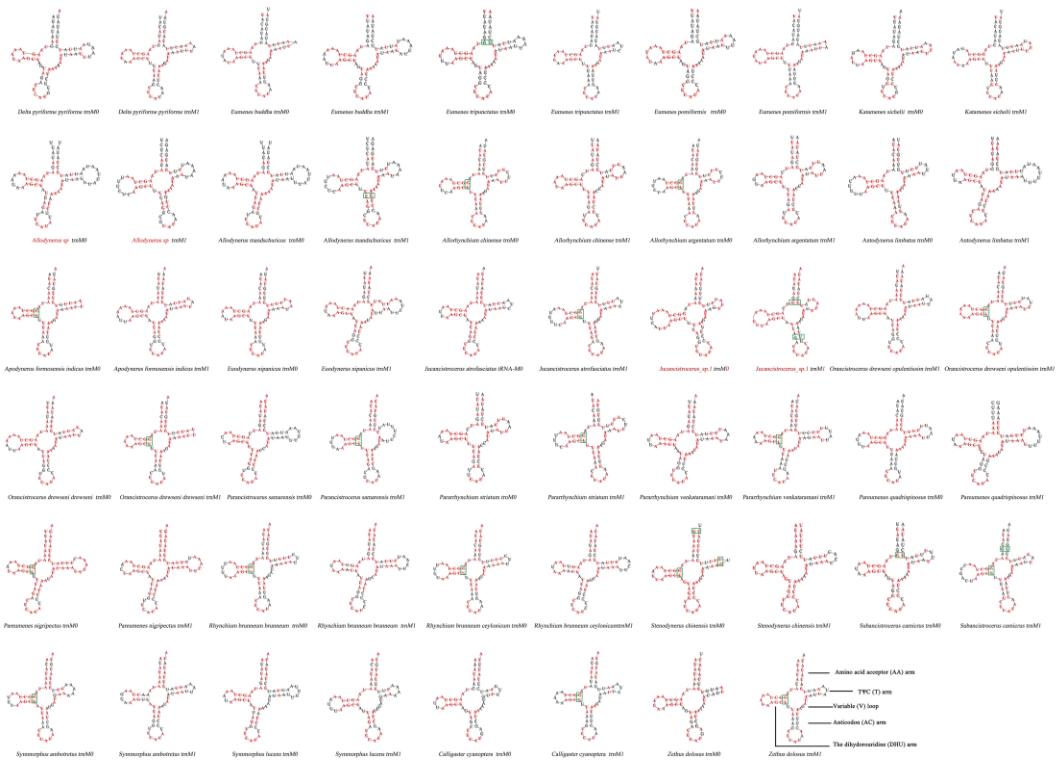


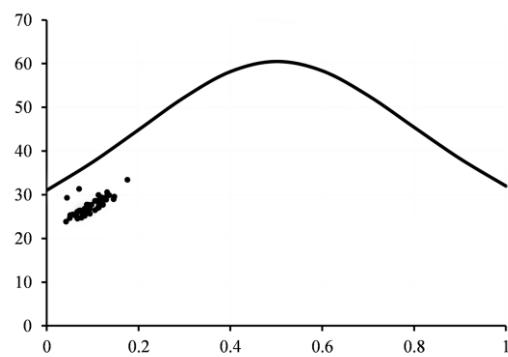
## Ancestral

*Delta campaniforme esuriens*  
*Delta pyriforme pyriforme*  
*Eumenes buddha Cameron*  
*Eumenes tripunctatus*  
*Eumenes pomiformis*  
*Katamenes sichelii sichelii*  
*Oreumenes decoratus*  
*Abispa ephippium*  
*Allodynerus delphinalis*  
*Allodynerus mandschuricus*  
*Allorhynchium chinense*  
*Allorhynchium radiatum*  
*Allorhynchium argentatum*  
*Ancistrocerus renimaculus*  
*Ancistrocerus tussaci*  
*Antepipona sp*  
*Antepipona ovalis*  
*Anterhynchium abdominale*  
*Anterhynchium coracinum*  
*Anterhynchium flavomarginata*  
*Anterhynchium mellyi*  
*Apodynerus protuberantus*  
*Ectopiglossa sanban*  
*Ectopiglossa sublaevis*  
*Ectopiglossa taiwana*  
*Euodynerus dantici*  
*Euodynerus nipanicus*  
*Jucancistrocerus atrofasciatus*  
*Jucancistrocerus angustifrons*  
*Labus pusillus*  
*Labus angularis*  
*Leptochilus sp*  
*Orancistrocerus aterrimus*  
*Orancistrocerus drewseni*  
*Paralepomenes sp*  
*Parancistrocerus samarensis*  
*Pararrhynchium striatum*  
*Pararrhynchium septemfasciatum*  
*Pareumenes quadrispinosus*  
*Pseudepipona kozhevnikovi*  
*Pseudepipona przewalskyi*  
*Pseudozumia indosinensis*  
*Pseumenes depressus*  
*Rhynchium quinquecinctum*  
*Rhynchium brunneum brunneum*  
*Rhynchium brunneum ceylonicum*  
*Stenodynerus frauenfeldi*  
*Stenodynerus chinensis*  
*Subancistrocerus sichelii*  
*Subancistrocerus camicrus*  
*Symmorphus ambotretus*  
*Symmorphus lucens*  
*Calligaster cyanoptera*  
*Discoelius zonalis*  
*Zethus dolosus*

**Figure S1.** The gene order of eumenine mitogenomes used in this study.



**Figure S2.** Inferred secondary structures of duplicated *trnM*. The substitutions in *trnM0* and *trnM1* compared with each other are indicated by red color.



**Figure S3.** ENC-GC<sub>3s</sub> plot of total PCGs in 54 eumenine mitogenomes, the black curve shows the relationship between ENC values and GC<sub>3s</sub> under random codon usage assumption.

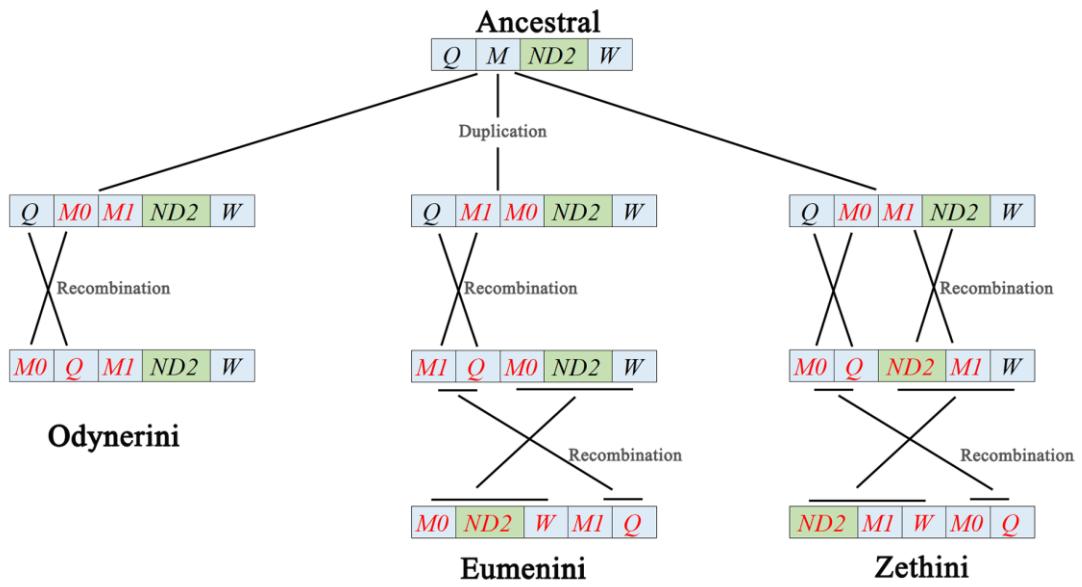
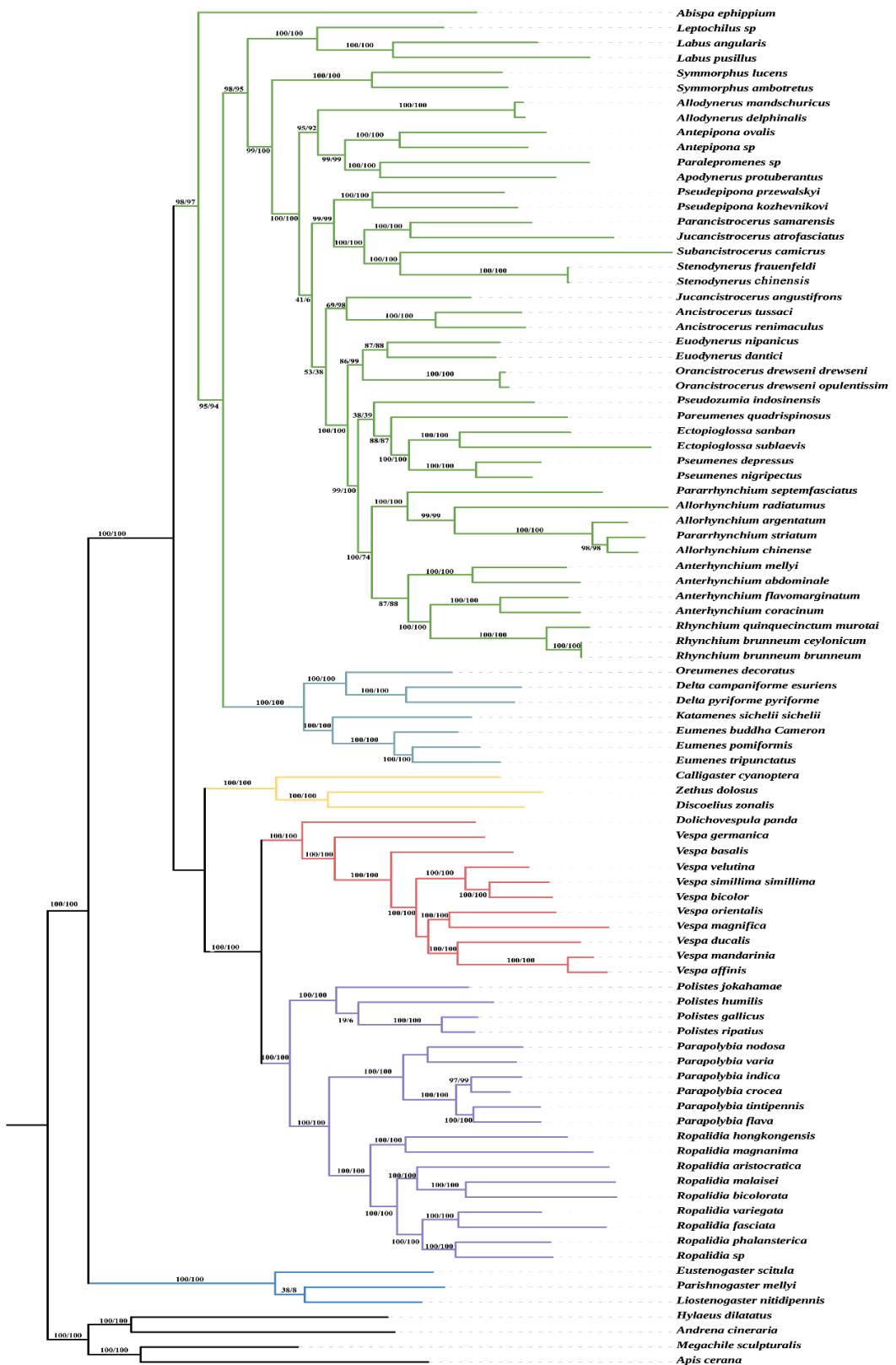


Figure S4. The hypothesized pathway of the translocated inversion derived by recombination and duplication in three tribes of the subfamily Eumeninae. The red genes represent their positions changed.



**Figure S5.** Phylogenetic trees of the Vespidae inferred from PCG and PCGR by ML. Each nod shows the bootstrap support (BS) values.



**Figure S6.** Phylogenetic trees of the Vespidae. A): Phylogenetic tree of the Vespidae inferred from PCG by MP. B): Phylogenetic tree of the Vespidae inferred from PCGR by MP. Each nod shows the bootstrap support values.

**Table S1.** Mitochondrial genomes used for phylogenetic analysis in this study.

Family	Subfamily	Species	Accession No.	Group
Vespidae	Stenogastrinae	<i>Eustenogaster scitula</i>	KY856830	Ingroup
		<i>Liostenogaster nitidipennis</i>	MH910629	
		<i>Parishnogaster mellyi</i>	MH910630	
	Vespinae	<i>Vespa germanica</i>	KR703583	
		<i>Vespa mandarinia</i>	NC_027172	
		<i>Vespa bicolor</i>	KJ735511	
		<i>Vespa velutina</i>	NC_035146	
		<i>Vespa orientalis</i>	KY563657	
		<i>Vespa magnifica</i>	MT137097	
		<i>Vespa ducalis</i>	KX950825	
		<i>Vespa basalis</i>	MK440075	
		<i>Vespa affinis</i>	NC_039134	
		<i>Vespa simillima simillima</i>	NC_046020	

		<i>Dolichovespula panda</i>	NC_036067	
		<i>Ropalidia</i> sp	MK034142	
		<i>Ropalidia phalansterica</i>	MK034143	
		<i>Ropalidia bicolorata</i>	MK034144	
		<i>Ropalidia fasciata</i>	MK034145	
		<i>Ropalidia magnanima</i>	MK034146	
		<i>Ropalidia hongkongensis</i>	MK034147	
		<i>Ropalidia variegata</i>	MK034148	
		<i>Ropalidia malaisei</i>	MK034149	
		<i>Ropalidia aristocratica</i>	MK034150	
	Polistinae	<i>Parapolybia varia</i>	MH065751	
		<i>Parapolybia tintipennis</i>	MH065754	
		<i>Parapolybia flava</i>	MH065755	
		<i>Parapolybia nodosa</i>	MH065756	
		<i>Parapolybia indica</i>	MH065757	
		<i>Parapolybia crocea</i>	KY679828	
		<i>Polistes humilis</i>	EU024653	
		<i>Polistes jokahamae</i>	KR052468	
		<i>Polistes riparius</i>	LC519884	
		<i>Polistes gallicus</i>	ON017795	
		<i>Abispa ephippium</i>	EU302588	
		<i>Allodynerus delphinialis</i>	ON024142	
		<i>Allodynerus mandschuricus</i>	ON012816	
		<i>Allorhynchium chinense</i>	MK051021	
		<i>Allorhynchium argentatum</i>	MK051022	
	Eumeninae	<i>Allorhynchium radiatumus</i>	ON055163	
		<i>Ancistrocerus renimaculus</i>	ON045342	
		<i>Ancistrocerus tussaci</i>	ON012815	
		<i>Antepipona</i> sp	ON012817	
		<i>Antepipona ovalis</i>	ON012818	
		<i>Anterhynchium abdominale</i>	MK051029	
		<i>Anterhynchium coracinum</i>	MK051028	
		<i>Anterhynchium flavomarginatum</i>	MK051026	
		<i>Anterhynchium mellyi</i>	ON012812	
		<i>Apodynerus protuberantus</i>	ON045341	
		<i>Calligaster cyanoptera</i>	ON012814	
		<i>Delta pyriforme pyriforme</i>	ON076029	
		<i>Delta campaniforme esuriens</i>	ON055486	
		<i>Discoelius zonalis</i>	ON076025	
		<i>Eumenes buddha Cameron</i>	ON076024	
		<i>Eumenes tripunctatus</i>	ON045343	
		<i>Eumenes pomiformis</i>	ON076031	
		<i>Ectopioglossa sublaevis</i>	ON045340	

		<i>Ectopioglossa sanban</i>	ON012813	
		<i>Euodynerus dantici</i>	ON076022	
		<i>Euodynerus nipanicus</i>	ON076021	
		<i>Jucancistrocerus atrofasciatus</i>	ON045348	
		<i>Jucancistrocerus angustifrons</i>	ON012819	
		<i>Katamenes sichelii sichelii</i>	ON076027	
		<i>Labus pusillus</i>	ON076026	
		<i>Labus angularis</i>	ON076030	
		<i>Leptochilus sp</i>	ON045339	
		<i>Orancistrocerus aterrimus aterrimus</i>	KY941926	
		<i>Orancistrocerus drewseni drewseni</i>	ON045338	
		<i>Oreumenes decorates</i>	ON076028	
		<i>Paralepromenes sp</i>	ON045337	
		<i>Parancistrocerus samarensis</i>	ON076023	
		<i>Pararrhynchium striatum</i>	ON045347	
		<i>Pararrhynchium septemfasciatus</i>	ON055487	
		<i>Pareumenes quadrispinosus acutus</i>	ON076020	
		<i>Pseudepipona kozhevnikovi</i>	ON076019	
		<i>Pseudepipona przewalskyi</i>	ON024141	
		<i>Pseudozumia indosinensis</i>	ON045335	
		<i>Pseumenes nigripectus</i>	ON045336	
		<i>Pseumenes depressus</i>	ON045346	
		<i>Rhynchium quinquecinctum murotai</i>	MK051030	
		<i>Rhynchium brunneum brunneum</i>	MK051031	
		<i>Rhynchium brunneum ceylonicum</i>	MK051032	
		<i>Stenodynerus frauenfeldi</i>	ON045334	
		<i>Stenodynerus chinensis</i>	ON045345	
		<i>Subancistrocerus camicrus</i>	ON045344	
		<i>Symmorphus ambotretus</i>	ON076018	
		<i>Symmorphus lucens</i>	ON076017	
		<i>Zethus dolosus</i>	ON076016	
Apoidea	Megachilidae	<i>Megachile sculpturalis</i>	NC028017	Outgroup
	Andrenidae	<i>Andrena cineraria</i>	KT164628	
	Colletidae	<i>Hylaeus dilatatus</i>	NC026468	
	Apidae	<i>Apis cerana</i>	NC014295	

**Table S2.** The best partitioning scheme selected by PartitionFinder for different data matrices.

Data matrices	Optimal Partition	Model	Initial Partition
PCG-codon partition	Partition 1	GTR+I+G	<i>a6p1, c1p1, cbp1, c3p1, a8p1, c2p1, n1p1, n5p1, n4p1, n4lp1</i>
	Partition 2	GTR+I+G	<i>c1p3, n3p3, c2p3, c3p3, cbp3, a6p3, a8p3, n1p3, n4p3, n5p3</i>
	Partition 3	GTR+I+G	<i>a8p2, n4p2, n1p2, n5p2, a6p2, n3p2, c1p2, c2p2, c3p2, cbp2</i>
	Partition 4	GTR+G	<i>n2p1, n6p2, n2p2, n3p1, n6p1, n4lp2, n2p3, n6p3, n4lp3</i>
PCGR-codon partition	Partition 1	GTR+I+G	<i>c3p2, cbp2, a6p2, a8p2, n4p2, n1p2, c1p2, n3p2, c2p2, n5p2</i>
	Partition 2	GTR+I+G	<i>a6p1, c3p1, a8p1, c2p1, c1p1, cbp1, n1p1, n5p1, n4p1</i>
	Partition 3	GTR+I+G	<i>c3p3, cbp3, a6p3, a8p3, n4p3, n1p3, c1p3, n3p3, c2p3, n5p3</i>
	Partition 4	GTR+G	<i>n3p1, n6p1, n4lp1, n2p1, n6p2, n2p2, n4lp2, n6p3, n4lp3, n2p3</i>
	Partition 5	GTR+G	<i>rrnl, rrns</i>

*a6/8: atp6/8, c1/2/3: cox1/2/3, n1/2/3/4/4l/5/6: nd1/2/3/4/4l/5/6, cb: cyt b*

**Table S3.** Base composition, total length (bp) and AT-skew of complete Eumeninae mitogenomes.

Species	bp	A	C	G	T	GC%	AT%	AT-skew
<i>Allodynerus delphinalis</i>	16932	40.3	11.7	5.9	42.1	17.6	82.4	-0.022
<i>Allodynerus mandschuricus</i>	17449	40.3	11.5	5.9	42.2	17.4	82.5	-0.023
<i>Allorhynchium chinense</i>	16909	39	11.1	6.1	43.8	17.2	82.8	-0.058
<i>Allorhynchium argentatum</i>	17972	38.8	11.5	6.3	43.4	17.8	82.2	-0.056
<i>Antepipona</i> sp	19040	41.6	11.7	6.7	40	18.4	81.6	0.020
<i>Anterhynchium mellyi</i>	18692	39.9	11.8	7	41.4	18.8	81.3	-0.018
<i>Calligaster cyanoptera</i>	16316	40.9	11.9	6.4	40.8	18.3	81.7	0.001
<i>Discoelius zonalis</i>	15435	41.5	14.2	6.6	37.7	20.8	79.2	0.048
<i>Eumenes pomiformis</i>	16520	41.8	9.8	5.5	42.9	15.3	84.7	-0.013
<i>Ectopioglossa sanban</i>	16454	41.1	12.3	6.8	39.7	19.1	80.8	0.017
<i>Euodynerus dantici</i>	17493	40	10.2	6.7	43.1	16.9	83.1	-0.037
<i>Euodynerus nippanicus</i>	22088	39.1	9.8	7.7	43.4	17.5	82.5	-0.052
<i>Jucancistrocerus atrofasciatus</i>	18848	37.7	15	7.5	40.2	22.5	77.9	-0.032
<i>Jucancistrocerus angustifrons</i>	19867	39.5	9.3	6.6	44.6	15.9	84.1	-0.061
<i>Oreumenes decorates</i>	15563	42.4	9.5	5.9	42.2	15.4	84.6	0.002
<i>Parancistrocerus samarensis</i>	17773	39.5	11.3	6.3	42.9	17.6	82.4	-0.041
<i>Pararrhynchium striatum</i>	20403	37.1	10.5	8.4	44	18.9	81.1	-0.085
<i>Para.septemfasciatus</i>	18003	39.6	11.8	8.3	40.3	20.1	79.9	-0.008
<i>Pareumenes quadrifasciatus</i>	17426	40.9	11.6	8.2	39.2	19.8	80.1	0.021
<i>Pseudepipona przewalskyi</i>	20281	38.1	8.8	8.4	44.7	17.2	82.8	-0.080
<i>Pseumenes nigripectus</i>	17773	39.6	11.4	5.9	43.1	17.3	82.7	-0.042
<i>Pseumenes depressus</i>	16677	43.2	11.2	6.1	39.6	17.3	82.8	0.043
<i>Rhynchium brunneum</i>	23251	36.7	9.3	7.7	46.3	17	83	-0.116
<i>Rh. brunneum ceylonicum</i>	23122	36.8	9.4	7.6	46.2	17	83	-0.113
<i>Stenodynerus chinensis</i>	17194	40.1	12.8	6.3	40.9	19.1	81	-0.010
<i>Subancistrocerus camicrus</i>	18035	39	14.7	6.7	39.6	21.4	78.6	-0.008
<i>Symmorphus ambotretus</i>	17280	40.2	14.3	6.5	39	20.8	79.2	0.015
<i>Symmorphus lucens</i>	17865	40	13.7	6.3	40.1	20	80.1	-0.001
<i>Zethus dolosus</i>	16306	41.1	13.3	6.5	39.1	19.8	80.2	0.025

**Table S4.** Substitution saturation test results.

Data partition	Iss	Iss.cSym†	Psym‡	Iss.cAsym§	Pasym¶
PCG	0.387	0.7532	0.0000	0.5684	0.0000
PCG+RNA	0.342	0.7489	0.0000	0.5640	0.0000

†Index of substitution saturation assuming a symmetrical true tree.

‡Probability of significant difference between Iss and Iss.cSym (two-tailed test).

§Index of substitution saturation assuming an asymmetrical true tree.

¶Probability of significant difference between Iss and Iss.cAsym (two-tailed test).