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Next-generation sequencing of the mitochondrial genome of *Dolichovespula panda* (Hymenoptera: Vespidae) with a phylogenetic analysis of Vespidae

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ABSTRACT

For the first time the mitochondrial genome of a *Dolichovespula* species, *D. panda* Archer (Hymenoptera: Vespidae), was sequenced with a next-generation sequencing approach. The sequenced mitochondrial genome is 17137 bp long and consists of 13 protein-coding, 22 tRNA and two rRNA genes, as well as a partial A + T-rich region. Twenty-two of the genes are encoded on the majority strand and 15 genes on the minority strand. All protein-coding genes start with ATN codons and have a TAA termination codon, except for one with a TA codon. Compared with the putative ancestral arrangement of insects, the *D. panda* mitochondrial genome shows the shuffling of *trnN* and *trnE*, and of *trnQ* and *trnM*, the translocation of *trnY* to upstream of *trnI*, and of *trnL1* to the region between *trnS2* and *nad1* and a reversal of *trnS1*. A phylogenetic tree within the Vespidae was reconstructed using the 13 protein-coding mitochondrial genes. This shows a sister group relationship between *Dolichovespula* and a clade formed by *Vespa* and *Vespula*. It also corroborated the position of *Eumeninae* as sister group of the clade Polistinae + Vespinae.

Introduction

The typical animal mitochondrial genome is a closed-circular and double-stranded DNA molecule, approximately 16 kb long (Boore, 1999). It usually consists of 37 genes, including 13 protein-coding genes, 22 tRNA genes, two rRNA genes and an A + T-rich region. The features of maternal inheritance (Avise, 1986), conserved gene components (Curole and Kocher, 1999), rare recombination (Boore, 1999) and rapid evolutionary rate make the mitochondrial genome an ideal molecular marker for population genetics, species identification, phylogenetics and molecular evolution (Knudsen et al., 2006).

Mitochondrial genomes of Hymenoptera usually have high A + T content (Gotzek et al., 2010; Wei et al., 2009) and frequent rearrangement of gene positions (Dowton and Austin, 1999; Dowton et al., 2003). Representative mitochondrial genomes have been sequenced for most of the higher taxonomic levels within the Hymenoptera (Mao et al., 2015; Wei et al., 2014), and even for lower levels (Li et al., 2016; Wei et al., 2010b) in certain groups. An increasing number of sequenced mitochondrial genomes provides information for the study of genome evolution (Kaltenpoth et al., 2012; Mao et al., 2014a; Mao et al., 2014b; Oliveira et al., 2008; Tang et al., 2015; Xiao et al., 2011), phylogeny (Dowton et al., 2009; Mao et al., 2015; Oliveira et al., 2008) and biodiversity (Tang et al., 2015).

The subfamily Vespinae (Hymenoptera), which includes the yellowjackets (*Vespula* and *Dolichovespula*) and hornets (*Vespa* and *Provespa*), are the most well known as a highly developed eusocial member of the family Vespidae because of their pugnacious defense of their conspicuous enveloped nests (Tan et al., 2015). Up to the present, the phylogenetic relationships among genera remain unclear (Lopez-Osorio et al., 2017; Tan et al., 2015). Carpenter (1987) using morphological and behavioral characters, provided the first comprehensive cladistic treatment of supraspecific taxa in the Vespinae. The analyses supported yellowjackets (*Dolichovespula* + *Vespula*) as a clade sister to *Provespa*, placing *Vespa* sister to the remaining vespine genera. Pickett and Carpenter (2010) basing on molecular, morphological, and behavioral data, found that *Provespa* was sister to the yellowjackets, which agreed with the result of Carpenter (1987). Perrard et al. (2013) after studying morphological and molecular characters, confirm the monophyly of the genus *Vespa*. Perrard et al. (2016) inferred the phylogeny within Vespinae using the wing venation. However, the conclusion on the genus *Vespa* is the sister-group to the other Vespine genera support Carpenter (1987), but contradict Pickett and Carpenter (2010). Lopez-

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Osorio et al. (2014) investigated the evolutionary history of yellowjackets on the basis of mitochondrial and nuclear markers. Their results indicate that a yellowjacket clade is either weakly supported (parsimony) or rejected (Bayesian inference). They concluded that the use of molecular characters to elucidate the evolutionary history of yellowjackets has been limited and, for the most part, peripheral. Based on transcriptomic (RNA-seq) data, Lopez-Osorio et al. (2017) found the results of their phylogenomic analyses recover *Dolichovespula* as more closely related to *Vespa* than to *Vespula*, therefore challenging the prevailing hypothesis of yellowjacket (Vespula + Dolichovespula) monophyly. This suggests that traits such as large colony size and high paternity arose in the genus *Vespula* following its early divergence from the remaining vespine genera.

Mitochondrial genomes have been sequenced from two genera of Vespinae: *Vespula* and *Vespa* (Cameron et al., 2008; Chen et al., 2016a; Zhou et al., 2016). Rearrangements of tRNA genes were found in all sequenced mitochondrial genomes of Vespinae (Cameron et al., 2008; Chen et al., 2016a; Zhou et al., 2016). The mitochondrial genome of the third genus of Vespinae, *Dolichovespula* Thomson, is unknown.

For this study we sequenced the mitochondrial genome of a *Dolichovespula* species, *D. panda* Archer, with a next-generation sequencing approach. The features of the newly sequenced mitochondrial genome are reported and the sequence is used to analyze the phylogenetic relationships within the Vespidae.

Material and methods

Sample preparation and DNA extraction

The specimen of *D. panda* was collected at Daocheng (Sichuan, China) July 2006 and identified by Jiang-Li Tan. The specimen was stored at − 20 °C before DNA extraction. Total genomic DNA was extracted from two legs with the DNeasy tissue kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Voucher DNA was deposited in the entomological collections of Beijing Academy of Agriculture and Forestry Sciences. The DNA concentration was quantified by Qubit 3.0 (Invitrogen, Life technologies, Carlsbad, CA, USA).

Mitochondrial genome sequencing and assembly

The mitochondrial genome sequence was obtained by next-generation sequencing. The library was sequenced by BerryGenomics Company (Beijing, China) using Illumina Hiseq 2500 with the strategy of 250 paired-ends, which was constructed with two indexes using the Illumina TruSeq@ DNA PCR-Free HT Kit. The target insert size is 500 bp. Adapter sequences were removed and low-quality bases were trimmed using Trimmomatic version 0.36 (Bolger et al., 2014). Considering short reads produced in this study, the putative mitochondrial targets were determined by BLAST searching (BLASTn version 2.2.27 +) from the raw reads, which is based on a database containing Hymenoptera mitochondrial sequences with an E value of 1×10^{-5} and maximum target sequences of 1. The targeted mitochondrial reads were extracted using a Perl script (FastqExtract.pl) (Crampton-Platt et al., 2015). Celera Assembler version 8.3rc2 and IDBA_UD version 1.1.1 were used to assemble mitochondrial reads into contigs. The de novo assembly of the mitochondrial contigs was conducted by Geneious version 9.1.4 (Kearse et al., 2012).

Mitochondrial genome annotation and analysis

Initially the sequence was annotated online by Mitos Web Server (Bernt et al., 2013) with the genetic code of Invertebrate Mitochondria. The tRNA genes and their anticodons were identified online using tRNAscan-SE Search Server version 1.21, with a Cove cutoff score of 5 and a source with Mito/Chloroplast (Lowe and Eddy, 1997). The protein-coding genes were determined by alignment against their homologs in the Vespidae, using MEGA version 6 (Tamura et al., 2013). The rRNA genes and A + T-rich region were identified through the boundary of their neighboring genes.

Table 1
Mitochondrial genomes used in this study.

Species	Family	Accession number
Dolichovespula panda	Vespidae	KY293679
Vespula germanica	Vespidae	KR703587
Vespa mandarinia	Vespidae	KR059904
Vespa bicolor	Vespidae	KJ735511
Vespidae sp. MT 2004	Vespidae	KM244667
Polistes jokahamae	Vespidae	KR052468
Polistes humilis	Vespidae	EU024653
Abispa ephippium	Vespidae	NC011520
Solenopsis richteri	Formicidae	HQ215539
Myrmica scabrinodis	Formicidae	LN607806

The base compositions were calculated in MEGA version 6. The AT-skew and GC-skew were computed according to the following formulas: AT-skew = (A – T%) / (A + T%) and GC-skew = (G – C%) / (G + C%) (Hassanin et al., 2005). The codon usage of 13 protein-coding genes was calculated by CodonW (written by John Peden, University of Nottingham, UK). The gene arrangement was compared with the putative ancestral arrangement of the insect mitochondrial genome (Dowton and Austin, 1999).

Phylogenetic analysis

The phylogenetic relationships within the Vespidae were analyzed using the Bayesian inference method implemented in MrBayes version 3.2.5 (Ronquist et al., 2012) and Maximum likelihood implemented in RAxML version 7.9.6 (Stamatakis, 2015) based on the nucleotide sequences and amino acids sequences of the 13 protein-coding genes, respectively. Eight species from Eumeninae, Polistinae and Vespinae were used for phylogenetic inference (Table 1). Two species of ants, *Solenopsis richteri* and *Myrmica scabrinodis* (*Formicidae*) were used as outgroups (Babbucci et al., 2014). MAFFT version 7.205 (Katoh and Standley, 2013) was used to align the amino acid sequences of the genes. Nucleotide sequence alignment was guided by the alignment of amino acid sequences. PartitionFinder version 1.1.1 (Lanfear et al., 2012) was used to determine the best scheme of data partition and the suitable substitution models (Table 2). Four independent Markov chains were operated with 10 million metropolis-coupled generations, tree sampling occurring every 1000 generations and a burn-in of 25% trees.

In the name of initial partition, a6, a8, c1–c3, cb, n1–n6 indicate the 13 protein-coding genes, while p1, p2 and p3 indicate the three codon positions of each protein-coding gene.

Table 2
The best schemes of partition and substitution models used for each partition.

Optimal partition	Model	Initial partition
Partition 1	GTR + I + G	a6p1, c1p1, c2p1, c3p1, cbp1, n3p1
Partition 2	HKY + I + G	a8p1, n2p1, n6p1
Partition 3	GTR + G	a6p2, c2p2, c3p2, cbp2, n3p2
Partition 4	GTR + G	n1p2, n4lp2, n4p2, n5p2
Partition 5	F81 + I	c1p2
Partition 6	GTR + G	a8p2, n2p2, n6p2
Partition 7	HKY + G	a8p3, n2p3, n6p3
Partition 8	GTR + I + G	n1p1, n4lp1, n4p1, n5p1
Partition 9	HKY + G	n1p3, n4lp3, n4p3, n5p3
Partition 10	HKY + I + G	a6p3, c1p3, c2p3, c3p3, cbp3, n3p3

Results and discussion

General features of the genome

In total 4,519,872 clean reads were sequenced. The assembled mitochondrial genome of *D. panda* (GenBank accession No. KY293679) is 17137 bp long, covering about 97.2% of the complete genome, and consists of 13 protein-coding, 22 tRNA genes and two rRNA genes, with a partial A + T-rich region. Twenty-two of the genes are encoded on the majority strand and 15 genes on the minority strand. All protein-coding genes start with ATN codons and have a TAA termination codon except one with a TA codon.

The AT-skew, GC-skew and A + T content were used to reveal the base composition behavior of mitochondrial genomes (Crozier and Crozier, 1993; Wei et al., 2010a). The mitochondrial genome of *D. panda* consists of A = 42.80%, *T* = 41.81%, G = 5.39% and C = 10.00% (AT-skew = 0.01 and GC-skew = − 0.30) (Table 1). The A + T content (84.61%) is significantly higher than the GC content (15.39%), which is consistent with the base composition of mitochondrial genomes of other Vespidae species (Chen et al., 2016b).

Protein-coding genes

The total length of the 13 protein-coding genes in *D. panda* was 11259 bp, accounting for 65.70% of the whole mitochondrial genome (Table 3). Nine of 13 protein-coding genes were encoded on the majority strand and four protein-coding genes were encoded on the minority strand. All 13 protein-coding genes were initiated by three types of start codon (ATG, ATA and ATT). Three protein-coding genes (*cox1*, *atp6* and *nad4*) started from the ATG codon, three genes (*cox3*, *nad3* and *cob*) from ATA, and seven genes (*nad2*, *cox2*, *atp8*, *nad5*, *nad4l*, *nad6* and *nad1*) from ATT. All of the 13 protein-coding genes ended with a typical stop codon (TAA), except for *nad4* that ended with the incomplete termination codon TA.

The codon usage values in the mitochondrial genome of *D. panda* reflected a significant bias towards A and T nucleotides (Table 4). Leu, Ile, Phe and Met were the four most frequently used amino acids and TTA (Leu), ATT (Ile), TTT (Phe) and ATA (Met) were the most frequent codons, as in other mitochondrial genomes of Hymenoptera (Mao et al., 2012; Song et al., 2016b). In comparison, almost all of the frequently used codons consisted of A/T and ended with A/T, which may lead to the high A + T content in the mitochondrial genome.

tRNA and rRNA genes

The mitochondrial genome of *D. panda* contained 22 tRNA genes with lengths ranging from 63 bp (*trnS1*) to 73 bp (*trnL1*), and they were distributed among protein-coding genes and rRNA genes. The total length of the *D. panda* mitochondrial genome encoding for tRNA genes was 1506 bp, with an A + T content of 86.32%, which was similar to other species of Hymenoptera (Song et al., 2016b). The *rrnS* and *rrnL* were 762 and 1364 bp long, respectively. The *rrnS* was located between

Table 3
Annotation of the *Dolichovespula panda* mitochondrial genome.

Gene	Strand	Position	Length (bp)	Base composition (%)				Start codon	Stop codon	Intergenic nucleotides (bp)
				T	C	A	G			
trnY	N	1–67	67	37.74	3.77	49.06	9.43			21
trnI	J	89–159	71	33.96	9.43	41.51	15.09			399
trnM	J	559–626	68	30.19	15.09	45.28	9.43			216
trnQ	N	843–909	67	37.74	3.77	43.40	15.09			42
nad2	J	952–2001	1050	47.90	9.46	39.34	3.30	ATT	TAA	1
trnW	J	2003–2073	71	32.08	7.55	54.72	5.66			− 7
trnC	N	2067–2132	66	35.85	1.89	54.72	7.55			2
cox1	J	2135–3670	1536	41.14	11.71	35.44	11.71	ATG	TAA	14
trnL2	J	3685–3756	72	32.08	7.55	49.06	11.32			0
cox2	J	3757–4440	684	41.05	12.33	38.35	8.27	ATT	TAA	25
trnK	J	4466–4535	70	33.96	11.32	41.51	13.21			133
trnD	J	4669–4734	66	41.51	5.66	50.94	1.89			0
atp8	J	4735–4896	162	46.15	6.51	44.38	2.96	ATT	TAA	− 7
atp6	J	4890–5555	666	43.99	14.26	36.19	5.56	ATG	TAA	− 4
cox3	J	5552–6343	792	45.20	10.51	35.89	8.41	ATA	TAA	− 2
trnG	J	6342–6408	67	47.17	7.55	43.40	1.89			0
nad3	J	6409–6783	375	45.51	10.82	36.73	6.94	ATA	TAA	10
trnA	J	6794–6846	71	49.06	3.77	39.62	7.55			− 1
trnR	J	6846–6933	70	41.51	9.43	41.51	7.55			46
trnE	J	6980–7044	65	41.51	5.66	50.94	1.89			43
trnS1	N	7088–7150	63	33.96	7.55	50.94	7.55			3
trnN	J	7154–7223	70	37.74	5.66	45.28	11.32			117
trnF	N	7341–7408	68	32.08	5.66	45.28	16.98			3
nad5	N	7412–9088	1677	51.20	6.31	31.53	10.96	ATT	TAA	− 3
trnH	N	9086–9152	67	33.96	1.89	50.94	13.21			42
nad4	N	9195–10,513	1319	51.80	5.41	30.93	11.86	ATG	TA	− 7
nad4l	N	10,507–10,800	294	53.00	2.06	35.58	9.36	ATT	TAA	49
trnT	J	10,850–10,916	67	37.74	5.66	50.94	5.66			22
trnP	N	10,939–11,006	68	32.08	1.89	52.83	13.21			14
nad6	J	11,021–11,603	619	46.71	8.51	41.09	3.69	ATT	TAA	0
cob	J	11,604–12,725	1122	44.29	10.06	35.59	10.06	ATA	TAA	11
trnS2	J	12,737–12,804	68	32.08	3.77	54.72	9.43			1
trnL1	N	12,806–12,878	73	43.40	3.77	37.74	15.09			404
nad1	N	13,283–14,245	963	47.45	6.46	33.93	12.16	ATT	TAA	37
rrnL	N	14,283–15,646	1364	40.69	4.77	44.13	10.41			71
trnV	N	15,718–15,788	71	47.17	1.89	47.17	3.77			1
rrnS	N	15,790–16,551	762	41.31	4.84	42.75	11.11			0
A + T-rich region		16,552–17,137		32.42	0.34	67.24	0			

In strand column, J indicates the majority strand while N stands for the minority strand.

Table 4
Codon usage in the mitochondrial genome of *Dolichovespula panda*.

AA	Codon	No.	RSCU	AA	Codon	No.	RSCU	AA	Codon	No.	RSCU
Phe	UUU	397	1.9	Ser	UCU	82	1.96	Tyr	UAU	174	1.91
	UUC	20	0.1		UCC	1	0.02		UAC	8	0.09
Leu	UUA	469	4.95		UCA	153	3.65	Cys	UGU	38	2
	UUG	27	0.28		UCG	2	0.05		UGC	0	0
	CUU	39	0.41	Pro	CCU	61	2.22	His	CAU	62	1.91
	CUC	1	0.01		CCC	3	0.11		CAC	3	0.09
	CUA	32	0.34		CCA	46	1.67	Gln	CAA	51	1.92
	CUG	1	0.01		CCG	0	0		CAG	2	0.08
Ile	AUU	459	1.94	Thr	ACU	60	1.88	Asn	AAU	231	1.83
	AUC	15	0.06		ACC	0	0		AAC	21	0.17
Met	AUA	332	1.91		ACA	68	2.13	Lys	AAA	142	1.92
	AUG	15	0.09		ACG	0	0		AAG	6	0.08
Val	GUU	79	2.57	Ala	GCU	45	2.57	Asp	GAU	55	1.86
	GUC	1	0.03		GCC	2	0.11		GAC	4	0.14
	GUA	42	1.37		GCA	23	1.31	Glu	GAA	74	1.92
	GUG	1	0.03		GCG	0	0		GAG	3	0.08
Arg	CGU	10	0.98	Ser	AGU	27	0.64	Gly	GGU	51	1.23
	CGC	0	0		AGC	1	0.02		GGC	1	0.02
	CGA	30	2.93		AGA	67	1.6		GGA	110	2.65
	CGG	1	0.1		AGG	2	0.05		GGG	4	0.1
Trp	UGA	82	1.93								
	UGG	3	0.07								

AA: amino acid; No.: number; RSCU: relative synonymous codon usage.

trnV and the A + T-rich region, with the *rrnL* upstream *rrnS* between *nad1* and *trnV*.

Gene rearrangements

According to the location of rearranged genes, the rearrangement events were classified into transposition, gene shuffling, local inversion or remote inversion (Dowton and Austin, 1999; Dowton et al., 2003). At least five rearrangement events occurred in the *D. panda* mitochondrial genome (Fig. 1) compared with the putative ancestral arrangement of the mitochondrial genome in insects: translocation of *trnY* to upstream of *trnI* (as in *Vespa mandarinia* Smith), translocation of *trnL1* to the region between *trnS2* and *nad1* (as in *Vespa* and *Polistes*), shuffling of *trnN* and *trnE*, shuffling of *trnQ* and *trnM* and reversal of *trnS1*. The reversal of *trnS1* does not occur in any other species used in this study. The protein-coding gene rearrangement in other species from the Vespidae (Chen et al., 2016b) has not been found in the *D. panda* mitochondrial genome.

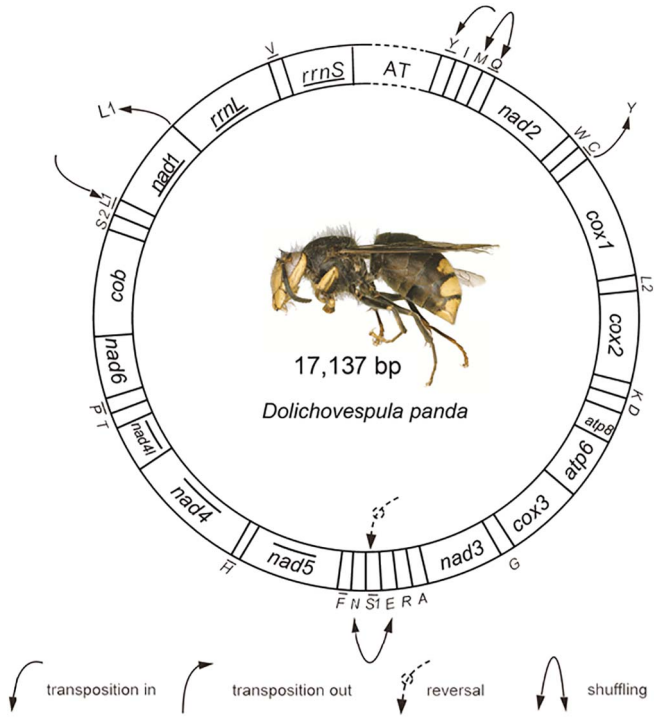

Figure 1. Mitochondrial genome map of *Dolichovespula panda*. Five gene rearrangement events were indicated in the genome map. AT indicates the A + T-rich region. Dashed lines indicates the unsequenced region. Underlined genes are coded on the minority strand, while the others are coded on the majority strand.

Phylogenetic relationships

A phylogenetic tree for the Vespidae was reconstructed using 13 protein-coding genes from the mitochondrial genomes (Fig. 2). Vespidae sp. MT 2014 was identified as a species from the genus of *Polistes* based on phylogenetic analysis (Song et al., 2016a; Zhou et al., 2016). By adding the species of *D. panda* in the Vespidae, our study corroborates a relationship of Eumeninae + (Polistinae + Vespinae) within the Vespidae. The phylogenetic analysis showed a sister group relationship between *Vespa* (*V. mandarinia* + *V. bicolor* Fabricius) and *Vespula* (*V. germanica* (Fabricius)), a relationship also found by other researchers based on mitochondrial genomes (Chen et al., 2016a; Chen et al., 2016b; Zhou et al., 2016) and wing venation (Perrard et al., 2016).

Conclusions and discussion

The mitochondrial genome of *D. panda* was successfully sequenced for the first time. Five rearrangement events were recognized in the *D. panda* mitochondrial genome. Phylogenetic analysis based on mitochondrial genome sequences showed that *Vespa* is more closely related to *Vespula* than to *Dolichovespula*, which is different from the result of Lopez-Osorio et al. (2017) based on transcriptomic data (Lopez-Osorio et al., 2017), but congruent with Perrard et al. (2016). This study was challenging the result in which yellowjackets (*Vespula* and *Dolichovespula*) is a monophyletic group (Carpenter, 1987). Mitochondrial genomes from more species are needed to validate the phylogenetic relationships within Vespidae.

Conflicts of interest

The authors declare no conflict of interest.

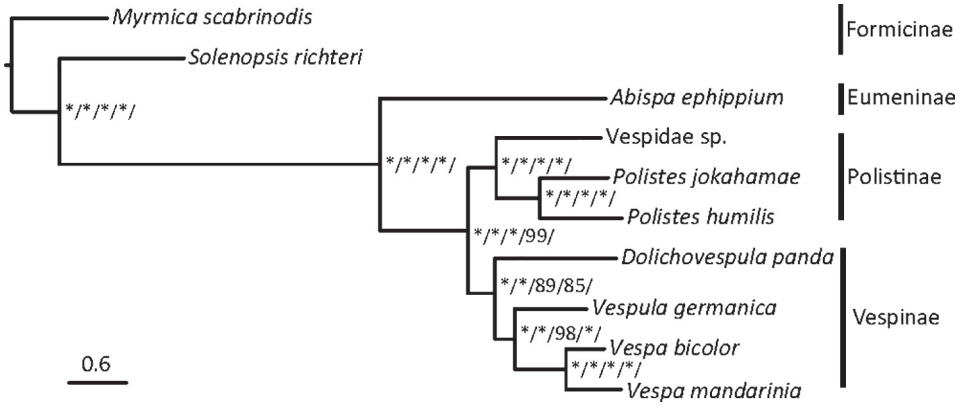

Fig. 2. Phylogenetic relationships within Vespidae based on nucleotide sequences and amino acid sequences of 13 protein-coding genes based on Bayesian inference and Maximum Likelihood methods. Values separated by "/" near the corresponding nodes indicates the posterior probabilities of Bayesian inferences based on nucleotide and amino acid sequences and bootstrap values of ML trees based on nucleotide and amino acid sequences, respectively. * indicates the full support of a node by the corresponding method.

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