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New insights into the molecular phylogeny and taxonomy of the family Issidae (Hemiptera: Auchenorrhyncha: Fulgoroidea)

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ABSTRACT

The phylogenetic relationships among major lineages of the planthopper family Issidae were explored by analyzing a molecular dataset of nine fragments (COI, CytB, 12S, H3, 16S, 18SII, 18SIII, 28S D3–D5, 28S D6–D7) and 48 terminal taxa. Bayesian and Maximum likelihood analyses yielded similar and mostly well-resolved trees with moderate to high support for most branches. The obtained results suggest subdivision of the family Issidae Spinola into two subfamilies, Issinae Spinola, 1839 (= Thioniinae Melichar, 1906, = Hemisphaeriinae Melichar, 1906) and Hysteropterinae Melichar, 1906. The Issinae was clustered into the tribes Issini Spinola, 1839, with the subtribes Issina Spinola, 1839 and Thioniina Melichar, 1906, Sarimini Wang, Zhang et Bourgoïn, 2016, Parahiraciini Cheng et Yang, 1991, Hemisphaeriini Melichar, 1906, and Kodaianellini Wang, Zhang et Bourgoïn, 2016. The Hysteropterinae incorporates the rest of Western Palaearctic taxa except Issina. Chimetopini Gnezdilov, 2017, stat. nov. is elevated to tribe from the subtribal level. Most well-supported clades showed clear geographical patterning. Newly obtained data contradicts the scenario of an early split of American Thioniinae from other Issidae and possible origin of the family in the New World, while the combination of Palaearctic *Issus* Fabricius and *Latissus* Dlabola with Oriental and American taxa in one well supported clade may serve as an evidence for a common ancestor for extant Oriental, American, and Palaearctic issids.

Key words: Hyteropterinae, Issinae, Issina, Issini, molecular phylogeny, taxonomy, Thioniina

Новый взгляд на молекулярную филогению и систематику семейства Issidae (Hemiptera: Auchenorrhyncha: Fulgoroidea)

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РЕЗЮМЕ

Выявлены филогенетические отношения среди основных групп семейства Issidae по результатам анализа 9 генных фрагментов (COI, CytB, 12S, H3, 16S, 18SII, 18SIII, 28S D3–D5, 28S D6–D7) и 48 видов. Использование Байесова анализа и анализа максимального правдоподобия позволили получить схожие и, в основном, хорошо разрешенные деревья с умеренной или высокой поддержкой большинства ветвей. Полученные результаты позволяют подразделить семейство Issidae Spinola на два

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подсемейства – Issinae Spinola, 1839 (= Thioniinae Melichar, 1906, = Hemisphaeriinae Melichar, 1906) и Hysteropterinae Melichar, 1906. Подсемейство Issinae в свою очередь распадается на трибы Issini Spinola, 1839, с подтрибами Issina Spinola, 1839 и Thioniina Melichar, 1906, Sarimini Wang, Zhang et Bourgoïn, 2016, Parahiraciini Cheng et Yang, 1991, Hemisphaeriini Melichar, 1906 и Kodaianellini Wang, Zhang et Bourgoïn, 2016. Подсемейство Hysteropterinae объединяет все западнопалеарктические таксоны за исключением Issina. Chimetopini Gnezdilov, 2017, stat. nov. повышена в ранге до трибы. Клады с наибольшей поддержкой показывают явные географические паттерны. Полученные данные противоречат сценарию раннего отделения американских Thioniinae от других Issidae и возможному возникновению семейства в Новом Свете, в то время как комбинация палеарктических *Issus* Fabricius и *Latissus* Dlabola с ориентальными и американскими таксонами в составе одной, хорошо поддержанной клады Issinae, свидетельствует в пользу существования общего предка для современных ориентальных, американских и палеарктических иссид.

Key words: Hysteropterinae, Issinae, Issina, Issini, молекулярная филогения, систематика, Thioniina

INTRODUCTION

The family Issidae Spinola, 1839 is a worldwide distributed group of planthoppers with more than 1000 species described in nearly 200 genera (Gnezdilov 2013a, 2016a; Bourgoïn 2019) authentically known since Eocene (Gnezdilov and Bourgoïn 2016). The Western Palaearctic and Oriental regions harbour the richest faunas of the family while the Afrotropical issid fauna is poor and Australian one is still mostly undescribed (Gnezdilov 2013a, 2016a). Apparently rich Neotropical issid fauna is still in its initial stage of discovering (Gnezdilov 2018b, 2019a; Gnezdilov and Bartlett 2018). In dry habitats of Western Palaearctic region issid species are associated with trees and shrubs, e.g. *Quercus*, *Astragalus*, *Amygdalus*, *Atraphaxis*, *Spiraea*, and *Echinospartum* species, and grasses, e.g. *Alhagi*, *Artemisia*, *Festuca*, *Tanacetum* species etc. (Emeljanov 1969, 1978; Dlabola 1980; Mitjaev 2002; Gnezdilov and Aguin-Pombo 2014; Gnezdilov et al. 2019). In tropical areas issids inhabit forest canopies (Meng et al. 2013; Gnezdilov 2015; Gnezdilov et al. 2010; Gnezdilov and Bartlett 2018; Barringer et al. 2019), small trees and shrubs in the forests or cereals in opened places (Gnezdilov 2013c, 2016b). Some species, e.g. *Agalmatium bilobum* (Fieber, 1877) and *Thabena brun-nifrons* (Bonfils, Attie et Reynaud, 2001), are widely polyphagous and were easily distributed across the world (Gnezdilov and O'Brien 2006; Chan et al. 2013). Many issid species are peculiarly subbrachypterous, with beetle-shaped forewings (Gnezdilov et al. 2014), and flightless which makes this group of particular importance for historic biogeography and evolution of terrestrial biota.

The classification of the family since the group was established by Spinola (1839) was developed by Melichar (1906), Fennah (1954), Dlabola (1987), and Gnezdilov (2002, 2003, 2007, 2009, 2013a, 2016c). Particularly Gnezdilov (2013a) treated the family Issidae comprising one subfamily Issinae Spinola, 1839 with three tribes – Issini Spinola, 1839, Hemisphaeriini Melichar, 1906, and Parahiraciini Cheng et Yang, 1991. The tribe Thioniini Melichar, 1906 was placed in synonymy under Issini (Gnezdilov 2009). Later Gnezdilov (2016c, 2017a) resurrected the subtribe Thioniina Melichar, 1906 in the tribe Issini and erected the subtribe Chimetopina Gnezdilov, 2017 to accommodate African taxa with well-developed hind wings. Finally Gnezdilov and Bartlett (2018) and Gnezdilov (2018a, 2018b, 2019a) resurrected the subfamily Thioniinae Melichar, 1906, with the tribes Thioniini, comprising three subtribes (Thioniina, Oronoquina Gnezdilov, 2018, Waoraniina Gnezdilov et Bartlett, 2018), Guianaphrynini Gnezdilov, 2018, and Cordelini Gnezdilov, 2019 (Table 1).

Despite considerable progress in taxonomic studies, no phylogenetic treatment of the group had been published until recently. Starting from 2015 several studies appeared dealing with phylogeny of Issidae based on morphological (Gnezdilov 2016a, 2016c) and on molecular data (Gnezdilov et al. 2015; Sun et al. 2015; Wang et al. 2016). Before these studies some species of the family Issidae were involved in the molecular analysis devoted to the phylogeny of Fulgoroidea as a whole or issidoid group of families comprising Issidae, Caliscelidae, Tropiduchidae, Nogodinidae, and Acanaloniidae (Yeh et al. 1998, 2005; Yeh and Yang 1999; Bourgoïn et al. 1997; Urban and Cryan 2007; Song and Liang 2013).

Table 1. Current classification of the family Issidae.

Subfamily Issinae Spinola, 1839	Subfamily Hysteropterinae Melichar, 1906
Tribe Issini Spinola, 1839 Subtribe Issina Spinola, 1839 Subtribe Thioniina Melichar, 1906 Subtribe Oronoquina Gnezdilov, 2018, Subtribe Waoraniina Gnezdilov et Bartlett, 2018 Tribe Chimetopini Gnezdilov, 2017 Tribe Guianaphryni Gnezdilov, 2018 Tribe Cordelini Gnezdilov, 2019 Tribe Sarimini Wang, Zhang et Bourgoin, 2016 Tribe Kodaianellini Wang, Zhang et Bourgoin, 2016 Tribe Hemisphaeriini Melichar, 1906 Subtribe Hemisphaeriina Melichar, 1906 Subtribe Mongolianina Wang, Zhang et Bourgoin, 2016 Tribe Parahiraciini Cheng et Yang, 1991	Groups of genera recognized by Gnezdilov (2016a, 2016c). Phylogenetic analysis is in progress.

Sun with coauthors (Sun et al. 2015) built the first phylogenetic tree of Issidae based on sequences of 18S and Wg of 34 species from 20 genera using Bayesian analysis. In this study the monophyly of Issidae was weakly supported (0.61), but three well supported clades were recognized within the family which corresponds to Issini *sensu* Gnezdilov (2009) or Sarimini + Kodaianellini *sensu* Wang et al. (2016), Hemisphaeriini, and Parahiraciini. The two latter clades were sister groups on the tree with a support 0.84. Unfortunately several terminal taxa were misidentified by the authors, e.g. the species identified as *Sivaloka* Distant, 1906 in fact belongs to the genus *Kodaianella* Fennah, 1956, *Jagannata* sp.1 and *Jagannata* sp.2 belong to the genus *Eusarima* Yang, 1994, while *Kodaiana* sp. in fact belongs to the genus *Thabena* Stål, 1866. Correct identifications were made by the senior author during his visit to North-West A&F University in Yangling (Shaanxi, China) (unpublished).

Gnezdilov with coauthors (Gnezdilov et al. 2015) published phylogenetic study of issidoid families of Fulgoroidea *sensu* Gnezdilov (2013b) based on sequences of COI, 28S (D4, D5, and D6), and 18S (helix 17 – helix 50) of 32 species from 29 genera of Issidae, Caliscelidae, Tropiduchidae, Nogodinidae, Ricaniidae, Dictyopharidae, Flatidae, and Aphrophoridae as an outgroup. Seventeen issid species from 14 genera were involved in this study. Parsimony analysis revealed polyphyly of the genus *Bubastia* Emeljanov, 1975. Soon after Gnezdilov (2016a) performed a Bayesian analysis on the same dataset and revealed sister positions of the genera *Agalma-*

tium Emeljanov, 1971 and *Hysteropterum* Amyot et Serville, 1843 with a support 90, which resulted later in synonymization of Hysteropterina Melichar, 1906 and Agalmatiina Gnezdilov, 2002 (Gnezdilov 2016c).

Wang et al. (2016) provided new phylogenetic analysis and classification of the family Issidae based on 18S, two parts of 28S (D3–D5, D6–D7), COI and CytB genes sequences from 79 species belonging to 50 genera using both Maximum likelihood and Bayesian analyses. According to the resulting classification, the family Issidae was divided into three subfamilies with seven tribes. In particular, the subfamily Thioniinae, with the tribe Thioniini, was reestablished to accommodate Neotropical issids as an independent lineage sister to all other Issidae including the subfamilies Issinae, with the tribes Issini and Hysteropterini, for Palaearctic issids, and Hemisphaeriinae, with the tribes Hemisphaeriini, Kodaianellini Wang, Zhang et Bourgoin, 2016, Sarimini Wang, Zhang et Bourgoin, 2016, and Parahiraciini, for Oriental, Australian, and African issids. American genus *Picumna* Stål, 1864 was provisionally placed in the Hemisphaeriinae as well (Wang et al. 2016). However three years later Zhao et al. (2019) describing new genus of Hemisphaeriini suggested another topology for the family recovering subfamily rank for Hysteropterinae – Thioniinae, Hysteropterinae, Issinae + Hemisphaeriinae (Zhao et al. 2019, fig. 22). In the same paper reassessment of the subtribal division of the Hemisphaeriini proposed by Wang with coauthors (Wang et al. 2016) was suggested. Based mainly on the same data Bourgoin with coauthors (Bourgoin et al. 2018) proposed a calibrated molecu-

lar tree of Issidae and suggested early Cretaceous origin of the group (110 Mya) with a basal split of the family between Neotropical taxa (Thioniinae) and other Issidae (Issinae + Hemisphaeriinae) which is congruent with the opening of the South Atlantic Ocean separating South America and Africa.

Gnezdilov (2016a, 2016b) based on the analysis of morphological and biogeographical data proposed a phylogeny of the subtribe Issina Spinola (all Western Palaearctic taxa included) and suggested Eocene origin of Issidae in the Oriental Region with the subsequent dispersal to the Palaearctic region, Africa, and Australia, and to the New World via Beringia.

Aiming to get a better understanding of phylogenetic relationships within Issidae and to test previously suggested phylogenetic hypotheses we assemble a new molecular dataset that includes 48 terminals representing all major issid clades and data on nine fragments (COI, CytB, 12S, H3, 16S, 18SII, 18SIII, 28S D3–D5, 28S D6–D7).

MATERIAL AND METHODS

Taxon sampling

This study incorporates 48 species out of 43 genera comprising 46 ingroup taxa representing main tribes of the family Issidae and two outgroups from the families Fulgoridae and Kinnaridae. Thirty two species were directly sequenced by us for nine markers, including four mitochondrial (COI, CytB, 12S, 16S), and five nuclear (H3, 18SII, 18SIII, 28S D3–D5, 28S D6–D7) fragments. Sequences of CytB, H3, 18S, 28S D3–D5, and 28S D6–D7 for 16 included species were downloaded from NCBI and were mainly received by Wang et al. (2016). Voucher specimens sequenced in this study are retained in the Auchenorrhyncha collection of the Zoological Institute of the Russian Academy of Sciences in Saint Petersburg (see Table 2).

DNA sequencing and alignment

Total genomic DNA was extracted from thoracic musculature of the specimens preserved in 96% alcohol using a Thermo Scientific GeneJET Genomic DNA Purification Kit with the standard protocol.

Primer pairs used for amplification are provided in Table 3. PCRs were performed in a 20 μ L reaction mixture contacting 1–2 μ L of genomic DNA

template with the following protocol: an initial denaturation 5' at 94 °C for 5 min, followed by 35 cycles of denaturation in 40 s at 94 °C, 40 s annealing at 48–58° (see Table 3), 1 min elongation at 72 °C, and a final elongation for 10 min at 72 °C. The amplification was performed with 0.4 μ M of each primer using a ScreenMix reaction mixture (Evrogen, Russia) containing DNA polymerase, dNTP, MgCl₂ and enhancers at optimal concentrations. Amplified fragments were purified with a PCR purification kit (Evrogen, Russia). Purified PCR products were sequenced in both directions at Evrogen Inc.

All sequences were checked using BLAST through the NCBI database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Forward and reverse sequences were concatenated and manually verified with Geneious Prime 2019.03 (<https://www.geneious.com>). The 18S gene was sequenced in two overlapping parts using the coupled primers 3F-Bi and A2–9R and subsequently spliced into one sequence. Obtained sequences were aligned with data on 16 species taken from Genbank. The accession numbers for all sequences are provided in Table 2. Sequence summary statistics are given in Table 4 including information on total/average length and base frequencies. Alignment was completed using the Muscle algorithm (Edgar 2004) and subsequently checked in Geneious. All genes were concatenated in Sequence Matrix 1.7.8 (Vaidya et al. 2011) to create a master alignment of 5587 bp.

Phylogenetic analysis

Bayesian estimation search (BI) was performed using MrBayes (Ronquist et al. 2011) on the CIPRES Science Gateway V3.1 Portal. Two runs with 12 chains were running simultaneously for 12×10^6 generations with 0.1 temperature setting; burn-in was set at 25%. We applied the most complex model GTR+G+I applied to each partition, as it usually provides a better fit for real data (Arenas 2015; Abadi et al. 2019). Chains were sampled every 1000 generations and the respective trees written to a tree file. After the analysis the stdout file was checked to ensure that the average standard deviation of split frequencies was below 0.01. Fifty-percent majority-rule consensus trees and posterior probabilities of clades were calculated using the trees sampled after the chains converged. The posterior probability supports are provided on Fig. 1.

Table 2. Taxa and molecular partitions used in phylogenetic analysis (generic groups in Hysteropterinae are named after Gnezdilov 2016a, 2016c).

Species	Locality	Taxonomy	Specimen identifier	COI	CytB	H3	12S	16S	18S	28S D3-D5	28S D6-D7	Source
<i>Agalmatium flavescens</i> (Olivier, 1791)	Russia	Hysteropterum-group	ZISP_ISSID G025	MN194180	MN191521	MN267374	MN219666	MN227704	MN165781	MN266987	MN266956	Present study
<i>Anatolodus musivus</i> Diabola, 1982	Turkey	Bubastia-group	ZISP_ISSID G005	MN194181				MN227705	MN165782	MN266988	MN266957	Present study
<i>Baldiza una</i> (Ball, 1910)	Mexico	Issini, Thioniina	ZISP_ISSID G017		MN191522	MN267376	MN219665	MN227706	MN165783	MN266989	MN266958	Present study
<i>Bootheca taurus</i> (Oshanin, 1870)	Bulgaria	Kervillea-group	ZISP_ISSID G034	MN194182	MN191523	MN267377	MN219667	MN227707	MN165784	MN266990	MN266959	Present study
<i>Bubastia josifovi</i> Diabola, 1980	Bulgaria	Bubastia-group	ZISP_ISSID G020		MN191524		MN219668	MN227708	MN165785	MN266991	MN266960	Present study
<i>Bubastia</i> sp.	Greece	Bubastia-group	ZISP_ISSID G018		MN191525	MN267378		MN227709	MN165786	MN266992	MN266961	Present study
<i>Cheiloceps argo</i> (Fennah, 1949)	USA	Issini, Thioniina				DQ532696			DQ532543			Urban & Cryan, 2007
<i>Conosimus coelatus</i> Mulsant et Rey, 1855	France	Conosimus-group	ZISP_ISSID G012	MN194183	MN191526	MN267379	MN219669	MN227710	MN165787	MN266993	MN266962	Present study
<i>Dactylissus armillarius</i> Gnezdilov et Soulier-Perkins, 2014	Vietnam	Sarimini			KX702879				KX702829			Wang et al., 2016
<i>Darwallia barbata</i> Gnezdilov et Bourgoin, 2014	Vietnam	Sarimini			KX702888				KX702838	KX761410	KX702864	Wang et al., 2016
<i>Dorysarthrus mobilicornis</i> Puton, 1895	UAE	Fulgoridae	ZISP_ISSID G028	MN194184			MN267380	MN219670	MN165788	MN266994	MN266963	Present study
<i>Eurovenus vaysieresii</i> (Bonfils, Attié et Reynaud, 2001)	Reunion	Sarimini	ZISP_ISSID G004				MN267381	MN219671	MN165789	MN266995	MN266964	Present study
<i>Euxaldar lenis</i> Gnezdilov, Bourgoin et Wang, 2017	Vietnam	Hemisphaerini							KX761565	KX761412		Wang et al., 2016
<i>Falcidius limbatus</i> (A. Costa, 1864)	Italy	Bubastia-group	ZISP_ISSID G016	MN194185			MN267375	MN219672	MN165790	MN266996	MN266965	Present study
<i>Flacina hainana</i> (Wang et Wang, 1999)	China	Parahiracini			KX702912				KX702824	KX761453		Wang et al., 2016
<i>Thabena litaoensis</i> (Yang, 1994)	China	Parahiracini			KX702911				KX702823	KX761452	KX702811	Wang et al., 2016

Table 2. Continued.

Species	Locality	Taxonomy	Specimen identifier	COI	CytB	H3	12S	16S	18S	28S D3-D5	28S D6-D7	Source
<i>Hemisphaerius coccinelloides</i> (Burmeister, 1834)	Philippines	Hemisphaerini			KX702884				KX702834	KX761405	KX702861	Wang et al., 2016
<i>Hysteropterum dolichotum</i> Gnezdilov et Mazzoni, 2004	France	Hysteropterum-group	ZISP_ISSID G013				MN219673	MN227713	MN165791	MN266997	MN266966	Present study
<i>Issus coleopratus</i> (Fabricius, 1781)	Italy	Issini, Issina	ZISP_ISSID G030	MN194186	MN191527	MN267383	MN219674	MN227714	MN165792	MN266998	MN266967	Present study
<i>Issus lauri</i> Ahrens, 1814	Italy	Issini, Issina	ZISP_ISSID G019		MN191528	MN267384	MN219675	MN227715	MN165793	MN266999	MN266968	Present study
<i>Kervillea conspurcata</i> (Spinola, 1839)	Slovenia	Kervillea-group	ZISP_ISSID G024	MN194187	MN191529		MN219676	MN227716	MN165794	MN267000	MN266969	Present study
<i>Kodaianella bicinctifrons</i> Fennah, 1956	China	Kodaianellini			KX702902				KX702814	KX761441	KX702802	Wang et al., 2016
<i>Latematium latifrons</i> (Fieber, 1877)	Bulgaria	Kervillea-group	ZISP_ISSID G009	MN194188	MN191530	MN267385	MN219677	MN227717	MN165795	MN267001	MN266970	Present study
<i>Latilica antalyica</i> (Diabola, 1986)	Greece	Bubastia-group	ZISP_ISSID G022						MN165796	MN267002	MN266971	Present study
<i>Latilica oertzeni</i> (Matsumura, 1910)	Greece	Bubastia-group	ZISP_ISSID G033		MN191531	MN267386	MN219679					Present study
<i>Latissus dilatatus</i> (Fourcroy, 1785)	Slovenia	Issini, Issina	ZISP_ISSID G031		MN191532	MN267387		MN227719	MN165797	MN267003	MN266972	Present study
<i>Macrodaruma pertinax</i> Fennah, 1978	Vietnam	Hemisphaerini			KX702882				KX702832	KX761402	KX702859	Wang et al., 2016
<i>Macrodarumoides petalinus</i> Che, Zhang et Wang, 2012	China	Parahiracini			KX702880				KX702827		KX702856	Wang et al., 2016
<i>Mongoliana triangularis</i> Che, Wang et Chou, 2003	China	Hemisphaerini			KX761510				KX761561	KX761528		Wang et al., 2016
<i>Mycterodus drosopoulovi</i> Diabola, 1982	Greece	Mycterodus-group	ZISP_ISSID G006	MN194189	MN191533		MN219680		MN165798	MN267004	MN266973	Present study
<i>Mycterodus goricus</i> (Diabola, 1958)	Armenia	Mycterodus-group	ZISP_ISSID G001	MN194190	MN191534	MN267388	MN219681		MN165799	MN267005	MN266974	Present study
<i>Ophthalmosphaerius triobolus</i> (Che, Zhang et Wang, 2006)	China	Hemisphaerini			KX702914				KX702826	KX761455	KX702813	Wang et al., 2016

Table 2. Continued.

Species	Locality	Taxonomy	Specimen identifier	COI	CytB	H3	12S	16S	18S	28S D3-D5	28S D6-D7	Source
<i>Palmallorus punctulatus</i> (Rambur, 1840)	Portugal	Bubastia-group	ZISP_ISSID G010		MN191535	MN267389	MN219682	MN227720	MN165800	MN267006	MN266975	Present study
<i>Peloma brunnescens</i> (Emeljanov, 1984)	UAE	Kinnaridae	ZISP_ISSID G029	MN194192	MN191536	MN267391		MN227722	MN165802	MN267008	MN266977	Present study
<i>Picumna</i> sp.	Mexico	Issinae	ZISP_ISSID G027	MN194191		MN267390	MN219683	MN227721	MN165801	MN267007	MN266976	Present study
<i>Proteissus bitimeki</i> Fowler, 1904	Mexico	Issini, Thioniina	ZISP_ISSID G002	MN194193	MN191537	MN267392	MN219684		MN165803	MN267009	MN266978	Present study
<i>Sarima bifurca</i> Meng et Wang, 2016	China	Sarimini			KX761552				KX702819	KX761447	KX702808	Wang et al., 2016
<i>Scorlupaister heptapotamicum</i> Mitjavec, 1971	Kazakhstan	Phasmena-group	ZISP_ISSID G011			MN267393		MN227723		MN267010	MN266979	Present study
<i>Scorlupella discolor</i> (Germar, 1821)	Bulgaria	Kervillea-group	ZISP_ISSID G008			MN267394	MN219685	MN227724	MN165804	MN267011	MN266980	Present study
<i>Tetricissus philo</i> (Fennah, 1978)	Vietnam	Kodaianellini			KX702889				KX702839			Wang et al., 2016
<i>Tetricodes songae</i> Zhang et Chen, 2009	China	Parahiracini			KX702916				KX702841	KX761457		Wang et al., 2016
<i>Tetricodissus pandineus</i> Wang, Bourgoin et Zhang, 2015	China	Parahiracini			KX702907				KX702817	KX761445	KX702807	Wang et al., 2016
<i>Thalassana ephialtes</i> (Linnavuori, 1971)	Turkey	Mycterodus-group	ZISP_ISSID G015	MN194194	MN191538	MN267396	MN219686		MN165805	MN267012	MN266981	Present study
<i>Tingissus guadarumense</i> (Melichar, 1906)	Portugal	Hysteropterum-group	ZISP_ISSID G021			MN267397	MN219687	MN227725	MN165806	MN267013	MN266982	Present study
<i>Traxus fulvus</i> Metcalf, 1923	Mexico	Issini, Thioniina	ZISP_ISSID G014	MN194195	MN191539	MN267398	MN219688	MN227726	MN165807	MN267014	MN266983	Present study
<i>Tshurtshurnella bicolorata</i> Gnezdilov et Oezgen, 2018	Turkey	Mycterodus-group	ZISP_ISSID G007	MN194196	MN191540				MN165808	MN267015	MN266984	Present study
<i>Tshurtshurnella zelleri</i> (Kirschbaum, 1868)	Italy	Mycterodus-group	ZISP_ISSID G032		MN191541	MN267399	MN219689	MN227728	MN165809	MN267016	MN266985	Present study
<i>Zopherisca penelopae</i> (Diabola, 1974)	Greece	Mycterodus-group	ZISP_ISSID G003						MN165810	MN267017	MN266986	Present study

Table 3. Primer sequences and annealing temperatures.

Region	Primer	Direction	Sequence	Source	Tm
COI	2183	Fwd	CAACATTTATTTTGATTTTTTGG	Simon et al. (1994)	48
	UEA8	Rev	AAAAATGTTGAGGGAAAAATGTTA	Lunt et al. (1996)	
CytB	Cytb_F	Fwd	GTTCTACCTTGAGGTCAAATATC	Song & Liang (2013)	56
	Cytb_R	Rev	TTCTACTGGTCGTGCTCCAATTCA		
H3	AF	Fwd	ATGGCTCGTACCAAGCAGACVGC	Ogden & Whiting (2003)	48
	AR	Rev	ATATCCTTRGGCATRATRGTGAC		
12S	ai	Fwd	AAACTAGGATTAGATACCTATTAT	Simon et al. (1994)	48
	bi	Rev	AAGAGCGACGGGCGATGTGT		
16S	Full_16S_F	Fwd	CCGGTTTGAAGCTCAGATCATGTAA	Song & Liang (2013)	48
	Full_16S_R	Rev	ATTTATTGTACCTTTTGTATCAG		
18S II	3F	Fwd	GTTTCGATCCGGAGAGGGA	Giribet et al. (1996)	56
	Bi	Rev	GAGTCTCGTTCGTTATCGGA	Urban & Cryan (2007)	
18S III	A2	Fwd	ATGGTTGCAAAGCTGAAAC	Urban & Cryan (2007)	58
	9R	Rev	GATCCTTCCGCAGGTTACCTAC	Giribet et al. (1996)	
28S D3-D5	28S Ai	Fwd	GACCCGTCTTGAAACACG	Belshaw & Quicke (2002)	54
	28S D4D5r	Rev	GTTACACACTCCTTAGCGGA		
28S D6-D7	28S EE	Fwd	CCGCTAAGGAGTGTGTAA	Cryan et al. (2000)	54
	28S MM	Rev	GAAGTTACGGATCTARTTG		

Table 4. Summary statistics of genes used for phylogeny.

Region	Number of taxa	Sequence length			Identical sites, %	Pairwise identity, %	Base frequencies			
		Longest	Shortest	Average			%A	%T	%C	%G
COI	17	584	550	571.5	60.6	85.1	36.0	34.3	15.9	13.8
CytB	35	639	592	610.3	40.4	78.9	38.1	33.5	18.6	9.6
H3	27	370	249	344.8	64.3	88.3	23.0	16.7	31.8	28.0
12S	25	346	260	319.5	20.8	78.4	23.9	47.5	7.0	15.6
16S	25	583	397	500.5	51.8	84.1	29.3	44.4	9.1	17.1
18S	46	1856	651	1475.8	31.5	87.5	22.5	23.7	24.5	28.4
28S D3-D5	43	712	611	677.3	58.9	94.1	23.1	18.6	25.3	32.1
28S D6-D7	40	810	671	752.8	60.1	90.6	20.4	19.2	26.9	33.2

Maximum Likelihood (ML) analysis was performed using RAxML (Stamatakis 2016) via the CIPRES Science Gateway V. 3.3 (http://www.phylo.org/sub_sections/portal/) (Miller et al., 2010). We used RAxML-HPC BlackBox tool with 10000 boot-

strap iterations and a subsequent thorough ML search, using the General-Time-Reversible (GTR) algorithm with gamma distributed substitution rates and invariable sites (GTR+I+G) for each partition independently. The bootstrap supports are provided on Fig. 2.

RESULTS

Tree topologies recovered by both BI and ML analyses were largely congruent (Figs 1, 2). The ML tree shows less resolution and did not recover Issini and Parahiraciini as monophyletic groups, while the BI analysis recovering both tribes with high support (93% and 100% respectively). Nodes of the major clades are numbered from 1 to 12.

Node 1 (BI: 100; ML: 77) supports the monophyly of the subfamily Issinae (= Thioniinae, = Hemisphaeriidae Melichar, 1906) *sensu* Gnezdilov (2009, 2013a). This clade includes Issini, Thioniinae and Hemisphaeriinae *sensu* Wang et al. (2016) or Issina *sensu* Gnezdilov (2002) + Hemisphaeriini + Parahiraciini *sensu* Gnezdilov (2013a) (Table 5). In this combination of taxa the subfamily Issinae is defined for the first time. Many taxa of this subfamily are characterized by furcating *CuA* on forewings and well-developed hind wings.

Node 2 (BI: 100; ML: 69) represents the subfamily Hysteropterinae and comprises Hysteropterini *sensu* Wang et al. (2016) or Issina, excluding *Issus* Fabricius, 1803 and *Latissus* Dlabola, 1974, *sensu* Gnezdilov (2016a, 2016c), or Hysteropterina + Agalmatiina *sensu* Gnezdilov (2002). This clade includes Western Palaearctic taxa with rudimentary anal lobe of hind wings, without vannal cleft or with reduced hind wings (Gnezdilov 2016a).

Node 3 (BI: 93; not recovered in ML) forms the tribe Issini and combines Western Palaearctic *Issus* Fabricius and *Latissus* Dlabola together with all American taxa involved in the current analysis *viz.*, *Balduza* Gnezdilov et O'Brien, 2006, *Proteinissus* Fowler, 1904, *Cheiloceps* Uhler, 1895, and *Traxus* Metcalf, 1923. In this combination of taxa the tribe Issini is defined for the first time. The composition of this clade may serve as a confirmation of the synonymy of Issini and Thioniini proposed by Gnezdilov (2009) since *Cheiloceps* Uhler belongs to the subtribe Thioniina Melichar *sensu stricto* (Gnezdilov 2018a) and was previously treated in *Thionia sensu lato*. However the clade is not supported by morphological data and requires further study.

Node 4 (BI: 100; ML: 100) corresponds to the subtribe Issina *sensu* Gnezdilov (2002) and *Issus* group of genera *sensu* Gnezdilov (2016a, 2016c). This clade includes two genera *Issus* Fabricius and *Latissus* Dlabola which are morphologically related by a unique synapomorphy – the presence of paired

digitate processes on the inner side of the dorsolateral lobes of the phallobase (Gnezdilov 2016c, figs 1–4). This group retained many ancestral characters within Western Palaearctic Issidae (Gnezdilov 2016a, 2017a) including bi-lobed hind wings, with vannal cleft only and anal lobe reduced to a small appendage with simple second anal vein. *Issus pospisili* Dlabola, 1958 has hind wing with partly fused *Pcu* and anterior branch of first anal vein (Gnezdilov 2017a, Fig. 22) which apparently relates Issina to Oriental issid taxa and to American subtribe Thioniina *sensu* Gnezdilov (2018a).

Node 5 (BI: 100; ML: 78) corresponds to the subtribe Thioniina *sensu* Gnezdilov (2018a) with inclusion of American taxa, characterized by reduced or rudimentary hind wings, and to the subfamily Thioniinae *sensu* Wang et al. (2016) (Table 5).

Node 6 (BI: 100; ML: 100) the tribe Sarimini *sensu* Wang et al. (2016) was not recovered in our analysis (Figs 1, 2). However all genera currently assigned to this group (Wang et al. 2016; Gnezdilov 2019b) are characterized by tri-lobed hind wings with deep cubital cleft and often with *CuA* and *Cup* fusing apically with flattening. The genus *Euroxenus* Gnezdilov, 2009 also shares these characters and might belong to this group from the morphological standpoint.

Node 7 (BI: 100; not recovered in ML) corresponds to Parahiraciini *sensu* Wang et al. (2016) and Gnezdilov (2017b). Most included genera are characterized by bi-lobed hind wing, with deep cubital cleft and more or less reduced anal lobe.

Node 8 (BI: 90; ML: 88) represents the tribe Hemisphaeriini *sensu* Wang et al. (2016) and contains genera with hemispherical fore wings and single-lobed or rudimentary hind wings.

Node 9 (BI and ML: 93) forms the tribe Kodai-anellini. Members of this tribe are united by the three-lobed hind wings, with large remigial lobe and small remigio-vannal and anal lobes (Wang et al. 2016).

Node 10 (BI: 100; ML: 92) corresponds to the *Kervillea* group of genera *sensu* Gnezdilov (2016a, 2016c). These genera are characterized by a peculiar structure of the phallobase with a pair of long folds which frequently conceals ventrally its ventral lobe and separated lobes of gonoplares (Gp 1 and Gp 2) (Gnezdilov 2016a, 2016c).

Node 11 (BI: 69; ML: 59) represents the *Mycterodus* group of genera *sensu* Gnezdilov (2016a, 2016c). This group is united by the structure of penis with

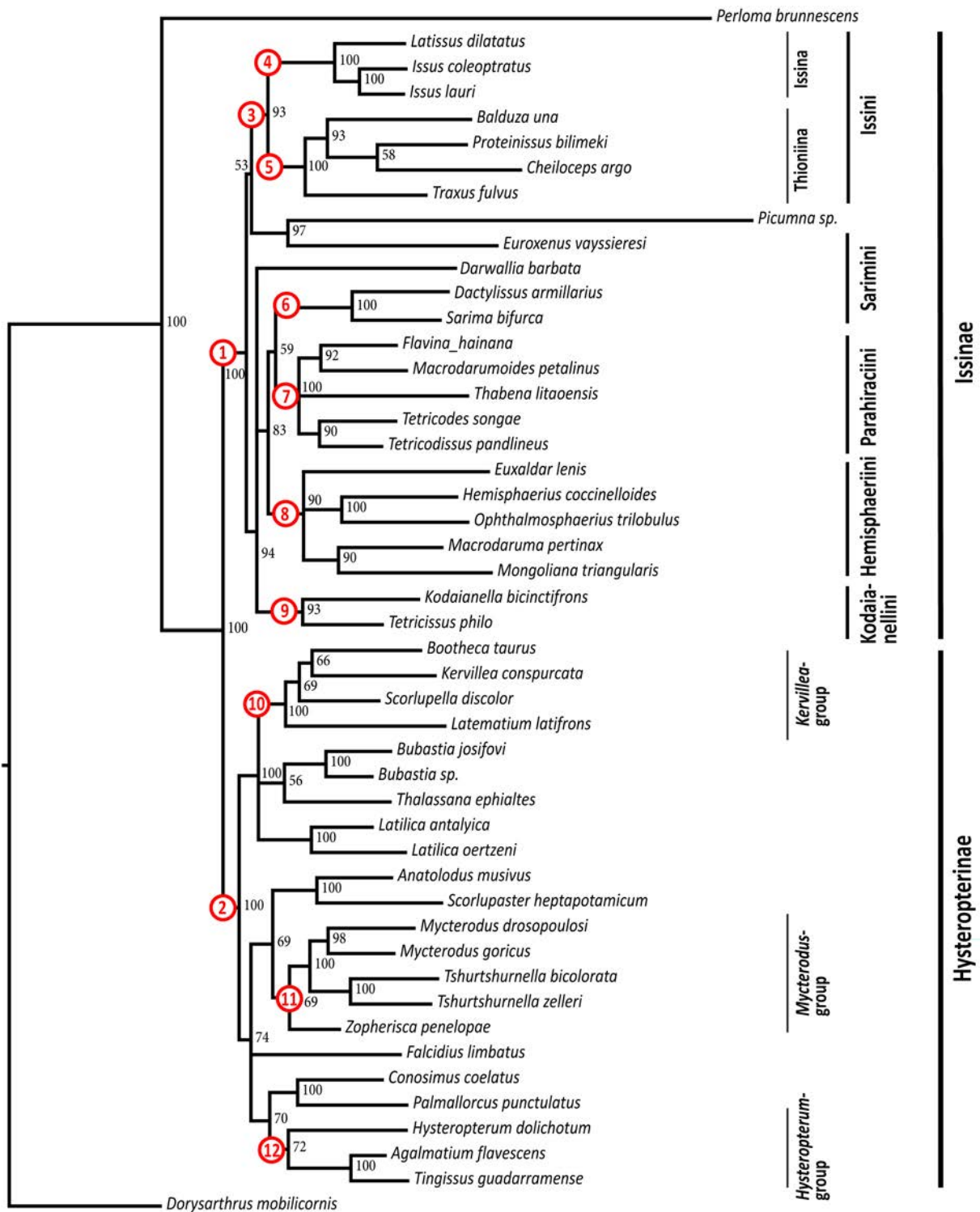


Fig. 1. Bayesian 50% consensus tree based on combined dataset (BI). Nodes of the major clades are numbered and refer to text. Each node is documented with its posterior probability supports.

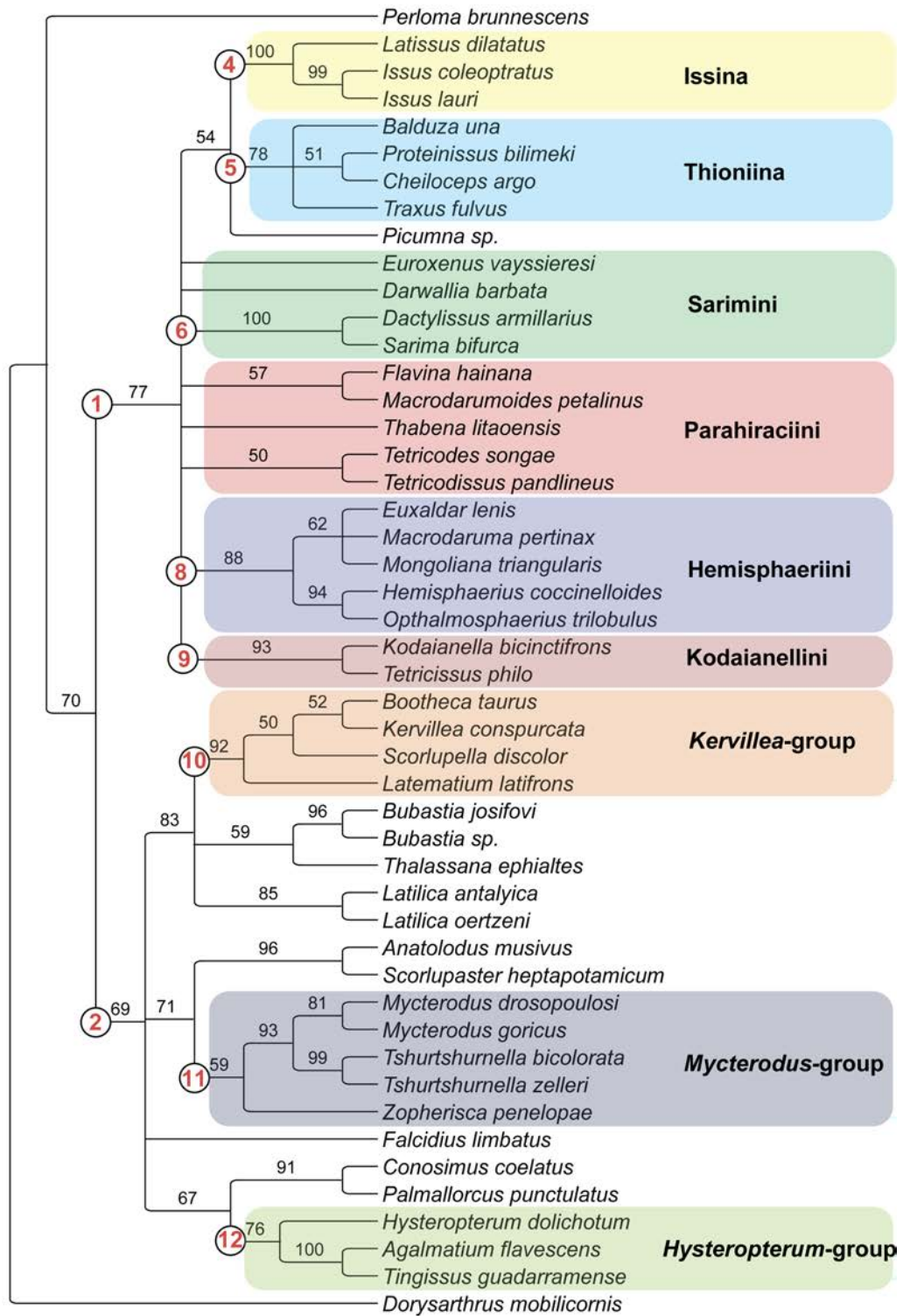


Fig. 2. Maximum Likelihood (ML) tree estimated from the combined dataset. At each node, values indicate bootstrap supports.

Table 5. Taxonomic groups matching in recent publications and current data.

Gnezdilov 2013a, 2016c	Wang et al. 2016	Current data
Issini	Issinae, Thioniinae, Hemisphaeriinae (part)	Issinae (=Thioniinae, =Hemisphaeriinae), Hysteropterinae
Issina	Issinae (Issini, Hysteropterini)	Issinae (Issini), Hysteropterinae
Thioniina	Thioniinae (Thioniini), Hemisphaeriinae (Kodaianellini, Sarimini)	Issinae (Thioniini, Kodaianellini, Sarimini)
Parahiraciini	Hemisphaeriinae (Parahiraciini)	Issinae (Parahiraciini)
Hemisphaeriini	Hemisphaeriinae (Hemisphaeriini: Hemisphaeriina, Mongolianina)	Issinae (Hemisphaeriini)

the aedeagus not entirely concealed by the phallobase and clearly visible from the outside and the fused apical aedeagal processes forming cylinder (Gnezdilov 2016a, 2016c).

Node 12 (BI: 72; ML: 76) corresponds to the *Hysteropterum* group of genera *sensu* Gnezdilov (2016a, 2016c). According to Kusnezova and Aguin-Pombo (2015) the genera *Hysteropterum* Amyot et Serville, 1843 and *Agalmatium* Emeljanov, 1971 are similar in having nucleolus organizer regions (NORs) located sub-terminally in the largest pair of autosomes although this character occur in other groups of Auchenorrhyncha as well.

DISCUSSION

Our study involves more molecular markers than previously and recovered a mostly well-resolved tree with moderate to high support for most branches.

The combination of Palaearctic *Issus* Fabricius and *Latisus* Dlabola with Oriental and American taxa in one well-supported clade of Issinae (Node 1) confirms the existence of a common ancestor for extant Oriental, American, and Palaearctic issids earlier hypothesized by Gnezdilov (2016a). Thus newly obtained data contradicts the scenario of an early split of American Thioniinae from other Issidae and possible origin of the family in the New World recently suggested by Wang et al. (2016) and Bourgoin et al. (2018). At the same time monophyly of the tribe Kodaianellini erected by Wang et al. (2016) is confirmed by our study while monophyly of Sarimini Wang, Zhang et Bourgoin, 2016 was not supported by our data.

Within the subfamily Issinae five clades are well supported (Fig. 1) and correspond to the tribes

Hemisphaeriini (*Euxaldar* Fennah, 1978 – *Hemisphaerius* Schaum, 1850 – *Ophthalmosphaerius* Gnezdilov, 2018 – *Macrodaruma* Fennah, 1978 – *Mongoliana* Distant, 1909) (Node 8), Kodaianellini (*Tetricissus* Wang, Bourgoin et Zhang, 2017 – *Kodaianella* Fennah, 1956) (Node 9), Sarimini (*Dactylissus* Gnezdilov et Bourgoin, 2014 – *Sarima* Melichar, 1903) (Node 6), and Thioniina (*Traxus* Metcalf, 1923 – *Cheiloceps* Uhler, 1895 – *Balduza* Gnezdilov et O'Brien, 2006 – *Proteinissus* Fowler, 1904) (Node 5) and Issina (*Issus* Fabricius – *Latisus* Dlabola) (Node 4) of the tribe Issini (Node 3). Sixth clade corresponding to the tribe Parahiraciini (Node 7) is well supported in the Bayesian tree and forms a sister group to Sarimini with a support 59 (Fig. 1), but was not recovered in RAXML analysis (Fig. 2). The relationships of these tribes within Issinae are still not clear. The tribe Hemisphaeriini forms a sister group to Sarimini + Parahiraciini in the BI analysis with a support of 83. Close relationships of Sarimini, Parahiraciini, Hemisphaeriini, and Kodaianellini revealed by Wang et al. (2016) were also confirmed in the BI with a support 94 of this clade (Fig. 1), although it was not recovered in the ML tree (Fig. 2).

Our concept of Issinae includes all taxa with well-developed hind wings. This feature was treated by Gnezdilov (2016a, 2016b, 2016c, 2017a) as an ancestral trait in comparison to Hysteropterinae having reduced or rudimentary hind wings. Most part of American taxa involved in the current analysis are combined in one clade (Figs 1, 2) which supports the assumption of the existence of a common ancestor for extant Nearctic and Neotropical issid taxa even the relationships of North American *Picumna* Stål, 1864 to other American and Oriental taxa is still unclear.

In accordance with the above treatment of the subfamily Issinae and the taxonomic ranks of other groups under study, the subtribe Chimetopina Gnezdilov, 2017 from tropical Africa (Gnezdilov 2017a) should be treated as a group of the tribal level – Chimetopini Gnezdilov, 2017, stat. nov.

Our data suggest that the subtribe Issina *sensu* Gnezdilov (2002), comprising *Issus* Fabricius and *Latissus* Dlabola, is combined with Oriental and American taxa and is not related to other Western Palaearctic Issidae belonging to Hysteropterinae. The last group is represented on the trees (Figs 1, 2) by three distinct clades (BI: 69–100; ML: 67–83): (1) *Hysteropterum* group + *Conosimus* Mulsant et Rey, 1855 + *Palmallorcus* Gnezdilov, 2003; (2) *Kervillea* group + *Bubastia* Emeljanov, 1975 + *Thalassana* Gnezdilov, 2016 + *Latilica* Emeljanov, 1971; (3) *Mycterodus* group + *Scorlupaster* Emeljanov, 1971 + *Anatolodus* Dlabola, 1982. The genus *Falcidius* Stål, 1866 occupies separate position on the trees.

The *Bubastia* group of genera *sensu* Gnezdilov (2016a, 2016c) which includes the genera *Bubastia* Emeljanov, *Falcidius* Stål, and *Latilica* Emeljanov is rendered as non-monophyletic. Close relationships of *Conosimus* Mulsant et Rey and *Palmallorcus* Gnezdilov (BI: 100; ML: 91) and association of this clade with *Hysteropterum* group (Node 12) as well as close relationships of *Anatolodus* Dlabola and *Scorlupaster* Emeljanov (BI: 100; ML: 96) and association of this clade with *Mycterodus* group (Node 11) are revealed for the first time. *Thalassana* Gnezdilov, previously treated as a member of *Mycterodus* group of genera by Gnezdilov (2016a, 2016c), forms a sister group to *Bubastia* Emeljanov, although support for this clade is very low (BI: 56; ML: 59).

CONCLUSIONS

The BI and ML trees obtained in our study (Figs 1, 2) suggest the subdivision of the family Issidae into two subfamilies – Issinae and Hysteropterinae. The combination of all taxa with developed hind wings (ancestral condition) in the subfamily Issinae favors the concept of a common ancestor for Oriental and American issids previously hypothesized by Gnezdilov (2016a, 2019a). The obtained results well demonstrate a geographical pattern of Issidae already pointed out by Wang et al. (2016) e.g., Hysteropterinae are restricted in distribution to Western Palaearctic region while the clade Sarimini +

Parahiraciini + Hemisphaeriini + Kodaianelini is distributed in the Oriental Region.

More taxa from the Neotropics, tropical Africa, and Australia have to be involved in further analysis to clarify the tribal positions of the genera not included in the current study, to elucidate the taxonomic status of currently recognized tribes and subtribes, and to test the hypothesis of the origin and dispersal of Issidae from the Oriental region to Africa and New World proposed by Gnezdilov (2016a, 2016b, 2019a).

Morphological data suggest inclusion of substantial number of American and tropical African taxa not accessible for current analysis in the subfamily Issinae (Gnezdilov 2013a, 2016a, 2016b, 2016d, 2017a, 2019a). Our study corroborates the placement of some American taxa within the tribe Issini (Table 1, Figs 1, 2). Australian issid fauna, as currently known, is largely derived from the Orient region (Gnezdilov 2013a; Gnezdilov and Fletcher 2010) which may suggest close relationships of Australian taxa with the tribe Sarimini. Perhaps the status of main groups recognized above within the subfamily Hysteropterinae is of tribal level, but a much broader sample of Western Palaearctic taxa is clearly needed for testing and this issue will be addressed in the subsequent study.

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