UC Riverside

UC Riverside Electronic Theses and Dissertations

Title

Genomics and Mitogenomics of Ampulex compressa

Permalink

https://escholarship.org/uc/item/82k1w7w9

Author

Khazaali, Mahziar

Publication Date

2024

Peer reviewed|Thesis/dissertation

eScholarship.org Powered by the California Digital Library
University of California

UNIVERSITY OF CALIFORNIA
RIVERSIDE

Genomics and Mitogenomics of Ampulex compressa

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Master of Science

in

Cell, Molecular, and Developmental Biology

by

Mahziar Khazaali

June 2024

Thesis Committee:
Dr. Michael E. Adams, Chairperson
Dr. Morris Maduro
Dr. Howard Judelson

Copyright by
Mahziar Khazaali
2024

The Thesis of Mahziar Khazaali is approved:

Committee Chairperson

University of California, Riverside

ACKNOWLEDGEMENTS

I want to dedicate my work to my children, Nora, Taha, and Sadra, who are my whole world. I want to thank my parents, Hassan Khazaali and Monir Abbasvand, for their love and support throughout my life, my brothers, Danial, Dinyar, and Mahyar, and my wife, Asma. I want to thank Dr. Michael E. Adams, for supporting me during my stay at the University of California, Riverside. I would also like to thank Dr. Morris Maduro and Dr. Howard Judelson, my other two thesis committee members, for reading this manuscript and providing constructive feedback. I am very thankful to Dr. Patrick Ferree (Claremont College, Claremont, CA) for allowing me to attend his lab and teaching me how to do karyotype. Among scholars at the University of California, Riverside, I thank Dr. Naoki Yamanaka, Dr. Alan Brelsford, and Dr. Martin Riccomagno for their collaboration and technical advice. I also thank post-docs and graduate students Dr. Mitchel Masterson, Dr. Daiki Fujinaga, Dr. Eisuke Imura, Dr. German Laguna-Robles, and Dr. Teresa Ubina for their technical help and expertise.

ABSTRACT OF THE THESIS

Genomics and Mitogenomics of Ampulex compressa

by

Mahziar Khazaali

Master of Science, Graduate Program in Cell, Molecular, and Developmental Biology
University of California, Riverside, June 2024
Dr. Michael E. Adams, Chairperson

Ampulex compressa, also known as the emerald jewel wasp, is a parasitoid wasp that exhibits unique behavior in incapacitating its cockroach prey. The species is notable for its neurologically active venom, which contains a complex mixture of peptides, primarily ampulexins, which facilitate behavioral manipulation of its host.

We conducted comprehensive genomic and mitogenomic analyses of a male *Ampulex compressa* to assemble and investigate the nuclear and mitochondrial DNA genome. The study employed advanced bioinformatic tools for sequence assembly, annotation, and phylogenetic analysis, including SPAdes, Augustus, Geneious, and phylogenetic inference using RAxML and MrBayes based on mitochondrial protein-coding genes.

The assembled nuclear genome measured 277.7 Mbp across 11,507 scaffolds, while the mitochondrial genome was 17,097 bp long, including 13 protein-coding genes, 22 tRNA genes, and two rRNA genes. Phylogenetic analysis positioned *Ampulex compressa* closely with other aculeate parasitoids, corroborating the taxonomic placement within Apoidea. Gene annotation revealed three ampulexin genes within a single scaffold, suggesting a potential gene family. Additionally, karyotyping identified 11 chromosomes (n=11), aligning with the expected number for other Spheciformes species.

This study provides the first complete mitochondrial genome and an extensive nuclear genome assembly for *Ampulex compressa*. Findings confirm the conserved nature of mitochondrial genomes across Apoidea and support ongoing research into the evolutionary adaptations associated with parasitoidism, such as venom production. Further comparative genomic studies are encouraged to explore gene functions and evolutionary trajectories within Hymenoptera.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Introduction

Background on Ampulex compressa

Ampulex compressa, commonly known as the emerald jewel wasp, is a member of the Ampulicidae family within the Apoidea superfamily of Hymenoptera. This superfamily encompasses a diverse group of insects, including both sphecid wasps and bees. Ampulex compressa is renowned for its unique parasitoid behavior and striking metallic green coloration. Found across equatorial regions of Southeast Asia, Africa, and the Pacific Ocean islands, this species has also been spotted in South America and introduced to Hawaii for biological control (Pires et al. 2014).

Biology and Behavior

The emerald jewel wasp exhibits a fascinating and unique life cycle involving manipulating prey, typically cockroaches. Female wasps incapacitate their prey by delivering a highly specialized sting directly to the cockroach's central nervous system. This sting results in a hypokinetic state, rendering the cockroach zombified and compliant. (Arvidson et al. 2018) The wasp then leads the immobilized cockroach to a suitable location and lays an egg on its abdomen. Upon hatching, the wasp larva feeds on the cockroach’s hemolymph before eventually consuming internal organs and tissues, completing its development within the host remains. (Arvidson et al. 2019)

The venom of Ampulex compressa contains a complex mixture of neuroactive peptides, with ampulexins being the most abundant. These peptides likely induce hypokinesia through interactions with the cockroach's dopamine receptors, manipulating the host's behavior to the wasp's advantage. (Arvidson et al. 2019) Understanding the composition and function of these venom components provides valuable insights into neurochemical processes and potential applications in neurobiology and pest control.

Phylogenetic Position and Comparative Genomics

Recent phylogenomic analyses suggest that the Ampulicidae family is paraphyletic to other Apoidea. (Peters et al. 2017) The Dolichurus genus, also within the Ampulicidae, and the Trypoxylon species from the Crabronidae family exhibit similar parasitic behaviors, targeting cockroaches and spiders, respectively. (Bohart et al. 1976) Additionally, the Pompilidae and Scoliidae families within the Aculeata subclade include parasitoids of spiders and beetles, highlighting a convergent evolution of parasitic strategies across diverse taxa. (Johnson et al. 2013)

Comparative genomics allows the inference of protein and gene functions by homology. The conserved nature of mitochondrial genomes makes phylogenetic trees based on mitogenomic data instrumental in resolving classification issues and understanding evolutionary relationships within Hymenoptera. (Sann et al. 2018) Phylogenetic studies using mitochondrial DNA (mtDNA) have proven effective in uncovering the evolutionary history of various insect taxa, including bees, wasps, and ants. (X.-Y. Zheng et al. 2021; Imai, Crozier, and Taylor 1977)

Significance of Mitochondrial and Nuclear Genomics

Mitochondrial genomes (mitogenomes) in insects typically range from 15,000 to 20,000 base pairs and contain a conserved set of 37 genes, including 13 protein-coding genes, 22 tRNA genes, and two rRNA genes. (Boore 1999) Mitogenomes' structure and gene order are often highly conserved within taxonomic groups, making them useful for phylogenetic studies. (Cameron 2014) In this study, we aim to assemble and annotate the complete mitochondrial genome of Ampulex compressa and explore its phylogenetic placement within the Aculeata subclade.

Nuclear genomes provide a broader genetic landscape, encompassing numerous genes across multiple chromosomes. The nuclear genome assembly offers insights into gene content, structural variations, and evolutionary adaptations. Gene annotation can reveal the presence and organization of gene families, such as those encoding venom components like ampulexins. (King and Hardy 2013) Additionally, studying the nuclear genome enables the identification of chromosomal features and karyotype analysis, contributing to our understanding of genetic diversity and evolutionary processes within Hymenoptera. (Imai et al. 1988)

Research Objectives

The specific goals of this project are to assemble and annotate the complete mitochondrial genome and the extensive nuclear genome of Ampulex compressa. The study aims to:

1. Investigate the phylogenetic position of A. compressa within the Aculeata subclade using mitochondrial and nuclear genomic data.
2. Identify and characterize venom-related genes, particularly ampulexin genes, within the nuclear genome.
3. Perform karyotype analysis to determine the chromosomal composition of A. compressa.

By achieving these objectives, this study will enhance our understanding of the genetic and evolutionary mechanisms underlying the unique biology of A. compressa. The findings will also contribute to broader comparative genomic studies within Hymenoptera, providing insights into parasitoid insects' evolutionary trajectories and functional adaptations.

Materials and Methods

A male wasp from our insect-rearing laboratory was used for this study. The insects are maintained under standard temperature and humidity conditions at the University of California, Riverside. (Arvidson et al. 2018) A male was chosen because the haploid-diploid sex determination system makes the male genome smaller and thus simpler to analyze. A head was also used to minimize contamination from gut microbiota.

DNA extraction was performed in a collaborative laboratory (Dr. Alan Brelsford) at the University of California, Riverside. The genomic core at UCR performed DNA quality checks and library preparations, and sequencing was conducted at the University of California, Davis, using a NovaSeq 6000 machine. (Baym et al. 2015; Henderson and Brelsford 2020)

The computational work was conducted on the High-Performance Computing Center at the University of California, Riverside, using installed modules and software for assembly and phylogenetic analysis. (https://hpcc.ucr.edu/)

For the karyotype analysis, ovaries from an adult female were macerated under a microscope using dissection needles, spread on a slide, and fixed using three fixative solutions with varying concentrations of glacial acetic acid, absolute ethanol, and distilled water as prescribed by Gokhman and Imai. (V. E. Gokhman 2009; Imai et al. 1988) The slides were stained with Aceto-Orcein (Fisher Science Education™ Aceto-Orcein Solution 2%) for 3 hours and examined under a phase-contrast light microscope. (Vladimir E. Gokhman et al. 2019; Zeiss 2000)

We first anesthetized an adult female to harvest the ovary using Co2 and ice. (Gilbertson and Wyatt 2016) Then, we pinned and dissected the adult's metasoma in a hypotonic solution containing colchicine. We approached from the dorsal side of the insect, and after cutting the cuticle, we dissected two ovaries into a glass slide with a shallow hole. The specimen was incubated at room temperature in the hypotonic solution for half an hour before fixation.

We tried other tissues at different developmental stages using different stains but could not find the chromosomes. We first used the squashed testis samples from 3-4 weeks pupa. We followed the DAPI stain technique developed by Dr. Patrick Ferree for Karyotyping and fluorescent in-situ hybridization. (Ferree and Barbash 2009; Kakazu et al. 1999) However, we could not find the chromosomes because we needed to capture a very narrow development window to catch the mitotic chromosomes. Also, because the tissue differs from Nasonia, we might need a tailored fixation protocol.

To prepare karyotypes for male Ampulex compressa using the DAPI staining, male wasp pupae in early development were selected based on their smaller pupal size and weight. (Arvidson et al. 2018) The fixative mixture comprising glacial acetic acid, water, and 20% paraformaldehyde (450 µL, 425 µL, and 125 µL, respectively) was prepared in an Eppendorf tube. The dissection was conducted on a Petri dish lid under a dissecting microscope using 1xPBT buffer as the medium.

The bottom of the abdomen was pulled using a fine tweezer to dissect the genitalia and reveal the testes, which were then transferred to the PBT solution. (figure 1) Subsequently, 12 µL of the prepared fixative was applied onto a coverslip, and the testes were moved onto this drop, waiting about three minutes to avoid over-fixing. After fixation, a frosted glass slide was brought close to the coverslip to attach it.

The coverslip was gently blotted and pressed firmly to adhere to the slide without sliding. The slide was then frozen in liquid nitrogen for a couple of minutes until the nitrogen stopped boiling. After freezing, the slide was removed, and the coverslip was detached using a razor blade. The tissue and chromosomes were now attached to the glass slide and dehydrated in an ethanol-filled Coplin jar for 10-15 minutes. The slide was left to dry at room temperature. For staining, 12 µL of VECTASHIELD mounting medium with DAPI was added to the slide, covered with a coverslip, and sealed with nail polish, drying in a dark drawer for at least half an hour. The prepared slide was then ready for observation under an epifluorescent microscope.

Computational Methods

The genomic DNA was extracted using the QIAGEN DNeasy Blood & Tissue Kit. The entire wasp was ground with a pestle in a 1.7 ml tube in liquid nitrogen and incubated overnight with 180µL buffer ATL and 20µL proteinase K at 56°C. The supernatant was transferred to the QIAcube HT/QIAxtractor robot to complete the extraction, and the DNA was eluted in 100 µL of buffer Tris-HCl (pH 8.0). The DNA extract was stored at -20°C and sent to the UCR core genomics for short-read Illumina sequencing. (Henderson and Brelsford 2020) Nanodrop and Qubit were used to check the quality of the DNA extraction, and the library preparation was then produced. The specimen was sequenced using a NovaSeq 6000 sequencer at the University of California, Davis.

We trimmed the whole genome paired-read sequences using the bbduk program from the BBTools suite (Bushnell, n.d.) Then we used GetOrganelle v1.7.7.0 software (SPAdes version 3.13.0) (Dong, n.d.) to assemble the mitochondrial genome and annotate the genes. The average animal mitochondrial kmer coverage was 925.5, and the average animal mitochondrial base coverage was 3017.9. We used KmerGenie software (kmergenie/1.7051) (Chikhi and Medvedev 2014) to find the best kmer for genome de novo assembly. The analysis predicted that kmer 117 was optimal for assembly.

We used SPAdes version 3.15.5 for genome assembly and utilized the draft reference genome as a trusted source and one available genome as untrusted. The assembly was filtered with seqkit software (seqkit/2.4.0) (Shen et al. 2016) to remove sequences below 200bp. We also used NCBI-BLAST software (ncbi-blast/2.14.1+) (Altschul et al. 1990) Kitto exclude the mitochondrial genome from the nuclear genome. The coverage for this assembly was 104x and contained 11,200 scaffolds.

The completeness of the Ampulex compressa genome assembly was evaluated using the Benchmarking Universal Single-Copy Orthologs (BUSCO) tool, version 5.5.0. BUSCO (Seppey, Manni, and Zdobnov 2019) assesses genome assembly quality by searching for near-universal single-copy orthologs within the genome. The insecta_odb10 lineage dataset, which contains 1367 single-copy orthologs specific to insects, was employed for this analysis. The genome assembly was analyzed in 'genome' mode, optimized for raw genome sequences. The BUSCO analysis was conducted with a high-performance computing cluster, utilizing 32 CPUs to expedite the process. The results, including the percentages of complete, fragmented, and missing BUSCOs, were subsequently summarized to assess the genome assembly's completeness and quality comprehensively.

Gene annotation was performed using Augustus/3.5.0 software (Stanke and Waack 2003) with Nasonia vitripennis as the model. The annotation file in gff3 format was converted to an sqn file using ncbi-table2asn software (version 1.0.883) and submitted to NCBI. We used Geneious software (v2024.0.5) (Kearse et al. 2012) to annotate the genome and perform BLAST searches for the Ampulexin genes. We also utilized the Berkeley Drosophila Genome Project to predict promoters for each gene using a neural network-based program. (Fig 5)

We searched the NCBI database for complete mitogenomes within the Aculeata subclade and included three outgroup species to construct phylogenetic trees. We excluded six from the 35 mitogenomes found due to different annotations or significant annotation overlap that could affect the analysis. (Table.1) We extracted protein-coding genes from the remaining 29 mitogenomes using Clustal Omega and MAFFT software (Katoh 2002; Katoh and Standley 2013) for multiple sequence alignments of each gene. We used Geneious software to concatenate the alignments, ensuring each sequence contained only protein-coding genes. Phylogenetic trees were constructed using MrBayes (Ronquist et al. 2012) and RAxML software for Bayesian and maximum likelihood methods, respectively. (Stamatakis 2014) (Figure 3 and 4)

Results

Sequencing and Assembly

We obtained 500,828,020 paired reads from the short-read Illumina sequencer. After trimming, 500,772,062 reads remained for downstream analysis. These trimmed reads were used to assemble the mitochondrial genome using the GetOrganelle program. The mitochondrial genome, measuring 17,097 bp, contains 22 tRNA genes, 2 rRNA genes, and 15 protein-coding genes. The regulatory region of the mitochondrion is AT-rich. The annotated mitogenome (NCBI Reference Sequence: PP341299.1) is illustrated in Figure 4. The nuclear genome was assembled, submitted to NCBI, and published in GenBank under the reference GCA_038496175.1. It comprises 11,507 scaffolds and spans a total length of 277.7 Mbp. (Table 2) The gene annotation for the whole genome has also been submitted to NCBI.

BUSCO Analysis Results

The completeness of the Ampulex compressa genome assembly was assessed using BUSCO v5.5.0 with the insecta_odb10 lineage dataset, comprising 1367 single-copy orthologs. The analysis revealed that 98.4% of the BUSCOs were identified as complete, of which 98.2% were single-copy and 0.2% were duplicated. Additionally, 0.8% of the BUSCOs were found to be fragmented, and 0.8% were missing. These results indicate a high-quality genome assembly with minimal fragmentation and duplication. The detailed results are summarized in Table 3.

Identification and Characterization of Ampulexin Genes

The genome was subjected to BLAST searches for ampulexin mRNAs and proteins. three ampulexin genes were identified on scaffold number 2235 (JBCFXI010002235.1), spanning approximately 4.8 Kbp. Two venom extracts (13 and 17) were also localized to this region. A similar genome assembly (GCA_019049445.1) on NCBI displayed the same annotations on scaffold 3. The venom-specific region of the genome contains five genes: Ampulexin 1, Ampulexin 4, venom isolates 13 and 17, and a gene coding for both Ampulexin 2 and 3. (Figure 5)

We predicted the promoter regions upstream of each gene with 95-100% confidence (Table 4). Each core promoter featured a TATA box region for transcription factor binding and measured approximately 50 bp in length. The distance between the promoter and the transcription start site was about 30 or 100 bp. (Figure 5)

Phylogenetic Analysis

We searched the NCBI database for complete mitogenomes within the Aculeata subclade, excluding six mitogenomes due to different annotations or significant overlaps that could affect the analysis. From the remaining 29 mitogenomes, three were considered outgroups: Nasonia giraulti, Diadegma semiclausum, and Cotesia vestalis. We could not find a suitable mitogenome for Thiphioidea as all available partial genomes lacked several mitochondrial genes. Additionally, no mitogenomes were found for Thynnoidea. The phylogenetic analysis results based on both Bayesian and maximum likelihood methods are presented in Figures 6 and 7. Furthermore, we predicted a tree based only on 16S rRNA gene. (see figure 8)

Phylogenetic trees correctly grouped Anthophila, Crabronidae, Sphecidae, and Formicidae. Ampulex compressa was shown to be closely related to Pompiloidae and Scoliidae. The trees also suggest that Ampulex compressa is paraphyletic to Apoidae.

Anthophila, Crabronidae, and Sphecidae are monophyletic. Also, chrysoidae, mutilidae, ,Formicidae, and Vespidae are monophyletic. Ampulex is closely related to Pompilidae and Scoliidae. However, the Ampulex compressa is farther from Apoidea than predicted before.

These relationships are very novel regarding systematics and need a closer look and study.Although the phylogenetic trees correlate well with predictions based on the nuclear genome (Peters et al. 2017), there are some differences which is related to inherent differences between mitogenomics and genomic approach. (see discussion)

Karyotype Analysis

The karyotype analysis of Ampulex compressa revealed that it possesses 11 chromosomes (n=11), aligning with the bimodal distribution of chromosome numbers in the Apocrita (n=6 and n=11) described by Gokhman. (V. E. Gokhman 2009) Each ovary has three ovarioles, which converge into an oviduct. The germarium is the youngest oocyte at the tip of the ovariole. (Eastin, Huang, and Ferree 2020) (Figure 9) Although we could not capture metaphysic chromosomes, we believe that with further study and more slide preparation, we could find metaphysic chromosomes to better understand chromosome structure. Using fluorescent in-situ hybridization also could help to find the rearrangements and indels that help classify the Ampulicidae species.

Discussion

Mitogenome Assembly and Phylogenetic Analysis

This study represents the first comprehensive assembly of the complete mitogenome of Ampulex compressa, including the regulatory region and all 37 genes. Our findings align with previous studies, such as those by Zheng et al. (B.-Y. Zheng et al. 2018), which noted significant rearrangements in the mitochondrial genomes within the Apoidea superfamily. Specifically, in A. compressa, we observed the rearrangement of the ATP8 gene and tRNA-Asp, forming a unique rnaL-tRNAV-rnaS cluster, a feature not commonly seen within this superfamily. (Beckenbach and Joy 2009) This unique arrangement can provide insights into the evolutionary pressures and genetic mechanisms underlying the adaptation of A. compressa to its parasitoid lifestyle.

The phylogenetic trees constructed using Bayesian and maximum likelihood methods confirmed the monophyly of major groups such as Anthophila, Crabronidae, Sphecidae, and Formicidae. Our analysis suggests that A. compressa is closely related to Pompilidae and Scoliidae, supporting the hypothesis that Ampulicidae is paraphyletic to other Apoidea. (Johnson et al. 2013) This finding is consistent with the phylogenomic analysis by Peters et al. (Peters et al. 2017), which used an extensive dataset of protein-coding genes to classify Hymenoptera based on evolutionary history. Our study further emphasizes the utility of mitogenomic data in resolving complex phylogenetic relationships within this diverse group.

Discrepancies Between Mitogenomic and Nuclear-Based Phylogenetic Trees

In this study, the phylogenetic tree generated using mitogenomic data for 29 samples of Aculeata showed some discrepancies when compared to the available trees based on nuclear genome data. While the overall topology of the mitogenomic tree aligns closely with the nuclear-based trees, certain divergences are evident. These discrepancies can be attributed to the inherent differences in the evolutionary dynamics of mitochondrial and nuclear genomes.

Mitochondrial DNA (mtDNA) is maternally inherited and does not undergo recombination, simplifying its evolutionary history and making it susceptible to lineage sorting and genetic drift. As a result, mtDNA may reflect recent evolutionary events more prominently than nuclear DNA (nucDNA), which is inherited from both parents and undergoes recombination. This can lead to a more comprehensive and balanced representation of an organism's phylogeny in nuclear-based trees. (Ballard and Whitlock 2004) Additionally, selective sweeps and population bottlenecks can affect mtDNA more profoundly, potentially obscuring true phylogenetic signals. (Galtier et al. 2009) These factors collectively contribute to the observed discrepancies between mitogenomic and nuclear-based phylogenies.

Furthermore, nuclear genomes represent a broader genetic background, encompassing numerous genes from different chromosomes, providing a more robust dataset for phylogenetic inference. (Funk and Omland 2003) On the other hand, the mitochondrial genome, being a single genetic locus, may present a biased picture of evolutionary relationships, particularly in cases where introgression or hybridization events have occurred. (Rubinoff and Holland 2005) Therefore, while mitogenomic data is invaluable for resolving recent evolutionary relationships due to its high mutation rates, integrating mitochondrial and nuclear data in a concatenated or multi-locus approach is crucial for a more comprehensive understanding of evolutionary histories. (Degnan and Rosenberg 2009)

Recent studies have highlighted the importance of integrating multi-locus data to resolve phylogenetic relationships accurately. For instance, analyses combining mitochondrial and nuclear genomes have provided deeper insights into the evolutionary histories of complex taxa, such as the stinging wasps within Aculeata. (X.-Y. Zheng et al. 2021) These integrated approaches can mitigate the limitations of single-locus data and enhance our understanding of the evolutionary processes shaping biodiversity.

Genomic Insights and Ampulexin Genes

Our genome assembly revealed a total length of 277.4 Mb with a non-gapped length comprising 11,507 scaffolds. The identification and characterization of ampulexin genes within the genome highlight their significance in the venom of A. compressa. These genes clustered in a region approximately 4.5 Kbp in length suggest a gene family. The presence of these genes in a haploid male without a venom apparatus implies they may be inactive in males and active in diploid females, potentially requiring dimerization to function. (Arvidson et al. 2019; 2018)

Blasting the ampulexin genes in the NCBI database yielded no homologs in other organisms within the aculeate family, indicating a unique evolutionary path for these proteins in A. compressa. Transcriptomic and proteomic analyses of tissue-specific venom glands further support the tissue-specific expression of these proteins, which are not detectable in whole-body transcriptomics due to their lower mRNA concentrations. (Crampton-Platt et al. 2015) Also, this region may be a supergene that controls the different components of the venom. This type of supergene has been described in ants and proposed to regulate the organism's eusociality. (Lagunas-Robles, Purcell, and Brelsford 2021)

The evolutionary uniqueness of the ampulexin genes suggests that A. compressa has developed specialized venom components for its parasitoid lifestyle. Comparative genomics of venomous insects, such as other Hymenoptera, can provide insights into the evolutionary pressures shaping venom composition and function. (Drukewitz and Von Reumont 2019; McKenzie, Oxley, and Kronauer 2014) Understanding these evolutionary adaptations can also have practical applications in biotechnology and medicine, where venom peptides are being explored for therapeutic uses. (King and Hardy 2013)

Furthermore, the detailed study of venom genes can uncover potential targets for pest control. For example, exploring the molecular mechanisms underlying the neurotoxic effects of ampulexins could lead to the development of novel biopesticides that mimic these natural compounds, offering environmentally friendly alternatives to chemical pesticides. (Ayilara et al. 2023)

Karyotype Analysis

The karyotype analysis of A. compressa revealed that it possesses 11 chromosomes (n=11), consistent with the bimodal distribution of chromosome numbers in the Apocrita (n=6 and n=11) described by Gokhman. (V. E. Gokhman 2009) This study is the first to describe the chromosome number of a species from the Ampulicidae family, providing valuable cytogenetic data that aligns with the evolutionary history of other stinging and parasitoid wasps.

Karyotype analysis in insects has proven to be a powerful tool for understanding chromosomal evolution and speciation. The chromosomal diversity observed in Hymenoptera results from various chromosomal rearrangements, including fusions, fissions, and inversions, contributing to their evolutionary adaptability. (Imai et al. 1988) The karyotype data from A. compressa adds to the growing body of knowledge on insect cytogenetics and highlights the importance of chromosomal studies in elucidating evolutionary relationships.

According to Gokhman's comprehensive review, the distribution of parasitic wasp species by chromosome number is bimodal, with two obvious modes at n = 6 and n = 11, characteristic of most members of the superfamilies Chalcidoidea and Ichneumonoidea, respectively. (Vladimir E. Gokhman 2022) The karyotype of A. compressa with its 22 chromosomes aligns it more closely with these groups, suggesting that the evolutionary pathways of these wasps involve significant chromosomal rearrangements. Gokhman emphasizes that these chromosomal changes are not merely incidental but are indicative of broader evolutionary trends within parasitoid Hymenoptera, where reductions in chromosome number and karyotypic desymmetrization are common evolutionary processes.

Recent advancements in molecular cytogenetics, such as chromosome painting and fluorescent in situ hybridization (FISH), have provided more profound insights into chromosomal evolution in Hymenoptera. (Rens et al. 2006) These techniques can be applied to A. compressa to explore further chromosomal organization and its implications for species evolution and adaptation.

Comparative Genomics and Future Research

Comparative genomics allows for the inference of protein and gene functions by homology. Due to the conserved nature of mitochondrial genomes, phylogenetic trees based on mitogenomic data are instrumental in resolving classification issues within Hymenoptera. (Sann et al. 2018) Our study demonstrates the utility of mitogenomics in providing a precise picture of evolutionary relationships, corroborating results obtained from nuclear genome analyses with fewer computational resources.

Future research should focus on the experimental and genetic study of ampulexin proteins, including comparative genomics, to identify possible homologs in closely related species such as Dolichurus, Pompiloidae, and Scoliidae. This study lays the foundation for further investigations into gene rearrangement, evolutionary adaptations, and the functional roles of the ampulexin genes in A. compressa. Additionally, advances in gene editing technologies, such as CRISPR/Cas9, offer exciting opportunities to manipulate these genes and experimentally study their functions in vivo. (Jinek et al. 2012; Zhang et al. 2024)

The insights gained from this study not only enhance our understanding of the genetic and evolutionary mechanisms underlying the unique biology of A. compressa but also provide a framework for future research on other parasitoid wasps. By integrating genomic, transcriptomic, and proteomic data, researchers can further elucidate the complex interactions between these insects and their hosts, shedding light on the evolutionary strategies that have enabled their success as parasitoids.

Moreover, expanding the scope of comparative genomics to include more parasitoid species could reveal broader evolutionary patterns and adaptive traits within Hymenoptera. This integrative approach could also uncover genetic determinants of host specificity and venom potency, providing valuable information for developing targeted biological control agents. (Whitfield 2003; Jalali, Ojha, and Venkatesan 2015)

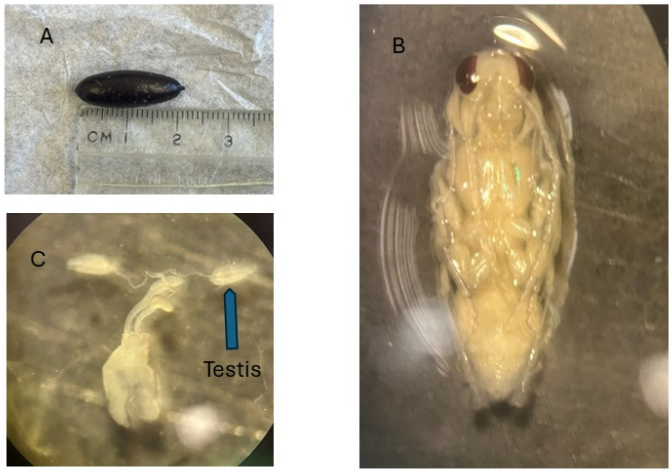

Fig 1. A. a Pupa extracted from the carcass of a cockroach, B. a 4-week larva extracted from the pupa, C. Male genitalia including testis and accessory glands

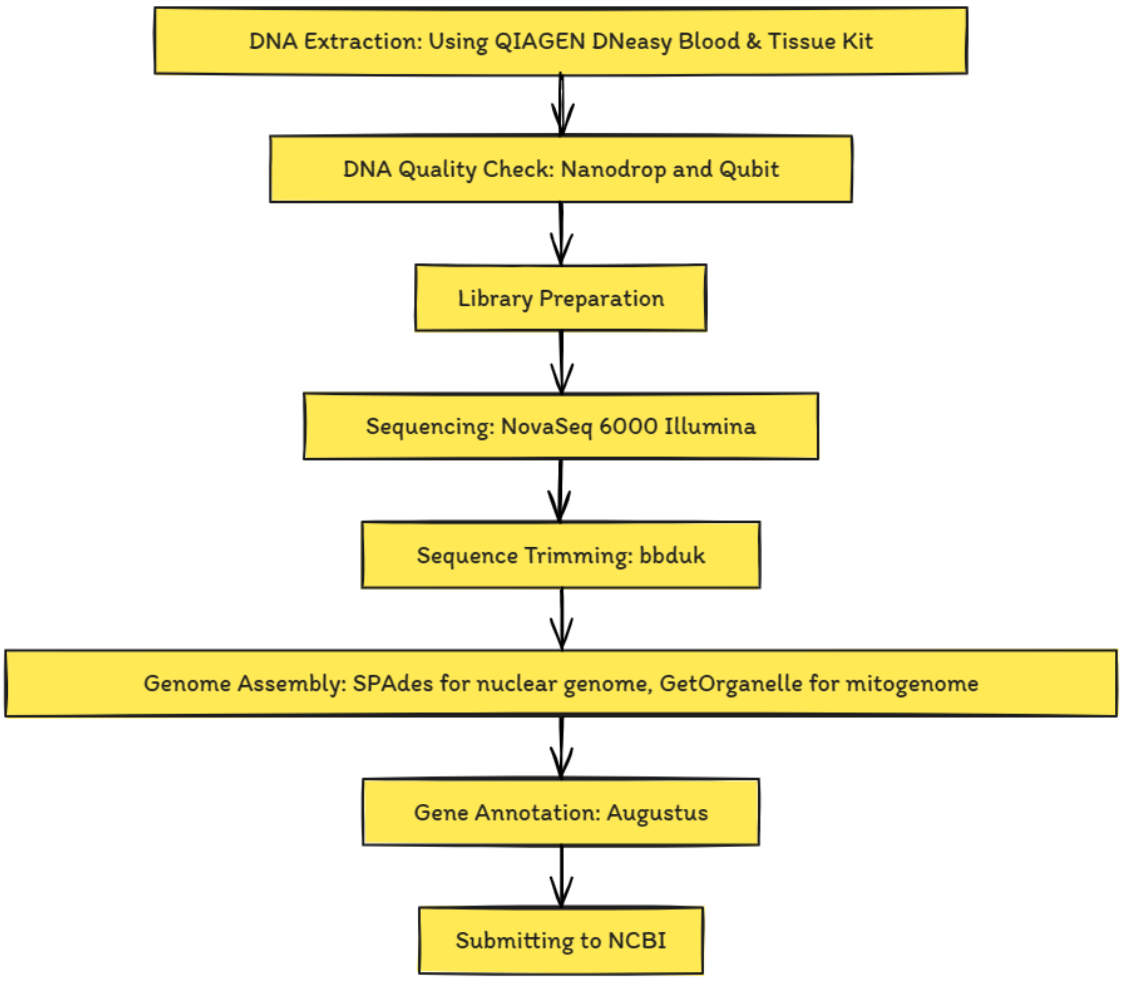

Figure 2. Genomic workflow.

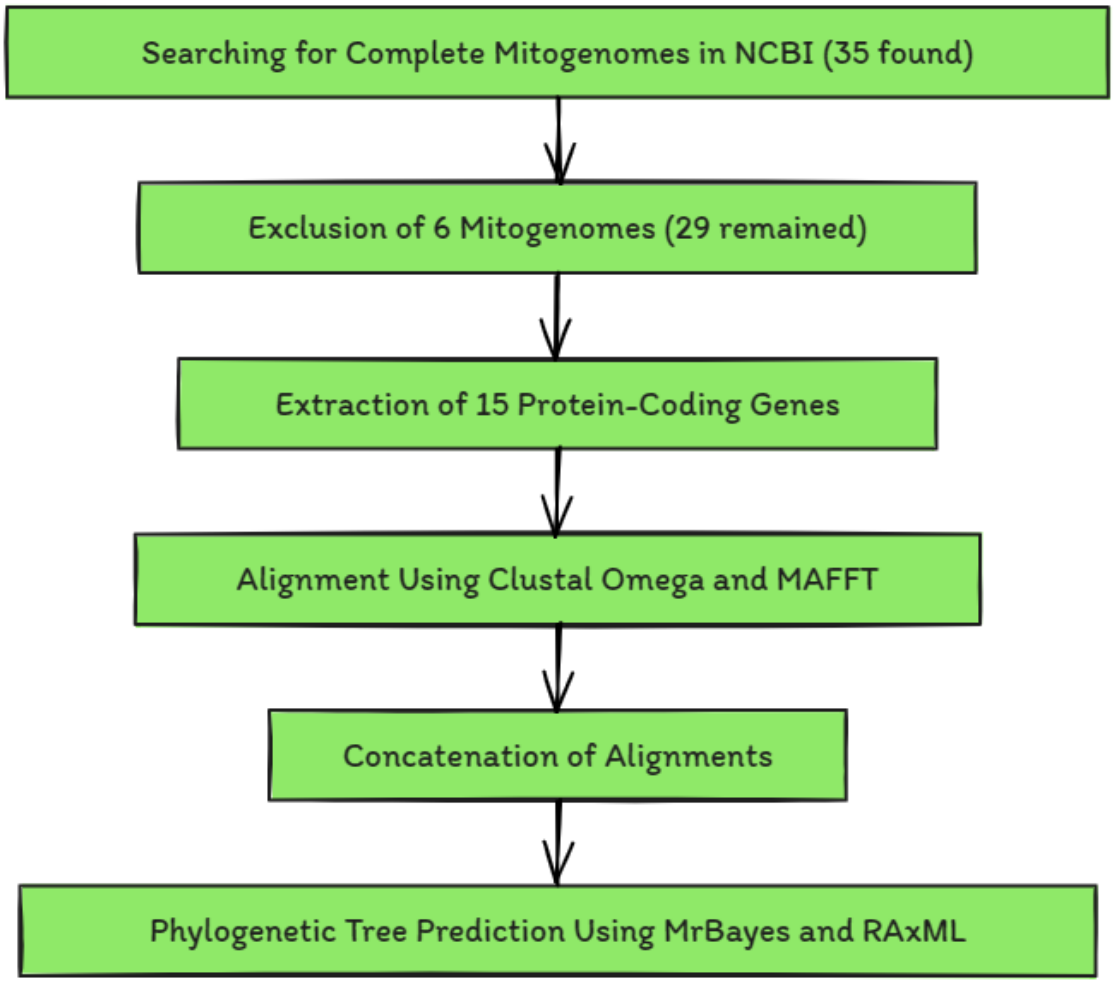

Figure 3. Phylogenetic workflow.

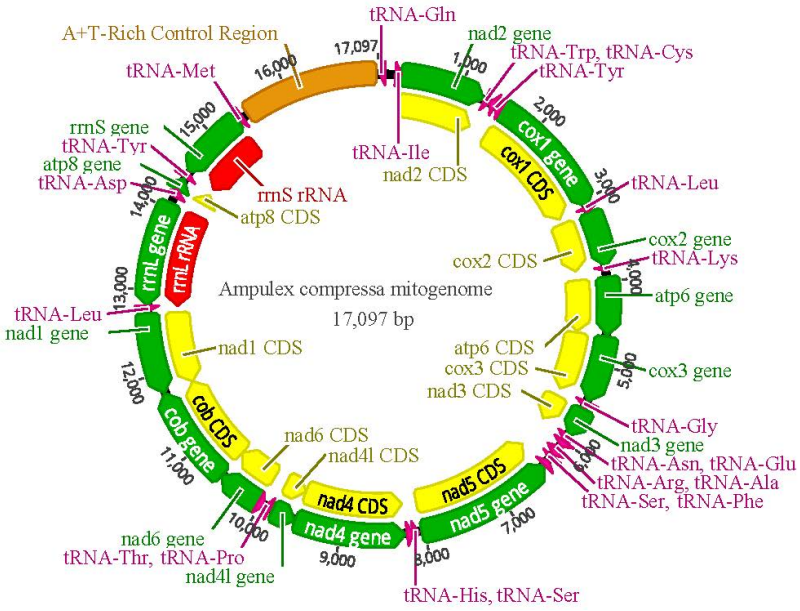

Figure 4. The complete mitogenome of the Ampulex compressa. The control region is a repetitive sequence crucial for mitochondrial gene regulation.

Fig 5. A. Structure of Genes in the venom-specific region of Ampulex compressa. Promoters are depicted in green, and the coding sequences are shown in blue. Ampulexin 2 and 3 have the same mRNA sequence, while ampulein 2 has more length. **B.** BLASTing ampulexin mRNA sequences against GCA_019049445.1 localizes the queries on JAHFVI010000003.1. Ampulexin 1 and 4 have two exons and one introns. Ampulexin 2 and 3 are different splices of the same gene, with three exons and two introns with varying initiation sites inside the gene.

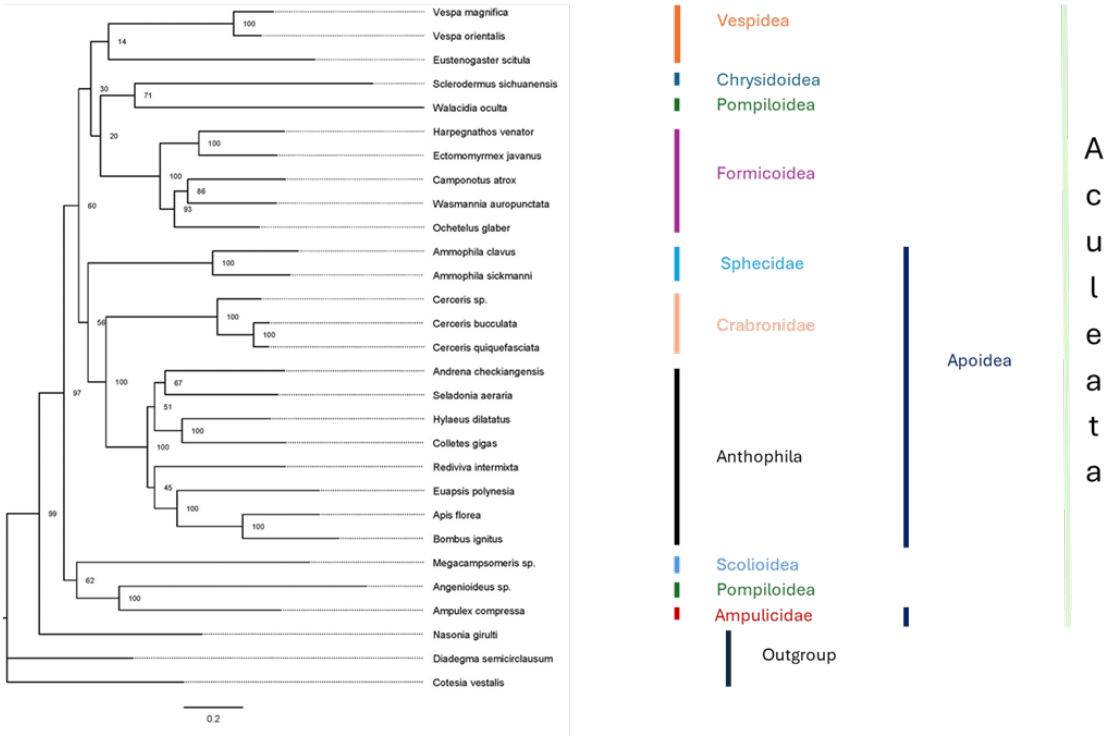

Fig 6. Maximum Likelihood phylogenetic tree of 29 Aculeata samples based on the alignment of 15 protein-coding genes of the mitochondrial genome. The tree was generated using RAxML-NG with the GTR+G substitution model and 2000 bootstrap replicates. The numbers on the nodes represent bootstrap support values, indicating the robustness of each clade.

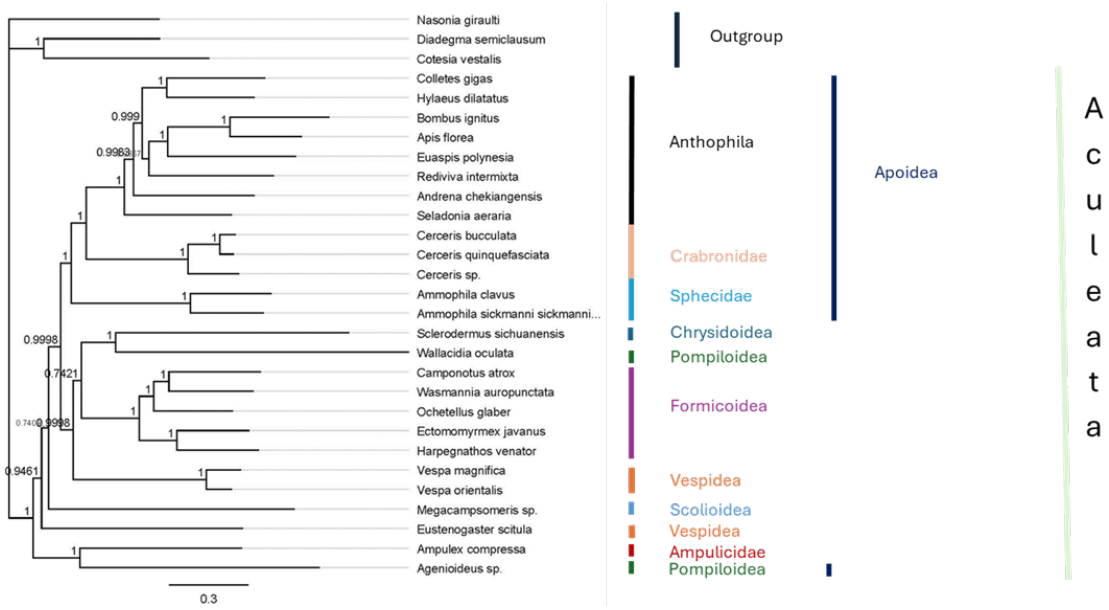

Fig. 7. Phylogenetic tree based on the concatenated alignment of 29 mitogenomes obtained by MrBayes software. Branch labels are posterior probabilities. Geneious version 2024.0.5 (https://www.geneious.com)

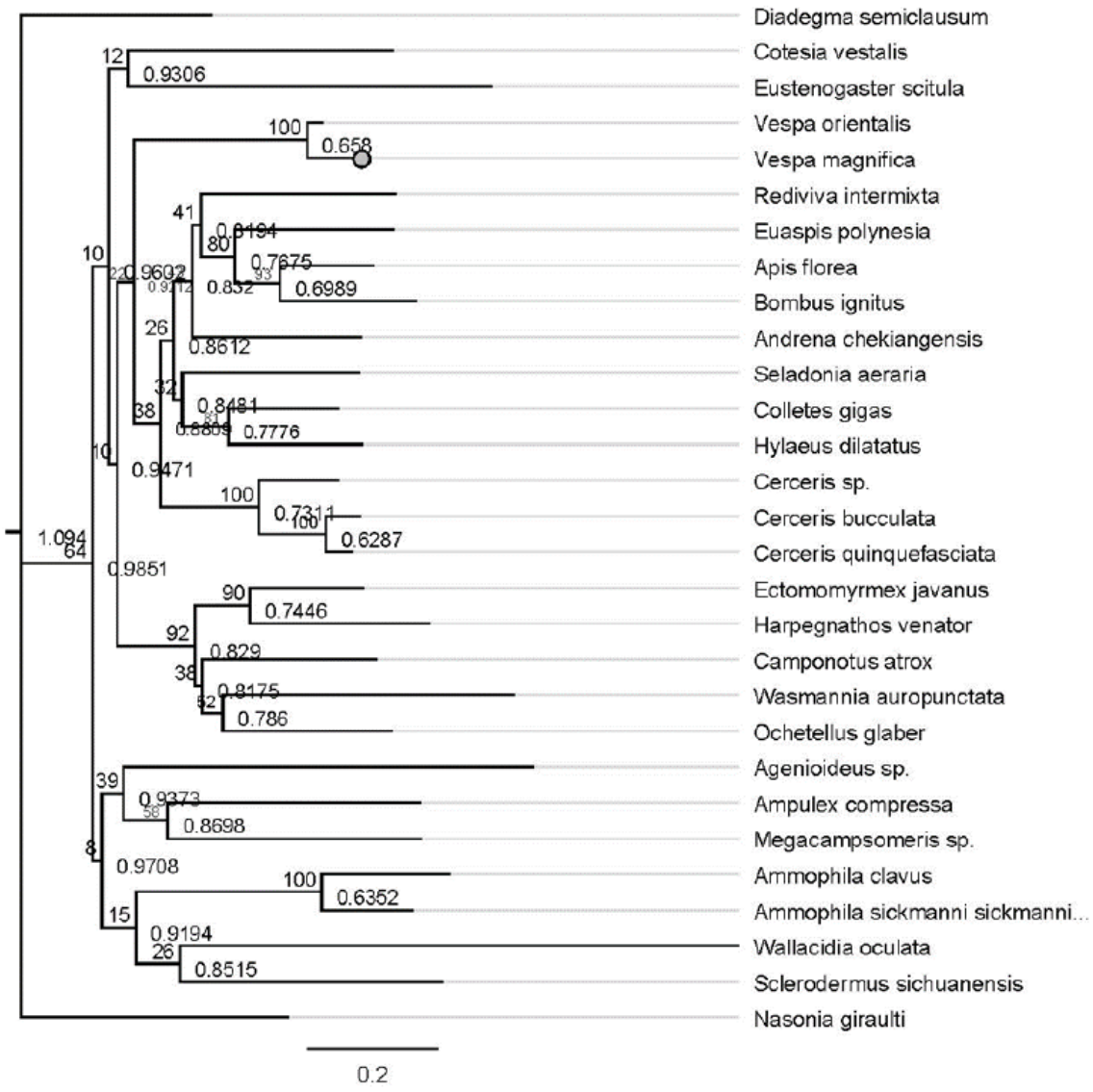

Fig. 8. A phylogenetic tree based on the 16S rRNA alignment of 29 organisms was constructed using MAFFT v7.490 and RAxML v8.0. Includes 1000 bootstrap replicates, with branch labels showing bootstrap support and node labels indicating clade heights.

A B C

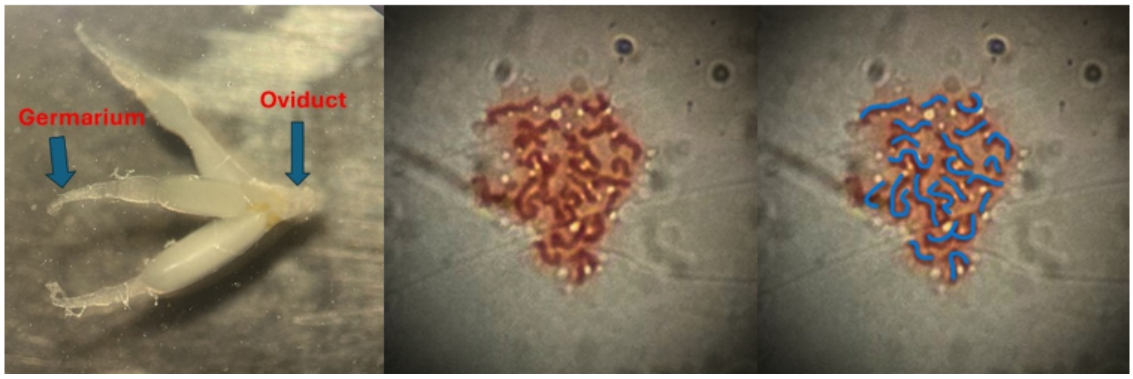

Fig 9. A. Structure of an ovary in an adult female. **B and C.** Karyotype of Ampulex compressa. 2n=22; Stained with Aceto-Orcein; X100 magnification. Chromosomes are marked with blue lines.

Infraorder/Subclade	Superfamily	Subgroup/Family	Organism	Accession number	Length (bp)	Common name
Aculeata	Apoidea	Anthophila	Colletes gigas	KM978210	15,855	
			Hylaeus dilatatus	KP126800.1	15,475	Dilated Yellow-faced Bee
			Bombus ignitus	NC_010967.1	16,434	Chinese Honey Bee
			Apis florea	NC_021401.1	17,694	Dwarf Honeybee
			Rediviva intermixta	NC_030284.1	16,875	
			Andrena chekiangensis	NC_042768.1	15,804	
			Euaspis polynesia	NC_056370.1	17,682	
			Seladonia aeraria	NC_057084.1	15,410	
		Crabronidae	Cerceris bucculata	MW376475.1	16,178	digger wasp
			Cerceris quinquefasciata	MW402864.1	16,188	digger wasp
			Cerceris sp.	OM215185.1	16,790	
		Sphecidae	Ammophila clavus	NC_086963.1	16,154	thread-waisted wasps
			Ammophila sickmanni sickmanni	OM220041.1	17,401	thread-waisted wasps
		Ampulicidae	Ampulex compressa	PP341299.1	17,097	Emerald jewel wasp
	Chrysidoidea	Chrysidoidae	Sclerodermus sichuanensis	NC_065376.1	15,491	flat wasps
	Formicoidea	Formicidae	Camponotus atrox	KT159775.1	16,540	carpenter ant
			Wasmannia auropunctata	NC_030541.1	16,362	Little Fire Ant
			Ectomomyrmex javanus	NC_042678.1	15,512	stinging ant
			Ochetellus glaber	NC_049860.1	16,259	Glabrous crazy ant
			Harpegnathos venator	NC_084286.1	16,089	hunter ant
	Pompiloidea	Mutillidae	Wallacidia oculata	FJ611801.1	18,442	
		Pompiloidae	Agenioideus sp. SJW-2017	KX584356.1	16,596	spider wasps
	Scolidioidea	Scoliidae	Megacampsomeris sp. 1 YJY-2023a	OM142776.1	20,649	scoliid wasps
	Vespoidea	Vespidae	Eustenogaster scitula	NC_044146.1	17,867	hover wasp
			Vespa magnifica	NC_064062.1	16,731	Magnificent Hornet
			Vespa orientalis	OR367661.1	16,092	Oriental Hornet
Proctotrupomorpha	Chalcidoidea	Pteromalidae	Nasonia girault	NC_066199.1	16,415	jewel wasp
Ichneumonoidea	Ichneumonidae	Campopleginae	Diadegma semiclausum	EU871947.1	18,728	parasitoid wasp
Ichneumonoidea	Braconidae	Microgastrinae	Cotesia vestalis	FJ154897.1	15,543	parasitoid wasp

Table 1. Mitogenomes that are used for this study.

Parameter	Value
Genome size	277.4 Mb
Total ungapped length	277.4 Mb
Number of scaffolds	11,507
Scaffold N50	141.4 kb
Scaffold L50	556
Number of contigs	11,950
Contig N50	131.3 kb
Contig L50	589
GC percent	42.5
Genome coverage	101.0x
Assembly level	Scaffold

Table 2. Statistics of the whole genome assembly.

Category	Count	Percentage
Complete BUSCOs (C)	1345	98.4%
- Complete and single-copy (S)	1342	98.2%
- Complete and duplicated (D)	3	0.2%
Fragmented BUSCOs (F)	11	0.8%
Missing BUSCOs (M)	11	0.8%
Total BUSCO groups searched	1367	100%

Table 3: BUSCO analysis results for the Ampulex compressa genome assembly using the insecta_odb10 lineage dataset. Most BUSCOs were identified as complete, with a very low percentage of fragmented and missing BUSCOs, indicating a high-quality genome assembly.

Gene	Promoter	Location	Probability
Ampullexin 1	TCAGTTAACATAAATAACACTCGCCCTTTGGTATTTGCAGTGACGTAATT	-149 to -99	0.95
Ampullexin 4	AATTTATTTTTAAAAAACGCCCACAAACTTATTGTGATTCTAAAAGCTAG	-83 to -32	1
Venom 13	GCTTACCGAATATAAATACCTTGCGTCTTTGGGCTTTTGCGGTAGTGCTA	-81 to -30	0.97
Venom 17	CTACTCGGTATATATATCTTCCGCATTTTGGGCTTTGAGGAAGTATTGTC	-85 to -34	0.99
Ampullexin 2&3	ATTTTATTTTTAAAAAACGCCCACATACTTATTATGATTCAGAAGCTAG	-149 to -98	1

Table 4. Promoter prediction by the Berkeley Drosophila Genome Project. TATA boxes are shaded in yellow.

References

Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman. 1990. “Basic Local Alignment Search Tool.” *Journal of Molecular Biology* 215 (3): 403–10. https://doi.org/10.1016/S0022-2836(05)80360-2.

Arvidson, Ryan, Maayan Kaiser, Sang Soo Lee, Jean-Paul Urenda, Christopher Dail, Haroun Mohammed, Cebrina Nolan, et al. 2019. “Parasitoid Jewel Wasp Mounts Multipronged Neurochemical Attack to Hijack a Host Brain.” *Molecular & Cellular Proteomics* 18 (1): 99–114. https://doi.org/10.1074/mcp.RA118.000908.

Arvidson, Ryan, Victor Landa, Sarah Frankenberg, and Michael E. Adams. 2018. “Life History of the Emerald Jewel Wasp Ampulex Compressa.” *Journal of Hymenoptera Research* 63 (April):1–13. https://doi.org/10.3897/jhr.63.21762.

Ayilara, Modupe S., Bartholomew S. Adeleke, Saheed A. Akinola, Chris A. Fayose, Uswat T. Adeyemi, Lanre A. Gbadegesin, Richard K. Omole, Remilekun M. Johnson, Qudus O. Uthman, and Olubukola O. Babalola. 2023. “Biopesticides as a Promising Alternative to Synthetic Pesticides: A Case for Microbial Pesticides, Phytopesticides, and Nanobiopesticides.” *Frontiers in Microbiology* 14 (February):1040901. https://doi.org/10.3389/fmicb.2023.1040901.

Ballard, J. William O., and Michael C. Whitlock. 2004. “The Incomplete Natural History of Mitochondria.” *Molecular Ecology* 13 (4): 729–44. https://doi.org/10.1046/j.1365-294X.2003.02063.x.

Baym, Michael, Sergey Kryazhimskiy, Tami D. Lieberman, Hattie Chung, Michael M. Desai, and Roy Kishony. 2015. “Inexpensive Multiplexed Library Preparation for Megabase-Sized Genomes.” Edited by Stefan J. Green. *PLOS ONE* 10 (5): e0128036. https://doi.org/10.1371/journal.pone.0128036.

Beckenbach, Andrew T., and Jeffrey B. Joy. 2009. “Evolution of the Mitochondrial Genomes of Gall Midges (Diptera: Cecidomyiidae): Rearrangement and Severe Truncation of tRNA Genes.” *Genome Biology and Evolution* 1 (January):278–87. https://doi.org/10.1093/gbe/evp027.

Bohart, Richard M., Richard Mitchell Bohart, Arnold S. Menke, A. S. Menke, and H. S. Court. 1976. *Sphecid Wasps of the World: A Generic Revision*. Berkeley, Calif.: Univ. of California Press.

Boore, J. L. 1999. “Animal Mitochondrial Genomes.” *Nucleic Acids Research* 27 (8): 1767–80. https://doi.org/10.1093/nar/27.8.1767.

Bushnell, Brian. n.d. “BBTools: A Suite of Fast, Multithreaded Bioinformatics Tools Designed for Analysis of DNA and RNA Sequence Data.” Joint Genome Institute. Accessed July 20, 2023. https://jgi.doe.gov/data-and-tools/bbtools/.

Cameron, Stephen L. 2014. “Insect Mitochondrial Genomics: Implications for Evolution and Phylogeny.” *Annual Review of Entomology* 59 (1): 95–117. https://doi.org/10.1146/annurev-ento-011613-162007.

Chikhi, Rayan, and Paul Medvedev. 2014. “Informed and Automated *k* -Mer Size Selection for Genome Assembly.” *Bioinformatics* 30 (1): 31–37. https://doi.org/10.1093/bioinformatics/btt310.

Crampton-Platt, Alex, Martijn J.T.N. Timmermans, Matthew L. Gimmel, Sujatha Narayanan Kutty, Timothy D. Cockerill, Chey Vun Khen, and Alfried P. Vogler. 2015. “Soup to Tree: The Phylogeny of Beetles Inferred by Mitochondrial Metagenomics of a Bornean Rainforest Sample.” *Molecular Biology and Evolution* 32 (9): 2302–16. https://doi.org/10.1093/molbev/msv111.

Degnan, James H., and Noah A. Rosenberg. 2009. “Gene Tree Discordance, Phylogenetic Inference and the Multispecies Coalescent.” *Trends in Ecology & Evolution* 24 (6): 332–40. https://doi.org/10.1016/j.tree.2009.01.009.

Dong, Jinbo. n.d. “GetOrganelle v1.7.7.0.” Accessed August 20, 2024. https://github.com/Kinggerm/GetOrganelle.

Drukewitz, Stephan Holger, and Björn Marcus Von Reumont. 2019. “The Significance of Comparative Genomics in Modern Evolutionary Venomics.” *Frontiers in Ecology and Evolution* 7 (May):163. https://doi.org/10.3389/fevo.2019.00163.

Eastin, Katherine J., Austin P. Huang, and Patrick M. Ferree. 2020. “A Novel Pattern of Germ Cell Divisions in the Production of Hymenopteran Insect Eggs.” *Biology Letters* 16 (5): 20200137. https://doi.org/10.1098/rsbl.2020.0137.

Ferree, Patrick M., and Daniel A. Barbash. 2009. “Species-Specific Heterochromatin Prevents Mitotic Chromosome Segregation to Cause Hybrid Lethality in Drosophila.” Edited by Mohamed A. F. Noor. *PLoS Biology* 7 (10): e1000234. https://doi.org/10.1371/journal.pbio.1000234.

Funk, Daniel J., and Kevin E. Omland. 2003. “Species-Level Paraphyly and Polyphyly: Frequency, Causes, and Consequences, with Insights from Animal Mitochondrial DNA.” *Annual Review of Ecology, Evolution, and Systematics* 34 (1): 397–423. https://doi.org/10.1146/annurev.ecolsys.34.011802.132421.

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 (22): 4541–50. https://doi.org/10.1111/j.1365-294X.2009.04380.x.

Gilbertson, Cody R., and Jeffrey D. Wyatt. 2016. “Evaluation of Euthanasia Techniques for an Invertebrate Species, Land Snails (Succinea Putris).” *Journal of the American Association for Laboratory Animal Science: JAALAS* 55 (5): 577–81.

Gokhman, V. E. 2009. *Karyotypes of Parasitic Hymenoptera*. Dordrecht: Springer.

Gokhman, Vladimir E. 2022. “Comparative Karyotype Analysis of Parasitoid Hymenoptera (Insecta): Major Approaches, Techniques, and Results.” *Genes* 13 (5): 751. https://doi.org/10.3390/genes13050751.

Gokhman, Vladimir E., Marcelo de Bello Cioffi, Christian König, Marie Pollmann, Cornelia Gantert, Lars Krogmann, Johannes L. M. Steidle, Nadezda Kosyakova, Thomas Liehr, and Ahmed Al-Rikabi. 2019. “Microdissection and Whole Chromosome Painting Confirm Karyotype Transformation in Cryptic Species of the Lariophagus Distinguendus (Förster, 1841) Complex (Hymenoptera: Pteromalidae).” *PloS One* 14 (11): e0225257. https://doi.org/10.1371/journal.pone.0225257.

Henderson, Elisa C., and Alan Brelsford. 2020. “Genomic Differentiation across the Speciation Continuum in Three Hummingbird Species Pairs.” *BMC Evolutionary Biology* 20 (1): 113. https://doi.org/10.1186/s12862-020-01674-9.

Imai, Hirotami T., Ross H. Crozier, and Robert W. Taylor. 1977. “Karyotype Evolution in Australian Ants.” *Chromosoma* 59 (4): 341–93. https://doi.org/10.1007/BF00327974.

Imai, Hirotami T., Robert W. Taylor, Michael W.J. Crosland, and Ross H. Crozier. 1988. “Modes of Spontaneous Chromosomal Mutation and Karyotype Evolution in Ants with Reference to the Minimum Interaction Hypothesis.” *The Japanese Journal of Genetics* 63 (2): 159–85. https://doi.org/10.1266/jjg.63.159.

Jalali, S. K., Rakshit Ojha, and T. Venkatesan. 2015. “DNA Barcoding for Identification of Agriculturally Important Insects.” In *New Horizons in Insect Science: Towards Sustainable Pest Management*, edited by Akshay Kumar Chakravarthy, 13–23. New Delhi: Springer India. https://doi.org/10.1007/978-81-322-2089-3_2.

Jinek, Martin, Krzysztof Chylinski, Ines Fonfara, Michael Hauer, Jennifer A. Doudna, and Emmanuelle Charpentier. 2012. “A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity.” *Science* 337 (6096): 816–21. https://doi.org/10.1126/science.1225829.

Johnson, Brian R., Marek L. Borowiec, Joanna C. Chiu, Ernest K. Lee, Joel Atallah, and Philip S. Ward. 2013. “Phylogenomics Resolves Evolutionary Relationships among Ants, Bees, and Wasps.” *Current Biology* 23 (20): 2058–62. https://doi.org/10.1016/j.cub.2013.08.050.

Kakazu, Naoki, Masafumi Taniwaki, Shigeo Horiike, Kazuhiro Nishida, Toyoshi Tatekawa, Masami Nagai, Takayuki Takahashi, et al. 1999. “Combined Spectral Karyotyping and DAPI

Banding Analysis of Chromosome Abnormalities in Myelodysplastic Syndrome." *Genes, Chromosomes and Cancer* 26 (4): 336–45. https://doi.org/10.1002/(SICI)1098-2264(199912)26:4<336::AID-GCC8>3.0.CO;2-H.

Katoh, K. 2002. "MAFFT: A Novel Method for Rapid Multiple Sequence Alignment Based on Fast Fourier Transform." *Nucleic Acids Research* 30 (14): 3059–66. https://doi.org/10.1093/nar/gkf436.

Katoh, K., and D. M. Standley. 2013. "MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability." *Molecular Biology and Evolution* 30 (4): 772–80. https://doi.org/10.1093/molbev/mst010.

Kearse, Matthew, Richard Moir, Amy Wilson, Steven Stones-Havas, Matthew Cheung, Shane Sturrock, Simon Buxton, et al. 2012. "Geneious Basic: An Integrated and Extendable Desktop Software Platform for the Organization and Analysis of Sequence Data." *Bioinformatics* 28 (12): 1647–49. https://doi.org/10.1093/bioinformatics/bts199.

King, Glenn F., and Margaret C. Hardy. 2013. "Spider-Venom Peptides: Structure, Pharmacology, and Potential for Control of Insect Pests." *Annual Review of Entomology* 58 (1): 475–96. https://doi.org/10.1146/annurev-ento-120811-153650.

Lagunas-Robles, German, Jessica Purcell, and Alan Brelsford. 2021. "Linked Supergenes Underlie Split Sex Ratio and Social Organization in an Ant." *Proceedings of the National Academy of Sciences of the United States of America* 118 (46): e2101427118. https://doi.org/10.1073/pnas.2101427118.

McKenzie, Sean K, Peter R Oxley, and Daniel Jc Kronauer. 2014. "Comparative Genomics and Transcriptomics in Ants Provide New Insights into the Evolution and Function of Odorant Binding and Chemosensory Proteins." *BMC Genomics* 15 (1): 718. https://doi.org/10.1186/1471-2164-15-718.

Peters, Ralph S., Lars Krogmann, Christoph Mayer, Alexander Donath, Simon Gunkel, Karen Meusemann, Alexey Kozlov, et al. 2017. "Evolutionary History of the Hymenoptera." *Current Biology* 27 (7): 1013–18. https://doi.org/10.1016/j.cub.2017.01.027.

Pires, Em, Ak Campos, Mr Pereira, Rm Nogueira, Lao Campos, Psa Moreira, and Ma Soares. 2014. "First Report of 'Jewel Wasp' Ampulex Compressa (Fabricius, 1781) (Hymenoptera: Ampulicidae) in the Amazon Biome of Brazil." *Brazilian Journal of Biology* 74 (3 suppl 1): S233–34. https://doi.org/10.1590/1519-6984.05913.

Rens, Willem, Beiyuan Fu, Patricia C M O'Brien, and Malcolm Ferguson-Smith. 2006. "Cross-Species Chromosome Painting." *Nature Protocols* 1 (2): 783–90. https://doi.org/10.1038/nprot.2006.91.

Ronquist, Fredrik, Maxim Teslenko, Paul Van Der Mark, Daniel L. Ayres, Aaron Darling, Sebastian Höhna, Bret Larget, Liang Liu, Marc A. Suchard, and John P. Huelsenbeck. 2012.

"MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space." *Systematic Biology* 61 (3): 539–42. https://doi.org/10.1093/sysbio/sys029.

Rubinoff, Daniel, and Brenden S. Holland. 2005. "Between Two Extremes: Mitochondrial DNA Is Neither the Panacea nor the Nemesis of Phylogenetic and Taxonomic Inference." Edited by Vincent Savolainen. *Systematic Biology* 54 (6): 952–61. https://doi.org/10.1080/10635150500234674.

Sann, Manuela, Oliver Niehuis, Ralph S. Peters, Christoph Mayer, Alexey Kozlov, Lars Podsiadlowski, Sarah Bank, et al. 2018. "Phylogenomic Analysis of Apoidea Sheds New Light on the Sister Group of Bees." *BMC Evolutionary Biology* 18 (1): 71. https://doi.org/10.1186/s12862-018-1155-8.

Seppey, Mathieu, Mosè Manni, and Evgeny M. Zdobnov. 2019. "BUSCO: Assessing Genome Assembly and Annotation Completeness." In *Gene Prediction*, edited by Martin Kollmar, 1962:227–45. Methods in Molecular Biology. New York, NY: Springer New York. https://doi.org/10.1007/978-1-4939-9173-0_14.

Shen, Wei, Shuai Le, Yan Li, and Fuquan Hu. 2016. "SeqKit: A Cross-Platform and Ultrafast Toolkit for FASTA/Q File Manipulation." Edited by Quan Zou. *PLOS ONE* 11 (10): e0163962. https://doi.org/10.1371/journal.pone.0163962.

Stamatakis, Alexandros. 2014. "RAxML Version 8: A Tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies." *Bioinformatics* 30 (9): 1312–13. https://doi.org/10.1093/bioinformatics/btu033.

Stanke, Mario, and Stephan Waack. 2003. "Gene Prediction with a Hidden Markov Model and a New Intron Submodel." *Bioinformatics* 19 (suppl_2): ii215–25. https://doi.org/10.1093/bioinformatics/btg1080.

Whitfield, J. 2003. "Phylogenetic Insights into the Evolution of Parasitism in Hymenoptera." In *Advances in Parasitology*, 54:69–100. Elsevier. https://doi.org/10.1016/S0065-308X(03)54002-7.

Zeiss. 2000. "Axioskop [Phase Contrast Light Microscope]." Zeiss.

Zhang, Xinmi, Anabhra Singh, Kassandra Soriano Martinez, and Patrick M. Ferree. 2024. "Direct Parental (DIPA) CRISPR in the Jewel Wasp, Nasonia Vitripennis." *G3 (Bethesda, Md.)*, May, jkae095. https://doi.org/10.1093/g3journal/jkae095.

Zheng, Bo-Ying, Li-Jun Cao, Pu Tang, Kees Van Achterberg, Ary A. Hoffmann, Hua-Yan Chen, Xue-Xin Chen, and Shu-Jun Wei. 2018. "Gene Arrangement and Sequence of Mitochondrial Genomes Yield Insights into the Phylogeny and Evolution of Bees and Sphecid Wasps (Hymenoptera: Apoidea)." *Molecular Phylogenetics and Evolution* 124 (July):1–9. https://doi.org/10.1016/j.ympev.2018.02.028.

Zheng, Xiao-Yu, Li-Jun Cao, Peng-Yan Chen, Xue-Xin Chen, Kees Van Achterberg, Ary Anthony Hoffmann, Jing-Xian Liu, and Shu-Jun Wei. 2021. “Comparative Mitogenomics and Phylogenetics of the Stinging Wasps (Hymenoptera: Aculeata).” *Molecular Phylogenetics and Evolution* 159 (June):107119. https://doi.org/10.1016/j.ympev.2021.107119.