

# Molecular Systematics of the Issidae (Hemiptera: Fulgoroidea) from China Based on *Wingless* and *18S rDNA* Sequence Data

Yanchun SUN, Rui MENG, Yinglun WANG<sup>①</sup>

Key Laboratory of Plant Protection Resources and Pest Management of the Ministry of Education; Entomological Museum, Northwest A&F University, Yangling, Shaanxi 712100, China

**Abstract:** In this study, phylogenetic relationships of 33 species belonging to 19 genera of Issidae and 1 outgroup (*Paravarcia decapterix*) were analyzed using maximum parsimony (MP) and Bayesian inference (BI) methods based on partial sequences of the nuclear *18S rDNA* and *wingless* genes (*Wg*). The topologies of the phylogenetic trees generated from different methods were quite similar. The phylogenetic analysis divided Issidae into five subfamilies: Caliscelinae, Tonginae, Parahiraciinae, Hemisphaeriinae and Issinae. The results also supported that the genus *Eusudasina* should be transferred from Issinae to Hemisphaeriinae.

**Key words:** Fulgoromorpha; Phylogeny; topology

基于 *18S rDNA* 和无翅基因的中国瓢蜡蝉科分子系统发育关系研究 (半翅目: 蜡蝉总科)

孙艳春, 孟 瑞, 王应伦<sup>①</sup>

植保资源与病虫害治理教育部重点实验室, 西北农林科技大学昆虫博物馆, 陕西 杨凌 712100

**摘要:** 研究测定了 19 属 33 种中国瓢蜡蝉和 1 种外群 (*Paravarcia decapterix*) 的 *18S rDNA* 及无翅基因 (*Wg*) 部分 DNA 序列, 并利用最大简约法 (maximum parsimony, MP) 和贝叶斯法 (bayesian inference, BI) 进行系统发育关系分析。研究结果表明: 采用最大简约法和贝叶斯法 2 种分析方法获得的瓢蜡蝉科 (Issidae) 系统发育树的拓扑结构基本一致, 将该科分为 5 个亚科: 杯瓢蜡蝉亚科 (Caliscelinae)、汤瓢蜡蝉亚科 (Tonginae)、帕瓢蜡蝉亚科 (Parahiraciinae)、球瓢蜡蝉亚科 (Hemisphaeriinae) 和瓢蜡蝉亚科 (Issinae)。并支持将角唇瓢蜡蝉属 (*Eusudasina*) 归入球瓢蜡蝉亚科 (Hemisphaeriinae)。

**关键词:** 蜡蝉次目; 系统发育; 拓扑结构

## Introduction

The family Issidae Spinola *s. l.* is one of the largest families of fulgoroid planthoppers with approximately 270 genera and 1200 species. All species in this family are phytophagous, of which some are important pests of fruits and damage crops by sucking and spawning (Chou *et al.* 1985; Yan *et al.* 2005; Che 2006; Meng *et al.* 2011).

The Issidae was first established as a subfamily of the family Fulgoridae in 1839 by Spinola. Schaum (1850) promoted it to the family level. The history of notions about supergeneric system of the family Issidae has more than one hundred years (Spinola 1839;

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<sup>①</sup>Corresponding author, E-mail: yinglunw@nwsuaf.edu.cn

Distant 1906, 1916; Kirkaldy 1906, 1907; Melichar 1906; Fennah 1954; Muir 1923, 1930; Schaum 1850; Chan & Yang 1994). Melichar (1906) considered three subfamilies in the family Issidae (Issinae Spinola, Hysteropterinae Melichar, and Thioniinae Melichar) and treated Calicelidae Amyot & Serville and Hemisphaeriidae Melichar as separate families by morphological characteristics of the body configuration, the relative length of fore wing, fore wing with claval suture or without, and protibiae of the male expanding foliaceous or not. According to the classification system proposed by Muir (1930), the family Issidae consists of three subfamilies: Issinae, Caliscelinae and Hemisphaeriinae. Muir (1930) recognized in the subfamily Issinae three tribes: Issini, Hysteropterini and Thioniini. However, Fennah (1954) included in Issidae five subfamilies: Issinae, Tonginae, Trinopinae, Caliscelinae, and Acanaloniinae on base of synthetical characteristics. Metcalf (1958) revised the classification system of family Issidae proposed by Melichar(1906), Muir (1923, 1930) and Fennah (1954), divided the family Issidae into five subfamilies and six tribes: Caliscelinae (Ahomocnemiellini, Caliscelini, Omamatidiotini), Hemisphaerinae, Issinae (Hysteropterini, Issini, Thionini) and Tonginae.

Since then, classification and systematics of the family Issidae has undergone considerable changes which included some genera and species previously listed within Issidae (Metcalf 1958) were transferred to the other family or that previously listed within the other family transferred to Issidae. Relevant researches mainly included the following aspects in the group.

Dlabola(1980) erected a tribe Adenissini in the subfamily Issinae for the genera *Adenissus* Linnavuori, 1973 and *Anissussis* Dlabola, 1980. Cheng & Yang (1991) on the basis of larval features erected the subfamily Paraheiaciinae for the genus *Parahiracia* Ôuchi, 1940, which was transferred to Issidae from Tropicuchidae by Fennah (1982) and was downgraded to tribe by Gnezdilov (2003a). According to the phylogenetic research of Issidae, Emeljanov (1990) considered the family Issidae *s. l.* included Issinae, Tonginae, Trienoppinae, Caliscelinae, Acanaloniinae, and Bladininae, and upgraded Acanaloniinae Amyot et Serville and Caliscelinae Amyot et Serville to family level. However, a study of the phylogenetic relationship of 38 genera of Issidae from China based on morphological characteristics supported that Caliscelinae was a subfamily of the family Issidae (Che 2006).

Gnezdilov (2002) treated Hysteropterini as a subtribe of the Issini and erected a new subtribe Agalmatiina after the study of the ovipositor structure. Soon after, he erected again a tribe Colpopterini based on the features of the ovipositor structure and hind wings and arranged in the subfamily Issinae, and definitely proposed that the family Issidae *s. str.* included the nominotypical subfamily Issinae with five tribes Hemisphaeriini Melichar, Parahiraciini Cheng & Yang, Issini Spinola, Thioniini Melichar and Colpopterini Gnezdilov (Gnezdilov 2003a). The Thioniini was erected by Melichar (1906), as a subfamily Thioniinae of the family Issidae, based on a well developed trilobed hind wings but the taxon was treated as a tribe in subfamily Issinae by (Muir 1930; Gnezdilov 2003a), according to the relevant research, the condition of the hind wing cannot be treated as apomorphies of Issini and Thioniini, thus, the tribe Thioniini Melichar, 1906 was treated as a junior synonym of Issini Spinola, 1839 (Gnezdilov 2009; Gnezdilov & Fletcher 2010). Gnezdilov and Wilson (2006) transferred the tribe Adenissini Dlabola and the genera *Pterilia* Stål and *Coinquenda* Distant from Issidae to Calicelidae and proposed a tribal system of the family. The subfamily

Trienopinae Fennah, 1954 was transferred to the family Tropicuchidae Stål, 1866, while the subfamily Tonginae Kirkaldy, 1907 and the tribe Colpopterini Gnezdilov, 2003 were again transferred to the family Nogodinidae Melichar, 1898 with their ranks changed to those of a tribe and a subfamily by male and female genitalia characteristics (Gnezdilov 2007, 2012, 2013). Recently Gnezdilov (2013) suggested the scope of the family Issidae *s. str.* was reduced to three tribes: Issini Spinola, 1839; Hemisphaeriini Melichar, 1906; Parahiraciini Cheng et Yang, 1991, included into the nominotypical subfamily with 161 genera and 997 species and subspecies.

From the above it is not difficult to see that there are many different opinions about the classification of the family Issidae based on morphological data, even same author has different views in the different periods on a group. Meanwhile, recent studies using molecular evidence about phylogenetic analysis of Fulgoroidea also questioned the monophyly of Issidae (Bourgoin *et al.* 1997; Yeh *et al.* 1998, 2005; Urban & Cryan 2007; Song & Liang 2013).

The *18S rDNA* gene is a commonly used marker in molecular phylogenetic investigations due to its universality and evolutionary rate heterogeneity. This gene is easily detected for most insect taxa and therefore often used to address relationships among a wide variety of taxonomic levels (Hillis & Dixon 1991; Hamby & Zimmer 1992; Cryan *et al.* 2000). Similarly, the nuclear protein coding gene *wingless* is useful for phylogenetic analysis of insect relationships at levels of divergence from species to subfamilies (Brower *et al.* 1998).

At the present time, molecular study has not yet been conducted within the family Issidae. To go further in our understanding of the phylogenetic relationships of the family and to better assess the monophyly of the family, the subfamilies, we carried molecular phylogenetic analyses using data from the partial sequences of the *18S rDNA* and the *wingless* gene in 33 species belonging to 19 genera of Issidae and 1 outgroup (*Paravaricia decapterix*). The phylogenetic relationships among taxa were reconstructed using both BI and MP inference methods. The main questions we intend to answer in this paper are: (1) how many subfamilies of Issidae are there? and (2) are the subfamilies monophyletic?

## Material and methods

### Taxon sampling

Insect specimens (Table 1) were collected and immersed in 95–100% ethanol and were stored at  $-20^{\circ}\text{C}$  in the Entomological Museum, Northwest A&F University. The 33 ingroup specimens included in this analysis represent 19 genera of Issidae, and 1 species of Nogodinidae, which is a sister group of Issidae, was chosen as outgroup taxon in consideration of Urban and Cryan (2007) and Gnezdilov (2013b).

### DNA extraction and PCR amplification

Genomic DNA was extracted from the legs of adults. Legs were homogenized in a 1.5  $\mu\text{L}$  centrifuge tube in 600  $\mu\text{L}$  extracting solution that contained 0.5M NaCl, 0.1M EDTA, 0.05M Tris (PH = 7.0–8.5), 5% SDS and 2 mg/ml proteinase K. This mixture was incubated at  $50^{\circ}\text{C}$  for 4 hours, then extracted with phenol-chloroform-isoamylol. Extracted crude DNA was precipitated with absolute ethanol and eluted in 800  $\mu\text{L}$  70% ethanol. Finally, the crude DNA was dissolved in 30  $\mu\text{L}$  TE buffer and stored in  $-20^{\circ}\text{C}$  to be used as the DNA template in the

following amplification reaction.

The polymerase chain reaction (PCR) was employed to amplify the *18S rDNA* and *wingless* gene fragments. The primers for amplification the *18S rDNA* gene were designed based on the conserved nucleotide sequences of tettiometrid *T. sulphurea* (Bourgoin *et al.* 1997); the forward primer was 18S-mid, 5'-GAT ACC GCC CTA GTT CTA ACC-3' and the reverse primer was 18S-2200, 5'-CGG CAG GTT CAC CTA CGG-3'. The primers for amplification of the *wingless* gene were developed by Urban and Cryan (2007), and the forward primer was Wg 1A, 5'-GAR TGY AAR TGY CAY GGY ATG TCT GG-3' and the reverse primer was Wg DelR1, 5'-GTC CTG TAR CCR CGK CCA CAA CAC AT-3'.

Amplification was carried out for 35 cycles in a 25  $\mu$ L volume reaction that contained 10 $\times$ PCR buffer, 25 mmol/L MgCl<sub>2</sub>, 2.5 mmol/L of each dNTP, 10  $\mu$ mol/L primers, 5 U/ $\mu$ L Taq polymerase and 1  $\mu$ L DNA template. All PCR sets included a negative control reaction tube in which all reagents were included except the template DNA. And the temperature profile included: initial denaturation for 3 min at 94°C, denaturation for 1 min at 94°C, annealing for 1 min at 51–55°C and extension for 1 min at 72°C. Amplified DNA was visualized using 1% agarose gel electrophoresis with ethidium-bromide staining, and the DNA products were sequenced directly.

#### Sequence alignment and phylogenetic analysis

All sequence chromatogram were visually inspected, edited and assembled in contiguous sequences using BioEdit 7.0.9 (Hall 1999). The *wingless* sequences were further checked after the derived amino acid sequences were obtained. Multiple sequence alignment was performed using Clustal X 1.83 (Thompson *et al.* 1997). Highly variable regions of *18S rDNA* that differed in base composition and sequence length across the sampled issid taxa were excluded from phylogenetic analysis due to the extreme ambiguity involved in any possible alignment, following that of previous studies using this marker (Sanderson & Shaffer 2002; Cryan *et al.* 2004; Urban *et al.* 2010). Within the aligned regions, gaps in *18S rDNA* sequences were coded as missing data. Multiple sequence alignment of the nuclear protein coding gene *wingless* was unambiguous, and no gaps detected. Codon position was determined by comparison of amplified *wingless* sequences with sequences available on GenBank for which the amino acid identity and reading frame had been determined (Urban & Cryan 2007).

Aligned DNA sequences were analyzed using MEGA5.05 (Tamura *et al.* 2011) for calculating the average content of A, T, C, G variable sites, conserved sites and parsimony-informative sites.

Analyses of the combined data were conducted under the criterion of maximum parsimony (MP) using Paup\*4.0b10 (Swofford 2002). Heuristic tree searches were performed using 1000 replications of random additions with the tree bisection and reconnection (TBR) option. Bootstrap analyses were conducted using 1000 standard replicates to provide an estimate of support for individual nodes in parsimony-based strict consensus tree.

Bayesian analysis (BI) was conducted for the combined data using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The best-fitting model SYM+I+G was determined by Modeltest3.7 based on results of the Akaike information criterion (AIC; Akaike 1974). Bayesian analysis was run for 2 million generations, with four chains (one cold and three heated) and flat priors. Trees were sampled at intervals of 100 generations, and a total of

20,000 sampled trees were finally obtained. The first 5000 sampled trees were discarded as burn-in, and the remaining trees were used to construct a 50% majority rule consensus tree.

**Table 1. Taxa sampled and the collected data**

Subfamily	Genus	Species	Collection locality	Accession no.		
				<i>18S rDNA</i>	<i>wingless</i>	
Hemisphaeriinae	<i>Gergithus</i> Stål	<i>Gergithus carbonarius</i>	Hainan: Bawangling	JX196138	JX196179	
		<i>Gergithus spinosus</i>	Hainan: Jianfengling	JX196134	JX196180	
		<i>Gergithus tessellatus</i>	Zhejiang: Fengyangshan	JX196140	JX196185	
	<i>Mongoliana</i> Distant	<i>Mongoliana sinuata</i>	Yunnan: Lvchun	JX196139	JX196181	
		<i>Mongoliana triangularis</i>	Yunnan: Menglun	JX196141	JX196182	
		<i>Mongoliana recurrens</i>	Hunan: Mangshan	JX196137	JX196183	
	<i>Gergithoides</i> Schumacher	<i>Mongoliana serrata</i>	Zhejiang: Qingliangfeng	JX196160	JX196184	
		<i>Gergithoides undulatus</i>	Zhejiang: Qingliangfeng	JX196162	JX196168	
		<i>Gergithoides carinatifrons</i>	Hainan: Bawangling	JX196167	JX196170	
	<i>Neogengithoides</i> Sun <i>et al.</i>	<i>Gergithoides rugulosus</i>	Hunan: Mangshan	JX196163	JX196169	
		<i>Neogengithoides tubercularis</i>	Hainan: Diaoluoshan	JX196166	JX196171	
		<i>Hemisphaerius trilobulus</i>	Yunnan: Longmen	JX196161	JX196187	
	<i>Hemisphaerius</i> Schaum	<i>Hemisphaerius testaceus</i>	Hainan: Jianfengling	JX196135	JX196186	
		<i>Eusudasina nantouensis</i>	Yunnan: Lvchun	JX196136	JX196173	
	<i>Choutagus</i> Zhang <i>et al.</i>	<i>Choutagus longicephalus</i>	Hainan: Jianfengling	JX196154	JX196172	
	Issinae	<i>Eusarima</i> Yang	<i>Eusarima</i> sp.1	Hunan: Mangshan	JX196146	JX196192
			<i>Eusarima</i> sp.2	Hainan: Yinggeling	JX196147	JX196191
			<i>Eusarima</i> sp.3	Hainan: Yinggeling	JX196148	JX196198
			<i>Eusarima</i> sp.4	Hainan: Jianfengling	JX196164	JX196193
<i>Eusarima</i> sp.5			Hainan: Diaoluoshan	JX196145	JX196195	
<i>Eusarima</i> sp.6			Zhejiang: Qingliangfeng	JX196143	JX196194	
<i>Kodaiana</i> Distant		<i>Kodaiana</i> sp.	Fujian: Wuyishan	JX196151	JX196178	
<i>Thabena</i> Stål		<i>Thabena hainanensis</i>	Hainan: Bawangling	JX196152	JX196177	
<i>Kodaianella</i> Distant		<i>Kodaianella damnosus</i>	Yunnan: Yexianggu	JX196156	JX196189	
		<i>Kodaianella bicinctifrons</i>	Zhejiang: Qingliangfeng	JX196155	JX196190	
<i>Jagannata</i> Distant		<i>Jagannata</i> sp. 1	Hainan: Limuling	JX196142	JX196196	
		<i>Jagannata</i> sp. 2	Hainan: Diaoluoshan	JX196157	JX196175	
Parahiracinae		<i>Fortunia</i> Distant	<i>Fortunia byrrhoides</i>	Yunnan: Menglun	JX196158	JX196176
	<i>Flavina</i> Stål	<i>Flavina hainana</i>	Hainan: Jianfengling	JX196159	JX196174	
	<i>Mincopius</i> Distant	<i>Mincopius</i> sp.	Hainan: Yinggeling	JX196150	JX196200	
Caliscelinae	<i>Caliscelis</i> de Laporte	<i>Caliscelis rhabdocladis</i>	Hebei: Langfang	JX196149	JX196201	
	<i>Symplanella</i> Fennah	<i>Symplanella unipuncta</i>	Hainan: Yinggeling	JX196165	JX196188	
Tonginae	<i>Tonga</i> Kirkaldy	<i>Tonga westwoodi</i>	Hainan: Jianfengling	JX196153	JX196199	
Outgroup	<i>Paravarcia</i> Schmidt	<i>Paravarcia decapterix</i>	Hainan: Yinggeling			

## Results and Discussion

### Sequence variation

Approximately 1078 bp in length of the combined *18S rDNA* and *wingless* gene sequence were analyzed for 34 taxa (33 ingroup and 1 outgroup). Among these sites, 221 were variable, 853 were conserved and 120 were parsimony-informative. The average nucleotide composition proportions for the combined data partition were: T, 23.9%; C, 23.5%; A, 25.1%; G, 27.5%. Regression analysis of total substitutions versus transitions (Ts) and transversions (Tv) revealed that the substitution saturation due to multiple hits has not yet occurred in issids examined in this study.

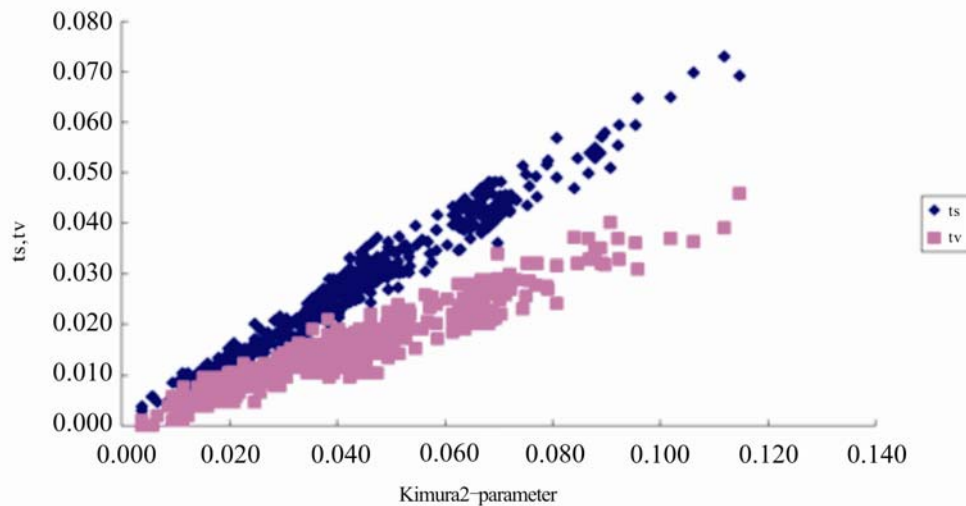


Figure 1. Regression analysis of total substitutions versus transitions (Ts) and transversions (Tv) in the combined genes of *18S rDNA* and *wingless* of Issidae.

### Phylogenetic analysis

Figures 2 and 3 show the phylogram of the issids examined in this study recovered from the MP and Bayesian analyses respectively. Both trees show generally concordant topology in the pattern of main groups representing different subfamilies. The phylogenetic relationships of them show both similarities and differences compared with previous studies based on morphology. Both trees show Issidae is a monophyletic group. The most basal lineage is Caliscelinae Amyot et Serville, as is sister to the remaining subfamilies with strong nodal support. Therefore, the placement of the Caliscelinae as a subfamily within the family Issidae was well supported in this study, herein, consistent with the hypothesis of Metcalf (1958). However, it is clear that these results above do not support Emeljanov's (1999) placement of the Caliscelidae as a family outside of Issidae based on morphology and specifically the female and male genitalia-based hypotheses (Gnezdilov 2003b; Gnezdilov & Wilson 2006).

The subfamily Parahiraciinae, represented in these analyses by three genera, is treated as sister to the Hemisphaeriinae + Tonginae (Figs. 2, 3). This result concurs with that of Cheng and Yang (1991), rather than the placement of the Parahiraciini as a tribe of Issinae as proposed by the ovipositor characteristic-based hypotheses (Gnezdilov 2003).

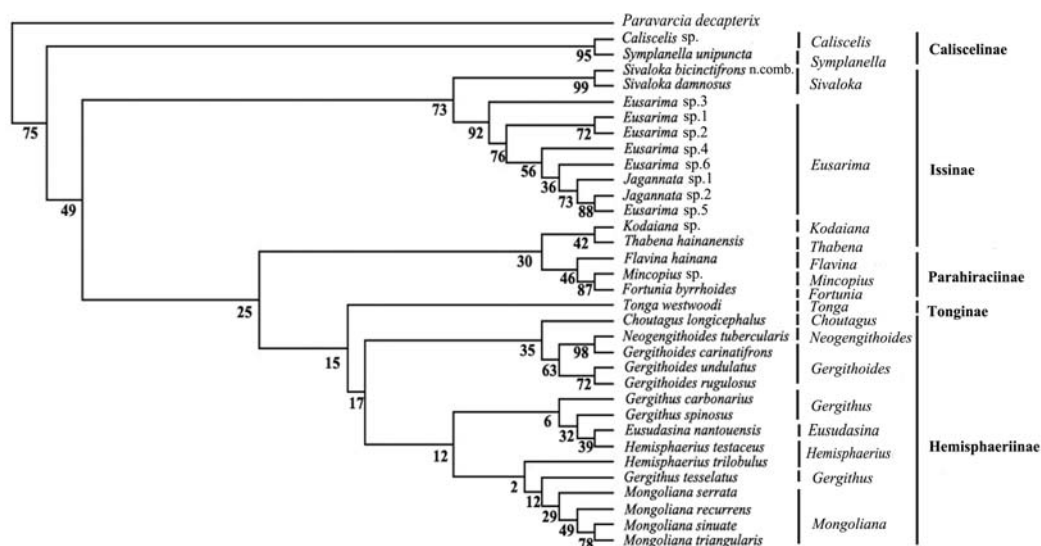


Figure 2. Maximum parsimony tree (MP) based on analysis of combined data *18S rDNA* + *wingless* genes among 33 species of Issidae and Ioutgroup. The number on the node indicates bootstrap value.

Until now, morphological data had been unable to resolve the placement of Tonginae. Tonginae was erected and placed in the family Flatidae due to the unique morphology and its uncertain classification (Kirkaldy 1907). Baker (1927), Fennah (1954) and Metcalf (1958) proposed that Tonginae should be placed in the family Issidae. However, Tonginae was transferred to Nogodinidae based on features of the male and female genitalia (Gnezdilov 2007). In this investigation, the placement of Tonginae as a subfamily of Issidae was supported by both parsimony and Bayesian trees except that the related nodes are lowly supported (Figs. 2, 3).

The monophyly of Issinae was not supported by either parsimony or Bayesian analyses, with the two genera *Kodaiana* and *Thabena*, both formerly included within Issinae, being placed in a clade sister to the parahiraiciid clade in the current study. And Gnezdilov (2003) degraded the subfamily Parahiraicinae to Parahiraiciini as a tribe of the Issinae based on the characters of the ovipositor. So, more detailed investigation is needed to test the monophyly of Issinae and its affinity to Parahiraicinae.

In both parsimony and Bayesian analyses, a *Choutagus* + *Neogengithoides* + *Gergithoides* clade was supported within the Hemisphaeriinae group. Based on morphological characters, they share certain common characters: body hemispherical, median carina in frons and pronotum, wing well-developed, translucent and netlike. Both molecular and morphological data support *Choutagus* Zhang, Wang et Che, *Neogengithoides* Sun, Meng et Wang and *Gergithoides* Schumacher having close relationships.

Three species representing the genus *Gergithus* were mixed with two species which represents the genus *Hemisphaerius* in these analyses. The morphologies used to distinguish these two genera are mainly based on the ratio of the size of tegmen and wing (e.g. wing longer than half length of tegmen in *Gergithus*, but shorter than half length of tegmen in *Hemisphaerius*). However, this feature has limitations in distinguishing between species, and

therefore additional data are clearly needed to more thoroughly resolve placement of the genera *Gergithus* and *Hemisphaerius*.

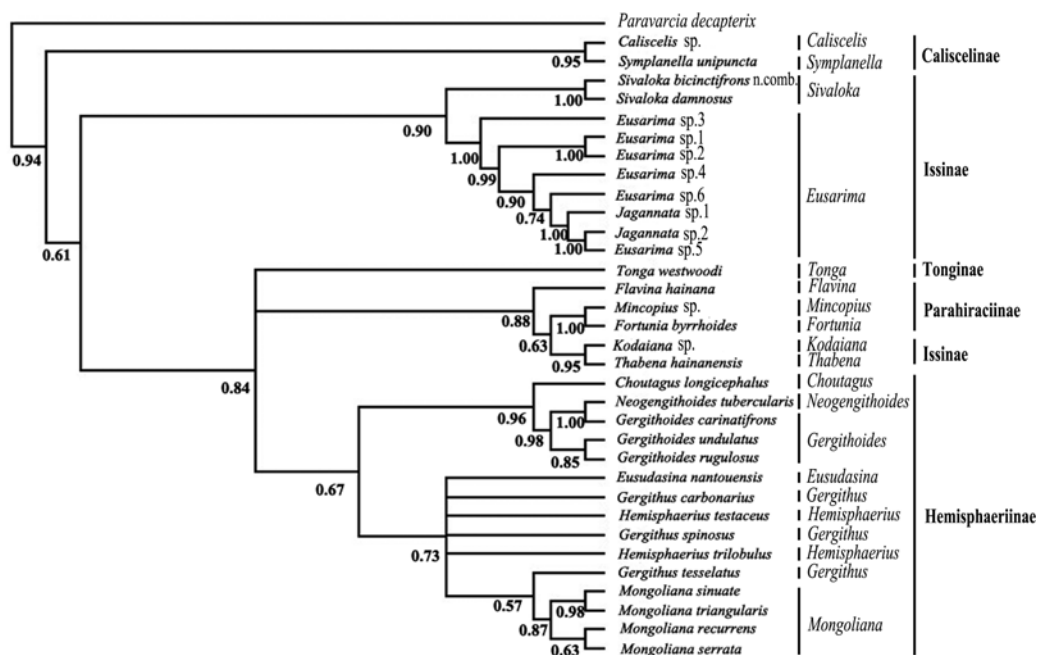


Figure 3. Bayesian tree (BI) resulting from analysis of combined data *18S rDNA* + *wingless* genes among 33 species of Issidae and 1 outgroup. The number on the node indicate posterior probability value.

Both parsimony and Bayesian trees show that *Eusudasina* are sister to the genus *Hemisphaerius* in the Hemisphaeriinae clade which accord with the result of phylogenetic relationship of Issidae from China based on morphological characteristics (Che 2006). This result rejects the hypothesis of putting the *Eusudasina* into Issinae (Yang & Fang 1993).

As previously described, the two species *Jagannata* sp. 1 and *Jagannata* sp. 2 were in the genus *Jagannata*. But, in both parsimony and Bayesian topologies, *Jagannata* sp. 1 and *Jagannata* sp. 2 are placed within the genus *Eusarima*. In reexamining their morphology (tegmen with Sc + R diverging near base, “Y” shorter than clavus, aedeagus somewhat U-shaped with sword processes), their features are more coincidence with the taxonomic characteristics of *Eusarima*. Both molecular and morphological data support *Jagannata* sp. 1 and *Jagannata* sp. 2 belonging within the genus *Eusarima*. Based on molecular data, the genus *Jagannata* Distant is closely related to the genus *Eusarima* Yang, as is different to the results based morphological study. The relationship between these two genera needs to be further addressed based on combined molecular and morphological data.

## Conclusions

The following conclusions were supported after analyzing both parsimony and Bayesian trees constructed based on *18S rDNA* and *wingless* combined data.

- (1) The placement of Caliscelinae as a subfamily of Issidae is strongly supported by the



molecular data.

(2) Subfamily Tonginae is placed within Issidae by both parsimony and Bayesian trees.

(3) The tribe Parahiraciini should be promoted to the subfamily Parahiraciinae outside of Issinae.

(4) *Eusudasina* should be transferred to Hemisphaeriinae and is supported by both parsimony and Bayesian trees.

Our limited taxon sampling only can provide a preliminary phylogenetic picture of Issidae, and the research results confirms that the *18S rDNA* and the *wingless* genes are available in resolving phylogenetic relationships of Issidae. Obviously, further research that integrates more taxon sampling including more outgroups, and data from other molecular markers would provide greater insight into the evolution of Issidae.

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### References

- Akaike H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, 19(6): 716–723.
- Baker CF. 1927. Spolia Mentawiensia: Homoptera-Fulgoroidea. *Philippine Journal of Science*, 32(3): 391–411.
- Bourgoin T, Steffen-Campbell JD & Campbell BC. 1997. Molecular phylogeny of Fulgoromorpha (Insecta, Hemiptera, Archaeorrhyncha). The enigmatic Tettigometridae: evolutionary affiliations and historical biogeography. *Cladistics*, 13(3): 207–224.
- Brower AVZ & Salle RD. 1998. Patterns of mitochondrial versus nuclear DNA sequence divergence among nymphalid butterflies: the utility of wingless as a source of characters for phylogenetic inference. *Insect Molecular Biology*, 7(1): 73–82.
- Chan ML & Yang CT. 1994. *Issidae of Taiwan (Homoptera: Fulgoroidea)*. Chen Chung Book Press, Taipei, 168 pp.
- Che YL. 2006. The taxonomic study and phylogeny of Issidae from China (Hemiptera: Fulgoroidea). Dissertation for Doctor Degree, Northwest A&F University, Yangling, Shaanxi, 245 pp.
- Cheng CL & Yang CT. 1991. Nymphs of Issidae of Taiwan (Homoptera) (II). *Chinese Journal of Entomology*, 11(3): 232–241.
- Chou I, Lu JS, Huang J & Wang SZ. 1985. *Economic Insect Fauna of China, Fasc. 36*. Science Press, Beijing, 152 pp.
- Cryan JR, Wiegmann BM, Deitz LL & Dietrich CH. 2000. Phylogeny of the treehoppers (Insecta: Hemiptera: Membracidae): Evidence from two nuclear genes. *Molecular Phylogenetics and Evolution*, 17(2):

- 317–334.
- Cryan JR, Wiegmann BM, Deitz LL, Dietrich CH & Whiting MF. 2004. Treehopper trees: phylogeny of Membracidae (Hemiptera: Cicadomorpha: Membracoidea) based on molecules and morphology. *Systematic Entomology*, 29: 441–454.
- Distant WL. 1906. *The fauna of British India including Ceylon and Burma. Rhynchota–Vol. III (Heteroptera Homoptera)*. Taylor and Francis, London, 503 pp.
- Distant WL. 1916. *The fauna of British India including Ceylon and Burma. Rhynchota–Vol. VI (Homoptera: appendix)*. Taylor and Francis, London, 115 pp.
- Dlabola J. 1980. Tribus-Einteilung, neue Gattungen und Arten der subf. Issinae in der Eremischen Zone (Homoptera: Auchenorrhyncha). *Sboranik Narodního Muzea v Praze (Rada B, Přírodovědecká)*, 36(4): 173–248.
- Emeljanov AF. 1990. An attempt of construction of a phylogenetic tree of the planthoppers (Homoptera: Cicadina). *Entomologicheskoe Obozrenie*, 69(2): 353–356.
- Emeljanov AF. 1999. Note on delimitation of families of the Issidae group with description of a new species of Caliscelidae belonging to a new genus and tribe (Homoptera: Fulgoroidea). *Zoosystematica Rossica*, 8(1): 61–72.
- Fennah RG. 1954. The higher classification of the family Issidae (Homoptera: Fulgoroidea) with descriptions of new species. *Transactions of the Royal Entomological Society of London*, 105: 455–474.
- Fennah RG. 1982. A tribal classification of the Tropiduchidae (Homoptera: fulgoroidea), with the description of a new species on tea in Malaysia. *Bulletin of Entomological Research*, 72: 631–643.
- Fennah RG. 1987. A recharacterisation of the Ommatidiotini (Hem.–Hom., Fulgoroidea, Issidae, Caliscelinae) with the description of two new genera. *The Entomologist's Monthly Magazine*, 123: 243–247.
- Gnezdilov VM. 2002. Morphology of the ovipositor in the subfamily Issinae (Homoptera, Cicadina, Issidae). *Entomological Review*, 82(8): 957–974.
- Gnezdilov VM. 2003a. A new tribe of the family Issidae with comments on the family as a whole (Homoptera: Cicadina). *Zoosystematica Rossica*, 11(2), 25: 305–309.
- Gnezdilov VM. 2003b. Review of the family Issidae (Homoptera, Cicadina) of the European fauna, with notes on the structure of ovipositor in planthoppers. *Chteniya pamyati N.A. Kholodkovskogo (Meetings in memory of N.A. Cholodkovsky)*, St. Petersburg, 56(1): 1–145.
- Gnezdilov VM. 2007. On the systematic positions of the Bladinini Kirkaldy, Tonginae Kirkaldy, and Trienopinae Fennah (Homoptera, Fulgoroidea). *Zoosystematica Rossica*, 15(2): 293–297.
- Gnezdilov VM. 2009. Revisionary Notes on Some Tropical Issidae and Nogodinidae (Hemiptera: Fulgoroidea). *Acta Entomologica Musei Nationalis Pragae*, 49(1): 75–92.
- Gnezdilov VM. 2012. Revision of the Tribe Colpopterini Gnezdilov, 2003 (Homoptera, Fulgoroidea: Nogodinidae). *Entomologicheskoe obozrenie*, 91(4): 757–774. English translation published in *Entomological Review*, 2013, 93(3): 337–353.
- Gnezdilov VM. 2013a. Modern classification and the distribution of the family Issidae Spinola (Homoptera, Auchenorrhyncha, Fulgoroidea). *Entomologicheskoe obozrenie*, 92(4): 724–738. English translation published in *Entomological Review*, 2014, 94(5): 687–697.
- Gnezdilov VM. 2013b. “Issidisation” of Fulgoroid Planthoppers (Homoptera, Fulgoroidea) as a Case of Parallel Adaptive Radiation. *Entomologicheskoe Obozrenie*, 92(1): 62–69. English translation published in *Entomological Review*, 2013, 93(7): 825–830.
- Gnezdilov VM & Aguin-Pombo D. 2014. A new species of the planthopper genus *Conosimus* associated with an endemic shrub in southern Spain. *Journal of Insect Science*, 14(92): 1–12.
- Gnezdilov VM, Bourgoin T & Soulier-Perkins A. 2014. Vietnamese Issidae (Hemiptera, Fulgoroidea): new taxa, new records and new distribution data. *Zootaxa*, 3847(1): 80–96.

- Gnezdilov VM & Fletcher MJ. 2010. A review of the Australian genera of the planthopper family Issidae (Hemiptera: Fulgoromorpha) with description of an unusual new species of *Chlamydopteryx* Kirkaldy. *Zootaxa*, 2366: 35–45.
- Gnezdilov VM & Wilson MR. 2006. Systematic notes on tribes in the family Caliscelidae (Hemiptera: Fulgoroidea) with the description of new taxa from Palaearctic and Oriental Regions. *Zootaxa*, 1359: 1–30.
- Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41: 95–98.
- Hamby RK & Zimmer E. 1992. Ribosomal RNA as a phylogenetic tool in plant systematics. In: Soltis PS, Soltis DE & Doyle JJ (Eds.), *Molecular Systematics of Plants*. Chapman & Hall, New York, pp. 50–91.
- Hillis DM & Dixon MT. 1991. Ribosomal DNA: Molecular evolution and phylogenetic inference. *The Quarterly Review of Biology*, 66: 411–453.
- Kirkaldy GW. 1906. Leaf-hoppers and their natural enemies (Hemiptera). *Bulletin Division of Entomology. Report of the Work of the Experiment Station of the Hawaiian Sugar Planters' Association*, 1(9): 1–186.
- Kirkaldy GW. 1907. Leafhoppers supplement (Hemiptera). *Bulletin Division of Entomology. Report of the Work of the Experiment Station of the Hawaiian Sugar Planters' Association*, 3: 1–186.
- Melichar DL. 1906. Monographie der Issiden (Homoptera). *Abhandlungen der Zoologisch-Botanischen Gesellschaft in Wien*, 3: 1–327.
- Metcalf ZP. 1958. *General Catalogue of the Homoptera. Fasc. IV Fulgoroidea. Part 15 Issidae*. North Carolina State College, Raleigh, North Carolina, 561 pp.
- Muir F. 1923. On the classification of the Fulgoroidea (Homoptera). *Proceeding of the Hawaiian Entomological Society*, 5: 205–247.
- Muir F. 1930. On the classification of the Fulgoroidea. *The Annals and Magazine of Natural History*, 10(6): 461–478.
- Meng R, Che YL, Yan JH & Wang YL. 2011. Study on the reproductive system of *Sivaloka damnosus* Chou et Lu (Hemiptera: Issidae). *Entomotaxonomia*, 33(1): 12–22.
- Ronquist F & Huelsenbeck JP. 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19: 1572–1574.
- Sanderson MJ & Shaffer HB. 2002. Troubleshooting molecular phylogenetic analyses. *Annual Review Ecology, Evolution, Systematics*, 33: 49–72.
- Schaum HR. 1850. Fulgorellae. Allgemeine Encyklopädie der Wissenschaften und Künste in alnhaberischen folge von Genannten Schriftstellern bearbeitet und herausgegeben von I.S. Ersch und I.G. Gruber mit Kupfern und Charten. *Erster Section A-G*, 51: 58–73.
- Song N & Liang AP. 2013. A preliminary molecular phylogeny of planthoppers (Hemiptera: Fulgoroidea) based on nuclear and mitochondrial DNA sequences. *PLoS ONE*, 8(3): 1–11.
- Spinola M. 1839. Essai sur les Fulgorelles, sous-tribu de la tribu des Cicadaïres, ordre des Rhyngotes. *Annales de la Société Entomologique de France*, 8: 133–337.
- Swofford DL. 2002. *PAUP\*. Phylogenetic Analysis Using Parsimony (\* and other methods), version 4*. Sunderland, MA: Sinauer Association.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M & Kumar S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28(10): 2731–2739.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F & Higgins DG. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 25: 4876–4882.
- Urban JM & Cryan JR. 2007. Evolution of the planthoppers (Insecta: Hemiptera: Fulgoroidea). *Molecular*

- Phylogenetics and Evolution*, 42(2): 556–572.
- Urban JM, Bartlett CR & Cryan JR. 2010. Evolution of Delphacidae (Hemiptera: Fulgoroidea): combined-evidence phylogenetics reveals importance of grass host shifts. *Systematic Entomology*, 35(4): 678–691.
- Yan JH, Xia MH & Wang HQ. 2005. Bionomics of *Sivaloka damnosus*. *Chinese Bulletin of Entomology*, 42(6): 708–710.
- Yang CT & Fang SJ. 1993. Phylogeny of Fulgoromorpha nymphs, first results. In: Drosopolus S, Petrakis PV, Claridge MF & de Virjer PWF (Eds.), *Proceedings of the 8th Auchenorrhyncha Congress*; 9–13 Aug.; Delphi, Greece, pp. 25–26.
- Yeh WB, Yang CT & Hui CF. 1998. Phylogenetic relationships of the Tropicuchidae-group (Homoptera: Fulgoroidea) of planthoppers inferred through nucleotide sequences. *Zoological Studies*, 37(1): 45–55.
- Yeh WB, Yang CT & Hui CF. 2005. A molecular phylogeny of planthoppers (Hemiptera: Fulgoroidea) inferred from mitochondrial 16S rDNA sequences. *Zoological Studies*, 44: 519–535.