

Molecular phylogeny of the fungus gnat family Diadocidiidae and its position within the infraorder Bibionomorpha (Diptera)

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The molecular phylogeny of the family Diadocidiidae (Diptera: Sciaroidea) is reconstructed based on the combined analysis of four mitochondrial (12S, 16S, COI, cytB) and two nuclear (28S, ITS2) gene markers. All the analyses strongly support Diadocidiidae as a monophyletic group. Genus *Diadocidia* Ruthe, 1831 includes monophyletic subgenera *Diadocidia* s. str. and *Taidocidia* Papp and Ševčík (*Acta Zoologica Academiae Scientiarum Hungaricae*, 51, 2005b, 329). The monophyly of *Adidocidia* Laštovka & Matile, 1972 was not confirmed. The position of Diadocidiidae and relationships of the families within the infraorder Bibionomorpha are demonstrated in the analyses based on three gene markers (28S, 12S and 16S). The Bayesian and maximum likelihood analyses of 10 families of Bibionomorpha revealed Sciaridae as the closest relative of Diadocidiidae. Most of the currently recognised extant families of Bibionomorpha proved to be monophyletic. The family Keroplatidae revealed as paraphyletic, with the genera of Macrocerinae being more related to Cecidomyiidae, but the support is low.

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Introduction

Fungus gnats of the family Diadocidiidae (Diptera) belong to the superfamily Sciaroidea (cf. Söli *et al.* 2000) comprising also eight other families (Bolitophilidae, Cecidomyiidae, Ditomyiidae, Keroplatidae, Lygistorrhinidae, Mycetophilidae, Rangomaramidae and Sciaridae) and several enigmatic genera not yet assigned to a family. Although Sciaroidea as a whole represents one of the most abundant and diverse groups of insects, diadocidiids

are a rather uniform and small family comprising 26 described extant species in the world (Bechev & Chandler 2011). Their biology still remains mostly unknown, except for the common European species *Diadocidia ferruginosa* (Meigen, 1830), whose larvae live within slime or silky tubes on the surface of fruiting bodies of the wood-decaying fungus *Peniophora* sp. (see Chandler 2010; Jakovlev 2011, 2012). The adults can be found in forest habitats and usually aggregate with other fungus gnats along banks

of streams, fallen tree trunks, and similar moist and shady places.

The phylogeny of the family Diadocidiidae has not yet been studied and also its position within the Sciaroidea or Bibionomorpha is still unclear. Most of the studies based on the morphology of adult gnats placed Diadocidiidae close to Keroplatidae (Matile 1990, 1997; Söli 1997; Hippa & Vilkkamaa 2006; Amorim & Rindal 2007). Molecular studies of the entire Diptera have usually included only one species of Diadocidiidae (e.g. Bertone *et al.* 2008; Wiegmann *et al.* 2011), or this family has been used as the outgroup to Mycetophilidae (Rindal *et al.* 2009; Ševčík *et al.* 2013). In Wiegmann *et al.* (2011), *Diadocidia ferruginosa* appeared as the sister group to all other Sciaroidea, excluding Ditomyiidae and “Manotidae” (actually Manotinae; see their Table S1), all represented by only one species in their analysis. Rindal *et al.* (2009) used two species of Diadocidiidae as the outgroup for Mycetophilidae, but their sampling was not sufficient enough to establish the position of the family convincingly, nor its relationships among the other families of Sciaroidea. Both the taxon and gene sampling in the previous unpublished molecular analysis of Sciaroidea by Baxter (1999) were even less comprehensive.

The family Diadocidiidae currently includes only one extant genus *Diadocidia* Ruthe, 1831, divided into three subgenera, viz. *Adidocidia* Laštovka & Matile, 1972, *Diadocidia* s. str., and *Taidocidia* Papp & Ševčík 2005 (see Laštovka & Matile 1972, Papp & Ševčík 2005b, Bechev & Chandler 2011). However, the characters separating these subgenera have recently been questioned (Jaschhof & Jaschhof 2007; Vockeroth 2009) because they apparently apply mainly to Holarctic species of the genus and some species remain not assigned to any subgenus. The genus *Diadocidia* is also represented by one species in the fossil record. An additional fossil genus *Docidiadia* was described by Blagoderov & Grimaldi (2004).

The adult diadocidiids are morphologically rather uniform, with relatively few characters other than terminalia being used to separate individual taxa. Therefore, the aim of this paper was to provide molecular evidence for the phylogeny and affiliation of this family within the superfamily Sciaroidea and the entire infraorder Bibionomorpha.

Material and methods

Sampling

We have selected 57 species belonging to 10 families of Bibionomorpha (Table 1, S1) and used four species from the families Anisopodidae and Scatopsidae as the outgroup. Several taxa were taken from the GenBank database. The samples were collected throughout the world, usually by means of Malaise traps, in the years 1996–2013.

DNA extraction, amplification and sequencing

All the material used for DNA analysis was alcohol preserved (70% or 99.9% ethanol) except a specimen of *Diadocidia fissa* Zaitzev, which was preserved in glycerol. The DNA was extracted using DNeasy Blood & Tissue Kit (QIAGEN, Valencia, CA, USA) following the manufacturer’s protocol. Individual flies or tissue portions were rinsed in PBS, placed in sterile Eppendorf tubes and incubated overnight at 56 °C with proteinase K. PCRs (total volume = 20 µL) were performed using primers published in Cook *et al.* (2004) (ribosomal 12S), Roháček *et al.* (2009) (ribosomal 16S), Folmer *et al.* (1994) (protein-encoding COIa), Simon *et al.* (1994) (protein-encoding COIb) and Su *et al.* (2008) (protein-encoding CytB) for four mitochondrial genes (the COI was amplified in two fragments) as well as two nuclear genes (ribosomal 28S) (Belshaw *et al.* 2001), and ITS2 (region containing partial 5.8S rRNA and ITS2 spacer), the latter from Beebe & Saul (1995). Amplified products were purified using the QIAquick PCR purification kit (QIAGEN) or GenElute™ PCR clean-up kit. Sequencing was carried out with BIGDYE TERMINATOR ver.3.1 (Applied Biosystems, Foster, CA, USA) on an ABI 3100 genetic analysis sequencer (Perkin Elmer Applied Biosystems, Norwalk, CT, USA), or PCR products were sequenced by MacroGen Europe (Netherlands). All sequences were assembled and edited in SEQUENCHER 5.0 (Gene Codes Corporation, Ann Arbor, MI, USA). GenBank accession numbers for the sequences are listed in Table 1.

Sequence alignment and analyses

The ribosomal genes 12S, 16S, 28S and ITS2 and protein coding genes Cytb and COI were aligned using MAFFT version 7 (Katoh & Standley 2013) on MAFFT server (<http://mafft.cbrc.jp/alignment/server/>) with default settings and then manually inspected. The protein-encoding genes Cytb and COI sequences were checked based on amino-acid translations and yielded indel-free nucleotide alignments. All sequence alignments were edited in GBLOCKS 0.91b program to remove unreliably aligned regions (Castresana 2000) on Gblocks server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html). We used the least stringent conditions – allowed smaller blocks, allowed gap positions within the final blocks and allowed less strict flanking positions.

We made a comprehensive alignment of 52 taxa to comprise sufficient diversity of the infraorder Bibionomorpha. This dataset is available upon request. Then, we created two final datasets with different taxon sampling and different number of molecular markers. For the first one, we selected representatives of diverse lineages in each family to make-up a balanced dataset consisting of 47 taxa and

Table 1 List of specimens used for DNA extraction, with GenBank accession numbers. More information about the specimens is listed in the Supporting information. All the specimens are males

Taxa	GenBank accession numbers						Availability
	12S	16S	28S	COI	CytB	ITS2	
Anisopodidae							
<i>Sylvicola fenestralis</i>	KC177473	KC177461	KC177637	n/a	n/a	n/a	Wiegmann et al. (2011)
Bibionidae							
<i>Bibio marci</i>	KJ136689	KJ136724	KJ136761	n/a	n/a	n/a	present study
<i>Bibio xanthopus</i>	KC177468	n/a	n/a	n/a	n/a	n/a	Wiegmann et al. (2011)
<i>Plecia nearctica</i>	KJ136690	KJ136725	KJ136762	n/a	n/a	n/a	present study
<i>Plecia</i> sp.	n/a	JQ613956	JQ613760	n/a	n/a	n/a	Shin et al. (2013)
Bolitophilidae							
<i>Bolitophila cinerea</i>	KJ136677	KJ136712	KJ136749	n/a	n/a	n/a	present study
<i>Bolitophila hybrida</i>	n/a	FJ172002	n/a	n/a	n/a	n/a	Rindal et al. (2009)
<i>Bolitophila occlusa</i>	KJ136678	KJ136713	KJ136750	n/a	n/a	n/a	present study
<i>Bolitophila</i> sp.	n/a	FJ172021	n/a	n/a	n/a	n/a	Rindal et al. (2009)
Cecidomyiidae							
<i>Lestremia cinerea</i>	KJ136693	KJ136728	KJ136765	n/a	n/a	n/a	present study
<i>Lestremiinae</i> gen. sp.	n/a	n/a	KC177648	n/a	n/a	n/a	Wiegmann et al. (2011)
<i>Mikiola fagi</i>	KJ136694	KJ136730	KJ136767	n/a	n/a	n/a	present study
<i>Oligotrophini</i> gen. sp.	n/a	KJ136729	KJ136766	n/a	n/a	n/a	present study
Diadocidiidae							
<i>Diadocidia</i> (<i>D.</i>) <i>ferruginosa</i>	KC435526	KC435562	KC435598	KC435634	KC435680	KC435705	Ševčík et al. (2013)
<i>Diadocidia</i> (<i>A.</i>) <i>fissa</i>	n/a	KJ136747	KJ136782	n/a	n/a	n/a	present study
<i>Diadocidia</i> (<i>T.</i>) <i>globosa</i>	KJ136702	KJ136738	n/a	n/a	n/a	KJ136787	present study
<i>Diadocidia</i> (<i>D.</i>) <i>bruneicola</i>	KJ136708	KJ136744	KJ136779	KJ136805	KJ136815	KJ136793	present study
<i>Diadocidia</i> (<i>T.</i>) sp.	KJ136709	KJ136745	KJ136780	n/a	n/a	KJ136794	present study
<i>Diadocidia hybrida</i>	KJ136703	KJ136739	KJ136774	n/a	KJ136811	KJ136788	present study
<i>Diadocidia</i> (<i>A.</i>) <i>queenslandensis</i>	KJ136704	KJ136740	KJ136775	KJ136801	KJ136812	KJ136789	present study
<i>Diadocidia</i> (<i>A.</i>) <i>borealis</i>	KJ136705	KJ136741	KJ136776	KJ136802	KJ136813	KJ136790	present study
<i>Diadocidia</i> (<i>A.</i>) <i>trispinosa</i>	KJ136700	KJ136736	KJ136772	KJ136799	KJ136809	KJ136786	present study
<i>Diadocidia</i> (<i>A.</i>) <i>valida</i>	KJ136699	KJ136735	KJ136771	n/a	n/a	KJ136785	present study
<i>Diadocidia</i> (<i>A.</i>) <i>stanfordensis</i>	KJ136706	KJ136742	KJ136777	KJ136803	n/a	KJ136791	present study
<i>Diadocidia</i> (<i>D.</i>) <i>cizeki</i>	KJ136707	KJ136743	KJ136778	KJ136804	KJ136814	KJ136792	present study
<i>Diadocidia</i> (<i>D.</i>) <i>spinosa</i>	KJ136701	KJ136737	KJ136773	KJ136800	KJ136810	n/a	present study
<i>Diadocidia</i> (<i>D.</i>) sp. (Sabah)	KJ136710	KJ136746	KJ136781	n/a	n/a	KJ136795	present study
<i>Diadocidia</i> (<i>D.</i>) sp. (Thailand)	KJ136711	KJ136748	KJ136783	KJ136806	KJ136816	KJ136796	present study
Ditomyiidae							
<i>Ditomyia fasciata</i>	KJ136698	KJ136734	KJ136770	n/a	n/a	n/a	present study
<i>Symmerus annulatus</i>	n/a	FJ172005*	KC177643**	n/a	n/a	n/a	*Rindal et al. (2009) **Wiegmann et al. (2011)
<i>Symmerus nobilis</i>	KJ136679	KJ136714	KJ136751	n/a	n/a	n/a	present study
Keroplastidae							
<i>Arachnocampa flava</i>	JN861748*	JN861748*	KC177644**	n/a	n/a	n/a	*Beckenbach (2012) **Wiegmann et al. (2011)
<i>Chiasmoneura anthracina</i>	KJ136682	KJ136717	KJ136754	n/a	n/a	n/a	present study
<i>Keroplastus testaceus</i>	KJ136683	KJ136718	KJ136755	n/a	n/a	n/a	present study
<i>Macrocera</i> sp.	n/a	FJ172018	n/a	n/a	n/a	n/a	Rindal et al. (2009)
<i>Rutylapa ruficornis</i>	KJ136684	KJ136719	KJ136756	n/a	n/a	n/a	present study
Lygistorrhinidae							
<i>Asiorrhina parasiatica</i>	KJ136680	KJ136715	KJ136752	n/a	n/a	n/a	present study
<i>Lygistorrhina sanctaecatherinae</i>	n/a	n/a	KC177645	n/a	n/a	n/a	Wiegmann et al. (2011)
<i>Lygistorrhina</i> sp.	n/a	FJ172007	n/a	n/a	n/a	n/a	Rindal et al. (2009)
<i>Matileola</i> sp.	KJ136681	KJ136716	KJ136753	n/a	n/a	n/a	present study
Mycetophilidae							
<i>Exechia seriata</i>	KJ136688	KJ136723	KJ136760	n/a	n/a	n/a	present study
<i>Mycetophila alea</i>	KJ136687	KJ136722	KJ136759	KJ136798	KJ136808	n/a	present study
<i>Mycomya circumdata</i>	KJ136685	KJ136720	KJ136757	KJ136797	KJ136807	KJ136784	present study

Table 1 Continued

Taxa	GenBank accession numbers						Availability
	12S	16S	28S	COI	CytB	ITS2	
<i>Novakia scatopsiformis</i>	KC435548	KC435584	KC435620	n/a	n/a	n/a	Ševčík et al. (2013)
<i>Neoempheria winnertzi</i>	KJ136686	KJ136721	KJ136758	n/a	n/a	n/a	present study
<i>Sciophila thoracica</i>	KC435559	KC435595	KC435631	n/a	n/a	n/a	Ševčík et al. (2013)
Pachyneuridae							
<i>Cramptonomyia spenceri</i>	JN861747*	JN861747*	KC177653**	n/a	n/a	n/a	*Beckenbach (2012) **Wiegmann et al. (2011)
Scatopsidae							
<i>Coboldia fuscipes</i>	KJ136692	KJ136727	KJ136764	n/a	n/a	n/a	present study
<i>Scatopse notata</i>	KJ136691	KJ136726	KJ136763	n/a	n/a	n/a	present study
<i>Coboldia</i> sp.	n/a	JQ613954	JQ613758	n/a	n/a	n/a	Shin et al. (2013)
Sciaridae							
<i>Bradysia amoena</i>	GQ387651*	GQ387651*	FJ040522**	n/a	n/a	n/a	*Beckenbach (2012) **Bertone et al. (2008)
<i>Bradysia distincta</i>	KJ136696	KJ136732	KJ136769	n/a	n/a	n/a	present study
<i>Cratyna nobilis</i>	n/a	JQ613975	JQ613778	n/a	n/a	n/a	Shin et al. (2013)
<i>Dolichosciara flavipes</i>	KJ136695	KJ136731	KJ136768	n/a	n/a	n/a	present study
<i>Sciara humeralis</i>	n/a	JQ613912	JQ613716	n/a	n/a	n/a	Shin et al. (2013)
<i>Trichosia edwardsi</i>	n/a	JQ613980	JQ613783	n/a	n/a	n/a	Shin et al. (2013)
Incertae sedis							
<i>Nepaetricha sigma</i>	KJ136697	KJ136733	n/a	n/a	n/a	n/a	present study

1198 characters: 12S – 342 bp, 16S – 339 bp, 28S – 517 bp. The second one consists of species from the family Diadocidiidae with Mycetophilidae as the outgroup, altogether 18 taxa and 3590 characters: 12S – 354 bp, 16S – 340 bp, COI – 1300 bp, CytB – 653 bp, 28S – 560 bp, 5.8S – 87 bp, ITS2 – 296 bp.

The dataset was analysed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) to explore strength of phylogenetic signal under different optimality criteria.

Parsimony analyses of the datasets were performed using TNT v.2.0 (Goloboff et al. 2008) with the following parameters: New technology search, level 50, initial addseqs = 9, find minimum tree length five times. Analyses were carried out with gaps coded as fifth character states and as missing data. Nodal support was assessed by jackknife resampling (JK, 250 replicates with 36.8% character deletion).

Trees were rooted by the outgroup taxa, *Sylvicola fenestralis* and *Sciophila thoracica*, respectively. In the second tree (Fig. 2), we initially used the family Sciaridae as the outgroup, but this topology appeared to suffer from the long-branch attraction artefact, so we have chosen Mycetophilidae as the outgroup.

To evaluate the best fit model for the BI and ML analyses, the concatenated dataset was partitioned into seven sets: seven gene regions (12S, 16S, 28S, Cytb, COI, 5.8S and ITS2). Each of the partitions was evaluated in MRMODELTEST v.2.2 (Nylander 2004) using both hierarchi-

cal likelihood ratio tests (hLRTs) and Akaike information criterion (AIC). We used model GTR + Γ + I (Rodriguez et al. 1990) for Bayesian inference and GTR + Γ for ML analysis.

The partitioned Bayesian inference of 10 million generations on the concatenated dataset was implemented in MRBAYES, version 3.2.2 (Huelsenbeck & Ronquist 2001) and carried out on the CIPRES computer cluster (Cyberinfrastructure for Phylogenetic Research; San Diego Supercomputing Center, Miller et al. 2010).

The ML analyses were conducted on CIPRES computer cluster using RAXML-HPC BLACKBOX 7.6.3 (Stamatakis 2006) employing automatic bootstrapping on partitioned dataset.

Datasets are available at TREEBASE (treebase.org, accession URL: <http://purl.org/phylo/treebase/phylovs/study/TB2:S15613>).

Results

The results based on the Bayesian, likelihood and parsimony analyses of the dataset are summarised in Figs 1, S1 and 2. The trees presented are Bayesian topologies with node support values from the Bayesian, ML and MP (indels treated as fifth character state) analyses. For the Bayesian analyses, we used a burn in of 30% and the standard deviation of split frequencies was in all cases < 0.004.

The log likelihood values for the best tree of the datasets were –19451.66 and –17249.74, respectively. Both MP

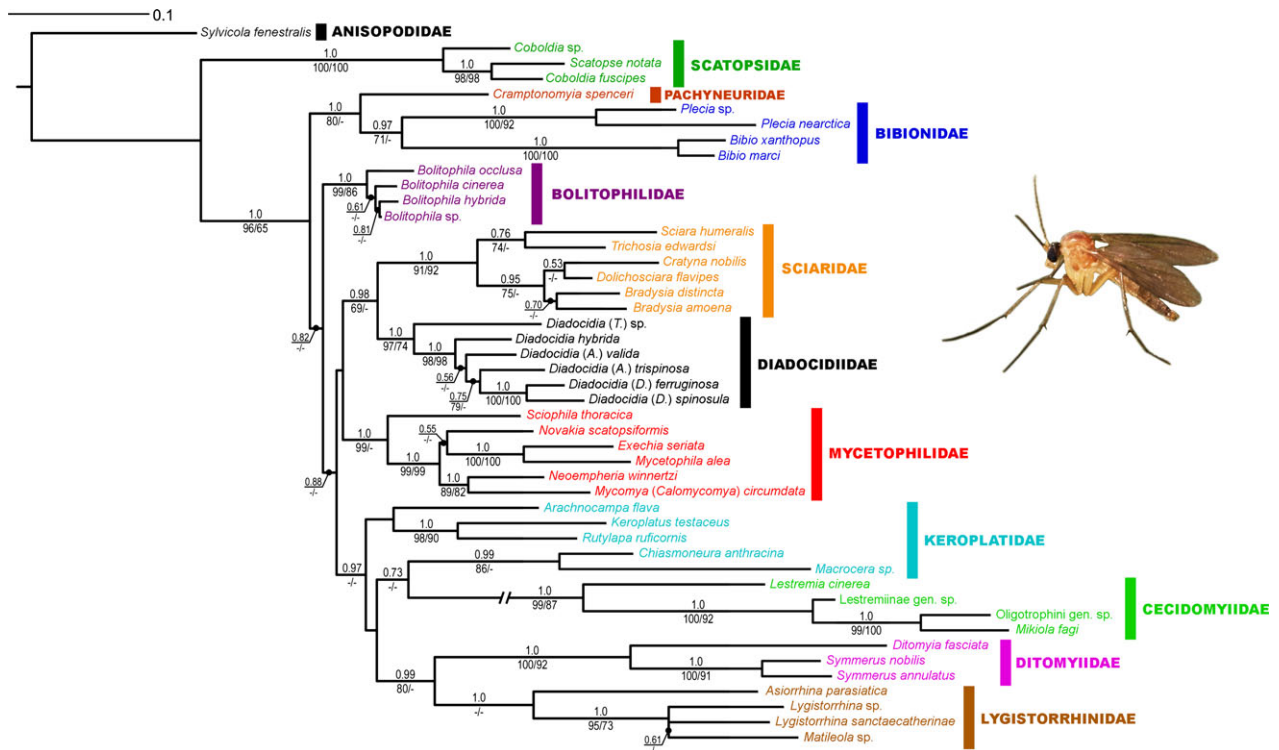


Fig. 1 Bayesian hypothesis for relationships among selected taxa of Bibionomorpha based on DNA sequence data (12S, 16S and 28S), 1198 characters, including additional data from GenBank. Above node number = posterior probability (PP) over 0.5; below node left = bootstrap support for maximum likelihood (ML); below node right = JK support for maximum parsimony (MP). The branch leading to Cecidomyiidae clade has been shortened to its half to fit into the graphic. The image is of *Diadocidia ferruginosa*, a representative of the family Diadocidiidae.

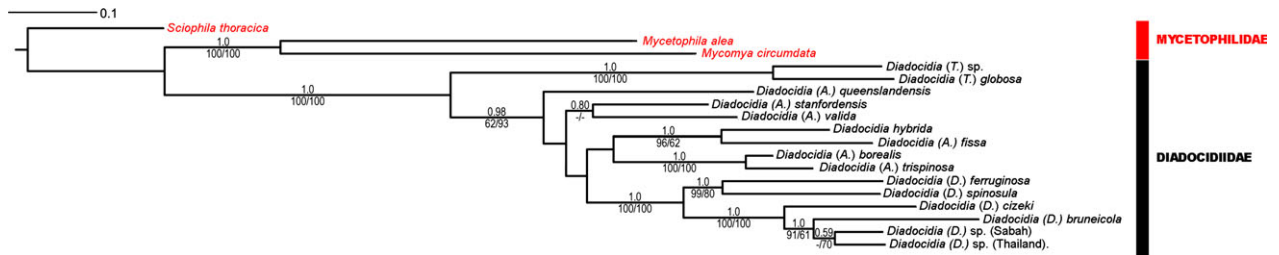


Fig. 2 Bayesian hypothesis for Diadocidiidae relationships based on combined DNA sequence data (12S, 16S, COI, cytB, 28S, ITS2), 3590 characters. Above node number = posterior probability (PP) over 0.5; below node left = bootstrap support for maximum likelihood (ML); below node right = JK support for maximum parsimony.

analyses of the datasets (with gaps coded as a fifth character state) resulted in four and two most parsimonious trees, respectively (See supplementary data).

Relationships among the families of Bibionomorpha and position of Diadocidiidae

The monophyly of the infraorder Bibionomorpha was established with high support (posterior probability, PP = 1.00, ML = 96, JK = 65) in both the parsimony and model-based analyses (Figs 1 and S1). The mono-

phyly of the superfamily Sciaroidea is less supported (PP = 0.82).

Most of the currently recognised families of Sciaroidea proved to be monophyletic (Fig. 1). The family Keroplatidae revealed as paraphyletic, with the genera of Macrocerinae being more related to Cecidomyiidae than to the other taxa of Keroplatidae. This relationship is, however, poorly supported.

Both the Bayesian and likelihood analyses of 10 families of Bibionomorpha revealed Sciaridae as the closest relative

of Diadocidiidae (PP = 0.98, ML = 69), with Mycetophilidae as a sister group to this clade. The well-supported (PP = 0.99, ML = 80) clade (Ditomyiidae + Lygistorrhinidae) forms, together with (Cecidomyiidae + Macrocerinae), a moderately supported (PP = 0.97) group, containing also the other taxa of Keroplatidae (Fig. 1).

When we included *Nepalectricha* Chandler 2002 to the dataset, as a representative of the enigmatic Sciaroidea incertae sedis, most of the support node values decreased (Fig. S1) and this genus occupied a rather isolated position within Sciaroidea.

Monophyly and phylogeny of Diadocidiidae

All the analyses strongly support Diadocidiidae as a monophyletic group (Fig. 1: PP = 1.0, ML = 97, JK = 74; Fig. 2: PP = 1.0, ML = 100, JK = 100). Within this clade, the well-supported *Taidocidia* branch separates as a sister group to all the other taxa of Diadocidiidae with maximum node support values (PP = 1.0, ML = 100, JK = 100, Figs 1 and 2).

The Oriental *Diadocidia* s. str. (*D. cizeki* Ševčík 2003 and related species) form a monophyletic group (Fig. 2) with high node support values (PP = 1.00, ML = 100, JK = 100), being the sister group to the two common Palearctic species (*D. ferruginosa* + *D. spinosula*), also with high support (PP = 1.00, ML = 100, JK = 100). The closely related species *Diadocidia* (*A.*) *borealis* and *D.* (*A.*) *trispinosa* form a sister group to the clade (*D. hybrida* + *D. fissa*). The other relationships are not strongly supported (Fig. 2).

Discussion

Comparison of the Bayesian, likelihood and parsimony analyses

All three methods (BI, ML, MP) yielded mostly congruent nodes, and well-supported relationships were consistent across all trees. Incongruence between MP and model-based methods was observed mainly with regard to the relationships among the families of Bibionomorpha where MP analysis provided almost no significant results. Also the ML analysis yielded a tree with low to moderate node support values for these taxa (Figs 1 and S1).

Phylogeny of Bibionomorpha

The phylogeny of Bibionomorpha, especially of the families of Sciaroidea, has been subject of many studies and discussions during the last 10 years (for a summary see Jaschhof 2011). Most of them were based on morphological characters because a comprehensive molecular phylogeny of this infraorder has not yet been available. As the material of some peculiar and phylogenetically important taxa (especially the genera of Sciaroidea incertae sedis) is still not available for molecular studies, the results presented in

this paper are mostly based on taxa from traditionally recognised families.

This study confirms Bibionomorpha as a monophyletic clade, supporting the findings of most previous authors since Hennig (1954) to Wiegmann *et al.* (2011). Almost all of the currently recognised extant families of Sciaroidea (except for Keroplatidae, see below) proved to be monophyletic in the present study. On the other hand, the monophyly of the entire superfamily Sciaroidea is poorly supported (Figs 1 and S1).

The relationships among the families of Sciaroidea, as revealed in this study, differ markedly from those proposed in the literature. Notably, the close (sister) relationship of the families Diadocidiidae and Sciaridae has never been postulated. In the phylogenetic studies based on morphology, diadocidiids have usually been placed close to Keroplatidae (see e.g. Matile 1990, 1997; Chandler 2002; Hippa & Vilkkamaa 2006; Amorim & Rindal 2007). Molecular studies, if they included any Diadocidiidae at all, put this family either close to Mycetophilidae (Bertone *et al.* 2008; Rindal *et al.* 2009) or as the sister group to all the other Sciaroidea, excluding Ditomyiidae and Manotinae (Wiegmann *et al.* 2011). The recent molecular study of Sciaridae by Shin *et al.* (2013) unfortunately did not include any representative of the family Diadocidiidae.

Another surprising finding is the placement of Ditomyiidae in the same and well-supported clade with Lygistorrhinidae. These two families have hitherto been considered unrelated, and they usually occupied quite distant branches of the tree (e.g. Matile 1990, 1997; Chandler 2002; Hippa & Vilkkamaa 2006). Zaitzev (1983, 1984) concluded in his comparative studies of larval characters that Ditomyiidae possess the most primitive type of mouthparts, similar to those of lower Bibionomorpha (Hesperinidae and Pachyneuridae), suggesting their close relationship. These findings are in stark contrast with molecular data presented here. Anyway, molecular phylogeny and affiliation of Ditomyiidae will be the subject of a separate study which is now under preparation.

Lygistorrhinids have usually been considered as a sister group to Mycetophilidae (Matile 1997; Chandler 2002; Hippa & Vilkkamaa 2006; Amorim & Rindal 2007). This relationship is, however, not confirmed by the present study, where this family nested in the same clade with Keroplatidae, Cecidomyiidae and Ditomyiidae (Fig. 1). Only Tuomikoski (1966) hypothesised a possible relationship of Lygistorrhinidae with Keroplatidae, although his views were criticised by Thompson (1975). Within the family Lygistorrhinidae, *Asiorrhina parasiatica* Blagoderov *et al.* 2009 branched as a sister group to the other Lygistorrhinidae (Fig. 1), in accordance with the phylogenies proposed by Blagoderov *et al.* (2009) and Hippa *et al.* (2005).

Interestingly, the family Keroplatidae revealed as paraphyletic, although the relationship of Macrocerinae with Cecidomyiidae is poorly supported and it may also be a result of the long-branch attraction or any other artefact from the analyses rather than representation of true evolutionary relationship. Macrocerinae is usually treated as a subfamily of Keroplatidae (e.g. Matile 1990), but several authors considered it as a distinct family (e.g. Krivosheina & Mamaev 1988; Papp & Schumann 2000). The limited number of taxa included in this study does not currently allow reaching a final conclusion, but our further studies will be focused also on the phylogeny of this family, including the recently described genera (e.g. Ševčík 2012) and the peculiar subfamily Sciarokeroplatinae (cf. Papp & Ševčík 2005a).

The exact position of Bolitophilidae remained unconfirmed in our analyses. Its separation from the rest of Sciaroidea is only weakly supported (Fig. 1). On the contrary, this family nested quite apically within the Bibionomorpha clade in the tree presented by Wiegmann *et al.* (2011). Rindal *et al.* (2009) suggested Bolitophilidae as a sister group to (Diadocidiidae + Mycetophilidae), but the relationship of this clade with (Keroplatidae + Lygistorrhinidae) and Ditomyiidae was unresolved in their trees.

Cecidomyiidae is a huge family and deserves separate attention which is beyond the scope of this paper. However, even with our limited sampling, this family clearly forms a much derived, apomorphic group, as indicated by its long branch in the tree (Fig. 1).

Nepaetricha is the first representative of the *Heterotricha* group of Chandler (2002), or the Sciaroidea *incertae sedis* of Jaschhof (2011), of which molecular data are currently available. Chandler (2002) put this genus among the common clade with Sciaridae and several genera not assigned to family. As can be seen from Fig. S1, a close relationship of *Nepaetricha* with Sciaridae is not supported and more likely it forms a separate branch within the other Sciaroidea.

Phylogeny of Diadocidiidae

At present, Diadocidiidae are considered to include only one extant genus *Diadocidia* (cf. Bechev & Chandler 2011). Although Sasakawa (2004) described a new genus (*Palaecodocidia*) with a single species from Japan, it was based on the misinterpretation of the number of antennal segments and this species actually belongs to *Diadocidia*, subgenus *Adidocidia* (see Bechev & Chandler 2011).

In this study, we do not raise the three subgenera (*Adidocidia*, *Diadocidia* s. str., and *Taidocidia*) to generic level pending a further sampling of additional taxa, especially those not assigned to a subgenus yet, and further morphological studies. However, *Taidocidia* clade forms a relatively

long sister branch to all the other Diadocidiidae and this topology is well supported in both MP and model-based analyses, so it may well be considered as a distinct genus. This subgenus was described by Papp & Ševčík (2005b) to include a single species from Taiwan and Thailand, but it probably represents a group of closely related species (J. Ševčík, unpublished data), of which one (undescribed) was included in this study (Table 1).

The nominotypical subgenus *Diadocidia* is well supported in our analyses (Figs 1 and 2) and includes two Palaearctic and four Oriental species of the dataset. Among the oriental species included in our analysis, two are still undescribed, but morphologically similar to *D. bruneicola* Ševčík in Papp & Ševčík, 2005b. The common and widely distributed *Diadocidia cizeki* Ševčík 2003 is considered by Bechev & Chandler (2011) a possible junior synonym of *D. sinica* Wu, 1995, but the situation is complicated by the fact that the type of *D. sinica* is not available to study and that this taxon probably represents a group of sibling species differing only in details on the aedeagal complex (M. Jaschhof, pers. comm.).

Several species belonging to subgenus *Adidocidia* grouped together in pairs, while *Diadocidia* (*A.*) *queenslandensis* revealed as the sister group to all the other *Diadocidia* s. str. and *Adidocidia*. The species *Diadocidia hybrida* Jaschhof & Jaschhof 2007; hitherto not assigned to subgenus, grouped with *Diadocidia* (*A.*) *fissa*, and this relationship is well supported (Fig. 2), indicating that the former species may actually belong to *Adidocidia*. Nevertheless, some of the other species not assigned to subgenus have either not been available for this study or the extraction of DNA was not successful, as was the case of *D. setistylus* Papp, 2003.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Bayesian hypothesis for relationships among selected taxa of Bibionomorpha based on DNA sequence data (12S, 16S and 28S), 1198 characters, including additional data from GenBank and *Nepaleticrha sigma*.

Table S1. List of specimens included in the phylogenetic analysis.

Table S1: List of specimens included in the phylogenetic analysis

Species	Authors	Sampling locality and year
<i>Asiorrhina parasiatica</i>	Blagoderov, Hippa & Ševčík 2009	Thailand, 2008
<i>Bibio marci</i>	(Linnaeus, 1758)	Czech Republic, 2013
<i>Bolitophila (Bolitophila s. str.) cinerea</i>	Meigen, 1818	Slovakia, 2012
<i>Bolitophila (Cliopisa) occlusa</i>	Edwards, 1913	Slovakia, 2012
<i>Bradysia distincta</i>	(Stæger, 1840)	Slovakia, 2013
<i>Coboldia fuscipes</i>	(Meigen, 1830)	Czech Republic, 2011
<i>Diadocidia (Adidocidia) borealis</i>	Coquillett, 1900	USA, 2009
<i>Diadocidia (Adidocidia) fissa</i>	Zaitzev, 1994	Czech Republic, 2005
<i>Diadocidia (Adidocidia) queenslandensis</i>	Jaschhof & Jaschhof, 2007	Australia, 1996
<i>Diadocidia (Adidocidia) stanfordensis</i>	Arnaud & Hoyt, 1956	USA, 2008
<i>Diadocidia (Adidocidia) trispinosa</i>	Polevoi, 1996	Slovakia, 2009
<i>Diadocidia (Adidocidia) valida</i>	Mik, 1874	Slovakia, 2010
<i>Diadocidia (Diadocidia s. str.) bruneicola</i>	Ševčík in Papp & Ševčík, 2005	Brunei, 2013
<i>Diadocidia (Diadocidia s. str.) cizeki</i>	Ševčík, 2003	Thailand, 2007
<i>Diadocidia (Diadocidia s. str.) spinosula</i>	Tollet, 1948	Slovakia, 2009
<i>Diadocidia (Diadocidia s. str.) sp. /cf. bruneicola/</i>	undescribed	Sabah, 2007
<i>Diadocidia (Diadocidia s. str.) sp.</i>	undescribed	Thailand, 2008
<i>Diadocidia (Taidocidia) globosa</i>	Papp & Ševčík, 2005	Thailand, 2006
<i>Diadocidia (Taidocidia) sp. /cf. globosa/</i>	undescribed	Sulawesi, 2010
<i>Diadocidia hybrida</i>	Jaschhof & Jaschhof, 2007	Costa Rica, 2003
<i>Ditomyia fasciata</i>	(Meigen, 1818)	Czech Republic, 2010
<i>Dolichosciara flavipes</i>	(Meigen, 1804)	Slovakia, 2012
<i>Exechia seriata</i>	(Meigen, 1830)	Slovakia, 2012
<i>Chiasmoneura anthracina</i>	Meijere, 1913	Thailand, 2009
<i>Keroplatus testaceus</i>	Dalman, 1818	Slovakia, 2012
<i>Lestremia cinerea</i>	Macquart, 1826	Slovakia, 2012
<i>Matileola sp.</i>	unidentified to species	Thailand, 2007
<i>Mikiola fagi</i>	(Hartig, 1839)	Slovakia, 2013
<i>Mycetophila alea</i>	Laffoon, 1965	Slovakia, 2012
<i>Mycomya (Calomycomya) circumdata</i>	(Staeger, 1840)	Slovakia, 2012
<i>Neoempheria winnertzi</i>	Edwards, 1913	Slovakia, 2012
<i>Nepaetricha sigma</i>	Hippa & Ševčík, 2014	India, 2012
Oligotrophini gen. sp.	unidentified to genus	Slovakia, 2012
<i>Plecia nearctica</i>	Hardy, 1940	USA, 2013
<i>Rutylapa ruficornis</i>	(Zetterstedt, 1851)	Turkey, 2011
<i>Scatopse notata</i>	(Linnaeus, 1758)	Czech Republic, 2011
<i>Symmerus nobilis</i>	Lackschewitz, 1937	Slovakia, 2012

Figure S1: Bayesian hypothesis for relationships among selected taxa of Bibionomorpha based on DNA sequence data (12S, 16S and 28S), 1198 characters, including additional data from GenBank and *Nepaetricha sigma*. Above node number = posterior probability (PP) over 0.5; below node left = bootstrap support for ML; below node right = JK support for MP. The branch leading to Cecidomyiidae clade has been shortened to its half to fit it into the graphic. The image is of *Diadocidia ferruginosa*, a representative of the family Diadocidiidae.

