

Gain and loss: energetic cost of plant camouflage in Mantodea

Ling-Yun Song¹, Chun-Xiang Liu², Chao Wu², Guang-Ping Huang^{1,*}, Fu-Wen Wei^{1,2,3,*}

¹Jiangxi Provincial Key Laboratory of Conservation Biology, College of Forestry, Jiangxi Agricultural University, Nanchang, China

²Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China

³University of Chinese Academy of Sciences, Beijing 100049, China

*Correspondence: weifw@ioz.ac.cn; huanggp@ioz.ac.cn

ABSTRACT

Many praying mantis species exhibit remarkable femoral modification representing a key adaptive morphological camouflage in natural environments. To estimate the evolution of the femoral lobe in Mantodea, we performed the analyses of internal phylogenetic relationships, ancestral state reconstruction and estimate species divergence times using 175 mitochondrial genomes spanning 20 families and 93 genera. The findings revealed that the phenotype of the femoral lobe originated during the mid-Cretaceous. Subsequently, this trait was retained and further diversified within the Galinthiadidae, Hymenopodidae and Empusidae, whereas it was lost in the family Mantidae. Additionally, analyses of molecular evolutionary rates revealed that seven mitochondrial protein-coding genes (*Nd2*, *Nd5*, *Nd6*, *Atp6*, *Cox2*, *Cox1* and *Cytb*) underwent accelerated evolution in mantis lineages possessing femoral lobes, which might be attributed to the inability of the rigid femoral lobes to contract, resulting in increased energy requirements for mantis locomotion. Further, transcriptomic analysis of leg tissues in *Hymenopus coronatus*, a species with enlarged femur demonstrated that energy metabolic capacity increases during ontogeny. Metabolomic comparisons between *Tenodera sinensis* representing femur-absent taxa and *H. coronatus* revealed that *H. coronatus* exhibits enhanced capacities for energy metabolism and conversion. Collectively, these findings indicated a trade-off between plant camouflage and energy metabolism during the evolution of Mantodea.

Key words: Mantodea; Plant camouflage; Origin and evolution; Energy metabolism

INTRODUCTION

Camouflage constitutes an essential and widespread survival strategy in nature, exerting profound impacts on the survival and reproductive success of species (Briolat et al., 2021; Duarte



et al., 2018; Stoddard et al., 2016). Investigating animal camouflage facilitates our understanding of animal morphological adaptations. Animals employ a diverse array of camouflage strategies, among which crypsis and masquerade are the two significant forms (Huang et al., 2024). Crypsis is not a passive hiding behavior but an active strategy, which enables prey exposed to predators' visual field to evade detection via adjustments to body coloration and behavior (Stevens & Merilaita, 2009). Masquerade refers to predators misidentify prey as inanimate environmental objects (e.g., stones, branches and fallen leaves) (Skelhorn et al., 2010).

Praying mantises (Mantodea) serve as typical examples of crypsis and masquerade, utilizing color patterns and specialized structures to blend seamlessly with vegetation, thereby aiding in predator avoidance (Huang et al., 2023; Viana et al., 2023). Among the diverse camouflage structures of mantises, the femoral lobe is a key trait that enhances camouflage (O'hanlon, 2016). Typically, the femoral lobe is located on the ventral side of the mantis femur. However, certain taxa (e.g., Toxoderini) display considerable expansion on both the dorsal and ventral sides (Thornthan et al., 2019). Additionally, the femoral lobe exhibits interspecific variation in size. For instance, *Hymenopus coronatus* possesses larger petal-shaped femoral lobes (Mizuno et al., 2014; O'hanlon et al., 2014), whereas species of the genus *Popa* feature smaller, leaf-like projections (Lombardo, 1995). Recent studies have explored the biological functions and developmental mechanisms underlying the petal-shaped femoral lobes of *H. coronatus* (Huang et al., 2024; Huang et al., 2023; Zhao et al., 2024). Structurally, the petal-shaped femoral lobes have evolved aerodynamic properties that enable gliding (Zhao et al., 2024). Molecular evidence indicates that femoral lobes are primarily composed of cuticle, and their morphogenesis is regulated by the Wnt signaling pathway (Huang et al., 2023).

Despite significant advances in understanding the function and development of the femoral lobe phenotype, its evolutionary history remains largely unexplored. To reconstruct this historical trajectory, [it is essential to build robust phylogenetic relationships](#). To date, the monophyly of Mantodea and its status as a sister group to Blattodea have been consistently supported by both morphological classification and molecular phylogeny (Deitz et al., 2003; Klass & Meier, 2006; Svenson & Whiting, 2004; Yager & Svenson, 2008). However, the internal phylogenetic relationships within Mantodea remain incompletely resolved. The most species-rich Mantodea phylogenetic dataset was built covering 288 species from all families and 45 subfamilies via nine



molecular loci (12S rRNA, 16S rRNA, 18S rRNA, 28S rRNA, histone 3, *Cox1*, *Cox2*, *Nd4* and *Wingless*), which revealed that only half of these taxa were monophyletic while others were polyphyletic or paraphyletic (Svenson & Whiting, 2009). This also exposed discrepancies between molecular phylogeny and traditional morphological classification. Therefore, more scientific and reasonable classification criteria for mantises have been continuously explored (Wieland, 2013; Yager & Svenson, 2008). Recently, the latest taxonomic reference for Mantodea was updated by integrating male genital morphology, karyotyping, phenotypic traits and molecular data (Schwarz & Roy, 2019). On this basis, Ma et al. (2023) inferred the phylogeny of 98 mantis species across 15 families and 30 subfamilies using 13 mitochondrial protein-coding genes and two rRNA genes (12S rRNA and 16S rRNA). The resulting topology is highly consistent with the latest classification framework, further verifying its reliability. We further expanded taxon sampling to cover 93 genera from 20 Mantodea families, thereby constructing a more comprehensive phylogenetic tree to explore the evolutionary trajectory of femoral lobe morphological traits during mantis diversification.

Mitochondria, often termed the “energy factories” of eukaryotic cells, are responsible for converting nutrients into chemical energy (ATP) to fuel biological activities (Casanova et al., 2023). They possess their own genetic material (the mitochondrial genome), which typically comprises 22 transfer RNAs (tRNAs), 2 ribosomal RNAs (12S and 16S rRNAs) and 13 protein-coding genes (D'souza & Minczuk, 2018; Taanman, 1999). These genes are involved in the oxidative phosphorylation process, which is crucial for energy production and transformation (Kummer & Ban, 2021; Nolfi-Donagan et al., 2020). Numerous studies have indicated that the evolution of mitochondrial genomes was not merely driven by neutral evolution, and the energy requirements corresponding to the locomotor functions of species have had a profound impact on the evolutionary trajectory of mitochondrial genomes (Da Fonseca et al., 2008; Zhang et al., 2024). For example, relaxed selective constraints on avian mitochondrial DNA have been documented following the degeneration of flight ability (Shen et al., 2009). The origin of flight in bats involved positive selection on oxidative respiratory chain-related genes to meet the increased energy requirements of flight (Shen et al., 2010). Similar adaptive evolution of mitochondrial genes linked to energy metabolism has been documented in numerous invertebrates (Kang & Hu, 2023; Mitterboeck & Adamowicz, 2013; Sun et al., 2018; Zhang et al., 2019). For instance, intertidal spiders (*Desis*



jiaxiangi) (Li et al., 2021) and spider mites (*Tetranychus*) (Sun et al., 2018) exhibit strong positive selection on mitochondrial genes, facilitating adaptation to the metabolic requirements of diverse environments. Moreover, mitogenomic evolutionary rates in bilateria are influenced by parasitic lifestyle and locomotory capacity (Jakovlic et al., 2023). Collectively, these findings demonstrate that the varying energy requirements of animals result in differing levels of selection pressure on mitochondrial genes.

Unlike the retractable wing membranes and webs of vertebrates, the femoral lobe of the mantis is composed of rigid, non-retractable cuticle. Studies on *H. coronatus* have revealed that its expanded femoral lobes play a crucial role in gliding during flight and jumping (Zhao et al., 2024). Given the functional divergence in locomotion associated with femoral lobes, it remains unclear whether divergent energy requirements for locomotion between mantis species with and without femoral lobes have shaped selection pressure on mitochondrial protein-coding genes.

In this study, we utilized topological relationships from a newly constructed dataset (encompassing 20 families and 93 genera of mantises) to investigate the origin and evolution of the femoral lobe phenotype. Divergence time estimation was used to infer the evolutionary drivers of this trait, with results indicating that the femoral lobe originated during the mid-Cretaceous. We propose that avian predation and the diversification of flowering plants may have provided the key selective pressure and environmental context for the evolution of this trait. Furthermore, analyses of molecular evolutionary rates across 13 mitochondrial protein-coding genes revealed significantly accelerated evolution in mantises possessing femoral lobes. Consistently, the metabolomic and transcriptomic analyses showed significant up-regulation and enrichment of energy metabolism pathways in mantises with femoral lobes. Our findings provide a novel perspective: the evolution of complex camouflage structures in animals may be accompanied by corresponding changes in energy metabolic capacity. Accordingly, we propose a hypothesis regarding the evolutionary trade-off between plant camouflage and energy metabolism.

MATERIALS AND METHODS

Taxon sampling and DNA sequencing

We performed de novo sequencing of the complete mitogenomes of 46 mantis individuals, which were integrated with previously published mitogenomic data to construct an expanded dataset.

Our taxon sampling included 175 individuals across the order Mantodea, covering 20 families and 93 genera (Supplementary Table S1). Moreover, mitogenomic sequences of four Blattodea species (*Cryptocercus kyebangensis*, *Eupolyphaga sinensis*, *Macrotermes barneyi* and *Termes hospes*) were retrieved from the NCBI database to serve as outgroup taxa in the phylogenetic analyses. [All newly sequenced mitogenomes in the present study are marked with an asterisk \(*\) in Supplementary Table S1.](#)

Fresh mantis [specimens](#) for sequencing were collected and preserved at -80°C prior to DNA extraction. Genomic DNA was isolated from the leg muscle tissue of each sample using DNeasy Blood and Tissue Kits (Qiagen, Germany), strictly following the manufacturer's standard protocol. Approximately 0.2 µg of DNA per sample was used for library construction. Sequencing libraries were prepared with the NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA; Catalog #: E7370L) strictly following the manufacturer's recommendations, with unique index codes assigned to individual samples for subsequent identification. In brief, genomic DNA samples were sonicated to achieve a fragment size of 350 base pairs, followed by end-polishing, A-tailing and ligation with full-length adapters for Illumina sequencing. The libraries were further enriched via Polymerase Chain Reaction (PCR) amplification, and [the resultant PCR products were purified](#) using the AMPure XP system (Beverly, USA). Library quality was evaluated using the Agilent 5400 system (Agilent, USA), and quantification was performed by quantitative Polymerase Chain Reaction (qPCR). The qualified libraries were then pooled and sequenced on Illumina platforms using a [Paired-End 150 bp sequencing \(PE150\)](#) strategy at Novogene Bioinformatics Technology Co., Ltd. (Beijing, China), based on the effective library concentration and the required data [yield](#).

Mitogenome assembly and annotation

Mitobin v.1.9.1 (Hahn et al., 2013) and GetOrganelle v.1.7.7 (Jin et al., 2020) were used for the [preliminary mitogenome assembly](#). The position edges of each mitochondrial gene, the secondary structure of tRNAs and the control region were predicted using the Mitos Web Server with the invertebrate mitochondrial genetic code (Bernt et al., 2013). Subsequently, annotation information was [cross-checked against](#) other reference mitogenomes of mantises obtained from the NCBI Organelle Genome Resources database using Geneious Prime (Kearse et al., 2012) to enhance accuracy.



Dataset selection and preparation

After excluding the control region, intergenic spacers, and poorly informative tRNA genes, only protein-coding genes (PCGs) and rRNA genes were retained for multiple sequence alignment.

The nucleotide sequence of PCGs were aligned using the invertebrate mitochondrial codon strategy in MACSE v_{2.06} (Ranwez et al., 2011), and the two rRNA genes (rRNAs) were aligned using the G-INS-i algorithm in MAFFT v_{7.505} (Kato et al., 2002). We employed GBLOCKS v_{0.91b} (Castresana, 2000) to remove poorly aligned regions from the alignments. PCGs and rRNAs were trimmed respectively using “codons” and “DNA” mode, both with half gaps allowed (b5=with half), and other parameters at default settings. All aligned PCGs and rRNAs (PCGs+rRNAs) were concatenated to facilitate subsequent phylogenetic analyses. To account for potential phylogenetic biases caused by wobble at the third codon position (Gibson, 2005), we generated an additional dataset (PCG12S+rRNAs) by excluding third codon positions of PCGs using MEGA v_{1.1} (Tamura et al., 2021). DnaSP v_{5.0} (Librado & Rozas, 2009) was utilized to estimate both the synonymous substitution rate (d_s) and the non-synonymous substitution rate (d_n) across the 13 PCGs. Furthermore, we assessed the heterogeneity of sequence composition in mitogenomes for each dataset using AliGROOVE (Kuck et al., 2014).

Phylogeny reconstruction and tree topology test

Phylogenetic relationships were inferred using two methods: maximum likelihood (ML) and Bayesian inference (BI). For ML analyses, RAxML v_{8.2.12} (Stamatakis, 2014) was employed to construct phylogenetic trees for both the “PCGs+rRNAs” and “PCG12s+rRNAs” datasets. Branch support was evaluated via the bootstrap approximation method (Hoang et al., 2018) with 1000 bootstrap replicates. For BI analyses, optimal substitution models for each partition were selected using PartitionFinder v_{2.0.0} (Lanfear et al., 2017) based on the Bayesian Information Criterion (BIC), separately for the “PCGs+rRNAs” and “PCG12s+rRNAs” datasets (Supplementary Tables S2–S3). Posterior distributions were subsequently estimated using Markov Chain Monte Carlo (MCMC) sampling in MrBayes v_{3.2.7a} (Ronquist et al., 2012). We ran four simultaneous MCMC chains for 20 million generations, with sampling every 2000 generations, until the average standard deviation of split frequencies fell below 0.01. Convergence was verified using Tracer v_{1.6} (Rambaut et al., 2018), and the top 25% of trees were discarded before summarization.

To assess the optimal phylogenetic topology, we performed Shimodaira-Hasegawa (SH) (Shimodaira & Hasegawa, 1999), Kishino-Hasegawa (KH) (Kishino & Hasegawa, 1989), weighted KH (WKH), weighted SH (WSH) and approximately unbiased (AU) (Shimodaira, 2002) topology tests in IQ-TREE v.1.6.9 (Nguyen et al., 2015) using 10,000 RELL (Kishino et al., 1990) resamplings (Supplementary Table S4). Additionally, Four-cluster likelihood mapping (FcLM) was employed (Strimmer & Von Haeseler, 1997) to test alternative phylogenetic hypotheses for the placements of Haaniidae, Acanthopidae, Gonypetoidea and Thespidae. The input consisted of five clusters: (i) Haaniidae, (ii) Acanthopidae, (iii) Gonypetoidea, (iv) Thespidae and (v) the remaining species used in the phylogenetic analyses, with the option “IGNORED” (Supplementary Table S5).

Divergence time estimation

Divergence time estimation was conducted using MCMCTREE in the PAML v.4.9 package (Dos Reis et al., 2014; Yang, 2007), which performs Bayesian estimation of species divergence times under relaxed molecular clock models with soft fossil constraints, and uses approximate likelihood calculation to reduce the computational burden. Ten fossil-calibrated time points (Supplementary Table S6) were obtained from the TimeTree database (Kumar et al., 2017). Convergence of the MCMC chains was assessed using Tracer v.1.7.1 (<http://beast.community/tracer>), with effective sample size (ESS) values >200 considered to indicate reliable convergence.

Ancestral state reconstruction

A femoral lobe is a leaf-like flap projecting from the integument of the insect femur, serving as a specialized adaptive structure for camouflage. We examined images and records of mantises from literature and online databases (GBIF, <https://www.gbif.org/>; iNaturalist, <https://www.inaturalist.org/>) to score the presence or absence of the femoral lobe trait, which served as the input for ancestral state reconstruction. After excluding genus-level identifications, the final dataset included 118 valid species, of which 33 (27.97%) exhibited the femoral lobe phenotype.

Ancestral characterization of the femoral lobe was performed by Phytools and the Ape package in the R statistical software (Revell, 2012). To determine the most appropriate model for reconstructing the ancestral state, we tested the applicability of the following models: the equal rates model (ER), the all-rates-different model (ARD) and the symmetrical rates model (SYM). Model



fit was assessed using the Akaike Information Criterion (AIC). The corresponding AIC values for ER, SYM and ARD were 97, 97 and 93, respectively (Supplementary Table S7). Since the ARD model yielded the lowest AIC score, it was selected as the best-fitting model. Accordingly, we performed ancestral state reconstruction of the femoral lobe phenotype using the ARD model.

Rapid evolutionary analysis

The branch model was employed in PAML to assess the evolutionary rates of 13 mitochondrial protein-coding genes in mantises possessing the femoral lobe phenotype, using the invertebrate mitochondrial codon (icode=4) and allowing evolutionary rates to vary among branches (clock=0). Genes with a likelihood-ratio test statistic ($2\Delta\ln L$)>3.841 and a significantly higher nonsynonymous-to-synonymous substitution ratio (d_N/d_S) on the foreground branch than on background branches were identified as undergoing significantly accelerated evolution in the focal lineage.

Weighted gene co-expression network analysis

Previous studies have demonstrated that femoral lobes in *H. coronatus* exhibit significant allometric growth during ontogeny, with their relative size increasing disproportionately (Zhao et al., 2024). Building on this development pattern, we performed weighted gene co-expression network analysis (WGCNA) (Langfelder & Horvath, 2008) using leg transcriptomes from different instars of *H. coronatus* to identify gene modules associated with two phenotypic gradients: the presence or absence of femoral lobes (1st instar vs. other instars) and the relative size of femoral lobes (adult>5th instar>1st instar). In the correlation heatmap, Pearson correlation coefficients approaching 1 or -1 indicated strong positive or negative associations between gene modules and the trait, respectively. The statistical significance of these correlations was assessed using a two-tailed Student's *t*-test, with *P*-value<0.05 considered statistically significant. Finally, functional enrichment analysis was performed on gene modules significantly correlated with the increase in femoral lobe size.

Metabolites extraction, identification and quantification

Biological samples were lyophilized and ground into a fine powder. A 100 mg powder was extracted with 1.2 mL of 70% methanol, subjected to six cycles of vortexing and then incubated



overnight at 4°C. After centrifugation, the supernatant was filtered through a microporous membrane and transferred to an injection vial for analysis by ultra-performance liquid chromatography (UPLC) (Shimadzu Nexera X2, Japan) coupled with tandem mass spectrometry (MS/MS) (Applied Biosystems QTRAP, USA). Experimental parameters were set following established protocols (Wang et al., 2019).

Metabolites were identified against a self-constructed spectral library using quadratic regression-based spectral matching. Quantification was performed using multiple reaction monitoring (MRM) mode on a triple quadrupole mass spectrometer. Chromatographic peaks were integrated and corrected using MultiaQuant software v_3.0.3, with the resulting peak areas serving as quantitative indicators of relative metabolite abundance.

Differential metabolite screening and enrichment

Principal component analysis (PCA) and Spearman correlation analysis were used to evaluate intragroup repeatability and intergroup differences among samples. The function annotation of metabolite was performed against the KEGG, HMDB and LipidMaps databases. Orthogonal projections to latent structures-discriminant analysis (OPLS-DA) analysis was performed using R package ropls to eliminate orthogonal variation unrelated to the experimental groups, with 200 permutation tests to verify the reliability of the model. Based on the OPLS-DA model, differential metabolites were screened using Variable Importance in Projection (VIP) values combined with univariate analysis, with the criteria of $VIP \geq 1$, fold change (FC) > 1 and t -test P -value < 0.05. The screened differential metabolites were further subjected to KEGG pathway enrichment analysis, and significance was calculated using the hypergeometric distribution test.

RESULTS

Origin and evolution of the femoral lobe in Mantodea

The femoral lobe phenotype is a key morphological trait in the camouflage strategy of praying mantises, however, its origin and diversification across the mantis phylogeny remain poorly understood. To comprehend this process, we analyzed mantis mitogenomes to reconstruct phylogenetic relationships and investigate the evolutionary history of this trait. Initial analysis of base composition across these mitogenomes revealed a uniformly high adenine and thymine (A+T) content, ranging from 70% to 80% across all species (Figure 1A). Subsequently, assessments of



nonsynonymous-to-synonymous substitution rate ratios (d_N/d_S) for the 13 mitochondrial protein-coding genes revealed substantial variation in evolutionary rates among genes: *Atp8* exhibited the fastest evolutionary rate, while *Cox1* was the most conserved (Figure 1B). Additionally, analyses of sequence heterogeneity across different datasets (PCGs+rRNAs and PCG12s+rRNAs) revealed that the PCGs+rRNAs dataset (Supplementary Figure S1A) exhibited higher levels of sequence heterogeneity compared to the PCG12s+rRNAs dataset (Supplementary Figure S1B).

The ML tree and BI tree were inferred using both the PCGs+rRNAs and the PCG12s+rRNAs datasets (Supplementary Figures S2–S5). In both ML and BI topologies, the majority of nodes received strong statistical support, with bootstrap values >90 and Bayesian posterior probabilities >0.9. Nevertheless, topological discrepancies were observed between the BI and ML trees, within the early-diverging lineages of Mantodea, including Haaniidae, Thespidae, Acanthopidae and Gonypetidae. Topological reliability assessments showed that the ML tree of PCGs+rRNAs received significant support across all tests (SH, KH, WKH, WSH, AU) (Supplementary Table S4). We further compared our ML tree with the latest taxonomic references for Mantodea (Ma et al., 2023; Schwarz & Roy, 2019), and found that Haaniidae diverged earlier than its closely related families Acanthopidae and Coptopterygidae (Figure 1C). To address the phylogenetic placement controversy of Haaniidae, we proposed three alternative topological hypotheses (a, b, c) focusing on Haaniidae. The FcML result indicated that the sequences from the PCGs+rRNAs and PCG12s+rRNAs datasets did not show an obvious preference for all of the three topologies (Figure 1D).

We performed ancestral state reconstruction of the femoral lobe phenotype using the best fitting ARD model (Supplementary Table S7). The results revealed that most species within Deroplatyidae, Galinthiidae, Empusidae and Hymenopodidae exhibited protrusion or enlargement of the femoral ventral region, whereas this trait was largely absent in Mantidae (Figure 2). Within Toxoderidae, the femoral lobe phenotype was predominantly observed in the subfamily Toxoderinae and among Acanthopidae, only *Callibia diana* displayed this phenotype. Based on these findings, we speculated that the femoral lobe phenotype originated independently in Toxoderidae, Acanthopidae and Deroplatyidae, respectively. Meanwhile, the femoral lobe phenotype in Galinthiidae, Empusidae and Hymenopodidae was derived from the ancestral trait of Deroplatyidae and was lost in Mantidae species. Additionally, we estimated divergence times by

integrating molecular sequences of extant species with fossil calibration points. The results revealed that mantises originated in the Late Jurassic, with most lineages diversifying during the Cretaceous. Notably, Deroplatyidae, Galinthiadidae and Hymenopoidea (Empusidae + Hymenopodidae) emerged during the middle to late Cretaceous (Figure 2).

Mitochondrial protein-coding genes evolved rapidly in praying mantises possessing the femoral lobe phenotype

Femoral lobes represent a key camouflage phenotype in mantises. Previous studies have demonstrated that this structure facilitates gliding (Zhao et al., 2024), and such locomotor specialization may drive shifts in energy requirements. As the primary energy generating organelles of eukaryotic cells, mitochondria play an indispensable role in mediating energy metabolism, with their protein-coding genes being tightly linked to the efficiency of this physiological process. To explore the potential evolutionary relationships between mitochondrial [protein-coding](#) genes and the camouflage phenotype, we investigated the evolutionary rates of 13 mitochondrial protein-coding genes across mantis lineages. The results revealed a notable trend: mantis species possessing the femoral lobe exhibited accelerated evolutionary rates across 13 mitochondrial protein-coding genes compared to those lacking this trait. Of these, seven genes (*Nd2*, *Cytb*, *Cox2*, *Cox1*, *Nd5*, *Nd6* and *Atp6*) displayed statistically significant rapid evolution (Figure 3A; Supplementary Table S8). Given that foliate pronotum represents another camouflage structure functionally convergent with femoral lobes in mantises, we further found that *Nd2* and *Nd5* also evolved rapidly in species possessing only foliate pronotum (Supplementary Figure S6; Supplementary Table S9). These findings indicate that the complex camouflage structure may have driven relaxed selective constraints on genes related to mitochondrial energy metabolism.

Praying mantises with larger size of femoral lobes exhibited enhanced energy metabolism

The growth of *H. coronatus* nymphs was accompanied by the rounding of the femoral lobes and a disproportionate increase in their area relative to body size (Zhao et al., 2024). We performed WGCNA using leg tissue transcriptome data from *H. coronatus* across three developmental instars (1st instar, 5th instar and adult) to identify gene modules associated with femoral lobe size. Our results revealed a strong positive correlation between the MElightgreen module and femoral lobe size, with a correlation coefficient of 0.88 (Figure 3B). Expression levels of the module genes



showed a gradual up-regulation in leg tissues during ontogeny (Figure 3C). Moreover, functional enrichment analysis revealed that pathways associated with energy metabolism were significantly enriched among the module genes, including the citrate cycle, carbon metabolism, amino acid metabolism, oxidative phosphorylation, fatty acid metabolism and glycolysis/gluconeogenesis (Figure 3C; Supplementary Table S10). Notably, carbohydrate, lipid and amino acid metabolism ultimately converged on the citrate cycle to participate in energy production (Figure 3D). Additionally, in order to exclude the possibility that this difference in energy metabolism is caused by overcoming greater self-weight with advancing instars, we selected two species of similar body size at the same instar: *H. coronatus* (possessing femoral lobe phenotype) and *T. sinensis* (lacking femoral lobe phenotype) for comparative metabolomics analysis. A total of 1,269 metabolites were identified. Repeated correlation analysis and principal component analysis revealed strong within-group reproducibility and clear between-group differences, confirming the high reliability of the acquired data (Supplementary Figures S7A–S7B). The KEGG database was used to annotate the metabolites, revealing their predominant enrichment in pathways related to membrane transport, amino acid metabolism and carbohydrate metabolism (Supplementary Figure S7C). OPLS-DA yielded $R^2Y=1$ and $Q^2Y=0.994$, demonstrating that the established model efficiently discriminated *H. coronatus* and *T. sinensis* based on metabolic profiles, and was suitable for differential metabolite screening (Figure 4A). The results of differential metabolite screening showed that 313 metabolites were significantly up-regulated and 413 metabolites were significantly down-regulated in *H. coronatus* compared with *T. sinensis* (Supplementary Figure S8). Enrichment analysis of all up-regulated metabolites showed that energy metabolism-associated pathways were significantly enriched, including the citrate cycle, central carbon metabolism, the glucagon signaling pathway and amino acid metabolism pathways (Figure 4B; Supplementary Table S11). Among these, organic acids involved in the citrate cycle (e.g., succinic acid, citric acid, malic acid) (Figure 4C) and carbohydrates involved in carbon metabolism (e.g., cellobiose, gluconic acid, lactose) (Figure 4D) were significantly up-regulated in *H. coronatus* compared with *T. sinensis*. The down-regulated metabolites in *H. coronatus* were mainly enriched in ABC transports pathway et al (Supplementary Figure S9). In summary, praying mantises possessing femoral lobes exhibited enhanced energy metabolism.



DISCUSSION

The phylogenetic relationship within the Mantodea

Consistent with previous studies (Ma et al., 2023; Schwarz & Roy, 2019), our phylogenetic results also displayed a polyphyletic trait in Eremiaphilidae, Deroplatyidae and Toxoderidae. This is attributed to the loss of ancestral traits and convergent evolution—two key factors that have led to inaccurate morphological classifications (Svenson & Whiting, 2009). Therefore, many studies integrated evidence from anatomical, neurophysiological and morphological traits, including exoskeleton structure, fossil record, behavioral and individual developmental observations, to reconstruct the phylogenetic relationships of praying mantises (Wieland, 2013; Yager & Svenson, 2008). More recently, Schwarz & Roy (2019) introduced a phylogenetic framework incorporating male genital structure, chromosome analysis, morphology and molecular data, representing the latest classification reference for Mantodea.

In our phylogenetic tree, Haaniidae diverged earlier than the clade comprising Coptopterygidae and Acanthopidae, a pattern inconsistent with the latest taxonomic framework. Notably, our study expanded taxon sampling within Haaniidae to include *Haania vitalisi*, *Arria pallida*, *Sinomiopteryx grahami*, *Caliris sp.* JZ-2017, *Haania hainanensis* and *Caliris melli*. By contrast, the latest taxonomic reference (Schwarz & Roy, 2019) included only *Haania sp.* and *Caliris sp.* as representatives of this family. The FcLM results indicate that the resolution provided by the sequence information of 13 mitochondrial protein-coding genes and two rRNAs is insufficient to provide stable phylogenetic support for Haaniidae. Therefore, there is an urgent need to incorporate additional nuclear gene sequences to elucidate the phylogenetic relationships within Mantodea. Despite these uncertainties, this family occupies a basal phylogenetic position in Mantodea and diverged mainly during the Early Cretaceous, a time when early lineages generally lacked the femoral lobe phenotype. Therefore, the controversial phylogenetic placement of Haaniidae does not compromise our subsequent ancestral state reconstruction and evolutionary rate analyses.

Evolutionary drivers of camouflage phenotype in Mantodea

Mantis originated in the Late Jurassic and most lineages developed in the Cretaceous period (Ma et al., 2023). During this historical period, the bird predators of the mantis flourished (Cooper & Penny, 1997; Luis & Gareth, 2002), while flowering plants underwent adaptive radiation,

gradually asserting dominance among terrestrial plants (De Boer et al., 2012; Ramirez-Barahona et al., 2020; Wu et al., 2024). Consequently, the combined selective pressures from natural predators and the favorable habitats provided by flowering plants may have promoted the evolution of the plant camouflage phenotypes in mantises. A recent study indicated that orchids originated in the Late Cretaceous (Perez-Escobar et al., 2024), a timescale that largely overlaps with the divergence period of *H. coronatus*. This finding may lend support to the hypothesis that the orchid mantis mimics orchids.

The mechanistic link between femoral lobe and energy expenditure in mantises

In this study, mantises with the femoral lobe phenotype exhibited accelerated evolution and enhanced expression activity of energy metabolism-related genes. We therefore discuss in depth the selective pressures that the femoral lobe phenotype may exert on energy metabolism.

First, previous studies have shown that applying a load to the femur or tibia of locusts can increase the moment of inertia of the femur-tibia joint (Matheson & Durr, 2003). In butterflies, despite their relatively low wing mass, changes in moment of inertia during flight strongly influence flight manoeuvres (Lin et al., 2012). Therefore, from the perspective of locomotor biomechanics, the moment of inertia is positively correlated with mass, as described by the formula $I = mr^2$ (where I = moment of inertia, m = mass, and r = the distance from the axis of rotation). Accordingly, in mantises, the additional mass of the rigid femoral lobes may increase moment of inertia during crawling and predation. This suggests that leg muscles may need to generate greater force to drive limb movement, thereby increasing energy expenditure. Consistent with this, metabolomic analyses revealed more active energy metabolic pathways in *H. coronatus* (possessing femoral lobes) than in *T. sinensis* (lacking femoral lobes). Future studies incorporating a broader range of mantis species with and without femoral lobes will be needed to further validate the relationship between this phenotypic trait and energy metabolism.

Second, the non-contractible femoral lobe impedes locomotion in complex microhabitats. When moving through dense vegetation, the inflexible structure easily colliding with surrounding objects (e.g., leaf edges, small branches) and thus increase energy consumption. Meanwhile, studies on *Anagyrus pseudococci* show that air resistance reduces the jumping distance of this insect by 49%, and the magnitude of air resistance acting on an insect is influenced by its mass to area ratio



(Urca & Ribak, 2018). According to the aerodynamic formula $F = \frac{C\rho SV^2}{2}$ (where C =drag coefficient, ρ =air density, S =windward area, V =velocity), air resistance is positively correlated with the frontal area. Compared with lineages without femoral lobes, the presence of femoral lobes increases the body surface area of mantises, which may require overcoming greater air resistance during jumping and flighting, and thus increase energy consumption.

In addition to the direct biomechanical constraints on locomotion, the development and maintenance of the tissues and organs also require additional energy input (Mueller et al., 2015). The femoral lobe, as an extension of the chitinous exoskeleton, requires the synthesis and deposition of additional chitin and cuticular proteins during development. Therefore, energy investment may be increasing in development of femoral lobes. Furthermore, mitochondrial encoded genes often show pronounced adaptive evolution associated with distinct life strategies. For instance, mitochondrial genes in Pteromalidae and Eulophidae wasps exhibit adaptive evolution to high altitude, low temperature and hypoxic environments (Kang & Hu, 2023). In Tephritidae fruit flies, the NADH dehydrogenase gene family evolves rapidly during adaptation to diverse hosts and environmental shifts (Medina et al., 2025). Both the femoral lobes and foliate pronotum serve as key camouflage structures in mantises, and the mitochondrial protein-coding genes show signals of accelerated evolution, suggesting a potential coevolutionary relationship between complex camouflage strategies and energy metabolism.

In summary, while femoral lobes enhance camouflage in mantises, they may require overcoming increased moment of inertia, environmental resistance and developmental costs. This drives active responses in energy metabolism related genes, including relaxed selection and elevated expression. Mantid lineages unable to sustain such energy requirements tend to reduce or lose femoral lobes, instead employing alternative camouflage strategies such as background matching. This suggests an evolutionary trade-off between camouflage and energy metabolism (Figure 5). Given the limited ecological data on mantises, future studies should account for confounding factors (such as body size, life history and locomotor mode) to better clarify the evolutionary correlation between mitochondrial protein-coding genes and camouflage phenotypes.

CONCLUSION



437 This study reveals that the femoral lobe, a key camouflage structure in mantises, originated
438 approximately in the mid-Cretaceous, and its evolution maybe driven by predatory birds and
439 flowering plants. Meanwhile, lineages possessing this camouflage phenotype exhibit accelerated
440 evolutionary rates and elevated expression activity in energy metabolism related genes. Our findings
441 provide novel insights into the evolution of complex camouflage structures in animals is also
442 accompanied by the co-evolution of energy metabolism. Based on these findings, we attempt to
443 propose an evolutionary trade-off hypothesis between mantis camouflage and energy metabolism.
444 Future studies may employ biomechanical modeling to quantify the effects of femoral lobe
445 morphology and size on leg motion and energy consumption. Meanwhile, further molecular
446 analyses can then identify the key genes and pathways underlying this trade-off, clarifying the
447 molecular basis of balance in development and evolution.



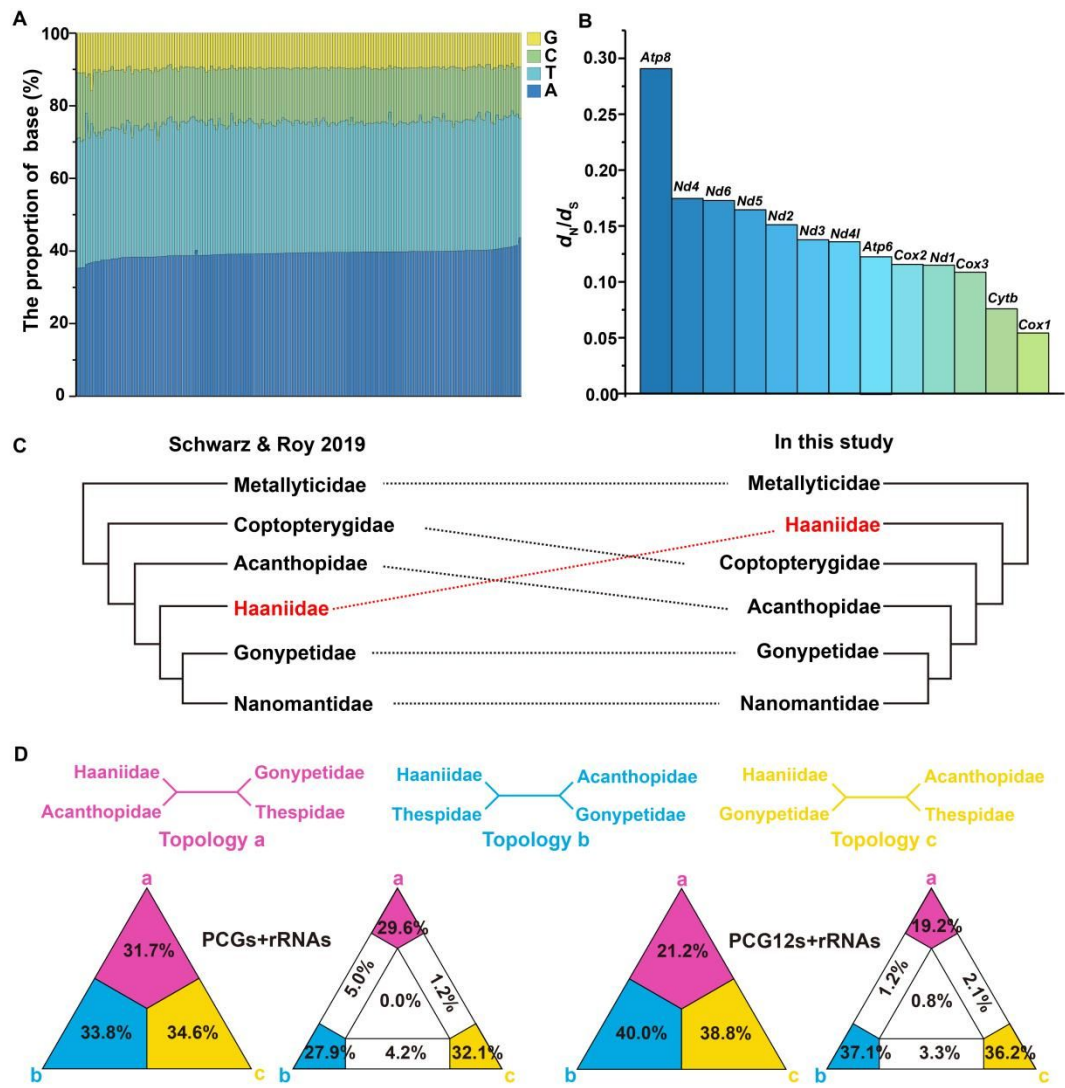


Figure 1 The mitogenome and phylogeny of mantises

A: Mitochondrial genome A+T base content. Bases A, T, C and G were distinguished by dark blue, light blue, green, yellow. The ordinate represented the proportion of bases in the whole genome. B: d_N/d_S comparison of each mitochondrial protein-coding gene among 175 individuals of Mantodea. The 13 mitochondrial protein-coding genes were distinguished by different colors, and the ordinate represented non-synonymous mutations/synonymous mutations. C: Comparison of phylogenetic relationships among early-diverging mantis lineages in light of the latest classification framework (Schwarz & Roy, 2019). The inconsistency in the phylogenetic position of Haaniidae is highlighted in red. D: FcLM analysis of tree topology. The support ratios of the three topological hypotheses (a, b, and c) for Haaniidae were estimated by FcLM. Topologies a, b and c were distinguished by red, blue and yellow, respectively.



463

464

465

466

467

468 were shown at each node. The timescale (Ma) was indicated at the bottom, with the Quaternary (Q),
469 Neogene (N), Paleogene (Pg), Cretaceous, Jurassic and Triassic periods shaded in different colors.
470 The red arrow marked the divergent nodes of the Deroplatyidae, Galinthiidae, Hymenopoda
471 (Hymenopodidae and Empusidae) and Mantoidea. Families with non-monophyletic characteristics
472 were highlighted in red.
473

uncorrected proof



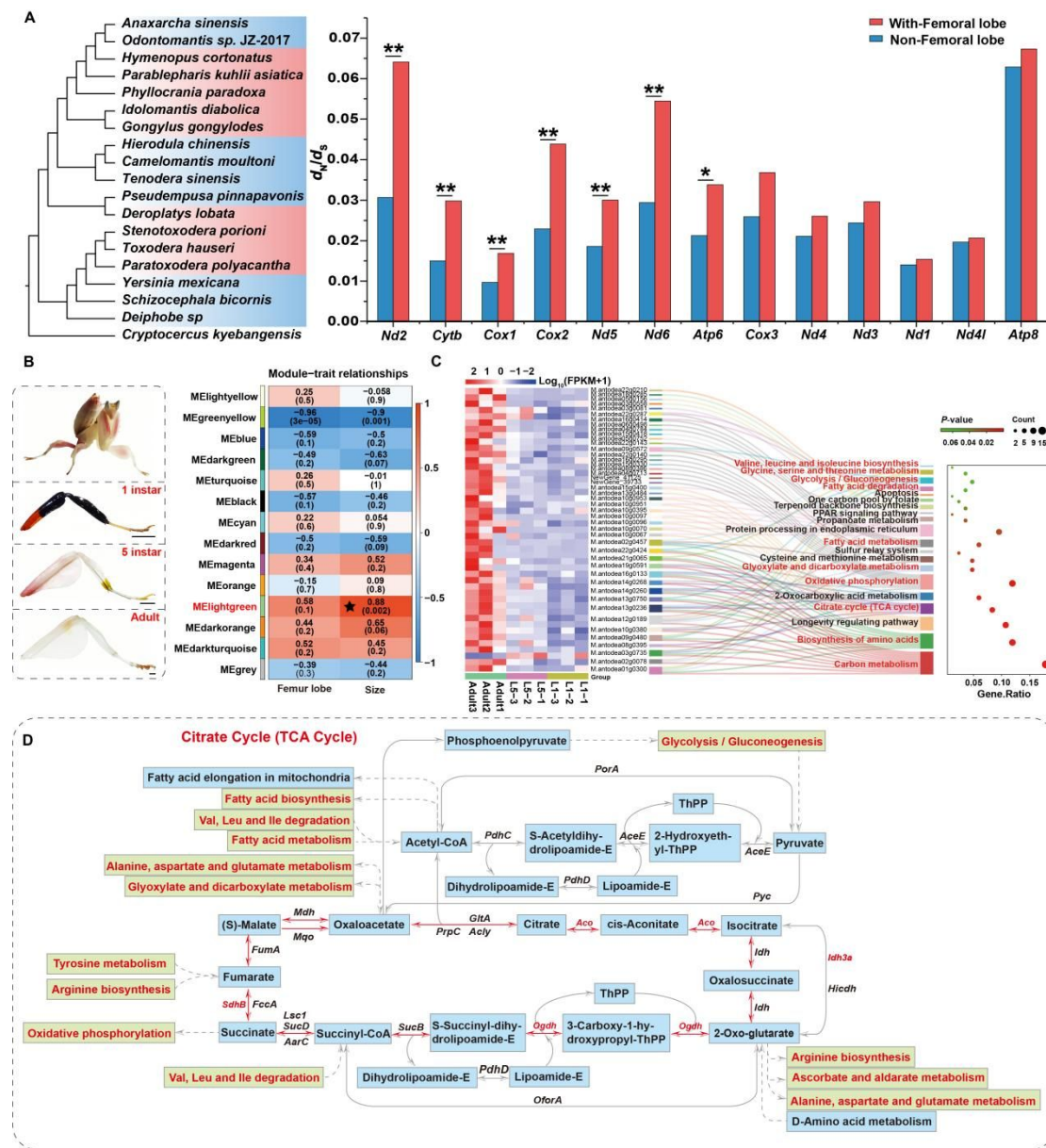


Figure 3 Genomic and transcriptomic insights into the relationship between the femoral lobe and energy metabolism in mantises

A: Rapid evolutionary analysis of 13 mitochondrial protein-coding genes in mantises. The abscissa showed 13 mitochondrial protein-coding gene, and the ordinate represented the evolutionary rate (non-synonymous mutation rate/synonymous mutation rate). Mantises with and without femoral lobes were distinguished in red and blue, respectively. Statistical significance was assessed using the chi-square test. **: a likelihood ratio test statistic ($2\Delta\ln L > 6.635$), *: $2\Delta\ln L > 3.841$. **B:** Correlation analysis between femoral lobe size and gene expression in the leg of *H. coronatus* across developmental instars. Three developmental instars (1st-instar nymph, 5th-instar nymph and adult) were sampled with three biological replicates per group. The abscissa represented two phenotypic

variables: the presence/absence of the femoral lobe and the relative size of the femoral lobe. The coordinates represented the different gene modules, which were colored differently. In each grid cell, the top value showed the Pearson correlation coefficient of traits and gene modules, and the value in parentheses below indicated the corresponding *t*-test *P*-value. Correlations with *P*-value<0.05 were considered statistically significant. Red and blue indicated distinguish positive and negative correlations, respectively. C: Enrichment analysis and expression heatmap of the MELightgreen module. The heatmap showed gene expression profiles of the MELightgreen module in leg tissues of *H. coronatus* at three developmental stages: the 1st-instar nymph (1L), 5th-instar nymph (5L) and adult. All FPKM values were normalized using $\log_{10}(\text{FPKM}+1)$ transformation. Red and blue represented high and low expression levels, respectively. The Sankey diagram displayed the functional enrichment of genes in the MELightgreen module; circle size indicated gene number, and red and blue corresponded to higher and lower *P*-values, respectively. D: The tricarboxylic acid cycle pathway. Enriched pathways and up-regulated genes in the MELightgreen module were labeled in red.



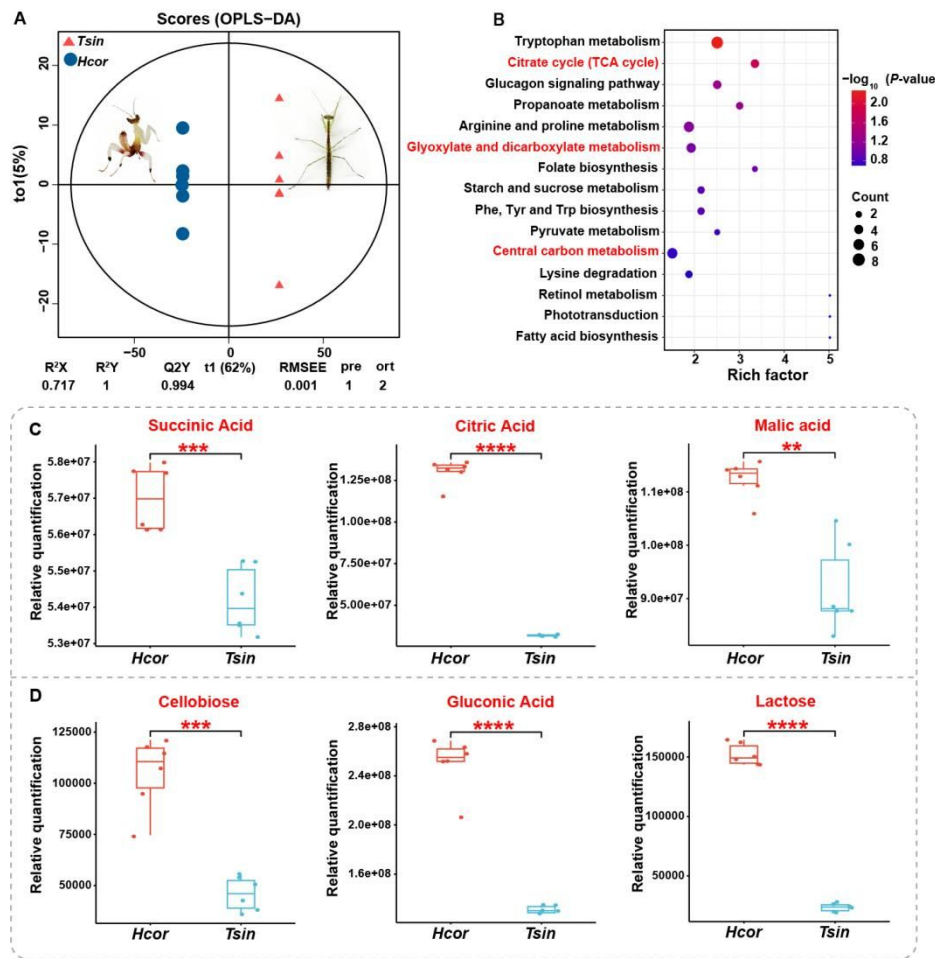


Figure 4 Cross-species metabolomics comparison revealed the relationship between femoral lobe and energy metabolism in *H. coronatus* and *T. sinensis*

A: OPLS-DA metabolome analysis of *H. coronatus* and *T. sinensis* at young nymphs of the same instar. Six biological replicates were set for each group. The red triangle represented *T. sinensis*, and the blue circle represented *H. coronatus*. The abscissa represented the difference between groups, and the ordinates represented within-group differences. B: Enrichment analysis of up-regulated metabolites in *H. coronatus* compared with *T. sinensis*. The size of the circle indicated the number of metabolites, and the red and blue indicated the low and high values of the *P*-value, respectively. C: The comparative analysis of relative abundances of tricarboxylic acid cycle organic acids between *H. coronatus* and *T. sinensis*. D: The comparative analysis of the relative abundances of carbohydrates involved in carbon metabolism between *H. coronatus* and *T. sinensis*. The abscissa was two species groups (*Hcor*, *H. coronatus*; *Tsin*, *T. sinensis*.), and the ordinate was the relative quantification of metabolites. The *P*-value was obtained by a two-sided student *t*-test and **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$.

uncorrected proof



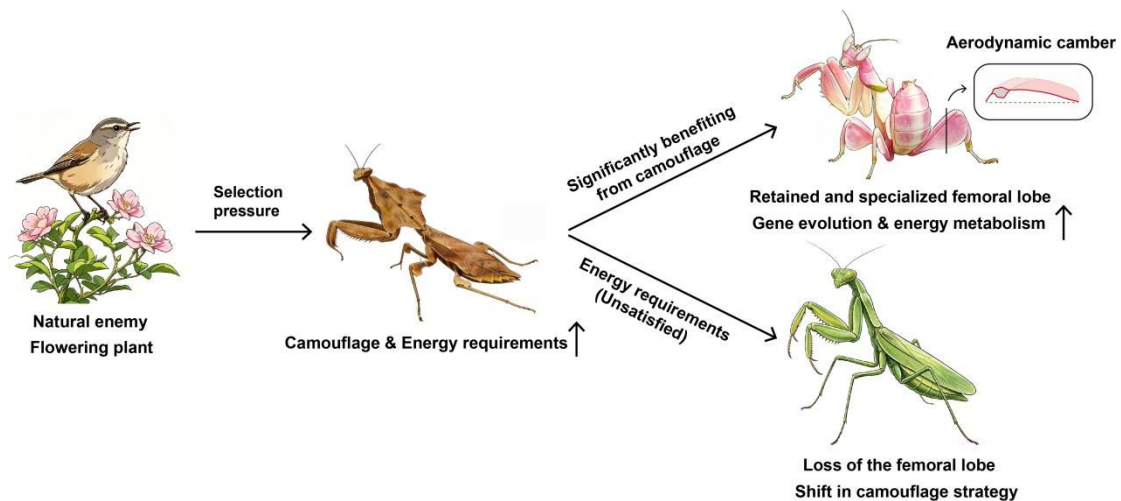


Figure 5 Trade-off between camouflage phenotype and energy requirements

The femoral lobe phenotype appeared in the mantis species with mimetic dead leaves during the mid-Cretaceous. At this time, the prosperity of natural enemies (birds) and angiosperms provided selection pressure for femoral lobe evolution. However, the femoral lobe could not be retracted, which increased the camouflage effect and also increased the energy consumption during locomotion. In subsequent evolution, mantis lineages that derived significant benefits from camouflage—such as those in the families Hymenopodidae and Empusidae—continued to retain and further elaborate the femoral lobe phenotype. Meanwhile, these lineages actively matched the accompanying increase in energy requirements, via optimization of the aerodynamic camber of the lobe structure, accelerated evolution of mitochondrial protein-coding genes, and elevated energy metabolism at the expression level. In contrast, most species within Mantoidea that could not cope with the elevated energy requirements evolved smaller femoral lobes or even lost them entirely, instead adopting survival strategies based on background matching.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

F.W.W. conceived the project, participated in result discussion, supervised the research and secured research funding; G.P.H. collected samples, discussed experimental results, revised and edited the manuscript; C.X.L. and C.W. accomplished mantis species identification; L.Y.S. performed sample collection, data analysis and figure visualization, drafted the original manuscript as well as revised and edited the paper. All authors read and approved the final version of the manuscript.

ACKNOWLEDGEMENTS

This study is supported by the Major Program of the Natural Science Foundation of Jiangxi Province of China (20233ACB209001, 20242BAB24004), and Jiangxi Provincial Key Laboratory of Conservation Biology (2023SSY02081). We thank Xin Huang for providing the suggestions and scripts.

DATA AVAILABILITY

Genome resequencing data of 46 mantis individuals reported in this study were deposited in the Genome Sequence Archive (GSA) at National Genomics Data Center (NGDC), China National Center for Bioinformation/Beijing Institute of Genomics and Chinese Academy of Sciences (accession number CRA035085), Science Data Bank (DOI: <https://doi.org/10.57760/sciencedb.39314>), and National Center for Biotechnology Information (BioProjectID PRJNA1475783). Transcriptomic data of *H. coronatus* leg tissue and NGS data for mitochondrial genome assembly of *H. coronatus* and *Deroplatys lobata* were obtained from the GSA of the National Genomics Data Center under accession number CRA010804.

REFERENCES

- Bernt M, Donath A, Jühling F, et al. 2013. MITOS: Improved metazoan mitochondrial genome annotation. *Molecular Phylogenetics and Evolution*, **69**(2): 313–319.
- Briolat ES, Arenas LM, Hughes AE, et al. 2021. Generalist camouflage can be more successful than microhabitat specialisation in natural environments. *BMC Ecology and Evolution*, **21**(1): 151.
- Casanova A, Wevers A, Navarro-Ledesma S, et al. 2023. Mitochondria: It is all about energy. *Frontiers in Physiology*, **14**: 1114231.
- Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, **17**(4): 540–552.
- Cooper A, Penny D. 1997. Mass survival of birds across the Cretaceous-Tertiary boundary: molecular evidence. *Science*, **275**(5303): 1109–1113.
- D'souza AR, Minczuk M. 2018. Mitochondrial transcription and translation: overview. *Essays in Biochemistry*, **62**(3): 309–320.
- Da Fonseca RR, Johnson WE, O'Brien SJ, et al. 2008. The adaptive evolution of the mammalian mitochondrial genome. *BMC Genomics*, **9**: 119.
- De Boer HJ, Eppinga MB, Wassen MJ, et al. 2012. A critical transition in leaf evolution facilitated the Cretaceous angiosperm revolution. *Nature Communications*, **3**: 1221.
- Deitz LL, Nalepa C, Klass KD. 2003. Phylogeny of the Dictyoptera re-examined (Insecta). *Entomologische Abhandlungen (Dresden)*, **61**(1): 69–91.
- Dos Reis M, Zhu T, Yang Z. 2014. The impact of the rate prior on bayesian estimation of divergence times with multiple loci. *Systematic Biology*, **63**(4): 555–565.
- Duarte RC, Stevens M, Flores AaV. 2018. The adaptive value of camouflage and colour change in a polymorphic prawn. *Scientific Reports*, **8**: 16028.
- Gibson RS. 2005. Principles of Nutritional Assessment. 2nd Edition. Oxford University Press Inc.
- Hahn C, Bachmann L, Chevreaux B. 2013. Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads—a baiting and iterative mapping approach. *Nucleic Acids Research*, **41**(13): e129.
- Hoang DT, Chernomor O, Von Haeseler A, et al. 2018. UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution*, **35**(2): 518–522.
- Huang G, Zhang Y, Zhang W, et al. 2024. Genetic mechanisms of animal camouflage: an interdisciplinary perspective. *Trends in Genetics*, **40**(7): 613–620.
- Huang GP, Song LY, Du X, et al. 2023. Evolutionary genomics of camouflage innovation in the orchid mantis. *Nature Communications*, **14**(1): 4821.
- Jakovlic I, Zou H, Ye T, et al. 2023. Mitogenomic evolutionary rates in bilateria are influenced by parasitic lifestyle and locomotory capacity. *Nature Communications*, **14**(1): 6307.
- Jin JJ, Yu WB, Yang JB, et al. 2020. GetOrganelle: A fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biology*, **21**(1): 241.
- Kang N, Hu H. 2023. Adaptive evidence of mitochondrial genes in Pteromalidae and Eulophidae (Hymenoptera: Chalcidoidea). *PLoS One*, **18**(11): e0294687.
- Katoh K, Misawa K, Kuma K, et al. 2002. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, **30**(14): 3059–3066.
- Kearse M, Moir R, Wilson A, et al. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**(12): 1647–1649.



- Kishino H, Hasegawa M. 1989. Evaluation of the maximum-likelihood estimate of the evolutionary tree topologies from DNA-sequence data, and the branching order in hominoidea. *Journal of Molecular Evolution*, **29**(2): 170–179.
- Kishino H, Miyata T, Hasegawa M. 1990. Maximum-likelihood inference of protein phylogeny and the origin of chloroplasts. *Journal of Molecular Evolution*, **31**(2): 151–160.
- Klass KD, Meier R. 2006. A phylogenetic analysis of Dictyoptera (Insecta) based on morphological characters. *Entomologische Abhandlungen* (Dresden), **63**(1–2): 3–50.
- Kuck P, Meid SA, Gross C, et al. 2014. AliGROOVE--visualization of heterogeneous sequence divergence within multiple sequence alignments and detection of inflated branch support. *BMC Bioinformatics*, **15**(1): 294.
- Kumar S, Stecher G, Suleski M, et al. 2017. Timetree: A resource for timelines, timetrees, and divergence times. *Molecular Biology and Evolution*, **34**(7): 1812–1819.
- Kummer E, Ban N. 2021. Mechanisms and regulation of protein synthesis in mitochondria. *Nature Reviews Molecular Cell Biology*, **22**(5): 307–325.
- Lanfear R, Frandsen PB, Wright AM, et al. 2017. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, **34**(3): 772–773.
- Langfelder P, Horvath S. 2008. WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*, **9**: 559.
- Li F, Lv Y, Wen Z, et al. 2021. The complete mitochondrial genome of the intertidal spider (*Desis jiaxiangi*) provides novel insights into the adaptive evolution of the mitogenome and the evolution of spiders. *BMC Ecology and Evolution*, **21**(1): 72.
- Librado P, Rozas J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**(11): 1451–1452.
- Lin T, Zheng L, Hedrick T, et al. 2012. The significance of moment-of-inertia variation in flight manoeuvres of butterflies. *Bioinspiration & Biomimetics*, **7**(4): 044002.
- Lombardo F. 1995. A review of the genus *Popa* Stål 1856 (Insecta Mantodea). *Tropical Zoology*, **8**(2): 257–267.
- Luis MC, Gareth JD. 2002. The Mesozoic radiation of birds. *Annual Review of Ecology Evolution and Systematics*, **33**: 91–124.
- Ma Y, Zhang LP, Lin YJ, et al. 2023. Phylogenetic relationships and divergence dating of Mantodea using mitochondrial phylogenomics. *Systematic Entomology*, **48**(4): 644–657.
- Matheson T, Durr V. 2003. Load compensation in targeted limb movements of an insect. *The Journal of Experimental Biology*, **206**(Pt 18): 3175–3186.
- Medina NS, Moreno-Carmona M, Canal NA, et al. 2025. Mitochondrial genome variations and possible adaptive implications in some tephritid flies (Diptera, Tephritidae). *International Journal of Molecular Sciences*, **26**(12).
- Mitterboeck TF, Adamowicz SJ. 2013. Flight loss linked to faster molecular evolution in insects. *Proceedings of the Royal Society B: Biological Sciences*, **280**(1767): 20131128.
- Mizuno T, Yamaguchi S, Yamamoto I, et al. 2014. Double-trick" visual and chemical mimicry by the juvenile orchid mantis *Hymenopus coronatus* used in predation of the oriental honeybee *Apis cerana*. *Zoological Science*, **31**(12): 795–801.
- Mueller CA, Eme J, Manzon RG, et al. 2015. Embryonic critical windows: Changes in incubation temperature alter survival, hatchling phenotype, and cost of development in lake whitefish (*Coregonus*



clupeaformis). *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, **185**(3): 315–331.

Nguyen LT, Schmidt HA, Von Haeseler A, et al. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, **32**(1): 268–274.

Nolfi-Donagan D, Braganza A, Shiva S. 2020. Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement. *Redox Biology*, **37**: 101674.

O'hanlon JC. 2016. Orchid mantis. *Current Biology*, **26**(4): 145–146.

O'hanlon JC, Holwell GI, Herberstein ME. 2014. Pollinator deception in the orchid mantis. *American Naturalist*, **183**(1): 126–132.

Perez-Escobar OA, Bogarin D, Przelomska NaS, et al. 2024. The origin and speciation of orchids. *New Phytologist*, **242**(2): 700–716.

Rambaut A, Drummond AJ, Xie D, et al. 2018. Posterior summarization in bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, **67**(5): 901–904.

Ramirez-Barahona S, Sauquet H, Magallon S. 2020. The delayed and geographically heterogeneous diversification of flowering plant families. *Nature Ecology & Evolution*, **4**(9): 1232–1238.

Ranwez V, Harispe S, Delsuc F, et al. 2011. MACSE: Multiple alignment of coding sequences accounting for frameshifts and stop codons. *PLoS One*, **6**(9): e22594.

Revell LJ. 2012. phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, **3**(2): 217–223.

Ronquist F, Teslenko M, Van Der Mark P, et al. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**(3): 539–542.

Schwarz CJ, Roy R. 2019. The systematics of Mantodea revisited: an updated classification incorporating multiple data sources (Insecta: Dictyoptera). *Annales De La Societe Entomologique De France*, **55**(2): 101–196.

Shen YY, Liang L, Zhu ZH, et al. 2010. Adaptive evolution of energy metabolism genes and the origin of flight in bats. *Proceedings of the National Academy of Sciences of the United States of America*, **107**(19): 8666–8671.

Shen YY, Shi P, Sun YB, et al. 2009. Relaxation of selective constraints on avian mitochondrial DNA following the degeneration of flight ability. *Genome Research*, **19**(10): 1760–1765.

Shimodaira H. 2002. An approximately unbiased test of phylogenetic tree selection. *Systematic Biology*, **51**(3): 492–508.

Shimodaira H, Hasegawa M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution*, **16**(8): 1114–1116.

Skelhorn J, Rowland HM, Speed MP, et al. 2010. Masquerade: camouflage without crypsis. *Science*, **327**(5961): 51–51.

Stamatakis A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**(9): 1312–1313.

Stevens M, Merilaita S. 2009. Animal camouflage: Current issues and new perspectives. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **364**(1516): 423–427.

Stoddard MC, Kupan K, Eyster HN, et al. 2016. Camouflage and clutch survival in plovers and terns. *Scientific Reports*, **6**: 32059.



- Strimmer K, Von Haeseler A. 1997. Likelihood-mapping: A simple method to visualize phylogenetic content of a sequence alignment. *Proceedings of the National Academy of Sciences of the United States of America*, **94**(13): 6815–6819.
- Sun JT, Jin PY, Hoffmann AA, et al. 2018. Evolutionary divergence of mitochondrial genomes in two Tetranychus species distributed across different climates. *Insect Molecular Biology*, **27**(6): 698–709.
- Svenson GJ, Whiting MF. 2004. Phylogeny of Mantodea based on molecular data: evolution of a charismatic predator. *Systematic Entomology*, **29**(3): 359–370.
- Svenson GJ, Whiting MF. 2009. Reconstructing the origins of praying mantises (Dictyoptera, Mantodea): the roles of Gondwanan vicariance and morphological convergence. *Cladistics*, **25**(5): 468–514.
- Taanman JW. 1999. The mitochondrial genome: structure, transcription, translation and replication. *Biochimica et Biophysica Acta*, **1410**(2): 103–123.
- Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, **38**(7): 3022–3027.
- Thornthan U, Yudthana S, Weeyawat J. 2019. New records of praying mantis (Mantodea) from Thailand. *Far Eastern Entomologist*, **395**: 23–32.
- Urca T & Ribak G. 2018. The effect of air resistance on the jump performance of a small parasitoid wasp, Anagyrus pseudococci (Encyrtidae). *Journal of Experimental Biology*, **221**(Pt 7).
- Viana JVD, Duarte RC, Vieira C, et al. 2023. Crypsis by background matching and disruptive coloration as drivers of substrate occupation in sympatric Amazonian bark praying mantises. *Scientific Reports*, **13**(1): 19985.
- Wang J, Feng J, Xu L, et al. 2019. Ionic liquid-based salt-induced liquid-liquid extraction of polyphenols and anthraquinones in Polygonum cuspidatum. *Journal of Pharmaceutical and Biomedical Analysis*, **163**: 95–104.
- Wieland F. 2013. The phylogenetic system of mantodea (Insecta: Dictyoptera). Universitätsverlag Göttingen.
- Wu S, Rheindt FE, Zhang J, et al. 2024. Genomes, fossils, and the concurrent rise of modern birds and flowering plants in the Late Cretaceous. *Proceedings of the National Academy of Sciences of the United States of America*, **121**(8): e2319696121.
- Yager DD, Svenson GJ. 2008. Patterns of praying mantis auditory system evolution based on morphological, molecular, neurophysiological and behavioural data. *Biological Journal of the Linnean Society*, **94**(3): 541–568.
- Yang Z. 2007. PAML 4: Phylogenetic analysis by maximum likelihood. *Molecular Biology and Evolution*, **24**(8): 1586–1591.
- Zhang J, Shu L, Peng Z. 2024. Adaptive evolution of mitochondrial genomes in Triplophysa cavefishes. *Gene*, **893**: 147947.
- Zhang QL, Yang XZ, Zhang L, et al. 2019. Adaptive evidence of mitochondrial genomes in Dolycoris baccarum (Hemiptera: Pentatomidae) to divergent altitude environments. *Mitochondrial DNA A DNA Mapp Seq Anal*, **30**(1): 9–15.
- Zhao X, Liu JX, Charles Dominique T, et al. 2024. Petal-shaped femoral lobes facilitate gliding in orchid mantises. *Current Biology*, **34**(1): 183–189.



730 **题目：**增益与损耗：螳螂植物伪装的能量权衡

731 宋凌云¹，刘春香²，吴超²，黄广平¹，魏辅文^{1,2,3}

732 ¹ 江西农业大学林学院，江西省保护生物学重点实验室，中国南昌

733 ² 中国科学院动物研究所，北京 100101，中国

734 ³ 中国科学院大学，北京 100049，中国

735 **摘要**

736 股叶是螳螂重要的适应性形态伪装结构，可帮助其规避天敌、提升生存适合度。为探明螳螂股叶性状的演
737 化规律，本研究基于涵盖 20 科 93 属的螳螂线粒体基因组数据，开展系统发育重建、祖先性状溯源与物种
738 分化时间推算。结果显示，股叶表型最早出现于白垩纪中期模拟枯叶的螳螂中，此时，天敌鸟类兴起、被
739 子植物辐射演化可能为股叶起源与演化提供了选择压力及条件。后续演化进程中，该性状在姬螳科
740 （*Galinthiidae*）、花螳科（*Hymenopodidae*）和锥螳科（*Empusidae*）中被保留并不断分化，却在螳科
741 （*Mantidae*）中发生丢失。分子进化速率分析表明，7 个线粒体蛋白编码基因（*Nd2*、*Nd5*、*Nd6*、*Atp6*、
742 *Cox2*、*Cox1*、*Cytb*）在具股叶的螳螂支系中发生加速进化。转录组和代谢组的结果进一步佐证，拥有股叶
743 或股叶相对面积越大的螳螂个体表现出能量代谢能力更强。据此我们推断：伪装收益突出的螳螂支系选择
744 保留并特化股叶表型，通过优化叶状形态、驱动线粒体编码基因加速演化、上调能量代谢水平，代偿伪装
745 带来的额外能耗；而难以维系高额能量消耗的螳螂类群，则逐步退化股叶、甚至彻底丢失该性状，转而采
746 用基于背景匹配的生存策略。综上所述，螳螂复杂的植物伪装结构的演化与机体能量代谢间可能存在进化
747 权衡关系。

748 **关键词：**螳螂目；植物伪装；起源与演化；能量代谢

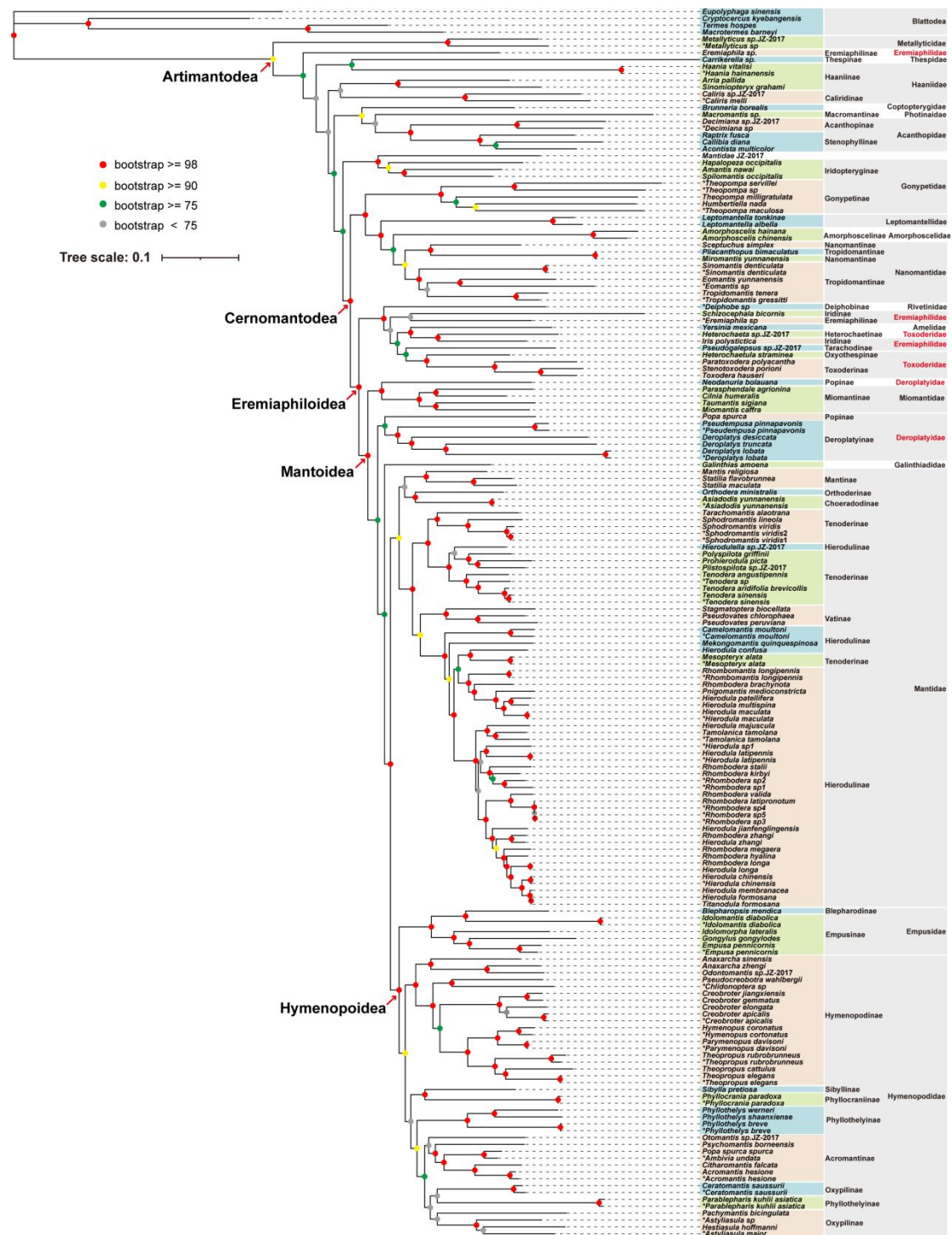
749



753

754

755



Supplementary Figure S2 Maximum likelihood phylogeny of Mantodea

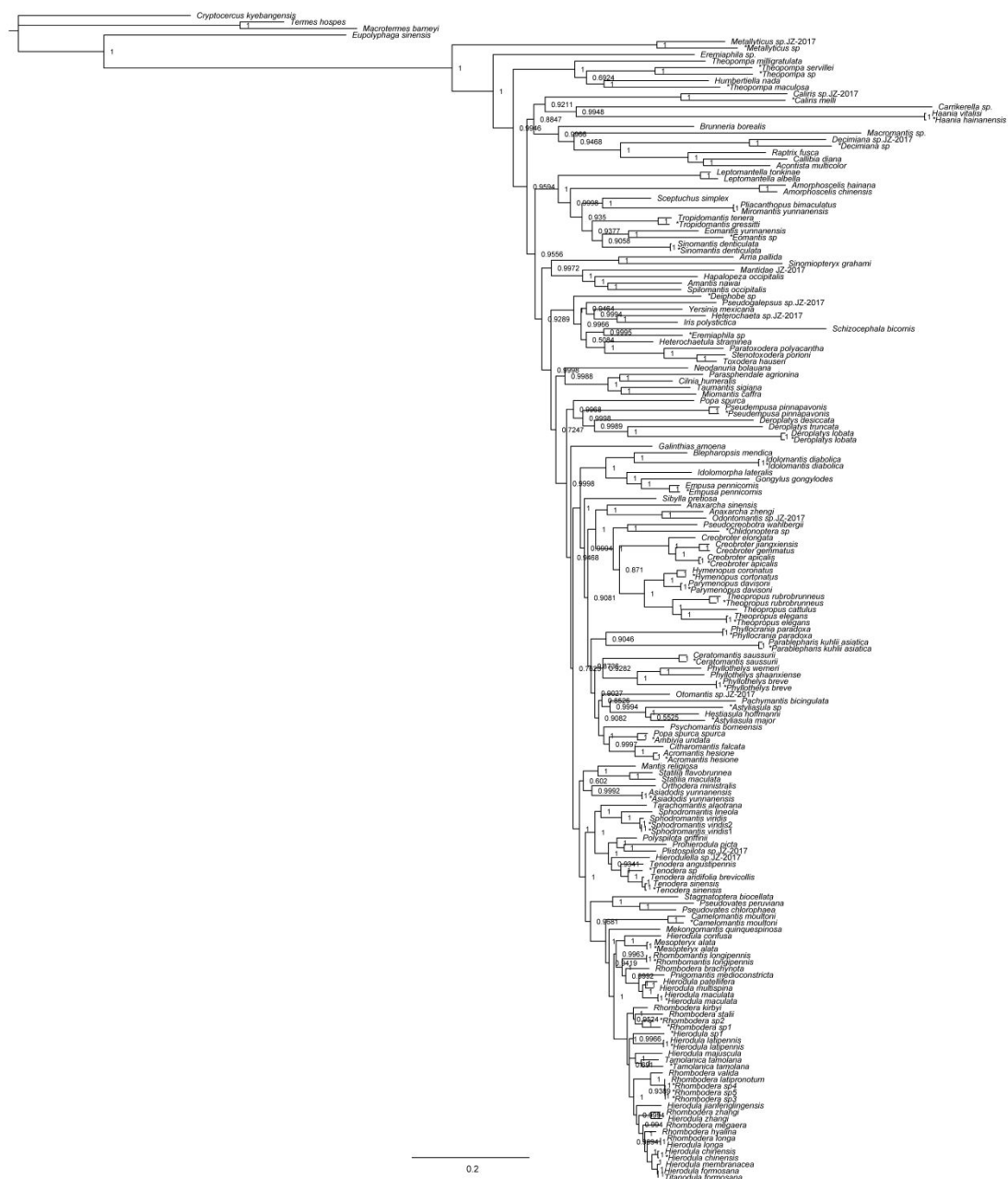
ML tree based on the PCGs+rRNAs dataset. The families of the non-monophyletic group were marked in red. Bootstraps were distinguished by dots of different colors: red, ≥ 98 ; yellow, ≥ 90 ; green, ≥ 75 ; gray, < 75 .



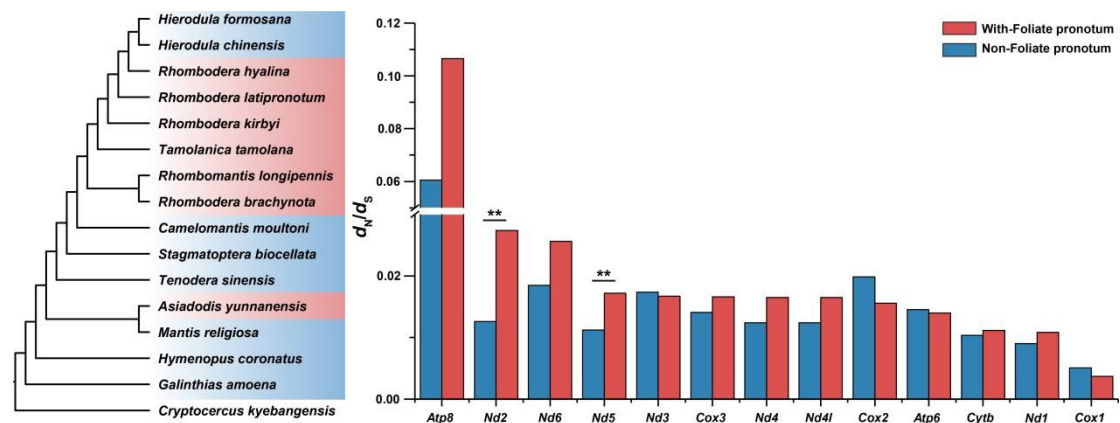
Supplementary Figure S3 Maximum likelihood phylogeny of Mantodea inferred from the PCG12s+RNAs dataset



Supplementary Figure S4 Bayesian phylogeny of Mantodea inferred from the PCGs+RNAs dataset

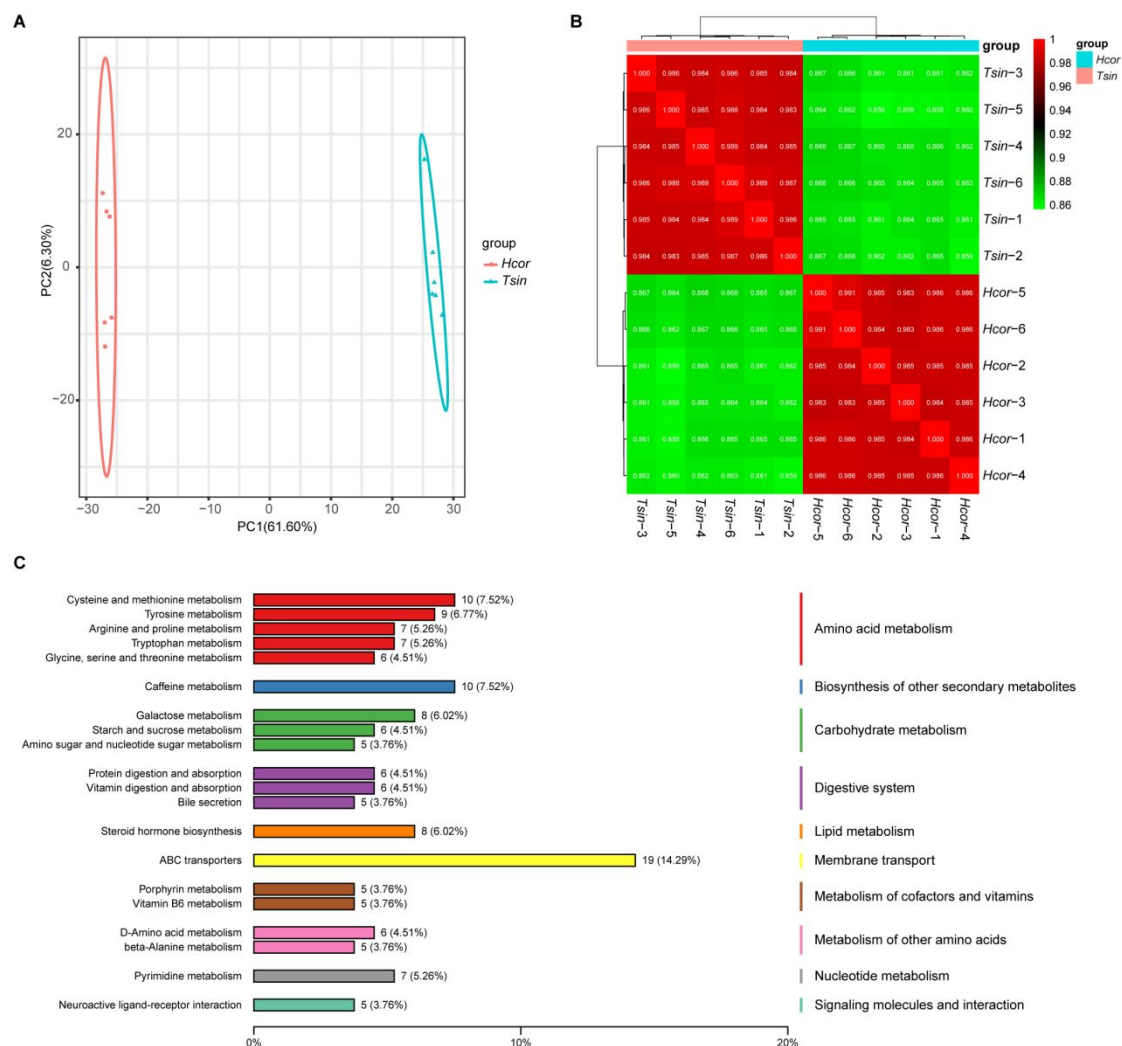


Supplementary Figure S5 Bayesian phylogeny of Mantodea inferred from the PCG12s+RNAs dataset



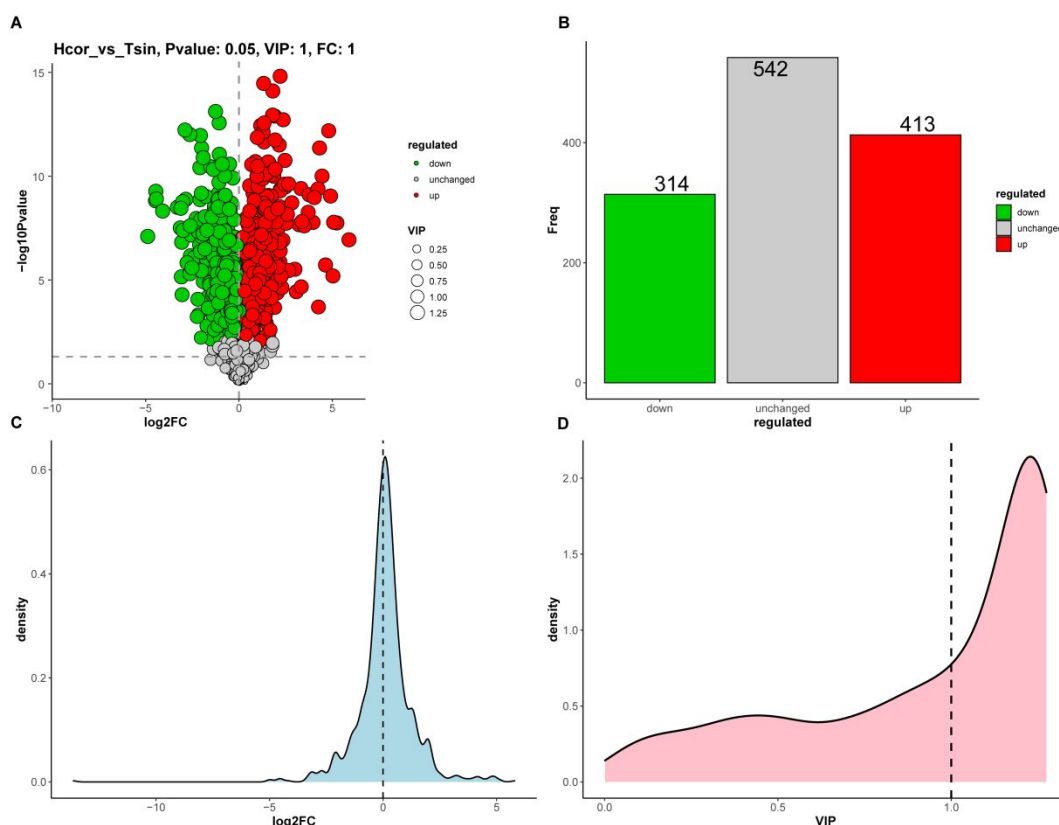
Supplementary Figure S6 Rapid evolutionary analysis of 13 mitochondrial protein-coding genes in mantis with foliate pronotum

The abscissa was 13 mitochondrial protein coding gene, and the ordinate was the evolutionary rate (non-synonymous mutation rate/synonymous mutation rate). Mantises with and without foliate pronotum were distinguished by red and blue, respectively. Statistical significance was assessed using the chi-square test, **: $2\Delta\ln L > 6.635$.



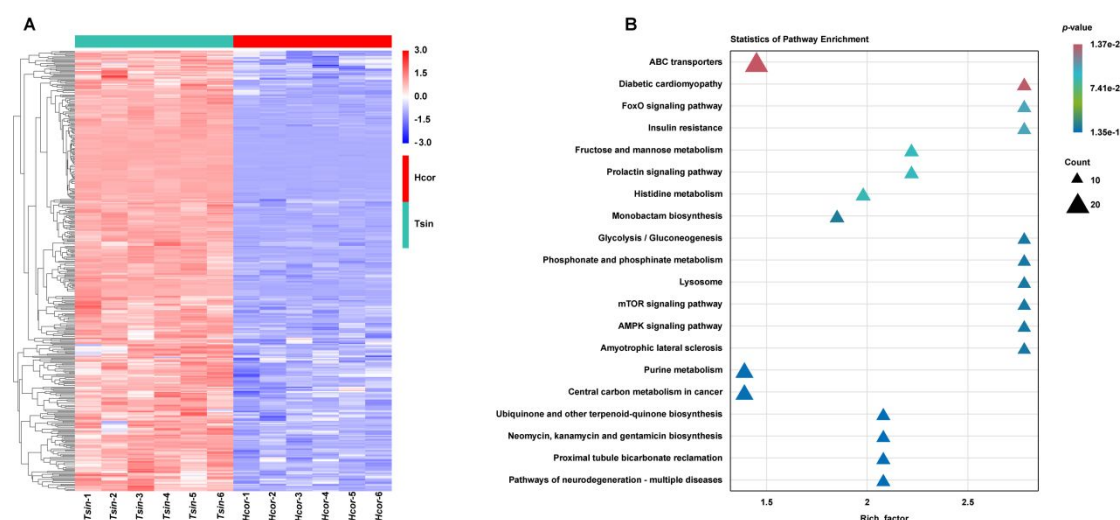
Supplementary Figure S7 Metabolomics data quality assessment and annotation

A: Principal component analysis of *H. coronatus* (*Hcor*) and *T. sinensis* (*Tsin*), $n=6$. The X-axis represented the first principal component and the Y-axis represented the second principal component, with the percentages on the axes indicating the contribution of each principal component to the observed sample variation. Each point in the plot represented an individual sample; *Hcor* was shown in red and *Tsin* in cyan. B: Correlation analysis and evaluation between samples. The horizontal and vertical axes represented sample names, and the color intensity indicated the magnitude of the correlation coefficient r . Samples were grouped as *Hcor* (red) and *Tsin* (cyan). C: The metabolites were annotated using the KEGG database. The entries under the same box in the figure represented the hierarchical classification annotation of the KEGG pathway. The column length represented the count and relative proportion of metabolites annotated to each pathway.



Supplementary Figure S8 Statistics of differential metabolites of *H. coronatus* and *T. sinensis*

A: Volcanic diagram of differential metabolites between *H. coronatus* and *T. sinensis*. Significantly up-regulated and down-regulated metabolites were shown in red and green, respectively and metabolites with no significant change were shown in gray. Differential metabolite screening conditions: differential screening fold change $\log_2FC > 1$, *t*-test *P*-value < 0.05 , variable projection importance $VIP > 1$. B: Statistics of the number of differential metabolites. Density distributions of metabolites for C: $\log_2FC$ and D: VIP .



Supplementary Figure S9 Significantly down-regulated metabolites pathway enrichment in *H. coronatus* compared to *T. sinensis*

A: Heatmap of significantly downregulated metabolites in *H. coronatus* compared with *T. sinensis*.
 B: The size of the triangle indicated the number of metabolites and the red and blue indicated the low and high values of the *P*-value, respectively.

Supplementary Table S1 175 praying mantis and outgroup species basic information

Supplementary Table S2 The best partitioning scheme and model were selected by PartitionFinder for phylogenetic analyses based on the PCGs+rRNAs dataset

Matrix	Partitions	Models	Genes
PCGs+rRNAs	P1	GTR+I+G	16Srna,12Srna
	P2	GTR+I+G	<i>Cox3_p1, Atp6_p1</i>
	P3	GTR+I+G	<i>Cytb_p2, Cox3_p2, Nd3_p2, Atp6_p2</i>
	P4	HKY+G	<i>Atp6_p3</i>
	P5	HKY+I+G	<i>Atp8_p1</i>
	P6	GTR+I+G	<i>Cox2_p1, Cox2_p3, Atp8_p2, Cox2_p2</i>
	P7	HKY+I+G	<i>Nd6_p3, Atp8_p3, Nd3_p3</i>
	P8	GTR+I+G	<i>Cytb_p1, Cox1_p1</i>
	P9	GTR+I+G	<i>Cox1_p2</i>
	P10	GTR+I+G	<i>Cytb_p3, Cox3_p3, Cox1_p3</i>
	P11	GTR+I+G	<i>Nd1_p1</i>
	P12	GTR+I+G	<i>Nd1_p2, Nd5_p2</i>
	P13	HKY+I+G	<i>Nd1_p3</i>
	P14	GTR+I+G	<i>Nd2_p1, Nd3_p1, Nd6_p1</i>
	P15	GTR+I+G	<i>Nd6_p2, Nd2_p2</i>
	P16	GTR+G	<i>Nd2_p3</i>
	P17	GTR+I+G	<i>Nd4L_p1, Nd4_p1</i>
	P18	GTR+I+G	<i>Nd4_p2</i>
	P19	GTR+I+G	<i>Nd4_p3</i>
	P20	GTR+G	<i>Nd4L_p3, Nd4L_p2</i>
	P21	GTR+I+G	<i>Nd5_p1</i>
	P22	GTR+G	<i>Nd5_p3</i>

Supplementary Table S3 The best partitioning scheme and model were selected by PartitionFinder for phylogenetic analyses based on the PCG12s+rRNAs dataset

Matrix	Partitions	Models	Genes
PCG12s+rRNAs	P1	GTR+I+G	<i>Nd3_p1, Nd6_p1, 16Srna, 12Srna</i>
	P2	GTR+I+G	<i>Atp6_p1, Cox3_p1</i>
	P3	GTR+I+G	<i>Cox3_p2, Cytb_p2, Atp6_p2, Nd3_p2</i>
	P4	HKY+I+G	<i>Atp8_p1</i>
	P5	GTR+I+G	<i>Cox2_p1, Atp8_p2, Cox2_p2</i>
	P6	GTR+I+G	<i>Cox1_p1, Cytb_p1</i>
	P7	GTR+I+G	<i>Cox1_p2</i>
	P8	GTR+I+G	<i>Nd1_p1</i>
	P9	GTR+I+G	<i>Nd5_p2, Nd1_p2</i>
	P10	GTR+I+G	<i>Nd2_p1</i>
	P11	GTR+I+G	<i>Nd2_p2, Nd6_p2</i>
	P12	GTR+I+G	<i>Nd4L_p1, Nd4_p1</i>
	P13	GTR+I+G	<i>Nd4_p2, Nd4L_p2</i>
	P14	GTR+I+G	<i>Nd5_p1</i>

847

848



Supplementary Table S4 Statistical confidence (*P*-values) for alternative hypotheses based on PCGs+rRNAs data and PCG12s+rRNAs data

Dataset	Tree	logL	deltaL	bp-RELL	p-KH	p-SH	p-WKH	p-WSH	c-ELW	p-AU
PCGs+rRNAs	RAxML.tree	-610103.23	0	0.998 +	0.999 +	1 +	0.999 +	0.999 +	0.998 +	0.999 +
	MrBayes.tree	-610182.1001	78.87	0.0018 -	0.001 -	0.001 -	0.001 -	0.001 -	0.00201 -	0.00118 -
PCG12s+rRNAs	RAxML.tree	-307779.085	0	0.827 +	0.823 +	1 +	0.823 +	0.823 +	0.826 +	0.812 +
	MrBayes.tree	-307811.752	32.667	0.173 +	0.177 +	0.177 +	0.177 +	0.177 +	0.174 +	0.188 +

Supplementary Table S5 Taxon group clusters for FcLM analyses



Supplementary Table S6 Fossil-calibrated time points used when constructing phylogenetic tree and calculating divergence

Node	Node minimum age (Ma)	Node maximum age (Ma)
Eupolyphaga sinensis-Cryptocercus kye bangensis split	131.4	258.8
Cryptocercus kye bangensis-Termes hospes split	130	185.1
Termes hospes-Macrotermes barneyi split	41.9	92.8
Miomantis caffra-Taumantis sigiana split	40.8	75.6
Ambivia undata-Citharomantis falcata split	70.2	117.3
Gongylus gongylodes-Empusa pennicornis split	0	32.0
Blepharopsis mendica-Idolomantis diabolica split	37.8	57.4
Deroplatys desiccata-Deroplatys truncata split	54.1	87.4
Yersinia mexicana-Stenotoxodera porioni split	38.3	92.0
Cilnia humeralis-Miomantis caffra split	45	84.5

860

861

862



Supplementary Table S7 AIC-based model selection for trait evolution

Model	log-likelihood	AIC	AICc	free parameters
ER	-47.581632	97.163264	97.185863	1
SYM	-47.581632	97.163264	97.185863	1
ARD	-44.593571	93.187143	93.255325	2

863

864

865

uncorrected proof



Supplementary Table S8 Rapid evolutionary analysis of 13 mitochondrial genes in mantis with the femoral lobe phenotype

Gene	lnL_One	np_One	omega_one	lnL_Two	np_Two	omega_ B	omega_ F	2ΔlnL
<i>Nd2</i>	-10816.94101	38	0.04422	10807.15051	39	0.03068	0.06409	19.580986
<i>Cytb</i>	-8893.232485	38	0.02037	8883.583811	39	0.015	0.02981	19.297348
<i>Cox1</i>	-11017.29403	38	0.01209	11010.21109	39	0.00972	0.01689	14.16589
<i>Cox2</i>	-5589.893168	38	0.02899	5582.985892	39	0.02292	0.04386	13.814552
<i>Nd5</i>	-13813.18894	38	0.02258	13806.74188	39	0.01858	0.03005	12.894124
<i>Nd6</i>	-5079.61569	38	0.03722	5075.557857	39	0.02941	0.05447	8.115666
<i>Atp6</i>	-5938.056118	38	0.0255	5934.877051	39	0.02124	0.0338	6.358134
<i>Cox3</i>	-6812.103922	38	0.02949	6810.213634	39	0.02592	0.03683	3.780576
<i>Nd4</i>	-11126.32122	38	0.02296	11125.14349	39	0.02108	0.02605	2.355466
<i>Nd3</i>	-3423.0124	38	0.02603	3422.875197	39	0.02438	0.02961	0.274406
<i>Nd1</i>	-7008.469767	38	0.0146	7008.345526	39	0.01404	0.01538	0.248482
<i>Nd4l</i>	-1989.093195	38	0.02006	1989.080307	39	0.01963	0.02067	0.025776
<i>Atp8</i>	-1574.166454	38	0.06505	1574.156952	39	0.06285	0.06731	0.019004

866

867

868



Supplementary Table S9 Rapid evolutionary analysis of 13 mitochondrial genes in mantis with the pronotum phenotype

Gene	lnL_One	np_One	omega_on e	lnL_Two	np_Two	omega_B	omega_F	2ΔlnL
<i>Nd5</i>	-8604.055464	32	0.01308	-8600.991453	33	0.01122	0.0172	6.12802199999715
<i>Cox3</i>	-4027.899967	32	0.01499	-4027.703094	33	0.01409	0.0166	0.393745999999737
<i>Nd3</i>	-1850.741993	32	0.01715	-1850.736763	33	0.01736	0.01671	0.010459999999966
<i>Nd6</i>	-2920.567814	32	0.02092	-2919.891952	33	0.01847	0.02564	1.3517240000001
<i>Cox2</i>	-3540.123552	32	0.0184	-3539.698602	33	0.01986	0.01558	0.849900000000162
<i>Nd1</i>	-4457.723498	32	0.00961	-4457.451855	33	0.00903	0.01086	0.543286000000008
<i>Atp8</i>	-1190.160372	32	0.07908	-1189.323249	33	0.06055	0.10652	1.674246000000004
<i>Nd4</i>	-6757.454225	32	0.01365	-6756.476477	33	0.01241	0.0165	1.955496000000049
<i>Cox1</i>	-7231.252839	32	0.00466	-7230.56202	33	0.00507	0.00369	1.381637999999893
<i>Nd2</i>	-6465.041244	32	0.01798	-6459.632485	33	0.01259	0.02739	10.8175179999998
<i>Atp6</i>	-3721.739125	32	0.01437	-3721.726481	33	0.01455	0.01399	0.025287999999818
<i>Cytb</i>	-5809.868594	32	0.0106	-5809.816685	33	0.01035	0.01117	0.103817999999592
<i>Nd4l</i>	-6757.454225	32	0.01365	-6756.476477	33	0.01241	0.0165	1.955496000000049

Supplementary Table S10 Lightgreen module genes enrichment

Supplementary Table S11 Up-regulation metabolite enrichment analysis in *H. coronatus*

