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INITIAL STUDY OF SOCIAL VESPID WASPS BY USING MOLECULAR APPROACH OF *28S* REGION

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ABSTRACT

The social wasps (Vespidae) display a wide range of diversity in their ecology and social organization, 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 has been grouped 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 is to make initial study relationship between the subfamily in the social vespid wasps (Stenogastrinae, Polistinae, and Vespinae) based on *28S* nuclear 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 Neighbour-Joining (NJ).

Keywords: Vespidae, *28S* region, monophyletic.

ABSTRAK

Tebuan sosial (Vespidae) mempunyai kepelbagaian dalam ekologi dan organisasi sosial. Tebuan sosial memainkan peranan penting dalam ekosistem, sebagai contoh, melalui perkhidmatan pendebungaan dan kawalan perosak mereka. Berbanding dengan serangga lain sosial (contohnya semut, anai-anai dan lebah), kurang kajian dilakukan keatas tebuan sosial. Tiga subfamili tebuan social vespid melimpah di kawasan oriental. Analisis kladistik data tingkah laku menunjukkan bahawa Stenogastrinae telah dikumpulkan bersama dengan Polistinae dan Vespinae. Walaubagaimanapun, Stenogastrinae lebih berkaitrapat dengan tebuan solitari; Eumeninae, jika dikumpulkan berdasarkan bentuk morfologi mereka. Tujuan kajian ini adalah untuk membuat kajian awal hubungan antara subfamili dalam tebuan vespid sosial (Stenogastrinae, Polistinae, dan Vespinae) berdasarkan jujukan penanda DNA 28S. Pembinaan pokok filogenetik menunjukkan klad monofiletik antara subfamili daripada Vespinae dan Polistinae dan subfamili daripada Stenogastrinae telah kumpulan sebagai klad beradik kepada tebuan sosial lain.

Kata kunci: Vespidae, kawasan28S, monofiletik.

INTRODUCTION

Vespidae is a cosmopolitan but predominantly tropical family which currently categorized into six subfamilies: Euparagiinae, Masarinae, Eumeninae, Stenogastrinae, Vespinae, and Polistinae (Yildirim and

Gusenleitner, 2012). These subfamilies are categorized based on morphological evidence and apparently they are monophyletic (Hines *et al.,* 2007). 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 & Nguyen, 2003). Colonies of social insects may consist of thousands of individuals living in close proximity and characterized by a reduced genetic variability (Barrachi *et al.,* 2012). 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 (Kojima *et al.*, 2009). 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 (Hunt, 2007) and the other two subfamily are pure social. Based on the lineage family of the Vespidae, it shows that this family evolve from the solitary behavior to the social behavior (Pickett & Carpenter, 2010). So understanding the phylogenetic within the social 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.

So, a molecular systematic studies and the construction of the Vespidae phylogeny on the basis of ribosomal DNA sequences from the nuclear genomes is proposed. The main goals in this study are to construct a phylogenetic relationship among the genera in the three subfamilies of social vespid wasps from Peninsular Malaysia. DNA sequence will be obtained from the nuclear 28S ribosomal DNA gene of social wasps.In this study, *28S* gene will be chosen due to its added value in the capability of estimating the relationships of the Hymenoptera order based on the previous study of ichneumonid wasps, evaniid wasps and chalcidoid wasps (Gillespie *et al*., 2010). Nuclear *28S* gene also are good tools in reconstructing the phylogeny of wasps and bees because the D2 and D3 segments of the rDNAcan solve almost any systematic question (Schmitz and Moritz, 1998)**.** Solitary wasps of the *Eumenes* spp (Vespidae) were used as outgroups. All the phylogenetic analysis will be conducted using Neighbor-Joining (NJ) methods that will be performed using the computer program MEGA 6.

METHOD

Sampling

The samples were collected throughout the peninsular of Malaysia using malaise trap. The inner forest was selected as the 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 the collection period. The collection bottles were filled with 70% ethyl alcohol for the purpose of specimen preservation within the short period. A total of 13 individual from three subfamilies were used in this research. Each of species were represent the genus in each of subfamily. List of species caught were list in the Table 1.

DNA extraction and amplification

Identified specimens were preserved in 95% ethanol at -20ºC. Samples (tissue) were extracted using DNAeasy Tissues Kit (Qiagen), following the DNAeasy protocol. In this study, the *28s* region were amplified by using MyTaq™ Red Mix. This kits is a ready-to-use 2x mix for fast, highly-specific PCR. 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 D2 part of the *28s*rDNA

Table 1. List of species of Social Vespid wasps

SAMPLEID	Subfamily	Species	State	Site
Vvel1	Vespinae	*Vespa velutina*	Pahang	Cameroon Highland
Vmoc1	Vespinae	*Vespa mocsayarana*	Pahang	Bukit Fraser
P. ana1	Vespinae	*Provespa anamola*	Pahang	Krau
R1	Postinae	*Polybiodes raphigastra*	Negeri Sembilan	Gunung Dato
PV1	Postinae	*Parapolybia varia*	Pahang	Bukit Fraser
Rf1	Polistinae	*Ropalidia flavopicta*	Pahang	Krau
Rs 1	Polistinae	*Ropalidia sumatrae*	Johor	Gunung Ledang
Pm 1	Stenogastrinae	*Parischonogaster melleyi*	Johor	Gunung Ledang
Puni1	Stenogastrinae	*Parischonogaster unicuspata*	Kedah	Langkawi
Lv1	Stenogastrinae	*Liostenogaster vechti*	Pahang	Kuala Tahan
Lf1	Stenogastrinae	*Liostenogaster flavolineata*	Pahang	Kuala Keniam
EC1	Stenogastrinae	*Eustenogaster calypodoma*	Negeri Sembilan	GunungDato
Eu	Eumininae	*Eumenes fraternus*	Perak	Ladang Koko Hilir Perak

DNA primer correspond to the nucleotide position 427 and 736 of the *V. crabro* sequence. Primer's sequences used in this study were (5'AAAGATCGAATGGGGAGATTC3') and (5'CACCGGGTCCG TACCTCC'3) (Schmitz and Moritz, 1998). Amplification for nuclear genome be performed with the following parameters: initial step at 94 C (3 min), denaturation at 95ºC for 1 minutes, annealing of *28s* gene primers at 60ºC for 1 minutes, extension of sequence at 72ºC for 10 minutes, for 35 cycles and final extantion at 70 C at 10 minutes. The PCR product later was purified by using Qiagen PCR purification. The DNA product that have been purified was sent to 1st Base Laboratories SDN BHD Malaysia for sequencing service. 5 µl of primer which included both reverse and forward was sent along for each of the sample that need to be sequenced.

Sequence and phylogenetic analysis

All of sequence that been obtained from 1st Base Laboratories Sdn Bhd (Malaysia) were validate using Sequence Similarity search by using GenBankBLASTn, and then align and edited by using Bioedit Sequence Alignment Editor. Align sequence were analyse by using MEGA 6 for nucleotide diversity and net nucleotide divergence and genetic distance.

Two phylogeny analysis were carried out which; Neighbor-Joining (distance-based method). 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.

RESULT AND DISCUSSION

Analysis has been done for 13 genetic samples which consist of 3 subfamilies of Stenogastrinae, Vespinae and Polistinae. Through *28s* sequence analysis, 433 base pairswere obtained, which 183 are conserved sites and 95 are parsimony informative as shown in Table 2.

Table 2. Analysis of *28S* sequences of Family of Vespidae

Character	Amount
Total character	433
Conserved sites	183
Variable sites	222
Parsimony informative	152

Table 3. Pairwise genetic distance between the subfamilies

	Outgroup	Stenogastrinae	Polistinae	Vespinae
Outgroup				
Stenogastrinae	0.302			
Polistinae	0.400	0.256		
Vespinae	0.341	0.232	0.132	

The pairwise distances (Table 3) were calculated to know the genetic distance between subfamilies of Vespidae. The number of base substitutions per site from averaging over all sequence pairs between groups are shown. Analyses were conducted using the Kimura 2-parameter model. The analyses involved 13 nucleotide sequences. There were a total of 273 positions in the final dataset. Evolutionary analyses were conducted in MEGA6. From the analysis, the pairwise distance relationship between *E. fraternus* (Outgroup) and subfamily Polistinae is the most distant with the value of 0.400. Subfamily Stenogastrinae has the closest distance with the outgroup (0.302) when compared with other two subfamilies.

Phylogeny tree was generated based on Neighbor Joining (NJ) method. The optimal tree with the sum of branch length = 0.68204310 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. 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

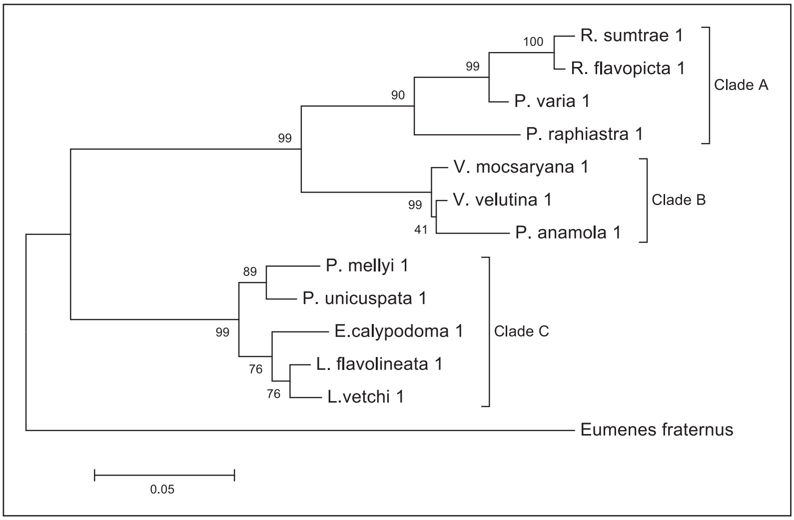

Figure 1. NJ tree topology that was generated based on Kimura-2-Parameter to show the relationship between the families of Vespidae. Bootstrap values indicated above the branch.

computed using the Kimura 2-parameter and are in the units of the number of base substitutions per site.

Based on the tree topology, there were three main clades generated; clade A, clade B and clade C. Those cladeswere generated based on the genus in each subfamily. The separation of clades show that the genus in each subfamily were clustered together successfully according to their subfamilies. Clade A shows that three main genus in subfamily Polistinae (*R. sumatrae, R. flavopicta., P. varia.,* and *R. raphigastra)* and clade B shows the cluster of two genus from subfamily Vespinae (*V. mocsaryana, V. velutina* and *P. anamola*), meanwhile clade C shows the three genus of subfamily Stenogastrinae clustered together. The topology of NJ tree shows that subfamily Polistinae formed a monophyletic clade with the subfamily of Vespinae. Meanwhile subfamily Stenogastrinae formed a sister clade between the main clade A and clade B making it a sister group between the pure social wasps.

Based on the genetic distance, subfamily Stenogastrinae shows that it has the closest distance toward to solitary wasps which is

Eumeninae. Thus it could be used to support the statement by (Pickett & Carpenter, 2010) that the evolution of social wasps are from the solitary wasps. This data was supported by the topology structure of NJ trees. This shows that subfamily Stenogastrinae is the interclade species between solitary wasps and social wasps. This molecular data is also supported by morphological and behavioral data. Based on the morphological data, Richard (1971) proposed that 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. However the morphology data by (Carpenter & Nguyen, 2003) suggested thatsocial wasps would have simple taxa and mesoscutum without the pretengula, meanwhile the solitary wasps would have *bifid* taxa and have the mesoscutum with the pratengula;making Stenogastrinaea member of social wasps group.

CONCLUSION

Phylogenetic study have been done on the three subfamilies of Vespidae namely Stenogastrinae, Polistinae and Vespinae by using 28S genetic marker of the D2 region. The result obtained from molecular analysis of 28S in three subfamilies of Vespidae concluded that Stenogastrinae are sister groups of social wasps, Polistinae+Vespinae. With these molecular data and few morphological support, it can be concluded that subfamily Stenogastrinaeis sister group with pure social wasps (Vespinae and Polistinae) and this subfamily is the intercladegroup between the social and solitary wasps.

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