



# Planthopper family Issidae (Insecta: Hemiptera: Fulgoromorpha): linking molecular phylogeny with classification



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## ABSTRACT

A molecular phylogeny of the planthopper family Issidae (Hemiptera, Fulgoroidea) is provided using both Maximum likelihood and Bayesian inference analyses. The phylogeny is based on 18S, two parts of 28S, COXI and Cytb genes from 50 genera and 79 ingroup species (including 8 species recently excluded from Issidae). As with the only few previous studies, an important taxonomic impediment is observed with the sampling; however for the first time, all analyses depict several fully supported lineages, which challenge the recent proposed “modern classification” of the family. It also highlights a strong coherence between these lineages and their respective geographical distribution. All previously excluded taxa are confirmed as not being part of the Issidae as recently defined which monophyly is confirmed. Accordingly, a new classification of the family is proposed with 3 subfamilies and 7 tribes as follows. Neotropical issid Thioniini in Thioniinae stat. rev. is re-established as an independent lineage sister to all other Issidae. Palearctic Issidae are weakly supported as a monophyletic lineage, Issinae stat. nov., including 2 tribes: Issini stat. nov. (genera *Issus* and *Latissus*) and Hysteropterini stat. rev. (all other Palearctic genera). Oriental Issidae form a strongly supported monophyletic subfamily group Hemisphaeriinae stat. rev. including 4 tribes: Kodaianellini trib. nov., Sarimini trib. nov., Parahiraciini Cheng & Yang, 1991, and Hemisphaeriini Melichar, 1906, the latter including 2 subtribes: Mongolianina s.trib. nov., and Hemisphaeriina Melichar, 1906. A Neotropical lineage including the genus *Picumna* is provisionally placed in *incertae sedis* within the Hemisphaeriinae stat. nov.

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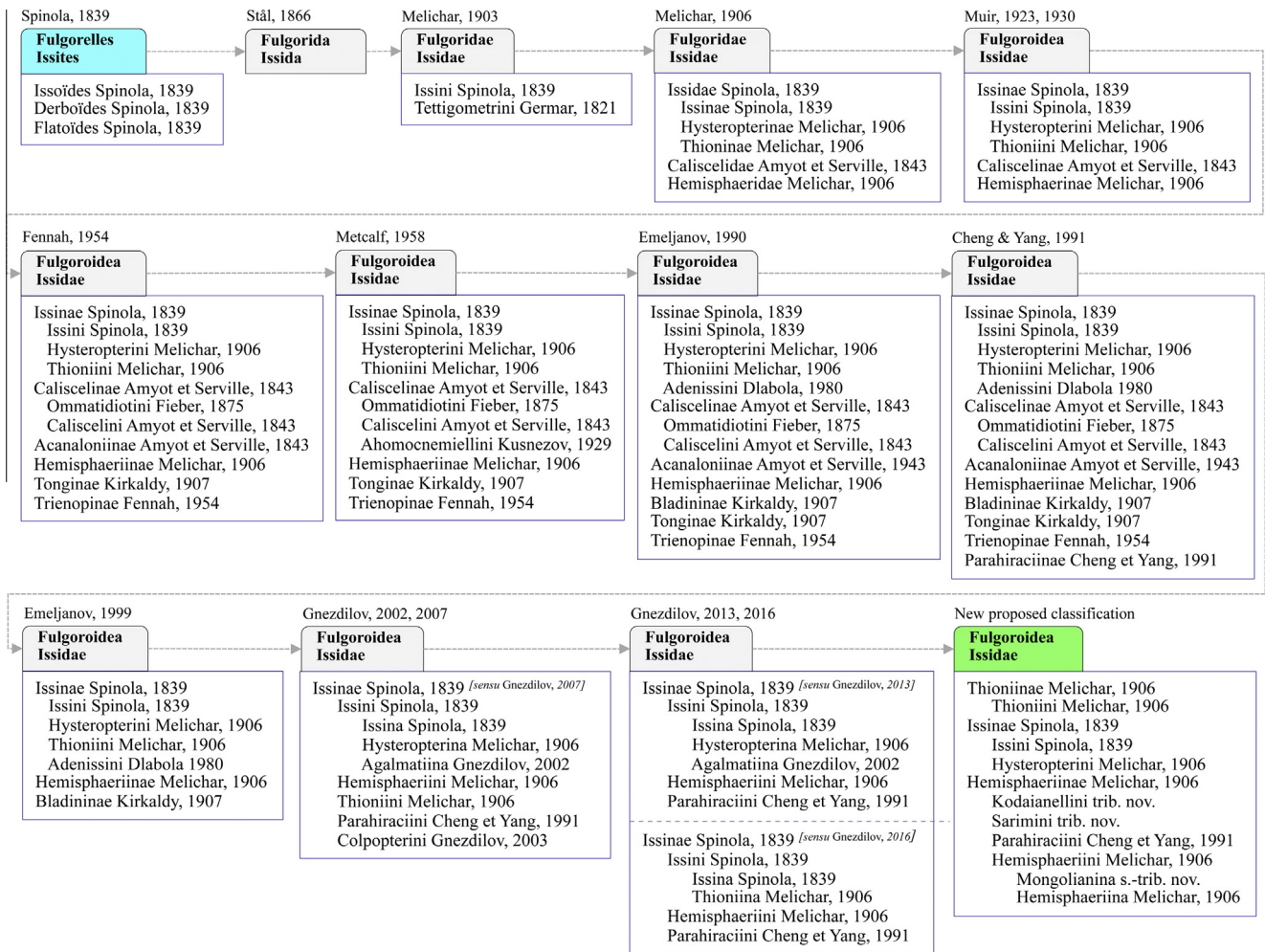
## 1. Introduction

The family Issidae Spinola, 1839 is one of the largest planthopper families (Hemiptera: Fulgoromorpha). It is distributed worldwide and currently includes 189 genera for some 1070 species (Bourgoïn, 2016). As with all planthoppers, Issidae are phytophagous insects, sucking sap (phloem) from a large variety of plants. Some species are invasive – such as *Agalmatium bilobum* (Fieber, 1877) adventive to California in USA (Gnezdilov and O'Brien, 2006) or *Thabena brunnifrons* (Bonfils, Attié & Reynaud, 2001) in La Réunion island (Chan et al., 2013) – and some others, polyphagous, have acquired a pest status such as *Dentatissus damnosus* (Chou & Lu, 1985), a well known fruit tree pest in China (Yan et al., 2005) or *Agalmatium flavescens* (Oliver, 1791) on olives in Turkey (Lodos and Kalkandelen, 1981).

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Issids were first recognized as a distinct taxonomic group, the Issoides, by Spinola (1839), who included them with what are now recognized as the independent families Derbidae Spinola, 1839 and Flatidae Spinola, 1839, within the planthoppers (as “Fulgores”). Since, both delineation and classification of Issidae was much debated. Diverse taxa from other families were including or excluding from Issidae in a manner that has been difficult to follow (Fig. 1). Formalized later by Muir (1923, 1930), Melichar (1906) published the first classification of the family based on the morphological characters of the shape and size of the forewings; clavus venation, and form of the male protibiae (foliated or not). He recognized three main ‘groups’: Caliscelidae Amyot & Serville, 1843, Hemisphaeridae Melichar, 1906, and Issidae, the latter with 3 ‘subgroups’: Hysteropterinae Melichar, 1906, Issinae and Thioniinae Melichar, 1906 (sic) according to hind wing development. After several additions of suprageneric taxa to the family (Kirkaldy, 1907; Baker, 1915; Kusnezov, 1929) such as Tonginae Kirkaldy, 1907, Augilinae Baker, 1915, Ahomocnemiellinae Kusnezov, 1929,



**Fig. 1.** Graphical display of the key steps in the evolution of the classification of the Issidae family according to the successive classification systems or additions of the authors depicting the clear shift from a broad taxonomic concept of Issidae (including 8 subfamilies) to a more restricted concept of "Issidae sensu stricto" (3, then only one subfamily) that occurred in the last fifteen years.

Fennah (1954) revised the higher classification of Issidae according to the shape of the antennae, rostrum proportion, metatibial spines and male and female genitalia. Forgetting to list the Hemisphaeriinae Melichar, 1906; Fennah (1954) divided the Issidae into 5 subfamilies: Caliscelinae Amyot & Serville, 1843, Issinae Spinola, 1839, Acanaloniinae Amyot & Serville, 1843, Trienopinae Fennah, 1954 and Tonginae. This system was followed and strengthened by Metcalf (1958) - who excluded Acanaloniinae for a valid separated family but included Hemisphaeriinae - and Dlabola (1980) with further recognition of additional tribes, particularly in Issinae, which he divided into four tribes: Hysteropterini Melichar, 1906, Issini, Thioniini Melichar, 1906 and Adenissini Dlabola, 1980. In 1990, Emeljanov added the subfamily Bladininae Kirkaldy, 1907, transferred from the Nogodinidae Melichar, 1898, and re-included Acanaloniinae as a subfamily, while Cheng and Yang (1991) erected a new one, Parahiraciinae Cheng & Yang, 1991.

However in the last 15 years, the family Issidae started to be regarded as a more restricted concept, mainly based on hind wing and genitalia patterns re-analysis (Emeljanov, 1999; Gnezdilov, 2003a,b, 2007) with consequent changes in the delineation and rank of several taxa (Fig. 1). Subsequently, Caliscelinae and Acanaloniinae were definitively discarded from Issidae and upgraded again to family rank (Emeljanov, 1999), Tonginae and Trienopinae were successively transferred to Acanaloniidae (Emeljanov, 1999) and then respectively to Nogodinidae and Tropiduchidae Stål, 1866

(Gnezdilov, 2007), and Bladininae were transferred back to Nogodinidae (Gnezdilov, 2007). The resulting classification included one subfamily and five tribes (Gnezdilov, 2007): Issinae including Hemisphaeriini, Parahiraciini, Colpopterini Gnezdilov, 2003, Thioniini, and Issini (itself divided into 3 subtribes). However some years later and based particularly on hind wing venation re-analysis, Thioniini was considered a synonym of Issini (Gnezdilov, 2009) and Colpopterini were transferred to Nogodinidae (Gnezdilov, 2012). Accordingly, Issidae "sensu stricto" currently contains only one subfamily Issinae, and 3 tribes: Issini, Hemisphaeriini and Parahiraciini (Gnezdilov, 2002, 2013). The tribe Issini which included three subtribes: Issina Spinola, 1839, Hysteropterina Melichar, 1906 and Agalmatiina Gnezdilov, 2002 (Gnezdilov, 2002, 2013) was modified again very recently by Gnezdilov (2016) who finally recognized two subtribes in Issini: Thioniina Melichar, 1906 which was re-established and Issina (sensu Gnezdilov, 2016) with which previous subtribes Hysteropterina and Agalmatiina were synonymized. Fig. 1 provides an overview of all these classification changes.

On their side, formal phylogenetic analyses offer only few and partial results. In a general study on planthopper families, Urban and Cryan (2007) confirmed Caliscelidae and Acanaloniidae as families independent from Issidae s.s., based on partial 18S, 28S, H3 and Wingless gene sequences. Similar results were subsequently reached by Song and Liang (2013). Concerning Issidae

s.s., Sun et al. (2015) published a molecular analysis of 19 genera for 33 species based on Wingless and partial 18S gene sequences. Unfortunately their analysis (1) considered only Chinese taxa, some misidentified (e.g., *Paravarcia decapterix* Schmidt, 1919, *Jagannata* sp. 1 and sp. 2, *Mincopius* sp.) or very probably contaminated (*Tonga* sp.), and (2) ended with unjustified conclusions such as Caliscelinae maintained as an issid subfamily - which could not be tested in their analysis - or Parahiraciinae recognized as a paraphyletic issid subfamily. Another phylogenetic analysis of Issidae, based on the genes COXI, 28S (D4–D6 regions) and part of 18S genes, was published by Gnezdilov et al. (2015). The aim of this study was to investigate the phylogenetic relationships among major lineages linked to Issidae (represented by 14 genera), and among the family Issidae s.s., to test family classification (with 13 genera and 18 species as ingroups) (Gnezdilov et al., 2015). Most probably due to taxon sampling too limited, the analysis did not provided clear results: Issidae and Caliscelidae monophyly were not always recovered - but Caliscelidae and Acanaloniidae were clearly excluded from the Issidae, all Western Palaearctic Issini appeared as a possible monophyletic taxon, and as well as subtribes Issina Spinola, 1839 and Hysteropterina Melichar, 1906 (*sensu* Gnezdilov, 2002, 2003b).

The investigation of issid phylogeny has proven to be a challenge in part because the taxonomic impediment remains important in the family: viz. not only many species are undescribed (Gnezdilov, 2013), but specimens used in previous analysis were often misidentified as many genera have been too superficially described and need revisionary treatments. Accordingly, while also testing the assumptions of inclusion or exclusion of these taxa recently proposed for the family, the major objective of this study was to investigate, within a molecular framework, the global phylogenetic structure and main lineages of the family Issidae *sensu stricto*. Our goal is to provide a vision of the evolution of the family based, for the first time, on a quantitatively derived, phylogeny-based classification; and to stimulate interest to develop research for new sets of morphological characters around the clades suggested by the molecular phylogeny.

## 2. Materials and methods

### 2.1. Taxon sampling

Specimens were selected from the collections of Muséum national d'Histoire naturelle (Paris, France) and Northwest A&F

University (Yangling, Shaanxi, China), including dry but recent specimens, and others stored in 75% ethyl alcohol. A total of 43 genera for 71 species of Issidae s.s. were selected and sequenced for the analysis. Additional sequences of five issid species were downloaded from Genbank. To test monophyly and recent classification changes in the Issidae, sampling was widened to the recent included/excluded lineages of two nogodinid taxa (*Bladina* sp., *Lollius* sp.), one acanaloniid (*Acanalonia* sp.), one tropiduchid (*Trienopa* sp.), and four caliscelids. Seven other species from 6 well defined other non-Issid families were also added and used as outgroups (1 Cixiidae, 1 Delphacidae, 1 Dictyopharidae, 1 Tropiduchidae, 2 Ricaniidae and 1 Flatidae). A summary of taxon sampling is provided in Appendix A.

### 2.2. DNA extraction, PCR amplification and DNA sequencing

Total genomic DNA was extracted from legs or thorax of the specimens, using the DNA exaction Blood and Tissue kit QIAGEN (Germany). Four genes were chosen for the molecular analysis: two chromosomal nuclear genes: 18S and 28S, respectively encoding for the small and the large subunit ribosomal RNA, and two mitochondrial genes: COXI and Cytochrome *b* (Cytb), both encoding proteins. Primers used for amplification are listed in Table 1. PCR reactions were prepared in 25 µl protocol, with the following procedures: an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation in 40 s at 94 °C, 40 s annealing at 45–58 °C (around 50 °C for 18S 1F-5R, 58 °C for 18S 3F-Bi, 52 °C for 18S A2-9R, 54 °C for 28S Ai-D4D5r, 55 °C for 28S EE-MM, 46 °C for Cytb), 1 min elongation at 72 °C, and a finally elongation for 10 min at 72 °C, except for the COXI, we used: an initial denaturation at 94 °C for 2 min, followed by 5 cycles of denaturation in 30 s at 94 °C, 40 s annealing at 45 °C, 1 min elongation at 72 °C, then 35 cycles of denaturation in 30 s at 94 °C, 40 s annealing at 51 °C, 1 min elongation at 72 °C, and a finally elongation for 10 min at 72 °C. The PCR product was detected by 1% agarose gel electrophoresis, then sent to Eurofines (Germany) for DNA sequencing. The 18S gene was sequenced into three parts using the coupled primers 1F-5B, 3F-Bi and A2-9R respectively starting on helices H1, H13 and H31. Original data from Gnezdilov et al. (2015) were also taken into account: all were rechecked on chromatograms (ab1 format), corrected when necessary, missing data fixed eventually and concatenated with our own data.

**Table 1**  
Primers used to amplify and sequencing.

Gene	Primer	Sequence (5'–3')	References
18S	1F	TACCTGGTTGATCCTGCCAGTAG	Giribet et al. (1996)
	5R	CTTGGCAAATGCTTTCCG	Giribet et al. (1996)
	3F	GTTTCGATTCCGGAGAGGGA	Giribet et al. (1996)
	Bi	GAGTCTCGTTCTGTTATCGGA	Urban and Cryan (2007)
	A2	ATGGTTGCAAAGCTGAAAC	Urban and Cryan (2007)
	9R	GATCCTTCCGACAGTTACCTAC	Giribet et al. (1996)
28S (D3–D5)	28S Ai	GACCCGCTTGAACACG	Litvaitis et al. (1994)
	28S D4D5r	GTTACACACTCCTTAGCGGA	Belshaw and Quicke (2002)
28S (D6–D7)	28S EE	CCGCTAAGGAGTGTGTAA	Cryan et al. (2000)
	28S MM	GAAGTTACGGATCTARITTTG	Cryan et al. (2000)
COXI	LCO1490puc_t1	TGTAAACGACGGCCAGTTTCAACWAATCATAAAGATATTGG	Cruaud et al. (2010)
	LCO1490Hem1_t1	TGTAAACGACGGCCAGTTTCACTAAAYCATAARGATATYGG	Germain et al. (2013)
	HCO2198puc_t1	CAGGAAACAGCTATGACTAAACTTCWGGRTGWCCAAARAATCA	Cruaud et al. (2010)
	HCO2198Hem1_t1	CAGGAAACAGCTATGACTAAACYTCDDGATGBCCAAARAATCA	Germain et al. (2013)
	HCO2198Hem2_t1	CAGGAAACAGCTATGACTAAACYTCAGGATGACCAAAAAYCA	Germain et al. (2013)
Cytb	Cytb_F	GTTTACTCTTGAGGTCAAATATC	Song and Liang (2013)
	Cytb_R	TTTACTGGTCGTGCTCAATCA	Song and Liang (2013)

### 2.3. Data processing

Software CodonCode Aligner v 5.1.5 (CodonCode Corporation, USA) was used to check sequence chromatograms and to splice partial sequences into one. Obscure bases were coded with an “N” symbol. All sequences were blasted in Genbank to detect potential contamination. Mega v 5.03 (Tamura et al., 2011) was used for sequence alignment using the method of align by ClustalW with default settings. Ambiguously aligned regions at the beginning and end were removed for each gene. The aligned sequences were meanwhile checked by inspection, and with only obvious mistakes corrected manually for the length polymorphism of 18S and 28S genes. Secondary structure for 18S and 28S genes was not taken into account in the alignment process and full sequences were used. In addition, absence of the termination codon in protein coding genes COXI and Cytb were verified in the same software. ClustalX v 2.1 (Larkin et al., 2007) and Mega v 5.03 were used to switch to different dataset formats in the different phylogeny analyses. Number of conserved and variable sites and base frequencies were calculated by Mega v 5.03.

### 2.4. Phylogenetic analyses

The phylogenetic analyses were primarily conducted for nuclear and mitochondrial genes datasets, respectively, to check for obvious artefacts and contaminations. Then a combined dataset of all the genes was used in the subsequent phylogeny tree establishment. Gaps and ambiguous characters were treated using the default setting (gaps as missing data) in phylogenetic tree reconstructed softwares. Maximum likelihood (ML) analysis was conducted in IQtree v1.4.1 (Lam-Tung et al., 2015) using the best-fit substitution model automatically selected by the software according to the Bayesian information criterion scores and weights (BIC) with partitions. The best-fit substitution model and partitions are listed in Appendix B. An ultrafast bootstrap (UFB) (Bui et al., 2013) of 1000 replications and the SH-aLRT test were used in the analysis to assess branch supports. Bayesian inference (BI) analysis was conducted with MrBayes v3.2.4 (Ronquist et al., 2012) using the models and partitions produced by IQtree v1.4.1. We did not consider the models that mixed invariant (I) with a gamma distribution shape parameters ( $\Gamma$ ) because they are strongly correlated, which could bias their estimation (Sullivan et al., 1999). Instead,  $nst = 6$  and  $rates = gamma$  were used unlinked in all the partitions so that each partition has its own set of parameters. Two independent runs were used, using 4 Markov chain Monte Carlo (MCMC) calculations, with run of 40 million generations, sampling every 100 generations, with generations added until results were convergent. The first 25% of samples were discarded as burn-in and the remaining samples were used to generate a 50% majority rule consensus tree. FigTree v1.1.2 (Rambaut, 2008) was used to view the tree obtained.

## 3. Results

### 3.1. Sequence characterization

In total, a matrix length of 4702 bp for the combined DNA sequences was obtained and used for the phylogenetic analyses.

**Table 2**  
Characterization of the genes used in this study.

Gene	nb bp	Conserved	Variable	Ratio (%)	T	C	A	G
18S	1898	1353	519	27.3	23	28.3	23.9	24.7
28S D3–D7	1517 (742 + 775)	837	631	41.6	19.3	28.8	21.6	30.4
COXI	681	317	364	53.4	32.9	18.6	34.7	13.8
Cytb	606	238	365	60.2	35.0	17.6	38.0	9.4

The length of sequences included 1898 bp of the 18S gene, 742 bp of the 28S D3–D5 region, 775 bp of the 28S D6–D7 region, 681 bp of the COXI gene and 606 bp of Cytb (Table 2).

### 3.2. Phylogenetic relationships

The ML (Fig. 2) and BI (Bayesian 50% majority rule consensus) (Fig. 3) topologies are fully compatible, only differing by five trichotomies (BI-tree) resolved in the ML-tree. Two basal trichotomies concern the old now rejected issid taxa with (1) an always fully supported monophyletic Caliscelidae lineage sister to a (Nogodinidae (*Lollius*) + Acanaloniidae (*Acanalonia*)) lineage, with both sister (ML-tree) or in trichotomy with Ricaniidae lineage (BI-tree), on one side and (2) a (Flatidae (*Metcalfa*) + Nogodinidae (*Bladina*)) lineage on the other side, either in-between the previous lineages and the Issidae (clade 1) (ML-tree) or forming a second trichotomy with these two (BI-tree).

Within the Issidae (clade 1), the ML-tree identifies 23 major Issidae lineages (Fig. 2) and only three of them (clades 4, 13, 21) are not fully recovered in the BI-tree topology (Fig. 3), where instead two trichotomies occur. The first and basal trichotomy is formed by clades 5, 6 and 7 (BI-tree) which is resolved as clade 4 (=clades 6 + 7) in the ML topology, having a sister relationship with (clade 5 + (clade 6 + clade 7)). The second trichotomy occurs between a new genus (clade 22) (BI-tree) that is resolved in the ML topology as belonging to a “*Hemisphaerius*” lineage (clade 21). Two more distal trichotomies occur in more distal clades: in clade 18 with the position of *Mongoliana serrata* within the “*Mongolianina*” lineage and in clade 8 with *Hysteropterum albaceticum* relative to *Latilica maculipes*.

Posterior probabilities (PP) values of BI-tree and ultrafast bootstrap (UFB) values of ML-tree are consistent in the two topologies and several major lineages are fully supported with PP = 1 or UFB = 100 such as the Caliscelidae group, and clades 6, 7, 10, 16, 17, 20, 23. Most other clades are also well supported with PP  $\geq 0.9$  or UFB  $\geq 90$ .

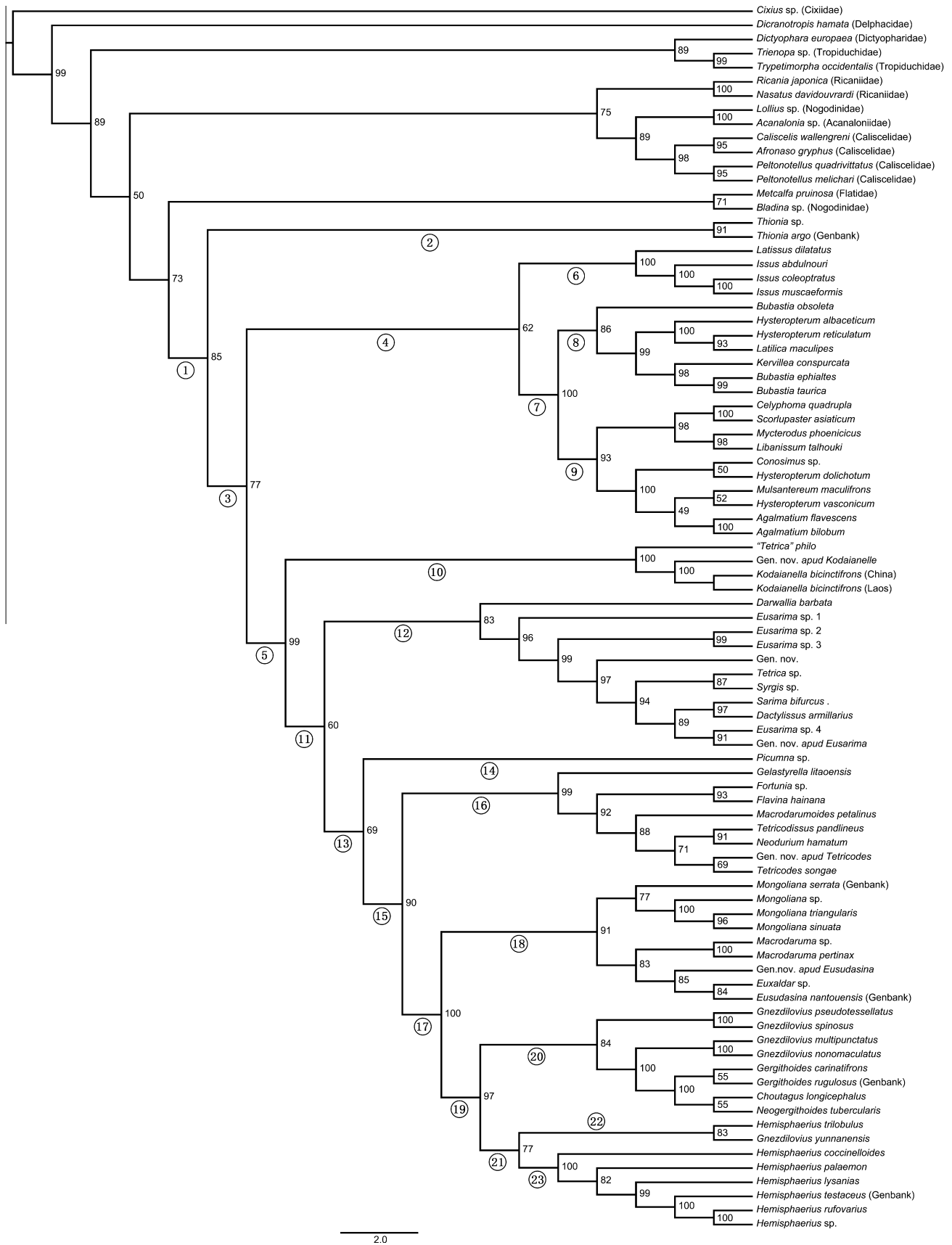
Less supported clades, although always recovered in our analyses, were observed with lower values particularly for clades 1, 3, 11 and 13. The first one (clade 1) concerns the monophyly of the Issidae, which is not well supported (PP = 0.57 and UFB = 85). Clade 3 groups together all Issidae except the *Thionia* species lineage (PP = 0.72 and UFB = 77). Clade 11, grouping most of the Oriental taxa (except the fully supported monophyletic branch clade 10), is the less supported of these lineages (PP = 0.53 and UFB = 60). This apparent inconsistency is probably due to the genus *Picumna* (clade 14), as discussed later, that comes either as sister to clade 10 in the BI-tree (PP = 0.51), or to (Hemisphaeriini + Parahiraciini), clade 15, in the ML-tree (UFB = 69).

## 4. Discussion

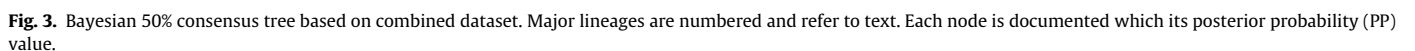
### 4.1. Representative sampling with proper taxonomy

Previous molecular studies on Issidae phylogeny failed to provide robust results because of some important weaknesses: (1) taxon sampling not sufficient enough to address the diversity and identity of the various issid lineages, as in Gnezdilov et al.





**Fig. 2.** Maximum-likelihood tree estimated from the combined dataset. Major lineages are numbered and refer to text. At each node, values indicate ML ultrafast bootstrap (UFB) support.



**Fig. 3.** Bayesian 50% consensus tree based on combined dataset. Major lineages are numbered and refer to text. Each node is documented which its posterior probability (PP) value.

(2015), (2) attempting to address phylogenetical issues with geographically restricted sampling (e.g., China-centred sampling in Sun et al., 2015), and (3) taxon sampling imprecisely identified or misidentified due to the taxonomic impediment (*Paravarcia decapterix* Schmidt, 1919, *Jagannata* sp. 1 and sp. 2, *Mincopius* sp. in Sun et al., 2015). Moreover contaminated sequences in these two papers were also detected during our own analysis. Other papers about issid evolution included non-testable arbitrary classification objectives (Gnezdilov, 2013).

Like previous workers, we also experienced taxonomic problems, even at the generic level for taxa not sufficiently well described. The species “*Tetrica*” *philo* doesn’t belong to *Tetrica* Stål, 1866 and predictably the two *Tetrica* were placed in two different clades (10 and 12). In clade 12, four species *a priori* placed in the genus *Eusarima* Yang, 1994, according to the diagnostic characters of the first fork of veins MP and CuA on forewing and the shape and carinae of frons, appear to belong to different lineages. *Eusarima*, as currently recognized, appears to be a polyphyletic genus. Finally, even the placement of some genera into tribes needs review: *Macrodarumoides* Che, Zhang & Wang, 2012 and *Gelastyrrella* Yang, 1994 currently belonging Issini are moved into Parahiraciini and two genera from Issini, *Eusudasina* Yang, 1994 and *Euxaldar* Fennah, 1978, belong in Hemisphaeriini (Mongolianina subtrib. nov.). We fully concur with Sun et al. (2015), that a careful re-examination of the species of these important Oriental clades is necessary to reallocate them into better defined genera.

At higher levels, several taxa which have recently been excluded from Issidae are confirmed as non issid lineages: Trienopini to Tropiduchidae (Gnezdilov, 2007), Bladinini Kirkaldy, 1907 to Nogodinidae (Gnezdilov, 2007) and Caliscelidae as a valid independent family (Emeljanov, 1999). Sun et al. (2015) was unable to test placement of Caliscelidae since they were the only other taxa within other issid taxa of their analysis. They provided no evidence to retain Caliscelidae as a subfamily of Issidae. Exclusion of these different taxa from Issidae is supported here; however, our sampling is too restricted to address more specifically this issue, which was not the main aim of this paper.

#### 4.2. Linking phylogeny with classification, toward a new classification of Issidae

Unexpectedly, although always recovered in our analysis, the monophyly of Issidae *sensu* Gnezdilov (2013) is not strongly supported in Bayesian analysis (PP = 0.57) (Fig. 3) and thus raises the question of whether clade 2 (*Thionia* species) belongs to the Issidae. In fact, even if in the ML analysis the monophyly of Issidae is better supported (UFB = 85) (Fig. 2), it seems that it is the monophyly of the Nearctic-Neotropical taxa itself that raises question. Indeed, all nodes of major issid lineages were better recovered with *Picumna* excluded (Fig. 4). In our analysis, *Picumna* includes only COXI, Cytb and a small part of 18S, but is missing the remaining sequences, suggesting that the placement of this taxon may be an artifact reflecting in fact the impact of its missing data in the analysis that does not anchor firmly the taxa in its right place. Accordingly, the relatively weak support for issid monophyly in our analysis may be just an artefact due to this biased sampling.

Palaeartic Issidae are represented by two fully supported monophyletic groups (clades 6 and 7), but monophyly of these clades is not supported in the BI-tree, although recovered in ML-tree (clade 4, UFB = 62 and slightly better when excluding *Picumna*: UFB = 72). In contrast, all Oriental Issidae form a strong, fully recovered monophyletic group (clade 5), either sister to Palaeartic Issidae (clade 4) or in trichotomy with the two major Palaeartic lineages (clades 6 and 7). Within these three major lineages (clades 1, 4 and 5), several other fully supported groups are also observed.

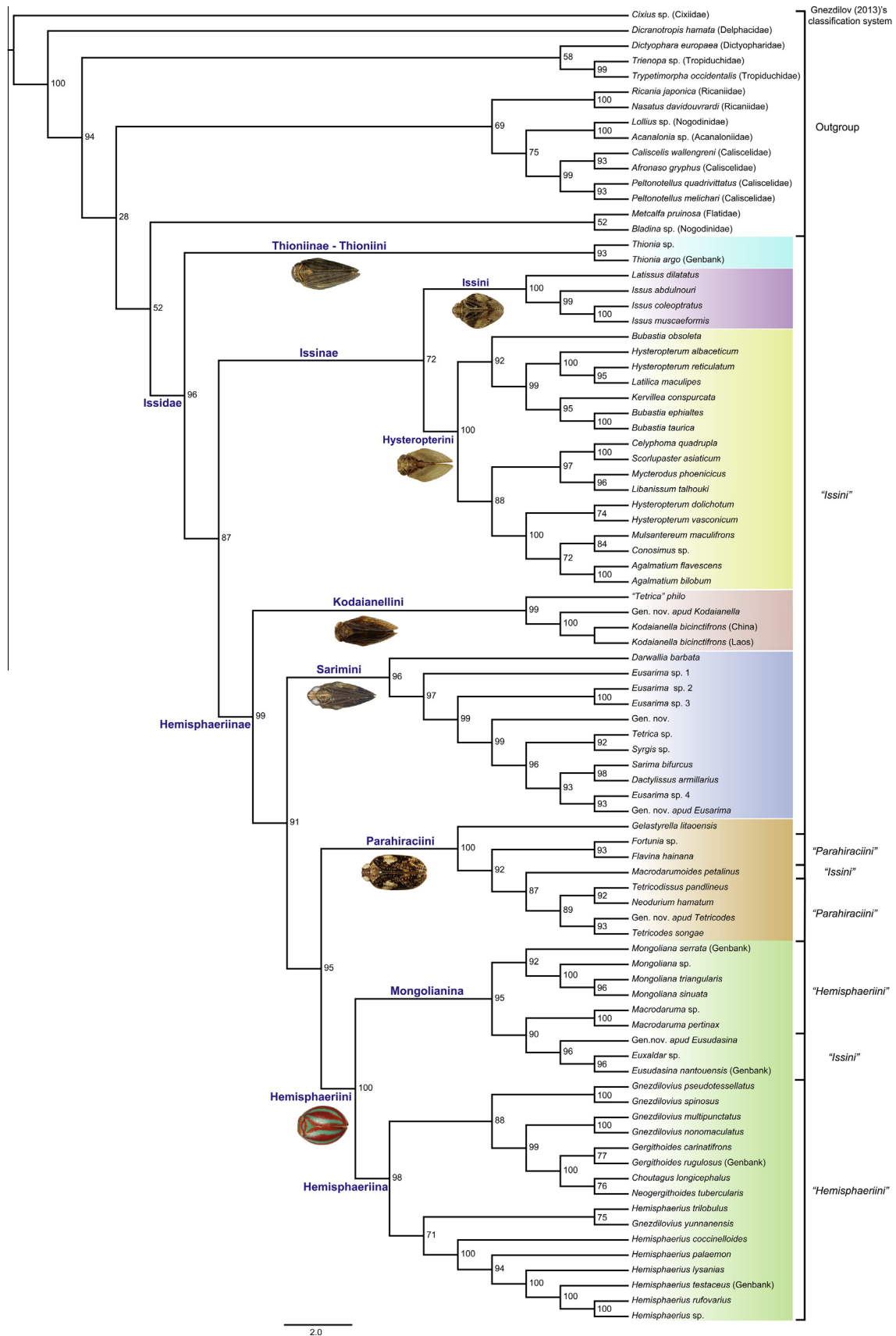
All our analyses challenge the current “modern classification” of Issidae (Gnezdilov, 2013) that depict the family as divided into three tribes: Issini, Hemisphaeriini and Parahiraciini. According to our results, this classification needs to take into account that: (1) both Issini *sensu* Gnezdilov (2013) and (2) Oriental Issini *sensu* Gnezdilov (2013) do not represent monophyletic groups; that (3) the monophyly of Palaeartic Issini is weakly supported; that (4) at least *Thionia* species tested here and probably most Neotropical Issidae taxa (with some lineages extending into the Nearctic area) form an independent lineage sister to all other Issidae; that (5) Oriental Issidae form a monophyletic group; and that (6) several more and fully supported monophyletic lineages of suprageneric ranks were also recognized in all our ML- and BI-analyses. Moreover, the results show that these strongly supported clades exhibit also a coherent geographical distribution through a clear geographic patterning. Accordingly, we consider that all these lineages are compatible with a new and well-supported classification of the family, including new subfamilies (particularly for the Oriental Issidae which deserve a subfamily rank) but also several strongly supported tribal and subtribal units. Consequently, these taxa are formally established here in Issidae *sensu* Gnezdilov (2013), here subdivided as 3 subfamilies (new or re-established), with revised or new tribes (clades 2, 6, 7, 10, 12, 16, and 17) or subtribes (clades 18, 19). Interestingly, these changes lead to a new Issidae classification reminiscent of Melichar (1906)’s pioneering work. A summary of this new Issidae classification is therefore here proposed in Table 3.

#### – Thioniinae Melichar, 1906, stat. rev.

This subfamily includes *Thionia* Stål and all Neotropical taxa (such as *Oronoqua* Fennah, 1947) with their hind wing having a A2 vein branched and PCu and A1 veins free, not partially fused. The two, independently sequenced, “*Thionia* sp.” always came as sister to all other Issidae from our analysis in both ML- and BI-trees. *Thionia* is a huge Neotropical and Nearctic genus of some 75 species (Bourgoin, 2016), with a large distribution from Canada to Chile and one invasive species reported from Italy in Europe (Gnezdilov and Poggi, 2014). Previously, Stroiński and Szewo (2008) noted the diversity of *Thionia* species particularly for the head capsule, pronotum and tegmina characters. Both their disparity of features and wide distribution suggest the need of a revision of *Thionia* and allied genera. In contrast to Gnezdilov (2013, 2016), we recognize this lineage (clade 2) as a valid tribe Thioniini Melichar, 1906 stat. rev. in their own subfamily, Thioniinae Melichar, 1906 stat. rev., and separated as sister to all other Issidae taxa.

#### – Issinae Spinola, 1839, stat. nov.

All Palaeartic issids genera are included in this group, represented by clades 6 and 7 as a diphyletic unit in Bayesian analysis or as a weakly supported monophyletic lineage in Maximum likelihood analysis. If Bayesian results are confirmed, the two lineages should gain recognition as subfamilies. We currently recognize these clades as tribes: Issini Spinola, 1839, stat. nov., corresponding to Issina *sensu* Gnezdilov (2003b) with genera *Issus* and *Latissus* – but *versus* Issina *sensu* Gnezdilov (2016); and Hysteropterini Melichar, 1906, which includes all other Palaeartic genera, both forming subfamily Issinae stat. nov. Issini are recognized by their hind wing fully developed, 3-lobed and a pair of inner side laterodorsal digitate process developed at the apex of phallobase (Gnezdilov, 2016). In Hysteropterini stat. nov., the lineages Agalmatiina Gnezdilov, 2002, and Hysteropterina Melichar, 1906, *sensu* Gnezdilov (2002, 2003b) are not supported. In contrast, two new groupings (clades 8 and 9) are supported *contra* the recent simplified cladogram provided by Gnezdilov (2016) for Issina *sensu* Gnezdilov 2016 (=Issinae stat. nov., this study) which is also not supported



**Fig. 4.** Phylogeny (ML-tree) of Issidae with new phylogeny-based proposed classification of the family (genus *Picumna* excluded). At each node, values denote ML ultrafast bootstrap (UFB) support. On the right side, groups following Gnezdilov (2013)'s classification are provided showing paraphyly of all these major groups previously recognized.



**Table 3**

New Issidae classification. Each supra-generic taxa is presented with its type genus, other included genera and diagnosis is provided for new taxonomic group proposed.

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**Family Issidae Spinola, 1839**
**– Thioniinae Melichar, 1906, stat. rev.**
**Thioniini Melichar, 1906, stat. rev.**

Type genus: *Thionia* Stål, 1859

Included genera: all Neotropical taxa with hindwing branched A2 vein such as in *Oronoqua* Fennah, 1947

**– Issinae Spinola, 1839, stat. nov.**

Restricted to all Palearctic genera

**Issini Spinola, 1839, stat. nov.**

Type genus: *Issus* Fabricius, 1803

Included genera: *Issus*, *Latissus* Dlabola, 1974 (=Issina sensu Gnezdilov, 2003b)

**Hysteropterini Melichar, 1906, stat. nov.**

Type genus: *Hysteropterum* Amyot & Serville, 1843

Included genera: *Hysteropterum* and probably all other Palearctic genera. Probably with two monophyletic subunits, but subtribes Agalmatiina Gnezdilov, 2002 and Hysteropterina Melichar, 1906 sensu Gnezdilov (2013) are not supported

**– Hemisphaeriinae Melichar, 1906, stat. rev.**

Included genera: all Oriental taxa, Australian taxa and tentatively all Afrotropical genera and at least Nearctic genus *Picumna*

**Kodaianellini trib. nov.**

Type genus: *Kodaianella* Fennah, 1956

Included genera: at least *Kodaianella* Fennah, 1956, “*Tetrica*” *philo* (as belonging to a new genus) and *Dentatissus* Cheng, Zhang & Chang, 2014. Also *Neokodaiana* Yang, 1994. This tribe is characterized by a 3-lobed hind wing with Pcu-A1 lobe distinctly thinner, less than half wide as ScP-R-MP-Cu lobe; A2 lobe with anterior and posterior margins subparallel and distinctly surpassing half length of Pcu-A1 lobe; Pcu single, submedially anastomosing with a 2-branched A1; A2 non-branched

**Sarimini trib. nov.**

Type genus: *Sarima* Melichar, 1903

Included genera: at least *Sarima* Melichar, 1903, *Eusarima* Yang, 1994, *Darwallia* Gnezdilov, 2010, *Syrgis* Stål, 1870, *Dactylissus* Gnezdilov & Bourgoin, 2014, *Tetrica* Stål, 1866, and several other genera. The taxon is characterized by a 3 lobed hind wing with Pcu-A1 lobe more or less as wide as ScP-R-MP-Cu lobe and Pcu single or branched. Pcu and A1 anastomosing on a short or longer distance. A2 non branched. A monophyletic taxa but still also a composite group with several subtaxa to be identified

**Parahiraciini Cheng & Yang, 1991**

Type genus: *Parahiracia* Ōuchi, 1940 (currently synonym of *Fortunia* Distant, 1909)

Included genera: At least *Gelastrella* Yang, 1994 (syn. of *Thabena* Stål, 1861 according to Gnezdilov, 2013), *Fortunia* Distant, 1909, *Flavina* Stål, 1861, *Macrodarumoides* Che, Zhang & Wang, 2012, *Tetricodissus* Wang, Bourgoin & Zhang, 2015, *Neodurium* Fennah, 1956, *Pseudochoutagus* Che, Zhang & Wang, 2011, and all currently described Parahiraciini genera characterized by a deep narrowed incision of hind wing with a distinctly wider Pcu-A1 lobe than ScP-R-MP-Cu lobe and a short, thin A2 lobe in which A2 is often absent; Pcu and A1 free, not partially fused

**Hemisphaeriini Melichar, 1906**
**Mongolianina subtrib. nov.**

Type genus: *Mongoliana* Distant, 1909

Included genera: At least *Mongoliana*, *Macrodaruma* Fennah, 1978, *Eusudasina* Yang, 1994, *Euxaldar* Fennah, 1978. Also *Bruneastrum* Gnezdilov, 2015.

Characterized by short and reduced but elongated hind wing with margins subparallel; venation distinct, CuA and CuP not apically merged but running parallel, CuP and A1 not partially merged, and anal lobe absent

**Hemisphaeriina Melichar, 1906**

Type genus: *Hemisphaerius* Schaum, 1850

Included genera: At least *Hemisphaerius*, *Gnezdilovius* Meng et al., 2016 (in press), *Gergithoides* Schumacher, 1915, *Neogergithoides* Sun, Meng & Wang, 2012, *Choutagus* Zhang, Wang & Che, 2006. But also genera such as *Rotundiforma* Meng, Wang & Qin, 2013, *Neohemisphaerius* Chen, Zhang & Chang, 2014, *Folifemurum* Che, Zhang & Wang, 2013. The subtribe is characterized by rudimentary hind wing with venation not evident or a drop-like hind wing, proximally thinner with costal and anal margins distinctly concave and a strongly reticulated venation; lacking anal lobe

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by our analysis. Because *Hysteropterum* is distributed paraphyletically within these two sub-lineages, it prevents a formal recognition of them. This raises the question of the current definition/identification of *Hysteropterum*, or the placement of species within it. Genus *Bubastia* Emeljanov, 1975 requires similar consideration.

**– Hemisphaeriinae Melichar, 1906, stat. rev.**

It is probable that New World Issidae form a paraphyletic group. According to our analysis, *Picumna* appears to be more closely related to the Oriental Issidae (clade 5) than to the *Thionia* group. We therefore place this taxon in *incertae sedis* within Hemisphaeriinae stat. rev. Other New World taxa with a non-branched A2 hind wing vein (as probably *Amphiscepa* Germar, 1830) might belong to this same lineage. If this diphyletic origin of New World Issidae is verified, it would be probably due to a latter dispersal event of these taxa from Asia to Nearctic. However, precise placement of this lineage within Oriental taxa remains unknown, very probably because of incomplete molecular sequence data. An improved sampling (taxa and genes) will be necessary to confirm its placement.

All Oriental Issidae form a strong and large monophyletic group that we recognized at the subfamily rank as Hemisphaeriinae. Currently we divide Hemisphaeriinae into four tribes, the monophyly

of which is strongly supported: Kodaianellini trib. nov. (clade 10), Sarimini trib. nov. (clade 12) and the two previously recognized Parahiraciini (clade 16) and Hemisphaeriini (clade 17); the latter separated into Mongolianina subtrib. nov. (clade 18) and Hemisphaeriina subtrib. nov. (clade 19). Other lineages within these tribes will probably emerge in the future with greater taxon sampling, particularly for Sarimini trib. nov. Indeed Sarimini is recognized as having a hind wing Pcu-A1 lobe well developed with a branched Pcu (*Darwallia*, *Tetrica*, *Parasarima* Yang, 1994, *Nikomiklukha* Gnezdilov, 2010, *Apsadaroptyx* Kirkaldy, 1907, ...) versus other genera with a non branched Pcu, partially fused medially with A1 (*Eusarima*, *Dactylissus*, *Sarimodes* Matsumura, 1916, *Chlamydoptyx* Kirkaldy, 1907, *Orbita* Meng & Wang, 2016, ...). Previously placed within Parahiraciini, *Scantinius* Stål, 1866 with a 3-lobed hind wing, Pcu-A1 lobe reticulated as wide as ScP-R-MP-Cu lobe, a thinner A2 lobe but still as large as others, deserves currently an *incertae sedis* position within Hemisphaeriinae. Within Hemisphaeriini, the two subtribes are well supported. Genus *Eusudasina* originally described in Hysteropterini (Chan and Yang, 1994), then transferred (Gnezdilov, 2014) to the Issini group sensu Gnezdilov (2013) is here moved to the tribe Hemisphaeriini, as previously suggested by Sun et al. (2015), together with genus *Euxaldar* into Mongolianina subtrib. nov. Within the subtribe

Hemisphaeriina, two clades (clades 20, 21) appear, but are not fully supported. Clade 21 mainly concerns the genus *Hemisphaerius* represented by different species, sister to clade 20 in which *Gnezdilovius* appears clearly as a paraphyletic taxon. Better delineation and revision of generic attribution for the species of these two paraphyletic genera are necessary.

The Hemisphaeriinae includes all Oriental taxa, plus most Australasian taxa – which have an Indo-Malayan origin according to Gnezdilov (2013) – and probably also Afrotropical taxa. Morphological features (e.g., hind wing 3-lobed, with Pcu and A1 free, not partially fused, Pcu branched and A2 single) suggest that the Afrotropical genera *Chimetopon* Schmidt, 1910 and *Ikonzia* Hesse, 1925, might belong to this subfamily but with *incertae sedis* status: molecular analyses are required to confirm this hypothesis and to precise their placement. Afrotropical Issidae are represented by only six genera and 20 endemic species (Bourgoin, 2016). It would particularly interesting to test Gnezdilov (2013)'s hypothesis that the Equatorial African genus *Chimetopon* Schmidt, 1910 represents a relict of African forest issids otherwise extinct due to glaciation 26–10 thousand years ago. Also the Oriental origin of Australian issids needs to be confirmed. More generally, adding more diversified taxa is needed to test our hypothesis of a paraphyletic origin of the new world Issidae.

## 5. Conclusion

This study presents the first reliable molecular phylogeny of the Issidae, including all major lineages that were identified in the past as part of the family but that we confirm to be excluded, and focused on issid phylogeny. We revealed several unexpected but well supported lineages for which a morphological assessment needs to be made. It also highlights a strong coherence between taxonomic lineage and their geographical distribution: such a geographic patterning at the tribal scale should be expected for relatively recent monophyletic lineages. As noted, wide representative samplings and careful taxonomic scrutiny are key factors in the robustness of the results. Further clarification of the phylogeny of Issidae will necessarily require more extensive sampling, including Afrotropical and Australian taxa, and especially New World taxa to test the basal placement of the Thioniinae, and therefore the monophyly of the Issidae as currently defined (*sensu* Gnezdilov, 2013). But a better understanding of the evolution of Issidae will come with the revision of non-monophyletic genera to devise less superficial taxonomic definitions. This pre-requisite would best be performed in parallel to molecular analyses, investigating novel morphological character systems to test and define a new morphological basis of these newly recognized issid lineages as they have been suggested by molecular studies.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2016.08.012>.

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