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PHYLOGENY STUDY OF SOCIAL VESPID WASPS INFERRED FROM CYTOCHROME OXIDASE 1 (*CO1*) LOCUS

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ABSTRACT

Social wasps (Vespidae) display a wide range of diversity in their ecology and social organisation, providing insights into the origins of simple societies and the elaboration and maintenance of complex societies. Social wasps play an important role in our ecosystems and economies, for example, through their pollination and pest control services. Compared with other social insects (e.g. ants, termites and bees), the social wasps are understudied. The social subfamilies within the Vespidae namely Stenogastrinae, Polistinae and Vespinae occurred together only in the oriental region. Cladistic analysis of behavioral data showed that Stenogastrinae have been grouped together with the social Polistinae and Vespinae in the family of Vespidae. However, it has been reported that Stenogastrinae are more closely related to the solitary wasps; Eumeninae than to the other social subfamilies, based on their morphological characters. The aim of this study was to clarify the relationship between the subfamily in the social vespid wasps (Stenogastrinae, Polistinae, and Vespinae) based on *CO1* mitochondrial DNA. Construction of phylogenetic tree shows a monophyletic clade between subfamily of Vespinae and Polistinae and subfamily of Stenogastrinae have been group as the sister clade to other social wasps.

Key words: Vespidae, Stenogastrinae, Cytochrome Oxidase 1 (*CO1*), Social wasps

INTRODUCTION

Vespidae is a cosmopolitan but predominantly tropical family which currently categorized into six subfamilies: Euparagiinae, Masarinae, Eumeninae, Stenogastrinae, Vespinae, and Polistinae. These subfamilies are categorized based on morphological evidence and apparently they are monophyletic. Vespidae is a group that has maintained the necessary transitional states to illustrate social evolution, including solitary, presocial, facultatively eusocial, and eusocial taxa (Hines *et al.,* 2007). One of the remarkable aspects of Vespidae is abundance in its social species, which are comprised of three social subfamilies, namely, Stenogastrinae, Polistinae and Vespinae which occur together only in Oriental and Papuan regions (Carpenter and Nguyen, 2003).

Information on the diversity and abundance of social wasps in Southeast Asia, including Peninsular Malaysia, is still very sparse despite their ecological importance not only as insect predators or being at the top of terrestrial insect food web but also as effective pollinators of various plants, and could be considered as one of the most important bio-indicator for environmental condition changes (Carpenter and Kojima, 1997). As such, many of them can be classified as the key stone species in that particular ecosystem.

Social wasps in the family of Vespidae play importance role in order to understanding the development evolution of the social habits. There would be two type of social behavior in the social wasps; facultative social and pure social. Facultative eusocial is only exhibit by the subfamily Stenogastrinae (Hines *et al.,* 2007) and the other two subfamilies are pure social. Based on the lineage family of the Vespidae, it shows that this family evolves from the solitary behavior to the social behavior (Pickett and Carpenter, 2010). So understanding the phylogenetic within the social

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wasps could be as the initial study for further evolution study in the family of Vespidae. Thus, this drawing the attention of the scientific research in evolution as they have the potential for understanding the evolution model.

Due to that, a molecular systematic studies and the construction of the Vespidae phylogeny on the basis of ribosomal DNA sequences sequence from the nuclear genomes is proposed. The main objective of this study is to construct dendograms and phylogenetic relationship among the genera in the three subfamilies of social vespids collected from Peninsular Malaysia. DNA sequences were obtained from the mitochodrial region of Cytochrome Oxidase 1 (CO1) DNA gene of social wasps**.** Solitary wasps of the *Eumenes* spp (Vespidae) were used as an outgroups. All of the phylogenetic analyses were conducted using the Neighbor-Joining (NJ) and Maximum Parsimony (MP) methods performed using the computer program of MEGA 6.

METHODS

Sampling

The samples were collected throughout peninsular Malaysia using malaise trap. The inner forest was selected as the specific target location for sample collection. The collection period was set up at three weeks interval. The malaise traps were left unattended throughout the collection period. Only the collecting bottle were taken and replaced with a new one at the end of each collection period. The collection bottles were filled with 70% ethyl alcohol for the purpose of specimen preservation. A total of 13 individual derived from 3 different subfamilies were successfully collected and used for the amplification of *CO1* gene in this research. Each species represents the genus in each of the subfamily. The identified species of social vespid wasps caught is listed in Table 1.

Table 1. List of wasps' species that have been successfully collected throughout peninsular Malaysia

SAMPLE ID	Subfamily	Genus	Species	State	Site
Vvel1	Vespinae	*Vespa*	*Velutina*	Pahang	Cameroon Highland
Vvel2	Vespinae	*Vespa*	*Velutina*	Pahang	Cameroon Highland
Va1	Vespinae	*Vespa*	*Affinis*	Pahang	Kuala keniam
Va2	Vespinae	*Vespa*	*Affinis*	Pahang	Kuala keniam
Vmoc1	Vespinae	*Vespa*	*Mocsayarana*	Pahang	Bukit fraser
Vmoc2	Vespinae	*Vespa*	*Mocsayarana*	Pahang	Bukit fraser
Vtro1	Vespinae	*Vespa*	*Tropica*	Johor	Gunung Ledang
Vtro2	Vespinae	*Vespa*	*Tropica*	Johor	Pulau Perhentian
P. ana1	Vespinae	*Provespa*	*Anamola*	Pahang	Krau
P. ana2	Vespinae	*Provespa*	*Anamola*	Pahang	Krau
P. n	Vespinae	*Provespa*	*Nocturna*	Pahang	Krau
P. n 2	Vespinae	*Provespa*	*Nocturna*	Pahang	Krau
Rfaci	Polistinae	*Ropalidia*	*Faciesta*	Pahang	Krau
Rfaci 2	Polistinae	*Ropalidia*	*Faciesta*	Pahang	Krau
Rf1	Polistinae	*Ropalidia*	*Flavopicta*	Pahang	Krau
Rf2	Polistinae	*Ropalidia*	*Flavopicta*	Pahang	Krau
Rs 1	Polistinae	*Ropalidia*	*Sumatrae*	Johor	Gunung Ledang
Rs 2	Polistinae	*Ropalidia*	*Sumatrae*	Johor	Gunung Ledang
Pm 1	Stenogastrinae	*Parischonogaster*	*Melleyi*	Johor	Gunung Ledang
Pm 2	Stenogastrinae	*Parischonogaster*	*Melleyi*	Johor	Gunung Ledang
Puni1	Stenogastrinae	*Parischonogaster*	*Unicuspata*	Kedah	Langkawi
Puni2	Stenogastrinae	*Parischonogaster*	*Unicuspata*	Kedah	Langkawi
Lv1	Stenogastrinae	*Liostenogaster*	*Vechti*	Pahang	Kuala Tahan
Lv2	Stenogastrinae	*Liostenogaster*	*Vechti*	Pahang	Kuala Tahan
Lf1	Stenogastrinae	*Liostenogaster*	*Flavolineata*	Pahang	Kuala Keniam
Lf 2	Stenogastrinae	*Liostenogaster*	*Flavolineata*	Pahang	Kuala Keniam
EC1	Stenogastrinae	*Eustenogaster*	*Calypodoma*	Negeri Sembilan	Gunung Dato
EC2	Stenogastrinae	*Eustenogaster*	*Calypodoma*	Negeri Sembilan	Gunung Dato
Eu	Eumininae	*Eumenes*	*Fraternus*	Perak	Ladang Koko Hilir Perak

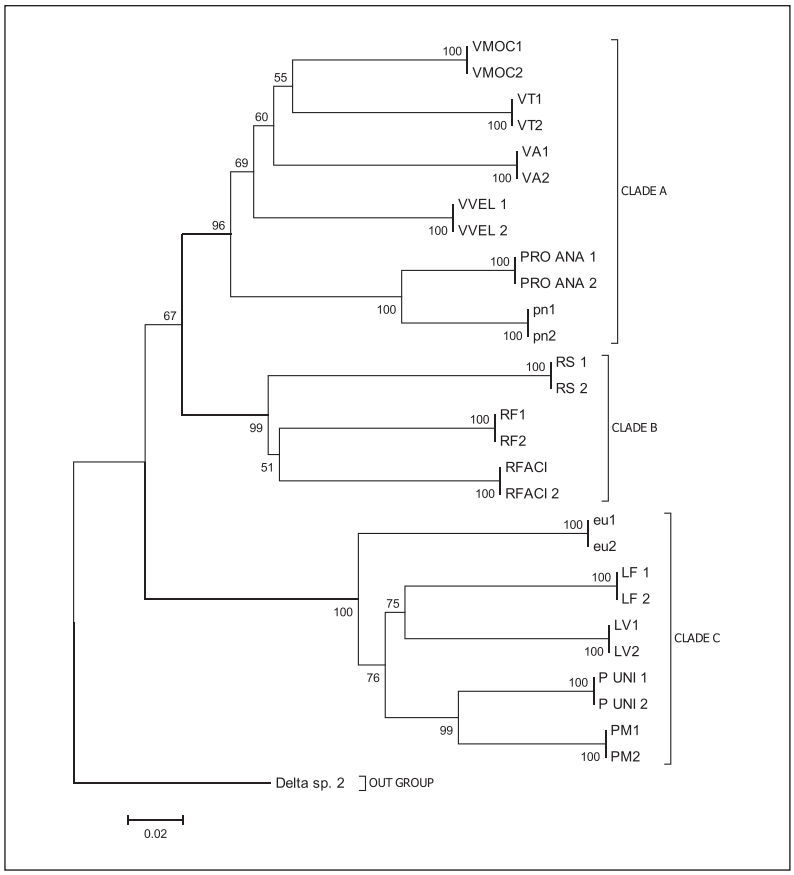

Fig. 1. NJ tree between subfamily of Vespidae. Subfamily Stenogastrinae have been placed as the sister group from both pure social group of wasps.

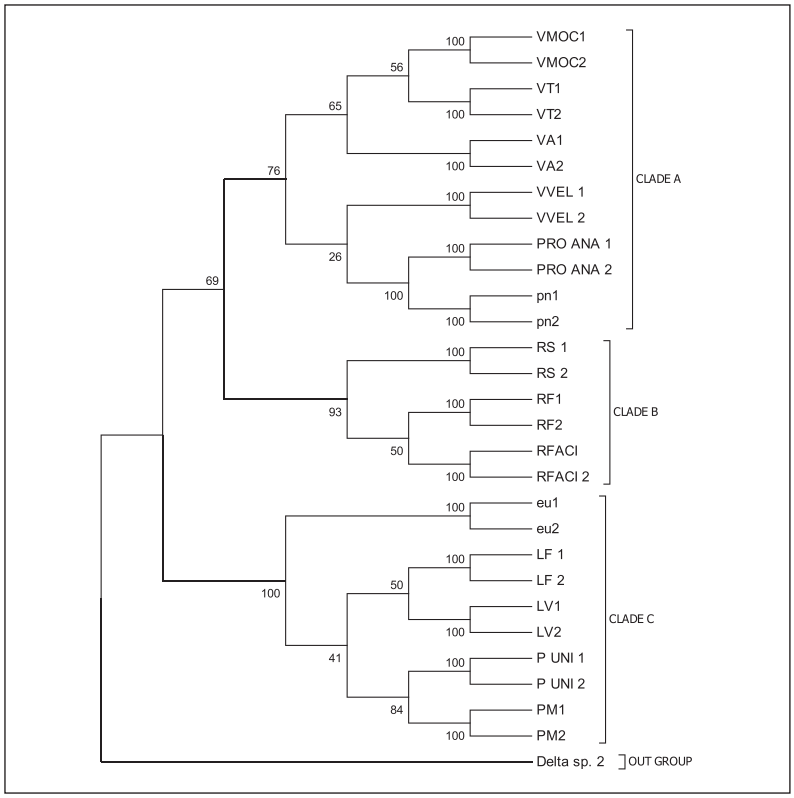

Fig. 2. MP tree between the subfamily of Vespidae. Subfamily Stenogastrinae have been placed as the sister group from both pure social group of wasps.

DNA extraction and amplification

Identified specimens were preserved in 95% ethanol at -20°C. Samples (tissue) were extracted using DNAeasy Tissues Kit (Qiagen) with minor protocol modification. In this study, the Cytochrome oxidase *(CO1)* was amplified by using MyTaq™ Red Mix PCR kits. This kit is a ready-to-use 2x mix for fast and highly specific PCR amplification. The advanced formulation of MyTaq Red Mix exhibits more robust amplification than other commonly used polymerases, delivering very high yield over a wide range of PCR templates and also at significantly faster PCR reaction times without compromising PCR specificity or yield. The mitochondrial DNA Cytochrome C Oxidase Subunit I gene (*COI)* were partially amplified from the total DNA genome using a pair of LCO2198 (forward) and HCO1490 (reverse) primers adapted for the bee (Folmer, 1994). Amplification of the nuclear genome was performed with the following parameters; initial step at 94^{o}C for 3 min, denaturation at 95^{o}C for 1 min, annealing at 47^{o}C for 1 min, extension of sequence at 72^{o}C for 10 min for 35 cycles and a final extension at 70^{o}C for 10 min. QIAquick PCR Purification Kit (Qiagen) was used to purify the PCR products and subsequently, the purified DNA product was sent to 1st Base Laboratories Sdn Bhd in Shah Alam, Selangor, Malaysia for sequencing process.

Sequence and phylogenetic analysis

All of the CO1 gene sequences obtained from 1st Base Laboratories Sdn Bhd (Malaysia) were validated using Sequence Similarity Search tool via NCBI GenBank BLASTn, and later aligned and edited by using Bioedit Sequence Alignment Editor. Two phylogeny analyses were carried out which are the Neighbor-Joining (distance-based method) and the Maximum Parsimony (Parsimony method) analysis. Phylogenetic tree were generated by using the MEGA 6 software. For NJ tree, the Kimura-2-Parameter model was selected for phylogenetic reconstructions. The analysis were constructed underwent 1000 bootstrap replication to obtain the bootstrap confident level.

RESULTS AND DISCUSSION

Topology of NJ tree

The evolutionary history was inferred using the Neighbor-Joining method (Felsenstein, 1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791 The optimal tree with the sum of branch length = 1.37506363 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Kimura, 1980). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Tamura *et al.,* 2013) and are in the units of the number of base substitutions per site.

The analysis involved 29 nucleotide sequences. All positions containing gaps and missing data were eliminated. Based on the topology, there would be three main clade formed; clade A, clade B and clade C. Those clades are formed based on the genus in each of subfamily; except for the clade B. The separation of clade shows that the genus in each of subfamily is successfully clustered together according to their subfamily.

Clade A shows that main genus of the subfamily Vespinae (*Vespa* sp. and *Provespa* sp.), and clade B shows the cluster of the species in the genus *Ropalidia* sp. from the subfamily of Polistinae. Meanwhile, clade C shows the three genus of the subfamily Stenogastrinae clustered together. Clade A formed by the value of 96% separation from two genus, while species in subfamily Polistinae formed a cluster in clade B with 99% bootstrap value and genus in subfamily Stenogastrinae formed a clade C with the 100% bootstrap values.

Topology of MP tree

The evolutionary history was inferred using the Maximum Parsimony method. The most parsimonious tree with the length value of 1019 is shown. The consistency index is 0.481151, the retention index is 0.787744, and the composite index is 0.383436 for all sites and parsimony-informative sites (in parentheses). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985).

The MP tree was obtained using the Tree-Bisection-Regrafting (TBR) algorithm (Kimura, 1980) with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). The analysis involved 29 nucleotide sequences. All positions containing gaps and missing data were eliminated. Same as NJ tree, there would be three major clades formed between the subfamily. This means that each of the subfamily is successfully grouped together within its own species and genus. Genus *Provespa* sp. and *Vespa* in the subfamily of Vespinae are forming the Clade A with the bootstrap value of 76%. While, clade B is clustered together by the species in the genus of *Ropalidia sp.* from the subfamily of Polistinae with a strong bootstraps value of 93% and subfamily Stenogastrine is clumped together in the clade C with the bootstrap value 100%.

The topology of NJ and MP tree shows that the subfamily of Polistinae formed a monophyletic cluster with the subfamily Vespinae. Meanwhile, subfamily Stenogastrinae formed a sister clade between the main clade A and clade B, and that makes the Stenogastrinae become the sister group between the pure social wasps. Thus, this was supported by the previous study on the evolutions of social wasps are from the solitary wasps (Pickett and Carpenter, 2010). Therefore, it could be said that the subfamily Stenogastrinae are the interclade species between the solitary wasps and social wasps.

This hypothesis could also be supported by the morphological and behavioral data. Based on the morphological data, it has been reported and proposed that the Stenogastrinae is a Eumenes-like solitary ancestor due to a long pointed clypeus, long narrow mandibles lying alongside it and abnormally placed first thoracic spiracles (Richard, 1971). But the morphology data based on earlier study that stated the social wasps would have simple taxa and mesoscutom without the pretengula, meanwhile the solitary wasps would have *bifid* taxa and have the mesoscutom with the pratengula; making the Stenogastrinae still categorized in the social wasps group (Carpenter and Nguyen, 2003).

However, based on the behavioral data, subfamily Stenogastrinae would have inhibited the facultative eusocial behavior (Hines *et al.,* 2007). Facultative eusocial wasps have a distinctive feature where there is no clear delineation between eusocial and solitary wasps (Hines *et al.,* 2007). Therefore, based on both morphological and behavioral data, it clearly supported the molecular data found in this study where the subfamily of Stenogastrinae is the sister clade of the social wasps.

CONCLUSIONS

Phylogenetic study was done on the three subfamilies of Vespidae namely as Stenogastrinae, Polistinae and Vespinae by using the genetic marker of *CO1* gene. The result obtained showed that the molecular analysis of *CO1* in three subfamily of Vespidae concluded that Stenogastrinae is the sister group of social wasps, Polistinae+Vespinae. With these molecular data, supported by the morphological and behavioral data from the previous studies, it could be concluded that subfamily Stenogastrinae is the sister group with the pure social wasps (Vespinae and Polistinae) and this subfamily is the interclade between the social and solitary wasps.

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