together, these chapters offer an entirely novel perspective on adaptive trait evolution in *Conus* and indicate that genes underlying olfaction respond to selection imposed by diet.

Taken as a whole, my dissertation presents a novel body of work that reveals previously undiscovered genetic drivers of diversification in Conidae and contributes to our understanding of the genetics of adaptive evolution more broadly.

CHAPTER 1

Reticulate evolution in Conidae: Evidence for Nuclear and Mitochondrial Introgression in *Virroconus*

with Thomas F. Duda Jr.

ABSTRACT

Conidae – "cone snails" – is a hyper-diverse family of marine snails that has many hallmarks of adaptive radiation. Hybridization and introgression may contribute to such instances of rapid diversification by generating novel combinations of genetic variation that facilitate exploitation of novel ecological niches. In this study, we ask whether these mechanisms have contributed to the evolutionary history of the subgenus *Virroconus*. *Virroconus* species frequently occur in sympatry – presenting opportunities for hybridization – and incongruence between shell morphology and phylogenies inferred from mitochondrial sequences hints at past introgression in the group. We use a phylogenomic dataset generated by sequencing venom-duct RNA of *Virroconus* species to i) infer a robust nuclear phylogeny, ii) assess mitochondrial and nuclear gene tree discordance, and iii) formally test for introgression of nuclear loci among *Virroconus* species. We find strong signals of introgression among several members of *Virroconus*,

demonstrating a history of hybridization in this lineage, and our nuclear phylogeny resolves the incongruence between mitochondrial data and shell morphologies from past studies. In particular we discover introgression of mitochondria and nuclear loci between ancestors of *Conus judaeus* and *Conus coronatus*, and mitochondrial introgression between *Conus fulgetrum* and *Conus abbreviatus*. We also find evidence for adaptive introgression of conotoxin venom loci between *Conus fulgetrum* and *Conus miliaris*. Together, our results demonstrate the viability of hybridization and introgression as contributing evolutionary mechanisms in the adaptive radiation of Conidae. They also add to a growing body of evidence showing that porous species boundaries facilitate the assemblage of novel genotypic variation that can fuel adaptive radiation and the origin of biodiversity.

INTRODUCTION

The origin of biodiversity may be described simplistically as a series of bifurcation events, wherein small changes from one generation to the next accumulate into isolating barriers between populations, producing distinct species. This staid thinking is rapidly giving way to more nuanced consideration of porous species (Abbott et al. 2013). This is due in large part to an improved understanding of how reticulate processes – primarily introgressive hybridization – enable the horizontal exchange of genes among otherwise independently evolving lineages and thereby impart network-like qualities to evolutionary histories. Lineages that diversify rapidly and in sympatry – as often happens during adaptive radiations – may be especially prone to introgressive hybridization (Seehausen 2004).

Reticulate processes produce genomes comprised of genes with discordant patterns of descent (Pease and Hahn 2015). Studies that generate phylogenetic hypotheses from few

genes therefore risk that chosen markers exhibit conflicting histories of descent. This problem is accentuated – especially in non-model systems lacking substantial nuclear genomic resources – by the sole use of mitochondrial DNA in phylogenetic reconstructions. Introgression of mtDNA is common, resulting in mito-nuclear discordance and unreliable phylogenetic inference, which has led the field to favor multi-locus data (Ballard and Whitlock 2004; Galtier et al. 2009; Toews and Brelsford 2012; Sloan et al. 2017). An increasing number of studies illustrate how these data-rich methods can effectively parse phylogenetic signal and correctly identify instances of introgressive hybridization, helping to adjust previously incomplete or erroneous interpretations of data, and adding nuance to our understanding of evolutionary processes. For example, evidence of ancient introgression between human and Neanderthal populations has modified our understanding of our own origins from a strict out-of-Africa hypothesis of modern human evolution to one that incorporates admixture between modern humans and archaic human lineages (Green et al. 2010; Durand et al. 2011). One of the few stark illustrations of sympatric speciation in nature was supposed to be found in the endemic radiations of cichlids in Cameroon's crater lakes, where mitochondrial phylogenies recovered the species assemblage in each lake as monophyletic, suggesting that each was the result of a single colonization event (Schliewen et al. 1994). However, recent studies using multi-locus approaches detected post-colonization gene flow among the disparate radiations and with ancestral riverine populations (Martin et al. 2015), suggesting some contribution of allopatric divergence, and casting doubt on assertions that these radiations occurred in sympatry. Finally, Fontaine et al (2015) utilize whole-genome resources to clarify the branching order of the malaria vector-containing *Anopheles gambiae* species complex,

revealing rampant introgression between the two most important vector lineages. This finding provides medically relevant insight into disease vector evolution and demonstrates how different gene regions tell different stories.

Conidae – "cone snails" – is a species rich (>800 species) (MolluscaBase 2019) family of predatory marine gastropods that has many hallmarks of adaptive radiation and exhibits potential for reticulate processes to have contributed to its diversification. Conidae – of which the genus *Conus* comprises some 85% of described species – has diversified rapidly over the past 55 million years, with the fastest diversification rates found in gastropods (Stanley 2007). *Conus* has a circumtropical distribution, with a center of diversity in the Indo-West pacific, where it is not uncommon to find numerous *Conus* species coexisting in the same habitat – 36 species were recorded on a single reef platform at Liang Island, Papua New Guinea (Kohn 2001) – and the genus is unusual in how frequently close relatives and sister species occur in sympatry (Röckel et al. 1995; Kohn 2001; Vallejo 2005). Niches are partitioned primarily by diet in these situations, with co-occurring *Conus* species specializing on distinct prey taxa (Kohn 1959; Leviten 1978; Duda et al. 2009a), which they subdue with complex venom cocktails delivered via a modified radular tooth. *Conus* venom is comprised of small neurotoxic peptides termed "conotoxins" which exhibit patterns of evolution and gene expression consistent with a role in facilitating prey specialization and niche partitioning (Duda 2008; Weese and Duda 2019).

The *Virroconus* clade comprises ten species that have diversified in the past ~10 million years (Duda and Kohn 2005), and stands out as a group in which it would be fruitful to examine the role of reticulate processes in the diversification of *Conus*. The clade includes some of the most widely distributed species in the genus, and many members can

be found coexisting in the same microhabitats (Röckel et al. 1995; Kohn 2001). Their relatively recent divergence and frequent sympatry suggest that opportunities for hybridization exist. Moreover, substantial incongruence between mitochondrial phylogenies and relationships inferred from shell color patterns raises suspicions of reticulate evolution (Figure 1). Three pairs of species are of particular note: *Conus judaeus – Conus ebraeus, Conus fulgetrum – Conus miliaris,* and *Conus aristophanes – Conus coronatus*. Initially described by Rudolph Bergh (1895) from a single specimen collected in the Philippines, *C. judaeus* was synonymized with *C. ebraeus* by subsequent taxonomists due to their indistinguishable shell morphology. Only after a comprehensive examination of radular morphology, dietary data, and sequence data, was *C. judaeus* again recognized as a distinct species (Duda et al. 2009b). Strangely, although similar morphology suggests close phylogenetic affinity of *C. ebraeus* and *C. judaeus*, mitochondrial gene sequences recovered from the two are quite divergent (and from *C. chaldaeus,* another morphologically similar species), and phylogenies produced from these data recover *C. judaeus* as sister to *C. coronatus*, a markedly distinct species (Remigio and Duda 2008; Puillandre et al. 2014). This relationship is also surprising given the morphological similarity of *C. coronatus* and *C. aristophanes*, two species which have also been historically synonymized (Kohn 1959) and do not occur as sister species in phylogenies derived from mitochondrial genes (Remigio and Duda 2008; Puillandre et al. 2014). A similar mismatch is apparent with *C. fulgetrum* and *C. miliaris*, the former having been identified at times as a subspecies of *C. miliaris*, which share remarkably similar shell morphology and coloration (Röckel et al. 1995). Yet these species do not resolve as sister to one another with mtDNA (Puillandre et al. 2014). Closer inspection of similar mismatches in other systems has frequently revealed that

mitochondrial introgression is responsible (e.g. Shaw 2002; Sota 2002; Babik et al. 2005; Renoult et al. 2009; Köhler and Deein 2010).

This study leverages high-throughput RNA sequencing to formally test for signatures of introgression among members of the *Virroconus* clade. Doing so clarifies our understanding of the evolutionary history of these species, providing important taxonomic resolution, but it also offers insight into the mechanisms that played a role more generally in the diversification of *Conus*. Furthermore, a broader understanding of when and how introgression shapes evolutionary trajectories has far-reaching applications, ranging from academic questions such as the origin of species or invasion biology to exercises in applied biology like improving crop yields (Tester and Langridge 2010), shaping conservation policy (Hamilton and Miller 2016), or the evolution of resistance and virulence (Arnold 2004).

METHODS

Taxon sampling

We sampled a total 38 individuals from ten species: eight members of the *Virroconus* subgenus (*Conus miliaris, Conus fulgetrum, Conus judaeus, Conus ebraeus, Conus aristophanes, Conus abbreviatus, Conus coronatus, Conus chaldaeus*), and two outgroup species (*Conus morderiae, Conus regonae*) from the Cape Verde species flock. We lacked appropriately preserved tissues from two additional *Virroconus* species (*Conus taeniatus* and *Conus doreensis*), and so did not include them in our analyses. For two species, *C. miliaris* and *C. coronatus*, we used sequencing data generated by Weese & Duda (2019). All tissues used in this study were retrieved from the University of Michigan Museum of

Zoology Mollusk Division collections, where tissues were stored in -80 C or long-term liquid nitrogen storage. Specimens obtained, collection locales, sex, and tissue types are presented in Table 1.

RNA extraction, library preparation, & sequencing

We followed workflows used by Weese & Duda (2019) to generate transcriptome data for *C. miliaris* and *C. coronatus*. Total RNA was extracted from whole venom ducts for all specimens except *C. chaldaeus*, for which the osphradium (the snail's olfactory organ) was utilized. In brief, tissues were pestle-homogenized and total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, USA) following the supplier instructions. RNA was submitted to the University of Michigan DNA Sequencing Core for quality assessment using a Bioanalyzer 2100 and for library preparation and indexing (Illumina Tru-Seq kit, San Diego, CA, USA). Samples were spread over three flowcell lanes on an Illumina HiSeq4000.

Read processing, transcriptome assembly, transcript filtering

It can be challenging to produce phylogenomic datasets from transcriptome data in non-model systems that lack well-developed genomic resources. Without these resources, establishing homology and orthology can be unreliable, so it is wise to use conservative read- and transcript-filtering parameters to minimize the downstream effects of contaminants or errors during extraction, sequencing, and assembly. We utilized a mixed approach in which we built a robust *de novo* transcriptome for *C. miliaris*, and then used a reference-based assembly method to produce transcriptomes of the remaining species for which we have less raw data. To generate a reliable set of orthologs for phylogenomic

analyses, we adapted the phylogenomic dataset construction pipeline established by Yang & Smith (Yang and Smith 2014) by modifying it to accommodate our reference-based assembly approach. A number of tools implemented in this pipeline are from Phyx (Brown et al. 2017). All analyses were performed on the University of Michigan Flux High Performance Computing core.

Raw Illumina reads were filtered in the same way for both *de novo* and reference-based assemblies. Potential sequencing errors were corrected with Rcorrector (Song and Florea 2015), and reads that could not be corrected were removed. Trimmomatic v0.36 (Bolger et al. 2014) was used with default parameters to remove Illumina sequencing adapters and low-quality sequences. Reads were binned as mitochondrial or nuclear DNA according to whether or not they mapped to a custom database comprised of nine complete *Conus* mitochondrial genomes using Bowtie2 v2.3.4.3 (Langmead and Salzberg 2012). Finally, FastQC v0.10.1 (Andrews 2010) was used to assess read quality and read-representation, and over-represented sequences were culled. Cleaned *C. miliaris* reads were then assembled *de novo* using Trinity v2.4.0 (Grabherr et al. 2011).

We then stringently filtered the *C. miliaris* transcriptome to serve as a reference for assembling transcriptomes of other species. Assembly quality was assessed using Transrate v1.0.3 (Smith-Unna et al. 2016) and poor-quality transcripts were removed using default settings. Chimeric transcripts were also removed using the Yang & Smith (2014) pipeline. Corset v1.07 (Davidson and Oshlack 2014) was used to cluster transcripts belonging to the same putative gene, and a single representative transcript – the largest in a given cluster – was identified. Open reading frames (ORFs) were predicted using TransDecoder v5.0.1 (Haas et al. 2013) with a BLASTp homology search included for ORF

retention criteria. Candidate ORFs were blasted against a custom reference database that included all *Conus*, *Lottia gigantia*, and *Alpysia californica* protein sequences from the NCBI non-redundant database; ORFs with BLASTp hits were retained. Finally, redundant transcripts were removed using CD-HIT-EST (Li and Godzik 2006; Fu et al. 2012), with a sequence identity threshold of 0.99.

To assemble transcriptomes for the remaining nine species, we used the read mapping program Stampy v1.0.32 (Lunter and Goodson 2011) with default settings to align reads from each species to the filtered *C. miliaris* reference. Although a wide variety of read-mapping tools are available, we chose Stampy for its high sensitivity and optimization for aligning reads with sequence variation relative to the reference (Thankaswamy-Kosalai et al. 2017). Output SAM files were then converted to coordinate-sorted BAM files using samtools v1.3.1 (Li et al. 2009), and assembled using Trinity v2.4.0 in genome-guided mode (Grabherr et al. 2011). To minimize potential cross-contamination introduced during sample preparation or sequencing, we ran all assemblies and fastq read files (excepting *C. miliaris* & *C. coronatus*, for which data were generated separately) through CroCo v1.1 (Simion et al. 2018) with default settings, which produces a meta-transcriptome from all samples, maps reads to it, and determines putative cross-contamination based on relative read-coverage. We removed all transcripts identified as contaminants. We then ran the remaining transcripts through the same filtering pipeline used on the *C. miliaris* reference transcriptome. Because conotoxins are central to Conidae feeding ecology and have likely played a role in the adaptive radiation of this family, we examined conotoxin loci separately from non-conotoxin ("housekeeping") loci to determine if they exhibit markedly different topologies or patterns of introgression suggestive of adaptive introgression. We therefore

separated the final transcript set into conotoxin and non-conotoxin loci by creating a local BLASTx database from all conotoxin sequences in the Conoserver (Kaas et al. 2008, 2012) database and extracting those transcripts that hit with an e-value of 1e-4 or lower.

Mitochondrial genes were assembled for each species from raw, unfiltered reads using NOVOPlasty (Dierckxsens et al. 2017). As seed sequences, we used the coding sequences of all 13 protein-coding mitochondrial genes of *Conus betulinus* and for a reference sequence, we used the complete *C. betulinus* mitochondrial genome (Genbank accession: NC_039922). We performed separate NOVOPlasty runs with each of the 13 genes as a seed sequence for each species. Default parameters were used except that read length was set to 151, insert size to 150, and K-mer size of 23. The identity of resultant contigs were confirmed using BLASTx, and assembled contigs were aligned to the appropriate seed sequence using MUSCLE (Edgar 2004) and manually edited in Seqotron (Fourment and Holmes 2016).

Ortholog Inference

Orthology was estimated using the pipeline developed by Yang & Smith (2014). First, homology inference was conducted for nuclear loci using coding sequences obtained during the transcript filtering step. Transcripts from all species were combined into a single FASTA file and subjected to all-by-all BLASTn (Altschul et al. 1990) to identify clusters of similar transcripts as candidate homologs. Raw BLASTn output was filtered according to a hit fraction cutoff of 50% coverage to produce tight clusters and high-quality alignments. The resulting clusters were input to MCL v.14-137 (Enright et al. 2002; van Dongen and Abreu-Goodger 2012) to refine clusters using a minusLogEvalue of one and an

inflation value of 1.5, with the output filtered to retain only homolog clusters with at least four species represented. Putative homolog clusters were then refined using the iterative tree-building and deep-paralog trimming method described by Yang & Smith (2014). We executed three rounds of homolog tree building with internal branch-length cutoffs of 0.5, 0.25, and 0.15 before building bootstrapped final homolog trees. Final orthologs were then inferred using the Maximum Inclusion approach, set to a minimum of four represented taxa. Final ortholog clusters were aligned using PRANK v.15080 (Löytynoja and Goldman 2008) and trimmed using Phyx (Brown et al. 2017) for a minimum column occupancy of 0.6.

Phylogenetic analyses

Mitochondrial

We selected eight genes (COI, ND1, ND2, ND3, ND4, ND4L, ND5, ATP6) that were recovered from all ten species to use as our final mitochondrial dataset, producing a single concatenated sequence for each species. We then aligned concatenated sequences using MUSCLE (Edgar 2004), and manually inspected and trimmed the alignment in Seqotron. We used ModelFinder (Kalyaanamoorthy et al. 2017) to select the best fit substitution model according to BIC (TPM3u+F+R2), and conducted phylogenetic inference using IQ-TREE (Nguyen et al. 2015) with 1000 bootstrap replicates.

Nuclear

We inferred a species tree from our nuclear loci using a concatenated supermatrix of non-conotoxin ("housekeeping") orthologs. Orthologs were included in the supermatrix if they

were recovered from at least six species and if the trimmed ortholog alignment was at least 100 nucleotides long. We partitioned the supermatrix by locus, allowing each partition to evolve under a different substitution model. Model selection was done separately for each partition using ModelFinder (Kalyaanamoorthy et al. 2017); selected models were then assigned to each partition in a NEXUS partition file. We then ran multi-locus partitioned tree inference with IQ-TREE (Nguyen et al. 2015) with 1000 bootstrap replicates. We carried out two additional analyses to assess the consistency of support across the phylogeny. First, we calculated gene concordance factors (gCF) and site concordance factors (sCF) using IQ-TREE concordance analysis (Minh et al. 2018). These metrics offer an alternative to bootstrap values for measuring branch support and provide a glimpse at gene tree discordance within the species tree. The former metric represents the proportion of genes trees containing a given branch and the latter the number of individual sites supporting a given branch. Both metrics account for variable taxon coverage among gene trees. Second, we randomly assigned the 2216 loci into 8 subsets of 277 loci each, concatenated and partitioned each subset, and estimated phylogenies with IQ-TREE in the same way that we did for the full-concatenation supermatrix. By assessing several subsamples of genes, we can determine how robust the relationships that we recover in our full concatenation tree are to random effects. The number of subsets was chosen arbitrarily.

Concordance analysis & tests of introgression

To test for introgression we used two analyses: Bayesian Concordance Analysis (BCA) and Patterson's D-statistic. We targeted these analyses based on patterns of discordance we

observed between our mitochondrial and nuclear trees, as well the IQ-TREE concordance factors.

Bayesian Concordance Analysis (BCA)

We carried out BCA with BUCKy v1.4.4 (Ané et al. 2007; Larget et al. 2010) to more closely examine the patterns of discordance suggested by the mitochondrial tree and by gCFs/sCFs calculated by IQ-TREE. By comparing the relative frequencies of topologies that are discordant with the primary concordance tree, it is possible to distinguish patterns of introgression from a null hypothesis of incomplete lineage sorting (ILS). Under ILS, discordant topologies are expected with equal frequency, whereas introgression should cause one discordant topology to occur with significantly higher frequency. Like with IQ-TREE concordance factor analysis, BUCKy uses individual locus trees as input and produces concordance factors (CF) for the primary tree and for common discordant topologies, but BUCKy requires that input loci share a common set of taxa (i.e., a gene that is represented by only nine of ten species will be excluded from the analysis). We therefore performed BCA separately on different taxon subsets to maximize the number of usable loci for each analysis. We targeted these analyses to detect evidence of introgression in three areas of discordance: within the '*ebraeus*' clade (subset: *C. ebraeus, C. judaeus,* and *C. chaldaeus*), between *C. judaeus* and *C. coronatus* (subset: *C. chaldaeus, C. judaeus, C. coronatus*), and within the '*miliaris*' clade (*C. miliaris, C. fulgetrum,* and *C. abbreviatus*). We restricted subset size to four taxa to maximize the number of shared loci and so we could calculate Patterson's D-statistic (which requires a four-taxon phylogeny) on the same subsets. *C. mordeirae* was used as the outgroup species for all analyses. We analyzed housekeeping

loci for all subsets and separately analyzed conotoxin loci for those subsets with sufficient loci available (*judaeus-coronatus* subset, and *miliaris* clade).

For each taxon subset, we extracted orthologs with all target taxa represented and produced separate Bayesian trees for each locus using MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) with two runs of four chains each, 10,000,000 generations, a sample frequency of 1000 and a burn-in fraction of 0.25. We then used BUCKy 1.4.4 (Ané et al. 2007; Larget et al. 2010) to summarize the output tree files for each locus and create input files for the BCA analysis. Concordance factors were estimated using BUCKy with four separate chains and 10,000,000 generations with the alpha parameter set to one.

Patterson's D-statistic

We formally tested for introgression by calculating Patterson's D-statistic (Patterson et al. 2012) with the software package DFOIL (Pease and Hahn 2015). Briefly, this works by counting bi-allelic sites in a set of four taxa, tallying the number that support each possible tree topology, and using those counts calculate the D-statistic and an accompanying p-value (Green et al. 2010; Durand et al. 2011). Significantly higher counts of one pattern over the other indicate introgression. We extracted ortholog alignments and concatenated them into a single supermatrix for each taxon subset using Phyx (Brown et al. 2017). Site counts were performed on concatenated alignments using the script fasta2dfoil.py, and Patterson's D-statistic analysis was run with the script dfoil.py in dstat mode. Taxon subsets were the same as those used for BCA.

RESULTS

Read-filtering, transcriptome assemblies, ortholog inference

Our *de novo C. miliaris* reference transcriptome assembly resulted in 238,284 unfiltered transcripts and 28,631 post-filtering transcripts. This is similar to the number of uniquely-annotated transcripts (25,131) discovered by Weese & Duda (Weese and Duda 2019) with the same dataset. The average number of transcripts post-filtering for the reference-assembled transcriptomes was 7,007.

We identified a total of 2,216 orthologs with coding sequences of at least 100nt long that were found in a minimum of six taxa. On average, species were represented by 69.9% of orthologs. This was reduced by relatively low representation in three species: *C. judaeus* (49.7%), *C. fulgetrum* (48.1%), and especially *C. ebraeus* (13.2%).

Phylogenetic analyses

Mitochondrial phylogeny

Our final mitochondrial alignment included eight genes (COI, ND1, ND2, ND3, ND4, ND4L, ND5, ATP6) that were recovered from all ten species, resulting in a final alignment length of 7,846 nucleotides. Our IQ-TREE maximum likelihood phylogeny (Figure 1) hypothesizes a similar topology as *Virroconus* mitochondrial trees in prior studies (Remigio and Duda 2008; Puillandre et al. 2014), separating the group into two main clades. These groupings produce the same incongruence between morphology and phylogeny – most obvious in the sister relationship between *C. judaeus* and *C. coronatus* – noted previously.

Nuclear phylogeny

The final nuclear supermatrix included 2,216 loci and the concatenated alignment is 1,687,663 characters, with overall site occupancy of 63.7%. The IQ-TREE maximum likelihood phylogeny is presented in Figure 2, and separates *Virroconus* into two main clades: the '*ebraeus*' clade and the remaining *Virroconus* species (the '*miliaris'* clade plus *C. coronatus* and *C. aristophanes*). Although bootstrap values indicate mostly high branch support (with the exception of the sister pairing of *C. judaeus* and *C. ebraeus*), gCF and sCF values are less consistent and suggest reasonably high levels of gene tree discordance in some areas of the tree, particularly within the *ebraeus* and *miliaris* clades. These values do however provide robust support for the branch splitting the *ebraeus* clade from other *Virroconus* members. The eight trees built with random 277-locus subsets exhibit uncertainty in the arrangement of the *ebraeus* clade, but otherwise broadly agree with the full-concatenation tree. Of the eight subsets four recover the same topology as the full-concatenation tree. Three recover *C. ebraeus* as sister to *C. chaldaeus* (otherwise identical), and one places *C. ebraeus* basal to the rest of the *Virroconus*.

In contrast to the mitochondrial tree (Figure 2), the nuclear tree groups together species that are morphologically similar. *C. judaeus* and *C. ebraeus* are recovered as sisters, as are *C. coronatus* and *C. aristophanes*, the latter pair falling out adjacent to the clade containing *C. miliaris, C. abbreviatus,* and *C. fulgetrum*.

Bayesian Concordance Analysis

We performed BCA to ask if introgression is responsible for patterns of gene tree discordance (as opposed to ILS) in three subsets of *Virroconus* species. We analyzed

housekeeping loci for all subsets, and separately analyzed conotoxin loci when possible. Results are summarized in Figure 3.

Our BCA did not produce evidence of introgression among *C. ebraeus, C. chaldaeus*, and *C. judaeus* (Figure 3a). Unlike our nuclear tree (Figure 2), the primary concordance tree generated by BCA recovers *C. chaldaeus* as sister to *C. ebraeus* as in the mitochondrial tree (Figure 1). However, the 95% confidence intervals of the concordance factors (CF) for all three possible topologies were substantially overlapping, suggesting that the analysis was unable to resolve a confident primary concordance topology with the available loci. Due to the lack of venom duct tissue from *C. chaldaeus*, we were unable to perform the corresponding BCA using conotoxin loci.

We found strong evidence of introgression between *C. judaeus* and *C. coronatus* (Figure 3b) among housekeeping genes. Unlike the *ebraeus* clade, BCA for this taxon subset confidently resolves a primary concordance tree in agreement with our nuclear tree (Figure 1), finding *C. chaldaeus* and *C. judaeus* to be sisters with a high CF (0.692) that does not overlap with discordant topologies. Moreover, the CF for the discordant *C. judaeus-C. coronatus* sister pairing (0.197) is higher than for *C. chaldaeus-C. coronatus* (0.093), with confidence intervals that barely overlap. This suggests that introgression between *C. judaeus* and *C. coronatus* and not ILS is responsible for the pattern of discordance we observe. When performed with conotoxin loci, the primary concordance tree again recovers *C. chaldaeus* and *C. judaeus* as sister species with a CF that does not overlap with the discordant topology. Due likely to the small number of shared conotoxin loci, only one of the possible discordant topologies was recovered, making it impossible to directly compare their frequencies. However, this topology has *C. judaeus* and *C. coronatus* as sister

species, which is consistent with the evidence for introgression observed with housekeeping loci.

In the *miliaris* clade, we do not see evidence of introgression among housekeeping genes (Figure 3c). The primary concordance tree recovers *C. miliaris* and *C. abbreviatus* as sister, consistent with our nuclear tree (Figure 1). Although the discordant topology pairing *C. fulgetrum* with *C. miliaris* has a higher CF (0.262) than the topology pairing *C. fulgetrum* with *C. abbreviatus* (0.211), the surrounding confidence intervals overlap substantially, failing to reject ILS as an explanation for the discordance. Our BCA using conotoxin loci for this subset produces a similar pattern as did the *judaeus-coronatus* taxon subset, recovering only one alternative topology, *C. fulgetrum* and *C. miliaris* as sister in this case. However, this topology is recovered as the primary concordance tree, with a higher CF than the tree pairing *C. miliaris* and *C. abbreviatus* as in the nuclear tree and BCA with housekeeping genes. Again, without the other discordant topology available, we cannot directly determine if introgression is responsible, but these results are consistent with the pattern we see in housekeeping genes and hint at a past history of introgression between *C. miliaris* and *C. fulgetrum*.

Patterson's D-statistic

To further examine the patterns observed in our concordance analyses and to formally test for introgression, we calculated Patterson's D-statistic for the same taxon subsets as for BCA. In general, the results of this test corroborate patterns apparent from BCA. The results are summarized in Figure 4.

We do not find evidence of introgression of housekeeping genes among members of the *ebraeus* clade (*D*=0.077; *p-value*=0.283) (Figure 4a). As with BCA, we were unable to calculate Patterson's D-statistic with conotoxin loci because we lacked venom duct tissue for *C. chaldaeus*. We detected strong evidence for introgression in the history of the *C. judaeus* and *C. coronatus* lineages (*D*=0.144; *p-value*=8.66E-05) (Figure 4b) as was suggested by patterns of discordance in both our mitochondrial tree and BCA. We attempted to detect introgression among conotoxin loci by substituting *C. ebraeus* for *C. chaldaeus*, but there were too few informative sites among the small number of shared loci to perform the analysis. We did not find introgression of housekeeping loci among members of the *miliaris* clade (*D*=-0.026; *p-value*=0.406) (Figure 4c), which is in line with the results of our BCA. We did, however, find evidence for introgression of conotoxin loci between *C. miliaris* and *C. fulgetrum* (*D*=-0.758; *p-value*=1.35E-05) (Figure 4d). This finding is also consistent with the pattern we observed with conotoxin loci in the BCA.

DISCUSSION

Our results indicate that hybridization has played an active role in the evolutionary history of this group and demonstrate the viability of introgressive hybridization as a mechanism for genetic exchange among *Virroconus* and Conidae more broadly. We used a phylogenomic dataset of 2,216 loci to clarify the *Virroconus* phylogeny, detected strong mito-nuclear discordance, and identified several patterns of nuclear introgression, including one suggestive of adaptive introgression of conotoxin genes. As we discuss below, these findings further inform our understanding of the group's rapid diversification.

Mito-nuclear discordance & mitochondrial introgression

The phylogeny of *Virroconus* that we inferred from a robust phylogenomic dataset is starkly discordant with the topology of trees produced from mitochondrial loci (Figure 2), both in this study and previously (Remigio and Duda 2008; Puillandre et al. 2014). Similar patterns of mito-nuclear discordance are clear when comparing these mitochondrial phylogenies with the nuclear tree generated by Phuong et al (2019). Most immediately apparent is the rearrangement of *C. coronatus* and *C. judaeus*. While mitochondrial data recover these species as sisters in a clade positioned next to *C. ebraeus* and *C. chaldaeus*, the nuclear tree places *C. coronatus* sister to *C. aristophanes*, and *C. judaeus* sister to *C. ebraeus* and exhibits strong support at the node separating the *ebraeus* clade from the other *Virroconus* species. This is strongly indicative of hybridization and subsequent introgression of mitochondrial DNA between ancestors of *C. coronatus* and *C. judaeus*. We also see discordance within the *miliaris* clade where the mitochondrial tree places *C. miliaris* outside sisters *C. abbreviatus* and *C. fulgetrum*. In the nuclear tree, *C. abbreviatus* switches sisters, falling out with *C. miliaris*. Both areas of mito-nuclear discordance – particularly *C. coronatus*-*C .judaeus* – suggest avenues by which other genetic variation, possibly adaptive, could have traversed species boundaries, a question we explore further in our concordance analyses.

Mitochondrial introgression is not uncommon across the tree of life and is frequently responsible for discordance between mitochondrial and nuclear phylogenies (Toews and Brelsford 2012). This process may be driven by selection or drift, depending on the specific circumstances of the taxa involved (Ballard and Whitlock 2004). Opportunities for hybridization certainly exist among many members of *Virroconus*. The

range of *C. coronatus* overlaps completely with the much smaller range of *C. judaeus* and the two species are found in similar microhabitats where they cooccur (Reichelt 1982; Kohn 1983; Duda et al. 2009b). Indeed, the restricted range of *C. judaeus* relative to its congeners offers a potential clue about what mechanism may be driving mitochondrial introgression. If its small geographic range corresponds to a smaller effective population size, the *C. judaeus* mitochondrial genome may be particularly susceptible to Muller's Ratchet (Muller 1964; Felsenstein 1974). This phenomenon describes a situation wherein deleterious mutations accumulate rapidly in nonrecombining DNA (such as mitochondrial genomes), a process that is further accelerated by low effective population sizes where selection is inefficient (Lynch and Lande 1998; Gemmell et al. 2004). Under such circumstances, when a mitochondrial genome with lower mutational load is introduced via hybridization, it could introgress rapidly, producing the discordance we observe. A similar scenario is documented by Llopart et al (2014) who investigate mitochondrial introgression between *Drosophila yakuba* and *Drosophila santomea*, the former having a larger effective population size than the latter. They detect two distinct introgression events – one past and one ongoing – suggestive of adaptive introgression of the *D. yakuba* mitochondrial genome into *D. santomea*.

To gauge the plausibility of this hypothesis in *Virroconus,* future work should clarify two areas of uncertainty. First, the range limits of *C. judaeus* should be established with higher confidence. Because *C. judaeus* was only recently identified as a distinct species from *C. ebraeus* (Duda et al. 2009b) and it is difficult to distinguish without close inspection of radular tooth morphology or DNA sequence data, currently reported collection locales likely do not reflect the true extent of its geographic distribution. Thorough range

delimitation would certainly require fresh collection efforts, but could also follow the example of similar studies (e.g. Goldstein and Desalle 2003) by leveraging existing museum collections to detect previously unnoticed variation using DNA-based methods. Second, the effective population size and demographic history of *C. judaeus* should be examined to determine its past potential for susceptibility to mitochondrial deterioration via Mueller's ratchet, or to the fixation of an introgressed foreign mitochondrial genome by random chance. Effective population sizes have been estimated for a number of *Virroconus* species (Duda and Lessios 2009; Duda et al. 2012), but not *C. judaeus.*

Morphology and discordance

As discussed above, our mitochondrial data largely recapitulate the topologies inferred by previous studies that conflict with morphological variation present in *Virroconus*. The most striking of these are sister species relationships between *C. judaeus* and *C. coronatus* and to a lesser degree, *C. fulgetrum* and *C. abbreviatus* (Figure 1). Various aspects of shell morphology and coloration distinguish *C. judaeus* from *C. coronatus* (Figure 1). Where the former is broadly conical, has a narrow aperture, and has a grid-like pattern of roughly parallelogram black spots, the latter is more ovate, with a wider aperture, and an irregular pattern of blotches of brown-black pigment punctuated with small spots that resemble dots and dashes (Röckel et al. 1995). *C. judaeus* and *C. ebraeus*, however, are indistinguishable from shell morphology alone, requiring inspection of radular teeth or DNA for proper identification. Likewise, *C. fulgetrum* and *C. abbreviatus* have quite distinct shell patterning, each bearing some characteristics more similar to *C. miliaris*.

A novice shell collector arranging *Virroconus* species at a glance would almost certainly arrive at a different conclusion than the mitochondrial data, and our nuclear dataset would likely confirm her intuition, at least in part. Our concatenated supermatrix resolves the incongruence between mitochondrial data and morphology, placing *C. judaeus* as sister to *C. ebraeus* in a clade with *C. chaldaeus,* and pairing *C. coronatus* with the similarly patterned *C. aristophanes* (Figure 2). It also rearranges the *miliaris* clade, with *C. miliaris* sister to *C. abbreviatus*, and *C. fulgetrum* placed outside, although these groupings are less obvious based on shell pattern alone. Although the taxon subsets are not identical, these results are concordant with the topology recovered by Phuong et al (2019).

While the most egregious morphological disparity present in the mitochondrial tree – the sister relationship between *C. judaeus* and *C. coronatus* – is reconciled by our nuclear data, the internal arrangement of the *ebraeus* clade (*C. chaldaeus*, *C. ebraeus*, and *C. judaeus*) remains somewhat uncertain. In the BCA primary concordance tree and in three random-locus subset nuclear trees, *C. ebraeus* and *C. chaldaeus* are recovered as sister species while our full-concatenation nuclear tree pairs *C. ebraeus* and *C. judaeus*. *C. chaldaeus* is easily distinguished from *C. ebraeus* and *C. judaeus* by the black axial stripes on its shell. Based on shell pattern alone, one would be tempted to side with the full-concatenation nuclear tree, but there appears to be some uncertainty in the genetic data, perhaps due to past hybridization or ILS.

These findings provide a useful roadmap that can be applied elsewhere in the Conidae, which contains several cryptic species complexes. *Conus flavidus, Conus frigidus*, and *Conus peaseii*, for example, exhibit mito-nuclear discordance, and would benefit from a treatment utilizing multi-locus nuclear data (Lawler and Duda 2017). More broadly, our

results reinforce the notion that uncritical acceptance of species relationships inferred from mitochondrial data is unwise, as it ignores a great deal of genetic complexity. They also suggest that morphological variation can provide clues about the more complex evolutionary history of lineages. As such, conflict between phylogenies (especially those that use limited genetic data, such as mitochondrial genes) and morphological variation should serve as a warning that a richer genetic dataset may be required to fully appreciate the evolutionary history of the group in question.

Nuclear introgression in Virroconus

Our concordance analyses and Patterson's D-statistic tests produced strong evidence for several patterns – both expected and unexpected – of nuclear introgression among *Virroconus* species. We targeted three species subsets based on initial patterns of discordance between our nuclear and mitochondrial phylogenies.

Our analyses of nuclear introgression between *C. judaeus* and *C. coronatus* reinforced the notion of introgression suggested by mitochondrial data. As discussed previously, our curiosity was piqued initially by the morphological incongruence of pairing *C. judaeus* and *C. coronatus* as sister species in the mitochondrial tree and was expanded when we saw such stark discordance between our mitochondrial and nuclear trees suggestive of mitochondrial introgression in the history of these lineages. Both our BCA and D-statistic test rejected ILS as an explanation for discordant topologies that pair *C. judaeus* and *C. coronatus* as sisters. The frequency of this topology is significantly higher than discordant trees pairing *C. chaldaeus* with *C. coronatus*. Inference of introgression among conotoxin loci in this taxon subset was limited by the paucity of *C. ebraeus*

conotoxin loci, but the results of our BCA with this set of loci are at least consistent with the pattern of introgression detected in housekeeping loci.

Such strong corroboration of the mitochondrial pattern of introgression from nuclear loci indicates hybridization between ancestors of these species. Instances of nuclear introgression occurring alongside mitochondrial introgression are sparse in the literature, but not unprecedented. In two hybridizing species of chipmunk, for example, Good et al (Good et al. 2015) discover evidence for nuclear introgression occurring at the same time as mitochondrial introgression. The geographic distribution of these species offers one possible explanation for this pattern. Vallejo (2005) suggests that *Conus* species underwent rapid speciation following the closure of the Tethys Sea, radiating eastwards from the Indo-West Pacific. *C. chaldaeus, C. ebraeus*, and *C. coronatus* all have widespread distributions ranging from the Indian Ocean to the central – and even eastern – Pacific (Röckel et al. 1995; MolluscaBase 2019). *C. judaeus*, however, is restricted to the Indo-West Pacific. Hybridization and periods of rapid diversification are thought to go hand in hand (Seehausen 2004), and our results support a scenario in which the Indian Ocean lineage of the common ancestor of the *ebraeus* clade hybridized with the ancestral lineage of *C. coronatus* during this period of rapid speciation, while no such hybridization occurred in the eastern ranges, initiating divergence that led to *C. judaeus* and *C. ebraeus*. *C. ebraeus* then dispersed back into the Indian Ocean, resulting in the present species distribution.

Within the *miliaris* clade, our results hinted at introgression among housekeeping loci, but failed to reject ILS as an explanation for patterns of gene tree discordance. Our BCA results strongly supported the topology of our nuclear species tree, pairing *C. miliaris* with *C. abbreviatus* with high confidence. Of the two discordant topologies, the one pairing *C.*

miliaris and *C. fulgetrum* had a higher CF, but its confidence intervals overlap with those of the tree pairing *C. abbreviatus* and *C. fulgetrum*. We did, however, find evidence of introgression with our conotoxin locus set, calculating a highly significant D-statistic indicating introgression between *C. miliaris* and *C. fulgetrum*. Unlike the *judaeus-ebraeus* taxon subset, for which nuclear data reinforce the pattern of introgression suggested by mitochondrial data, in this clade the mitochondrial data suggests introgression between one species pair (*C. abbreviatus* and *C. fulgetrum*) while the nuclear conotoxin loci show introgression between another (*C. fulgetrum* and *C. miliaris*).

Despite substantial discordance within the *ebraeus* clade, we do not find evidence of introgression of housekeeping loci among these three species. Interestingly, the primary concordance tree produced by our BCA for this clade matches the topology inferred from our mitochondrial dataset and three of the random locus subset trees we inferred, placing *C. ebraeus* sister to *C. chaldaeus*. Together with the low IQ-TREE gCF values, these results leave the arrangement of the *ebraeus* clade unsettled. Such strong discordance may result from incomplete lineage sorting. However, our ability to confidently infer the relationships in this clade was hindered by low ortholog recovery from the *C. ebraeus* transcriptome (13% ortholog representation). We would need to rerun our analyses with a more complete *C. ebraeus* transcriptome before making a conclusion about the mechanism responsible for the nuclear discordance we observe in the *ebraeus* clade.

Limitations and future directions

Nearly all of our analyses would be strengthened by two improvements to our dataset: a more complete *C. ebraeus* transcriptome, and a venom duct transcriptome for *C. chaldaeus*.

With these data we would be able to examine introgression of conotoxin loci in the *ebraeus* clade and do so with more confidence in the *judaeus-coronatus* taxon subset. It would also likely help resolve the remaining uncertainty about the relationships between *C. chaldaeus*, *C. ebraeus,* and *C. judaeus*.

Several limitations we encountered, however, are inherent to transcriptomic datasets. First, genes with low levels of expression or whose expression varies temporally may be missed. For the housekeeping genes that form the core of our phylogenomic dataset, this may not pose a problem, but conotoxin loci, whose expression is highly variable even intra-specifically, may not be captured reliably. Second, it is not possible to identify specific loci or genomic regions that have undergone introgression using a transcriptomic approach. Few individual loci have sufficient SNPs to evaluate introgression such as Patterson's D-statistic, making it necessary to concatenate many loci scattered throughout the genome to get a strong enough signal for these analyses.

For these reasons, whole genomes are a valuable asset when testing for introgression (e.g. Fontaine et al. 2015; Zhang et al. 2016), and would be a welcome addition to our toolkit for Conidae. Not only would they provide much longer stretches of DNA with a corresponding increase in the number of SNPs available to calculate the D-statistic, but such sites can be mapped back to a genome, and the genes where they cluster can be identified, offering clues about their biological significance (e.g. Baiz et al. 2019). Furthermore, a genomic approach would allow us to capture genes that may be present, but not expressed or expressed at a low level, such as some conotoxin loci that are differentially expressed among species, facilitating more granular investigations into adaptive introgression and the ecological and evolutionary drivers. Unfortunately, efforts

to assemble Conidae genomes have been stymied by highly fragmented genomes and large amounts of repetitive DNA, making it difficult to produce a reasonable number of scaffolds (Hu et al. 2011; Barghi et al. 2016). Researchers should consider applying promising new technologies such as Hi-C sequencing (Belton et al. 2012) to surmount these challenges.

Implications for diversification in Conidae

Introgression provides avenues by which adaptive genetic variation may be exchanged among closely related species. Hybridization may therefore stimulate diversification and adaptation to novel ecological niches by producing novel genetic combinations. The evolutionary impact of hybridization and introgression is highly context-dependent, however (Abbott et al. 2013), and the extent to which it affects broader macroevolutionary patterns and the generation of biodiversity is subject to debate (Seehausen 2004; Mallet 2007; Abbott et al. 2013). Empirical and theoretical work suggest that the novel combinations of genetic and phenotypic variation produced by these processes are often deleterious (Mayr 1963; Rieseberg 1995), but when hybrid offspring encounter novel ecological conditions, such combinations may confer a fitness advantage or open previously inaccessible niches, thereby driving diversification (Anderson and Stebbins 1954; Lewontin and Birch 1966; Seehausen 2004; Mallet 2007). The effects of this on evolutionary trajectories are increasingly well-documented and range from saltatory to incremental. Between 2% and 4% of angiosperm speciation events are estimated to have been the direct result of polyploidy resulting from hybridization, demonstrating the near instantaneous diversifying effects of hybridization (Otto and Whitton 2000). Less dramatically, but perhaps more widespread, adaptive introgression has been found to

combine beneficial alleles and accelerate diversification in a growing number of systems, including the Lake Victoria Region Superflock of cichlids (Meier et al. 2017), Caribbean pupfishes (Richards and Martin 2017), and *Heliconius* bufferflies (Pardo-Diaz et al. 2012).

The implications of introgressive hybridization among Conidae are interesting to consider. Venomous taxa are prime candidates in which to examine how introgressed genes confer novel phenotypes, especially because single genes, or small cassettes of genes, may open entirely new dietary niches. In Mojave rattlesnakes, for example, the presence or absence of five genes dictates the difference between neurotoxic or hemorrhagic venom, with corresponding differences in prey (Strickland et al. 2018). Among the Conidae, there are several interesting avenues to explore. Piscivory has independently evolved at least twice in the family, and there are some 30 extant fish-eating species of *Conus* (Duda and Palumbi 2004). Aman et al (2015) examine the venom of *Conus tessulatus* and identify a single conotoxin with a high degree of similarity to piscivorous cone snail venom, arguing that it could represent preadaptation to a piscivorous life history. If genes like those described in either the snake or the snail were to leak through porous species boundaries, they may open new ecological niches to their recipients. This illustrates the potential for introgression to contribute to the impressive adaptive radiation of Conidae, and to the origins of biodiversity more generally, and highlights the value of additional work in this field.

REFERENCES

Abbott, R., D. Albach, S. Ansell, J. W. Arntzen, S. J. E. Baird, N. Bierne, J. Boughman, A. Brelsford, C. A. Buerkle, R. Buggs, R. K. Butlin, U. Dieckmann, F. Eroukhmanoff, A. Grill, S. H. Cahan, J. S. Hermansen, G. Hewitt, A. G. Hudson, C. Jiggins, J. Jones, B. Keller, T. Marczewski, J. Mallet, P. Martinez-Rodriguez, M. Möst, S. Mullen, R. Nichols, A. W. Nolte, C. Parisod, K. Pfennig, A. M. Rice, M. G. Ritchie, B. Seifert, C. M. Smadja, R.

Stelkens, J. M. Szymura, R. Väinölä, J. B. W. Wolf, and D. Zinner. 2013. Hybridization and speciation. Journal of Evolutionary Biology 26:229–246.

Ache, B. W., and J. M. Young. 2005. Olfaction: Diverse Species, Conserved Principles. Neuron 48:417–430.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. Journal of Molecular Biology 215:403–410.

Aman, J. W., J. S. Imperial, B. Ueberheide, M.-M. Zhang, M. Aguilar, D. Taylor, M. Watkins, D. Yoshikami, P. Showers-Corneli, H. Safavi-Hemami, J. Biggs, R. W. Teichert, and B. M. Olivera. 2015. Insights into the origins of fish hunting in venomous cone snails from studies of Conus tessulatus. Proc Natl Acad Sci U S A 112:5087–5092.

Anderson, E., and G. L. Stebbins. 1954. Hybridization as an Evolutionary Stimulus. Evolution 8:378–388.

Andrews, S. 2010. FastQC: a quality control tool for high throughput sequence data. Available online at http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Ané, C., B. Larget, D. A. Baum, S. D. Smith, and A. Rokas. 2007. Bayesian Estimation of Concordance among Gene Trees. Mol Biol Evol 24:412–426.

Arnold, M. L. 2004. Transfer and Origin of Adaptations through Natural Hybridization: Were Anderson and Stebbins Right? The Plant Cell 16:562–570.

Audesirk, T., and G. Audesirk. 1985. 1 - Behavior of Gastropod Molluscs. Pp. 1–94 *in* A. O. D. Willows, ed. The Mollusca. Academic Press.

Audesirk, T. E. 1975. Chemoreception in Aplysia californica. I. behavioral localization of distance chemoreceptors used in food-finding. Behavioral Biology 15:45–55.

Babik, W., W. Branicki, J. Crnobrnja-Isailović, D. Cogălniceanu, I. Sas, K. Olgun, N. A. Poyarkov, M. Garcia-París, and J. W. Arntzen. 2005. Phylogeography of two European newt species — discordance between mtDNA and morphology. Molecular Ecology 14:2475–2491.

Baiz, M. D., P. K. Tucker, and L. Cortés-Ortiz. 2019. Multiple forms of selection shape reproductive isolation in a primate hybrid zone. Molecular Ecology 28:1056–1069.

Ballard, J. W. O., and M. C. Whitlock. 2004. The incomplete natural history of mitochondria. Molecular Ecology 13:729–744.

Barghi, N., G. P. Concepcion, B. M. Olivera, and A. O. Lluisma. 2016. Characterization of the complete mitochondrial genome of Conus tribblei Walls, 1977. Mitochondrial DNA A DNA Mapp Seq Anal 27:4451–4452.

Bargmann, C. I. 2006. Comparative chemosensation from receptors to ecology. Nature 444:295.

Bear, D. M., J.-M. Lassance, H. E. Hoekstra, and S. R. Datta. 2016. The Evolving Neural and Genetic Architecture of Vertebrate Olfaction. Current Biology 26:R1039–R1049.

Belton, J.-M., R. P. McCord, J. Gibcus, N. Naumova, Y. Zhan, and J. Dekker. 2012. Hi-C: A comprehensive technique to capture the conformation of genomes. Methods 58.

Bergh, R. 1895. Beiträge zur Kenntnis der Coniden. Nova Acta Kaiserl. Leopold.-Carol. deut. Akad. Naturf 65:67–214.

Bertmar, G. 1969. The Vertebrate Nose, Remarks on Its Structural and Functional Adaptation and Evolution. Evolution 23:131–152.

Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120.

Brand, P., and S. R. Ramírez. 2017. The Evolutionary Dynamics of the Odorant Receptor Gene Family in Corbiculate Bees. Genome Biology and Evolution 9:2023–2036.

Brawand, D., M. Soumillon, A. Necsulea, P. Julien, G. Csárdi, P. Harrigan, M. Weier, A. Liechti, A. Aximu-Petri, M. Kircher, F. W. Albert, U. Zeller, P. Khaitovich, F. Grützner, S. Bergmann, R. Nielsen, S. Pääbo, and H. Kaessmann. 2011. The evolution of gene expression levels in mammalian organs. Nature 478:343–348.

Brown, J. W., J. F. Walker, and S. A. Smith. 2017. Phyx: phylogenetic tools for unix. Bioinformatics 33:1886–1888.

Buck, L., and R. Axel. 1991. A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. Cell 65:175–187.

Cahill, A. E., and S. A. Koury. 2016. Larval settlement and metamorphosis in a marine gastropod in response to multiple conspecific cues. PeerJ 4.

Chang, D., and T. F. Duda. 2016. Age-related association of venom gene expression and diet of predatory gastropods. BMC Evolutionary Biology 16.

Chang, D., and T. F. Duda. 2012. Extensive and Continuous Duplication Facilitates Rapid Evolution and Diversification of Gene Families. Mol Biol Evol 29:2019–2029.

Chang, D., A. M. Olenzek, and T. F. Duda. 2015. Effects of geographical heterogeneity in species interactions on the evolution of venom genes. Proceedings of the Royal Society B: Biological Sciences 282:20141984–20141984.

Clifford, K. T., L. Gross, K. Johnson, K. J. Martin, N. Shaheen, and M. A. Harrington. 2003. Slime-trail tracking in the predatory snail, Euglandina rosea. Behavioral Neuroscience 117:1086–1095.

Copeland, M. 1918. The olfactory reactions and organs of the marine snails Alectrion obsoleta (Say) and Busycon canaliculatum (Linn.). Journal of Experimental Zoology 177–227.

Croll, R. P. 1983. Gastropod Chemoreception. Biological Reviews 58:293–319.

Cummins, S. F., D. Erpenbeck, Z. Zou, C. Claudianos, L. L. Moroz, G. T. Nagle, and B. M. Degnan. 2009. Candidate chemoreceptor subfamilies differentially expressed in the chemosensory organs of the mollusc Aplysia. BMC Biology 7:28.

Davidson, N. M., and A. Oshlack. 2014. Corset: enabling differential gene expression analysis for de novoassembled transcriptomes. Genome Biology 15:410.

Dierckxsens, N., P. Mardulyn, and G. Smits. 2017. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Res 45:e18–e18.

Dong, D., G. He, S. Zhang, and Z. Zhang. 2009. Evolution of Olfactory Receptor Genes in Primates Dominated by Birth-and-Death Process. Genome Biol Evol 1:258–264.

Duda Jr, T. F., and S. R. Palumbi. 2000. Evolutionary Diversification of Multigene Families: Allelic Selection of Toxins in Predatory Cone Snails. Molecular Biology and Evolution 17:1286–1293.

Duda, T. 2001. Origins of diverse feeding ecologies within Conus, a genus of venomous marine gastropods. Biological Journal of the Linnean Society 73:391–409.

Duda, T. F. 2008. Differentiation of Venoms of Predatory Marine Gastropods: Divergence of Orthologous Toxin Genes of Closely Related Conus Species with Different Dietary Specializations. J Mol Evol 67:315.

Duda, T. F., D. Chang, B. D. Lewis, and T. Lee. 2009a. Geographic variation in venom allelic composition and diets of the widespread predatory marine gastropod Conus ebraeus. PLoS ONE 4:e6245.

Duda, T. F., and A. J. Kohn. 2005. Species-level phylogeography and evolutionary history of the hyperdiverse marine gastropod genus Conus. Molecular Phylogenetics and Evolution 34:257–272.

Duda, T. F., A. J. Kohn, and A. M. Matheny. 2009b. Cryptic Species Differentiated in Conus ebraeus, a Widespread Tropical Marine Gastropod. The Biological Bulletin 217:292–305.

Duda, T. F., and T. Lee. 2009. Ecological Release and Venom Evolution of a Predatory Marine Snail at Easter Island. PLoS ONE 4:e5558.

Duda, T. F., and H. A. Lessios. 2009. Connectivity of populations within and between major biogeographic regions of the tropical Pacific in Conus ebraeus, a widespread marine gastropod. Coral Reefs 28:651–659.

Duda, T. F., and S. R. Palumbi. 2004. Gene expression and feeding ecology: evolution of piscivory in the venomous gastropod genus *Conus*. Proceedings of the Royal Society of London. Series B: Biological Sciences 271:1165–1174.

Duda, T. F., and S. R. Palumbi. 1999. Molecular genetics of ecological diversification: Duplication and rapid evolution of toxin genes of the venomous gastropod Conus. Proceedings of the National Academy of Sciences 96:6820–6823.

Duda, T. F., M. Terbio, G. Chen, S. Phillips, A. M. Olenzek, D. Chang, and D. W. Morris. 2012. Patterns of Population Structure and Historical Demography of Conus Species in the Tropical Pacific. American Malacological Bulletin 30:175–187.

Durand, E. Y., N. Patterson, D. Reich, and M. Slatkin. 2011. Testing for Ancient Admixture between Closely Related Populations. Mol Biol Evol 28:2239–2252.

Dutertre, S., A.-H. Jin, P. F. Alewood, and R. J. Lewis. 2014. Intraspecific variations in Conus geographus defence-evoked venom and estimation of the human lethal dose. Toxicon 91:135–144.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797.

Eltz, T., Y. Zimmermann, C. Pfeiffer, J. R. Pech, R. Twele, W. Francke, J. J. G. Quezada-Euan, and K. Lunau. 2008. An Olfactory Shift Is Associated with Male Perfume Differentiation and Species Divergence in Orchid Bees. Current Biology 18:1844–1848.

Enright, A. J., S. Van Dongen, and C. A. Ouzounis. 2002. An efficient algorithm for large-scale detection of protein families. Nucleic Acids Res 30:1575–1584.

Fay, J. C., and P. J. Wittkopp. 2008. Evaluating the role of natural selection in the evolution of gene regulation. Heredity 100:191–199.

Felsenstein, J. 1974. The Evolutionary Advantage of Recombination. Genetics 78:737–756.

Finn, R. D., A. Bateman, J. Clements, P. Coggill, R. Y. Eberhardt, S. R. Eddy, A. Heger, K. Hetherington, L. Holm, J. Mistry, E. L. L. Sonnhammer, J. Tate, and M. Punta. 2014. Pfam: the protein families database. Nucleic Acids Research 42:D222–D230.

Firestein, S. 2001. How the olfactory system makes sense of scents. Nature 413:211.

Floden, E. W., P. D. Tommaso, M. Chatzou, C. Magis, C. Notredame, and J.-M. Chang. 2016. PSI/TM-Coffee: a web server for fast and accurate multiple sequence alignments of regular and transmembrane proteins using homology extension on reduced databases. Nucleic Acids Res 44:W339–W343.

Fontaine, M. C., J. B. Pease, A. Steele, R. M. Waterhouse, D. E. Neafsey, I. V. Sharakhov, X. Jiang, A. B. Hall, F. Catteruccia, E. Kakani, S. N. Mitchell, Y.-C. Wu, H. A. Smith, R. R. Love, M. K. Lawniczak, M. A. Slotman, S. J. Emrich, M. W. Hahn, and N. J. Besansky. 2015. Extensive introgression in a malaria vector species complex revealed by phylogenomics. Science 347:1258524.

Fourment, M., and E. C. Holmes. 2016. Seqotron: a user-friendly sequence editor for Mac OS X. BMC Research Notes 9:106.

Fu, L., B. Niu, Z. Zhu, S. Wu, and W. Li. 2012. CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics 28:3150–3152.

Galtier, N., B. Nabholz, S. Glémin, and G. D. D. Hurst. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. Molecular Ecology 18:4541–4550.

Gardiner, A., D. Barker, R. K. Butlin, W. C. Jordan, and M. G. Ritchie. 2008. Drosophila chemoreceptor gene evolution: selection, specialization and genome size. Molecular Ecology 17:1648–1657.

Gemmell, N. J., V. J. Metcalf, and F. W. Allendorf. 2004. Mother's curse: the effect of mtDNA on individual fitness and population viability. Trends in Ecology & Evolution 19:238–244.

Gilad, Y., C. D. Bustamante, D. Lancet, and S. Pääbo. 2003. Natural Selection on the Olfactory Receptor Gene Family in Humans and Chimpanzees. The American Journal of Human Genetics 73:489–501.

Gilad, Y., A. Oshlack, G. K. Smyth, T. P. Speed, and K. P. White. 2006. Expression profiling in primates reveals a rapid evolution of human transcription factors. Nature 440:242.

Gish, W., and D. J. States. 1993. Identification of protein coding regions by database similarity search. Nature Genetics 3:266.

Givnish, T. J., and K. J. Sytsma. 2000. Molecular Evolution and Adaptive Radiation. Cambridge University Press.

Goldstein, P. Z., and R. Desalle. 2003. Calibrating phylogenetic species formation in a threatened insect using DNA from historical specimens. Molecular Ecology 12:1993–1998.

Good, J. M., D. Vanderpool, S. Keeble, and K. Bi. 2015. Negligible nuclear introgression despite complete mitochondrial capture between two species of chipmunks. Evolution 69:1961–1972.

Grabherr, M. G., B. J. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, L. Fan, R. Raychowdhury, Q. Zeng, Z. Chen, E. Mauceli, N. Hacohen, A. Gnirke, N. Rhind, F. di Palma, B. W. Birren, C. Nusbaum, K. Lindblad-Toh, N. Friedman, and A. Regev. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. 29:644–652.

Green, R. E., J. Krause, A. W. Briggs, T. Maricic, U. Stenzel, M. Kircher, N. Patterson, H. Li, W. Zhai, M. H.-Y. Fritz, N. F. Hansen, E. Y. Durand, A.-S. Malaspinas, J. D. Jensen, T. Marques-Bonet, C. Alkan, K. Prüfer, M. Meyer, H. A. Burbano, J. M. Good, R. Schultz, A. Aximu-Petri, A. Butthof, B. Höber, B. Höffner, M. Siegemund, A. Weihmann, C. Nusbaum, E. S. Lander, C. Russ, N. Novod, J. Affourtit, M. Egholm, C. Verna, P. Rudan, D. Brajkovic, Ž. Kucan, I. Gušic, V. B. Doronichev, L. V. Golovanova, C. Lalueza-Fox, M. de la Rasilla, J. Fortea, A. Rosas, R. W. Schmitz, P. L. F. Johnson, E. E. Eichler, D. Falush, E. Birney, J. C. Mullikin, M. Slatkin, R. Nielsen, J. Kelso, M. Lachmann, D. Reich, and S. Pääbo. 2010. A Draft Sequence of the Neandertal Genome. Science 328:710–722.

Ha, M., W.-H. Li, and Z. J. Chen. 2007. External factors accelerate expression divergence between duplicate genes. Trends in Genetics 23:162–166.

Haas, B. J., A. Papanicolaou, M. Yassour, M. Grabherr, P. D. Blood, J. Bowden, M. B. Couger, D. Eccles, B. Li, M. Lieber, M. D. MacManes, M. Ott, J. Orvis, N. Pochet, F. Strozzi, N. Weeks, R. Westerman, T. William, C. N. Dewey, R. Henschel, R. D. LeDuc, N. Friedman, and A. Regev. 2013. De novo transcript sequence reconstruction from RNA-Seq: reference generation and analysis with Trinity. Nat Protoc 8.

Hadfield, M. G. 2011. Biofilms and Marine Invertebrate Larvae: What Bacteria Produce That Larvae Use to Choose Settlement Sites. Annual Review of Marine Science 3:453–470.

Hadfield, M., and V. Paul. 2001. Natural Chemical Cues for Settlement and Metamorphosis of Marine-Invertebrate Larvae. Pp. 431–461 *in* J. McClinTOCk and B. Baker, eds. Marine Chemical Ecology. CRC Press.

Hahn, M. W., T. D. Bie, J. E. Stajich, C. Nguyen, and N. Cristianini. 2005. Estimating the tempo and mode of gene family evolution from comparative genomic data. Genome Res. 15:1153–1160.

Hamilton, J. A., and J. M. Miller. 2016. Adaptive introgression as a resource for management and genetic conservation in a changing climate. Conservation Biology 30:33–41.

Hansson, B. S., and M. C. Stensmyr. 2011. Evolution of Insect Olfaction. Neuron 72:698–711.

Haswell, P. M., K. A. Jones, J. Kusak, and M. W. Hayward. 2018. Fear, foraging and olfaction: how mesopredators avoid costly interactions with apex predators. Oecologia 187:573–583.

Hay, M. E. 2009. Marine Chemical Ecology: Chemical Signals and Cues Structure Marine Populations, Communities, and Ecosystems. Annual Review of Marine Science 1:193–212.

Hayden, S., M. Bekaert, T. A. Crider, S. Mariani, W. J. Murphy, and E. C. Teeling. 2010. Ecological adaptation determines functional mammalian olfactory subgenomes. Genome Res. 20:1–9.

Hildebrand, J. G., and G. M. Shepherd. 1997. MECHANISMS OF OLFACTORY DISCRIMINATION: Converging Evidence for Common Principles Across Phyla. Annual Review of Neuroscience 20:595–631.

Holloway, A. K., M. K. N. Lawniczak, J. G. Mezey, D. J. Begun, and C. D. Jones. 2007. Adaptive Gene Expression Divergence Inferred from Population Genomics. PLOS Genetics 3:e187.

Hu, H., P. K. Bandyopadhyay, B. M. Olivera, and M. Yandell. 2011. Characterization of the Conus bullatus genome and its venom-duct transcriptome. BMC Genomics 12:60.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

Hussain, A., L. R. Saraiva, and S. I. Korsching. 2009. Positive Darwinian selection and the birth of an olfactory receptor clade in teleosts. PNAS 106:4313–4318.

James, D., C. A. Prator, G. G. Martin, and J. R. Schulz. 2014. Morphology of sensory papillae on the feeding proboscis of cone snails (Mollusca, Gastropoda). Invertebrate Biology 133:221–231.

Kaas, Q., J.-C. Westermann, R. Halai, C. K. L. Wang, and D. J. Craik. 2008. ConoServer, a database for conopeptide sequences and structures. Bioinformatics 24:445–446.

Kaas, Q., R. Yu, A.-H. Jin, S. Dutertre, and D. J. Craik. 2012. ConoServer: updated content, knowledge, and discovery tools in the conopeptide database. Nucleic Acids Res 40:D325–D330.

Kalyaanamoorthy, S., B. Q. Minh, T. K. F. Wong, A. von Haeseler, and L. S. Jermiin. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14:587–589.

Kamio, M., and C. D. Derby. 2017. Finding food: how marine invertebrates use chemical cues to track and select food. Natural Product Reports 34:514–528.

Khan, I., Z. Yang, E. Maldonado, C. Li, G. Zhang, M. T. P. Gilbert, E. D. Jarvis, S. J. O'Brien, W. E. Johnson, and A. Antunes. 2015. Olfactory Receptor Subgenomes Linked with Broad

Ecological Adaptations in Sauropsida. Molecular Biology and Evolution 32:2832–2843.

Köhler, F., and G. Deein. 2010. Hybridisation as potential source of incongruence in the morphological and mitochondrial diversity of a Thai freshwater gastropod (Pachychilidae, Brotia H. Adams, 1866). Zoosystematics and Evolution 86:301–314.

Kohn, A. 2001. Maximal species richness in Conus: diversity, diet and habitat on reefs of northeast Papua New Guinea. Coral Reefs 20:25–38.

Kohn, A. 1990. Tempo and mode of evolution in Conidae. Malacologia 55–67.

Kohn, A. J. 1961. CHEMORECEPTION IN GASTROPOD MOLLUSCS. American Zoologist 1:291–308.

Kohn, A. J. 2014. Conus of the Southeastern United States and Caribbean. Princeton University Press.

Kohn, A. J. 1983. Microhabitat Factors Affecting Abundance and Diversity of Conus on Coral Reefs. Oecologia 60:293–301.

Kohn, A. J. 1956. PISCIVOROUS GASTROPODS OF THE GENUS CONUS. Proc. Natl. Acad. Sci. U.S.A. 42:168–171.

Kohn, A. J. 1959. The Ecology of Conus in Hawaii. Ecological Monographs 29:47–90.

Kohn, A. J., and J. W. Nybakken. 1975. Ecology of Conus on eastern Indian Ocean fringing reefs: Diversity of species and resource utilization. Marine Biology 29:211–234.

Langmead, B., and S. L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359.

Larget, B. R., S. K. Kotha, C. N. Dewey, and C. Ané. 2010. BUCKy: Gene tree/species tree reconciliation with Bayesian concordance analysis. Bioinformatics 26:2910–2911.

Lawler, A. J., and T. F. Duda. 2017. Molecular and morphometric data suggest the presence of a neglected species in the marine gastropod family Conidae. Molecular Phylogenetics and Evolution 109:421–429.

Leviten, P. J. 1978. Resource Partitioning by Predatory Gastropods of the Genus Conus on Subtidal Indo-Pacific Coral Reefs: The Significance of Prey Size. Ecology 59:614–631.

Lewontin, R. C., and L. C. Birch. 1966. Hybridization as a Source of Variation for Adaptation to New Environments. Evolution 20:315–336.

Li, B., and C. N. Dewey. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12:323.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. Durbin. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079.

Li, W., and A. Godzik. 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22:1658–1659.

Linn, C., J. L. Feder, S. Nojima, H. R. Dambroski, S. H. Berlocher, and W. Roelofs. 2003. Fruit odor discrimination and sympatric host race formation in Rhagoletis. Proceedings of the National Academy of Sciences 100:11490–11493.

Llopart, A., D. Herrig, E. Brud, and Z. Stecklein. 2014. Sequential adaptive introgression of the mitochondrial genome in Drosophila yakuba and Drosophila santomea. Mol Ecol 23:1124–1136.

Löytynoja, A., and N. Goldman. 2008. Phylogeny-Aware Gap Placement Prevents Errors in Sequence Alignment and Evolutionary Analysis. Science 320:1632–1635.

Lunter, G., and M. Goodson. 2011. Stampy: A statistical algorithm for sensitive and fast mapping of Illumina sequence reads. Genome Res 21:936–939.

Lynch, M., and R. Lande. 1998. The critical effective size for a genetically secure population. Animal Conservation 1:70–72.

Mallet, J. 2007. Hybrid speciation. Nature 446:279–283.

Martin, C. H., J. S. Cutler, J. P. Friel, C. D. Touokong, G. Coop, and P. C. Wainwright. 2015. Complex histories of repeated gene flow in Cameroon crater lake cichlids cast doubt on one of the clearest examples of sympatric speciation. Evolution 69:1406–1422.

Mayr, E. 1963. Animal Species and Evolution. Belknap Press of Harvard University Press, Cambridge, MA.

McBride, C. S. 2007. Rapid evolution of smell and taste receptor genes during host specialization in Drosophila sechellia. PNAS 104:4996–5001.

McCarthy, D. J., Y. Chen, and G. K. Smyth. 2012. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. Nucleic Acids Res 40:4288–4297.

Meier, J. I., D. A. Marques, S. Mwaiko, C. E. Wagner, L. Excoffier, and O. Seehausen. 2017. Ancient hybridization fuels rapid cichlid fish adaptive radiations. Nature Communications 8:14363.

Minh, B. Q., M. W. Hahn, and R. Lanfear. 2018. New methods to calculate concordance factors for phylogenomic datasets. bioRxiv 487801.

MolluscaBase. 2019. Conidae J. Fleming, 1822. Accessed through: World Register of Marine Species.

Mombaerts, P. 2004. Genes and ligands for odorant, vomeronasal and taste receptors. Nature Reviews Neuroscience 5:263.

Muller, H. J. 1964. The relation of recombination to mutational advance. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 1:2–9.

Myers, R. A., L. J. Cruz, J. E. Rivier, and B. M. Olivera. 1993. Conus peptides as chemical probes for receptors and ion channels. Chemical Reviews 93:1923–1936.

Nguyen, L.-T., H. A. Schmidt, A. von Haeseler, and B. Q. Minh. 2015. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Mol Biol Evol 32:268–274.

Niimura, Y., and M. Nei. 2005. Comparative evolutionary analysis of olfactory receptor gene clusters between humans and mice. Gene 346:13–21.

Notredame, C., D. G. Higgins, and J. Heringa. 2000. T-coffee: a novel method for fast and accurate multiple sequence alignment11Edited by J. Thornton. Journal of Molecular Biology 302:205–217.

O'Leary, N. A., M. W. Wright, J. R. Brister, S. Ciufo, D. Haddad, R. McVeigh, B. Rajput, B. Robbertse, B. Smith-White, D. Ako-Adjei, A. Astashyn, A. Badretdin, Y. Bao, O. Blinkova, V. Brover, V. Chetvernin, J. Choi, E. Cox, O. Ermolaeva, C. M. Farrell, T. Goldfarb, T. Gupta, D. Haft, E. Hatcher, W. Hlavina, V. S. Joardar, V. K. Kodali, W. Li, D. Maglott, P. Masterson, K. M. McGarvey, M. R. Murphy, K. O'Neill, S. Pujar, S. H. Rangwala, D. Rausch, L. D. Riddick, C. Schoch, A. Shkeda, S. S. Storz, H. Sun, F. Thibaud-Nissen, I. Tolstoy, R. E. Tully, A. R. Vatsan, C. Wallin, D. Webb, W. Wu, M. J. Landrum, A. Kimchi, T. Tatusova, M. DiCuccio, P. Kitts, T. D. Murphy, and K. D. Pruitt. 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res 44:D733–D745.

Olivera, B. M., C. Walker, G. E. Cartier, D. Hooper, A. D. Santos, R. Schoenfeld, R. Shetty, M. Watkins, P. Bandyopadhyay, and D. R. Hillyard. 1999. Speciation of Cone Snails and Interspecific Hyperdivergence of Their Venom Peptides: Potential Evolutionary Significance of Intronsa. Annals of the New York Academy of Sciences 870:223–237.

Otto, S. P., and J. Whitton. 2000. Polyploid Incidence and Evolution. Annual Review of Genetics 34:401–437.

Painter, S. D., S. F. Cummins, A. E. Nichols, D.-B. G. Akalal, C. H. Schein, W. Braun, J. S. Smith, A. J. Susswein, M. Levy, P. A. C. M. de Boer, A. ter Maat, M. W. Miller, C. Scanlan, R. M. Milberg, J. V. Sweedler, and G. T. Nagle. 2004. Structural and functional analysis of Aplysia attractins, a family of water-borne protein pheromones with interspecific attractiveness. Proc Natl Acad Sci U S A 101:6929–6933.

Pardo-Diaz, C., C. Salazar, S. W. Baxter, C. Merot, W. Figueiredo-Ready, M. Joron, W. O. McMillan, and C. D. Jiggins. 2012. Adaptive Introgression across Species Boundaries in Heliconius Butterflies. PLOS Genetics 8:e1002752.

Patterson, N., P. Moorjani, Y. Luo, S. Mallick, N. Rohland, Y. Zhan, T. Genschoreck, T. Webster, and D. Reich. 2012. Ancient Admixture in Human History. Genetics 192:1065–1093.

Pease, J. B., and M. W. Hahn. 2015. Detection and Polarization of Introgression in a Five-Taxon Phylogeny. Syst Biol 64:651–662.

Phuong, M. A., M. E. Alfaro, G. N. Mahardika, R. M. Marwoto, R. E. Prabowo, T. von Rintelen, P. W. H. Vogt, J. R. Hendricks, and N. Puillandre. 2019. Lack of Signal for the Impact of Conotoxin Gene Diversity on Speciation Rates in Cone Snails. Systematic Biology, doi: 10.1093/sysbio/syz016.

Proffit, M., B. Schatz, R. M. Borges, and M. Hossaert-Mckey. 2007. Chemical mediation and niche partitioning in non-pollinating fig-wasp communities. Journal of Animal Ecology 76:296–303.

Puillandre, N., P. Bouchet, T. F. Duda, S. Kauferstein, A. J. Kohn, B. M. Olivera, M. Watkins, and C. Meyer. 2014. Molecular phylogeny and evolution of the cone snails (Gastropoda, Conoidea). Mol. Phylogenet. Evol. 78:290–303.

Rafferty, N. E., and J. W. Boughman. 2006. Olfactory mate recognition in a sympatric species pair of three-spined sticklebacks. Behav Ecol 17:965–970.

Ramasamy, S., L. Ometto, C. M. Crava, S. Revadi, R. Kaur, D. S. Horner, D. Pisani, T. Dekker, G. Anfora, and O. Rota-Stabelli. 2016. The Evolution of Olfactory Gene Families in Drosophila and the Genomic Basis of chemical-Ecological Adaptation in Drosophila suzukii. Genome Biol Evol 8:2297–2311.

Rambaut, A. 2009. FigTree.

Ranwez, V., S. Harispe, F. Delsuc, and E. J. P. Douzery. 2011. MACSE: Multiple Alignment of Coding SEquences Accounting for Frameshifts and Stop Codons. PLOS ONE 6:e22594.

Reichelt, R. E. 1982. Space: A non-limiting resource in the niches of some abundant coral reef gastropods. Coral Reefs 1:3–11.

Remigio, E. A., and T. F. Duda. 2008. Evolution of ecological specialization and venom of a predatory marine gastropod. Molecular Ecology 17:1156–1162.

Renoult, J. P., P. Geniez, P. Bacquet, L. Benoit, and P.-A. Crochet. 2009. Morphology and nuclear markers reveal extensive mitochondrial introgressions in the Iberian Wall Lizard species complex. Molecular Ecology 18:4298–4315.

Richards, E. J., and C. H. Martin. 2017. Adaptive introgression from distant Caribbean islands contributed to the diversification of a microendemic adaptive radiation of trophic specialist pupfishes. PLOS Genetics 13:e1006919.

Rieseberg, L. H. 1995. The Role of Hybridization in Evolution: Old Wine in New Skins. American Journal of Botany 82:944–953.

Robertson, H. M., C. G. Warr, and J. R. Carlson. 2003. Molecular evolution of the insect chemoreceptor gene superfamily in Drosophila melanogaster. Proceedings of the National Academy of Sciences 100:14537–14542.

Robinson, M. D., D. J. McCarthy, and G. K. Smyth. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140.

Röckel, D., W. Korn, and A. J. Kohn. 1995. Manual of the Living Conidae: Indo-Pacific Region. Hemmen.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Rosenberg, G. 2014. A New Critical Estimate of Named Species-Level Diversity of the Recent Mollusca*. malb 32:308–322.

Schliewen, U. K., D. Tautz, and S. Pääbo. 1994. Sympatric speciation suggested by monophyly of crater lake cichlids. Nature 368:629.

Schluter, D. 2000. The Ecology of Adaptive Radiation. OUP Oxford.

Seehausen, O. 2004. Hybridization and adaptive radiation. Trends Ecol. Evol. (Amst.) 19:198–207.

Shaw, K. L. 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: What mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. Proc Natl Acad Sci U S A 99:16122–16127.

Simion, P., K. Belkhir, C. François, J. Veyssier, J. C. Rink, M. Manuel, H. Philippe, and M. J. Telford. 2018. A software tool 'CroCo' detects pervasive cross-species contamination in next generation sequencing data. BMC Biol 16.

Sloan, D. B., J. C. Havird, and J. Sharbrough. 2017. The on-again, off-again relationship between mitochondrial genomes and species boundaries. Molecular Ecology 26:2212–2236.

Smith, T. D., J. B. Rossie, and K. P. Bhatnagar. 2007. Evolution of the nose and nasal skeleton in primates. Evolutionary Anthropology: Issues, News, and Reviews 16:132–146.

Smith-Unna, R., C. Boursnell, R. Patro, J. M. Hibberd, and S. Kelly. 2016. TransRate: reference-free quality assessment of de novo transcriptome assemblies. Genome Res 26:1134–1144.

Song, L., and L. Florea. 2015. Rcorrector: efficient and accurate error correction for Illumina RNA-seq reads. GigaScience 4:48.

Sonnhammer, E. L. L. 1998. A Hidden Markov Model for Predicting Transmembrane Helices in Protein Sequences.

Sota, T. 2002. Radiation and reticulation: extensive introgressive hybridization in the carabid beetles Ohomopterus inferred from mitochondrial gene genealogy. Popul Ecol 44:0145–0156.

Spengel, J. W. 1881. Die Geruchsorgane und dasNervensystem der Mollusken. Z. Wiss. Zool 333–383.

Spengler, H. A., and A. J. Kohn. 1995. Comparative external morphology of the Conus osphradium (Mollusca: Gastropoda). Journal of Zoology 235:439–453.

Spielman, S. J., and C. O. Wilke. 2013. Membrane Environment Imposes Unique Selection Pressures on Transmembrane Domains of G Protein-Coupled Receptors. J Mol Evol 76:172–182.

Stanley, S. M. 2007. An Analysis of the History of Marine Animal Diversity. Paleobiology 33:1–55.

Stewart, J., and W. F. Gilly. 2005. Piscivorous Behavior of a Temperate Cone Snail, *Conus californicus*. The Biological Bulletin 209:146–153.

Strickland, J. L., C. F. Smith, A. J. Mason, D. R. Schield, M. Borja, G. Castañeda-Gaytán, C. L. Spencer, L. L. Smith, A. Trápaga, N. M. Bouzid, G. Campillo-García, O. A. Flores-Villela, D. Antonio-Rangel, S. P. Mackessy, T. A. Castoe, D. R. Rokyta, and C. L. Parkinson.

2018. Evidence for divergent patterns of local selection driving venom variation in Mojave Rattlesnakes (Crotalus scutulatus). Scientific Reports 8:17622.

Tait Cheyenne, Batra Srishti, Ramaswamy Sree Subha, Feder Jeffrey L., and Olsson Shannon B. 2016. Sensory specificity and speciation: a potential neuronal pathway for host fruit odour discrimination in Rhagoletis pomonella. Proceedings of the Royal Society B: Biological Sciences 283:20162101.

Tester, M., and P. Langridge. 2010. Breeding Technologies to Increase Crop Production in a Changing World. Science 327:818–822.

Thankaswamy-Kosalai, S., P. Sen, and I. Nookaew. 2017. Evaluation and assessment of read-mapping by multiple next-generation sequencing aligners based on genome-wide characteristics. Genomics 109:186–191.

Toews, D. P. L., and A. Brelsford. 2012. The biogeography of mitochondrial and nuclear discordance in animals. Molecular Ecology 21:3907–3930.

Vallejo, B. 2005. Inferring the mode of speciation in Indo-West Pacific Conus (Gastropoda: Conidae). Journal of Biogeography 32:1429–1439.

van Dongen, S., and C. Abreu-Goodger. 2012. Using MCL to Extract Clusters from Networks. Pp. 281–295 *in* J. van Helden, A. Toussaint, and D. Thieffry, eds. Bacterial Molecular Networks: Methods and Protocols. Springer New York, New York, NY.

Walderon, M. D., K. J. Nolt, R. E. Haas, K. N. Prosser, J. B. Holm, G. T. Nagle, and J. G. Boal. 2011. Distance chemoreception and the detection of conspecifics in Octopus bimaculoides. J Molluscan Stud 77:309–311.

Weese, D. A., and T. F. Duda. 2019. Effects of Predator-Prey Interactions on Predator Traits: Differentiation of Diets and Venoms of a Marine Snail. Toxins 11:299.

Williams, S. T., and T. F. Duda. 2008. Did tectonic activity stimulate oligo-miocene speciation in the Indo-West Pacific? Evolution; international journal of organic evolution 62:1618–1634.

Wong, E. S. W., and K. Belov. 2012. Venom evolution through gene duplications. Gene 496:1–7.

Yang, Y., and S. A. Smith. 2014. Orthology Inference in Nonmodel Organisms Using Transcriptomes and Low-Coverage Genomes: Improving Accuracy and Matrix Occupancy for Phylogenomics. Mol Biol Evol 31:3081–3092.

Yang, Z. 2007. PAML 4: Phylogenetic Analysis by Maximum Likelihood. Mol Biol Evol 24:1586–1591.

Yu, G., D. K. Smith, H. Zhu, Y. Guan, and T. T.-Y. Lam. 2017. ggtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods in Ecology and Evolution 8:28–36.

Zhang, W., K. K. Dasmahapatra, J. Mallet, G. R. P. Moreira, and M. R. Kronforst. 2016. Genome-wide introgression among distantly related Heliconius butterfly species. Genome Biology 17:25.

FIGURES AND TABLES

Table 1.1. Summary of Conidae taxa sequenced.

Species	n	Tissue	Sex	Collection locale(s)
Conus miliaris	22	Venom duct	12♂, 10♀	Easter Island; Guam; American Samoa
Conus coronatus	2	Venom duct	2♀	American Samoa
Conus ebraeus	2	Venom duct	1♂, 1♀	Okinawa
Conus judaeus	2	Venom duct	1♂, 1♀	Okinawa
Conus chaldaeus	1	Osphradium	1♀	Okinawa
Conus abbreviatus	1	Venom duct	1♂	Hawaii
Conus aristophanes	2	Venom duct	1♂, 1♀	American Samoa
Conus fulgetrum	2	Venom duct	2♀	Okinawa
Outgroup species				
Conus morderiae	2	Venom duct	1♂, 1♀	Cape Verde
Conus regonae	2	Venom duct	1♂, 1♀	Cape verde

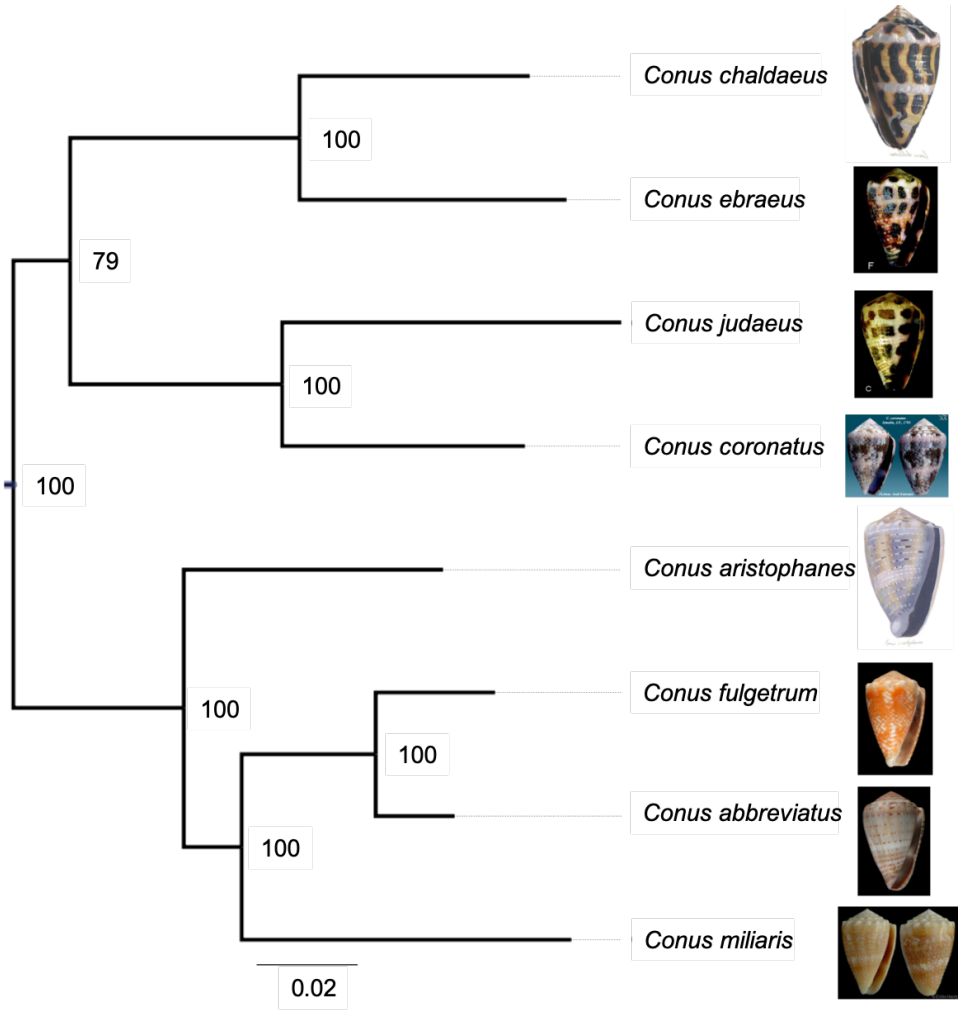

Figure 1.1. Maximum likelihood IQ-TREE phylogeny constructed from eight concatenated mitochondrial genes. Bootstrap support values are presented at each node. Tree is rooted using *C. regonae* and *C. morderiae* as outgroups (not shown). Shell illustrations by John Megahan.

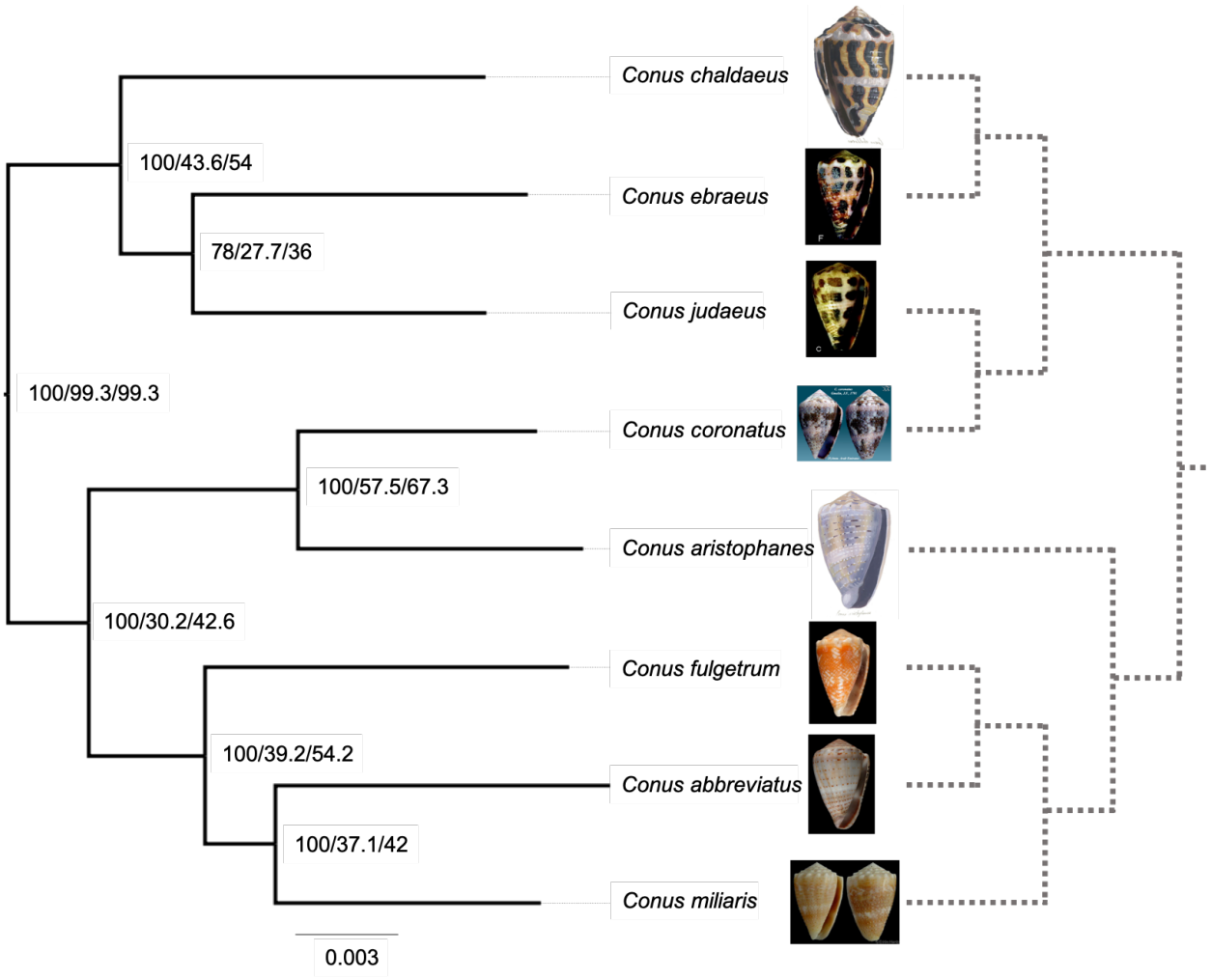

Figure 1.2. Maximum likelihood IQ-TREE phylogeny constructed from concatenated supermatrix of 2216 nuclear loci. Branch support values are indicated at each node (Bootstrap/gene concordance factor/site concordance factor). The mitochondrial tree shown in gray dashed lines to illustrate discordance between mitochondrial and nuclear topologies.

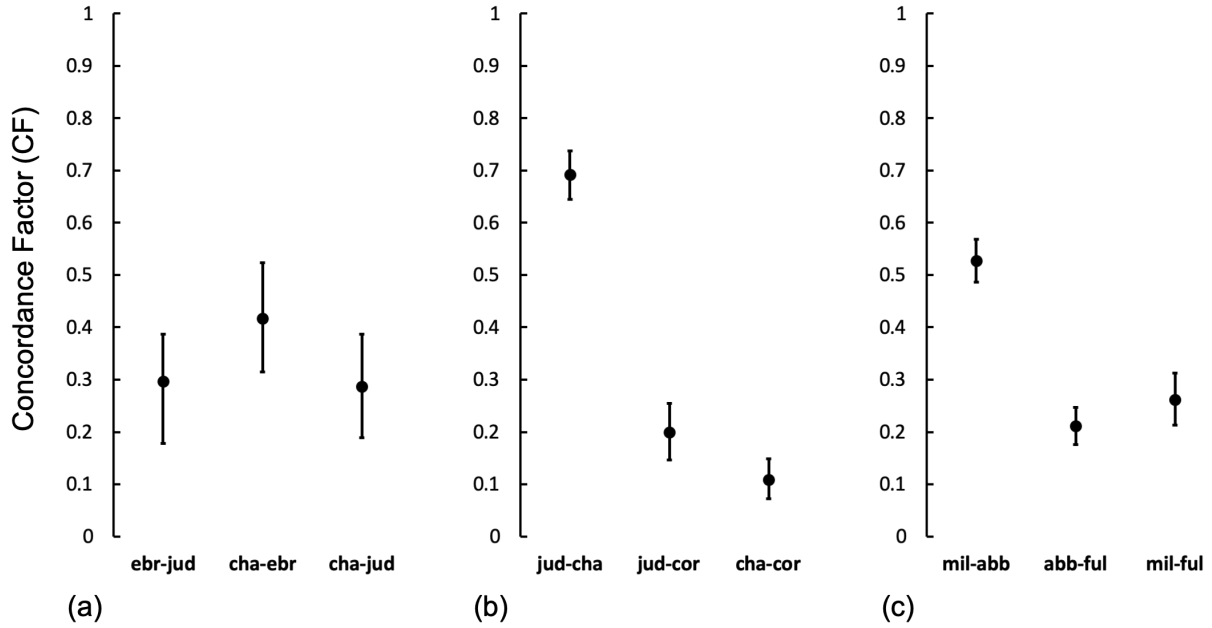

Figure 1.3. Results of Bayesian concordance analysis conducted with BUCKy. Error bars show 95% confidence intervals (CI). **(a)** CFs of 111 housekeeping loci for the *ebraeus* clade. Overlapping confidence intervals for all topologies (including primary concordance tree) in the *ebraeus* clade show widespread discordance and no signal of introgression. **(b)** CFs of 470 housekeeping loci for the *judaeus-coronatus* taxon subset. High CF (CF=0.692; CI: *0.645-0.738*) for the *C. judaeus-C. chaldaeus* pairing confirms the results of the nuclear phylogeny, and nearly non-overlapping CIs for the *C. judaeus-C. coronatus* (CF=0.199; CI: *0.146-0.255*) and *C. chaldaeus-C. coronatus* (CF=0.109; CI: *0.072-0.149*) pairings strongly suggest introgression between *C. judaeus* and *C. coronatus*. **(c)** CFs of 669 housekeeping loci for the *miliaris* clade. A distinctly higher CF (CF=0.527; CI: *0.486-0.568*) for the *C. miliaris-C. abbreviatus* pairing confirms the results of the nuclear phylogeny. The CF for the *C. miliaris-C. fulgetrum* pairing (CF=0.262; CI: *0.213-0.313*) is higher than for the *C. abbreviatus-C. fulgetrum* pairing (CF=0.211; CI: *0.176-0.247*), but their CIs overlap substantially, and therefore do not strongly indicate introgression. All BCA used *C. mordeirae* as the outgroup species for tree-building.

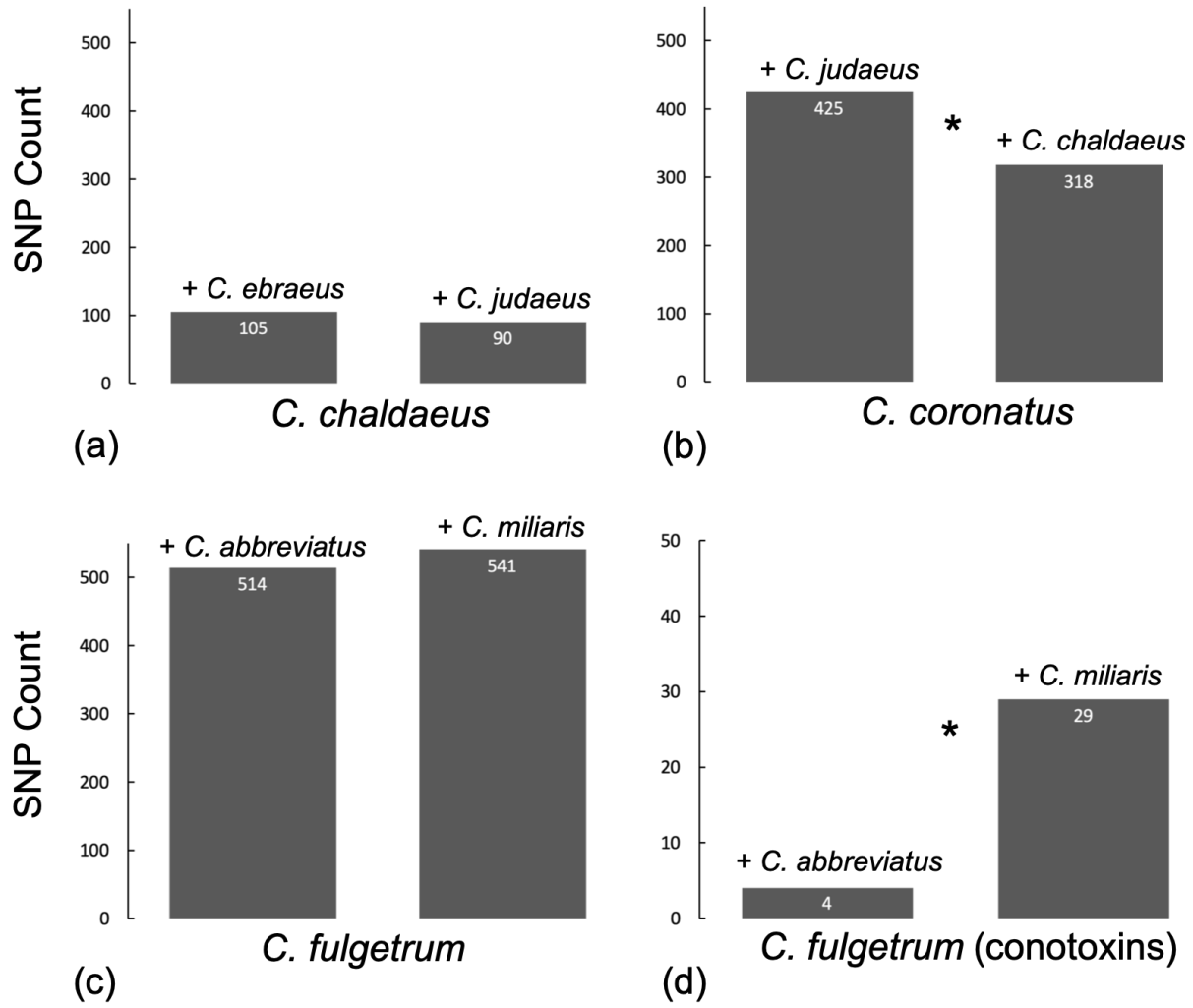

Figure 1.4. D-statistic tests of introgression. Counts of SNPs with ABBA-BABA patterns are shown for each taxon subset are shown. The *ebraeus* clade **(a)** exhibits no evidence of introgression from *C. chaldaeus* among housekeeping loci (*D*=0.077; *p-value*=0.283). In the *judaeus-coronatus* subset **(b)** we recovered a significant D-statistic (*D*=0.144; *p-value*=8.66E-05) revealing introgression between *C. judaeus* and *C. coronatus*. The *miliaris* clade **(c)** shows no evidence of introgression from *C. fulgetrum* among housekeeping loci (*D*=-0.026; *p-value*=0.406). Among conotoxin loci, the *miliaris* clade **(d)** exhibits significant evidence of introgression between *C. fulgetrum* and *C. miliaris* (*D*=-0.758; *p-value*=1.35E-05). *C. mordeirae* was used as the outgroup species for all D-statistic calculations.

CHAPTER 2

The Olfactory Gene Repertoire of *Conus ebraeus*

with Thomas F. Duda Jr.

ABSTRACT

Sensory biology is a critical component of organismal fitness, and olfaction is one of the most prominent sensory modalities. Olfaction plays an important role in the ecological specialization of many taxa and has been identified as an adaptive trait contributing to species divergence. Such may be the case in predatory marine snails in the family Conidae – "cone snails" - where olfaction likely plays a complementary role to venom in prey capture and dietary specialization as part of a broader predatory phenotype. In this study we sequence RNA from the primary olfactory organ of *Conus ebraeus*, one of the most widely distributed species of cone snail to characterize genes underlying olfaction and gain insight into the contribution of olfaction to diversification of this species rich group. We find a diverse gene family of 88 candidate olfactory receptor genes organized in two main subfamilies. We test several hypotheses regarding rates of evolution throughout this gene family, finding evidence for variable rates throughout the tree. Finally, we characterize the expression profile of the gene family, revealing a pattern of heterogeneous gene expression. Our thorough characterization of olfactory receptor genes in *C. ebraeus* provides novel

insights into adaptive trait evolution and its contribution to ecological specialization of cone snails, while laying a solid foundation for future work.

INTRODUCTION

An organism's ability to detect and interpret information from its environment to find mates, secure food resources, identify suitable habitat, and avoid predators is a critical component of fitness. Of the various sensory modalities, chemosensation – the detection of chemical signals – may encounter the most diverse information landscape since all organisms must interact with an extremely wide array of chemical compounds whose ecological relevance depends on their identity, concentration, cooccurrence with other compounds, and any number of other contextual cues (Bargmann 2006). Chemosensory systems must sample these inputs and extract relevant information for the organism to react appropriately (Firestein 2001). Chemoreception is commonly subdivided into gustation (contact chemoreception) and, the focus of this study, olfaction (distance chemoreception). Studies of olfaction not only enhance our understanding of organismal ecology and life history, but also inform our knowledge of broader concepts in ecology and evolutionary biology. Olfactory cues can contribute to population- and community-level structuring as is the case with induced behaviors caused by predator or competitor chemical signaling (Hay 2009; Haswell et al. 2018). Olfactory systems are also critical to evolutionary processes such as speciation, where they often play roles in conspecific recognition (Rafferty and Boughman 2006; Walderon et al. 2011) or niche partitioning (Proffit et al. 2007; Tait et al. 2016). And finally, they facilitate adaptation to novel niches thereby driving ecological diversification (Hayden et al. 2010; Khan et al. 2015).

Anatomical structures associated with olfaction have been key to understanding its function and evolution (Bertmar 1969; Smith et al. 2007), but because the operative scale is molecular, a granular understanding of olfaction requires a focus on olfactory receptor

(OR) proteins and the genes that encode them. The OR gene superfamily was first characterized by Buck & Axel (1991) in rats, and subsequent studies quickly expanded our appreciation of the scale and complexity of the superfamily. OR genes are remarkably similar across the tree of life, with near universal reliance on 7-transmembrane domain G-protein coupled receptor (GPCR) proteins that initiate signal cascades when odorant molecules bind to extracellular domains (Hildebrand and Shepherd 1997; Ache and Young 2005). The importance of OR genes was revealed by initial studies in mammals, where they comprise ~2% of protein coding genes in mammalian genomes (Mombaerts 2004). The number of OR genes can vary dramatically among vertebrate taxa, with humans and mice possessing 388 and 1037 functional genes, respectively, likely reflecting the relative importance of these ORs to the sensory ecology of the species (Niimura and Nei 2005). Invertebrate OR genes originated independently from those of vertebrates, but share many structural and functional characteristics (Bargmann 2006).

OR gene families exhibit strong evidence of adaptive evolution across the tree of life. Rapid gene turnover and birth and death gene family evolution are common, along with elevated rates of non-synonymous substitutions indicative of positive selection (Gilad et al. 2003; Gardiner et al. 2008; Dong et al. 2009; Hussain et al. 2009; Ramasamy et al. 2016). Most studies of invertebrate OR genetics have been carried out with *Drosophila* and *Caenorhabditis*, with arthropods overall receiving the most attention (Robertson et al. 2003; Hansson and Stensmyr 2011). Large taxonomic gaps remain, however, especially among non-model systems. With ~75,000 described species, Mollusca is the second most diverse phylum of animals (Rosenberg 2014) and relies heavily on chemosensation (Kohn 1961) yet the genetic basis of olfaction in this group is poorly studied. This is changing

slowly with the application of high-throughput sequencing, which has produced valuable information about OR gene content and gene family organization from a few select taxa, including the California sea hare, *Aplysia californica* (Cummins et al. 2009).

Conidae – "cone snails" – is a hyperdiverse family of marine gastropods comprised of ~800 species (MolluscaBase 2019). Cone snails are predatory, and are well known for their use of venom to subdue prey and potentially defend against predators. Their circumtropical distribution has a center of exceptionally high diversity in the reef-rich waters of the Indo-West pacific, with most species placed in the genus *Conus* (Röckel et al. 1995). Originating in the vicinity of what is now France, Conidae has experienced several bursts of diversification (Kohn 1990), the most dramatic of which may have been triggered ~15 million years ago by the closure of the Tethys sea, an ancient sea that united the Mediterranean basin with the Indian ocean (Vallejo 2005; Williams and Duda 2008). Cone snails often occur with high species richness, and in sympatry with close relatives (Kohn 2001; Vallejo 2005), and under these conditions they finely partition prey resources, likely permitting coexistence. Their rapid diversification and ecological specialization make them a good example of adaptive radiation (Givnish and Sytsma 2000; Schluter 2000). Most attention to date has focused on the role of venom in facilitating this ecological specialization. However, olfactory adaptations that determine prey and habitat preference are likely to play equally important roles. Studies of the genetic basis of olfaction are therefore likely to enhance our understanding of *Conus* diversification.

Olfaction is central to the sensory ecology of *Conus.* Cone snails likely do not rely on vision while foraging, as their eye-structures are very primitive and gastropod vision generally is thought to be poor (Audesirk and Audesirk 1985). Furthermore, *Conus* species

typically spend daylight hours buried under substrate with only their siphon protruding, emerging for active hunting only at night (Kohn 1959). Indeed, olfaction is typically the foremost sense employed by marine invertebrates to detect food resources (Kohn 1961; Audesirk 1975; Kamio and Derby 2017). Mating behavior is not well understood in *Conus*, but chemosensation is critical to this behavior in other gastropods (Croll 1983; Clifford et al. 2003; Painter et al. 2004). Finally, most Conidae species disperse during a planktonic larval stage, as is common among marine invertebrates, and require appropriate chemical cues to settle out of the water column in a favorable habitat to undergo metamorphosis (Hadfield and Paul 2001; Hadfield 2011; Cahill and Koury 2016). To perform these olfactory functions most gastropods, including Conidae, rely on a highly-enervated bipectinate structure called the osphradium, first described by Spengel (1881), that is the primary organ in this group responsible for distance chemoreception (Copeland 1918; Kohn 1961; Croll 1983). Early studies by Copeland (1918) of whelks in the genus *Busycon* found evidence that cauterizing the osphradium prevented individuals from sensing prey cues introduced into the siphon opening. The osphradium in *Conus* is positioned in the mantle cavity at the base of the of the siphon next to the ctenidium (gill), directly in the path of the inhalant water flow (Spengler and Kohn 1995). *Conus* osphradium morphology exhibits substantial interspecific variation correlated with habitat and diet, suggesting some degree of ecological adaptation (Spengler and Kohn 1995). Olfaction has long been recognized to mediate cone snail predatory behavior, which can be initiated by introducing a snail to water from an aquarium housing prey, without the prey itself being present (Kohn 1956, 1959, 1961; Stewart and Gilly 2005). Moreover, prey choice trials demonstrate that snails can distinguish preferred prey items at a distance, and do so with

high taxonomic resolution (Kohn 1959). No studies, however, have characterized the genes encoding olfactory receptors that are expressed in the osphradium and which ultimately mediate behavioral responses to chemical cues in the environment.

To understand the genetic basis of olfaction in Conidae and to evaluate evolutionary mechanisms that contribute to genetic diversification we characterized the olfactory receptor genes that underlie distance chemoreception in one of the most widely distributed cone snail species, *Conus ebraeus*. Because olfaction likely functions synergistically with venom to facilitate adaptation to novel ecological niches in *Conus*, understanding the genetic basis of this trait will offer insights into the remarkable diversification of Conidae. We have three primary goals. First, we seek to identify candidate OR genes and reconstruct the evolutionary history of this gene family in *C. ebraeus*. Second, we aim to characterize the expression profile of these genes, examine individual variation in expression, and test for the differential expression of genes between males and females. And third, we assess patterns of nucleotide substitutions across the gene family tree to determine if *Conus* OR genes have undergone adaptive evolution as has been observed in other taxa.

METHODS

Taxon sampling, RNA-extraction, and sequencing

We collected *Conus ebraeus* individuals in Okinawa, Japan during the summer of 2015. Field-collected snails were brought back to the lab and placed in cups with 200mL seawater for up to ~48 hours, at which point they were sacrificed and dissected. Osphradia were placed in RNAlater (Invitrogen, Carlsbad, CA, USA) and stored temporarily in a refrigerator before being stored long-term at -80 C at the University of Michigan.

Specimens and associated tissues were deposited in the Mollusk Division collections at the University of Michigan Museum of Zoology. We selected a mixture of adult males and females for this study to evaluate any potential sex-based differences in OR gene expression. Information about *C. ebraeus* individuals is presented in Table 1. We pestle-homogenized osphradium tissue, and extracted total RNA using Trizol (Invitrogen, Carlsbad, CA, USA) following the supplier instructions. We then submitted RNA to the University of Michigan DNA Sequencing Core for quality assessment using a Bioanalyzer 2100 and for library preparation and indexing (Illumina Tru-Seq kit, San Diego, CA, USA). Samples were run on a single flowcell lane on an Illumina HiSeq4000.

Read processing, transcriptome assembly, and transcript filtering

We filtered raw data using the read processing and transcript filtering pipeline developed by Yang & Smith (2014). Reads were processed for each individual separately. Rcorrector (Song and Florea 2015) was used to correct suspected sequencing errors, with uncorrectable reads removed, followed by Trimmomatic v0.36 (Bolger et al. 2014) with default parameters to remove Ilumina sequencing adapters and low-quality reads. We binned reads as mitochondrial or nuclear DNA by mapping them to a custom database of nine complete *Conus* mitochondrial genomes with Bowtie2 v2.3.4.3 (Langmead and Salzberg 2012), and used FastQC v0.10.1 (Andrews 2010) to assess read-quality and read representation, and to cull over-represented sequences. We then pooled the filtered reads from all five individuals and assembled them *de novo* with Trinity v2.4.0 (Grabherr et al. 2011).

Before searching for candidate olfactory receptor genes, we filtered the *de novo* transcriptome to remove low-quality transcripts and transcripts lacking open reading frames (ORF). We used Transrate v1.0.3 (Smith-Unna et al. 2016) with default settings to determine assembly quality and remove poor-quality transcripts and applied custom scripts from the Yang & Smith (2014) pipeline to remove chimeric transcripts. We produced clusters of transcripts belonging to the same putative gene using Corset v1.07 (Davidson and Oshlack 2014) and selected the longest transcript from each cluster as the representative transcript for that gene. We predicted ORFs using TransDecoder v5.0.1 (Haas et al. 2013), setting minimum ORF length to 100 amino acids, and including homology searches to increase sensitivity for ORFs with functional significance. Two homology searches were included: a BLASTp search (Altschul et al. 1990) against a custom reference database comprised of all *Conus*, *Lottia gigantia*, and *Alpysia californica* protein sequences from the NCBI non-redundant database, and a search of the PFAM database (Finn et al. 2014) to identify homology with common protein motifs, including transmembrane domains. TransDecoder combines the results of these searches with ORF predictions to determine which transcripts should be retained. Finally, we reduced transcript redundancy for predicted coding sequences with CD-HIT-EST and a sequence identity cutoff of 0.99, and for predicted peptide sequences with CD-HIT and a sequence identity cutoff of 0.98 (Li and Godzik 2006; Fu et al. 2012).

OR gene identification

To identify candidate olfactory receptor genes, we applied criteria similar to those utilized by Cummins et al (2009) to identify OR genes from the gastropod *Aplysia californica*. These

include, (i) limited predicted peptide sequence similarity to previously characterized molluscan chemosensory receptor genes, (ii) presence of at least six transmembrane domains, and (iii) a full-length coding sequence with an initiating methionine and terminating stop codon. We first submitted the filtered transcripts to a BLASTx search (Gish and States 1993) as queries against a custom database of 1,830 putative molluscan OR protein sequences retrieved from the GenBank RefSeq database (O'Leary et al. 2016). We used a liberal e-value of 1e-4 to ensure that we captured sequences with even relatively low sequence identity. We then used TMHMM v2.0 (Sonnhammer 1998) to predict the number and location of helical transmembrane (TM) domains in the predicted peptide sequences of BLASTx hits and retained transcripts with six or more predicted TM domains. To obtain our final set of candidate genes, we extracted transcripts with an initiating methionine codon and terminating stop codon.

Phylogenetic inference

We examined the relationships of candidate OR genes by aligning their predicted peptide sequences and inferring a maximum-likelihood phylogeny. To build a phylogeny of the entire set of genes, we aligned predicted peptide sequences, along with sequences of three *Aplysia californica* candidate OR genes – one from each subfamily identified by Cummins (2009) – using the aligner T-COFFEE in PSI/TM mode (Notredame et al. 2000; Floden et al. 2016). This method utilizes a reference database of transmembrane proteins to inform the alignment of predicted transmembrane proteins. We produced a matching nucleotide alignment using MACSE v2.03 (Ranwez et al. 2011), which uses the coding sequence that corresponds to a predicted peptide sequence to back-translate a protein alignment to a

nucleotide alignment. We selected best-fit substitution models for each dataset (nucleotide and protein) using ModelFinder (Kalyaanamoorthy et al. 2017) according to BIC, and inferred phylogenies using IQ-TREE (Nguyen et al. 2015) with 1000 bootstrap replicates. We produced both an unrooted star phylogeny and a rooted phylogram.

Substantial divergence among some *C. ebraeus* candidate OR genes and from *Aplysia* outgroup genes resulted in alignments with numerous gaps that necessitated removal of a large number of sites, reducing the number of phylogenetically informative sites available to resolve relationships within clades of more recently diverged OR genes. We therefore extracted sequences of genes comprising each of the two main clades in the full tree, realigned these subsets separately, and inferred trees for each clade using the same methodology as described above. This combined method allowed us to maximize the number of informative sites while retaining the larger scale topological features inferred from the comprehensive alignment.

Gene expression

We evaluated gene expression patterns of candidate OR genes using transcript quantification scripts packaged with Trinity (Haas et al. 2013). We performed two separate analyses, one in which we quantify expression with reads pooled from all individuals, and one with the reads from each individual assessed separately. We mapped filtered read fastq files to the filtered *C. ebraeus* transcriptome using the Perl script align_and_estimate_abundance.pl with *--est_method* set to RSEM (Li and Dewey 2011) and *--aln_method* set to Bowtie2 (Langmead and Salzberg 2012). We used the R package ggtree (Yu et al. 2017) to produce heatmaps illustrating patterns of gene expression and link them

to the OR gene phylogeny. We performed two differential expression analyses, one between males and females, and one between individuals from the two different collection sites, Cape Bise and Sesoko Station. We used the Perl script run_DE_analysis.pl (also packaged with Trinity) with the edgeR method (Robinson et al. 2010; McCarthy et al. 2012) and individuals from different sexes or sites designated as biological replicates.

Rates of evolution

We used the codeml program in PAML v4.9 (Yang 2007) to evaluate different scenarios of *C. ebraeus* OR gene family evolution. PAML measures the proportion of synonymous substitutions per synonymous site (d_S) and the proportion of nonsynonymous substitutions per nonsynonymous site (d_N) along branches of the gene tree and then calculates the ratio of d_N/d_S (ω) for a given branch. By calculating the likelihood of different models with ω estimated for different combinations of branches, and then performing likelihood ratio tests (LRT) comparing those models, it is possible to discern patterns of evolution experienced by different parts of the gene family. We evaluated three sets of models, which are summarized in Table 2. One set focuses on the whole tree, including outgroup sequences. The other two interrogate patterns of evolution within each subfamily and were considered separately to take advantage of higher quality sequences comprised of more closely related sequences. We utilized our previously generated nucleotide alignments for codeml analysis, but manually trimmed them to remove full codons that were not represented by most OR genes to minimize the number of gaps. We used FigTree v1.4.3 (Rambaut 2009) to designate branches for which different ω values should be estimated.

In the whole-tree model set, the M0 represents a null hypothesis with a uniform rate of evolution across the tree by estimating a single ω for all branches. M1 estimates two ω values, one for *Aplysia* sequences, and one for *C. ebraeus*. M2 estimates two additional ω values – one each subfamily. Comparisons among these models will reveal whether *C. ebraeus* genes are evolving at a different rate from *Aplysia* and whether the two subfamilies are evolving at different rates from one another. The models for each subfamily test if the overall rate of evolution for each subfamily is consistent with positive or purifying selection, and if different clades exhibit different rates of evolution . Subfamily M0 estimates a single ω for all branches, M1 fixes ω at a value of one for all branches, and M2 estimates a different ω for each designated sub-clade in a subfamily (four in subfamily one, and three in subfamily 2).

RESULTS

Read processing, transcriptome assembly, transcript filtering, and OR gene prediction

We produced a high-quality transcriptome from the osphradia of five *C. ebraeus* individuals. The average number of raw reads per individual was 15,350,522. Following read-processing we were left with an average of 7,800,517 reads per individual and a total of 40,400,587 reads to pool for our Trinity assembly. Assembled *de novo*, these reads produced a raw transcriptome of 429,975 transcripts, which was reduced by subsequent filtering steps to a final dataset of 35,143 transcripts. From this final transcript set we recovered a total of 4,234 with significant BLASTx hits to protein sequences in our database of molluscan OR genes. We predicted at least six TM domains in 308 transcripts.

Of these, 88 contained an initiating methionine and terminating stop codon, thereby satisfying our criteria for inclusion as candidate OR genes.

Phylogenetic analyses

To evaluate the relationships among candidate OR genes, we constructed maximum likelihood phylogenies from predicted amino acid dataset using IQ-TREE (Figure 1). The tree topology is characterized by two major clades: subfamily one comprising the majority (57) of candidate loci, and subfamily two with the remainder (31). The concentration of loci and short branch lengths observed in subfamily one suggests rapid diversification relative to subfamily two, which is sparser and has longer branch lengths. Most nodes exhibit high bootstrap support, with one exception being the first node of subfamily one.

Gene Expression

To characterize expression patterns of the 88 candidate *C. ebraeus* OR genes, we performed two analyses, one with reads pooled for all individuals and one with the individuals analyzed separately (Figure 3). The expression profile produced from pooled reads shows heterogeneous expression across the tree, with more genes that are highly expressed clustering in subfamily one, particularly within its largest subclade, and fewer highly expressed genes in subfamily 2 (Figure 4). The highest expression values calculated from pooled reads for subfamily one and subfamily two are 33.37 transcripts per million (TPM) and 29.71 TPM respectively, and the lowest are 1.13 TPM and 1.33 TPM. The mean expression values are 7.75 TPM (stdev: 6.72) and 6.07 TPM (stdev: 5.07) for subfamilies one and two respectively, with the large standard deviations highlighting wide variability.

Expression varies among individuals, but broadly recapitulates the pattern observed from pooled reads. No qualitative difference is apparent between male and female expression profiles, and differential expression analysis did not identify any loci that were expressed at significantly different levels between the sexes (no loci meet $p<0.05$ threshold).

Rates of evolution

The likelihood and omega values calculated from our codeml analysis for each model are reported in Table 2, and LRT comparing models are reported in Table 3. For our hypotheses regarding the whole OR gene tree we find that M1, which estimates separate ω values for the *C. ebraeus* OR gene family and *A. californica* sequences, is significantly better at explaining the data than the model (M0) estimating a single ω for the whole tree. However, our model estimating separate ω values for subfamilies one and two (M2) was no better at explaining the data than M1.

Our subfamily focused analyses reveal heterogeneous rates of evolution within each gene family. For subfamily one, the model estimating a single ω value for all branches (M0) does significantly worse than the model estimating a separate ω value for each major clade (M2). The model fixing ω at one for all branches also does significantly worse than M2. We see the same overall pattern for subfamily two, although the comparison of M0 and M2 has a higher p-value.

DISCUSSION

In this study, we characterize the OR gene repertoire of *Conus ebraeus* by sequencing osphradium RNA from five individuals, revealing a diverse set of loci. We document

heterogeneous patterns of gene expression across this newly characterized gene family, and examine several evolutionary scenarios across the tree, finding varying rates of evolution in different regions. Olfaction plays a central role in cone snail ecology, ranging from conspecific recognition, habitat selection, and prey specialization, and this study provides a much-needed genetic perspective, offering rich opportunities to explore adaptive trait evolution in Conidae and the contributions of olfaction to its remarkable diversification.

OR genes of Conus ebraeus

The OR gene family of *C. ebraeus* is organized into two main subfamilies, one representing the majority of candidate loci (57) and exhibiting signs of rapid diversification (subfamily one), and the other comprised of the remaining 31 loci with much longer branch lengths. The number of candidate loci (88) that we identified is comparable to that found for the gastropod *Aplysia californica* (90) (Cummins et al. 2009). When we performed a BLASTx search using only these *A. californica* genes as a reference database, we recovered only about half of the 88 loci that we identified when using a database of all molluscan OR genes retrieved from Genbank. The three *Aplysia* genes that we include in our phylogeny remain separate from the *C. ebraeus* candidate OR genes, forming their own distinct clade (Figure 1a). Due to the rapid evolution of these genes and the age of the most recent common ancestor of *A. californica* and *C. ebraeus*, any signal of sequence similarity may simply have been lost. Birth and death dynamics of gene family evolution common to OR gene families may have also reorganized the OR gene diversity to produce the tree structure we observe. The OR gene families of vertebrates, insects, and nematodes were recruited independently

from other membrane-bound signaling proteins (Bargmann 2006), but subsequent divergence of gene family composition and organization is likely due to processes such as gene duplication and loss. As such, it is unlikely the relationship between *C. ebraeus* and *A. californica* OR gene families is the result of an independent origin of these genes in either taxon, but rather the outcome of millions of years of separate – probably rapid – gene family evolution.

OR gene family evolution

A qualitative examination of the gene family suggests that *C. ebraeus* OR genes are diversifying most rapidly in subfamily one. This clade not only contains the majority of candidate loci, but the branch lengths are much shorter than in subfamily two. Heterogeneous patterns of diversification are expected in a gene family that performs a variety of functions, from mate and prey recognition to detection of settlement cues. Genes that facilitate adaptation to novel ecological conditions may undergo bursts of diversification and strong selection, while more genes with more conserved functions evolving more slowly. The rapid diversification apparent in subfamily one suggests these genes as potential candidates for ecological adaptation. Our quantitative analysis of rates of evolution did not support different rates of evolution for subfamily one and two, nor did they show evidence of positive selection among any subclade within these subfamilies (Tables 2 & 3). We do see evidence in both subfamilies of variable rates of evolution among their respective subclades, particularly in subfamily one, consistent with the notion that subfamily one is the more evolutionarily dynamic region of the tree.

Our analyses using codeml were likely limited in power due to the large and divergent sets of genes we tested. Under such circumstances, alignment quality can be compromised by numerous gaps and signals of selection can become swamped by long periods of divergence as has been observed in other studies of OR gene family evolution (Hussain et al. 2009). Furthermore, unlike conotoxin genes which are short and exhibit rapid substitution rates across the majority of the coding sequence(Duda 2008), OR genes may only experience strong positive selection in small regions, such as extracellular domains (Spielman and Wilke 2013).

Because a transcriptomic approach will inevitably miss unexpressed and some low-expression loci, there are likely genes in the *C. ebraeus* OR repertoire that we did not detect. In addition to incomplete sampling of OR gene diversity, this also has implications for our ability to detect patterns of selection that may be apparent from comparisons of recently duplicated genes. Duplicated genes can undergo rapid expression divergence, especially in response to selection imposed by ecological factors (Ha et al. 2007), and if one paralog experiences dramatic down-regulation, it may go undetected. Without sequences of recently diverged loci for which there has not been a saturation of nonsynonymous substitutions, signals of positive selection may be missed. For these reasons, a genome is critical tool for the study of gene family evolution (Hahn et al. 2005), and one that we currently lack for *C. ebraeus*. Cummins et al (2009), for example, find differential expression of OR genes among *Aplysia californica* chemosensory organs, and likely would not have recovered the full complement of genes without their genomic approach. The certainty offered by a genome – with regard to both the presence/absence of genes and their relative physical location in the genome – provides opportunities to detect gene copy

number, pseudogenes, rates of gene turnover, and birth-and-death gene family evolution. Such dynamics are commonly observed in OR gene families in other taxa (Ramasamy et al. 2016) and have been documented among conotoxin genes in *Conus*, where they facilitate rapid evolution, and presumably ecological adaptation (Chang and Duda 2012).

Expression levels

We measured expression levels of candidate OR genes using both pooled reads and reads from each individual separately (Figure 2). The OR expression profile derived from the pooled-read dataset reveals a cluster of genes with higher expression relative to the rest of the tree located in subfamily one, the clade undergoing rapid diversification. Although the mean expression values are similar between subfamilies, subfamily two has fewer loci that exhibit unusually high expression (Figure 3). Individual profiles exhibit some variability in gene expression, but all five reproduce the same general pattern observed from pooled reads. We do not detect any differentially expressed loci between male and female *C. ebraeus*, but this may be due to the small number of individuals sampled and a follow-up study using more individuals would increase confidence in these results.

Changes in gene expression can produce major shifts in phenotype and likely contribute to adaptive evolution (Gilad et al. 2006; Holloway et al. 2007; Fay and Wittkopp 2008). For a trait such as olfaction, where the phenotype is comprised of direct gene products (i.e. membrane-bound olfactory receptor proteins) and tightly linked to organismal ecology, small changes in expression may have dramatic fitness effects. Deeper studies of OR gene expression are therefore likely to yield valuable insights into the diversification of *Conus*. For example, Chang and Duda (2016) detected age-related shifts in

conotoxin gene expression in *C. ebraeus* using qPCR. A similar study focused on OR gene expression at different life stages would reveal whether their expression shifts in parallel with conotoxin genes, lending support to the hypothesis that these two traits play a concerted role in predation. Studies of OR gene expression in the planktonic life stage of *Conus* species could provide clues about which genes are important for detecting settlement cues, knowledge with applications not only to the biogeography of Conidae, but many other marine taxa that disperse in the plankton.

Implications for ecological adaptation in Conus

Our results provide a scaffold for future work investigating the role of olfaction in ecological diversification of Conidae. The pipeline we developed can be easily and fruitfully applied to other *Conus* species for comparative studies of OR gene diversity, evolution and expression, and our expression and selection analyses point to candidate loci that warrant closer inspection in a comparative context. Because selection imposed by diet is known to impact venom composition and evolution in cone snails (Remigio and Duda 2008; Duda and Lee 2009; Chang et al. 2015), it would be particularly instructive to compare the OR gene repertoires of close *Conus* relatives with distinct diets to see if similar patterns are apparent in olfaction. Differences in OR gene composition, gene expression, or patterns of selection would reinforce the notion that olfaction plays a complementary role to venom in ecological adaptation.

It would also be useful to examine candidate OR gene expression in other putative chemosensory organs in *Conus* similar to Cummins et al (2009); the tip of the proboscis is one such structure. Microscopy reveals sensory papillae at the tip of the proboscis whose

morphology appear to differ based on prey preferences (James et al. 2014). Because the proboscis is deployed late in the chronology of prey capture, chemosensory receptor genes expressed there may differ from those we characterized in the osphradium, which detects chemical signals at a distance.

Until now researchers have focused almost exclusively on venom as the adaptive trait facilitating niche partitioning among cone snails. This study provides an entirely new perspective on adaptive trait evolution in Conidae by evaluating the genetic basis of another trait that likely contributes to dietary specialization in cone snails. We reveal the phylogenetic organization of the OR gene family in conus and interrogate patterns of gene family evolution and gene expression. In addition to the novel results generated, this study offers a new framework on which further comparative and experimental work exploring the broader predatory phenotype in Conidae may be built.

REFERENCES

Abbott, R., D. Albach, S. Ansell, J. W. Arntzen, S. J. E. Baird, N. Bierne, J. Boughman, A. Brelsford, C. A. Buerkle, R. Buggs, R. K. Butlin, U. Dieckmann, F. Eroukhmanoff, A. Grill, S. H. Cahan, J. S. Hermansen, G. Hewitt, A. G. Hudson, C. Jiggins, J. Jones, B. Keller, T. Marczewski, J. Mallet, P. Martinez-Rodriguez, M. Möst, S. Mullen, R. Nichols, A. W. Nolte, C. Parisod, K. Pfennig, A. M. Rice, M. G. Ritchie, B. Seifert, C. M. Smadja, R. Stelkens, J. M. Szymura, R. Väinölä, J. B. W. Wolf, and D. Zinner. 2013. Hybridization and speciation. Journal of Evolutionary Biology 26:229–246.

Ache, B. W., and J. M. Young. 2005. Olfaction: Diverse Species, Conserved Principles. Neuron 48:417–430.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. Journal of Molecular Biology 215:403–410.

Aman, J. W., J. S. Imperial, B. Ueberheide, M.-M. Zhang, M. Aguilar, D. Taylor, M. Watkins, D. Yoshikami, P. Showers-Corneli, H. Safavi-Hemami, J. Biggs, R. W. Teichert, and B. M.

Olivera. 2015. Insights into the origins of fish hunting in venomous cone snails from studies of Conus tessulatus. Proc Natl Acad Sci U S A 112:5087–5092.

Anderson, E., and G. L. Stebbins. 1954. Hybridization as an Evolutionary Stimulus. Evolution 8:378–388.

Andrews, S. 2010. FastQC: a quality control tool for high throughput sequence data. Available online at http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Ané, C., B. Larget, D. A. Baum, S. D. Smith, and A. Rokas. 2007. Bayesian Estimation of Concordance among Gene Trees. Mol Biol Evol 24:412–426.

Arnold, M. L. 2004. Transfer and Origin of Adaptations through Natural Hybridization: Were Anderson and Stebbins Right? The Plant Cell 16:562–570.

Audesirk, T., and G. Audesirk. 1985. 1 - Behavior of Gastropod Molluscs. Pp. 1–94 *in* A. O. D. Willows, ed. The Mollusca. Academic Press.

Audesirk, T. E. 1975. Chemoreception in Aplysia californica. I. behavioral localization of distance chemoreceptors used in food-finding. Behavioral Biology 15:45–55.

Babik, W., W. Branicki, J. Crnobrnja-Isailović, D. Cogălniceanu, I. Sas, K. Olgun, N. A. Poyarkov, M. Garcia-París, and J. W. Arntzen. 2005. Phylogeography of two European newt species — discordance between mtDNA and morphology. Molecular Ecology 14:2475–2491.

Baiz, M. D., P. K. Tucker, and L. Cortés-Ortiz. 2019. Multiple forms of selection shape reproductive isolation in a primate hybrid zone. Molecular Ecology 28:1056–1069.

Ballard, J. W. O., and M. C. Whitlock. 2004. The incomplete natural history of mitochondria. Molecular Ecology 13:729–744.

Barghi, N., G. P. Concepcion, B. M. Olivera, and A. O. Lluisma. 2016. Characterization of the complete mitochondrial genome of Conus tribblei Walls, 1977. Mitochondrial DNA A DNA Mapp Seq Anal 27:4451–4452.

Bargmann, C. I. 2006. Comparative chemosensation from receptors to ecology. Nature 444:295.

Bear, D. M., J.-M. Lassance, H. E. Hoekstra, and S. R. Datta. 2016. The Evolving Neural and Genetic Architecture of Vertebrate Olfaction. Current Biology 26:R1039–R1049.

Belton, J.-M., R. P. McCord, J. Gibcus, N. Naumova, Y. Zhan, and J. Dekker. 2012. Hi-C: A comprehensive technique to capture the conformation of genomes. Methods 58.

Bergh, R. 1895. Beiträge zur Kenntnis der Coniden. Nova Acta Kaiserl. Leopold.-Carol. deut. Akad. Naturf 65:67–214.

Bertmar, G. 1969. The Vertebrate Nose, Remarks on Its Structural and Functional Adaptation and Evolution. Evolution 23:131–152.

Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120.

Brand, P., and S. R. Ramírez. 2017. The Evolutionary Dynamics of the Odorant Receptor Gene Family in Corbiculate Bees. Genome Biology and Evolution 9:2023–2036.

Brawand, D., M. Soumillon, A. Necsulea, P. Julien, G. Csárdi, P. Harrigan, M. Weier, A. Liechti, A. Aximu-Petri, M. Kircher, F. W. Albert, U. Zeller, P. Khaitovich, F. Grützner, S. Bergmann, R. Nielsen, S. Pääbo, and H. Kaessmann. 2011. The evolution of gene expression levels in mammalian organs. Nature 478:343–348.

Brown, J. W., J. F. Walker, and S. A. Smith. 2017. Phyx: phylogenetic tools for unix. Bioinformatics 33:1886–1888.

Buck, L., and R. Axel. 1991. A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. Cell 65:175–187.

Cahill, A. E., and S. A. Koury. 2016. Larval settlement and metamorphosis in a marine gastropod in response to multiple conspecific cues. PeerJ 4.

Chang, D., and T. F. Duda. 2016. Age-related association of venom gene expression and diet of predatory gastropods. BMC Evolutionary Biology 16.

Chang, D., and T. F. Duda. 2012. Extensive and Continuous Duplication Facilitates Rapid Evolution and Diversification of Gene Families. Mol Biol Evol 29:2019–2029.

Chang, D., A. M. Olenzek, and T. F. Duda. 2015. Effects of geographical heterogeneity in species interactions on the evolution of venom genes. Proceedings of the Royal Society B: Biological Sciences 282:20141984–20141984.

Clifford, K. T., L. Gross, K. Johnson, K. J. Martin, N. Shaheen, and M. A. Harrington. 2003. Slime-trail tracking in the predatory snail, Euglandina rosea. Behavioral Neuroscience 117:1086–1095.

Copeland, M. 1918. The olfactory reactions and organs of the marine snails Alectrion obsoleta (Say) and Busycon canaliculatum (Linn.). Journal of Experimental Zoology 177–227.

Croll, R. P. 1983. Gastropod Chemoreception. Biological Reviews 58:293–319.

Cummins, S. F., D. Erpenbeck, Z. Zou, C. Claudianos, L. L. Moroz, G. T. Nagle, and B. M. Degnan. 2009. Candidate chemoreceptor subfamilies differentially expressed in the chemosensory organs of the mollusc Aplysia. BMC Biology 7:28.

Davidson, N. M., and A. Oshlack. 2014. Corset: enabling differential gene expression analysis for de novoassembled transcriptomes. Genome Biology 15:410.

Dierckxsens, N., P. Mardulyn, and G. Smits. 2017. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Res 45:e18–e18.

Dong, D., G. He, S. Zhang, and Z. Zhang. 2009. Evolution of Olfactory Receptor Genes in Primates Dominated by Birth-and-Death Process. Genome Biol Evol 1:258–264.

Duda Jr, T. F., and S. R. Palumbi. 2000. Evolutionary Diversification of Multigene Families: Allelic Selection of Toxins in Predatory Cone Snails. Molecular Biology and Evolution 17:1286–1293.

Duda, T. 2001. Origins of diverse feeding ecologies within Conus, a genus of venomous marine gastropods. Biological Journal of the Linnean Society 73:391–409.

Duda, T. F. 2008. Differentiation of Venoms of Predatory Marine Gastropods: Divergence of Orthologous Toxin Genes of Closely Related Conus Species with Different Dietary Specializations. J Mol Evol 67:315.

Duda, T. F., D. Chang, B. D. Lewis, and T. Lee. 2009a. Geographic variation in venom allelic composition and diets of the widespread predatory marine gastropod Conus ebraeus. PLoS ONE 4:e6245.

Duda, T. F., and A. J. Kohn. 2005. Species-level phylogeography and evolutionary history of the hyperdiverse marine gastropod genus Conus. Molecular Phylogenetics and Evolution 34:257–272.

Duda, T. F., A. J. Kohn, and A. M. Matheny. 2009b. Cryptic Species Differentiated in Conus ebraeus, a Widespread Tropical Marine Gastropod. The Biological Bulletin 217:292–305.

Duda, T. F., and T. Lee. 2009. Ecological Release and Venom Evolution of a Predatory Marine Snail at Easter Island. PLoS ONE 4:e5558.

Duda, T. F., and H. A. Lessios. 2009. Connectivity of populations within and between major biogeographic regions of the tropical Pacific in Conus ebraeus, a widespread marine gastropod. Coral Reefs 28:651–659.

Duda, T. F., and S. R. Palumbi. 2004. Gene expression and feeding ecology: evolution of piscivory in the venomous gastropod genus *Conus*. Proceedings of the Royal Society of London. Series B: Biological Sciences 271:1165–1174.

Duda, T. F., and S. R. Palumbi. 1999. Molecular genetics of ecological diversification: Duplication and rapid evolution of toxin genes of the venomous gastropod Conus. Proceedings of the National Academy of Sciences 96:6820–6823.

Duda, T. F., M. Terbio, G. Chen, S. Phillips, A. M. Olenzek, D. Chang, and D. W. Morris. 2012. Patterns of Population Structure and Historical Demography of Conus Species in the Tropical Pacific. American Malacological Bulletin 30:175–187.

Durand, E. Y., N. Patterson, D. Reich, and M. Slatkin. 2011. Testing for Ancient Admixture between Closely Related Populations. Mol Biol Evol 28:2239–2252.

Dutertre, S., A.-H. Jin, P. F. Alewood, and R. J. Lewis. 2014. Intraspecific variations in Conus geographus defence-evoked venom and estimation of the human lethal dose. Toxicon 91:135–144.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797.

Eltz, T., Y. Zimmermann, C. Pfeiffer, J. R. Pech, R. Twele, W. Francke, J. J. G. Quezada-Euan, and K. Lunau. 2008. An Olfactory Shift Is Associated with Male Perfume Differentiation and Species Divergence in Orchid Bees. Current Biology 18:1844–1848.

Enright, A. J., S. Van Dongen, and C. A. Ouzounis. 2002. An efficient algorithm for large-scale detection of protein families. Nucleic Acids Res 30:1575–1584.

Fay, J. C., and P. J. Wittkopp. 2008. Evaluating the role of natural selection in the evolution of gene regulation. Heredity 100:191–199.

Felsenstein, J. 1974. The Evolutionary Advantage of Recombination. Genetics 78:737–756.

Finn, R. D., A. Bateman, J. Clements, P. Coggill, R. Y. Eberhardt, S. R. Eddy, A. Heger, K. Hetherington, L. Holm, J. Mistry, E. L. L. Sonnhammer, J. Tate, and M. Punta. 2014. Pfam: the protein families database. Nucleic Acids Research 42:D222–D230.

Firestein, S. 2001. How the olfactory system makes sense of scents. Nature 413:211.

Floden, E. W., P. D. Tommaso, M. Chatzou, C. Magis, C. Notredame, and J.-M. Chang. 2016. PSI/TM-Coffee: a web server for fast and accurate multiple sequence alignments of regular and transmembrane proteins using homology extension on reduced databases. Nucleic Acids Res 44:W339–W343.

Fontaine, M. C., J. B. Pease, A. Steele, R. M. Waterhouse, D. E. Neafsey, I. V. Sharakhov, X. Jiang, A. B. Hall, F. Catteruccia, E. Kakani, S. N. Mitchell, Y.-C. Wu, H. A. Smith, R. R. Love, M. K. Lawniczak, M. A. Slotman, S. J. Emrich, M. W. Hahn, and N. J. Besansky. 2015. Extensive introgression in a malaria vector species complex revealed by phylogenomics. Science 347:1258524.

Fourment, M., and E. C. Holmes. 2016. Seqotron: a user-friendly sequence editor for Mac OS X. BMC Research Notes 9:106.

Fu, L., B. Niu, Z. Zhu, S. Wu, and W. Li. 2012. CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics 28:3150–3152.

Galtier, N., B. Nabholz, S. Glémin, and G. D. D. Hurst. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. Molecular Ecology 18:4541–4550.

Gardiner, A., D. Barker, R. K. Butlin, W. C. Jordan, and M. G. Ritchie. 2008. Drosophila chemoreceptor gene evolution: selection, specialization and genome size. Molecular Ecology 17:1648–1657.

Gemmell, N. J., V. J. Metcalf, and F. W. Allendorf. 2004. Mother's curse: the effect of mtDNA on individual fitness and population viability. Trends in Ecology & Evolution 19:238–244.

Gilad, Y., C. D. Bustamante, D. Lancet, and S. Pääbo. 2003. Natural Selection on the Olfactory Receptor Gene Family in Humans and Chimpanzees. The American Journal of Human Genetics 73:489–501.

Gilad, Y., A. Oshlack, G. K. Smyth, T. P. Speed, and K. P. White. 2006. Expression profiling in primates reveals a rapid evolution of human transcription factors. Nature 440:242.

Gish, W., and D. J. States. 1993. Identification of protein coding regions by database similarity search. Nature Genetics 3:266.

Givnish, T. J., and K. J. Sytsma. 2000. Molecular Evolution and Adaptive Radiation. Cambridge University Press.

Goldstein, P. Z., and R. Desalle. 2003. Calibrating phylogenetic species formation in a threatened insect using DNA from historical specimens. Molecular Ecology 12:1993–1998.

Good, J. M., D. Vanderpool, S. Keeble, and K. Bi. 2015. Negligible nuclear introgression despite complete mitochondrial capture between two species of chipmunks. Evolution 69:1961–1972.

Grabherr, M. G., B. J. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, L. Fan, R. Raychowdhury, Q. Zeng, Z. Chen, E. Mauceli, N. Hacohen, A. Gnirke, N. Rhind, F. di Palma, B. W. Birren, C. Nusbaum, K. Lindblad-Toh, N. Friedman, and A. Regev. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. 29:644–652.

Green, R. E., J. Krause, A. W. Briggs, T. Maricic, U. Stenzel, M. Kircher, N. Patterson, H. Li, W. Zhai, M. H.-Y. Fritz, N. F. Hansen, E. Y. Durand, A.-S. Malaspinas, J. D. Jensen, T. Marques-Bonet, C. Alkan, K. Prüfer, M. Meyer, H. A. Burbano, J. M. Good, R. Schultz, A. Aximu-Petri, A. Butthof, B. Höber, B. Höffner, M. Siegemund, A. Weihmann, C. Nusbaum, E. S. Lander, C. Russ, N. Novod, J. Affourtit, M. Egholm, C. Verna, P. Rudan, D. Brajkovic, Ž. Kucan, I. Gušic, V. B. Doronichev, L. V. Golovanova, C. Lalueza-Fox, M. de la Rasilla, J. Fortea, A. Rosas, R. W. Schmitz, P. L. F. Johnson, E. E. Eichler, D. Falush, E. Birney, J. C. Mullikin, M. Slatkin, R. Nielsen, J. Kelso, M. Lachmann, D. Reich, and S. Pääbo. 2010. A Draft Sequence of the Neandertal Genome. Science 328:710–722.

Ha, M., W.-H. Li, and Z. J. Chen. 2007. External factors accelerate expression divergence between duplicate genes. Trends in Genetics 23:162–166.

Haas, B. J., A. Papanicolaou, M. Yassour, M. Grabherr, P. D. Blood, J. Bowden, M. B. Couger, D. Eccles, B. Li, M. Lieber, M. D. MacManes, M. Ott, J. Orvis, N. Pochet, F. Strozzi, N. Weeks, R. Westerman, T. William, C. N. Dewey, R. Henschel, R. D. LeDuc, N. Friedman, and A. Regev. 2013. De novo transcript sequence reconstruction from RNA-Seq: reference generation and analysis with Trinity. Nat Protoc 8.

Hadfield, M. G. 2011. Biofilms and Marine Invertebrate Larvae: What Bacteria Produce That Larvae Use to Choose Settlement Sites. Annual Review of Marine Science 3:453–470.

Hadfield, M., and V. Paul. 2001. Natural Chemical Cues for Settlement and Metamorphosis of Marine-Invertebrate Larvae. Pp. 431–461 *in* J. McClinTOCk and B. Baker, eds. Marine Chemical Ecology. CRC Press.

Hahn, M. W., T. D. Bie, J. E. Stajich, C. Nguyen, and N. Cristianini. 2005. Estimating the tempo and mode of gene family evolution from comparative genomic data. Genome Res. 15:1153–1160.

Hamilton, J. A., and J. M. Miller. 2016. Adaptive introgression as a resource for management and genetic conservation in a changing climate. Conservation Biology 30:33–41.

Hansson, B. S., and M. C. Stensmyr. 2011. Evolution of Insect Olfaction. Neuron 72:698–711.

Haswell, P. M., K. A. Jones, J. Kusak, and M. W. Hayward. 2018. Fear, foraging and olfaction: how mesopredators avoid costly interactions with apex predators. Oecologia 187:573–583.

Hay, M. E. 2009. Marine Chemical Ecology: Chemical Signals and Cues Structure Marine Populations, Communities, and Ecosystems. Annual Review of Marine Science 1:193–212.

Hayden, S., M. Bekaert, T. A. Crider, S. Mariani, W. J. Murphy, and E. C. Teeling. 2010. Ecological adaptation determines functional mammalian olfactory subgenomes. Genome Res. 20:1–9.

Hildebrand, J. G., and G. M. Shepherd. 1997. MECHANISMS OF OLFACTORY DISCRIMINATION: Converging Evidence for Common Principles Across Phyla. Annual Review of Neuroscience 20:595–631.

Holloway, A. K., M. K. N. Lawniczak, J. G. Mezey, D. J. Begun, and C. D. Jones. 2007. Adaptive Gene Expression Divergence Inferred from Population Genomics. PLOS Genetics 3:e187.

Hu, H., P. K. Bandyopadhyay, B. M. Olivera, and M. Yandell. 2011. Characterization of the Conus bullatus genome and its venom-duct transcriptome. BMC Genomics 12:60.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

Hussain, A., L. R. Saraiva, and S. I. Korsching. 2009. Positive Darwinian selection and the birth of an olfactory receptor clade in teleosts. PNAS 106:4313–4318.

James, D., C. A. Prator, G. G. Martin, and J. R. Schulz. 2014. Morphology of sensory papillae on the feeding proboscis of cone snails (Mollusca, Gastropoda). Invertebrate Biology 133:221–231.

Kaas, Q., J.-C. Westermann, R. Halai, C. K. L. Wang, and D. J. Craik. 2008. ConoServer, a database for conopeptide sequences and structures. Bioinformatics 24:445–446.

Kaas, Q., R. Yu, A.-H. Jin, S. Dutertre, and D. J. Craik. 2012. ConoServer: updated content, knowledge, and discovery tools in the conopeptide database. Nucleic Acids Res 40:D325–D330.

Kalyaanamoorthy, S., B. Q. Minh, T. K. F. Wong, A. von Haeseler, and L. S. Jermiin. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14:587–589.

Kamio, M., and C. D. Derby. 2017. Finding food: how marine invertebrates use chemical cues to track and select food. Natural Product Reports 34:514–528.

Khan, I., Z. Yang, E. Maldonado, C. Li, G. Zhang, M. T. P. Gilbert, E. D. Jarvis, S. J. O'Brien, W. E. Johnson, and A. Antunes. 2015. Olfactory Receptor Subgenomes Linked with Broad Ecological Adaptations in Sauropsida. Molecular Biology and Evolution 32:2832–2843.

Köhler, F., and G. Deein. 2010. Hybridisation as potential source of incongruence in the morphological and mitochondrial diversity of a Thai freshwater gastropod (Pachychilidae, Brotia H. Adams, 1866). Zoosystematics and Evolution 86:301–314.

Kohn, A. 2001. Maximal species richness in Conus: diversity, diet and habitat on reefs of northeast Papua New Guinea. Coral Reefs 20:25–38.

Kohn, A. 1990. Tempo and mode of evolution in Conidae. Malacologia 55–67.

Kohn, A. J. 1961. CHEMORECEPTION IN GASTROPOD MOLLUSCS. American Zoologist 1:291–308.

Kohn, A. J. 2014. Conus of the Southeastern United States and Caribbean. Princeton University Press.

Kohn, A. J. 1983. Microhabitat Factors Affecting Abundance and Diversity of Conus on Coral Reefs. Oecologia 60:293–301.

Kohn, A. J. 1956. PISCIVOROUS GASTROPODS OF THE GENUS CONUS. Proc. Natl. Acad. Sci. U.S.A. 42:168–171.

Kohn, A. J. 1959. The Ecology of Conus in Hawaii. Ecological Monographs 29:47–90.

Kohn, A. J., and J. W. Nybakken. 1975. Ecology of Conus on eastern Indian Ocean fringing reefs: Diversity of species and resource utilization. Marine Biology 29:211–234.

Langmead, B., and S. L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359.

Larget, B. R., S. K. Kotha, C. N. Dewey, and C. Ané. 2010. BUCKy: Gene tree/species tree reconciliation with Bayesian concordance analysis. Bioinformatics 26:2910–2911.

Lawler, A. J., and T. F. Duda. 2017. Molecular and morphometric data suggest the presence of a neglected species in the marine gastropod family Conidae. Molecular Phylogenetics and Evolution 109:421–429.

Leviten, P. J. 1978. Resource Partitioning by Predatory Gastropods of the Genus Conus on Subtidal Indo-Pacific Coral Reefs: The Significance of Prey Size. Ecology 59:614–631.

Lewontin, R. C., and L. C. Birch. 1966. Hybridization as a Source of Variation for Adaptation to New Environments. Evolution 20:315–336.

Li, B., and C. N. Dewey. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12:323.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. Durbin. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079.

Li, W., and A. Godzik. 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22:1658–1659.

Linn, C., J. L. Feder, S. Nojima, H. R. Dambroski, S. H. Berlocher, and W. Roelofs. 2003. Fruit odor discrimination and sympatric host race formation in Rhagoletis. Proceedings of the National Academy of Sciences 100:11490–11493.

Llopart, A., D. Herrig, E. Brud, and Z. Stecklein. 2014. Sequential adaptive introgression of the mitochondrial genome in Drosophila yakuba and Drosophila santomea. Mol Ecol 23:1124–1136.

Löytynoja, A., and N. Goldman. 2008. Phylogeny-Aware Gap Placement Prevents Errors in Sequence Alignment and Evolutionary Analysis. Science 320:1632–1635.

Lunter, G., and M. Goodson. 2011. Stampy: A statistical algorithm for sensitive and fast mapping of Illumina sequence reads. Genome Res 21:936–939.

Lynch, M., and R. Lande. 1998. The critical effective size for a genetically secure population. Animal Conservation 1:70–72.

Mallet, J. 2007. Hybrid speciation. Nature 446:279–283.

Martin, C. H., J. S. Cutler, J. P. Friel, C. D. Touokong, G. Coop, and P. C. Wainwright. 2015. Complex histories of repeated gene flow in Cameroon crater lake cichlids cast doubt on one of the clearest examples of sympatric speciation. Evolution 69:1406–1422.

Mayr, E. 1963. Animal Species and Evolution. Belknap Press of Harvard University Press, Cambridge, MA.

McBride, C. S. 2007. Rapid evolution of smell and taste receptor genes during host specialization in Drosophila sechellia. PNAS 104:4996–5001.

McCarthy, D. J., Y. Chen, and G. K. Smyth. 2012. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. Nucleic Acids Res 40:4288–4297.

Meier, J. I., D. A. Marques, S. Mwaiko, C. E. Wagner, L. Excoffier, and O. Seehausen. 2017. Ancient hybridization fuels rapid cichlid fish adaptive radiations. Nature Communications 8:14363.

Minh, B. Q., M. W. Hahn, and R. Lanfear. 2018. New methods to calculate concordance factors for phylogenomic datasets. bioRxiv 487801.

MolluscaBase. 2019. Conidae J. Fleming, 1822. Accessed through: World Register of Marine Species.

Mombaerts, P. 2004. Genes and ligands for odorant, vomeronasal and taste receptors. Nature Reviews Neuroscience 5:263.

Muller, H. J. 1964. The relation of recombination to mutational advance. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 1:2–9.

Myers, R. A., L. J. Cruz, J. E. Rivier, and B. M. Olivera. 1993. Conus peptides as chemical probes for receptors and ion channels. Chemical Reviews 93:1923–1936.

Nguyen, L.-T., H. A. Schmidt, A. von Haeseler, and B. Q. Minh. 2015. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Mol Biol Evol 32:268–274.

Niimura, Y., and M. Nei. 2005. Comparative evolutionary analysis of olfactory receptor gene clusters between humans and mice. Gene 346:13–21.

Notredame, C., D. G. Higgins, and J. Heringa. 2000. T-coffee: a novel method for fast and accurate multiple sequence alignment11Edited by J. Thornton. Journal of Molecular Biology 302:205–217.

O'Leary, N. A., M. W. Wright, J. R. Brister, S. Ciufo, D. Haddad, R. McVeigh, B. Rajput, B. Robbertse, B. Smith-White, D. Ako-Adjei, A. Astashyn, A. Badretdin, Y. Bao, O. Blinkova, V. Brover, V. Chetvernin, J. Choi, E. Cox, O. Ermolaeva, C. M. Farrell, T. Goldfarb, T. Gupta, D. Haft, E. Hatcher, W. Hlavina, V. S. Joardar, V. K. Kodali, W. Li, D. Maglott, P. Masterson, K. M. McGarvey, M. R. Murphy, K. O'Neill, S. Pujar, S. H. Rangwala, D. Rausch, L. D. Riddick, C. Schoch, A. Shkeda, S. S. Storz, H. Sun, F. Thibaud-Nissen, I. Tolstoy, R. E. Tully, A. R. Vatsan, C. Wallin, D. Webb, W. Wu, M. J. Landrum, A. Kimchi, T. Tatusova, M. DiCuccio, P. Kitts, T. D. Murphy, and K. D. Pruitt. 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res 44:D733–D745.

Olivera, B. M., C. Walker, G. E. Cartier, D. Hooper, A. D. Santos, R. Schoenfeld, R. Shetty, M. Watkins, P. Bandyopadhyay, and D. R. Hillyard. 1999. Speciation of Cone Snails and Interspecific Hyperdivergence of Their Venom Peptides: Potential Evolutionary Significance of Intronsa. Annals of the New York Academy of Sciences 870:223–237.

Otto, S. P., and J. Whitton. 2000. Polyploid Incidence and Evolution. Annual Review of Genetics 34:401–437.

Painter, S. D., S. F. Cummins, A. E. Nichols, D.-B. G. Akalal, C. H. Schein, W. Braun, J. S. Smith, A. J. Susswein, M. Levy, P. A. C. M. de Boer, A. ter Maat, M. W. Miller, C. Scanlan, R. M. Milberg, J. V. Sweedler, and G. T. Nagle. 2004. Structural and functional analysis of Aplysia attractins, a family of water-borne protein pheromones with interspecific attractiveness. Proc Natl Acad Sci U S A 101:6929–6933.

Pardo-Diaz, C., C. Salazar, S. W. Baxter, C. Merot, W. Figueiredo-Ready, M. Joron, W. O. McMillan, and C. D. Jiggins. 2012. Adaptive Introgression across Species Boundaries in Heliconius Butterflies. PLOS Genetics 8:e1002752.

Patterson, N., P. Moorjani, Y. Luo, S. Mallick, N. Rohland, Y. Zhan, T. Genschoreck, T. Webster, and D. Reich. 2012. Ancient Admixture in Human History. Genetics 192:1065–1093.

Pease, J. B., and M. W. Hahn. 2015. Detection and Polarization of Introgression in a Five-Taxon Phylogeny. Syst Biol 64:651–662.

Phuong, M. A., M. E. Alfaro, G. N. Mahardika, R. M. Marwoto, R. E. Prabowo, T. von Rintelen, P. W. H. Vogt, J. R. Hendricks, and N. Puillandre. 2019. Lack of Signal for the Impact of Conotoxin Gene Diversity on Speciation Rates in Cone Snails. Systematic Biology, doi: 10.1093/sysbio/syz016.

Proffit, M., B. Schatz, R. M. Borges, and M. Hossaert-Mckey. 2007. Chemical mediation and niche partitioning in non-pollinating fig-wasp communities. Journal of Animal Ecology 76:296–303.

Puillandre, N., P. Bouchet, T. F. Duda, S. Kauferstein, A. J. Kohn, B. M. Olivera, M. Watkins, and C. Meyer. 2014. Molecular phylogeny and evolution of the cone snails (Gastropoda, Conoidea). Mol. Phylogenet. Evol. 78:290–303.

Rafferty, N. E., and J. W. Boughman. 2006. Olfactory mate recognition in a sympatric species pair of three-spined sticklebacks. Behav Ecol 17:965–970.

Ramasamy, S., L. Ometto, C. M. Crava, S. Revadi, R. Kaur, D. S. Horner, D. Pisani, T. Dekker, G. Anfora, and O. Rota-Stabelli. 2016. The Evolution of Olfactory Gene Families in Drosophila and the Genomic Basis of chemical-Ecological Adaptation in Drosophila suzukii. Genome Biol Evol 8:2297–2311.

Rambaut, A. 2009. FigTree.

Ranwez, V., S. Harispe, F. Delsuc, and E. J. P. Douzery. 2011. MACSE: Multiple Alignment of Coding SEquences Accounting for Frameshifts and Stop Codons. PLOS ONE 6:e22594.

Reichelt, R. E. 1982. Space: A non-limiting resource in the niches of some abundant coral reef gastropods. Coral Reefs 1:3–11.

Remigio, E. A., and T. F. Duda. 2008. Evolution of ecological specialization and venom of a predatory marine gastropod. Molecular Ecology 17:1156–1162.

Renoult, J. P., P. Geniez, P. Bacquet, L. Benoit, and P.-A. Crochet. 2009. Morphology and nuclear markers reveal extensive mitochondrial introgressions in the Iberian Wall Lizard species complex. Molecular Ecology 18:4298–4315.

Richards, E. J., and C. H. Martin. 2017. Adaptive introgression from distant Caribbean islands contributed to the diversification of a microendemic adaptive radiation of trophic specialist pupfishes. PLOS Genetics 13:e1006919.

Rieseberg, L. H. 1995. The Role of Hybridization in Evolution: Old Wine in New Skins. American Journal of Botany 82:944–953.

Robertson, H. M., C. G. Warr, and J. R. Carlson. 2003. Molecular evolution of the insect chemoreceptor gene superfamily in Drosophila melanogaster. Proceedings of the National Academy of Sciences 100:14537–14542.

Robinson, M. D., D. J. McCarthy, and G. K. Smyth. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140.

Röckel, D., W. Korn, and A. J. Kohn. 1995. Manual of the Living Conidae: Indo-Pacific Region. Hemmen.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Rosenberg, G. 2014. A New Critical Estimate of Named Species-Level Diversity of the Recent Mollusca*. malb 32:308–322.

Schliewen, U. K., D. Tautz, and S. Pääbo. 1994. Sympatric speciation suggested by monophyly of crater lake cichlids. Nature 368:629.

Schluter, D. 2000. The Ecology of Adaptive Radiation. OUP Oxford.

Seehausen, O. 2004. Hybridization and adaptive radiation. Trends Ecol. Evol. (Amst.) 19:198–207.

Shaw, K. L. 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: What mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. Proc Natl Acad Sci U S A 99:16122–16127.

Simion, P., K. Belkhir, C. François, J. Veyssier, J. C. Rink, M. Manuel, H. Philippe, and M. J. Telford. 2018. A software tool 'CroCo' detects pervasive cross-species contamination in next generation sequencing data. BMC Biol 16.

Sloan, D. B., J. C. Havird, and J. Sharbrough. 2017. The on-again, off-again relationship between mitochondrial genomes and species boundaries. Molecular Ecology 26:2212–2236.

Smith, T. D., J. B. Rossie, and K. P. Bhatnagar. 2007. Evolution of the nose and nasal skeleton in primates. Evolutionary Anthropology: Issues, News, and Reviews 16:132–146.

Smith-Unna, R., C. Boursnell, R. Patro, J. M. Hibberd, and S. Kelly. 2016. TransRate: reference-free quality assessment of de novo transcriptome assemblies. Genome Res 26:1134–1144.

Song, L., and L. Florea. 2015. Rcorrector: efficient and accurate error correction for Illumina RNA-seq reads. GigaScience 4:48.

Sonnhammer, E. L. L. 1998. A Hidden Markov Model for Predicting Transmembrane Helices in Protein Sequences.

Sota, T. 2002. Radiation and reticulation: extensive introgressive hybridization in the carabid beetles Ohomopterus inferred from mitochondrial gene genealogy. Popul Ecol 44:0145–0156.

Spengel, J. W. 1881. Die Geruchsorgane und dasNervensystem der Mollusken. Z. Wiss. Zool 333–383.

Spengler, H. A., and A. J. Kohn. 1995. Comparative external morphology of the Conus osphradium (Mollusca: Gastropoda). Journal of Zoology 235:439–453.

Spielman, S. J., and C. O. Wilke. 2013. Membrane Environment Imposes Unique Selection Pressures on Transmembrane Domains of G Protein-Coupled Receptors. J Mol Evol 76:172–182.

Stanley, S. M. 2007. An Analysis of the History of Marine Animal Diversity. Paleobiology 33:1–55.

Stewart, J., and W. F. Gilly. 2005. Piscivorous Behavior of a Temperate Cone Snail, *Conus californicus*. The Biological Bulletin 209:146–153.

Strickland, J. L., C. F. Smith, A. J. Mason, D. R. Schield, M. Borja, G. Castañeda-Gaytán, C. L. Spencer, L. L. Smith, A. Trápaga, N. M. Bouzid, G. Campillo-García, O. A. Flores-Villela, D. Antonio-Rangel, S. P. Mackessy, T. A. Castoe, D. R. Rokyta, and C. L. Parkinson.

2018. Evidence for divergent patterns of local selection driving venom variation in Mojave Rattlesnakes (Crotalus scutulatus). Scientific Reports 8:17622.

Tait Cheyenne, Batra Srishti, Ramaswamy Sree Subha, Feder Jeffrey L., and Olsson Shannon B. 2016. Sensory specificity and speciation: a potential neuronal pathway for host fruit odour discrimination in Rhagoletis pomonella. Proceedings of the Royal Society B: Biological Sciences 283:20162101.

Tester, M., and P. Langridge. 2010. Breeding Technologies to Increase Crop Production in a Changing World. Science 327:818–822.

Thankaswamy-Kosalai, S., P. Sen, and I. Nookaew. 2017. Evaluation and assessment of read-mapping by multiple next-generation sequencing aligners based on genome-wide characteristics. Genomics 109:186–191.

Toews, D. P. L., and A. Brelsford. 2012. The biogeography of mitochondrial and nuclear discordance in animals. Molecular Ecology 21:3907–3930.

Vallejo, B. 2005. Inferring the mode of speciation in Indo-West Pacific Conus (Gastropoda: Conidae). Journal of Biogeography 32:1429–1439.

van Dongen, S., and C. Abreu-Goodger. 2012. Using MCL to Extract Clusters from Networks. Pp. 281–295 *in* J. van Helden, A. Toussaint, and D. Thieffry, eds. Bacterial Molecular Networks: Methods and Protocols. Springer New York, New York, NY.

Walderon, M. D., K. J. Nolt, R. E. Haas, K. N. Prosser, J. B. Holm, G. T. Nagle, and J. G. Boal. 2011. Distance chemoreception and the detection of conspecifics in Octopus bimaculoides. J Molluscan Stud 77:309–311.

Weese, D. A., and T. F. Duda. 2019. Effects of Predator-Prey Interactions on Predator Traits: Differentiation of Diets and Venoms of a Marine Snail. Toxins 11:299.

Williams, S. T., and T. F. Duda. 2008. Did tectonic activity stimulate oligo-miocene speciation in the Indo-West Pacific? Evolution; international journal of organic evolution 62:1618–1634.

Wong, E. S. W., and K. Belov. 2012. Venom evolution through gene duplications. Gene 496:1–7.

Yang, Y., and S. A. Smith. 2014. Orthology Inference in Nonmodel Organisms Using Transcriptomes and Low-Coverage Genomes: Improving Accuracy and Matrix Occupancy for Phylogenomics. Mol Biol Evol 31:3081–3092.

Yang, Z. 2007. PAML 4: Phylogenetic Analysis by Maximum Likelihood. Mol Biol Evol 24:1586–1591.

Yu, G., D. K. Smith, H. Zhu, Y. Guan, and T. T.-Y. Lam. 2017. ggtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods in Ecology and Evolution 8:28–36.

Zhang, W., K. K. Dasmahapatra, J. Mallet, G. R. P. Moreira, and M. R. Kronforst. 2016. Genome-wide introgression among distantly related Heliconius butterfly species. Genome Biology 17:25.

FIGURES AND TABLES

Table 2.1. *Conus ebraeus* individuals sequenced for this study.

Individual	UMMZ Accession #	Sex	Collection Locale	Collection Date
ebr_1	304691	♂	Sesoko Station, Okinawa, Japan	2015-07-24
ebr_2	304690	♀	Sesoko Station, Okinawa, Japan	2015-07-24
ebr_3	304692	♀	Sesoko Station, Okinawa, Japan	2015-07-24
ebr_4	304699	♂	Cape Bise, Okinawa, Japan	2015-07-24
ebr_5	304701	♀	Cape Bise, Okinawa, Japan	2015-07-24

Table 2.2. Models used to test hypotheses regarding rates of evolution for candidate *C. ebraeus* OR genes.

Whole tree	**-lnL**	$\omega_{Aplysia}$	ω_{Conus}	ω_{Sub_1}	ω_{Sub_2}
M0: Single ω for all branches	60301.12	0.218	0.218	0.218	0.218
M1: Separate ω, *Aplysia* & *Conus*: $\omega_{Aplysia} \neq \omega_{Conus}$	60289.40	0.008	0.219	0.219	0.219
M2: Separate ω, subfamilies: $\omega_{Sub\_1} \neq \omega_{Sub\_2}$	60288.66				
Subfamily one	**-lnL**	ω_{Clade_1}	ω_{Clade_2}	ω_{Clade_3}	ω_{Clade_4}
M0: Single ω estimated for all branches	37234.53	0.216	0.216	0.216	0.216
M1: ω = 1 for all branches	38827.32	[1]	[1]	[1]	[1]
M2: Separate ω estimated for subclades	37224.43	0.281	0.134	0.205	0.218
Subfamily two	**-lnL**	ω_{Clade_1}	ω_{Clade_2}	ω_{Clade_3}	-
M0: Single ω estimated for all branches	25749.63	0.216	0.216	0.216	-
M1: ω = 1 for all branches	26328.57	[1]	[1]	[1]	-
M2: Separate ω estimated for subclades	25744.68	0.009	0.211	0.221	-

Table 2.3. Hypothesis testing and LRT model comparison.

Hypothesis	Model comparison	LRT statistic	*p-value*
Whole tree			
$\omega_{Aplysia} = \omega_{Conus}$	M0 vs. M1	23.44	1.3×10^{-6}
$\omega_{Sub\_1} = \omega_{Sub\_2}$	M1 vs. M2	1.48	0.477
Subfamily one			
$\omega_{Clade1} = \omega_{Clade2} = \omega_{Clade3} = \omega_{Clade4}$	M0 vs. M2	20.19	4.58×10^{-4}
$1 = \omega_{Clade1} = \omega_{Clade2} = \omega_{Clade3} = \omega_{Clade4}$	M1 vs. M2	3205.77	~ 0
Subfamily two			

$\omega_{Clade1} = \omega_{Clade2} = \omega_{Clade3}$	M0 vs. M2	9.91	0.0194
$1 = \omega_{Clade1} = \omega_{Clade2} = \omega_{Clade3}$	M1 vs. M2	1167.79	~ 0

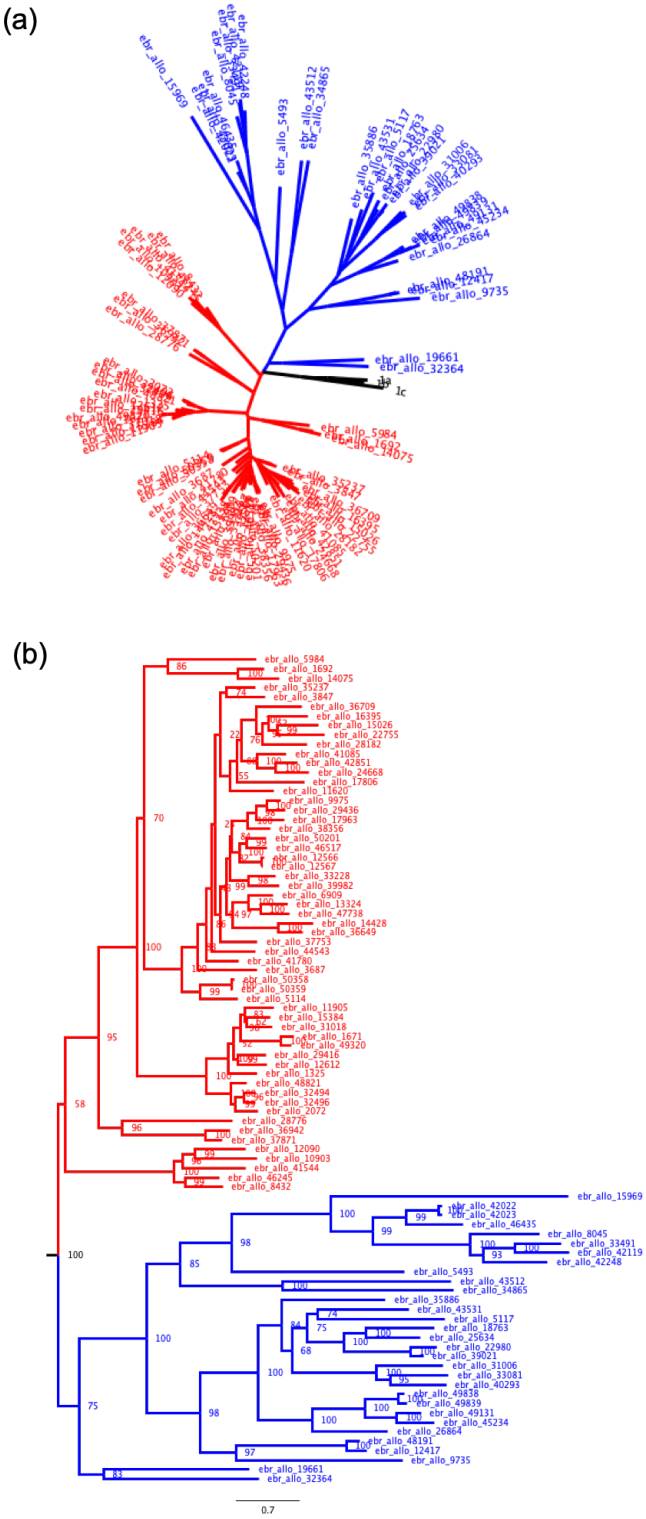

Figure 2.1. IQ-TREE maximum likelihood phylogeny of all *C. ebraeus* candidate OR genes. Subfamily one branches shown in red, subfamily two in blue. (a) Unrooted star phylogeny with *A. californica* OR genes (black) shown. (b) Rooted, with *A. californica* OR genes hidden and nodes labeled with bootstrap support values.

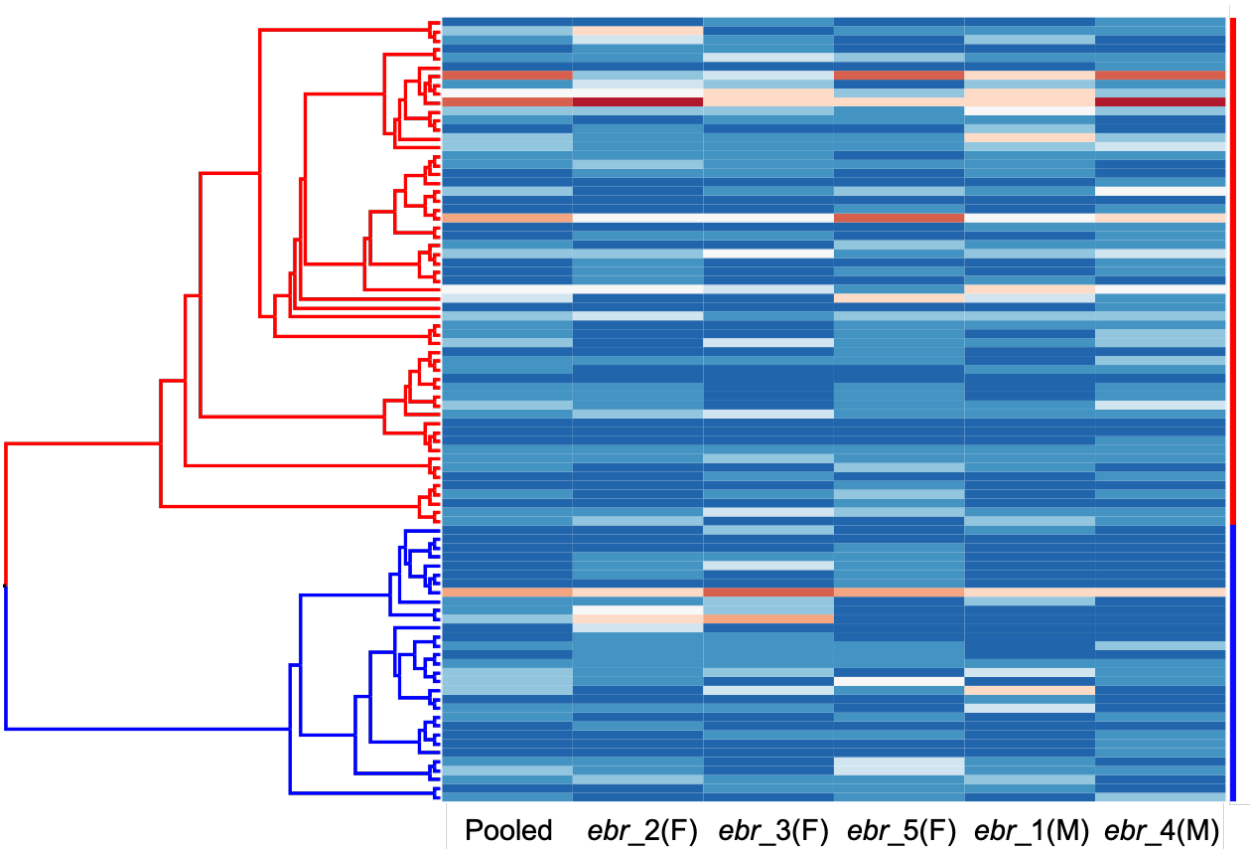

Figure 2.2. Gene expression profiles of candidate *C. ebraeus* OR genes. OR gene family tree is overlaid to the left, with subfamily one colored red and subfamily two colored blue. Dark blue cells represent lower relative expression values and dark red represents higher, spanning approximately two orders of magnitude.

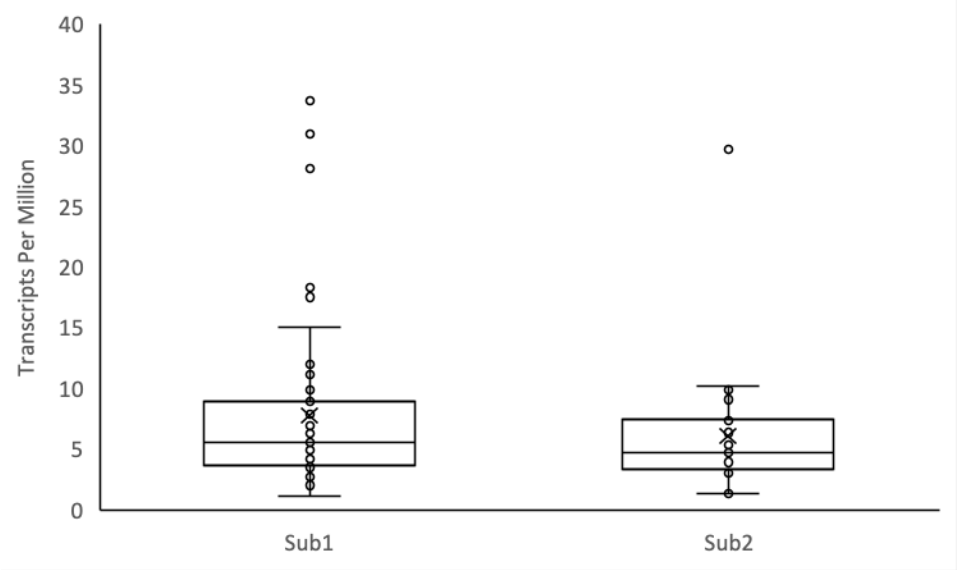

Figure 2.3. Histogram comparing expression values of transcripts between subfamily one and subfamily two in transcripts per million (TPM).

CHAPTER 3

Expanding the Predatory Phenotype: Olfactory Receptor Gene Evolution in *Conus judaeus* and *Conus ebraeus*

With Thomas F. Duda Jr.

ABSTRACT

Olfaction is a critical sensory modality for many taxa that contributes to ecological specialization and diversification. Predatory marine gastropods in the family Conidae – "cone snails" – rely on olfaction to navigate their environments and discriminate between prey items. It is therefore likely that olfaction contributes to the ecological specialization of these snails and plays a complementary role to venom in a broader adaptive predatory phenotype. In this study we compare the OR repertoires of the sister species *Conus ebraeus* and *Conus judaeus*, which co-occur frequently and exhibit highly distinct dietary specializations. We find broad scale differences in OR gene family composition of these species. To better understand mechanisms driving diversification of these genes, we compare rates of evolution among paralogs and orthologs, and test for positive selection among 33 pairs of orthologous loci. We find evidence that OR paralogs and orthologs evolve at similar rates and generally experience purifying selection, but we identify eight orthologs that exhibit evidence of positive selection. We also characterize the expression profiles of putative OR orthologs and identify four loci differentially

expressed between *C. ebraeus* and *C. judaeus* orthologs. These novel insights into the composition and evolution of OR gene families in *Conus* expand our understanding of factors that contribute to the stunning adaptive radiation of this group.

INTRODUCTION

Conidae – "cone snails" – is a species rich (~800 species) family of predatory marine gastropods with many hallmarks of adaptive radiation (Röckel et al. 1995; Puillandre et al. 2014). Approximately 85% of the diversity in Conidae is represented by the genus *Conus*, which originated some 55 MYA and boasts a rate of diversification double most marine gastropod genera (Kohn 1990, 2014). This breakneck pace highlights the rich potential for studies of *Conus* to contribute to our understanding of the origins of biodiversity, and has stimulated researchers to investigate aspects of *Conus* ecology and biology that facilitate such rapid diversification. Because adaptive radiations are characterized by adaptation to novel ecological niches, it is useful to understand how niches are differentiated among *Conus*. Members of the genus frequently occur in sympatry – often alongside close relatives – and as many as 36 species have been observed occupying the same reef in Papua New Guinea (Kohn 2001; Vallejo 2005). Niches are partitioned according to prey type and not microhabitat, a conclusion reinforced by both previous and subsequent findings indicating narrow dietary breadth in many conus species, especially those that prey on marine worms (Kohn 1959; Kohn and Nybakken 1975; Duda et al. 2009a). In Okinawa, for example, sister species *Conus ebraeus* and *Conus judaeus* occupy the same marine bench habitat, but adult dietary compositions are markedly distinct, with *C. ebraeus* preying almost exclusively on errant polychaetes in the family Eunicidae and *C. judaeus* on sedentary polychaetes in the

family Capitellidae (Duda et al. 2009b). The outlier nature of *C. judaeus*'s diet is reinforced when compared to additional members of the subgenus *Virroconus*, all of which share the errant polychaete preference exhibited by *C. ebraeus* (Duda 2001; Duda et al. 2009b). Such fine-scale interspecific partitioning of prey resources requires some trait or traits to facilitate differentiation.

The primary sensory modality for gastropods is chemosensation, which plays a central role in all aspects of gastropod life history, from habitat choice to mate choice (Kohn 1961; Croll 1983). Indeed, chemosensation has been recognized as the instigator of predatory behavior in *Conus* for many years (Kohn 1959, 1961). Predatory behavior can be initiated by introducing the snail to water from an aquarium containing prey, without the prey itself being present (Kohn 1956; Stewart and Gilly 2005). Also, prey choice experiments indicate that snails differentiate their preferred prey from other prey choices using chemical cues detected at a distance, and do so at fairly high taxonomic resolution (Kohn 1959). All neogastropods possess a prominent olfactory organ called an osphradium, which has attracted attention among malacologists since it was first investigated in detail by Spengel (1881), and is likely the most important sensory structure for this group of snails (Copeland 1918; Kohn 1961; Croll 1983). It is a highly enervated, bipectinate structure located adjacent to the ctenidium in the mantle cavity at the base of the siphon, sitting directly in the inhalant current of water (Spengler and Kohn 1995). Among *Conus*, it exhibits a high degree of interspecific morphological divergence, which varies significantly with habitat type and food type (Spengler and Kohn 1995).

The genetic basis of olfaction in cone snails has only recently been investigated, and does not yet include comparative studies of species with distinct ecological specializations.

Our own results from characterizing the OR gene repertoire of *C. ebraeus* reveal a diverse gene family organized into two main subfamilies (see Chapter 2) whose members share many of the same general characteristics of OR genes that have been reported from extensive studies of other taxa and which are highly conserved across metazoans (Hildebrand and Shepherd 1997; Ache and Young 2005). Broadly speaking, chemical cues are detected by a diverse suite of chemosensory receptor proteins, both at a distance (olfaction) and in contact (gustation), and signals are transduced and interpreted by the nervous system. These receptor proteins are primarily G-protein coupled receptors (GPCRs) with seven transmembrane domains (Buck and Axel 1991; Bargmann 2006), and the gene families to which they belong are among the richest found in many taxa, highlighting the central importance of chemosensory traits to organismal fitness (Mombaerts 2004). Indeed, olfaction plays well-documented roles at a range of biological scales, and has been found to contribute to species diversification via mechanisms such as mate recognition (Rafferty and Boughman 2006; Eltz et al. 2008) and ecological specialization (Linn et al. 2003; Ramasamy et al. 2016; Brand and Ramírez 2017). Extensively studied in vertebrates and some invertebrate groups such as insects, OR genes are not well characterized in mollusks. Cummins et al (2009), however, provided a foundation for OR gene work in gastropods by identifying 90 putative OR genes organized into three distinct subfamilies in *Aplysia californica*.

Because the functional units of olfaction are direct gene products (i.e. olfactory receptor proteins), the link between phenotype and genotype is much less convoluted than it is for many other adaptive traits. A single nonsynonymous substitution or shifts in gene expression may dramatically alter trait function, with potentially severe fitness

consequences for the organism. Sometimes, however, such changes may confer novel function that permits their carrier to exploit new prey and/or occupy previously inaccessible niches. By studying the molecular evolution and expression of genes underlying olfaction we can therefore learn how selection imposed by shifts in ecology contributes to differentiation of adaptive traits and to the diversification of species. Such studies of the adaptive evolution of cone snail venoms reveal markedly distinct venom compositions between even close species (Olivera et al. 1999) and implicate several processes driving their diversification. Conotoxins evolve extremely quickly under strong positive selection, in some cases making it difficult to align sequences from even closely related species (Duda and Palumbi 1999; Duda and Palumbi 2000). Evidence suggests that predator-prey interactions are the source of this strong selection, and numerous relationships have been documented between shifts in diet and feeding specialization and conotoxin evolution (Duda and Palumbi 2004; Duda and Lee 2009; Duda et al. 2009a; Dutertre et al. 2014). These patterns of evolution are mirrored by changes in conotoxin gene expression between species and populations that encounter distinct prey assemblages (Chang and Duda 2016; Weese and Duda 2019). In addition to strong positive selection, conotoxin gene families are constantly restructured by extensive gene duplication and birth-and-death dynamics (Chang and Duda 2012; Wong and Belov 2012). If olfaction plays a complementary role to venom in ecological specialization as part of a broader predatory phenotype, we may observe similar patterns of molecular evolution in *Conus* OR genes.

In this study, we use a transcriptomic approach to characterize the OR gene repertoires of *Conus ebraeus* and *Conus judaeus* – a pair of closely related and co-occurring

species that exhibit highly distinct dietary preferences – and examine differences and similarities in OR gene composition to better understand the role of olfaction in ecological specialization of *Conus*. We then examine several factors to determine what mechanisms contribute to the diversification of the OR gene family and to the evolution of ecological adaptation in *Conus* more broadly. First, we interrogate patterns of evolution between paralogous and orthologous loci to understand whether gene family diversification occurs within species or is driven by divergence between species. Next, we compare rates of evolution between orthologous pairs of loci to identify any genes that may be experiencing different selective pressure in the two species. Last, we test for differential expression of orthologs to assess how gene expression contributes to differences in OR repertoires in *C. ebraeus* and *C. judaeus*.

METHODS

Taxon sampling, RNA extraction, and sequencing

We collected *C. ebraeus* and *C. judaeus* individuals on an expedition to Okinawa, Japan during the summer of 2015. Upon collection, snails were brought back to Sesoko Station marine lab (University of the Ryukyus), placed in separate plastic vessels with 200 mL of seawater for up to 48 hours, and then sacrificed and dissected. Osphradium tissue was placed in cryovials with RNALater (Invitrogen, Carlsbad, CA, USA) and stored temporarily at 4°C in the field prior to transport and permanent storage at -80°C at the University of Michigan. Snails and their associated tissues were deposited with the Mollusk Division of the University of Michigan Museum of Zoology. We selected osphradia from five adult individuals of each species to sequence for this study; individual information is presented

in Table 1. Osphradium tissue was pestle-homogenized. Total RNA extracted with Trizol (Invitrogen, Carlsbad, CA, USA) according to manufacturer protocol and submitted to the University of Michigan DNA Sequencing Core for quality assessment with a Bioanalyzer 2100, followed by library preparation and indexing (Illumina Tru-Seq kit, San Diego, CA, USA). Libraries were sequenced with a larger batch of samples across two flowcell on an Illumina HiSeq4000.

Read processing, transcriptome assembly/filtering

To generate osphradium transcriptomes and identify candidate OR genes from *C. judaeus,* we utilized the same procedures that we already applied successfully to *C. ebraeus* (see Chapter 2). These procedures are described below. We followed the pipeline developed by Yang & Smith (2014) to process raw sequencing reads for each individual and filter raw transcriptomes. Reads were processed separately for each individual. We first used Rcorrector (Song and Florea 2015) to correct suspected sequencing errors and removed uncorrectable reads, and then ran Trimmomatic v0.36 (Bolger et al. 2014) with default parameters to remove Illumina sequencing adapters and low-quality reads. Reads were binned as mitochondrial or nuclear DNA by mapping them to a custom database of nine complete *Conus* mitochondrial genomes with Bowtie2 v2.3.4.3 (Langmead and Salzberg 2012). We used FastQC v0.10.1 (Andrews 2010) to assess read-quality and read representation, and to cull over-represented sequences. We then pooled the filtered reads of individuals from each species and assembled separate *de novo* transcriptomes for *C. ebraeus* and *C. judaeus* with Trinity v2.4.0 (Grabherr et al. 2011).

We filtered the *de novo* transcriptome to remove low-quality transcripts and transcripts lacking open reading frames (ORF). First, we ran Transrate v1.0.3 (Smith-Unna et al. 2016) with default settings to determine assembly quality and remove poor-quality transcripts, and then applied custom scripts from the Yang & Smith (2014) pipeline to remove chimeric transcripts. We produced clusters of transcripts belonging to the same putative gene using Corset v1.07 (Davidson and Oshlack 2014), selecting the longest transcript from each cluster to represent that gene. We predicted ORFs using TransDecoder v5.0.1 (Haas et al. 2013), setting minimum ORF length to 100 amino acids, and included homology searches to increase sensitivity for ORFs with functional significance. Two homology searches were included: a BLASTp search (Altschul et al. 1990) against a custom reference database comprised of all *Conus*, *Lottia gigantia*, and *Alpysia californica* protein sequences from the NCBI non-redundant database, and a search of the PFAM database (Finn et al. 2014) to identify homology with common protein motifs, including transmembrane domains. TransDecoder combines the results of these searches with ORF predictions to determine which transcripts should be retained. Finally, we reduced transcript redundancy for predicted coding sequences with CD-HIT-EST and a sequence identity cutoff of 0.99, and for predicted peptide sequences with CD-HIT and a sequence identity cutoff of 0.98 (Li and Godzik 2006; Fu et al. 2012).

Candidate OR gene prediction

We applied the same criteria used by Cummins et al (2009) to extract candidate OR genes from the filtered transcriptomes of each species. These include, (i) limited predicted peptide sequence similarity to previously characterized molluscan chemosensory receptor

genes, (ii) at least six predicted transmembrane domains, and (iii) a full-length coding sequence. We used the filtered transcriptomes of each species as queries in a BLASTx (Gish and States 1993) search (e-4) against a custom database of 1,830 predicted molluscan OR protein sequences gathered from the GenBank RefSeq database (O'Leary et al. 2016). We then predicted the number and location of helical transmembrane (TM) domains in the predicted peptide sequences of BLASTx hits using TMHMM v2.0 (Sonnhammer 1998), keeping those with six or more predicted TM domains. Finally, we extracted those loci containing both an initiating methionine and terminating stop codon.

Phylogenetics and ortholog inference

To compare the overall OR gene diversity of *C. ebraeus* and *C. judaeus* in a phylogenetic context, we combined the predicted peptide sequences of candidate OR genes from each species with sequences of three *Aplysia californica* OR genes identified by Cummins et al (2009) and aligned them using T-COFFEE in PSI/TM mode (Notredame et al. 2000; Floden et al. 2016). This program uses a reference database of TM proteins to inform the alignment of proteins with predicted TM domains. The best-fit model of substitution was selected using ModelFinder (Kalyaanamoorthy et al. 2017), and we inferred a phylogeny of all OR loci using IQ-TREE (Nguyen et al. 2015) with 1000 bootstrap replicates. Orthologous and paralogous loci were distinguished based on relative phylogenetic position.

Because highly divergent sequences can limit our ability to successfully estimate rates of evolution, we used a transcript clustering approach to remove candidate OR genes lacking a clear paralog or ortholog for subsequent analyses. We first concatenated candidate OR genes from both species into a single FASTA file and performed an all-by-all

BLASTn (Altschul et al. 1990) search with an e-value of e-10. Then, we applied the Markov clustering algorithm to cluster sequences into groups based on BLASTn results (Enright et al. 2002; van Dongen and Abreu-Goodger 2012). Remaining loci were used to produce three datasets: (i) loci belonging to subfamily one, (ii) loci belonging to subfamily two, and (iii) putative orthologous loci represented by both species. By separating loci into their respective subfamilies, we were able to produce alignments with fewer gaps, increasing the number of informative sites available for downstream analyses of evolutionary rates within each subfamily. For each of these three datasets, we used the same procedure described above to align and infer phylogenies.

Testing rates of evolution

To assess rates of evolution of paralogous and orthologous OR genes, we used the codeml program in the PAML v4.9 software package (Yang 2007) to calculate rates of nonsynonymous substitutions per nonsynonymous site (dN) and synonymous substitutions per synonymous site (dS) and their ratio (ω) across branches of the tree. Because tests of selection require nucleotide alignments that preserve codon position, we reverse-translated our peptide alignments using MACSE v2.03 (Ranwez et al. 2011) and the original nucleotide transcript sequences. We implemented several different models (Table 2) and compared their log-likelihood values using likelihood ratio tests (LRT) to determine which were more likely given the data. We performed all analyses separately for the two OR gene subfamilies to utilize more complete alignments for each. Our first model fixes ω at a value of one for all branches of the tree (Model A). Next, we estimate a single ω value for all branches of the tree (Model B). Comparing these two models allowed us to determine

whether OR genes (both paralogs and orthologs) are generally evolving under purifying or positive selection. Our third model (Model C) estimates three separate ω values: one for branches connecting paralogous loci, one for branches connecting putative orthologs, and one for the remainder of the tree. Comparing these models allowed us to determine if paralogs and orthologs are evolving at different rates. The fourth model (Model D) estimates one ω for branches connecting paralogs, fixes the ω for branches connecting orthologs to one, and estimates a third omega for the remaining branches. Comparison of models C and D allowed us to determine if orthologs are evolving under purifying or positive selection. Model E estimates a separate ω for each branch on the tree, which allows us to compare the lineage-specific branches leading to each ortholog for each species and identify those that may be evolving at different rates in *C. ebraeus* and *C. judaeus*.

Expression analysis

To identify differentially expressed loci between *C. ebraeus* and *C. judaeus* and reveal any relationship between the rate of OR ortholog evolution and gene expression, we estimated transcript abundance for each putative ortholog for both species and performed differential expression analysis using scripts packaged with Trinity (Haas et al. 2013). We mapped the processed-read fastq files from each individual to their respective species' filtered transcriptome using the Perl script align_and_estimate_abundance.pl with --*est_method* set to RSEM (Li and Dewey 2011) and --*aln_method* set to Bowtie2 (Langmead and Salzberg 2012). We then used the script abundance_estimates_to_matrix.pl to produce a cross-sample normalized abundance matrix for each species and extracted expression values for transcripts representing putative orthologs. We then used the R function

heatmap to produce heatmaps illustrating patterns of gene expression alongside a phylogeny of putative orthologs.

To detect orthologs differentially expressed between *C. ebraeus* and *C. judaeus*, we extracted the raw RNAseq fragment counts of putative orthologous transcripts, and assigned individuals of each species as biological replicates (i.e. five each). We then performed differential expression analyses using the Perl script run_DE_analysis.pl (also packaged with Trinity) with the edgeR method (Robinson et al. 2010; McCarthy et al. 2012), and a p-value cutoff of 0.05 to establish significance.

RESULTS

Read processing, transcriptome assembly/filtering, and OR prediction

The results of our read processing, transcript filtering and OR prediction pipeline, including the number of reads and transcripts remaining after various filtering steps, are summarized in Table 3. Starting from unfiltered transcriptomes of 429,975 transcripts for *C. ebraeus* and 370,330 for *C. judaeus*, we identified a total of 88 candidate OR genes from the former, and 118 from the latter after transcript filtering and OR gene prediction.

Phylogenetics and ortholog inference

The phylogeny of unfiltered candidate OR genes from both species is presented in Figure 1. As reported previously for *C. ebraeus* alone (see Chapter 2), the gene families of both species are organized into two well-resolved clades that we identify as subfamily one and subfamily two. *C. ebraeus* and *C. judaeus* are represented roughly equally in subfamily one with 52 and 54 loci, respectively, whereas *C. judaeus* has nearly double the number of

subfamily two loci (64) compared to *C. ebraeus* (36). Within each subfamily, loci are intermixed, and there are no distinct large clades comprised entirely of one species.

After filtering sequences lacking obvious paralogs or orthologs, we were left with 49 loci from *C. ebraeus* and 43 loci from *C. judaeus* for a total of 92. Subfamily one is composed of 68 genes with 25 putative ortholog pairs, and subfamily two is composed of 24 genes with 8 putative ortholog pairs. A tree of only putative orthologous loci is presented in Figure 2. The subfamilies are roughly even in representation of the two species with 37 *C. ebraeus* and 31 *C. judaeus* genes in subfamily one, and 12 *C. ebraeus* and 12 *C. judaeus* genes in subfamily two. The richness and shorter branch lengths observed in subfamily one indicates more recent and rapid divergence relative to subfamily two.

Rates of evolution

Our tree-wide codeml analyses suggest that both OR orthologs and paralogs are evolving under purifying selection. Model likelihood and estimated ω values for each subfamily are reported in Table 2 and model comparisons with LRT results in Table 4. Our second model estimates a single ω value of 0.25 and 0.26 in subfamily one and two respectively, and is significantly better than model A in both cases, suggesting that loci are evolving under purifying selection. Our third model, which estimates separate ω values for branches connecting orthologs and those connecting paralogs explains the data slightly better than model B, suggesting different rates of evolution for these classes of loci, although the estimated ω values are very similar (Table 1).

The lineage-specific dN and dS values calculated separately for each branch in model E are reported in Table 5 for the eight out of 33 orthologous loci that exhibited an

estimated $\omega > 1$. Five of these are in subfamily one and three in subfamily two; no ortholog under positive selection was recovered from both species, and we recovered four orthologs under positive selection each from *C. judaeus* and *C. ebraeus*.

Expression analysis

A heatmap illustrating the expression profiles of orthologous loci in each species and individual are presented in Figure 3. Overall expression patterns were similar between *C. ebraeus* and *C. judaeus*, with more highly expressed genes clustering in subfamily one. We recovered a total of four orthologs that were significantly differentially expressed between the two species, two from each subfamily. Both loci from subfamily two are characterized by extremely short branch lengths in both species. The differentially expressed orthologs in subfamily have longer branches leading to *C. ebraeus* than to *C. judaeus*. None of these four putative orthologs show evidence of positive selection, nor is there a consistent pattern of larger ω values in one versus the other species.

DISCUSSION

Comparisons of OR gene family composition can reveal evidence of adaptive trait evolution and point to genes that contribute to ecological specialization. Humans and chimpanzees, for example, have similar overall numbers of OR genes in their genomes but in humans only ~40% of these are functional due to accelerated pseudogenization while chimpanzees retain a much higher proportion of their OR genes intact, a difference due likely to a shift in lifestyle that altered the selective regime experience by humans (Gilad et al. 2003). *Drosophila suzukii* offers another example wherein small differences in OR gene family

composition are linked with a unique shift in dietary preference from fermenting substrates to fresh fruit (Ramasamy et al. 2016).

The OR gene families that we characterize from *C. ebraeus* and *C. judaeus* osphradia exhibit substantial similarities, both in terms of their broad structural organization and overall numbers of candidate genes. There are no large sub-clades consisting entirely of sequences from one species, and approximately 32% of all candidate OR genes belong to a putative ortholog pair. The most glaring difference is the asymmetric representation of species in subfamily two where we recover nearly twice as many genes from *C. judaeus* than *C. ebraeus*. This points to elevated diversification of subfamily two in *C. judaeus*, which could be associated with aspects of organismal ecology that are divergent from *C. ebraeus*. Smaller differences than this (e.g. single gene gain or loss) can still have significant impacts on ecological specialization as is demonstrated by other adaptive traits such as venom. For example, the presence or absence of a small venom gene cassette in Mojave rattlesnakes results in radically different hemorrhagic or neurotoxic venom types associated with distinct prey, and may represent the early stages of ecological speciation (Strickland et al. 2018). Definitively linking the observed differences in OR gene family composition between *C. ebraeus* and *C. judaeus* with unique aspects of their ecology will require additional work and an understanding of OR protein function in *Conus*. Furthermore, a comparative genomic approach will be necessary to confirm these differences and to make granular inferences of gene gain and loss since transcriptomes can miss unexpressed or low-expression loci.

Gene family diversification may be driven by a number of processes that operate in isolation and in concert. Extensive work with OR gene families has documented

mechanisms ranging from strong positive selection to rapid birth and death dynamics (Hansson and Stensmyr 2011; Bear et al. 2016). Neofunctionalization of paralogs and divergence of orthologous loci between species are both capable of driving gene family divergence and diversification. By estimating rates of synonymous and nonsynonymous substitutions along branches leading to both paralogous and orthologous loci, we can determine if they are evolving at different rates, and what their relative contribution to gene family divergence may be. Our results from performing this comparison on our filtered subset of 92 *C. ebraeus* and *C. judaeus* OR genes show that paralogous and orthologous loci exhibit slightly different rates of evolution, although both generally evolve under purifying selection (Table 3 & 4). Our model estimating separate ω values for orthologous and paralogous branches (Model C) does a significantly better job explaining our data than the model estimating a single ω value for all branches (Model B) for both subfamily one and subfamily two. Estimated ω values are very similar in subfamily one, however, at 0.24 and 0.23 for paralogs and orthologs respectively. The rates estimated for subfamily two are more divergent with $\omega_{paralog} = 0.22$ and $\omega_{ortholog} = 0.31$, which suggests more rapid divergence of orthologs than paralogs in subfamily two.

These results comport with studies of conotoxins that find no significant difference in rates of evolution of orthologs and paralogs. In a comparison of conotoxin evolution between *Conus miliaris* and *Conus abbreviatus* (close relatives of *C. ebraeus* and *C. judaeus*) Duda (2008) found that both classes of loci experience strong positive selection. Despite our finding of purifying selection across the OR gene family, it is possible our analyses were not calibrated to detect signals of positive selection that may exist. First, estimates of dN and dS for long branches and deep nodes can lose accuracy, and even when subdividing

candidate OR genes according to subfamilies and removing genes without obvious orthologs or paralogs, our final alignments still included some highly divergent sequences. Second, the candidate OR proteins that we recovered are much larger (~300 amino acids) than conotoxin peptides (~10-30 amino acids) (Myers et al. 1993), and may experience strong selection only in particular regions (e.g. extracellular domains) or a handful sites (Spielman and Wilke 2013). The ligand binding sites, for example, are good candidates for regions of strong selection and may exhibit substantial variation among species based on the odorant molecules emitted by different preferred prey items. Such heterogeneous patterns of selection have been documented in an OR gene family in teleost fishes where purifying selection experienced by most of the gene swamped signals of positive selection from a small handful of sites scattered associated with specific regions of the coding sequence (Hussain et al. 2009).

We also estimated the lineage-specific rates of evolution for each of the 33 pairs of putative orthologs we inferred. Although our results show the majority these loci evolving under purifying selection, we found a total of eight branches exhibiting an $\omega > 1$ – four leading to *C. ebraeus* and four to *C. judaeus*, none of them shared – indicating positive selection (Table 5). Because these eight orthologs are inferred to experience positive selection in one species and not the other, they represent promising candidates for further study to determine if they play a role in ecological specialization for either species. Differential rates of evolution in orthologs has frequently been linked to ecological adaptation and divergence (McBride 2007; Brand and Ramírez 2017).

Finally, differences in gene expression can point to genes that play a more important role in one species than another. Such differences can be the result of phenotypic plasticity,

but in many cases they are the product of evolution and are an important aspect of adaptive diversification (Brawand et al. 2011). We estimated the abundance of all 33 orthologs for both *C. ebraeus* and *C. judaeus* and find a pattern of heterogeneous expression across the tree (Figure 3) with a larger number of highly expressed orthologs found in subfamily one for both species. Furthermore, differences in overall gene family composition between *C. judaeus* and *C. ebraeus* suggest that some genes are expressed in one species and not expressed at all in the other. This is particularly noticeable in subfamily two, which is represented by nearly twice as many genes in *C. judaeus* than in *C. ebraeus*. We also performed differential expression analysis and identified four orthologs that were differentially expressed (Figure 3, green arrows). One ortholog was more highly expressed in *C. judaeus* and three were more highly expressed in *C. ebraeus*. None of these orthologs were among those we inferred to experience positive selection. These genes represent ideal candidates for future studies to determine what role they play in *Conus* ecology. Shifts in conotoxin gene expression have been linked to changes in prey assemblage in *Conus miliaris*, whose Easter Island population encounters a distinct prey assemblage from populations in the western Pacific (Weese and Duda 2019). A parallel study of population-level differences in OR gene expression could reveal a similar linkage with diet. Although we were able to identify four differentially expressed loci with our dataset, future studies comparing a larger number of individuals from each species may achieve higher resolution and capture more subtle variation. Differences in gene expression can stem from dosage effects resulting from multiple gene copies or from differences in regulatory regions upstream of gene coding sequences. As such, this is another area of inquiry where a genome would be a useful tool for understanding causality.

In this study, we present the first comparative study of OR gene families in cone snails. We illustrate differences between gene family composition between *C. ebraeus* and *C. judaeus* and investigate mechanisms that contribute to these differences and to the diversification of OR genes more broadly. Our results add a valuable new perspective to adaptive trait evolution in Conidae and contribute to a better understanding of their remarkable diversification.

REFERENCES

Abbott, R., D. Albach, S. Ansell, J. W. Arntzen, S. J. E. Baird, N. Bierne, J. Boughman, A. Brelsford, C. A. Buerkle, R. Buggs, R. K. Butlin, U. Dieckmann, F. Eroukhmanoff, A. Grill, S. H. Cahan, J. S. Hermansen, G. Hewitt, A. G. Hudson, C. Jiggins, J. Jones, B. Keller, T. Marczewski, J. Mallet, P. Martinez-Rodriguez, M. Möst, S. Mullen, R. Nichols, A. W. Nolte, C. Parisod, K. Pfennig, A. M. Rice, M. G. Ritchie, B. Seifert, C. M. Smadja, R. Stelkens, J. M. Szymura, R. Väinölä, J. B. W. Wolf, and D. Zinner. 2013. Hybridization and speciation. Journal of Evolutionary Biology 26:229–246.

Ache, B. W., and J. M. Young. 2005. Olfaction: Diverse Species, Conserved Principles. Neuron 48:417–430.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. Journal of Molecular Biology 215:403–410.

Aman, J. W., J. S. Imperial, B. Ueberheide, M.-M. Zhang, M. Aguilar, D. Taylor, M. Watkins, D. Yoshikami, P. Showers-Corneli, H. Safavi-Hemami, J. Biggs, R. W. Teichert, and B. M. Olivera. 2015. Insights into the origins of fish hunting in venomous cone snails from studies of Conus tessulatus. Proc Natl Acad Sci U S A 112:5087–5092.

Anderson, E., and G. L. Stebbins. 1954. Hybridization as an Evolutionary Stimulus. Evolution 8:378–388.

Andrews, S. 2010. FastQC: a quality control tool for high throughput sequence data. Available online at http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Ané, C., B. Larget, D. A. Baum, S. D. Smith, and A. Rokas. 2007. Bayesian Estimation of Concordance among Gene Trees. Mol Biol Evol 24:412–426.

Arnold, M. L. 2004. Transfer and Origin of Adaptations through Natural Hybridization: Were Anderson and Stebbins Right? The Plant Cell 16:562–570.

Audesirk, T., and G. Audesirk. 1985. 1 - Behavior of Gastropod Molluscs. Pp. 1–94 *in* A. O. D. Willows, ed. The Mollusca. Academic Press.

Audesirk, T. E. 1975. Chemoreception in Aplysia californica. I. behavioral localization of distance chemoreceptors used in food-finding. Behavioral Biology 15:45–55.

Babik, W., W. Branicki, J. Crnobrnja-Isailović, D. Cogălniceanu, I. Sas, K. Olgun, N. A. Poyarkov, M. Garcia-París, and J. W. Arntzen. 2005. Phylogeography of two European newt species — discordance between mtDNA and morphology. Molecular Ecology 14:2475–2491.

Baiz, M. D., P. K. Tucker, and L. Cortés-Ortiz. 2019. Multiple forms of selection shape reproductive isolation in a primate hybrid zone. Molecular Ecology 28:1056–1069.

Ballard, J. W. O., and M. C. Whitlock. 2004. The incomplete natural history of mitochondria. Molecular Ecology 13:729–744.

Barghi, N., G. P. Concepcion, B. M. Olivera, and A. O. Lluisma. 2016. Characterization of the complete mitochondrial genome of Conus tribblei Walls, 1977. Mitochondrial DNA A DNA Mapp Seq Anal 27:4451–4452.

Bargmann, C. I. 2006. Comparative chemosensation from receptors to ecology. Nature 444:295.

Bear, D. M., J.-M. Lassance, H. E. Hoekstra, and S. R. Datta. 2016. The Evolving Neural and Genetic Architecture of Vertebrate Olfaction. Current Biology 26:R1039–R1049.

Belton, J.-M., R. P. McCord, J. Gibcus, N. Naumova, Y. Zhan, and J. Dekker. 2012. Hi-C: A comprehensive technique to capture the conformation of genomes. Methods 58.

Bergh, R. 1895. Beiträge zur Kenntnis der Coniden. Nova Acta Kaiserl. Leopold.-Carol. deut. Akad. Naturf 65:67–214.

Bertmar, G. 1969. The Vertebrate Nose, Remarks on Its Structural and Functional Adaptation and Evolution. Evolution 23:131–152.

Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120.

Brand, P., and S. R. Ramírez. 2017. The Evolutionary Dynamics of the Odorant Receptor Gene Family in Corbiculate Bees. Genome Biology and Evolution 9:2023–2036.

Brawand, D., M. Soumillon, A. Necsulea, P. Julien, G. Csárdi, P. Harrigan, M. Weier, A. Liechti, A. Aximu-Petri, M. Kircher, F. W. Albert, U. Zeller, P. Khaitovich, F. Grützner, S. Bergmann, R. Nielsen, S. Pääbo, and H. Kaessmann. 2011. The evolution of gene expression levels in mammalian organs. Nature 478:343–348.

Brown, J. W., J. F. Walker, and S. A. Smith. 2017. Phyx: phylogenetic tools for unix. Bioinformatics 33:1886–1888.

Buck, L., and R. Axel. 1991. A novel multigene family may encode odorant receptors: A molecular basis for odor recognition. Cell 65:175–187.

Cahill, A. E., and S. A. Koury. 2016. Larval settlement and metamorphosis in a marine gastropod in response to multiple conspecific cues. PeerJ 4.

Chang, D., and T. F. Duda. 2016. Age-related association of venom gene expression and diet of predatory gastropods. BMC Evolutionary Biology 16.

Chang, D., and T. F. Duda. 2012. Extensive and Continuous Duplication Facilitates Rapid Evolution and Diversification of Gene Families. Mol Biol Evol 29:2019–2029.

Chang, D., A. M. Olenzek, and T. F. Duda. 2015. Effects of geographical heterogeneity in species interactions on the evolution of venom genes. Proceedings of the Royal Society B: Biological Sciences 282:20141984–20141984.

Clifford, K. T., L. Gross, K. Johnson, K. J. Martin, N. Shaheen, and M. A. Harrington. 2003. Slime-trail tracking in the predatory snail, Euglandina rosea. Behavioral Neuroscience 117:1086–1095.

Copeland, M. 1918. The olfactory reactions and organs of the marine snails Alectrion obsoleta (Say) and Busycon canaliculatum (Linn.). Journal of Experimental Zoology 177–227.

Croll, R. P. 1983. Gastropod Chemoreception. Biological Reviews 58:293–319.

Cummins, S. F., D. Erpenbeck, Z. Zou, C. Claudianos, L. L. Moroz, G. T. Nagle, and B. M. Degnan. 2009. Candidate chemoreceptor subfamilies differentially expressed in the chemosensory organs of the mollusc Aplysia. BMC Biology 7:28.

Davidson, N. M., and A. Oshlack. 2014. Corset: enabling differential gene expression analysis for de novoassembled transcriptomes. Genome Biology 15:410.

Dierckxsens, N., P. Mardulyn, and G. Smits. 2017. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Res 45:e18–e18.

Dong, D., G. He, S. Zhang, and Z. Zhang. 2009. Evolution of Olfactory Receptor Genes in Primates Dominated by Birth-and-Death Process. Genome Biol Evol 1:258–264.

Duda Jr, T. F., and S. R. Palumbi. 2000. Evolutionary Diversification of Multigene Families: Allelic Selection of Toxins in Predatory Cone Snails. Molecular Biology and Evolution 17:1286–1293.

Duda, T. 2001. Origins of diverse feeding ecologies within Conus, a genus of venomous marine gastropods. Biological Journal of the Linnean Society 73:391–409.

Duda, T. F. 2008. Differentiation of Venoms of Predatory Marine Gastropods: Divergence of Orthologous Toxin Genes of Closely Related Conus Species with Different Dietary Specializations. J Mol Evol 67:315.

Duda, T. F., D. Chang, B. D. Lewis, and T. Lee. 2009a. Geographic variation in venom allelic composition and diets of the widespread predatory marine gastropod Conus ebraeus. PLoS ONE 4:e6245.

Duda, T. F., and A. J. Kohn. 2005. Species-level phylogeography and evolutionary history of the hyperdiverse marine gastropod genus Conus. Molecular Phylogenetics and Evolution 34:257–272.

Duda, T. F., A. J. Kohn, and A. M. Matheny. 2009b. Cryptic Species Differentiated in Conus ebraeus, a Widespread Tropical Marine Gastropod. The Biological Bulletin 217:292–305.

Duda, T. F., and T. Lee. 2009. Ecological Release and Venom Evolution of a Predatory Marine Snail at Easter Island. PLoS ONE 4:e5558.

Duda, T. F., and H. A. Lessios. 2009. Connectivity of populations within and between major biogeographic regions of the tropical Pacific in Conus ebraeus, a widespread marine gastropod. Coral Reefs 28:651–659.

Duda, T. F., and S. R. Palumbi. 2004. Gene expression and feeding ecology: evolution of piscivory in the venomous gastropod genus *Conus*. Proceedings of the Royal Society of London. Series B: Biological Sciences 271:1165–1174.

Duda, T. F., and S. R. Palumbi. 1999. Molecular genetics of ecological diversification: Duplication and rapid evolution of toxin genes of the venomous gastropod Conus. Proceedings of the National Academy of Sciences 96:6820–6823.

Duda, T. F., M. Terbio, G. Chen, S. Phillips, A. M. Olenzek, D. Chang, and D. W. Morris. 2012. Patterns of Population Structure and Historical Demography of Conus Species in the Tropical Pacific. American Malacological Bulletin 30:175–187.

Durand, E. Y., N. Patterson, D. Reich, and M. Slatkin. 2011. Testing for Ancient Admixture between Closely Related Populations. Mol Biol Evol 28:2239–2252.

Dutertre, S., A.-H. Jin, P. F. Alewood, and R. J. Lewis. 2014. Intraspecific variations in Conus geographus defence-evoked venom and estimation of the human lethal dose. Toxicon 91:135–144.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797.

Eltz, T., Y. Zimmermann, C. Pfeiffer, J. R. Pech, R. Twele, W. Francke, J. J. G. Quezada-Euan, and K. Lunau. 2008. An Olfactory Shift Is Associated with Male Perfume Differentiation and Species Divergence in Orchid Bees. Current Biology 18:1844–1848.

Enright, A. J., S. Van Dongen, and C. A. Ouzounis. 2002. An efficient algorithm for large-scale detection of protein families. Nucleic Acids Res 30:1575–1584.

Fay, J. C., and P. J. Wittkopp. 2008. Evaluating the role of natural selection in the evolution of gene regulation. Heredity 100:191–199.

Felsenstein, J. 1974. The Evolutionary Advantage of Recombination. Genetics 78:737–756.

Finn, R. D., A. Bateman, J. Clements, P. Coggill, R. Y. Eberhardt, S. R. Eddy, A. Heger, K. Hetherington, L. Holm, J. Mistry, E. L. L. Sonnhammer, J. Tate, and M. Punta. 2014. Pfam: the protein families database. Nucleic Acids Research 42:D222–D230.

Firestein, S. 2001. How the olfactory system makes sense of scents. Nature 413:211.

Floden, E. W., P. D. Tommaso, M. Chatzou, C. Magis, C. Notredame, and J.-M. Chang. 2016. PSI/TM-Coffee: a web server for fast and accurate multiple sequence alignments of regular and transmembrane proteins using homology extension on reduced databases. Nucleic Acids Res 44:W339–W343.

Fontaine, M. C., J. B. Pease, A. Steele, R. M. Waterhouse, D. E. Neafsey, I. V. Sharakhov, X. Jiang, A. B. Hall, F. Catteruccia, E. Kakani, S. N. Mitchell, Y.-C. Wu, H. A. Smith, R. R. Love, M. K. Lawniczak, M. A. Slotman, S. J. Emrich, M. W. Hahn, and N. J. Besansky. 2015. Extensive introgression in a malaria vector species complex revealed by phylogenomics. Science 347:1258524.

Fourment, M., and E. C. Holmes. 2016. Seqotron: a user-friendly sequence editor for Mac OS X. BMC Research Notes 9:106.

Fu, L., B. Niu, Z. Zhu, S. Wu, and W. Li. 2012. CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics 28:3150–3152.

Galtier, N., B. Nabholz, S. Glémin, and G. D. D. Hurst. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. Molecular Ecology 18:4541–4550.

Gardiner, A., D. Barker, R. K. Butlin, W. C. Jordan, and M. G. Ritchie. 2008. Drosophila chemoreceptor gene evolution: selection, specialization and genome size. Molecular Ecology 17:1648–1657.

Gemmell, N. J., V. J. Metcalf, and F. W. Allendorf. 2004. Mother's curse: the effect of mtDNA on individual fitness and population viability. Trends in Ecology & Evolution 19:238–244.

Gilad, Y., C. D. Bustamante, D. Lancet, and S. Pääbo. 2003. Natural Selection on the Olfactory Receptor Gene Family in Humans and Chimpanzees. The American Journal of Human Genetics 73:489–501.

Gilad, Y., A. Oshlack, G. K. Smyth, T. P. Speed, and K. P. White. 2006. Expression profiling in primates reveals a rapid evolution of human transcription factors. Nature 440:242.

Gish, W., and D. J. States. 1993. Identification of protein coding regions by database similarity search. Nature Genetics 3:266.

Givnish, T. J., and K. J. Sytsma. 2000. Molecular Evolution and Adaptive Radiation. Cambridge University Press.

Goldstein, P. Z., and R. Desalle. 2003. Calibrating phylogenetic species formation in a threatened insect using DNA from historical specimens. Molecular Ecology 12:1993–1998.

Good, J. M., D. Vanderpool, S. Keeble, and K. Bi. 2015. Negligible nuclear introgression despite complete mitochondrial capture between two species of chipmunks. Evolution 69:1961–1972.

Grabherr, M. G., B. J. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, L. Fan, R. Raychowdhury, Q. Zeng, Z. Chen, E. Mauceli, N. Hacohen, A. Gnirke, N. Rhind, F. di Palma, B. W. Birren, C. Nusbaum, K. Lindblad-Toh, N. Friedman, and A. Regev. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. 29:644–652.

Green, R. E., J. Krause, A. W. Briggs, T. Maricic, U. Stenzel, M. Kircher, N. Patterson, H. Li, W. Zhai, M. H.-Y. Fritz, N. F. Hansen, E. Y. Durand, A.-S. Malaspinas, J. D. Jensen, T. Marques-Bonet, C. Alkan, K. Prüfer, M. Meyer, H. A. Burbano, J. M. Good, R. Schultz, A.

Aximu-Petri, A. Butthof, B. Höber, B. Höffner, M. Siegemund, A. Weihmann, C. Nusbaum, E. S. Lander, C. Russ, N. Novod, J. Affourtit, M. Egholm, C. Verna, P. Rudan, D. Brajkovic, Ž. Kucan, I. Gušic, V. B. Doronichev, L. V. Golovanova, C. Lalueza-Fox, M. de la Rasilla, J. Fortea, A. Rosas, R. W. Schmitz, P. L. F. Johnson, E. E. Eichler, D. Falush, E. Birney, J. C. Mullikin, M. Slatkin, R. Nielsen, J. Kelso, M. Lachmann, D. Reich, and S. Pääbo. 2010. A Draft Sequence of the Neandertal Genome. Science 328:710–722.

Ha, M., W.-H. Li, and Z. J. Chen. 2007. External factors accelerate expression divergence between duplicate genes. Trends in Genetics 23:162–166.

Haas, B. J., A. Papanicolaou, M. Yassour, M. Grabherr, P. D. Blood, J. Bowden, M. B. Couger, D. Eccles, B. Li, M. Lieber, M. D. MacManes, M. Ott, J. Orvis, N. Pochet, F. Strozzi, N. Weeks, R. Westerman, T. William, C. N. Dewey, R. Henschel, R. D. LeDuc, N. Friedman, and A. Regev. 2013. De novo transcript sequence reconstruction from RNA-Seq: reference generation and analysis with Trinity. Nat Protoc 8.

Hadfield, M. G. 2011. Biofilms and Marine Invertebrate Larvae: What Bacteria Produce That Larvae Use to Choose Settlement Sites. Annual Review of Marine Science 3:453–470.

Hadfield, M., and V. Paul. 2001. Natural Chemical Cues for Settlement and Metamorphosis of Marine-Invertebrate Larvae. Pp. 431–461 *in* J. McClinTOCk and B. Baker, eds. Marine Chemical Ecology. CRC Press.

Hahn, M. W., T. D. Bie, J. E. Stajich, C. Nguyen, and N. Cristianini. 2005. Estimating the tempo and mode of gene family evolution from comparative genomic data. Genome Res. 15:1153–1160.

Hamilton, J. A., and J. M. Miller. 2016. Adaptive introgression as a resource for management and genetic conservation in a changing climate. Conservation Biology 30:33–41.

Hansson, B. S., and M. C. Stensmyr. 2011. Evolution of Insect Olfaction. Neuron 72:698–711.

Haswell, P. M., K. A. Jones, J. Kusak, and M. W. Hayward. 2018. Fear, foraging and olfaction: how mesopredators avoid costly interactions with apex predators. Oecologia 187:573–583.

Hay, M. E. 2009. Marine Chemical Ecology: Chemical Signals and Cues Structure Marine Populations, Communities, and Ecosystems. Annual Review of Marine Science 1:193–212.

Hayden, S., M. Bekaert, T. A. Crider, S. Mariani, W. J. Murphy, and E. C. Teeling. 2010. Ecological adaptation determines functional mammalian olfactory subgenomes. Genome Res. 20:1–9.

Hildebrand, J. G., and G. M. Shepherd. 1997. MECHANISMS OF OLFACTORY DISCRIMINATION: Converging Evidence for Common Principles Across Phyla. Annual Review of Neuroscience 20:595–631.

Holloway, A. K., M. K. N. Lawniczak, J. G. Mezey, D. J. Begun, and C. D. Jones. 2007. Adaptive Gene Expression Divergence Inferred from Population Genomics. PLOS Genetics 3:e187.

Hu, H., P. K. Bandyopadhyay, B. M. Olivera, and M. Yandell. 2011. Characterization of the Conus bullatus genome and its venom-duct transcriptome. BMC Genomics 12:60.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755.

Hussain, A., L. R. Saraiva, and S. I. Korsching. 2009. Positive Darwinian selection and the birth of an olfactory receptor clade in teleosts. PNAS 106:4313–4318.

James, D., C. A. Prator, G. G. Martin, and J. R. Schulz. 2014. Morphology of sensory papillae on the feeding proboscis of cone snails (Mollusca, Gastropoda). Invertebrate Biology 133:221–231.

Kaas, Q., J.-C. Westermann, R. Halai, C. K. L. Wang, and D. J. Craik. 2008. ConoServer, a database for conopeptide sequences and structures. Bioinformatics 24:445–446.

Kaas, Q., R. Yu, A.-H. Jin, S. Dutertre, and D. J. Craik. 2012. ConoServer: updated content, knowledge, and discovery tools in the conopeptide database. Nucleic Acids Res 40:D325–D330.

Kalyaanamoorthy, S., B. Q. Minh, T. K. F. Wong, A. von Haeseler, and L. S. Jermiin. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14:587–589.

Kamio, M., and C. D. Derby. 2017. Finding food: how marine invertebrates use chemical cues to track and select food. Natural Product Reports 34:514–528.

Khan, I., Z. Yang, E. Maldonado, C. Li, G. Zhang, M. T. P. Gilbert, E. D. Jarvis, S. J. O'Brien, W. E. Johnson, and A. Antunes. 2015. Olfactory Receptor Subgenomes Linked with Broad Ecological Adaptations in Sauropsida. Molecular Biology and Evolution 32:2832–2843.

Köhler, F., and G. Deein. 2010. Hybridisation as potential source of incongruence in the morphological and mitochondrial diversity of a Thai freshwater gastropod (Pachychilidae, Brotia H. Adams, 1866). Zoosystematics and Evolution 86:301–314.

Kohn, A. 2001. Maximal species richness in Conus: diversity, diet and habitat on reefs of northeast Papua New Guinea. Coral Reefs 20:25–38.

Kohn, A. 1990. Tempo and mode of evolution in Conidae. Malacologia 55–67.

Kohn, A. J. 1961. CHEMORECEPTION IN GASTROPOD MOLLUSCS. American Zoologist 1:291–308.

Kohn, A. J. 2014. Conus of the Southeastern United States and Caribbean. Princeton University Press.

Kohn, A. J. 1983. Microhabitat Factors Affecting Abundance and Diversity of Conus on Coral Reefs. Oecologia 60:293–301.

Kohn, A. J. 1956. PISCIVOROUS GASTROPODS OF THE GENUS CONUS. Proc. Natl. Acad. Sci. U.S.A. 42:168–171.

Kohn, A. J. 1959. The Ecology of Conus in Hawaii. Ecological Monographs 29:47–90.

Kohn, A. J., and J. W. Nybakken. 1975. Ecology of Conus on eastern Indian Ocean fringing reefs: Diversity of species and resource utilization. Marine Biology 29:211–234.

Langmead, B., and S. L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359.

Larget, B. R., S. K. Kotha, C. N. Dewey, and C. Ané. 2010. BUCKy: Gene tree/species tree reconciliation with Bayesian concordance analysis. Bioinformatics 26:2910–2911.

Lawler, A. J., and T. F. Duda. 2017. Molecular and morphometric data suggest the presence of a neglected species in the marine gastropod family Conidae. Molecular Phylogenetics and Evolution 109:421–429.

Leviten, P. J. 1978. Resource Partitioning by Predatory Gastropods of the Genus Conus on Subtidal Indo-Pacific Coral Reefs: The Significance of Prey Size. Ecology 59:614–631.

Lewontin, R. C., and L. C. Birch. 1966. Hybridization as a Source of Variation for Adaptation to New Environments. Evolution 20:315–336.

Li, B., and C. N. Dewey. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12:323.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, and R. Durbin. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079.

Li, W., and A. Godzik. 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22:1658–1659.

Linn, C., J. L. Feder, S. Nojima, H. R. Dambroski, S. H. Berlocher, and W. Roelofs. 2003. Fruit odor discrimination and sympatric host race formation in Rhagoletis. Proceedings of the National Academy of Sciences 100:11490–11493.

Llopart, A., D. Herrig, E. Brud, and Z. Stecklein. 2014. Sequential adaptive introgression of the mitochondrial genome in Drosophila yakuba and Drosophila santomea. Mol Ecol 23:1124–1136.

Löytynoja, A., and N. Goldman. 2008. Phylogeny-Aware Gap Placement Prevents Errors in Sequence Alignment and Evolutionary Analysis. Science 320:1632–1635.

Lunter, G., and M. Goodson. 2011. Stampy: A statistical algorithm for sensitive and fast mapping of Illumina sequence reads. Genome Res 21:936–939.

Lynch, M., and R. Lande. 1998. The critical effective size for a genetically secure population. Animal Conservation 1:70–72.

Mallet, J. 2007. Hybrid speciation. Nature 446:279–283.

Martin, C. H., J. S. Cutler, J. P. Friel, C. D. Touokong, G. Coop, and P. C. Wainwright. 2015. Complex histories of repeated gene flow in Cameroon crater lake cichlids cast doubt on one of the clearest examples of sympatric speciation. Evolution 69:1406–1422.

Mayr, E. 1963. Animal Species and Evolution. Belknap Press of Harvard University Press, Cambridge, MA.

McBride, C. S. 2007. Rapid evolution of smell and taste receptor genes during host specialization in Drosophila sechellia. PNAS 104:4996–5001.

McCarthy, D. J., Y. Chen, and G. K. Smyth. 2012. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. Nucleic Acids Res 40:4288–4297.

Meier, J. I., D. A. Marques, S. Mwaiko, C. E. Wagner, L. Excoffier, and O. Seehausen. 2017. Ancient hybridization fuels rapid cichlid fish adaptive radiations. Nature Communications 8:14363.

Minh, B. Q., M. W. Hahn, and R. Lanfear. 2018. New methods to calculate concordance factors for phylogenomic datasets. bioRxiv 487801.

MolluscaBase. 2019. Conidae J. Fleming, 1822. Accessed through: World Register of Marine Species.

Mombaerts, P. 2004. Genes and ligands for odorant, vomeronasal and taste receptors. Nature Reviews Neuroscience 5:263.

Muller, H. J. 1964. The relation of recombination to mutational advance. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 1:2–9.

Myers, R. A., L. J. Cruz, J. E. Rivier, and B. M. Olivera. 1993. Conus peptides as chemical probes for receptors and ion channels. Chemical Reviews 93:1923–1936.

Nguyen, L.-T., H. A. Schmidt, A. von Haeseler, and B. Q. Minh. 2015. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Mol Biol Evol 32:268–274.

Niimura, Y., and M. Nei. 2005. Comparative evolutionary analysis of olfactory receptor gene clusters between humans and mice. Gene 346:13–21.

Notredame, C., D. G. Higgins, and J. Heringa. 2000. T-coffee: a novel method for fast and accurate multiple sequence alignment11Edited by J. Thornton. Journal of Molecular Biology 302:205–217.

O'Leary, N. A., M. W. Wright, J. R. Brister, S. Ciufo, D. Haddad, R. McVeigh, B. Rajput, B. Robbertse, B. Smith-White, D. Ako-Adjei, A. Astashyn, A. Badretdin, Y. Bao, O. Blinkova, V. Brover, V. Chetvernin, J. Choi, E. Cox, O. Ermolaeva, C. M. Farrell, T. Goldfarb, T. Gupta, D. Haft, E. Hatcher, W. Hlavina, V. S. Joardar, V. K. Kodali, W. Li, D. Maglott, P. Masterson, K. M. McGarvey, M. R. Murphy, K. O'Neill, S. Pujar, S. H. Rangwala, D. Rausch, L. D. Riddick, C. Schoch, A. Shkeda, S. S. Storz, H. Sun, F. Thibaud-Nissen, I. Tolstoy, R. E. Tully, A. R. Vatsan, C. Wallin, D. Webb, W. Wu, M. J. Landrum, A. Kimchi, T. Tatusova, M. DiCuccio, P. Kitts, T. D. Murphy, and K. D. Pruitt. 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res 44:D733–D745.

Olivera, B. M., C. Walker, G. E. Cartier, D. Hooper, A. D. Santos, R. Schoenfeld, R. Shetty, M. Watkins, P. Bandyopadhyay, and D. R. Hillyard. 1999. Speciation of Cone Snails and Interspecific Hyperdivergence of Their Venom Peptides: Potential Evolutionary Significance of Intronsa. Annals of the New York Academy of Sciences 870:223–237.

Otto, S. P., and J. Whitton. 2000. Polyploid Incidence and Evolution. Annual Review of Genetics 34:401–437.

Painter, S. D., S. F. Cummins, A. E. Nichols, D.-B. G. Akalal, C. H. Schein, W. Braun, J. S. Smith, A. J. Susswein, M. Levy, P. A. C. M. de Boer, A. ter Maat, M. W. Miller, C. Scanlan, R. M. Milberg, J. V. Sweedler, and G. T. Nagle. 2004. Structural and functional analysis of Aplysia attractins, a family of water-borne protein pheromones with interspecific attractiveness. Proc Natl Acad Sci U S A 101:6929–6933.

Pardo-Diaz, C., C. Salazar, S. W. Baxter, C. Merot, W. Figueiredo-Ready, M. Joron, W. O. McMillan, and C. D. Jiggins. 2012. Adaptive Introgression across Species Boundaries in Heliconius Butterflies. PLOS Genetics 8:e1002752.

Patterson, N., P. Moorjani, Y. Luo, S. Mallick, N. Rohland, Y. Zhan, T. Genschoreck, T. Webster, and D. Reich. 2012. Ancient Admixture in Human History. Genetics 192:1065–1093.

Pease, J. B., and M. W. Hahn. 2015. Detection and Polarization of Introgression in a Five-Taxon Phylogeny. Syst Biol 64:651–662.

Phuong, M. A., M. E. Alfaro, G. N. Mahardika, R. M. Marwoto, R. E. Prabowo, T. von Rintelen, P. W. H. Vogt, J. R. Hendricks, and N. Puillandre. 2019. Lack of Signal for the Impact of Conotoxin Gene Diversity on Speciation Rates in Cone Snails. Systematic Biology, doi: 10.1093/sysbio/syz016.

Proffit, M., B. Schatz, R. M. Borges, and M. Hossaert-Mckey. 2007. Chemical mediation and niche partitioning in non-pollinating fig-wasp communities. Journal of Animal Ecology 76:296–303.

Puillandre, N., P. Bouchet, T. F. Duda, S. Kauferstein, A. J. Kohn, B. M. Olivera, M. Watkins, and C. Meyer. 2014. Molecular phylogeny and evolution of the cone snails (Gastropoda, Conoidea). Mol. Phylogenet. Evol. 78:290–303.

Rafferty, N. E., and J. W. Boughman. 2006. Olfactory mate recognition in a sympatric species pair of three-spined sticklebacks. Behav Ecol 17:965–970.

Ramasamy, S., L. Ometto, C. M. Crava, S. Revadi, R. Kaur, D. S. Horner, D. Pisani, T. Dekker, G. Anfora, and O. Rota-Stabelli. 2016. The Evolution of Olfactory Gene Families in Drosophila and the Genomic Basis of chemical-Ecological Adaptation in Drosophila suzukii. Genome Biol Evol 8:2297–2311.

Rambaut, A. 2009. FigTree.

Ranwez, V., S. Harispe, F. Delsuc, and E. J. P. Douzery. 2011. MACSE: Multiple Alignment of Coding SEquences Accounting for Frameshifts and Stop Codons. PLOS ONE 6:e22594.

Reichelt, R. E. 1982. Space: A non-limiting resource in the niches of some abundant coral reef gastropods. Coral Reefs 1:3–11.

Remigio, E. A., and T. F. Duda. 2008. Evolution of ecological specialization and venom of a predatory marine gastropod. Molecular Ecology 17:1156–1162.

Renoult, J. P., P. Geniez, P. Bacquet, L. Benoit, and P.-A. Crochet. 2009. Morphology and nuclear markers reveal extensive mitochondrial introgressions in the Iberian Wall Lizard species complex. Molecular Ecology 18:4298–4315.

Richards, E. J., and C. H. Martin. 2017. Adaptive introgression from distant Caribbean islands contributed to the diversification of a microendemic adaptive radiation of trophic specialist pupfishes. PLOS Genetics 13:e1006919.

Rieseberg, L. H. 1995. The Role of Hybridization in Evolution: Old Wine in New Skins. American Journal of Botany 82:944–953.

Robertson, H. M., C. G. Warr, and J. R. Carlson. 2003. Molecular evolution of the insect chemoreceptor gene superfamily in Drosophila melanogaster. Proceedings of the National Academy of Sciences 100:14537–14542.

Robinson, M. D., D. J. McCarthy, and G. K. Smyth. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140.

Röckel, D., W. Korn, and A. J. Kohn. 1995. Manual of the Living Conidae: Indo-Pacific Region. Hemmen.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Rosenberg, G. 2014. A New Critical Estimate of Named Species-Level Diversity of the Recent Mollusca*. malb 32:308–322.

Schliewen, U. K., D. Tautz, and S. Pääbo. 1994. Sympatric speciation suggested by monophyly of crater lake cichlids. Nature 368:629.

Schluter, D. 2000. The Ecology of Adaptive Radiation. OUP Oxford.

Seehausen, O. 2004. Hybridization and adaptive radiation. Trends Ecol. Evol. (Amst.) 19:198–207.

Shaw, K. L. 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: What mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. Proc Natl Acad Sci U S A 99:16122–16127.

Simion, P., K. Belkhir, C. François, J. Veyssier, J. C. Rink, M. Manuel, H. Philippe, and M. J. Telford. 2018. A software tool 'CroCo' detects pervasive cross-species contamination in next generation sequencing data. BMC Biol 16.

Sloan, D. B., J. C. Havird, and J. Sharbrough. 2017. The on-again, off-again relationship between mitochondrial genomes and species boundaries. Molecular Ecology 26:2212–2236.

Smith, T. D., J. B. Rossie, and K. P. Bhatnagar. 2007. Evolution of the nose and nasal skeleton in primates. Evolutionary Anthropology: Issues, News, and Reviews 16:132–146.

Smith-Unna, R., C. Boursnell, R. Patro, J. M. Hibberd, and S. Kelly. 2016. TransRate: reference-free quality assessment of de novo transcriptome assemblies. Genome Res 26:1134–1144.

Song, L., and L. Florea. 2015. Rcorrector: efficient and accurate error correction for Illumina RNA-seq reads. GigaScience 4:48.

Sonnhammer, E. L. L. 1998. A Hidden Markov Model for Predicting Transmembrane Helices in Protein Sequences.

Sota, T. 2002. Radiation and reticulation: extensive introgressive hybridization in the carabid beetles Ohomopterus inferred from mitochondrial gene genealogy. Popul Ecol 44:0145–0156.

Spengel, J. W. 1881. Die Geruchsorgane und dasNervensystem der Mollusken. Z. Wiss. Zool 333–383.

Spengler, H. A., and A. J. Kohn. 1995. Comparative external morphology of the Conus osphradium (Mollusca: Gastropoda). Journal of Zoology 235:439–453.

Spielman, S. J., and C. O. Wilke. 2013. Membrane Environment Imposes Unique Selection Pressures on Transmembrane Domains of G Protein-Coupled Receptors. J Mol Evol 76:172–182.

Stanley, S. M. 2007. An Analysis of the History of Marine Animal Diversity. Paleobiology 33:1–55.

Stewart, J., and W. F. Gilly. 2005. Piscivorous Behavior of a Temperate Cone Snail, *Conus californicus*. The Biological Bulletin 209:146–153.

Strickland, J. L., C. F. Smith, A. J. Mason, D. R. Schield, M. Borja, G. Castañeda-Gaytán, C. L. Spencer, L. L. Smith, A. Trápaga, N. M. Bouzid, G. Campillo-García, O. A. Flores-Villela, D. Antonio-Rangel, S. P. Mackessy, T. A. Castoe, D. R. Rokyta, and C. L. Parkinson. 2018. Evidence for divergent patterns of local selection driving venom variation in Mojave Rattlesnakes (Crotalus scutulatus). Scientific Reports 8:17622.

Tait Cheyenne, Batra Srishti, Ramaswamy Sree Subha, Feder Jeffrey L., and Olsson Shannon B. 2016. Sensory specificity and speciation: a potential neuronal pathway for host fruit odour discrimination in Rhagoletis pomonella. Proceedings of the Royal Society B: Biological Sciences 283:20162101.

Tester, M., and P. Langridge. 2010. Breeding Technologies to Increase Crop Production in a Changing World. Science 327:818–822.

Thankaswamy-Kosalai, S., P. Sen, and I. Nookaew. 2017. Evaluation and assessment of read-mapping by multiple next-generation sequencing aligners based on genome-wide characteristics. Genomics 109:186–191.

Toews, D. P. L., and A. Brelsford. 2012. The biogeography of mitochondrial and nuclear discordance in animals. Molecular Ecology 21:3907–3930.

Vallejo, B. 2005. Inferring the mode of speciation in Indo-West Pacific Conus (Gastropoda: Conidae). Journal of Biogeography 32:1429–1439.

van Dongen, S., and C. Abreu-Goodger. 2012. Using MCL to Extract Clusters from Networks. Pp. 281–295 *in* J. van Helden, A. Toussaint, and D. Thieffry, eds. Bacterial Molecular Networks: Methods and Protocols. Springer New York, New York, NY.

Walderon, M. D., K. J. Nolt, R. E. Haas, K. N. Prosser, J. B. Holm, G. T. Nagle, and J. G. Boal. 2011. Distance chemoreception and the detection of conspecifics in Octopus bimaculoides. J Molluscan Stud 77:309–311.

Weese, D. A., and T. F. Duda. 2019. Effects of Predator-Prey Interactions on Predator Traits: Differentiation of Diets and Venoms of a Marine Snail. Toxins 11:299.

Williams, S. T., and T. F. Duda. 2008. Did tectonic activity stimulate oligo-miocene speciation in the Indo-West Pacific? Evolution; international journal of organic evolution 62:1618–1634.

Wong, E. S. W., and K. Belov. 2012. Venom evolution through gene duplications. Gene 496:1–7.

Yang, Y., and S. A. Smith. 2014. Orthology Inference in Nonmodel Organisms Using Transcriptomes and Low-Coverage Genomes: Improving Accuracy and Matrix Occupancy for Phylogenomics. Mol Biol Evol 31:3081–3092.

Yang, Z. 2007. PAML 4: Phylogenetic Analysis by Maximum Likelihood. Mol Biol Evol 24:1586–1591.

Yu, G., D. K. Smith, H. Zhu, Y. Guan, and T. T.-Y. Lam. 2017. ggtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods in Ecology and Evolution 8:28–36.

Zhang, W., K. K. Dasmahapatra, J. Mallet, G. R. P. Moreira, and M. R. Kronforst. 2016. Genome-wide introgression among distantly related Heliconius butterfly species. Genome Biology 17:25.

FIGURES AND TABLES

Table 3.1. Individuals of *Conus judaeus* and *Conus ebraeus* sequenced for this study.

Individual	UMMZ Accession #	Sex	Collection Locale	Collection Date
jud_1	304700	♀	Cape Bise, Okinawa, Japan	2015-07-24
jud_2	304704	♀	Cape Bise, Okinawa, Japan	2015-07-24
jud_3	304718	♀	Cape Bise, Okinawa, Japan	2015-07-24
jud_4	304721	♀	Cape Bise, Okinawa, Japan	2015-07-24
jud_5	304720	♂	Cape Bise, Okinawa, Japan	2015-07-24
ebr_1	304690	♀	Sesoko Station, Okinawa, Japan	2015-07-24
ebr_2	304692	♀	Sesoko Station, Okinawa, Japan	2015-07-24
ebr_5	304701	♀	Cape Bise, Okinawa, Japan	2015-07-24
ebr_4	304699	♂	Cape Bise, Okinawa, Japan	2015-07-24
ebr_5	304691	♂	Sesoko Station, Okinawa, Japan	2015-07-24

Table 3.2. Descriptions of models used to test evolution of evolution for orthologous and paralogous OR loci in *C. ebraeus* and *C. judaeus*. Model likelihoods and estimated omega values are provided to the right.

	Subfamily one			Subfamily two		
Model description	**-lnL**	**ω_{para}**	**ω_{ortho}**	**-lnL**	**ω_{para}**	**ω_{ortho}**
Model A: Fix $\omega = 1$, all branches	36175.3	[1]	[1]	14804.9	[1]	[1]
Model B: Estimate single ω for all branches	34862.0	0.25	0.25	14548.5	0.26	0.26
Model C: Estimate separate $\omega_{paralog}$ and $\omega_{ortholog}$	34858.0	0.24	0.23	14544.7	0.22	0.31
Model D: Estimate $\omega_{paralog}$ and fix $\omega_{ortholog} = 1$	35208.6	0.23	[1]	14574.1	0.21	[1]
Model E: Estimate separate ω for each branch	34725.0	-	-	14489.3	-	-

Table 3.3. Summary of read-processing and transcript-filtering steps.

Species	Raw reads (pooled)	Cleaned reads (pooled)	Unfiltered transcripts	Filtered transcripts	Candidate OR genes
C. ebraeus	76,752,610	40,400,587	429,975	35,143	88
C. judaeus	75,022,298	43,104,341	370,330	37,944	118

Table 3.4. Hypothesis testing and LRT model comparisons.

		Subfamily one		Subfamily two	
Hypothesis	**Model comparison**	**LRT**	***p-value***	***LRT***	***p-value***
$\omega_{paralog} = \omega_{ortholog} = 1$	A & B	2626.6	<0.001	512.8	<0.001
$\omega_{paralog} \neq \omega_{ortholog}$	B & C	1.48	<0.05	7.6	<0.05
$\omega_{paralog} \neq \omega_{ortholog} = 1$	C & D	701.28	<0.001	58.8	<0.001

Table 3.5. Lineage specific estimates of dN and dS for orthologous loci with $\omega > 1$.

Gene_ID	**Ortholog_ID**	**Subfamily**	**dN**	**dS**
jud_allo@32688	sub1_1	one	0.005448	0.003684
jud_allo@13162	sub1_15	one	0.007424	0.000091
ebr_allo@15026	sub1_12	one	0.001605	0.000008
ebr_allo@1692	sub1_20	one	0.005019	0.000008
ebr_allo@36942	sub1_2	one	0.013254	0.000013
ebr_allo@5493	sub2_8	two	0.003016	0.000003
jud_allo@14879	sub2_4	two	0.012092	0.000012
jud_allo@50661	sub2_3	two	0.001399	0.000001

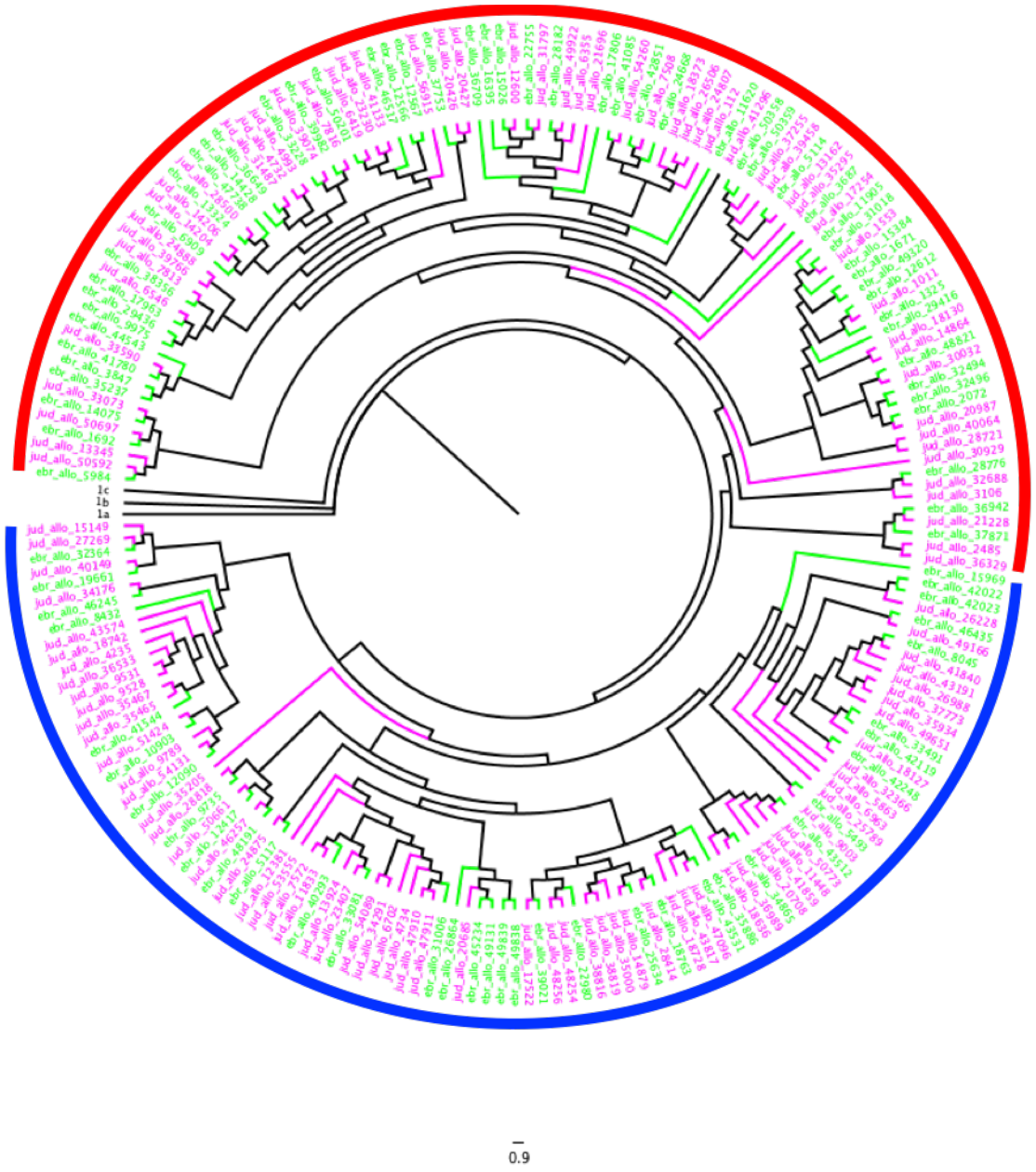

Figure 3.1. Circle cladogram of all candidate OR genes identified from *C. ebraeus* and *C. judaeus*. Maximum likelihood tree inferred using IQ-TREE and rooted to *Aplysia californica* OR gene sequences. Branches and tip labels are colored according to species (green = *C. ebraeus*, magenta = *C. judaeus*). Subfamilies are indicated by colored arcs around the tree circumference (red = subfamily one, blue = subfamily two).

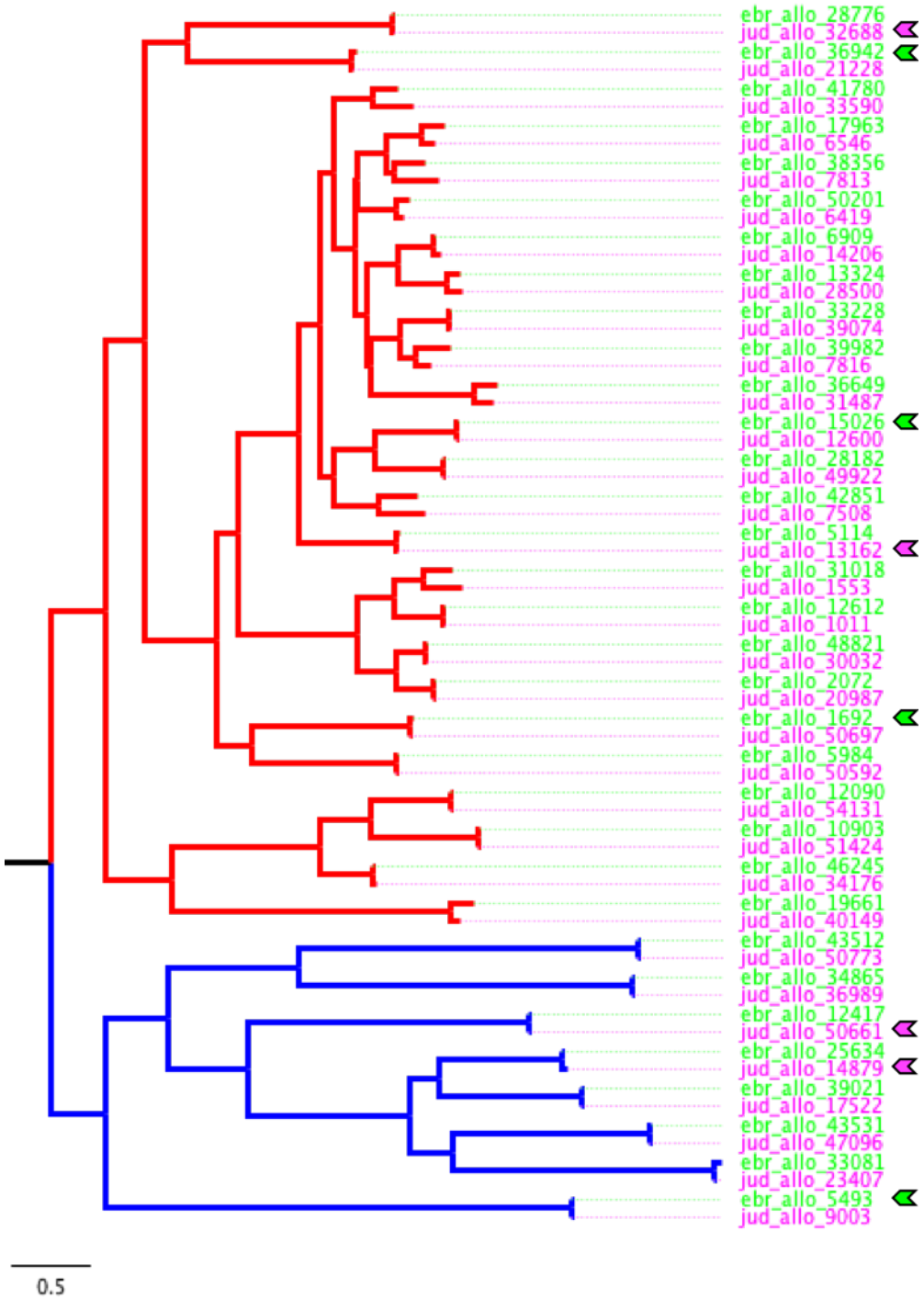

Figure 3.2. IQ-TREE maximum likelihood phylogeny of putative OR orthologs. Branches are colored according to subfamily (red = one, blue = two). Tips labels colored according to species (green = *C. ebraeus*, magenta = *C. judaeus*). Branches exhibiting ω > 1 indicated with arrows on the right.

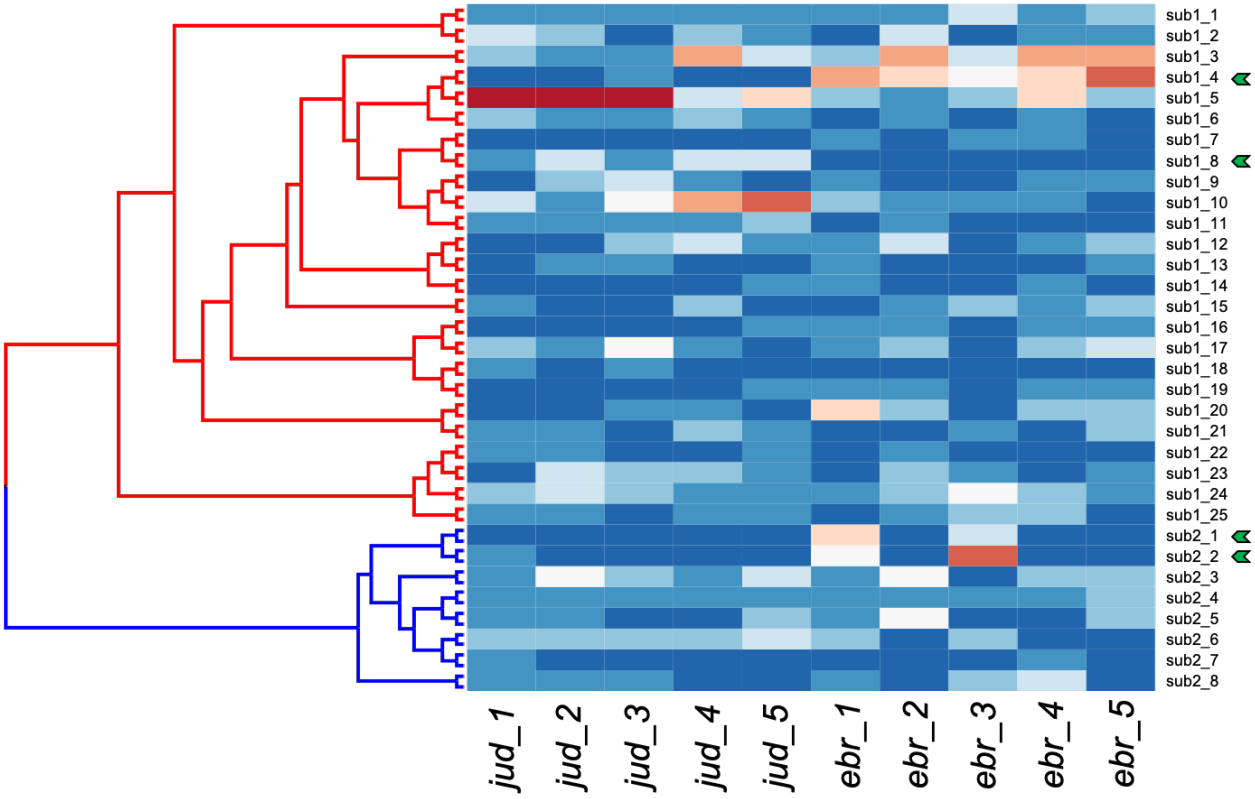

Figure 3.3. Expression heatmap of 33 orthologous loci. Cells are colored according to raw RNA fragment counts, with dark blue representing the lowest and dark red the highest expression levels. Each row of cells represents a single ortholog pair and is aligned to its respective tree position. Branches of the tree are colored by subfamily (one = red, blue = two). Ortholog identifiers are provided to the right, and green arrows indicate differentially expressed orthologs.

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