UNIVERSITY OF CALIFORNIA SAN DIEGO

Mitogenomics and the phylogeny of mantis shrimp [Crustacea: Stomatopoda]

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by

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The thesis of Cassandra Koga is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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ABSTRACT OF THE THESIS

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Stomatopoda, otherwise known as mantis shrimp, are a diverse group of marine crustaceans with some notable features. They have enlarged second maxillipeds encompassing the raptorial claw that distinguishes them into two groups: smashers and spearers. Previous studies have focused on morphology or a few gene trees, but only recently have there been whole mitochondrial genome-based phylogenies of mantis shrimp. However, there is only a small set of mantis shrimp taxa with their mitogenomes sequenced.

Nine new mitochondrial genomes were generated from genome skimming with a conserved gene order and a combined phylogenetic analysis with available data of the mitochondrial 13 protein coding genes, 12S, 16S, and nuclear 18S. Species from three out of the seven superfamilies were used in this study. Two different rooting options were used: 1) *Euphausia pacifica* (krill) and 2) *Hemisquilla californiensis* based on the current hypothesis that *Hemisquilla* is the sister group to the rest of Stomatopoda. The *H. californiensis* outgroup datasets had the same tree topology as the *E. pacifica* outgroup datasets with slight variation at low supported nodes. Squilloidea was found to be highly supported as monophyletic while Gonodactyloidea was non-monophyletic. The position of *H. californiensis* was found inside its superfamily, Gonodactyloidea, and grouped in a low supported clade containing *Odontodactylus havanensis* and *Lysiosquillina maculata* for the *E. pacifica* datasets. An ancestral state reconstruction was performed on the raptorial claw form to determine the evolution of one of its most characteristic features. The results exhibited spearers as the ancestral state with smashing evolving after.

Introduction

Mantis shrimp in the order Stomatopoda Latreille, 1817 are marine crustaceans well known for their raptorial claws and complex eyes. Stomatopods construct or occupy burrows in tropical and subtropical regions (Caldwell and Dingle, 1975). These marine carnivores capture prey by spearing or smashing depending on their distinctive second maxilliped appendages, the raptorial claws (Ahyong, 1997). Smashers strike with the heel of the dactyl (calcified tip of the claw shaped like a club) after energy is loaded in a saddle spring mechanism (Patek et al., 2004). This allows the claw to strike hard shelled prey. Spearers are ambush predators with elongated serrated raptorial claws enhanced for soft-bodied prey (DeVries et al., 2012). In addition to their claws, mantis shrimp are of research interest for their complex visual system due to their compound eyes with around 12-16 photoreceptors, capable of seeing ultraviolet, linear, and circular polarized light (Marshall, 1988; Thoen et al., 2014). Stomatopods have many important roles including: mantis shrimp fisheries, bioindicators of pollution, and their position as a predator in the marine ecosystem (Sukumaran, 1987; Abelló and Martín, 1993; Erdmann and Caldwell, 1997; Kodama et al., 2006; Ng et al., 2007; Antony et al., 2010).

Within Stomatopoda, the suborder, Unipeltata Latreille, 1825, contains all extant stomatopods, while the other two extinct suborders are Palaeostomatopodea and Archaeostomatopodea (Schram, 2007; Haug et al., 2010). Stomatopoda contains seven superfamilies, 17 families, and over 100 genera (Ahyong and Harling, 2000; Van Der Wal et al., 2017): Squilloidea Latreille, 1802; Gonodactyloidea Giesbrecht, 1910; Lysiosquilloidea Giesbrecht, 1910; Bathysquilloidea Manning, 1967; Eurysquilloidea Manning, 1977; Erythrosquilloidea Manning & Bruce, 1984; and Parasquilloidea Manning, 1995. The

majority of mantis shrimp species are contained within Squilloidea, Gonodactyloidea, and Lysioquilloidea (Ahyong and Jarman, 2009).

One of the larger superfamilies, Gonodactyloidea, contains the only four families of smashers out of all the superfamilies. The rest of the stomatopod superfamilies consist only of spearers. Gonodactyloidea has been found to be non-monophyletic in previous molecular studies due mainly to the position of the 'intermediate' raptorial claw family, Hemisquillidae Manning, 1980. Morphological data supports Hemisquillidae within Gonodactyloidea; however, molecular data has shown it as a sister group to all other superfamilies (Ahyong, 1997; Ahyong and Harling, 2000; Ahyong and Jarman, 2009; Porter et al., 2010; Van Der Wal et al., 2017). Furthermore, Pseudosquillidae Manning, 1977, one of the spearing families in Gonodactyloidea, has an unclear position. A couple of molecular studies have found Pseudosquillidae to be outside of the rest of the superfamilies, including Gonodactyloidea (Ahyong and Jarman, 2009; Porter et al., 2010), while Van Der Wal et al. (2017) showed Pseudosquillidae within Gonodactyloidea. Thus, no studies have resolved the non-monophyly of Gonodactyloidea regarding the positions of Hemisquillidae and Pseudosquillidae.

Certain evolutionary inferences can be made from the phylogenies pertaining to the charismatic features of the mantis shrimp such as the raptorial claw. There is an ongoing question on the evolutionary history of smashers and spearers and whether smashers and spearers diverged from the outset (Ahyong and Harling 2000; Ahyong and Jarman, 2009) or whether smashers evolved after a long history of spearers (Caldwell, 1991; Ahyong, 1997). The morphology-based phylogeny of Ahyong and Harling (2000), showed that Unipeltata deviated from the beginning with distinct clades of smashers and spearers, rather than a

grade of spearers with smashers deriving from them. The molecular phylogenies of Ahyong and Jarman (2009) and Porter et al. (2010) concur with the morphology-based phylogeny of Ahyong and Harling (2000), showing one evolutionary smashing event. The results of Van Der Wal et al. (2017) support both raptorial claw evolution hypotheses: smashers were nested deeply among spearers and there is a short branch on the node leading to the smashers indicating smashers evolved quickly. However, they lack strong nodal support at the origin for smashing and origin for spearing nodes in their phylogeny and the evolution of the raptorial claws is still in question (Van Der Wal et al., 2017).

Despite the diversity of mantis shrimp, only twelve different species' complete mitogenomes have been published. As next generation sequencing (NGS) technology such as genome skimming (shallow, low pass sequencing) becomes more accessible, studies using whole mitochondrial genomes in their phylogenies have had better resolution and support compared to those of single gene trees (Lin et al., 2012; Trevisan et al., 2019; Tilic et al., 2020). Stomatopoda phylogenetic studies using whole mitochondrial genomes (Kang et al., 2016; Zhang et al., 2020; Hwang et al., 2020) had fewer samples from three superfamilies, but some had higher support than Van Der Wal et al. (2017). Zhang et al. (2020) used maximum likelihood to build the phylogeny and used several members from Decapoda as the outgroups. This tree had high support throughout and the phylogeny matched those of Kang et al. (2016) and Hwang et al. (2020). However, more taxon sampling is needed to add more data to the mitochondrial genome phylogeny.

By sequencing whole mitochondrial genomes, we seek to fill these gaps of knowledge with better support and resolution. Genome skimming was used to obtain whole mitochondrial genome sequences of mantis shrimp from two superfamilies: Gonodactyloidea

and Squilloidea. We present nine newly sequenced mantis shrimp mitochondrial genomes and a Stomatopoda phylogeny, which includes existing published mitogenomes and available nuclear 18S gene data.

Materials and Methods

Sampling

Samples were collected from research cruises or from commercial aquarium suppliers (Table 1). The voucher specimens were fixed and preserved in 50% ethanol and deposited at the Scripps Institution of Oceanography, Benthic Invertebrate Collection, La Jolla, California, USA. Identification was determined by morphology based on the keys of Manning (1962) and Ahyong (2001) for SIO-BIC C14383 *Mesacturoides brevisquamatus*, SIO-BIC C12730 *Gonodactylus sp*., and SIO-BIC C12514 *Gonodactylellus sp*.

Table 1. Collection information, vouchers, GenBank accession numbers (mitogenome, 18S), and mitogenome length. New sequences are in bold.

Taxon	SIO-BIC Catalogue Number	Collection Locality	Mitochondrial Genome Genbank Accession Number	18S Genbank Accession Number	Mitochondrial Genome Length
Gonodactyloidea					
Hemisquillidae					
Hemisquilla californiensis	C14449	San Diego, CA	**MW867302**	HM138876	16,030
Odontodactylidae					
Odontodactylus havanensis	C14408	Florida Keys, Key Largo and vicinity	**MW867300**	HM138884	16,035
Gonodactylidae					
Neogonodactylus oerstedii	C14405	Florida Keys, Key Largo and vicinity	**MW867303**	HM138882	16,327
Neogonodactylus bredini	C14428	Gulf of Mexico, Florida, in the vicinity of New Port Richey	**MW867301**	HM138881	16,342
Gonodactylus chiragra		-	DQ191682	HM138870	-
Gonodactylaceus randalli		-	MW019425	-	
Gonodactylus sp.	C12730	Ghubbat North Channel, Red Sea	**MW867306**	-	16,032
Gonodactylellus sp.	C12514	Al-Fasar Reef, Red Sea	**MW867308**	-	16,011
Takuidae					
Mesacturoides brevisquamatus	C14383	Awol Marah, Red Sea	**MW867304**	-	16,151
Taku spinosocarinatus		-	MT672285	HM138899	-
Pseudosquillidae					
Pseudosquilla ciliata		-	AY947836	HM138888	-
Protosquillidae					
Chorisquilla orientalis		-	MT672286	-	-
Squilloidea					
Squillidae					
Oratosquilla oratoria		-	GQ292769	-	-
Squilla mantis		-	AY639936	GQ328958	-
Squilla empusa		-	DQ191684	HM138897	-
Squilla biformis	C13808	Quepos Slide, Costa Rica	**MW867305**	-	15,688
Squilloides leptosquilla		-	KR095170	-	-
Harpiosquilla harpax		-	AY699271	-	-
Lophosquilla costata		-	MT276143	-	-
Alima pacifica	C12719	Mubarak, Red Sea	**MW867307**	HM138858	15,678
Lysiosquilloidea					
Lysiosquillidae					
Lysiosquillina maculata		-	DQ191683	HM138878	-
Outgroup					
Euphausiidae					
Euphausia pacifica		-	EU587005	AY141010	-

DNA Extraction and Sequencing

DNA was extracted from claws, pleopods, and/or pereiopods using the Zymo Quick-DNA Miniprep plus kit, following the manufacturer's protocol. The mitochondrial COI gene was amplified using LCO1490(f) and HCO2198(r) primer set (Folmer et al., 1994). Samples were prepared with 8.5 µL of water, 12.5 µL of Apex 2X Taq RED Master Mix DNA polymerase (Genesee Scientific), 1 µL each of forward and reverse primers, and 2.0 µL of extracted DNA from specimens. The Eppendorf thermocycler was used to carry out the rest of the PCR with the temperature settings at: 94ºC/3min.; (94ºC/30s, 47ºC/45s, 72ºC/1min, 94ºC/30s, 52ºC/45s, 72ºC/1min) x35 cycles, 72ºC/5min.

Products were purified with 2 µL ExoSAP-IT and run in a thermocycler with settings: 37°C/20min and 80°C/15min. Sanger Sequencing was completed by Eurofins Genomics (Louisville, KY).

Mitochondrial Genome Assembly and Annotation

Extracted DNA was prepared and sequenced by Novogene (Sacramento, CA) using genome skimming, generating 2Gb worth of reads. Data statistics were checked with SeqKit v.0.13.2 (Shen et al., 2016) and the raw reads were trimmed with Trimmomatic v. 0.39 (Bolger et al., 2014). The mitochondrial genomes were assembled with Mitofinder v. 1.4 (Allio et al., 2020) using the Trimmomatic output files. Parameters chosen were Megahit metagenomic assembler v. 1.2.9 (Li et al., 2016) and tRNAs were annotated with Arwen v. 1.2.3 (Laslett and Canbäck, 2008). The Mitofinder contigs were checked with MITOS (Bernt et al., 2013) web server under the mitochondrial code for invertebrates and the annotations were manually edited in Geneious v 11.1.5 (Kearse et al., 2012) if necessary to reflect accurate positions.

Table 2. Models used for each gene in the datasets for maximum likelihood analyses. The models were chosen by ModelTest-NG.

Genes	*Euphausia* Outgroup Nucleotide Only Dataset	*Euphausia* Outgroup Amino Acid/Nucleotide Dataset	*Hemisquilla* Outgroup Nucleotide Only Dataset	*Hemisquilla* Outgroup Amino Acid/Nucleotide Dataset
ATP6	GTR+I+G	MTMAM	TVM+I+G	MTMAM
ATP8	TPM3uf+I+G	MTZOA	TPM3uf+I+G	MTZOA
COX1	GTR+I+G	MTZOA	TN93+I+G	MTZOA
COX2	GTR+I+G	MTZOA	GTR+I+G	MTZOA
COX3	TVM+I+G	MTZOA	TVM+I+G	MTZOA
CYTB	TPM2uf+I+G	MTZOA	TN93+I+G	MTZOA
NAD1	GTR+I+G	MTZOA	TVM+I+G	MTZOA
NAD2	GTR+I+G	MTMAM	TIM2+I+G	MTMAM
NAD3	TPM2uf+I+G	MTMAM	TPM2uf+I+G	MTZOA
NAD4	GTR+I+G	MTZOA	GTR+I+G	MTZOA
NAD4L	TVM+I+G	MTZOA	TVM+I+G	MTZOA
NAD5	GTR+I+G	MTZOA	GTR+I+G	MTZOA
NAD6	TVM+I+G	MTMAM	TVM+I+G	MTMAM
16S	TIM3+I+G	TIM3+I+G	TIM3+I+G	TIM3+I+G
12S	TIM2+I+G	TIM2+I+G	TIM1+I+G	TIM1+I+G
18S	TN93+I+G	TN93+I+G	TIM3+I+G	TIM3+I+G

Phylogenetic Analyses

Based on recent molecular studies of Malacostraca, Euphausiacea was found to be closely related to Stomatopoda (Schwentner et al., 2018; Lozano-Fernandez et al., 2019); thereby, *Euphausia pacifica* Hansen, 1911 was chosen as the outgroup. *Hemisquilla californiensis* Stephenson, 1967 was also chosen as a taxon to root Stomatopoda-only analyses because previous works (Ahyong and Jarman, 2009; Porter et al., 2010; Van Der Wal et al., 2017) found the position of *Hemisquilla* Hansen, 1895 as a sister group to a highly supported clade comprising the rest of Stomatopoda.

Four types of datasets were analyzed using DNA sequences for the 13 mitochondrial protein coding genes plus the 12S and 16S rRNA genes, and nuclear 18S rRNA gene with the outgroup of *Euphausia pacifica* or *Hemisquilla californiensis*. The analyses were conducted with nucleotide only sequences as well as amino acid sequences of the 13 protein coding genes and nucleotide sequences of the 3 rRNAs with different outgroups: 1) *E. pacifica* outgroup nucleotide only (EuphNuc); 2) *E. pacifica* outgroup mixed amino acid

(EuphAA); 3) *H. californiensis* outgroup nucleotide only (HemiNuc); and 4) *H. californiensis* outgroup mixed amino acid (HemiAA). The 13 protein coding genes were translated to amino acids to allow for control of saturation of the third codon position. The fraction of parsimony informative characters out of total characters for each dataset is shown in Table 3. *Chorisquilla orientalis* Hwang, Ahyong & Kim, 2018, was removed from the NADH6 nucleotide and amino acid gene alignments as well as the Cytochrome b gene amino acid alignment due to poor alignment caused by possible contamination. Gblocks v. 0.91b (Catresana, 2000) on the least stringent settings was used to remove poorly aligned regions of the 3 rRNA genes. The sequences were aligned with MAFFT v. 7.475 (Katoh and Standley, 2013) under the G-INSI-i method with 1000 iterations.

Table 3. Fraction of parsimony informative characters out of the total characters for each gene of the nucleotide and amino acid datasets.

Gene	Euphausia outgroup nucleotide dataset	Euphausia outgroup amino acid/nucleotide dataset	Hemisquilla outgroup nucleotide dataset	Hemisquilla outgroup amino acid/nucleotide dataset
ATP6	0.414	0.147	0.403	0.120
ATP8	0.528	0.462	0.491	0.442
COX1	0.358	0.068	0.354	0.064
COX2	0.385	0.135	0.370	0.114
COX3	0.383	0.136	0.370	0.122
CYTB	0.384	0.079	0.376	0.069
ND1	0.426	0.156	0.422	0.137
ND2	0.524	0.344	0.509	0.326
ND3	0.440	0.186	0.434	0.161
ND4	0.443	0.193	0.431	0.182
ND4L	0.367	0.152	0.357	0.111
ND5	0.451	0.208	0.437	0.189
ND6	0.485	0.335	0.470	0.305

Three phylogenetic analyses were performed on the datasets using maximum likelihood (ML), maximum parsimony (MP), Bayesian inference (BI). The ML analysis was performed in the raxmlGUI v. 2.0.2 (Edler et al., 2021) interface using RAxML-NG v. 1.0.1

(Kozlov et al., 2019). Gene sequences were concatenated in RAxML-NG and partitioned with variations of the substitution models determined by ModelTest-NG v. 0.1.6 (Darriba et al., 2020) (Table 2). The program parameters were set to ML+thorough bootstrap+consensus with 10 ML searches and 1000 bootstrap replicates for nucleotide datasets and 10 ML searches with 100 bootstrap replicates for mixed amino acid datasets. SequenceMatrix v. 1.8 (Vaidya et al., 2011) was used to concatenate the nucleotide datasets for the MP and BI analyses. Mesquite v. 3.61 (Maddison and Maddison, 2019) was used to concatenate the mixed amino acid and nucleotide datasets for the BI analyses. For the MP analysis, heuristic searches of concatenated datasets were run in PAUP* v. 4.0a168 (Swofford, 2003) with tree-bisection and reconnection (TBR) branch swapping of 100 random addition replicates and a bootstrap estimation using 1000 replicates. The BI analysis was conducted in MrBayes v. 3.2.7a (Ronquist, 2012) with the concatenated datasets. The GTR+I+G model was applied for the nucleotide partitions based on the models chosen with ModelTest-NG from the ML analyses and the WAG model was chosen for amino acid partitions in MrBayes. Parameters set for posterior distributions were under the Markov chain Monte Carlo (MCMC) sampling for 20,000,000 generations and 4 chains.

Likelihood and parsimonious ancestral reconstruction of the raptorial claw were mapped onto the outgroup *Euphausia pacifica*, ML nucleotide tree with the probability model, MK1, in Mesquite. The raptorial claws are classified as spearers or smashers based on the shape of the dactyl. The states are as follows, **0**:spearers, **1**:smashers, **0/1**:*Hemisquilla californiensis*, and **2**:no claw for the outgroup.

Results

Gene order and direction was conserved among newly sequenced stomatopod genomes and available GenBank sequences (Fig. 1). The phylogenetic trees produced from the analyses are shown in Figure 2 and 3 based on the four datasets. The nucleotide datasets had similar tree topologies to their respective mixed amino acid and nucleotide datasets, albeit the mixed datasets had lower support than the nucleotide datasets (Fig. 2, 3). Variations between datasets were due to a few nodes that had low support. The ML and BI trees were congruent in tree topology for the nucleotide datasets. Posterior probabilities were higher than ML bootstrap scores. Incongruence with the ML, BI, and MP analyses occurred at the low supported nodes (Fig. 2, 3). Most differences between trees were from the MP analyses compared to the others.

Figure 1. Gene order arrangement of the mitochondrial genome for all stomatopod species used in this study. Blue boxes represent protein coding genes, red boxes are rRNA genes, and green boxes are tRNA genes. The ND1, ND4, ND4L, ND5, rrnL, and rrnS genes were in the reverse direction and the rest were in the forward direction.

There were recurring patterns in all the dataset analyses: Squilloidea was found to be monophyletic and highly supported as a spearing clade for all datasets except for the ML and BI analyses with the *Euphausia pacifica* mixed amino acid and nucleotide tree dataset where it was non-monophyletic with low support (Fig. 2, 3). In addition to the spearing clade, there was high support for a clade of smashers within Gonodactyloidea containing the families: Gonodactylidae Giesbrecht, 1910, Protosquillidae Manning, 1980, and Takuidae Manning, 1980. The clade containing *Hemisquilla californiensis*, *Odontodactylus havanensis* Bigelow,

1893, and *Lysiosquillina maculata* Fabricius, 1793 was recovered for all *E. pacifica* datasets with low support and grouped with Gonodactyloidea; whereas, the *H. californiensis* datasets showed the three taxa forming a grade. Gonodactyloidea was non-monophyletic for all datasets. *Pseudosquilla ciliata* Fabricius, 1787, the spearing taxon in Gonodactyloidea, was found to be sister to the rest of the stomatopods in Gonodactyloidea with moderate support; however, it was grouped with Squilloidea in the *H. californiensis* outgroup datasets with low support for both the ML and BI analyses. The MP analyses were incongruent in all datasets for the position of *P. ciliata* (Fig. 2, 3).

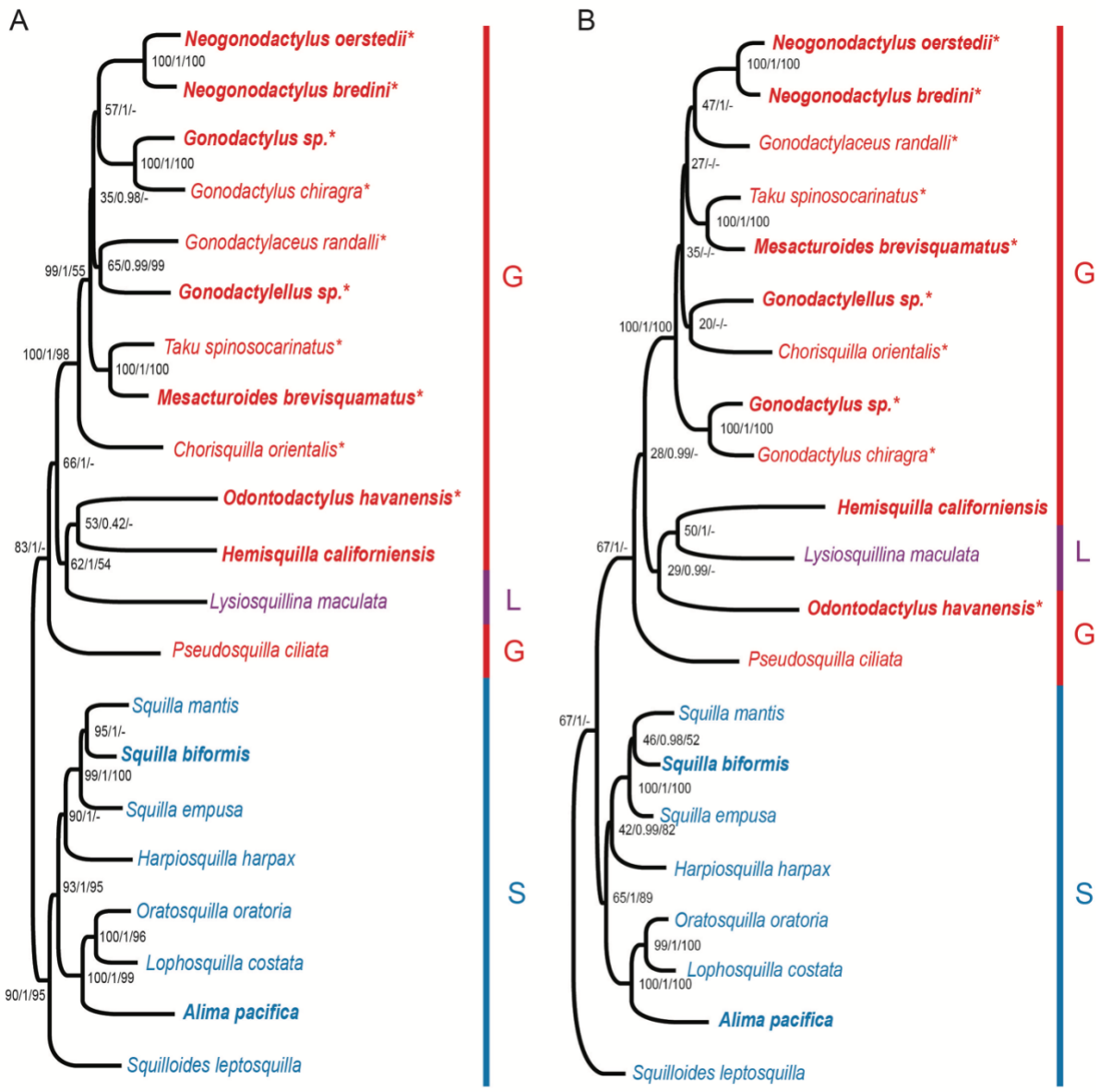

Figure 2. Stomatopoda phylogeny based on the concatenated mitochondrial genes and 18S. Newly sequenced species are in bold. Each superfamily is coded by color of the taxon name. Superfamily abbreviations are G: Gonodactyloidea, L: Lysiosquilloidea, S: Squilloidea. Asterisks in the taxon name denote smashers. Values at the nodes represent the bootstrap values of ML and MP and the posterior probability of BI in the format (ML/BI/MP). Hyphens represent nodes not recovered by the MP or BI analyses. The trees were rooted with the outgroup Euphausia pacifica (not shown in figure). (A) Euphausia outgroup maximum likelihood tree from the nucleotide dataset. (B) Euphausia outgroup maximum likelihood tree from the mixed amino acid and nucleotide dataset.

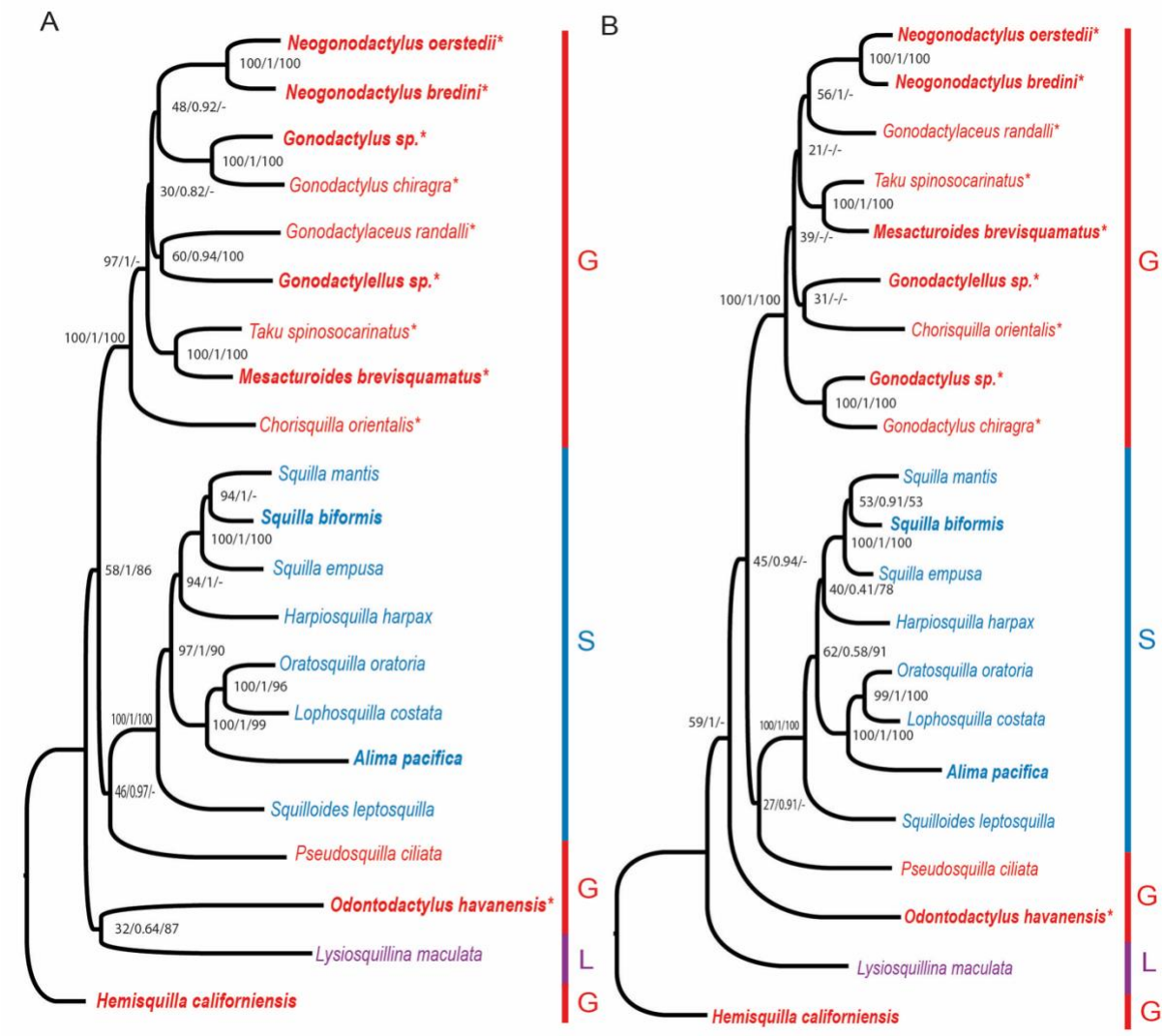

Figure 3. Stomatopoda phylogeny based on the concatenated mitochondrial genes and 18S. Newly sequenced species are in bold. Each superfamily is coded by color of the taxon name. Superfamily abbreviations are G: Gonodactyloidea, L: Lysiosquilloidea, S: Squilloidea. Asterisks in the taxon name denote smashers. Values at the nodes represent the bootstrap values of ML and MP and the posterior probability of BI in the format (ML/BI/MP). Hyphens represent nodes not recovered by the MP or BI analyses. (A) *Hemisquilla* outgroup maximum likelihood tree from the nucleotide dataset. (B) *Hemisquilla* outgroup maximum likelihood tree from the mixed amino acid and nucleotide dataset.

The relationships between datasets were similar; so, the EuphNuc tree was used for the remainder of the analysis and will be referred to for the rest of this paper unless stated otherwise. The likelihood ancestral state reconstruction of the raptorial claws found a high

proportional likelihood of 0.970 for spearers at the ancestral node (Fig. 4A). Three most parsimonious reconstructions (MPRs) suggest a few scenarios for the raptorial claw evolution: (1) one origin of spearing and two origins of smashing (in the clade of *Hemisquilla californiensis* + *Odontodactylus havanensis*, and for the clade of smashers in Gonodactyloidea); (2) one origin of spearing and two origins of smashing (in *O. havanensis* and for the clade of smashers in Gonodactyloidea); and (3) one origin of spearing and smashing with a reversal back to the ancestral state of spearing for *Lysiosquillina maculata* (Fig. 4B, C, D).

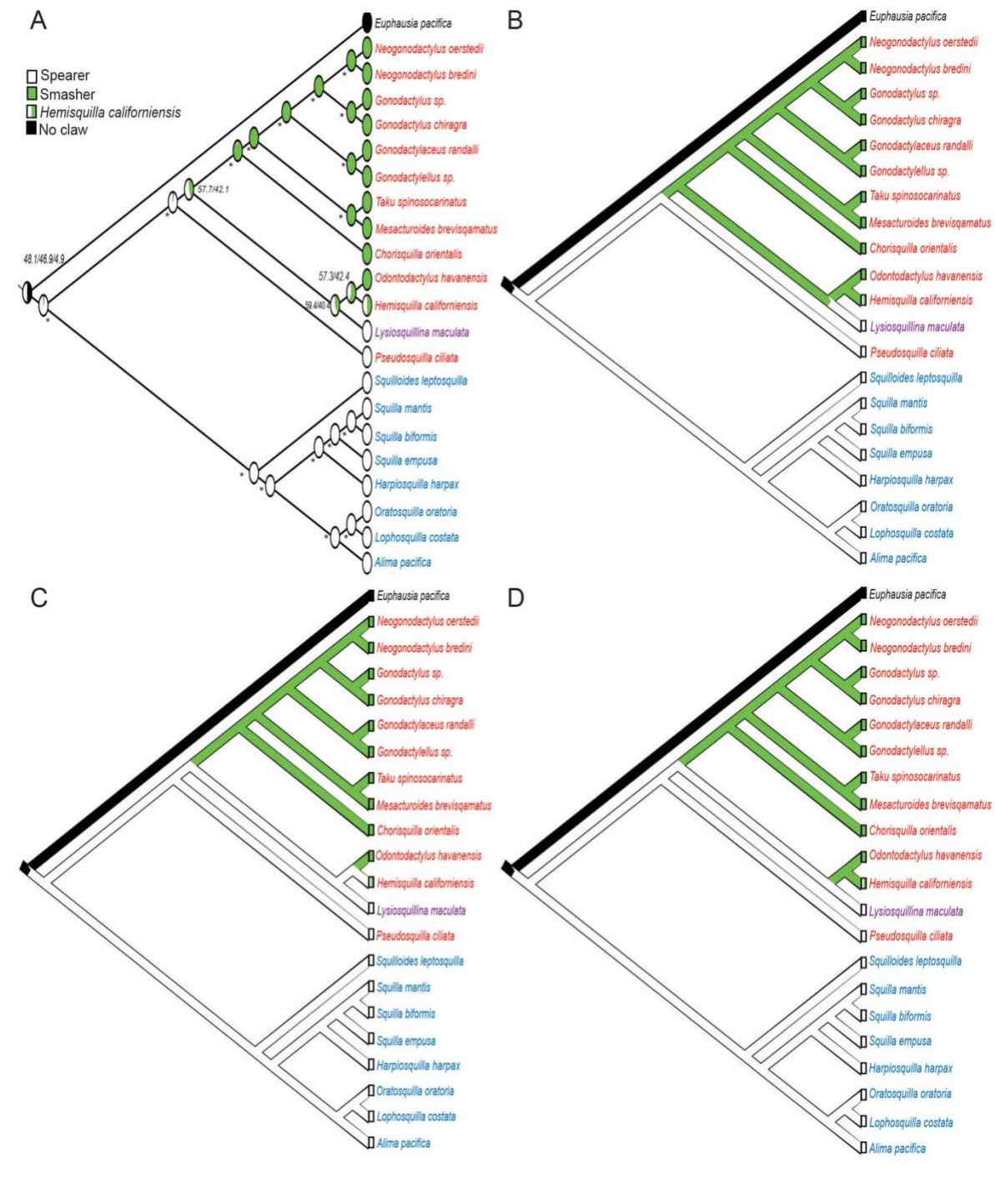

Figure 4. Ancestral state reconstruction of the raptorial claws mapped onto the nucleotide Euphausia outgroup maximum likelihood tree. (A) Maximum likelihood reconstruction. Asterisks represent nodes with proportional likelihood estimations of >95%. Other scores are provided in order of most likely states and separated with a forward slash. (B-D) Most parsimonious reconstructions. Coloring of taxa as in Fig. 2 and 3.

Discussion

This study added nine complete mitochondrial genomes to the 12 mantis shrimp mitogenomes available, bringing the total to 21. The gene order and direction was highly conserved among mantis shrimp species and followed the Crustacea ancestral state gene order (Boore et al., 1998; Kilpert and Podsiadlowski, 2006). The new sequences were combined with available mantis shrimp mitogenomes and 18S nuclear gene data to expand the phylogeny. This study added more taxa to the main superfamilies, Gonodactyloidea and Squilloidea, and added the first mitogenome data of *Hemisquilla californiensis* to the tree. From the expanded phylogeny, the ancestral state was determined as spearing with possible multiple origins for smashing evolving after.

This is the first whole mitochondrial genome phylogeny containing *Hemisquilla californiensis*, the taxon with the 'intermediate' claw form (Ahyong, 1997; Schram et al., 2013). Its proposed sister group position in relation to other stomatopods has brought attention to making Hemisquillidae into its own superfamily and it has produced a theory as a potential ancestral state with its claw form (Porter et al., 2010; Van Der Wal et al., 2017). However with outgroup rooting based on *Euphausia pacifica*, *H. californiensis* was found within Gonodactyloidea contrary to previous studies that found it outside of its superfamily (Ahyong and Jarman, 2009; Porter et al., 2010; Van Der Wal et al., 2017). Stating hemisquilloids are not true gonodactyloids may be premature with this newfound data. The clade found from the *E. pacifica* outgroup datasets containing *H. californiensis*, *Odontodactylus havanensis,* and *Lysiosquillina maculata* was not well supported; however, this is similar to the findings of Barber and Erdmann (2000) that found Hemisquillidae grouped with Odontodactylidae

Manning, 1980 and sister to the rest of the gonodactyloids. Furthermore, the other family, Pseudosquillidae, that caused paraphyly before with Gonodactyloidea (Ahyong and Jarman, 2009; Porter et al., 2010) was found to be clustered with Gonodactyloidea. With the moderately supported position of Pseudosquillidae grouped with Gonodactyloidea, the superfamily resolves its non-monophyly in regards to the two families-Hemisquillidae and Pseudosquillidae, but retains paraphyly from Lysiosquilloidea placed within.

In addition to outgroup choices being analyzed, the protein coding genes were translated to account for any saturation in the third codon position; yet, the little change with tree topology in the mixed amino acid and nucleotide datasets suggests there was little saturation. The mixed amino acid and nucleotide datasets had lower bootstrap support compared to the nucleotide-only dataset owing to around a 55% decrease of data when translated to amino acids. However, given the amount of nucleotide data from the whole mitochondrial genome and one nuclear gene, there was still low support within Gonodactyloidea due to internal conflict within Gonodactylidae. Despite the ambiguous relationships within Gonodactyloidea, a highly supported clade of smashers was recovered containing the families: Gonodactylidae, Takuidae, and Protosquillidae. Apart from the smashing clade, Squilloidea was recovered consistently as a highly-supported clade of spearers in the analyses (excluding the EuphAA dataset), confirming its monophyly in congruence to other phylogenies (Kang et al., 2016; Van Der Wal. et al., 2017; Zhang et al., 2020; Hwang et al., 2020).

One of the charismatic features of mantis shrimp are the raptorial claws that are used to group species into smashers and spearers based on the morphology and way they strike. The question of the evolution of the raptorial claw has thereby been a subject of discussion.

The reconstruction of the raptorial claw found spearers as the ancestral state which follows the views of Caldwell (1991) and Ahyong (1997), but was contrary to the findings of Ahyong and Harling (2000). In addition to the spearing ancestral state, the position of *H. californiensis* within Gonodactyloidea contradicts theories of a *Hemisquilla*-like ancestor based on its 'intermediate' claw form (Van Der Wal et al., 2017). With Lysiosquilloidea outside of Gonodactyloidea, the more likely evolutionary history would be one origin of spearing as the ancestral state and one origin for smashing. This makes sense for smashers to evolve later due to competition for soft-bodied prey and developing a modified claw suited for hard shelled prey that are not as sought after with spearers in the same environment. Spearing and smashing are more of a continuum and not a binary group noted by their diets being wide for both types. deVries et al. (2016) and deVries (2017) confirm spearers and smashers are capable of eating hard and soft bodied prey and in fact they have a more generalist diet than thought before. There could be an alternative explanation for their claw evolution, but we are limiting the groups as smashers and spearers, including Hemisquillidae, when they can strike with both a closed and open dactyl (Caldwell and Dingle, 1976; Ahyong and Jarman 2009).

Conclusion

Stomatopoda phylogeny was reassessed with whole mitogenome and nuclear data and built upon existing phylogenies that had minimal sampling. The prominent Hemisquillidae was added to the analyses and changed the *Hemisquilla*-like ancestor theory with its new position back into Gonoactyloidea. This analysis provides insight into the ancestral state of mantis shrimp raptorial claws lending credence to the hypothesis of spearing evolving first and smashing after. Understanding the phylogeny of Stomatopoda can resolve the history and

provide an explanation for how the raptorial claws became optimized and particularly how it is associated with the environment. To further explore the history, a molecular clock with fossil calibrations on the mitochondrial genome tree and comparison to geological events can be used to determine the estimated time of when these groups were established and diversified. The nine newly sequenced taxa and whole mitochondrial genome phylogeny of this study provide a base for future studies to build upon.

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