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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 *Echinopartum* 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 | | | | | | 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 | | | | 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 | | | 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 | | | | 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>Pertoma brunnescens</i> (Emeljanov, 1984) | UAE | Kinnaridae | ZISP_ISSID G029 | MN194192 | MN191536 | MN267391 | | MN227722 | MN165802 | MN267008 | MN266977 | Present study |
| <i>Picumma</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

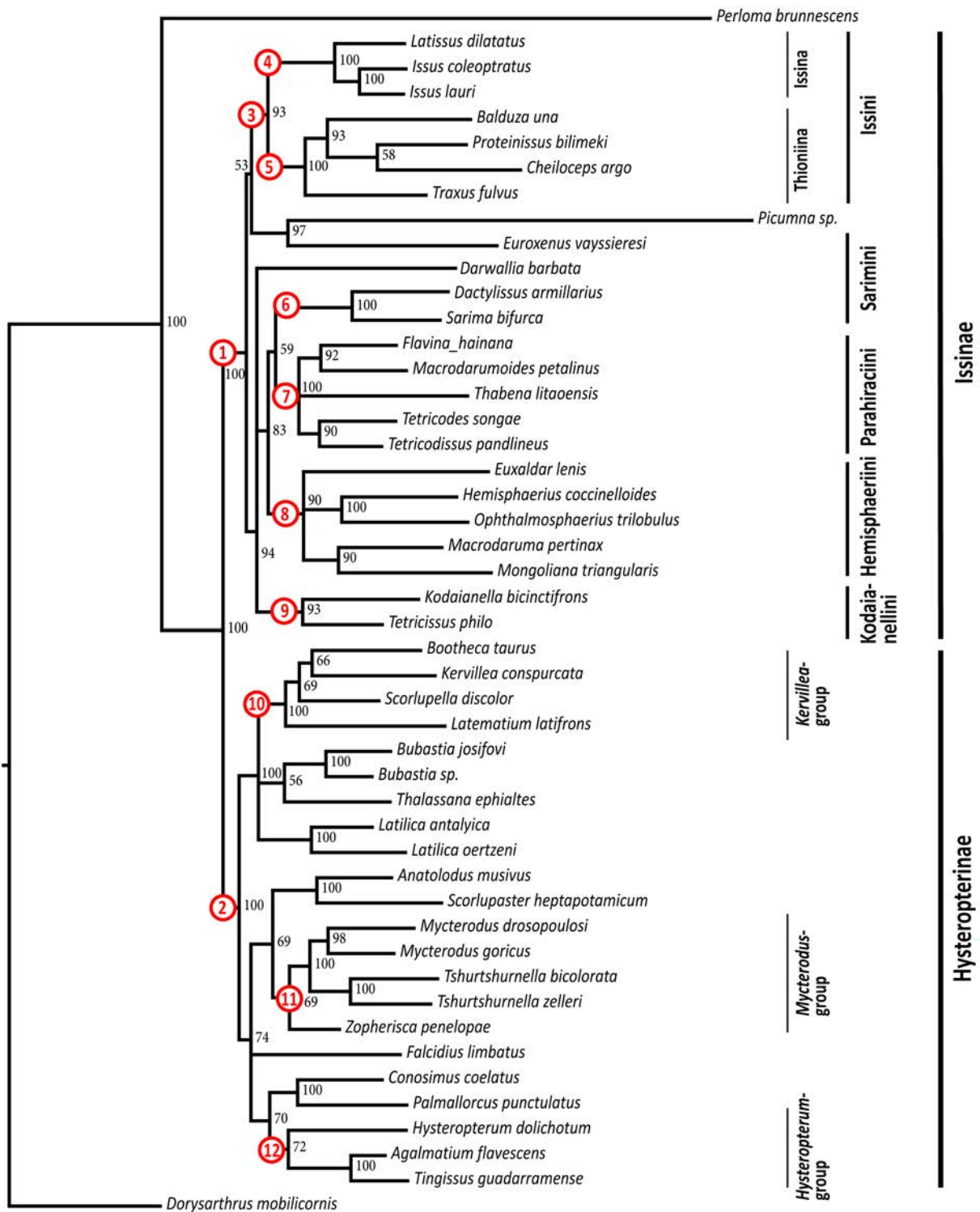


Fig. 1. Bayesian 50% consensus tree based on combined dataset (BI). Nodes of the major clades are numbered and refer to text. Each node is documented with its posterior probability supports.

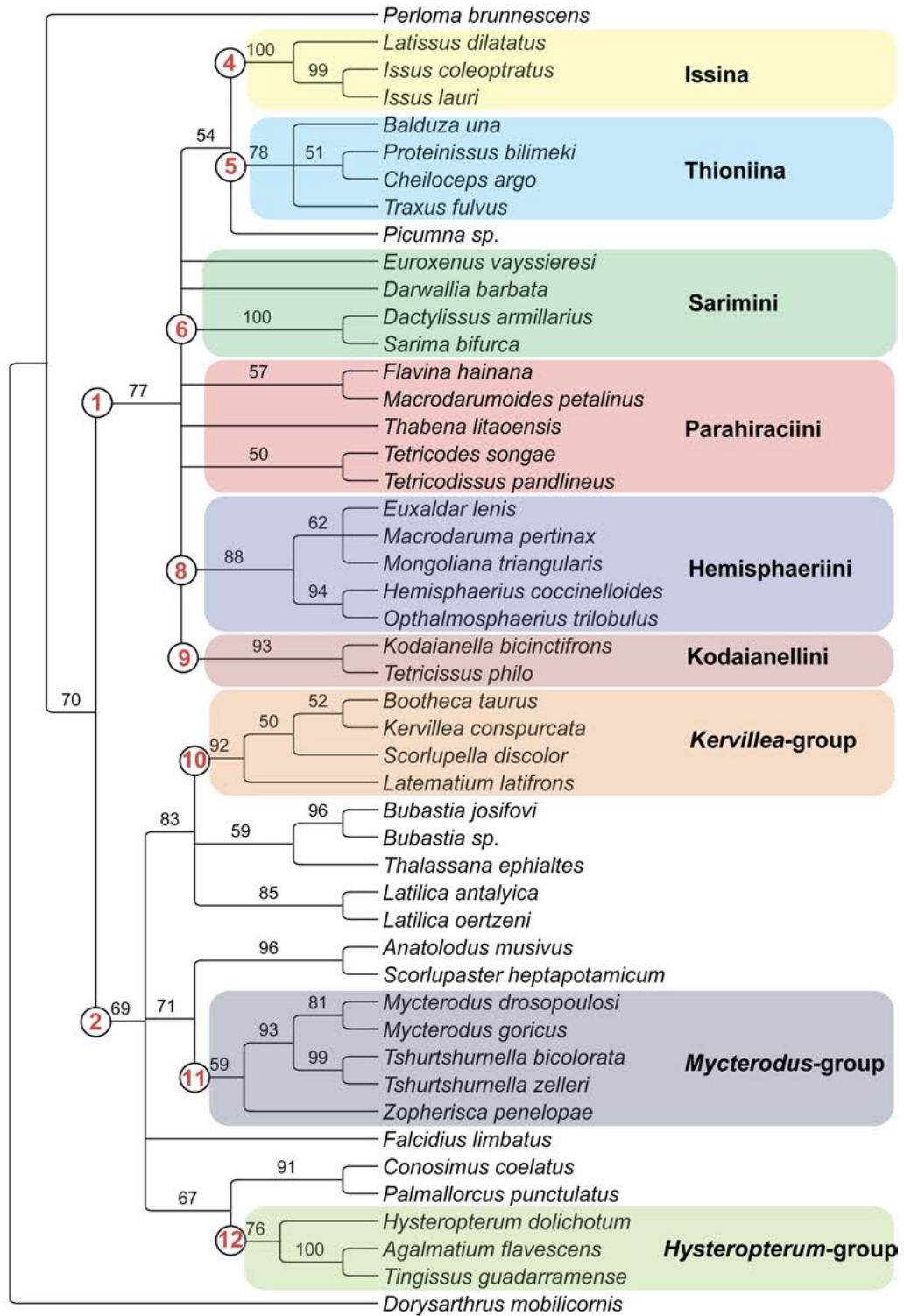


Fig. 2. Maximum Likelihood (ML) tree estimated from the combined dataset. At each node, values indicate bootstrap supports.

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 Palaeartic *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 Palaeartic 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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