Molecular phylogeny of the fungus gnat tribe Exechiini (Mycetophilidae, Diptera)

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The phylogenetic relationships within the fungus gnat tribe Exechiini have been left unattended for many years. Recent studies have not shed much light on the intergeneric relationship within the tribe. Here the first attempt to resolve the phylogeny of the tribe Exechiini using molecular markers is presented. The nuclear 18S and the mitochondrial 16S, and cytochrome oxidase subunit I (COI) genes were successfully sequenced for 20 species representing 15 Exechiini genera and five outgroup genera. Bayesian, maximum parsimony and maximum likelihood analyses revealed basically congruent tree topologies and the monophyly of Exechiini, including the genus *Cordyla*, is confirmed. The molecular data corroborate previous morphological studies in several aspects. *Cordyla* is found in a basal clade together with *Brachypeza*, *Pseudorymosia* and *Stigmatomeria*. The splitting of the genera *Allodiopsis* s.l. and *Brevicornu* s.l. as well as the sistergroup relationship of *Exechia* and *Exechiopsis* is also supported. The limited phylogenetic information provided by morphological characters is mirrored in the limited resolution of the molecular markers used in this study. Short internal and long-terminal branches obtained may indicate a rapid radiation of the Exechiini genera during a short evolutionary period.

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Introduction

Fungus gnats of the family Mycetophilidae are today commonly divided into two subfamilies, Mycetophilinae and Sciophilinae, although this classification is still under debate (for review, see, e.g., Søli *et al*. 2000). Both subfamilies are further divided into a number of tribes; Mycetophilinae into Mycetophilini and Exechiini.

Fungus gnats of the tribe Exechiini (Mycetophilidae) make up a small but relatively homogenous group. The tribe consists of 19 genera and approximately 620 recognised species. In terms of species richness, Exechiini is thus smaller than Mycetophilini, with 14 genera and approximately 1460 recognised species (numbers retrieved from an internal compilation, Natural History Museum, Oslo). Although representatives of Exechiini are known from all continents, the tribe seems to have a more restricted distribution than that of Mycetophilini. Little is known about the species life histories, but the larvae of the majority of species are polyphagous and live in the fruit bodies of soft fungi (see, e.g., Kurina 1994; Yakovlev 1994). In temperate regions adult specimens can be found throughout the year, but they are most active and abundant in late summer and autumn. Adults of several Exechiini genera are known to hibernate in caves during winter, for example, *Anatella*, *Exechia*, *Exechiopsis*, *Pseudexechia*, *Rymosia* and *Tarnania* (Kjærandsen 1993; Kurina 1996). Some *Exechia* species have also been reported to hibernate in umbelliferous stems (Väisänen 1981) and under the bark of snow covered logs (Hedmark 2000).

The phylogenetic relationship within Mycetophilinae is still poorly understood. Based on morphological characters Søli (1997) presented the first cladistic analysis of Mycetophilidae, primarily designed to address the phylogeny within Sciophilinae. Only three Mycetophilinae genera were included in that analysis, which revealed little information about the validity of the tribes Mycetophilini and Exechiini. A recent study by Rindal & Søli (2006) based on morphological

Table 1 Historic overview of the generic division of the tribe Exechiini Edwards (1925).

Before Tuomikoski (1966)	Tuomikoski (1966)	Current
Anatella	*Anatella*	*Anatella* Winnertz, 1863
Neoallodia	*Neoallodia*	*Neoallodia* Edwards, 1932
Cordyla	*Cordyla*	*Cordyla* Meigen, 1803
Rymosia	*Rymosia*	*Rymosia* Winnertz, 1863
	Tarnania	*Tarnania* Tuomikoski (1966)
	Pseudorymosia	*Pseudorymosia* Tuomikoski (1966)
	Allodiopsis	*Allodiopsis* s. str. Tuomikoski (1966)
	sg. *Gymnogonia*	*Synplasta* Skuse, 1890 (= *Gymnogonia* Tuomikoski 1966)
	sg. *Myrosia*	*Myrosia* Tuomikoski (1966)
	sg. *Notolopha*	*Notolopha* Tuomikoski (1966)
	sg. *Allodiopsis*	
Exechia	*Exechia*	*Exechia* Winnertz, 1863
	sg. *Pseudexechia*	*Pseudexechia* Tuomikoski (1966)
	sg. *Exechiopsis*	*Exechiopsis* Tuomikoski (1966)
		sg. *Exechiopsis* Tuomikoski (1966)
	sg. *Xenexechia*	sg. *Xenexechia* Tuomikoski (1966)
Allodia	*Allodia*	*Allodia* Winnertz, 1863
	sg. *Allodia*	sg. *Allodia* Winnertz, 1863
	sg. *Brachycampta*	sg. *Brachycampta* Winnertz, 1863
	Brevicornu	*Brevicornu* s. str. Marshall, 1896
	sg. *Brevicornu*	
	sg. *Stigmatomeria*	*Stigmatomeria* Tuomikoski (1966)
Brachypeza	*Brachypeza*	*Brachypeza* Winnertz, 1863
	sg. *Brachypeza*	sg. *Brachypeza* Winnertz, 1863
	sg. *Paracordyla*	sg. *Paracordyla* Tuomikoski (1966)
	Pseudobrachypeza	*Pseudobrachypeza* Tuomikoski (1966)
		Boraceomyia Lane, 1946

characters presented a cladistic analysis of the subfamily Mycetophilinae. The study revealed a relatively good resolution for the tribe Mycetophilini whereas the tribe Exechiini remained basically unresolved.

The classification within Exechiini varies much between different authors. The disagreement relates to (i) which genera should be included in the tribe, (ii) the delimitation of genera to be included, and (iii) the phylogenetic relationship of the genera within the tribe. A historic overview of the generic division of the tribe Exechiini is given in Table 1.

Tuomikoski (1966) provided the most comprehensive work on Exechiini until today. He scrutinised and discussed the validity of all genera within the tribe in a phylogenetic framework. After a proper delimitation only *Anatella*, *Cordyla* and *Neoallodia* were left unaltered. The considerations of Tuomikoski (1966) can be summarised as follows; most importantly, he reinstated *Brevicornu* Marshall, that Tonnoir & Edwards (1927) had included in *Allodia*, and raised six new genera and seven new subgenera (one emended). In his attempt to make Exechiini monophyletic he also transferred *Cordyla* from Mycetophilini to Exechiini, a view today adopted by most authors. Tuomikoski (1966) suggested a close relationship of *Cordyla* to *Brachypeza* and *Brevicornu* based on characters such as, for example, small male genitalia, setose metanepisternum, and the presence of more than one basal bristle on the hind coxa. He also suggested a close relationship of *Allodia* and *Allodiopsis* based on the oval shape of clypeus, a weak anal vein, and one or two pairs of longer bristles on the male 9th tergite. Within *Allodiopsis*, he considered the subgenus *Notolopha* closest to *Allodiopsis* s. str., differing only in antennae and male terminalia. He indicated that the subgenera within *Allodiopsis* and *Brevicornu* were likely to eventually receive generic status. This was accomplished by Vockeroth (1980) for *Brevicornu* and *Stigmatomeria*, and by Matile (1987) and Søli *et al*. (2000) for the four subgenera of *Allodiopsis*. Finally, based on the free-ending subcosta, the bare fork veins and the similar colouration of the abdomen, Tuomikoski (1966) supported with caution the suggestion by Edwards (1925) of a close relationship between *Exechia* and *Rymosia*.

The two most recent cladistic efforts to solve the phylogenetic relationships within Exechiini (Kjærandsen 2006; Rindal & Søli 2006) are both based on morphological characters, some of which were already applied in Tuomikoski's (1966) work. By scoring 65 morphological characters for 27 genera Rindal & Søli (2006) supported the monophyly of Exechiini *sensu* Tuomikoski as well as the tribe Mycetophilini and the subfamily Mycetophilinae. Within the tribe Exechiini, however, the phylogenetic relationships were poorly resolved with

Table 2 List of examined taxa with collecting data and GenBank accession numbers.

Taxa	Locality/coordinates	Collecting date	GenBank accession numbers		
			18S	16S	COI
Tribe Exechiini					
Anatella	Simadalen, Tveit, Norway 60°30′4.4″N 7°11′25.2″E	September/October 2004	DQ787911	DQ787936	DQ787886
Allodia	Geilo, Kikut, Norway UTM 60°29′24.3″N 8°14 42.6″E	August 2002	DQ787912	DQ787937	DQ787887
Allodiopsis rustica	Falsterbo, Sweden 55°23′28″ N 12°50′24″E	October 2003	DQ787913	DQ787938	DQ787888
Brazypeza bisignata	Frogn, Norway 59°41′41.9″N 10°43 17.6″E	May/June 2004	DQ787919	DQ787944	DQ787894
Brevicornu	Revetal, Våle, Norway 59°22′0.8″N 10°16 32.0″E	August 2002	DQ787915	DQ787940	DQ787890
Brevicornu	Geilo, Kikut, Norway 60°29′24.3″N 8°14 42.6″E	August 2002	DQ787914	DQ787939	DQ787889
Cordyla	Geilo, Kikut, Norway 60°29′24.3″N 8°14 42.6″E	August 2002	DQ787904	DQ787929	DQ787879
Exechia	Geilo, Kikut, Norway 60°29′24.3″N 8°14 42.6″E	August 2002	DQ787906	DQ787931	DQ787881
Exechiopsis	Grønnmo, Sølvdobla, Norway 59°50′37.4″N 10°52 45.0″E	September 2002	DQ787907	DQ787932	DQ787882
Exechiopsis sagitata	Bømlo, Skogafjell NR, Norway 59°38′43.3″N 5°12 53.9″E	January–March 2004	DQ787908	DQ787933	DQ787883
Exechiopsis subulata	Bergen, Gymmeland, Norway 60°18′13.1″N 5°27 8.5″E	April 2004	DQ787909	DQ787934	DQ787884
Notolopha cristata	Hovin, Spjeldset, Norway 59°56′29.5″N 9°2 15.3″E	May–July 2004	DQ787918	DQ787943	DQ787893
Pseudobrazypeza	Hovin, Spjeldset, Norway 59°56′29.5″N 9°2 15.3″E	May–July 2004	DQ787920	DQ787945	DQ787895
Pseudorymosia fovea	Revetal, Våle, Norway 59°22′0.8″N 10°16 32.0″E	October/November 2003	DQ787910	DQ787935	DQ787885
Rymosia	Revetal, Våle, Norway 59°22′0.8″N 10°16 32.0″E	September 2002	DQ787905	DQ787930	DQ787880
Stigmatomeria crassicornis	Bømlo, Vorland,Norway 59°36′27.3″N 5°12 50.0″E	October 2003	DQ787916	DQ787941	DQ787891
Synplasta gracilis	Falsterbo, Sweden 55°23′28″ N 12°50′24″E	August 2003	DQ787917	DQ787942	DQ787892
Tarnania	Grønnmo, Sølvdobla, Norway 59°50′37.4″N 10°52 45.0″E	September/October 2002	DQ787921	DQ787946	DQ787896
Tarnania fenestralis	Bergen, Gymmeland, Norway 60°18′13.1″N 5°27 8.5″E	April 2004	DQ787922	DQ787947	DQ787897
Tarnania dziedzickii	Bømlo, Lykling, Norway 59°42′51.6″N 5°10 30.5″E	October 2004	DQ787923	DQ787948	DQ787898
Tribe Mycetophilini					
Dynatosoma reciprocum	Hovin, Spjeldset, Norway 59°56′29.5″N 9°2 15.3″E	May–July 2004	DQ787903	DQ787928	DQ787878
Mycetophila fungorum	Geilo, Kikut, Norway 60°29′24.3″N 8°14 42.6″E	August 2002	DQ787902	DQ787927	DQ787877
Boletina	Grønnmo, Sølvdobla, Norway 59°50′37.4″N 10°52 45.0″E	September 2002	DQ787901	DQ787925	DQ787876
Docosia	Grønnmo, Sølvdobla, Norway 59°50′37.4″N 10°52 45.0″E	September/October 2002	DQ787900	DQ787926	DQ787875
Leia	Simadalen, Tveit, Norway 60°30′4.4″N 7°11 25.2″E	September/October 2003	DQ787899	DQ787924	DQ787874

most genera being left in a basal polytomy. The only well supported intergeneric relationships were the basal position of *Cordyla* followed by *Anatella*. The clade consisting of *Exechiopsis* and *Exechia* was also well supported; whereas the clade *Notolopha* (*Pseudorymosia*, *Brachypeza*) was only poorly supported. Kjærandsen (2006) focused on the *Rymosia* s. lat. group and scored for a total of 69 morphological characters in 9 genera, many of which related to the male terminalia. He found a close relationship between *Tarnania*, *Pseudorymosia* and *Rymosia*. He also found support for the splitting of *Allodiopsis* s.l. and considered the entire *Rymosia* s.l. group likely to be a polyphyletic assemblage of plesiomorphic genera.

While the monophyly of the tribe Exechiini as delimited by Tuomikoski (1966) now seems to be well supported by morphological data, there is still a poor understanding of the intergeneric relationships within the tribe.

Here, we present the first molecular phylogeny within the superfamily Sciaroidea that is based on nucleotide sequences of the nuclear 18S, the mitochondrial 16S and cytochrome oxidase subunit I (COI) genes in order to infer phylogenetic relationships among the genera of the tribe Exechiini.

Materials and methods

Sampling and species identification

Most specimens were collected at eight localities in Norway and Sweden (Table 2) using Malaise traps with 80% ethanol. The specimen's genitalia were dissected and stored for documentation. It was attempted to have representatives for all Exechiini genera, but fresh material suitable for molecular analyses representing all genera was not available. Museum material turned out to be unsuitable for the extraction of DNA of reasonable molecular weight. Whenever possible, more than one species per genus were analysed. The outgroup taxa were the same genera as those used by Rindal & Søli (2006): two representing Mycetophilini; namely, *Mycetophila* and *Dynatosoma*, and three representing Sciophilinae; namely, *Leia*, *Docosia* and *Boletina* (Table 2).

DNA isolation, PCR amplification and sequencing

DNA was extracted from parts of the specimens following the instructions of the Puregene kit (Gentra Systems, Minneapolis, MN, USA). For each specimen the abdomen was used for DNA extraction, while the genitalia were stored

Table 3 Primers used in this study for PCR amplifications and sequencing of the mitochondrial 16S and COI, and the nuclear 18S genes.

Primer	Sequence	Reference
16S		
LR-N-13398	CGCCTGTTTAACAAAAACAT	Simon *et al.* (1994)
LR-J-12887	CCGGTCTGAACTCAGATCACGT	Simon *et al.* (1994)
16S_FWD_39	CCAACATCGAGGTCGCAAAA	this study
16S_FWD_34	TTAATCCAACATCGAGGTCGC	this study
16S_REW_452	CCCACTGAATTAAAGGCTGCGGTAT	this study
COI		
TL2-N-3014	TCCAATGCACTAATCTGCCATATTA	Simon *et al.* (1994)
C1-J-2195	TTGATTTTTTGGTCACCCTGAAGT	Simon *et al.* (1994)
FWD_C1-J-2183	CAACATTTATTTTGATTTTTTGG	Simon *et al.* (1994)
18S		
18S_ai	CCTGAGAAACGGCTACCACATC	Whiting *et al.* (1997)
18S_a0.7	ATTAAAGTTGTTGCGGTT	Whiting *et al.* (1997)
18S_b5.0	TAACCGCAACAACTTTAAT	Whiting *et al.* (1997)
18S_bi	GAGTCTCGTTCGTTATCGGA	Whiting *et al.* (1997)

in glycerol in microvials as vouchers and deposited in the entomological collection of the Natural History Museum, Oslo. PCR amplification of the nuclear 18S and the mitochondrial 16S, and COI genes followed standard procedures. The amplification program for the 16S gene was 95 °C for 3 min, followed by 35 cycles of 95 °C for 1 min, 48 °C for 1 min and 72 °C for 1 min and 50 s, and a final extension step at 72 °C for 7 min. The amplification program for the COI gene was 94 °C for 3 min followed by 35 cycles of 94 °C for 30 s, 45 °C for 30 s and 72 °C for 1 min and 50 s, and a final extension step at 72 °C for 7 min. The amplification program for the 18S gene was 94 °C for 3 min; 35 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C 1 min and 50 s, and a final extension step at 72 °C for 7 min. All PCR amplifications were performed using the recombinant Taq polymerase of Roche (Switzerland). The primers used for PCR amplifications and sequencing are listed in Table 3.

DNA sequencing and sequence alignment

The obtained PCR products were purified with ExoSAP-IT (GE Healthcare, UK) according to the manufacturers instructions, and subsequently sequenced on an ABI3100 automatic sequencer (Applied Biosystems, Foster City, CA, USA) using the BigDye chemistry (Applied Biosystems, Foster City, CA, USA). Proofreading of the obtained nucleotide sequences and subsequent alignment was done using GeneTools 2.0 (Wishart & Fortin 2001). Variable regions in the 18S sequence, where the alignment was considered arbitrary due to the occurrence of indels, were omitted from the subsequent analyses.

Phylogenetic reconstructions

paup* 4 beta 10 win (Swofford 2003) was used to construct the most parsimonious (MP) cladograms using a heuristic search with 1 million replicates and treating gaps as a 5th character state. Bootstrap analyses were performed with 10 000 replicates with 10 searches within each bootstrap replicate. Bayesian analyses were conducted with an online version of MrBayes (Huelsenbeck & Ronquist 2001) implemented at the Bioportal at the University of Oslo (http://www.bioportal.uio.no). MrModeltest (Nylander 2004), a simplified version of Modeltest 3.06 (Posada & Crandall 1998), was used to estimate the best-fitting substitution model for the analyses. The best model of nucleotide substitution was the general time reversible model with a gamma distributed rate heterogeneity and a significant proportion of invariable sites (GTR + I + G). Bayesian inference analyses were performed under 4 000 000 generations and four Metropolis-coupled Markow chains, taking samples every 100 generations, with the first 4000 generation discarded as burn-in. From the resulting trees *a posteriori* probabilities for individual clades where assessed based on their observed frequencies. Maximum likelihood (ML) analysis was performed with the GTR + I + G model, and bootstrapping values were calculated for 1000 replicates. A 'Partition Homogeneity Test' in paup* was conducted with 100 replicates with 10 searches within each bootstrap replicate. Both pair-wise and total comparisons between the 18S, 16S and COI were performed.

To test for substitution saturation, a Xia *et al*. (2003) test was performed with Jukes–Cantor distances and Poisson + invariant used to calculate proportion of invariant sites. The test was performed in dambe (Xia & Xie 2001). Saturation plots were made using *p*-distances plotted against GTR + I + G distances in accordance with Sullivan & Joyce (2005).

Results

The mitochondrial 16S and COI, and the nuclear 18S genes were successfully amplified and sequenced from 25 species in 20 genera, 15 representing Exechiini and five representing outgroup genera (Table 2). The total alignment of all three genes consisted of 1882 bp including 502 (26.7%) variable positions out of which 386 (20.5%) were parsimony informative; 1380 bp were conserved sites (see Table 4 for further details). The strong AT bias normally found in insect mitochondrial DNA was also observed in the Exechiini sequences. The 18S, 16S and COI alignments were deposited in the EMBL database and can be retrieved electronically from <ftp://ftp.ebi.ac.uk/pub/databases/embl/align/>, accession nos ALIGN_001047–ALIGN_001049. Of the 282 variable positions of the COI alignment, 61 (21.6%) affect the first, 15 (5.3%) the second and 205 (72.7%) the third position of a codon, respectively, out of which 47, 9 and 188 positions are parsimony informative.

Table 4 Details on the alignment of the mitochondrial 16S and COI, and the nuclear 18S gene of the fungus gnat species listed in Table 2.

Gene	Length of alignment	AT content	Variable positions	Parsimony informative sites	Regions removed from analyses
COI	695 bp	70.7%	282 (40.6%)	245	—
16S	349 bp	56.8%	105 (30.1%)	74	—
18S	849 bp	52.8%	115 (13.7%)	67	11 bp (Leia)

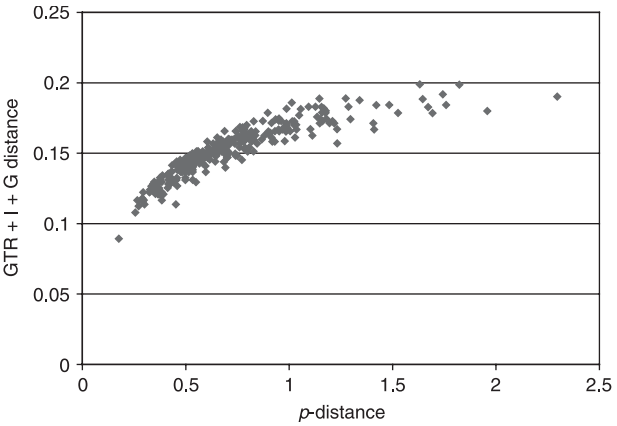

Fig. 1 Saturation plot for COI (all positions), pair-wise GTR + I + G distances are plotted against uncorrected *p*-distances.

The Partition Homogeneity Test showed a significant difference ($P = 0.04$) when all partitions were tested together, but when groups of two and two pairs of genes were tested against each other, no significant difference were found (18S and COI, $P = 0.27$; COI and 16S, $P = 0.15$; 16S and 18S, $P = 0.07$).

The saturation test by Xia *et al*. (2003) showed that there was no significant saturation for the COI gene ($P < 0.0001$). The saturation plot shows possible saturation for the COI gene (Fig. 1).

All phylogenetic analysis yielded trees (Figs 2–4) with Exechiini as a monophyletic group. The support for the monophyly of Exechiini was maximal in the Bayesian analysis (Fig. 3), somewhat lower in the ML analysis (Fig. 4) and below 50% in the MP analysis (Fig. 2). The two genera representing Mycetophilini were always the sister group of Exechiini.

Overall, little support was found for clades within Exechiini, but some interesting observations could be made. In both the MP trees (Fig. 2) and the ML tree (Fig. 4), *Cordyla* is found in a basal clade together with *Brachypeza*, *Pseudorymosia* and *Stigmatomeria*. In the Bayesian analysis, however, *Cordyla* resides in an unresolved bush for the entire Exechiini. Splitting of the genera *Allodiopsis* s.l. and *Brevicornu* s.l. is supported. *Brevicornu* and *Stigmatomeria* are well separated in all cladograms, as is *Brevicornu* s. str. as a separate entity from *Allodia*. Likewise is *Synplasta* well separated from *Notolopha* and *Allodiopsis*. In both the Bayesian tree (Fig. 3) and the ML tree (Fig. 4), *Anatella* forms the sister group of *Exechia* and *Exechiopsis*. The latter clade is supported with a posterior probability of 82 and a bootstrap of 57 on the ML tree (Fig. 4), but the clade is not found in the MP analysis (Fig. 2). The genera represented with more than one species (*Brevicornu* s. str., *Exechiopsis* and *Tarnania*), all group as monophyletic entities with strong support (typically 100%) in all analyses.

Discussion

The analyses of 18S, 16S and COI nucleotide sequences confirmed the monophyly of the fungus gnat tribe Exechiini, and thus support the findings of Rindal & Søli (2006) who also found Exechiini monophyletic on the basis of morphological characters. In addition, the analyses also support the monophyly of the subfamily Mycetophilinae, with the species representing the tribe Mycetophilini forming the sister group to Exechiini.

The molecular analyses support the inclusion of *Cordyla* in Exechiini; in agreement with Tuomikoski (1966) and Rindal & Søli (2006). This renders Exechiini with only two of the original characters used by Edwards (1925) as valid synapomorphies; that is, the rudimentary empodium and the short hind tibial comb (for a more detailed discussion of the morphological characters that delimit the two tribes within Mycetophilinae see Rindal & Søli 2006).

The 18S, 16S and COI nucleotide sequences belong to the standard toolbox of genes used in phylogenetic studies of insects that have in many cases been shown to be informative on species and generic levels. Unfortunately the molecular data set did not result in a greatly enhanced resolution of the phylogenetic relationships of the Exechiini genera. The two MP trees and the Bayesian tree collapse many genera into a basal polytomy, and the ML tree has only few well supported clades. We conclude that the obtained variability does not provide sufficient information for understanding the phylogenetic relationship within Exechiini. The low resolution can not be attributed to a particular method for phylogenetic reconstruction. Nevertheless, there are some common trends in results.

A basal clade consisting of the four genera *Brachypeza*, *Cordyla*, *Pseudorymosia* and *Stigmatomeria* was found both by the MP and the ML analyses, but not by the Bayesian analysis. Left aside *Pseudorymosia*, this is in agreement with Tuomikoski (1966) who suggested a close relationship between *Cordyla*, *Brachpeza* and *Brevicornu* (including *Stigmatomeria*). This basal clade was not found by Rindal & Søli (2006), who found

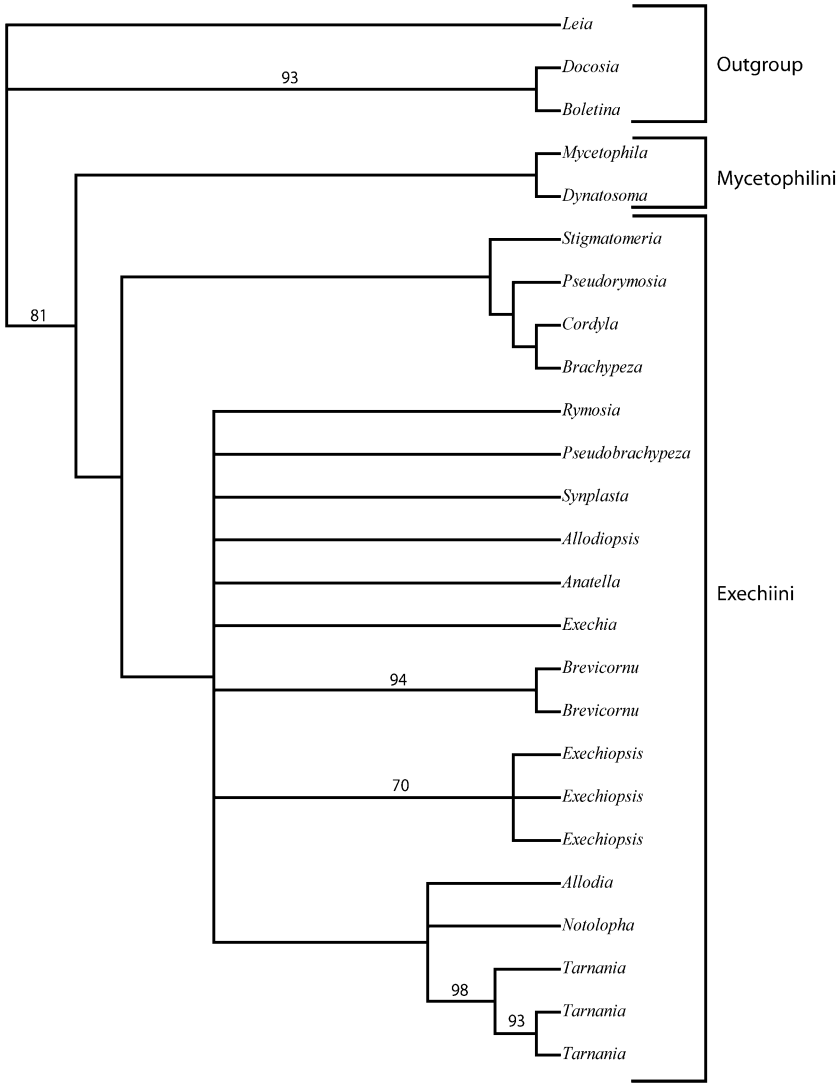

Fig. 2 Consensus of the two most parsimonious trees, based on heuristic search with 1 million replicates. Bootstrap values are based on 10 000 replicates with 10 reps within each search. *Mycetophila* and *Dynatosoma* representing Mycetophilini and *Leia*, *Docosia* and *Boletina* representing Sciophilinae serve as outgroups.

Cordyla in a sistergroup relationship with the rest of Exechiini. *Pseudorymosia* and *Brachypeza*, grouped together in the study of Rindal & Søli (2006), although not close to *Cordyla*.

The splitting of the genera *Allodiopsis* and *Brevicornu* is also supported by the molecular data which is in line with the morphological data (Kjærandsen 2006; Rindal & Søli 2006). The former subgenera of *Allodiopsis* are not found in a monophyletic group and the validity of *Allodiopsis* sensu Tuomikoski (1966) must be rejected. Only *Notolopha* and *Allodiopsis* group together, although only weakly supported, in the ML and Bayesian trees. This is noteworthy, since for all other genera with more than one representative, there is 100% support for the grouping of congeners (*Tarnania*, *Exechiopsis* and *Brevicornu*). The molecular data are thus supporting the results of Rindal & Søli (2006) who also found that *Allodiopsis* sensu Tuomikoski to be polyphyletic.

The sistergroup relationship of *Exechia* and *Exechiopsis* (Rindal & Søli 2006) is also supported in the ML and Bayesian analyses. However, this clade has less support in the molecular data than in the morphological data. The sistergroup relationship of *Anatella* to *Exechia* and *Exechiopsis* indicated in the ML and Bayesian trees is not in agreement with the morphological studies of Rindal & Søli (2006) who argued that *Anatella* belongs to a basal clade close to *Cordyla*.

The phylogenetic position of *Rymosia* within Exechiini remains unresolved. The close relationship of *Rymosia* and *Exechia* as suggested by Edwards (1925) and Tuomikoski (1966) is neither supported in the current analyses nor in Rindal & Søli (2006). We consider a close relationship of *Rymosia* to *Tarnania* as indicated in the ML tree and in the analysis of Kjærandsen (2006) more likely. Neither the present study nor Rindal & Søli (2006) recovered a monophyletic

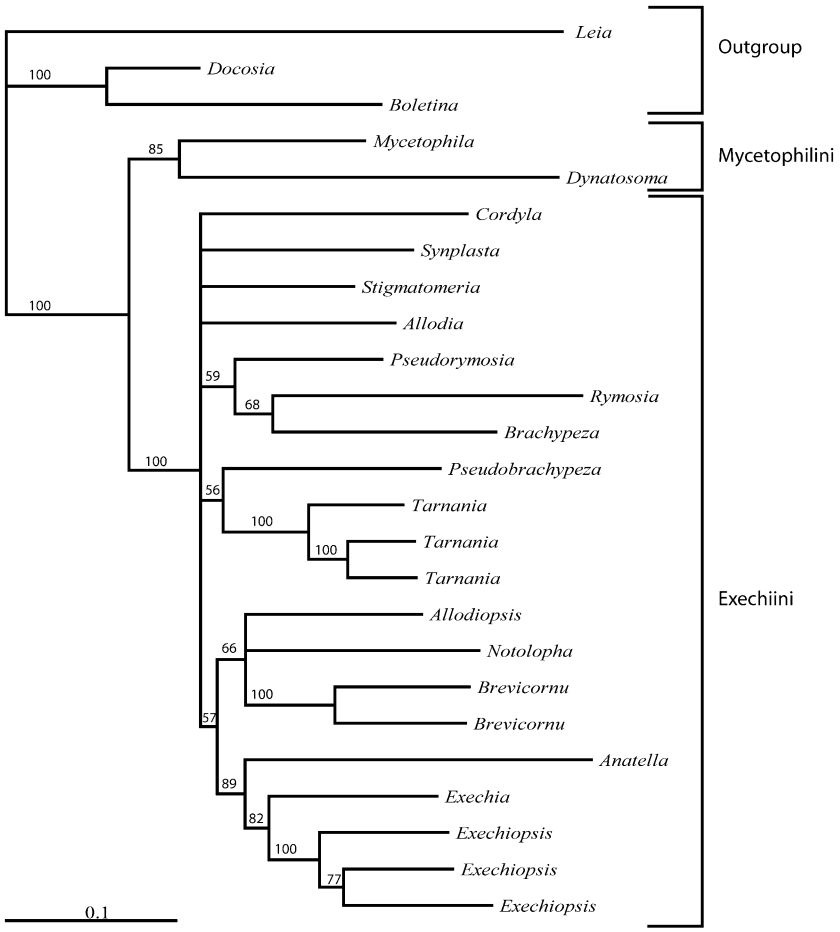

Fig. 3 Bayesian tree based on 4 million generations with the first 4000 generations discarded as burn-in. Posterior probabilities on branches. *Mycetophila* and *Dynatosoma* representing Mycetophilini and *Leia*, *Docosia* and *Boletina* representing Sciophilinae serve as outgroups.

Rymosia s.l. clade (see Table 1), which is in agreement with Tuomikoski (1966) and Kjærandsen (2006) who claimed that the *Rymosia* s.l. genus group is a polyphyletic assemblage of plesiomorphic genera.

The limited resolution of the molecular markers used in this study needs further consideration. There is some evidence (at least circumstantial) pointing towards Exechiini being a relatively young tribe. The oldest known fossil of any extant Exechiini genus, that is, *Anatella*, dates back to the Eocene; about the same age as the oldest extant species of Exechiini, *Synplasta crassicornis* (Meunier, 1904), known from the Eocene/Oligocene (Evenhuis 1994). In contrast, within the subfamily Sciophilinae, the genus *Synapha* was already present in the Cretaceous approximately 130 million years ago (Blagoderov & Martínez-Delclós 2001).

A comparison of the distribution of the Mycetophilinae genera adds further evidence for the relative young age of Exechiini. In a zoogeographical review of fungus gnats Bechev (2000) reported that only 10 of 16 (63%) Exechiini genera are represented in one or more of the Afrotropical, Neotropical or Australian regions, and only *Brevicornu* is found in all three regions. For Mycetophilini 12 of 14 (86%) genera occur in one or more of the Afrotropical, Neotropical or Australian regions, and six genera are found in all three regions. These regions represent parts of Gondwanaland and taxa represented in several of these regions might be considered old. If true, this interpretation indicates a younger age for the genera within Exechiini than for those within Mycetophilini.

As noted by Rindal & Søli (2006) there are better diagnostic characters for the genera within Mycetophilini than within Exechiini. The generic classification of fungus gnats has traditionally been strongly dependent on wing venation. However, characters relating to wing venation have been shown to be frequently homoplastic (Amorim & Rindal in press), and stable wing venation characters in taxa from the Holarctic region sometimes break down when representatives from tropical regions are included, as shown for some Exechiini genera by Kjærandsen (1994).

The limited resolution of the molecular markers used in this study mirrors the limited phylogenetic information

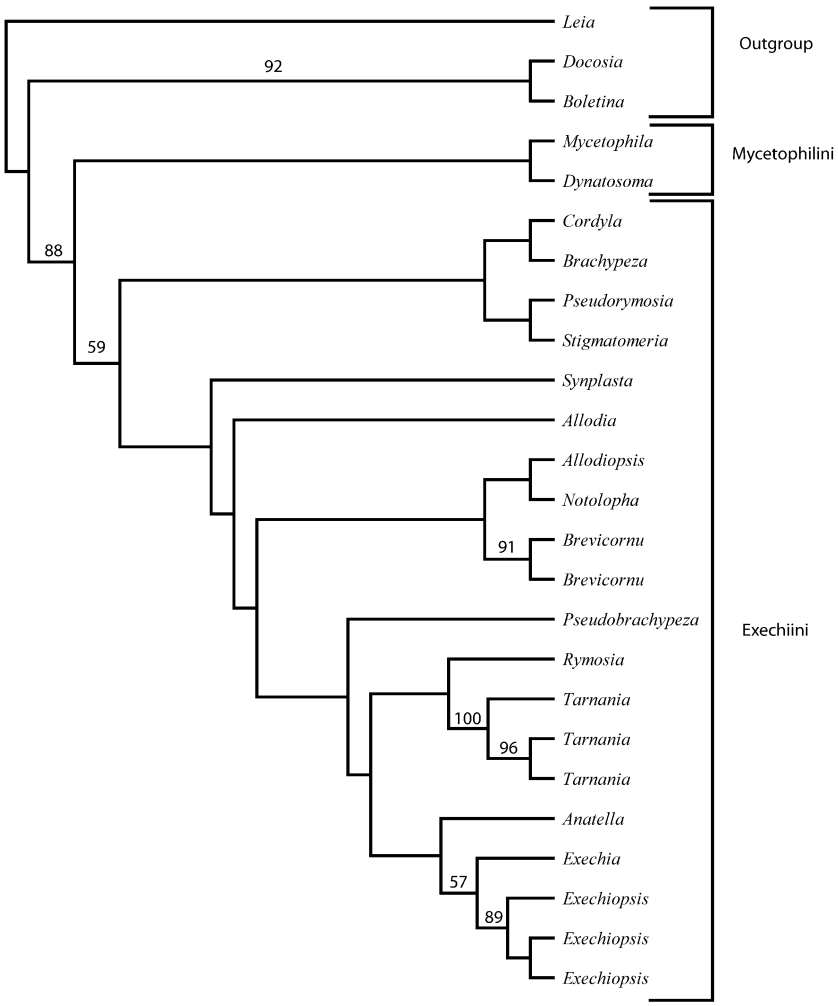

Fig. 4 Maximum likelihood tree, based on GTR + I + G, shape = 0.6832 and proportion of invariable sites = 0.6032. Bootstrap values are based on 100 replicates with 10 reps within each search. *Mycetophila* and *Dynatosoma* representing Mycetophilini and *Leia*, *Docosia* and *Boletina* representing Sciophilinae serve as outgroups.

provided by morphological characters. Interestingly when looking at the tree topology, the internal branches are very short (Fig. 3), at least when compared to the long-terminal branches. This could be an indication of a rapid radiation within Exechiini on the genus level, during a short evolutionary period. This makes a complete phylogenetic resolution of Exechiini difficult to achieve. A combined morphological and molecular study that includes more taxa and genes may solve this issue.

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