

# Molecular phylogeny of fungus gnats (Diptera: Mycetophilidae) revisited: position of Manotinae, Metanepsiini, and other enigmatic taxa as inferred from multigene analysis

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**Abstract.** The phylogeny of selected genera from four subfamilies of fungus gnats (Diptera: Mycetophilidae) – Manotinae, Leiinae, Sciophilinae and Gnoristinae (including Metanepsiini) – is reconstructed based on the combined analysis of five mitochondrial (*12S*, *16S*, *COI*, *COII*, *cytB*) and two nuclear (*28S*, *ITS2*) gene markers. Results of the different analyses all support Manotinae as a monophyletic group, with Leiinae as the sister group. *Allactoneura* DeMeijere is nested in the monophyletic and strongly supported clade of Leiinae. The tribe Metanepsiini is revealed as paraphyletic and the genera *Metanepsia* Edwards and *Chalastonepsia* Søli do not appear to be closely related. The genera *Docosia* Winnertz, *Ectrepesthoneura* Enderlein, *Novakia* Strobl and *Syntemna* Winnertz were placed with a group of genera included traditionally in the Gnoristinae. The monophyly of *Dziedzickia* Johannsen and *Phthinia* Winnertz is not supported. The genera of Sciophilinae (excluding *Paratinia* Mik but including *Eudicrana* Loew) form a monophyletic group in the Bayesian model.

## Introduction

Fungus gnats (Diptera: Mycetophilidae) represent one of the most abundant and diverse groups of insects in forest habitats. Their larvae are mostly associated with higher fungi or mycelia in rotting wood (for recent reviews see Chandler, 2010; Ševčík, 2010; Jakovlev, 2011, 2012). The adults can be found near the larval habitat or they aggregate along banks of streams, in cavities under tree roots, in caves and similar moist and shady places.

The phylogeny of this species-rich family and its relatives has been subject of several studies and discussions, especially during the last 20 years (cf. Søli, 1997; Hippa & Vilkkamaa, 2006; Amorim & Rindal, 2007; Rindal *et al.*, 2009; Jaschhof, 2011). The Mycetophilidae s.s. is widely accepted as belonging to the superfamily Sciaroidea (cf. Søli *et al.*, 2000;

Mycetophiliformia of Amorim & Rindal, 2007) which also contains the families Diadocidiidae, Ditomyiidae, Bolitophilidae, Keroplatidae, Lygistorrhinidae, Rangomaramidae, Sciaridae and Cecidomyiidae. The family Mycetophilidae usually is divided into six subfamilies; Mycomyiinae, Sciophilinae, Leiinae, Manotinae, Gnoristinae (with or without Metanepsiini) and Mycetophilinae, although some of these are ranked as tribes by some authors (see Gammelmo, 2004).

In the phylogenetic study by Rindal *et al.* (2009) using three molecular markers (nuclear *18S* and *28S*, and mitochondrial *16S* rRNA genes) and a range of taxa covering the Mycetophilidae, two groups of fungus gnats, Manotinae and Metanepsiini, both particularly well represented in the tropics, were not included, except for the European species *Manota unifurcata* (Lundström, 1913), which had a rather isolated position in the phylogenetic tree. In their maximum parsimony tree, *Manota* Williston appeared in a clade with *Leptomorphus* Curtis, a morphologically very different genus, probably because almost none of its potential relatives were included in that analysis, such as other genera of Manotinae or the Leiinae

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genera *Allactoneura* DeMeijere and *Sticholeia* Søli. These taxa were also not included in the unpublished molecular analysis of Sciarioidea by Baxter (1999).

The subfamily Manotinae has received recent attention in the number of new taxa described from tropical areas (e.g. Papp, 2004; Jaschhof & Hippa, 2005; Hippa, 2006; Hippa & Kurina, 2012). However, the relationships among the four extant genera included in Manotinae (*Manota*, *Eumanota* Edwards, *Paramanota* Tuomikoski and *Promanota* Tuomikoski) and the affinity of this peculiar subfamily to the other mycetophilid groups remains poorly understood. Phylogenetic relationships within Manotinae, based on morphological data, were discussed by Tuomikoski (1966); Zaitzev (1990), and Hippa *et al.* (2005). The only common, species-rich and widespread manotine genus is *Manota*, whereas the other taxa are rare and confined only to the Oriental and northern Australasian regions (cf. Papp, 2004; Hippa, 2010; Hippa & Ševčík, 2010). As *Manota* species clearly differ morphologically from the other mycetophilids, the manotines are accepted as a distinct subfamily (e.g. Tuomikoski, 1966; Matile, 1993; Søli, 1997; Søli *et al.*, 2000; Hippa *et al.*, 2005) and have even been treated as a separate family (e.g. Krivosheina & Mamaev, 1988).

The tribe Metanepsiini (or subfamily Metanepsiinae of some authors; cf. Väisänen, 1984) is a group of fungus gnats comprising the entirely tropical and mostly Oriental genera *Metanepsia* Edwards, *Chalastonepsia* Søli and *Pectinepsia* Ševčík & Hippa (see Ševčík & Hippa, 2010). It was established by Matile (1971) for one Oriental and two Afrotropical species of *Metanepsia*. Søli (1996a) proposed a new genus (*Chalastonepsia*) only for *C. orientalis* Søli, 1996 and provided a revised diagnosis of the tribe. Kallweit (1998) described additional species in both genera and noted the difficulty in separating the tribe Metanepsiini from Gnoristini as delimited by Väisänen (1986), who considered the two tribes of subfamilial rank. The subfamily rank of Metanepsiinae has been questioned by Oliveira & Amorim (2012) who noted that it may render the Gnoristinae paraphyletic. However, the same authors treated Allactoneurinae as a subfamily, although it is widely considered as a part of Leiinae (cf. Zaitzev, 1981; Matile, 1990; Søli, 1997).

The aim of this paper is to provide molecular evidence for the phylogeny of the genera in the Manotinae and this subfamily's affiliation to the other subfamilies, as well as elucidate the position of Metanepsiini genera, *Allactoneura*, *Eudicrana* Loew, *Paratinia* Mik and other enigmatic taxa.

## Material and methods

### Sampling

We sampled a total of 41 specimens belonging to 34 species of Mycetophilidae, representing all genera of Manotinae, the two genera in Metanepsiini and several potentially related genera of Gnoristinae, Leiinae, and Sciophilinae (Tables S1 and S4). The samples were collected in various zoogeographical

regions (see Table S3), usually using Malaise traps, from 2003 to 2012, and were preserved in 70% ethanol.

Several outgroup species were tested and eventually *Diadocidia ferruginosa* (Meigen, 1830), from the closely related family Diadocidiidae, was selected. Diadocidiidae were recovered as the closest relative of Mycetophilidae in the previous molecular studies by Rindal *et al.* (2007) and Bertone *et al.* (2008). Several other species of Mycetophilidae were added to the extended dataset using sequences obtained from GenBank (Table S2) to set our new findings into a wider context.

### DNA extraction, amplification and sequencing

All the material used for DNA analysis was alcohol-preserved. The DNA was extracted using DNeasy Blood & Tissue Kit (QIAGEN) 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 (Table S3) published in Roháček *et al.* (2009) (ribosomal *12S* and *16S*) and Su *et al.* (2008) (protein-encoding *COI*, *COII* and *CytB*) for five mitochondrial genes as well as two nuclear genes (ribosomal *28S* and *ITS2*, the latter from Beebe & Saul, 1995). The *COI* was amplified in two fragments (*COIa* and *COIb*). Amplified products were purified using the QIAquick PCR Purification Kit (QIAGEN), and cycle-sequenced with BigDye Terminator v3.1 (Applied Biosystems, Foster, CA). Direct sequencing was carried out on an ABI 3100 genetic analysis sequencer (Perkin Elmer Applied Biosystems, Norwalk, CT). All sequences were assembled and edited in SEQUENCHER v4.8 (Gene Codes Corporation, Ann Arbor, MI). GenBank accession numbers for the sequences are listed in Table S1.

### Sequence alignment and analyses

The protein encoding genes *Cytb*, *COI* and *COII* were aligned based on amino-acid translations and yielded indel-free nucleotide alignments. The ribosomal genes *12S*, *16S*, *28S* and *ITS2* were aligned in MEGA v5 using the incorporated ClustalW (Tamura *et al.*, 2011) using the default gap opening and extension costs (15:6.66). The aligned ribosomal and protein-encoding gene sequences were adjusted as described in Tóthová *et al.* (2012). The final molecular dataset consists of 35 taxa and 4733 characters: *12S* – 432, *16S* – 390, *COI* – 1332, *COII* – 597, *CytB* – 652, *28S* – 720, *ITS2* – 610 bp.

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

Parsimony analyses of the datasets were performed using TNT v2.0 (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 on *Diadocidia ferruginosa*.

In order to evaluate the best-fit model for the BI and ML analyses, the concatenated dataset was partitioned into eight sets: seven gene regions (*12S*, *16S*, *28S*, *Cytb*, *COI*, *COII* and *ITS2*). Each of the partitions was evaluated in MrModeltest v2.2 (Nylander, 2004) using both hierarchical likelihood ratio tests (hLRTs) and Akaike Information Criterion (AIC). The model GTR +  $\Gamma$  + I (Rodriguez *et al.*, 1990) was favoured for each of the individual gene regions.

The partitioned Bayesian inference of 10 million generations on the concatenated dataset was implemented in MrBayes (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 in Garli v2.0 (Zwickl, 2006). Two independent runs of 5 million generations using the default automated stopping criterion were carried out. Nodal support was assessed using a nonparametric bootstrap with 250 replicates.

## Results

The results based on the Bayesian, likelihood and parsimony analyses of the dataset are summarized in Fig. 1. Figure S1 is based also on additional data from GeneBank. The tree presented is a Bayesian topology with nodal support values from the Bayesian, ML and MP (indels treated as fifth character state) analyses indicated on the branches. For the Bayesian analyses we used a burn-in of 30%, and the standard deviation of split frequencies was < 0.009 in all cases.

The log likelihood value for the best tree was -55312.90. Both MP analyses of the dataset (with gaps coded as a fifth character state and as missing data) resulted in a single most parsimonious tree. The exclusion of third positions of protein encoding genes had no significant impact on the topologies from the various analyses.

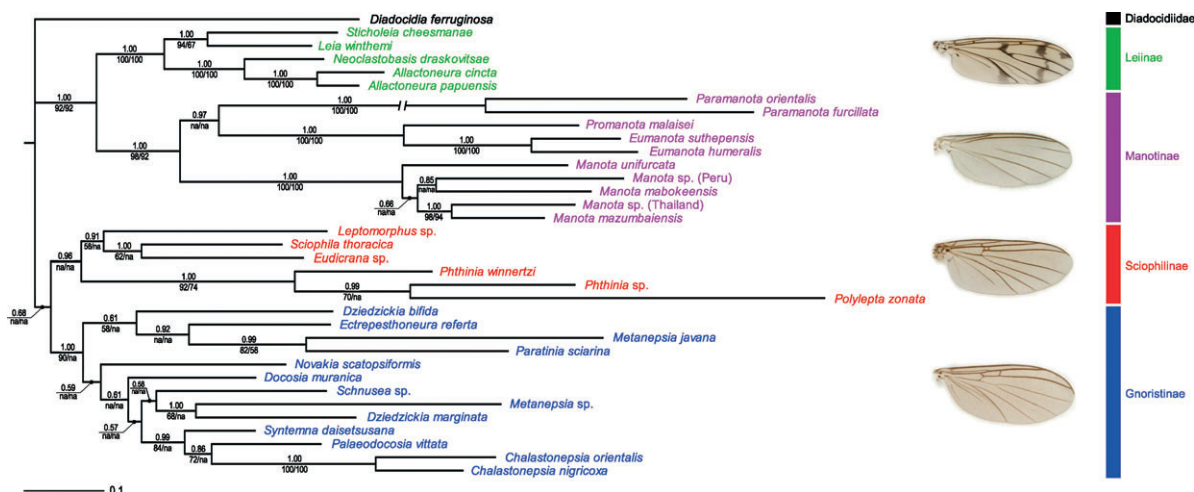
### Monophyly of Manotinae and Leiinae

The monophyly of the subfamily Manotinae had high support in both the parsimony and model-based analyses (Figs 1, S1; PP = 1.00, ML = 98, JK = 92). *Eumanota* proved to be a sister group to *Promanota* and this clade forms a sister group to the long branch containing the *Paramanota* species, which has a rather diverge position in the tree (Fig. 1). *Manota* is sister to all the other genera in the Manotinae. The sister group to Manotinae is the clade containing the Leiinae genera. The clade (Manotinae + Leiinae) forms a monophyletic group with high node support values (PP = 1.00, ML = 92, JK = 92).

*Allactoneura* is included in the monophyletic and strongly supported clade of Leiinae in all analyses, as the sister group to *Neoclastobasis* Ostroverkhova (PP = 1.00, ML = 100, JK = 100). The (*Sticholeia* + *Leia*) branch is sister to (*Allactoneura* + *Neoclastobasis*) with maximum node support values.

### Paraphyly of Metanepsiini

The tribe Metanepsiini has not proven to be a monophyletic group. The genera *Metanepsia* and *Chalastonepsia* appear not closely related (Figs 1, S1) and even the two species of *Metanepsia* included in the analysis nest in different



**Fig. 1.** Bayesian hypothesis for the relationships among selected taxa of Mycetophilidae based on DNA sequence data, 4733 characters. Above node number = posterior probability (PP) over 0.5; below node left = bootstrap support for Garli; below node right = JK support for MP. The long branch leading to *Paramanota* has been shortened by half to fit it into the graphic. Wing images show representatives of each clade/from top to bottom: *Leia winthemii* Lehmann, *Manota tumoae* Hippa & Kjerandsen, *Sciophila rufa* Meigen, *Dziedzickia marginata* (Dziedzicki).

clades. *Chalastonepsia orientalis* and *C. nigricoxa* form a well-supported clade which is the sister group to *Palaeodocosia* Meunier and *Syntemna* Winnertz. Both *Docosia* Winnertz, *Ectrepesthoneura* Enderlein, *Novakia* Strobl and *Syntemna* were placed within a group of genera placed traditionally in Gnoristini. *Paratinia* forms a well-supported branch with *Metanepsia javana* Edwards in all analyses and does not group with the Sciophilini genera.

The monophyly of *Dziedzickia* Johannsen s.l. is not supported, as the Oriental *Dziedzickia bifida* Ševčík, Bechev & Hippa appeared in a different clade than both *D. marginata* and *Schnusea* Edwards.

The traditional Sciophilinae genera (without *Paratinia*) form a well-supported (PP = 0.96) monophyletic group in the Bayesian analysis. Together, *Eudicrana* and *Sciophila* Meigen form a sister group to *Leptomorphus*. *Polylepta* Winnertz makes *Phthinia* Winnertz paraphyletic as it is placed in the clade containing the *Phthinia* species (Fig. 1).

## 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 taxa within the Gnoristinae and Sciophilinae 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 (Fig. 1).

### *Manotinae and Leiinae*

This study confirms the subfamily Manotinae as a monophyletic clade, supporting the findings of Hippa *et al.* (2005). The clade formed by Manotinae and Leiinae is monophyletic and strongly supported in all analyses, suggesting their close relationship. This relationship was already proposed by Edwards (1933) who mentioned that his new genus *Eumanota* forms a transition between Manotinae and Leiinae. Interestingly, studying the immature stages of *Manota unifurcata*, Zaitzev (1990) came to the conclusion that the larval structures of *Manota* do not indicate a relationship with Leiinae and placed the Manotinae as a sister group to Sciophilinae. This view is not supported in our analyses.

The placement of *Allactoneura* as a subfamily or tribe is not supported as it was recovered in the Leiinae in all analyses presented here. Originally Edwards (1925) put *Allactoneura* into the subfamily Manotinae but later Shaw & Shaw (1951) transferred it to a new tribe, Allactoneurini, and subsequently Tuomikoski (1966) placed it in the Leiini. Zaitzev (1981) pointed out the distinctions between the tribes Allactoneurini and Leiini but the separate status of the former tribe was

rejected by Söli (1996b). The placement of *Sticholeia* as sister to *Leia* Meigen shows it is not closely related to Manotinae and also not to *Allactoneura* (as suggested by Söli, 1996b).

The relationships among the genera within Manotinae proved to be the same as found by Hippa *et al.* (2005). The synonymy of *Promanota* with *Eumanota*, as proposed by Söli (2002), is not supported and these genera should be considered distinct, in accordance with Papp (2004) and Hippa *et al.* (2005). Although the ML and MP analyses have not resolved the sister group of *Paramanota* (*Eumanota* + *Promanota*), this relationship was strongly supported by the Bayesian analyses based on both the shorter (PP = 0.97) and extended (PP = 0.93) datasets (Figs 1, S1).

### *Gnoristinae and Sciophilinae*

The paraphyly of Gnoristinae and placement of several taxa outside of Sciophilinae has been noted by many authors (e.g. Söli, 1997). Väisänen (1986) discussed the possible relationships of several genera of uncertain position and transferred *Ectrepesthoneura*, *Syntemna* and *Tetragoneura* Winnertz into the Gnoristinae. *Ectrepesthoneura*, *Syntemna*, *Docosia* and *Novakia*, were clearly recovered within the clade containing traditional Gnoristinae genera in our analyses. The inclusion of *Docosia* in Gnoristinae was found also by Rindal *et al.* (2009).

The position of Metanepsiinae as a separate subfamily or even as a monophyletic clade within the Gnoristinae is not supported in our analyses. Rather, *Metanepsia* and *Chalastonepsia* appear to be local specialized apomorphic forms, probably derived from the genera related to *Dziedzickia* s.l. As proposed by several previous authors (e.g. Hutson, 1979; Vockeroth, 1980; Väisänen, 1986; Matile, 1992) and recently by Ševčík *et al.* (2011), *Dziedzickia* appears to be a heterogeneous and paraphyletic group of species, of which some apparently deserve separate generic status (as in the case of *Schnusea*). The latter genus is, however, considered by Oliveira & Amorim (2010) as a monophyletic subgroup within *Dziedzickia* s.l. and thus its junior synonym, in order to retain the entire *Dziedzickia* as monophyletic. This opinion is not supported by the present analyses, but further studies are still needed, covering further taxa of this group.

The common Oriental species *Metanepsia javana* surprisingly nested in a well-supported clade with *Paratinia sciarina* Mik, morphologically a rather different genus, whereas the other (undescribed) species of *Metanepsia* appeared as the closest relative of *Dziedzickia marginata* (Dziedzicki). *Paratinia* thus belongs to the Gnoristinae clade according to our analyses, supporting several previous opinions (e.g. Shaw & Shaw, 1951; Söli, 1997). Interestingly, when we set *Sciophila* as the root (data not shown) instead of *Diadocidia*, *Paratinia* grouped with *Ectrepesthoneura*, and *Metanepsia javana* with *Dziedzickia bifida*, respectively. There is also a possibility that the position of *Metanepsia javana* is affected by the missing data on the three mitochondrial genes (see Table S1). In the



broader analysis comprising sequences taken from GenBank that represent additional genera (Figure S1), *Paratinia* was not grouped with the supposedly related (cf. Kerr, 2011; Borkent & Wheeler, 2013) genus *Acomoptera* Vockeroth but still nested in the *Metanepsia javana* clade. This may, however, be attributed to the insufficient sequence data for *Acomoptera* (only 12S gene marker included) in relation to *Paratinia*. Borkent & Wheeler (2013) treated *Paratinia*, as well as *Acomoptera*, *Loicia* Vockeroth and *Drepanocercus* Vockeroth, among the Sciophilini, although in their analyses these taxa branched early amongst their included Sciophilini.

The limited number of Sciophilinae genera included in our analysis does not allow definite conclusions about their phylogeny but they constituted a satisfactorily supported monophyletic group, including both *Eudicrana* and an undescribed Oriental species of *Phthinia* without macrotrichia on the wing membrane. The separate subfamily Eudicraninae was proposed by Väisänen (1984) but he based his argumentation only on two morphological characters (number of ocelli and shape of anapleural suture). According to our analysis (Fig. 1), *Eudicrana* appears to be more closely related to *Sciophila* than to *Leptomorphus*, although in the extended tree (Figure S1) their relationship is less supported. The morphological phylogenetic analysis of Borkent & Wheeler (2013) suggests *Eudicrana* as a sister group to *Leptomorphus*, with *Polylepta* being the sister group to these genera. In our analyses, however, *Polylepta* appeared with high support as a sister group to the Oriental undescribed *Phthinia*, thus rendering *Phthinia* paraphyletic.

A comprehensive molecular analysis of Sciophilinae, Gnoristinae and also Mycomyiinae will be the subject of further studies, where more taxa will be included. This could shed further light on the relationships among these still little-known groups of fungus gnats.

## Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12023

Table S1. List of fungus gnat specimens used for DNA extraction, with specimen identification number and GenBank accession numbers. More information about the specimens is listed in the supporting information. All the specimens are males.

Table S2. List of additional taxa and sequences used in the extended dataset for Bayesian analysis of Mycetophilidae, with GenBank accession numbers.

Table S3. List of primers used for sequencing 12S ribosomal RNA, 16S ribosomal RNA, 28S ribosomal RNA, cytochrome oxidase subunit I (*COI*) and subunit II (*COII*), cytochrome b (*Cytb*) and internal transcribed spacer 2 (*ITS2*).

Table S4. List of specimens included in the phylogenetic analysis.

Figure S1. Bayesian hypothesis for Mycetophilidae relationships based on DNA sequence data, including additional data from GenBank. Values of posterior probability (PP) over 0.5 are given above node numbers. The long branch leading to *Paramanota* has been shortened by half to fit it into the graphic. The image is of *Manota unifurcata* (Lundström), a representative of the Manotinae.

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**Table S1:** List of specimens used for DNA extraction, with specimen identification number and GenBank accession numbers. All the specimens are males.

Taxa	Voucher code	GenBank accession numbers						
		12S	16S	28S	COI	COII	Cytb	ITS2
Diadocidiidae								
<i>Diadocidia ferruginosa</i>	SJ1	KC435526	KC435562	KC435598	KC435634	n/a	KC435680	KC435705
Mycetophilidae								
<i>Allactoneura cincta</i>	JSL9	KC435527	KC435563	KC435599	KC435635	n/a	n/a	KC435706
<i>Allactoneura papuensis</i>	JSL10	KC435528	KC435564	KC435600	KC435636	n/a	n/a	n/a
<i>Chalastonepsia nigricoxa</i>	JSM2	KC435529	KC435565	KC435601	KC435637	n/a	KC435681	KC435707
<i>Chalastonepsia orientalis</i>	JSM1	KC435530	KC435566	KC435602	KC435638	KC435665	KC435682	n/a
<i>Docosia muranica</i>	JSM10	KC435531	KC435567	KC435603	KC435639	n/a	KC435683	KC435708
<i>Dziedzickia bifida</i>	JSM8	KC435532	KC435568	KC435604	KC435640	KC435666	n/a	KC435709
<i>Dziedzickia marginata</i>	JSM7	KC435533	KC435569	KC435605	n/a	KC435667	KC435684	KC435710
<i>Ectrepesthoneura referta</i>	JSM11	KC435534	KC435570	KC435606	KC435641	n/a	KC435685	KC435711
<i>Eudicrana</i> sp.	JSS12	KC435535	KC435571	KC435607	KC435642	KC435668	KC435686	KC435712
<i>Eumanota humeralis</i>	JSL5	KC435536	KC435572	KC435608	KC435643	n/a	KC435687	KC435713
<i>Eumanota suthepensis</i>	JSL4	KC435537	KC435573	KC435609	KC435644	KC435669	KC435688	KC435714
<i>Leia winthemi</i>	JSL12	KC435538	KC435574	KC435610	KC435645	n/a	n/a	n/a
<i>Leptomorphus</i> sp.	JSS1	KC435539	KC435575	KC435611	KC435646	KC435670	KC435689	KC435715
<i>Manota mabokeensis</i>	JSS10	KC435540	KC435576	KC435612	KC435647	KC435671	KC435690	KC435716
<i>Manota mazumbaiensis</i>	JSS11	KC435541	KC435577	KC435613	KC435648	n/a	n/a	KC435717
<i>Manota unifurcata</i>	JSS8	KC435542	KC435578	KC435614	KC435649	KC435672	KC435691	KC435718
<i>Manota</i> sp.	JSL6	KC435543	KC435579	KC435615	KC435650	n/a	n/a	n/a
<i>Manota</i> sp.	JSL7	KC435544	KC435580	KC435616	KC435651	n/a	n/a	KC435719
<i>Metanepsia javana</i>	JSM3	KC435545	KC435581	KC435617	n/a	n/a	n/a	KC435720
<i>Metanepsia</i> sp.	JSM4	KC435546	KC435582	KC435618	KC435652	KC435673	KC435692	KC435721
<i>Neoclastobasis draskovitsae</i>	JSL11	KC435547	KC435583	KC435619	KC435653	KC435674	n/a	n/a
<i>Novakia scatopsiformis</i>	JSM12	KC435548	KC435584	KC435620	KC435654	KC435675	KC435693	KC435722
<i>Palaeodocosia vittata</i>	JSM6	KC435549	KC435585	KC435621	KC435655	n/a	KC435694	KC435723
<i>Paramanota furcillata</i>	JSL3	KC435550	KC435586	KC435622	KC435656	KC435676	KC435695	KC435724
<i>Paramanota orientalis</i>	JSL2	KC435551	KC435587	KC435623	KC435657	n/a	KC435696	KC435725
<i>Paratinia sciarina</i>	JSS6	KC435552	KC435588	KC435624	KC435658	n/a	KC435697	KC435726
<i>Phthinia</i> sp.	JSS3	KC435554	KC435590	KC435626	n/a	n/a	KC435699	KC435728
<i>Phthinia winnertzi</i>	JSS2	KC435555	KC435591	KC435627	KC435659	KC435677	KC435700	KC435729
<i>Polylepta zonata</i>	JSS9	KC435556	KC435592	KC435628	KC435660	n/a	KC435701	KC435730
<i>Promanota malaisei</i>	JSL1	KC435557	KC435593	KC435629	KC435661	n/a	KC435702	KC435731
<i>Schnusea</i> sp.	JSM9	KC435558	KC435594	KC435630	KC435662	KC435678	KC435703	KC435732
<i>Sciophila thoracica</i>	JSS4	KC435559	KC435595	KC435631	KC435663	n/a	KC435704	KC435733
<i>Sticholeia cheesmanae</i>	JSL8	KC435560	KC435596	KC435632	KC435664	KC435679	n/a	n/a
<i>Sytemna daisetsusana</i>	JSS5	KC435561	KC435597	KC435633	n/a	n/a	n/a	KC435734



**Table S2:** List of additional taxa and sequences used in the extended dataset for Bayesian analysis of Mycetophilidae, with GenBank accession numbers.

Taxa	GenBank accession numbers						References
	12S	16S	28S	COI	COII	Cytb	
<b>Mycetophilidae</b>							
<i>Acomoptera difficilis</i>	n/a	FJ172000	FJ171964	n/a	n/a	n/a	Rindal et al. (2009)
<i>Allocotocera pulchella</i>	n/a	FJ172034	FJ171999	n/a	n/a	n/a	Rindal et al. (2009)
<i>Anatella lenis</i>	n/a	DQ787936	n/a	n/a	n/a	n/a	Rindal et al. (2007)
<i>Azana</i> sp.	n/a	FJ172031	FJ171996	n/a	n/a	n/a	Rindal et al. (2009)
<i>Boletina erythropygia</i>	n/a	HQ230383	HQ230407	HQ230449	n/a	HQ230426	Martinsson et al. (2011)
<i>Boletina gripha</i>	n/a	HQ230380	HQ230403	HQ230446	n/a	HQ230424	Martinsson et al. (2011)
<i>Boletina sahlbergi</i>	n/a	HQ230384	HQ230408	n/a	n/a	HQ230427	Martinsson et al. (2011)
<i>Boletina sciarina</i>	n/a	HQ230367	HQ230390	HQ230433	n/a	HQ230413	Martinsson et al. (2011)
<i>Coelosia tenella</i>	n/a	FJ172026	FJ171991	n/a	n/a	n/a	Rindal et al. (2007, 2009)
<i>Cordyla</i> sp.	n/a	DQ787929	EU219586	DQ787879	n/a	n/a	Rindal et al. (2007, 2009)
<i>Dynatosoma reciprocum</i>	n/a	DQ787928	EU219597	DQ787878	n/a	n/a	Rindal et al. (2007, 2009)
<i>Epicypsa</i> sp.	n/a	EU219603	EU219579	n/a	n/a	n/a	Rindal et al. (2009)
<i>Exechia frigida</i>	n/a	DQ787931	n/a	n/a	n/a	n/a	Rindal et al. (2007)
<i>Gnoriste bilineata</i>	n/a	FJ172024	FJ171989	n/a	n/a	n/a	Rindal et al. (2009)
<i>Gnoriste longirostris</i>	n/a	HQ230376	HQ230398	HQ230442	n/a	HQ230421	Martinsson et al. (2011)
<i>Grzegorzekia collaris</i>	n/a	FJ172016	FJ171981	n/a	n/a	n/a	Rindal et al. (2009)
<i>Leia bilineata</i>	n/a	DQ787924	n/a	n/a	n/a	n/a	Rindal et al. (2007)
<i>Leptomorphus walkeri</i>	n/a	FJ172020	FJ171985	n/a	n/a	n/a	Rindal et al. (2009)
<i>Megalopelma nigroclavatum</i>	n/a	FJ172030	FJ171995	n/a	n/a	n/a	Rindal et al. (2009)
<i>Monoclona rufilatera</i>	n/a	FJ172029	FJ171994	n/a	n/a	n/a	Rindal et al. (2009)
<i>Mycetophila fungorum</i>	n/a	DQ787927	n/a	n/a	n/a	n/a	Rindal et al. (2007)
<i>Mycomya annulata</i>	n/a	FJ172011	FJ171976	n/a	n/a	n/a	Rindal et al. (2009)
<i>Neoempheria pictipennis</i>	n/a	FJ172032	FJ171997	n/a	n/a	n/a	Rindal et al. (2009)
<i>Phronia</i> sp.	n/a	EU219606	EU219585	n/a	n/a	n/a	Rindal et al. (2009)
<i>Phthinia humilis</i>	n/a	FJ172009	FJ171974	n/a	n/a	n/a	Rindal et al. (2009)
<i>Platurocypta</i> sp.	n/a	EU219601	EU219590	n/a	n/a	n/a	Rindal et al. (2009)
<i>Rondaniella dimidiata</i>	n/a	FJ172015	FJ171980	n/a	n/a	n/a	Rindal et al. (2009)
<i>Speolepta leptogaster</i>	n/a	FJ172033	FJ171998	n/a	n/a	n/a	Rindal et al. (2009)
<i>Synapha vitripennis</i>	n/a	FJ172001	FJ171965	n/a	n/a	n/a	Rindal et al. (2009)
<i>Syntenna stylata</i>	n/a	FJ172013	FJ171978	n/a	n/a	n/a	Rindal et al. (2009)
<i>Trichonta</i> sp.	n/a	EU219607	EU219588	EU219566	n/a	n/a	Rindal et al. (2009)
<b>Sciaridae</b>							
<i>Bradysia amoena</i>	GQ387651	GQ387651	n/a	GQ387651	GQ387651	GQ387651	Beckenbach & Joy (2009)

**Table S3:** List of primers used for sequencing 12S ribosomal RNA, 16S ribosomal RNA, 28S ribosomal RNA, cytochrome oxidase subunit I (COI) and subunit II (COII), cytochrome b (Cytb) and internal transcribed spacer 2 (ITS2)

Gene fragment	Primer sequences (5'→3')	Source
12S	CTGGGATTAGATACCCTGTTAT	Cook et al. 2004
	CAGAGAGTGACGGGCGATTTGT	Cook et al. 2004
16S	TAATCCAACATCGAGGTC	Roháček et al. 2009
	CGAAGGTAGCATAATCAGTAG	Roháček et al. 2009
28S	AGAGAGAGAGTTCAAGAGTACGTG	Belshaw <i>et al.</i> 2001
	TAGTTCACCATCTTTCGGGTC	Belshaw <i>et al.</i> 2001
COIa	GGTCAACAAATCATAAAGATATTGG	Su et al. 2008
	TAAACTTCAGGGTGACCAAAAAATCA	Su et al. 2008
COIb	CAACATTTATTTTGATTTTTTGG	Su et al. 2008
	TCCAATGCACTAATCTGCCATATTA	Su et al. 2008
COII	TAATATGGCAGATTAGTGCA	Su et al. 2008
	GTTTAACAGACCAGTACTT	Su et al. 2008
Cytb	TATGTTTTATGAGGACAAATATC	Su et al. 2008
	AAATTCTATCTTATGTTTCAAAC	Su et al. 2008
ITS2	TGTGAACTGCAGGACACAT	Beebe & Saul, 1995
	TATGCTTAAATTCAGGGGGT	Beebe & Saul, 1995

**Table S4:** List of specimens included in the phylogenetic analysis

<b>Species</b>	<b>Author</b>	<b>Sampling locality and year</b>
<i>Allactoneura cincta</i>	DeMeijere, 1907	Thailand, 2008
<i>Allactoneura papuensis</i>	Bechev, 1995	Papua New Guinea, 2009
<i>Diadocidia ferruginosa</i>	(Meigen, 1830)	Slovakia, 2010
<i>Docosia muranica</i>	Kurina & Ševčík, 2011	Slovakia, 2012
<i>Dziedzickia bifida</i>	Ševčík, Bechev & Hippa, 2011	Thailand, 2008
<i>Dziedzickia marginata</i>	(Dziedzicki, 1885)	Finland, 2003
<i>Ectrepesthoneura referta</i>	Plassmann, 1976	Czech Republic, 2007
<i>Eudicrana</i> sp.		Indonesia, 2010
<i>Eumanota humeralis</i>	Edwards, 1933	Malaysia, 2007
<i>Eumanota suthepensis</i>	Søli, 2002	Thailand, 2008
<i>Chalastonepsia nigricoxa</i>	Ševčík & Hippa, 2010	Thailand, 2007
<i>Chalastonepsia orientalis</i>	Søli, 1996	Thailand, 2008
<i>Leia winthemi</i>	Lehmann, 1822	Czech Republic, 2011
<i>Leptomorphus</i> sp.		Madagascar, 2009
<i>Manota maboakeensis</i>	Matile, 1972	Uganda, 2010
<i>Manota mazumbaiensis</i>	Søli, 1993	Uganda, 2010
<i>Manota</i> sp.		Peru, 2010
<i>Manota</i> sp.		Thailand, 2007
<i>Manota unifurcata</i>	(Lundström, 1913)	Slovakia, 2012
<i>Metanepsia javana</i>	Edwards, 1927	Thailand, 2007
<i>Metanepsia</i> sp.		Thailand, 2008
<i>Neoclastobasis draskovitsae</i>	Matile, 1978	Czech Republic, 2011
<i>Novakia scatopsiformis</i>	Strobl, 1893	Slovakia, 2011
<i>Palaeodocosia vittata</i>	(Coquillett, 1923)	Slovakia, 2010
<i>Paramanota furcillata</i>	Hippa, 2010	Thailand, 2008
<i>Paramanota orientalis</i>	Tuomikoski, 1966	Thailand, 2007
<i>Paratinia sciarina</i>	Mik, 1874	Slovakia, 2011
<i>Phthinia</i> sp.		Indonesia, 2010
<i>Phthinia winnertzi</i>	Mik, 1869	Slovakia, 2011
<i>Polylepta zonata</i>	(Zetterstedt, 1858)	Slovakia, 2011
<i>Promanota malaisei</i>	Tuomikoski, 1966	Thailand, 2007
<i>Sciophila thoracica</i>	Staeger, 1840	Slovakia, 2012
<i>Schnusea</i> sp.		Peru, 2010
<i>Sticholeia cheesmanae</i>	Søli, 1996	Papua New Guinea, 2009
<i>Syntemna daisetsusana</i>	Okada, 1938	Slovakia, 2010

Ševčík, J., Kaspřák, D. & Tóthová, A. (2013) Molecular phylogeny of fungus gnats (Diptera: Mycetophilidae) revisited: Position of Manotinae, Metanepsiini, and other enigmatic taxa as inferred from multigene analysis. Systematic Entomology.

**Figure S1:** Bayesian hypothesis for Mycetophilidae relationships based on DNA sequence data, including additional data from GenBank. Above node number = posterior probability (PP) over 0.5. The long branch leading to Paramanota has been shortened by half to fit it into the graphic. The image is of *Manota unifurcata* (Lundström), a representative of the Manotinae.

