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Phylogenetic relationships of tachinid flies in subfamily Exoristinae (Tachinidae: Diptera) based on 28S rDNA and elongation factor-1α

JOHN O. STIREMAN III
Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, U.S.A.

Abstract. The phylogenetic relationships within the largest subfamily of Tachinidae, Exoristinae, were explored using nucleotide sequences of two genes (EF-1α and 28S rDNA). A total of fifty-five and forty-three taxa were represented in the analyses for each gene, respectively, representing forty-three genera. Neighbour joining, parsimony and maximum likelihood inference methods were employed to reconstruct phylogenetic relationships in separate analyses of each gene, and parsimony was used to analyse the combined dataset. Although certain taxa were highly mobile, phylogenetic reconstructions generally supported recent classification schemes based on reproductive habits and genitalia. Generally, the monophyly of Tachinidae and Exoristinae was supported. Tribes Winthemiini, Exoristini and Blondeliini were repeatedly constructed as monophyletic groups, with the former two clades often occupying a basal position among Exoristinae. Goniini and Eryciini generally clustered together as a derived clade within Exoristinae; however, they were never reconstructed as two distinct clades. These results suggest that the possession of unembryonated eggs is plesiomorphic within the subfamily and that there may have been multiple transitions between microtype and macrotype egg forms.

Introduction

Tachinidae is generally regarded as a relatively recent, actively radiating clade of parasitic flies (Crosskey, 1976). Some authorities believe it may be the most species-rich family of Diptera (Crosskey, 1980), although currently it is outnumbered in described species (*c.* 8200 according to Cantrell & Crosskey, 1989) by Tipulidae. Tachinids are found on all continents and most major islands, being absent from only very high latitude polar regions and some small oceanic islands (Wood, 1987). All species with known life histories are internal (endo-) parasitoids of other insects and arthropods. In this regard, they often play significant roles as natural enemies of herbivorous insects in ecological communities (Sheehan, 1994; Stireman, 2001). The taxonomic diversity of tachinids is matched by the immense range of hosts they attack (at least fourteen orders of arthropods, from sawflies to spiders; Ferar, 1987; Williams *et al.*, 1990; Eggleton & Belshaw, 1993), and the wide variety of mechanisms by which they attack them (O'Hara, 1985). These oviposition strategies include planidial larvae that seek out hosts, minute eggs that are ingested by hosts and hatch in the gut, and membranous eggs that hatch shortly after deposition on a host, near a host or on a host's food plant.

Despite the taxonomic, morphological and ecological diversity that tachinids display, the family has been subject to almost no modern analyses of the relationships between taxa (though see O'Hara, 1989). In this paper, I present a molecular-based phylogenetic reconstruction of one subfamily of Tachinidae, Exoristinae (= Goniinae of Sabrosky & Arnaud, 1965), with reference to patterns of host use and oviposition strategy.

Tachinidae

It is quite likely that Tachinidae represent a monophyletic group based on a number of characteristics, including the presence of a swollen subscutellum, the reduced mandibles and fusion of the labrum to the cephalopharyngeal skeleton in first-instar larvae, and most notably the parasitic lifestyle

Correspondence: Department of Ecology and Evolutionary Biology, 310 Dinwiddie Hall, Tulane University, New Orleans, LA 70118, U.S.A. E-mail: jstirema@tulane.edu

that all known species exhibit (Wood, 1987; Pape, 1992). However, relationships between taxa within Tachinidae are ambiguous. Four subfamilies are generally recognized: Phasiinae, Dexiinae, Tachininae and Exoristinae (Fig. 1). Of these subfamilies, only Dexiinae can clearly be defined by a synapomorphy (i.e. hinged aedeagus, Tschorsnig, 1985; Wood, 1987). Each of the remaining subfamilies can only be defined on the basis of a series of characters, often with many exceptions (Tschorsnig, 1985; Wood, 1987), and their monophyly is uncertain at best.

All authorities of the family appear to agree that the 'taxonomy of the family is difficult and confused' (Sabrosky & Arnaud, 1965), they are 'one of the most difficult families of Diptera in which to make practical identifications' (Crosskey, 1976) and there is 'no consistent classificatory scheme within the family' (Wood, 1987). The current state of tachinid systematics and taxonomy can best be summarized with this quote from Wood's (1987) comprehensive treatment of Nearctic Tachinidae:

> '... Descriptions of new taxa, even today, sometimes neglect to mention unique features, but instead, present further combinations or recombinations of the same limited suites of characters. Furthermore, these character suites are usually not correlated with internal structure, life cycle, larval characters, or other character states ... Such convergence, multiplied many times over because of the vast number of superficially similar species, has resulted in a long history of incorrect identifications and misunderstanding of relationships, a trend that still continues ... even the most recent classifications still rely mainly on arrangements of bristles and probably contain few monophyletic taxa.'

In the past two decades, there has been a dramatic improvement in our understanding of the composition of tachinid genera and the relationships of species therein (e.g. Tschorsnig, 1985; Wood, 1985, 1987; Barraclough, 1992; O'Hara, 1994; O'Hara & Wood, 1998), but there are still few rigorous phylogenetic treatments of the deeper relationships between genera, tribes or subfamilies (though see treatments of Siphonini in O'Hara, 1989; Andersen, 1996).

*Exoristinae (*sensu *Herting, 1984)*

The phylogenetic reconstruction performed in this study is focused on the tribal and generic relationships in subfamily Exoristinae (this name is considered to have priority over Goniinae according to Sabrosky, 1999). Exoristinae comprise about one-half of all described tachinid species and the vast majority of species that are regularly recorded as parasites of insect pests (Crosskey, 1976, 1980). Members of this subfamily attack hosts belonging to at least eight orders of insects, including adult Coleoptera, Phasmida and Blattaria (O'Hara, 1985), but the majority of species use larval Lepidoptera as hosts. Although Exoristinae (or Goniinae) is recognized by most authors, its limits are rather difficult to delineate, especially with respect to Tachininae. Exoristinae are generally defined using a combination of several character states, none of which is exclusive of all other Tachinidae (Crosskey, 1973). Perhaps the most consistent shared character of the group is the presence of a haired prosternum, but there are several tachinines that exhibit this trait (e.g. *Hystricia*; Wood, 1987), in some exoristine species it can be variable, e.g. *Chetogena* (personal observation), and it may be plesiomorphic. Exoristinae are probably the most taxonomically difficult group of Tachinidae due to the great diversity of morphologically similar species.

A provisional morphology-based phylogeny of Exoristinae and other tachinid subfamilies is shown in Fig. 1. This 'consensus' phylogeny is based on the classifications and discussions of Herting (1984), Crosskey (1976), Tschorsnig (1985) and Wood (1987). Some of these more recent classificatory schemes (e.g. Herting, 1984) place considerable emphasis on the oviposition strategy and genital morphology as well as on host associations, in contrast to the aforementioned reliance on the arrangement of setae. Two well defined tribes, Acemyiini (which attack Orthoptera) and Siphonini, have been placed alternatively in Exoristinae or Tachininae by various authors (e.g. Herting, 1960; Sabrosky & Arnaud, 1965, respectively). The systematic position of Acemyini remains uncertain (Fig. 1), but most modern authors now place Siphonini in Tachininae (O'Hara, 1989; Andersen, 1996). The 'microtype' Exoristinae, which attack hosts by laying minute eggs on the host's food plant that is subsequently ingested, are presumed to form a monophyletic group based on this remarkable oviposition habit (Goniini). These forms were previously (Townsend, 1936–1941; Sabrosky & Arnaud, 1965) distributed among several mixed macrotype-microtype tribes due to their diverse appearance, and their relationships are not well understood. The composition of and relationships between a large assemblage of macrotype genera, Eryciini, are not well agreed upon and are left largely unresolved in the provisional tree. Blondeliini appear to form a clade based on host use (a predominance of Chrysomelidae and other coleopteran hosts) and a number of chaetotaxic and wing venation characteristics, including a small prealar bristle relative to the first postsutural dorsocentral bristle (probably plesiomorphic), divergent subapical bristles, and R_{4+5} and M ending near the wing tip, but the exact composition is still rather uncertain (Wood, 1985). Exoristini and Winthemiini, which tend to be rather well defined and relatively consistent in composition between authors, are placed basally due to their habit of laying unincubated eggs, which is thought to be the plesiomorphic state for the family (Wood, 1987).

In the present study, I evaluated the current understanding of relationships between tribes and genera within Exoristinae based upon morphological characters (Herting, 1984; Tschorsnig, 1985; Wood, 1987). By employing a modern numerical phylogenetic analysis using molecular sequence data, I hope to offer an alternative to the traditional morphological characters used in tachinid systematics and provide independent confirmation or refutation

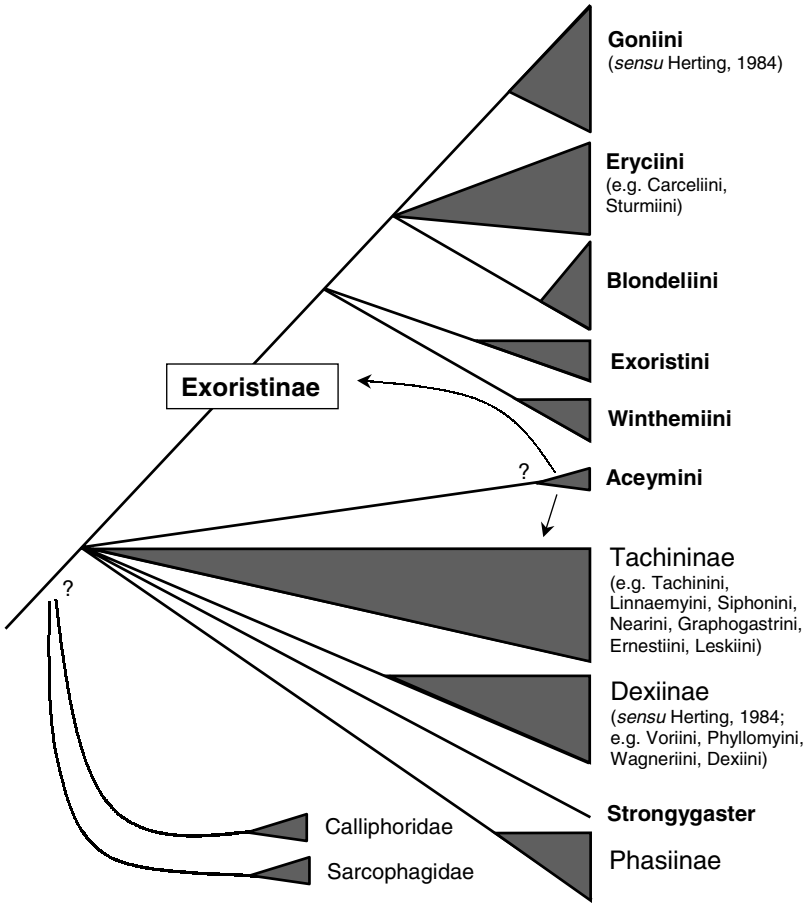

Fig. 1. A provisional phylogeny of Tachinidae and Exoristinae based on taxonomic classification schemes and discussion of Tschorsnig (1985), Herting (1960, 1984) and Wood (1987).

of proposed relationships. The resulting phylogenetic hypotheses are used to assess the monophyly and composition of this subfamily, to evaluate the phylogenetic utility of current classification systems and to examine species relationships within certain genera. The phylogenetic reconstructions are further used as a framework to examine the evolution of host associations and oviposition strategies within Tachinidae and evaluate their utility as indicators of the evolutionary relationships within this diverse and actively radiating clade.

Materials and methods

Taxa

Fifty-eight operational taxonomic units are represented in the current study (Table 1). Two of these are Sarcophagidae (*Metoposarcophaga* sp. and *Senotainia* sp.), the presumed sister group to Tachinidae (see Fig. 1; Pape, 1992, although see McAlpine, 1989), one is a calliphorid (*Phormia regina* Meigen), which represents another family of Oestroidea, and the rest are tachinids. The tachinid taxa represent forty genera, thirty-one to thirty-three of which are Exoristinae, along with two (or three) Dexiinae, two Phasiinae and three to four Tachininae (depending on the classification scheme), which are included to help determine the monophyly of Exoristinae (Table 1). This spans all of the currently recognized subfamilies of Tachinidae (Wood, 1987). Within Exoristinae, eleven of eighteen tribes of Sabrosky & Arnaud (1965) are represented, and all tribes recognized by Herting (1984) and Tschorsnig (1985), except Ethillini, are represented.

In some cases, multiple species of the same genus and multiple specimens of the same species are included in the analyses. The former are included to resolve relationships within certain genera and to aid in the correct placement of these genera. The latter represent species that were originally sequenced multiply due to availability and as secondary confirmations of sequence data. They are included in the analyses because they may aid in the correct placement of certain species, and they can be used to examine relative levels of divergence at different taxonomic levels.

Fifty-two of the taxa were collected or reared from hosts in southern Arizona from 1996 to 1998 (see Table 1), and were identified at least to genus using Wood's (1987) key to

Table 1. Included taxa and sequences, with collection date and locality or sources. Tribal classification follows Herting (1984), Wood (1985, Blondeliini) and Andersen (1988, *Phytomyptera*).

Classification	Species	EF-1a	28S	Date	Locality
Exoristinae					
Acemyini	*Ceracia dentata* (Coquillett)	AF364351	AF366657	16-VIII-98	USA: AZ, White Mts
Blondeliini	*Blondelia eufitchiae* (Townsend)	AF364349	AF366655	9-VIII-98	USA: AZ, Huachuca Mts
Blondeliini	*Cryptomeigenia* sp.n.	AF364358	AF366662	14-VIII-96	USA: AZ, Huachuca Mts
Blondeliini	*Cryptomeigenia* sp. 2	AF364359	–	1997?	CAN: ONT
Blondeliini	*Eucelatoria armigera* (Coquillett)	AF364362	AF366666	17-VI-97	USA: AZ, Santa Catalina Mts
Blondeliini	*Eucelatoria dimmocki* (Aldrich)	AF364363	–	23-VIII-98	USA: AZ, Rincon Mts
Blondeliini	*Myiopharus doryphorae* (Riley) (#1)	AF364379	–	14-VIII-98	USA: AZ, Santa Rita Mts
Blondeliini	*Myiopharus doryphorae* (Riley) (#2)	AF364380	AF366680	12-IX-98	USA: AZ, Arivaca
Blondeliini	*Myiopharus doryphorae* (Riley) (#3)	AF364381	–	28-VIII-98	USA: AZ, Santa Rita Mts
Blondeliini	*Myiopharus moestus* (Wulp)	AF364382	AF366661	17-VII-98	USA: AZ, Santa Rita Mts
Blondeliini	*Phyllophilopsis* sp.	AF364389	AF366686	1997?	CAN: ONT
Blondeliini	*Vibrissina aurifrons* (Curran)	AF364396	AF366690	9-VIII-98	USA: AZ, Huachuca Mts
Blondeliini	*Zaira arrisor* (Reinhard)	AF364398	–	17-VII-98	USA: AZ, Santa Rita Mts
Eryciini	*Ametadoria harrisinae* (Coquillett)	AF364345	AF366651	2-IX-98	USA: AZ, Pinaleno Mts
Eryciini	*Aplomya theclarum* (Scudder)	AF364346	AF366652	8-X-98	USA: AZ, Santa Catalina Mts
Eryciini	*Carcelia reclinata* (Aldrich & Webber)	AF364350	AF366656	26-VIII-98	USA: AZ, Rincon Mts
Eryciini	*Chrysoexorista* sp.	AF364357	–	18-3-99	USA: AZ, Santa Rita Mts
Eryciini	*Drino incompta* (Wulp)	AF364361	AF366665	5-VIII-97	USA: AZ, Santa Catalina Mts
Eryciini	*Frontiniella parancilla* Townsend	AF364365	AF366669	6-IX-98	USA: AZ, Santa Rita Mts
Eryciini	*Frontiniella regilla* (Reinhard)	AF364366	AF366664	26-VII-98	USA: AZ, Santa Rita Mts
Eryciini	*Lespesia archippivora* (Riley)	AF364372	AF366676	14-III-98	USA: AZ, Rincon Mts
Eryciini	*Lespesia cuculliae* (Webber)	AF364373	–	8-VIII-99	USA: AZ, Rincon Mts
Eryciini	*Lespesia cuculliae* (Webber) (#2)	AF364374	–	19-VIII-97	USA: AZ, Rincon Mts
Eryciini	*Lespesia datanarum* (Townsend)	AF364375	–	6-IX-98	USA: AZ, Atascosa Mts
Eryciini	*Patelloa facialis* (Coquillett)	AF364387	AF366683	18-III-99	USA: AZ, Santa Rita Mts
Eryciini	*Siphosturmia* sp.	AF364394	AF366688	1997	CAN: ONT
Eryciini	*Zizyphomyia crescentis* (Reinhard)	AF364399	AF366667	19-VIII-97	USA: AZ, Santa Rita Mts
Eryciini*	*Heliodorus cochisensis* (Reinhard)	AF364368	AF366673	18-III-99	USA: AZ, Santa Rita Mts
Exoristiini	*Austrophorocera* sp.	AF364347	AF366653	1997?	CAN: ONT
Exoristiini	*Chetogena edwardsi* (Williston)	AF364353	–	17-IV-98	USA: AZ, Rincon Mts

Exoristiini	*Chetogena edwardsi* (Williston) (#2)	AF364354	–	3-V-98	USA: AZ, Rincon Mts
Exoristiini	*Chetogena parvipalpis* (Wulp)	AF364355	AF366672	16-IV- 1998	USA: AZ, Santa Catalina Mts
Exoristiini	*Chetogena tachinomoides* (Townsend)	AF364356	AF366660	17-IV-98	USA: AZ, Rincon Mts
Exoristiini	*Exorista mella* (Walker)	AF364364	AF366668	28-IV-97	USA: AZ, Rincon Mts
Goniini	*Chaetogaedia monticola* (Bigot)	AF364352	AF366659	29-IX-96	USA: AZ, Santa Rita Mts
Goniini	*Gaediopsis setosa* Coquillett	–	AF366670	22-VII-98	USA: AZ, Huachuca Mts
Goniini	*Gonia brevipullvilli* Tothill	AF364367	AF366671	27-III-97	USA: AZ, Santa Catalina Mts
Goniini	*Hyphantrophaga hyphantriae* (Townsend)	AF364369	–	11-X-98	USA: AZ, Patagonia
Goniini	*Hyphantrophaga hyphantriae* (Townsend) (#2)	–	AF366650	10-VIII-97	USA: AZ, Santa Rita Mts
Goniini	*Hyphantrophaga virilis* (Aldrich & Webber)	AF364370	–	9-VIII-98	USA: AZ, Huachuca Mts
Goniini	*Leschenaultia adusta* (Loew)	AF364371	AF366675	18-III-99	USA: AZ, Santa Rita Mts
Goniini	*Mystacella frioensis* (Reinhard)	AF364383	AF366681	16-IV-99	USA: AZ, Santa Rita Mts
Goniini	*Pseudochaeta siminina* Reinhard	AF364391	AF366687	28-VI-98	USA: AZ, Patagonia Mts
Winthemiini	*Nemorilla pyste* (Walker)	AF364384	–	17-VI-99	USA: AZ, Santa Catalina Mts
Winthemiini	*Orasturmia vallicola* Reinhard	AF364385	AF366674	16-VII-98	USA: AZ, Santa Rita Mts
Winthemiini	*Orasturmia vallicola* Reinhard (#2)	AF364386	AF366682	12-IX-98	USA: AZ, Arivaca
Winthemiini	*Winthemia rufonotata* (Bigot)	AF364397	AF366691	22-VII-98	USA: AZ, Santa Rita Mts
Masiphyini	*Masiphya townsendi* Aldrich	AF364376	AF366677	18-III-99	USA: AZ, Santa Rita Mts
Dexiinae					
Phyllomyini	*Blepharomyia* sp.	AF364348	AF366654	1997?	CAN: ONT
Phyllomyini	*Muscopteryx* sp.	AF364378	AF366679	1997?	CAN: ONT
Tachininae					
Graphogastrini	*Phytomyptera longicornis* (Coquillett)	AF364390	–	28-VIII-98	USA: AZ, Santa Rita Mts
Siphonini	*Siphona* sp.n.	AF364393	AF366658	18-IV-99	USA: AZ, Santa Rita Mts
Tachinini	*Peleteria biangulata* Curran	AF364388	AF366684	22-IX-97	USA: AZ, Salt River
Phasiinae					
Tichopodiini	*Trichopoda indivisa* Townsend	AF364395	AF366689	28-VI-98	USA: AZ, Patagonia Mts
Cylindromyiini	*Cylindromyia euchenor* (Walker)	AF364360	AF366663	14-VIII-98	USA: AZ, Santa Rita Mts
Sarcophagidae					
	Metoposarcophaga sp.	AF364377	AF366678	18-IX-98	USA: AZ, Santa Rita Mts
	Semotainia sp.	AF364392	–	8-VI-98	USA: UT; San Rafael Swell
Calliphoridae					
	Phormia regina Meigen	–	AF366685	12-II-99	USA: AZ, Rincon Mts

*Based on O'Hara & Wood (unpublished classification); however, the placement of this genus is uncertain.

tachinid genera, the University of Arizona Insect Collection (UAIC) and the Canadian National Collection of insects (CNC). Identifications were confirmed in part by N. E. Woodley (SEL, USNM) and J. E. O'Hara (CNC). The other six taxa were donated by J. K. Moulton (University of Arizona), and were previously identified by D. M. Wood. Voucher specimens are deposited in the University of Arizona Insect Collection (UAIC).

Data collection

DNA was extracted from specimens frozen after collection, specimens in alcohol (70–95% ethanol) and a few pinned-dried specimens. Extractions were done following a modified protocol of Bender *et al.* (1983), in which individual flies were frozen in liquid N_2, pulverized with a pestle and incubated in a homogenization buffer (100 µl; 0.1 M NaCl, 0.2 M sucrose, 0.1 M Tris/HCL, 0.05 M EDTA, 0.5% SDS at pH 9.2). A salt solution (8 M KOH) was used to precipitate the DNA, which was then purified with a series of ethanol washes, dried and resuspended in low TE buffer (pH 8; 10 mM Tris, 0.1 mM EDTA). For most taxa, only the thorax was pulverized in this way; all other body parts were preserved in 70% ethanol. Occasionally, when the tachinid was very small, or was represented by multiple pinned vouchers in my personal collection, the entire flies were pulverized.

PCR amplification

For the nuclear protein coding gene elongation factor-1-alpha (EF-1α), the primers EFs175 (5′: GGAAATGGGAAAAGGCTCCTTCAAGTAYGCYTGGG) and EF2 (3′: ATGTGAGCAGTGTGGCAATCAA) were introduced with the genomic DNA into 10 µl polymerase chain reactions. These reactions consisted of 10 mM Tris-HCL (pH 8.3), 75 mM KCL, 2.0 mM $MgCl_2$, 2 pM of each primer, 1 mM dNTPs, 1 unit Taq polymerase (GibcoBRL) and approximately 5–10 ng genomic DNA. Double-stranded PCR reactions were conducted in a DNA thermocycler (PerkinElmer, Boston, U.S.A) using the following cycling parameters: first the reaction was heated to 94 °C for 2 min, then 35 cycles of 94 °C 1 min, 50 °C 1 min, 72 °C 1 min and finally 4 °C until the product was examined on an agarose gel. This resulted in the amplification of a fragment approximately 900 bp long. The same reactions were performed again at 100–150 µl volumes for sequencing with reagents scaled accordingly. PCR product was purified either using Qiagen© (Valencia, CA, U.S.A) spin columns, or, when alternate products were co-amplified, by running the products out on a polyacrylamide gel and cutting out the appropriate sized bands. Sequencing was accomplished by the Arizona Research Laboratory's automated sequencing facility located at the University of Arizona. Both 5′ and 3′ strands were sequenced to correct for degradation at the ends of sequence electropherograms.

Sequence for 28S ribosomal DNA was gathered in a similar manner using the conserved primers, 28y (5′: CAAGGATTCCCTTAGTAGCG) and 28b (3′: TCGGAAGGAACCAGCTACTA). These primers span approximately 1000 bp including the variable D1, D2 and D3 regions of the 28S subunit, as well as the intervening core and other more conserved regions (Hillis & Dixon, 1991; Schnare *et al.*, 1996). The PCR reactions for this gene consisted of 20 mM Tris-HCl (pH 8.3), 50 mM KCl, 3.0 mM $MgCl_2$, 2 pM of each primer, 1 mM dNTPs, 1 unit Taq polymerase (GibcoBRL) and approximately 5–10 ng genomic DNA. Reactions were conducted as above using the following cycling parameters: 94 °C for 2 min 30 s, 35 cycles of 94 °C 30 s, 55 °C 45 s, 72 °C 45 s and then 4 °C. Two pooled 50 µl reactions were used for sequencing.

Alignment

EF-1α sequences acquired from the automated sequencing facility in the form of electropherograms were aligned and examined thoroughly using the program Sequence Navigator© (Applied Biosystems, Foster City, CA, U.S.A) and corrected when errors were evident. Alignment for this fragment was unambiguous because very little protein evolution had occurred among the taxa examined and there was no evidence of insertions or deletions. Alignments were made against a previously published sequence for this gene from *Drosophila melanogaster* Meigen (Hovemann *et al.*, 1988) drawn from GenBank© (accession no. X06869). MacClade 4.0b2 (Maddison & Maddison, 2000) was used to translate the sequences to ensure that there were no stop codons present and to designate base positions.

The 28S rDNA sequences were received in a similar form and also aligned with reference to a previously published *D. melanogaster* sequence for this region (Tautz *et al.*, 1988; GenBank accession nos M21017, M29800) in Sequence Navigator. Alignments of the sequences, which contained numerous insertions and deletions across taxa, were constructed in two primary ways. First, eleven alignments were produced using a series of gap opening and gap extension penalties with the aid of the alignment program ClustalW 1.6 (Thompson *et al.*, 1996; penalty sets 30 : 5, 20 : 5, 15 : 7, 15 : 5, 10 : 3, 10 : 2, 7 : 3, 7 : 2, 5 : 2, 5 : 1, 3 : 1). Taxa were reordered randomly before each alignment was performed. All of these alignments together were concatenated into one dataset that was used in series of subsequent analyses (see below). This procedure, known as 'Elision' alignment (Wheeler *et al.*, 1995), weights character changes in relatively conserved regions more highly than those in areas that are more sensitive to alignment options.

To construct a manual alignment, simple neighbour joining trees were inferred for each of the above eleven alignments in PAUP* (Swofford, 1999), the taxa were reordered according to the resulting tree and the alignment was evaluated in MacClade 4.0b2 (Maddison & Maddison, 2000) with the taxon names hidden. The alignments were rated by summing the number of obvious blocks of misalignment

of five or more bases, whereby one taxon was shifted relative to the others, and by the number of columns of seemingly arbitrary nucleotides (*sensu* Maddison *et al*., 1999). Of the alignments examined, the 5 : 2 alignment scored lowest (best), though no alignment was found that did not exhibit these problems in at least some regions. This alignment was further refined by eye in MacClade 4.0b2 to correct problems identified above and, with reference to the published secondary structure of 28S rRNA in *D. melanogaster* (Rousset *et al*., 1991; Schnare *et al*., 1996), to assess whether the length-variable regions corresponded to expansion domains. I used this secondary structure to categorize all positions in the fragment of 28S rDNA amplified according to their structural position (Table 2). Extremely variable regions of insertions and deletions that could not be aligned were excluded in subsequent analyses.

Analysis

The reconstruction of the phylogenetic relationships between the taxa included in this study was accomplished using several inference methods, each with a number of variants. These are discussed below in reference to dataset (EF-1α or 28S rDNA) and inference method. All data were analysed in the form of NEXUS files and all analyses were performed with PAUP 3.11 (Swofford, 1993) or PAUP*4b2 (Swofford, 1999).

EF-1a. Uncorrected 'p' distances of independent taxon pairs (EF-1α, 25 pairs; 28S: 20 pairs) were plotted against maximum likelihood estimated distances (Modeltest analysis, see below) to examine whether variable sites appear to be saturated in the EF-1α and 28S rDNA datasets. As a conservative measure, uncorrected distances for EF-1α are estimated only using the highly variable third codon positions. Mean values of both of these distance measures were also plotted against taxonomic level as an additional measure of saturation. Independent taxon pairs were arbitrarily estimated on trees inferred by parsimony for EF-1α and a parsimony analysis using the Elision alignment (minus gaps10, see below) for 28S.

The EF-1α sequence was obtained for fifty-five taxa (Table 1). All analyses using this dataset were conducted using nucleotide data.

Table 2. The positions in the ClustalW+ manual alignment categorized according to their structural position in the 28S ribosomal RNA molecule (based on Schnare *et al*., 1996), with maximum likelihood estimated rates.

D1, D2, D3 variable regions

Stem-internal Est. rate: 1.084	Stem-external 1.189	Loop-internal 1.692	Loop-external 1.673
32–35, 38–47, 52–58, 139–156, 318–322, 327–333, 341–349, 355–359, 394–398, 405–413, 420–425, 428–436, 451–457, 464–467, 482–487, 516–521, 525–528, 535–541, 544–552, 564–572, 643–652, 659–664, 671–677, 684–688, 790–794, 797–799, 832–835, 839–843	1–8, 17–26, 65–74, 80–83, 91–94, 110–112, 120–122, 127–135, 364–372, 380–388, 491–498, 503–511, 575–580, 588–594, 602–605, 610–612, 616–618, 629–632, 802–809, 823–829, 845–853, 859–863, 898–912	27–31, 36, 37, 48–51, 59–64, 136–138, 323–326, 334–340, 350–354, 360–636, 389–393, 399–404, 414–419, 426, 427, 437–450, 458–463, 468–481, 488–490, 512–515, 522–524, 529–534, 542, 543, 553, 563, 573, 574, 595–601, 606–609, 619, 628, 633–642, 653–658, 665–670, 678–683, 795, 796, 800, 801, 830, 831, 836–838, 844, 854–858, 910	9–16, 75–79, 84–90, 95–109, 113–119, 123–126, 373–379, 499–502, 581–587, 613–615, 810–822, 864–897

Core and semi-conserved regions

Stem-internal Est. rate: 0.083	Stem-external 0.167	Loop-internal 0.603	Loop-external 0.390
211–214, 220–232, 261, 296, 302–311, 707–712, 717–729, 917–928, 932, 933, 939–944, 948–959, 1025–1037, 1057–1062	164–170, 179–185, 191–195, 204–208, 248–249, 254, 255, 732–740, 752–760, 961–964, 968–974, 979–985, 990–993, 996–1006, 1014–1022, 1040, 1041, 1049, 1050	157–163, 186–190, 209, 210, 215–219, 256–260, 262–270, 283–295, 297–301, 312–317, 689–706, 713–716, 730–731, 761–789, 913–916, 929–931, 934–938, 945–947, 960, 965–967, 986–989, 994, 995, 1001, 1004, 1023, 1024, 1026, 1038, 1039, 1051–1056	171–178, 196–203, 233–247, 250, 251–253, 741–751, 975–978, 1007–1014, 1042–1049

Two neighbour joining tree searches of the EF-1α nucleotide dataset were conducted in PAUP* using the uncorrected 'p' distance measure and an HKY85 model with rate variation (0% invariant, default $\Gamma = 0.5$; Hasegawa *et al*., 1985), respectively. The two sarcophagid taxa were designated as outgroup taxa.

An initial heuristic search using maximum parsimony (MP) was performed with 1000 replicates on the aligned dataset with equal character weighting and unordered character states (PAUP options including heuristic search, starting tree (start) = stepwise addition, addition sequence = random, branch swapping (swap) = TBR). The two Sarcophagidae (*Metoposarcophaga* sp., and a miltogramine, *Senotainia* sp.) were designated as outgroup taxa. Although the focus of these analyses was Exoristinae, tachinids belonging to the other subfamilies (especially Tachininae) were not designated as outgroup taxa because the monophyly of Exoristinae is questionable. The robustness of each branch was then evaluated by performing 1000 bootstrap replicates (ten addition replicates each with same PAUP options as the initial search except swap = SPR) and examining in how many replicates each clade was present. In addition, decay indices were calculated for each node.

An additional MP analysis was performed identical to that described above, except that weights were applied to codon positions (12, 22, 1). These weights are derived from the probability of change for each position estimated from a maximum likelihood analysis (described below).

In addition to these analyses, in which the goal was a general picture of the relationships among genera in Exoristinae, parallel analyses were conducted in the same manner with the exception that the species considered to be 'microtype' egg layers were constrained to be monophyletic. The presence of microtype egg was determined by dissection, literature references (e.g. Townsend, 1936–1941; Sabrosky & Arnaud, 1965; Wood, 1987) and personal communication from D. M. Wood. The most parsimonious trees, and trees with the highest likelihood found with this restrictive assumption, were compared to the unconstrained trees. As a conservative measure, *Heliodorus cochisenesis* was considered to possess microtype eggs (see Discussion).

Finally, a MP search was conducted in which three problematic taxa were omitted (hsearch: start = stepwise, replicates = 1000, swap = TBR). These three taxa, *Drino*, *Peleteria* and *Phyllophilopsis*, were among the most mobile taxa among analyses and their omission may allow better resolution of the relationships among the remaining taxa.

Maximum likelihood analyses were employed to allow a more complex model of nucleotide evolution, including substitution rates and probabilities of nucleotide change, to be developed and used to infer the most likely phylogenetic relationships among taxa. These parameters were estimated based on preliminary trees inferred by maximum likelihood (ML) using a succession of ever more complex models of rate variation among sites and character change probabilities. An alternative evaluation of models of nucleotide change was conducted using the program Modeltest (Posada & Crandall, 1998) that compares the likelihood of ever more complex models on an initial neighbour joining tree.

An initial tree was constructed using a simple model of evolution (i.e. HKY85: base frequencies and transition vs transversion ratios, tv/ts, estimated from the data, all sites assumed to evolve at equal rates; Hasegawa *et al*., 1985) with an abbreviated search strategy (hsearch, nni branch swapping, three replicates). The resulting tree, along with the estimated tv/ts was then used as a starting tree for a subsequent more powerful search (hsearch, spr branch swapping). This second resulting tree was then used to evaluate the likelihood of a series of models from the very simple (Jukes–Cantor + no rate variation; Swofford, 1999) to the most complex (General Time Reversible + rate variation by codon position, GTR + rv; Yang, 1994). The distribution of likelihood scores for the models evaluated is given Table 3 (expressed as –ln likelihood). As expected, the most complex model GTR + rv resulted in the highest likelihood score (= lowest –ln L).

A series of likelihood ratio tests were performed for each successive pair of models to select the least complex but most powerful model (Swofford *et al*., 1996; Sullivan & Swofford, 1997). The preliminary ML tree was significantly more likely under the GTR + rv model than any other model ($\chi^2 = 178$–3428, d.f. = 1–9 depending on which other model GTR + rv is compared to, and $P < 0.001$ for all comparisons). However, it should be noted that the application of a likelihood ratio test to compare ML models

Table 3. The likelihood scores (−ln likelihood) of the initial maximum likelihood tree for the EF-1α and 28S datasets (see text) under eight models of nucleotide evolution. Values reported are the increase in −ln likelihood relative to the most complex model (EF-1α, GTR + rate variation by codon position, −ln L = 9472.2; 28S, GTR + %I + Γ, −ln L = 4957.5). F_{81} = (Felsenstein, 1981), HKY = (Hasegawa *et al*., 1985), GTR = (Yang, 1994).

Dataset	Model of rate variation	Model of nucleotide substitution			
		Jukes–Cantor	F81	HKY85	GTR
EF-1α	Equal rates	+1714	+1682	+1205	+988
EF-1α	rates by codon position	+611	+576	+89	0
28S	Equal rates	+665	+546	+449	+416
28S	%I + Γ	+259	+134	+35	0

on phylogenetic trees (as in Sullivan & Swofford, 1997; Maddison *et al*., 1999) is controversial and the assumptions of the test may not be strictly met (Goldman, 1993). Given the vast difference between the calculated statistic and the critical values, it is unlikely that these criticisms affect the conclusion that the most complex model GTR + rv is significantly more likely than any other examined. The parameters estimated using the GTR + rv model were then used in subsequent more comprehensive ML analyses that utilized either the initial ML tree on which the model was based or the most parsimonious tree (MPT) with the highest likelihood as starting trees (hsearch, swap = TBR, basefreqs = estimated, rate matrix = A-C:2.94, A-G:5.97, A-T:2.04, C-G:0.997, C-T:12.32; siterates:pos1 : 0.2145, pos2 : 0.1209, pos3 : 2.6616). Due to the large size of this dataset, only one or two ML replicates were conducted in each analysis.

The Modeltest evaluation of ML models using the hierarchical likelihood ratio test criterion (essentially performed as above, though a larger number of models were tested; see Posada & Crandall, 1998) selected a TrN + I + Γ (Tamura & Nei, 1993) model with the following parameters (basefreqs = 0.3187, 0.2235, 0.1800, 0.2777; rate matrix = A-C:1, A-G:4.4087, A-T:1, C-G:1, C-T:7.2088, G-T:1; I = 0.5998, Γ = 1.0381). These parameters were used in two subsequent ML searches, one unconstrained and one with the microtype Goniini constrained to be monophyletic (hsearch, start = NJ, swap = TBR).

Finally, rate values under the GTR + rv model were translated according to codon position into weights by taking their inverse and scaling them so that the lowest weight equals 1.0. This was done to transform the rate values into weights that reflect how likely characters are to change and are relatively simple for PAUP* to incorporate into a parsimony analysis. This transformation function is relatively arbitrary. The resulting values, rounded to the nearest whole number were: position1 = 12, position2 = 22 and position3 = 1. These weights were then used in a less computationally demanding parsimony search (see above).

Several of the analyses described above resulted in trees that indicated relationships strongly contradictory to traditional classifications based on morphology and notions of how reproductive and ovipositional traits have evolved in Tachinidae (e.g. Herting, 1984; Wood, 1987). These included a polyphyletic Phasiinae (*Trichopoda* and *Cylindromyia*), a poly- or paraphyletic origin of taxa possessing microtype eggs (Goniini), placement of a *Peleteria-Cylindromyia* clade in various positions within Exoristinae, and a basal placement of *Drino* within Exoristinae (usually in some association with the previous clade).

To address whether these reconstructed relationships were due to long branch attraction or some other effect of tree shape, a parametric bootstrapping simulation test was performed (Huelsenbeck *et al*., 1995; Huelsenbeck, 1997). The null hypothesis was that recent morphological classifications based on reproductive characters reflect the true phylogeny, i.e. Phasiinae, Exoristinae and Goniini are monophyletic and *Drino* is a member of the Goniini-Eryciini, G-E, crown group. The test addresses the question: if the null hypothesis is true, how likely is the incorrect inference that these various taxa are polyphyletic or that *Drino* is basal to the G-E crown group? To evaluate this question the null hypothesis was expanded to include details concerning relationships, the lengths of branches and a model of molecular evolution inferred from the sequence data (Huelsenbeck *et al*., 1996a).

A model tree was selected by conducting a ML search using the parameters estimated from Modeltest (see above for parameters; TrN + I + Γ, start = NJ, swap = TBR), using a constraint tree in which all tribes and subfamilies (of Herting, 1984) are constrained to be monophyletic (see Fig. 1). One of the nine resulting trees (−lnL = 9213.93) with associated branch lengths was randomly chosen to serve as the model tree. One hundred simulated data matrices were then constructed by evolving characters up this tree under the inferred model of nucleotide evolution using the program Seq-Gen (Rambaut & Grassly, 1997). Each of these data matrices was subsequently used to infer trees using maximum parsimony (hsearch: start = stepwise, swap = SPR, replicates = 100). Strict consensus trees were constructed for each of the individual searches, and a majority rule consensus of these consensus trees was constructed to examine the frequency of various clades. If none of these trees match the observed trees, then the null hypothesis can be rejected. However, if the trees match in more than 5% of simulations with respect to the relationships under consideration, the model tree cannot be rejected.

28S rDNA. The analysis of the 28S rDNA gene was limited to a subset of the taxa (forty-three of fifty-seven) included in the EF-1α dataset due to difficulties encountered in gathering reliable sequence data for all taxa (Table 1). However, thirty-six of the total forty tachinid genera are represented, including *Gaediopsis* and the outgroup taxon *Phormia regina*, not present in the EF-1α dataset.

Two neighbour joining (NJ) analyses were conducted with the 28S dataset using the following distance measures: uncorrected p minus seventy-eight unalignable characters (= var2) and GTR + I + Γ -var2 (I = 0.56, derived from the estimated proportion of invariant characters from ML analyses (see below), and Γ = 0.5, as a default).

An initial unweighted MP analysis was conducted on the 28S rDNA dataset (hsearch, start = stepwise (random), swap = TBR, 1000 replicates, save all minimal trees) with *Metoposarcophaga* and *Phormia* defined as the outgroup. This and further analyses were conducted with the unalignable expansion domains excluded (five regions comprising a total of seventy-eight nucleotides). To maximize the number of aligned sites to be included, only the most questionably aligned nucleotide sites were removed. Gaps were coded as 'missing data' in most analyses, but I also performed an analysis employing the gaps equal fifth base option in PAUP. The robustness of nodes and branches of trees derived from the manual alignment was evaluated with bootstrap analysis and the calculation of decay indices as outlined for the EF-1α dataset (see above).

Analyses of the 10 840 character composite Elision alignment were conducted with gap runs of greater than ten nucleotide positions excluded (506 characters). Preliminary bootstrap analysis of a duplicated Elision dataset indicated that bootstrap values were artificially elevated over an unduplicated dataset. When 500 bootstrap replicates were calculated for both a single 5 : 2 28S alignment and for a 5 : 2 alignment duplicated ten times, bootstrap values were consistently higher for clades in the latter analysis. Therefore, a full bootstrap analysis was not performed for the Elision dataset.

In addition to these analyses, a MP was performed in which weights were assigned to characters based on their structural position in the 28S rRNA molecule (as described above). These weights were derived from probabilities of change estimated from maximum likelihood analyses (see below). The manually corrected (5 : 2) ClustalW alignment, with the unalignable regions removed (seventy-eight characters), was used as the dataset for this analysis.

Maximum likelihood analyses were performed on the 28S rDNA dataset using the same general procedure as for the EF-1α dataset, with an initial tree being reconstructed with rather cursory searches (nni, then spr branch swapping) and simple HKY85 models of nucleotide change (ts/tv ratio and base frequencies = estimated). To initially account for rate variation among sites, the proportion of invariant sites from the data on this initial tree were estimated, and a gamma distribution was used to estimate rates of change for the remaining characters (%I = estimated, Γ = estimated). As in the EF-1α analysis, the most complex model (GTR + %I + Γ) resulted in the lowest −ln L (Table 3; $\chi^2 = 70$–1330, d.f. = 1–7, $P < 0.001$ for all comparisons). Again, there is controversy concerning the application of this test (Goldman, 1993); however, the calculated test statistic was over ten times the critical value in the most marginal comparison and it is unlikely that the models are statistically equivalent. The parameters estimated with this model were used in a subsequent more thorough search using the initial estimated ML tree (spr) or the four MPTs from the parsimony analysis as starting trees (hsearch, swap = TBR, basefreqs = estimated, rate matrix = A-C:1.238, A-G: 6.028, A-T:3.060, C-G:0.3041, C-T:6.485;%I = 55.9, Γ = 0.703797).

An additional ML model was constructed for the 28S dataset using the program Modeltest (Posada & Crandall, 1998). The model chosen by this program was TVM + I + Γ, which is quite similar to the GTR model above. Parameters estimated by Modeltest were used in a single ML search with a NJ starting tree (hsearch; swap = TBR; basefreqs = A:0.3572, C:0.1353, G:0.1671, T:0.3404; rate matrix = A-C:0.884, A-G: 5.5019, A-T:2.967, C-G:0.2912, C-T: 5.5019;%I = 55.64, Γ = 0.6343). Throughout this analysis, the unalignable regions of the 28S dataset (seventy-eight characters) were excluded.

To produce a more informative estimate of the site to site rate variation, a character partition for PAUP* was produced that included information about structural position (e.g. variable region/core, stem/loop; Table 2). Once the characters were divided into these 'bins', their relative probabilities of change were estimated on a tree selected on the basis of an initial ML search as outlined above, and integrated into a GTR + rate variation model (exhibiting the lowest −lnL) to use in a more thorough search with the initial ML tree as a starting tree (hsearch; swap = TBR; basefreqs = empirical; rate matrix = A-C:0.8106 A-G: 4.133, A-T: 3.3509, C-G:0.28496, C-T:5.2172; see Table 2 for rate variation estimates).

Combined dataset. The question of whether multiple datasets should be combined into a single phylogenetic analysis or analysed separately constitutes a central debate in modern phylogenetic biology (de Queiroz *et al*., 1995; Huelsenbeck *et al*., 1996b). Opponents of combining generally argue that combining datasets may lead to erroneous phylogenetic estimation if datasets are heterogeneous (Miyamoto & Fitch, 1995), whereas proponents of combining datasets often argue that combining datasets can improve the probability of detecting real phylogenetic groups by increasing the ratio of signal to noise (Olmstead & Sweere, 1994; Wenzel & Siddall, 1999). Therefore, in addition to the independent analyses, a 'pure' parsimony search with the combined 28S rDNA and EF-1α datasets was performed. A partition homogeneity test was conducted using PAUP to examine the level of conflict among datasets. In this test, the sum of treelengths of the two trees calculated using the defined partition (EF-1α and 28S rDNA) is compared to a distribution of the sum of treelengths of trees calculated using random partitions of the two datasets. A treelength value of the defined partition that falls in the lowest 5% of the distribution of random partition treelengths indicates significant discordance between the two datasets (Farris *et al*., 1994). In the current study, 100 replicate MP searches were performed on the randomly partitioned datasets (hsearch, swap = TBR, start = stepwise addition, replicates = 10). No evidence of significant conflict among datasets was found ($P = 0.910$).

The parsimony analysis of the combined datasets (EF-1α and 28S manual alignment) was conducted with equal character weighting with the most unalignable regions (seventy-eight characters, including ends) of the 28S rDNA dataset excluded. Although the 28S dataset represents only about 75% of the taxa present in the EF-1α dataset, all taxa were included in the combined analysis despite the slight decrease in accuracy this might cause. As Wiens & Reeder (1995) pointed out, it is better to have a phylogenetic hypothesis for the incomplete taxa that is 'mostly right' than have none at all. A simple parsimony analysis (hsearch, stepwise addition = random, swap = TBR, 1000 replicates; with *Senotainia* sp., *Metoposarcophaga* sp. and *Phormia regina* forming the outgroup) of this dataset was conducted. Nodes were again evaluated using 1000 bootstrap replicates (performed as above) and by calculating decay indices.

Results and discussion

Datasets

EF-1α sequences varied in length from 866 to 899 bp. The nucleotide matrix consists of 899 aligned sites, of which 580 (64.5%) were invariant, fifty-five (6.1%) were parsimony uninformative and 264 (29.4%) were parsimony informative. Average nucleotide frequency among all taxa was A 0.257, T 0.267, C 0.243 and G 0.233. No significant variation in nucleotide frequencies was observed among taxa ($\chi^2 = 42.4$, d.f. = 162, $P = 1.0$). Two hundred and twenty-eight (86.4%) of parsimony informative sites occurred at third positions, which were near saturation with maximum divergences at these sites of 20–30%. Uncorrected distances between taxa at third position sites increased with divergence when mapped onto cladograms recovered through parsimony analyses for most nodes. In addition, uncorrected distances (using only third positions) plotted as a function of taxonomic level increased over deeper divergences, despite the fact that the largest distances approach 0.3 (Fig. 2). Only between the most divergent taxa did there appear to be saturation at these sites. Plots of uncorrected p distances (third position) between independent taxon pairs against ML (TrN + I + Γ) distances revealed little evidence of saturation (Fig. 3).

The 28S rDNA sequences ranged in length from 970 to 1007 bp (1046–1079 bp in the manually corrected 5 : 2

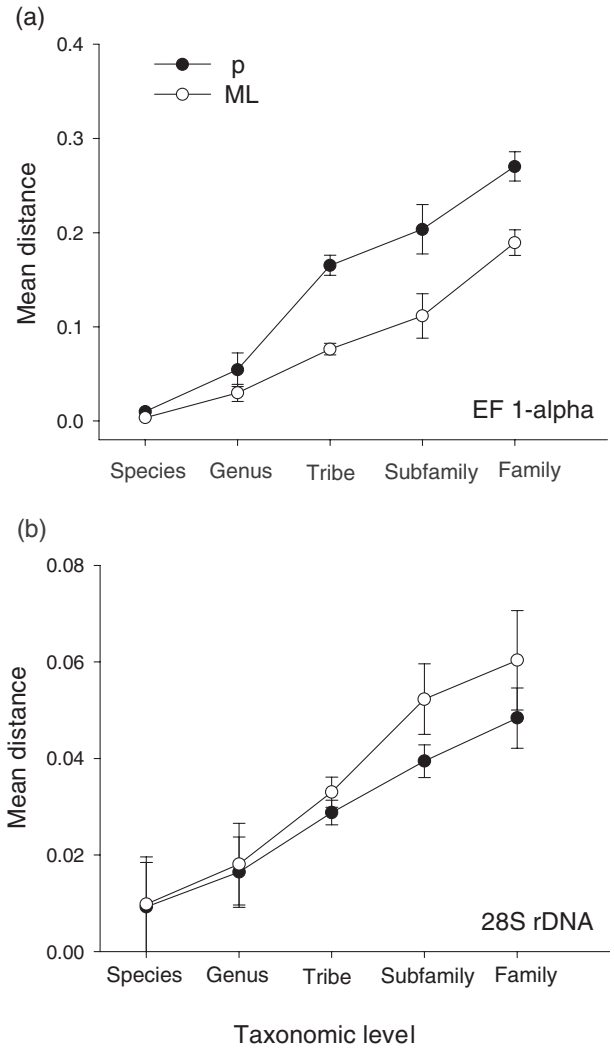

Fig. 2. Plots of uncorrected 'p' and maximum likelihood pairwise distance measures against taxonomic level with standard errors. Top panel, EF-1α (third position distances); bottom panel, 28S rDNA (all data). Taxonomic levels are based on Herting (1984) and Sabrosky & Arnaud (1965) for taxa not included in the former work.

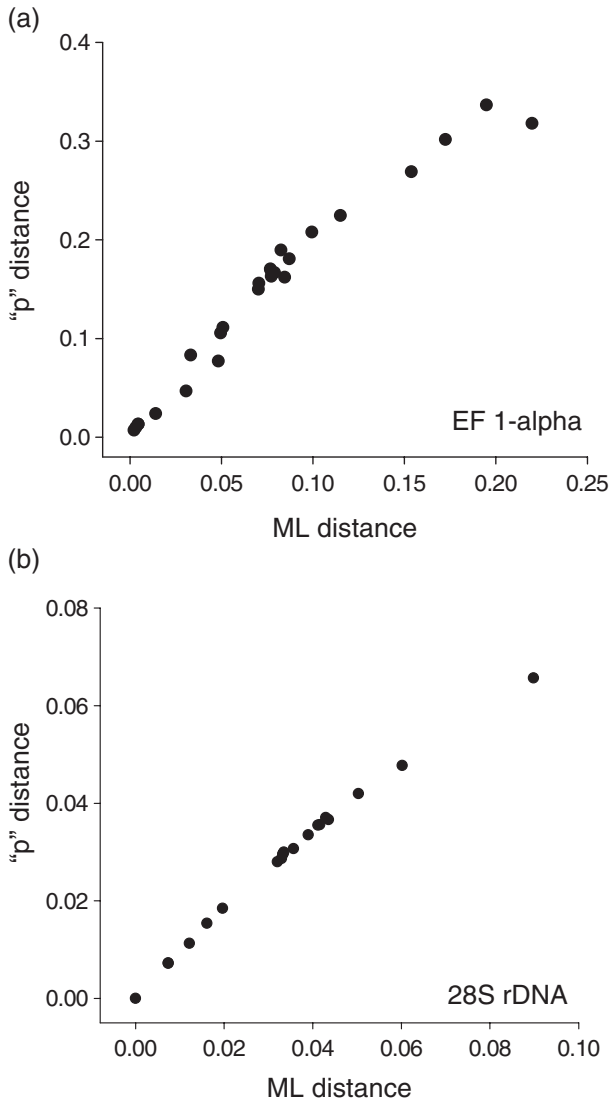

Fig. 3. Plots of uncorrected 'p' and maximum likelihood corrected pairwise distances. Top panel, EF-1α (third positions; twenty-five independent taxon pairs); bottom panel, 28S rDNA (all data; twenty independent taxon pairs).

Clustal W alignment). The truncated nucleotide matrix used in most analyses consisted of 1061 aligned sites, of which 777 (71.5%) were invariant, 125 (11.5%) were parsimony uninformative and 183 (16.8%) were parsimony informative. Average nucleotide frequency among all taxa was A 0.344, T 0.333, C 0.139 and G 0.184. As in the EF-1α dataset, no significant variation in base composition was observed among the taxa ($\chi^2 = 13.8$, d.f. = 126, $P = 1.0$). Plots of pairwise 'p' and ML (TVM + I + Γ) distances against taxonomic level for independent taxon pairs revealed no indication that variable sites are saturated (Fig. 2), as did plots of uncorrected p distances against ML distances (Fig. 3).

Analyses of the combined dataset utilized a data matrix of 1960 aligned sites, of which 1331 (68.1%) are invariant, 175 (9%) were parsimony uninformative and 448 (22.9%) were parsimony informative.

Phylogenetic inferences

The large number of separate analyses conducted in this study make it impractical and confusing to present each of the results as a separate tree. Representative trees for each of the two genes (EF-1α and 28S) based on neighbour joining, maximum parsimony and maximum likelihood are shown in Figs 4–9. These trees represent the simplest analyses that resulted in the most resolved trees. Results from all analyses described are summarized in Table 4

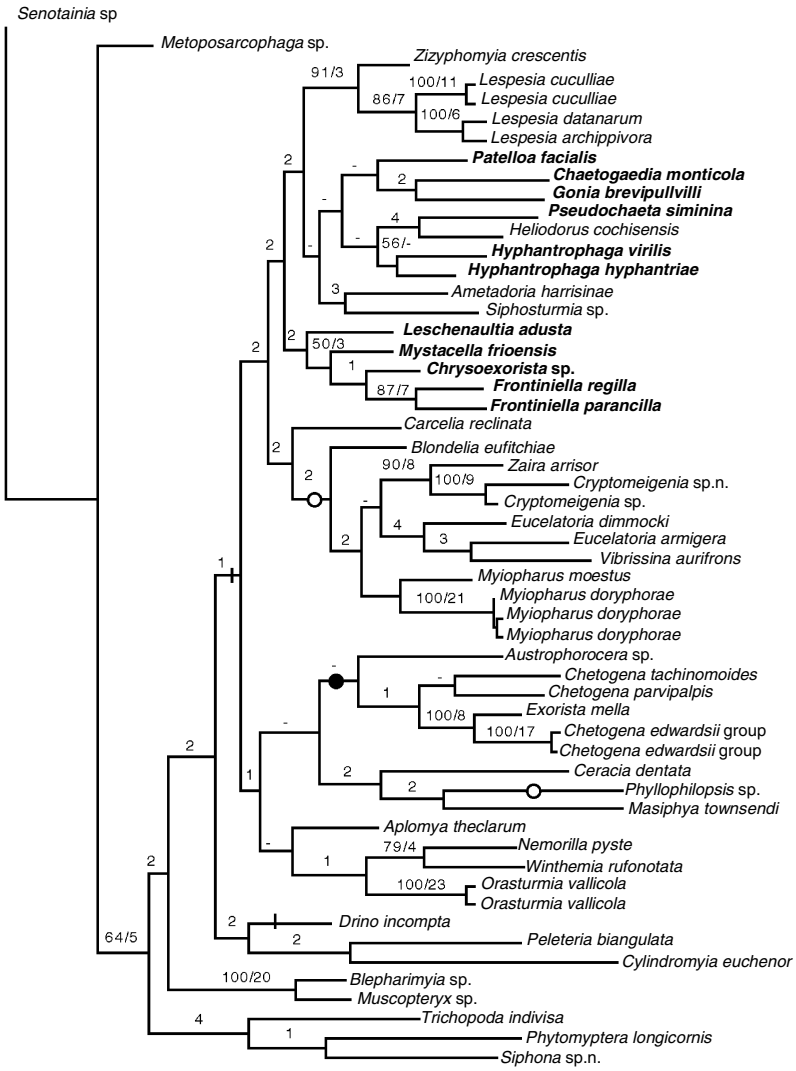

Fig. 4. One of twelve equally parsimonious trees inferred from the EF-1α nuclear gene by unweighted maximum parsimony (length = 1815). Numbers above branches indicate bootstrap support/decay indices. Bootstrap values are reported only for clades present in >50% of replicates. Branch lengths are proportional to the number of changes (ACCTRAN default). Microtype taxa are indicated by bold names and other groups of interest are indicated by symbols (● = Exoristini, ○ = Blondeliini, | = Exoristinae).

Table 4. A summary of the analyses of the EF-1a and 28S datasets with an indication of whether clades of interest were inferred and their placement (e.g. Winthemiini, see text). + = supported, e = equivocal, – = not supported. Numbers under the 'microtype clades' column indicate the inferred number of changes in this character. Exoristinae = + or – *Ceracia*, Blondeliini = – *Phyllophilopsis*; Winthemiini = – *Masiphya*.

Dataset	Analysis	No. trees	Score (length, −lnL, ME)	Tachinidae	Exoristinae	Exoristiini	Blondeliini	Winthemiini	Eryciini + Goniini	microtype clades*	Winthemiini basal
EF-1α	NJ – 'p'	1	1.757	+	+	+	+	+	+	4	+
EF-1α	NJ -HKY85 + Γ	1	2.160	+	+	+	+	+	+	3	+
EF-1α	MP -unwgt.	12	1815	+	+[1]	e	+	+	+[2]	3–4	e
EF-1α	MP w/ML wgt.	4	4143	–	–	–	–	–	–	6	–
EF-1α	MP Gon-mono	40	1827	+	+[1]	e	+	+	+[2]	1	e
EF-1α	MP -Drino, Phylo. & Pel.	8	1669	+	+[3]	+	+	–	+[2]	3	+
EF-1α	ML TrN + I + Γ	1	9143	+	–	–	+	–	+	4	+
EF-1α	ML GTR + rv	1	9472	+	–	–	–	+	–	3	+
28S	NJ -'p' (–var)	1	0.621	+	+[3]	+	+	+	+	5	+
28S	NJ -GTR + I + Γ	1	0.866	–	–	+	–	–	–	5	+
28S	MP (–var)	3064	711	+	e	+	+	+	–	4+	e
28S	MP (5th base-var)	208	811	+	+[3]	+	+	+	e	5–6	+
28S	MP + ML wgt	45	1372	e	–	–	+	+	–	8	–
28S	MP -Elision (– gaps10)	2	7577	+	e	+	+	+	e	6	+
28S	ML GTR + I + Γ	2	4957.5	–	+[3]	+	+	+	–	4	+
28S	ML TVM + I + Γ gonmono	6	5212.4	–	+	+	+	+	+	1	+
28S	ML TVM + I + Γ	1	5203	–	+[3]	+	+	+	+	5	+
28S	ML GTR + I + Struct wgts	1	5188	e	+[3]	+	–	+	+	5	+
EF-1α + 28S	MP combined data set	3	2644	+	+[1]	+	+	+	+[2]	3	+

[1]Except *Drino* (Pel-Cyl), [2]except *Aplomya*, *Carcelia*, [3]minus *Masiphya*.
*With *Heliodorus* considered a non-microtype eryciine.

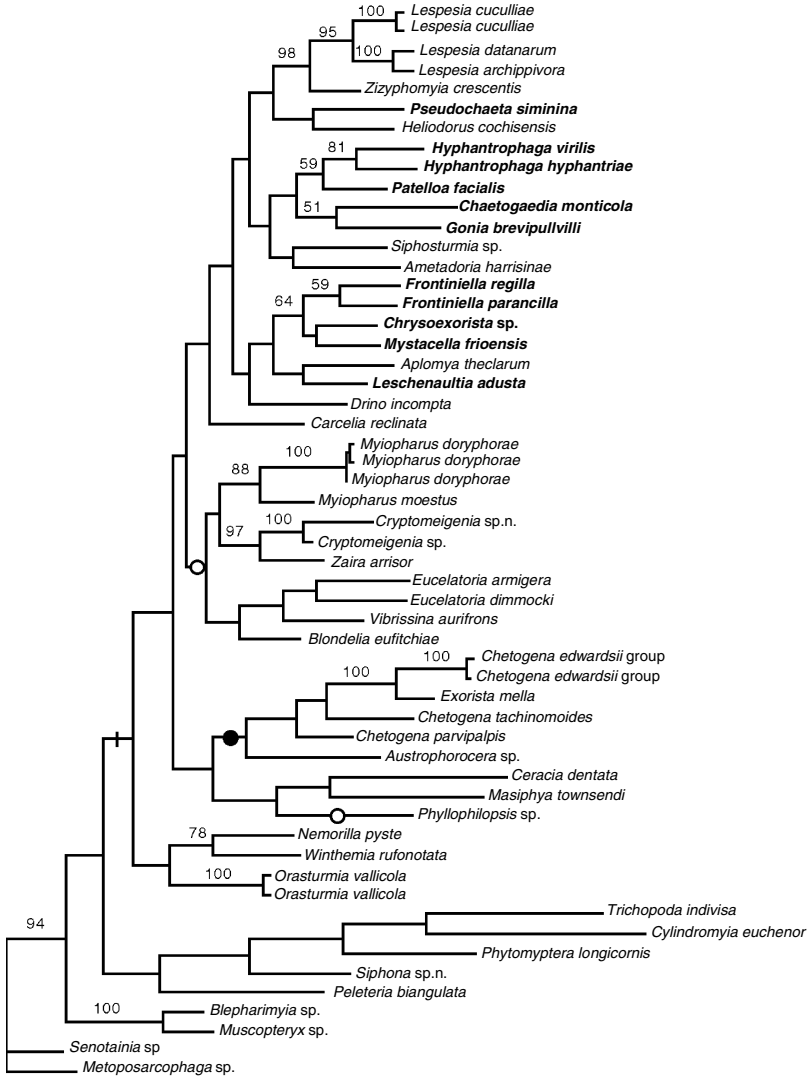

Fig. 5. Tree inferred by neighbour joining analysis of the EF-1α dataset employing uncorrected p distances. Numbers above branches indicate bootstrap support, and branch lengths are proportional to distance. See Fig. 4 for explanation of symbols.

by their statistics and their support for the most commonly observed clades and those indicated by the most recent taxonomic treatments (Herting, 1984; Tschorsnig, 1985).

Bootstrap values and decay indices are low for most clades across all analyses (Figs 4–9). Only certain, closely related terminal taxa such as *Cryptomeigenia* and *Zaira* are represented in 90% or more of bootstrap replicates (Fig. 4). Many of the deeper nodes are supported by decay indices of 1 or 2 (Fig. 4), suggesting that little confidence can be placed in some of the basal relationships. Given this, it is somewhat remarkable that most of the analyses resulted in trees that are essentially consistent with the taxonomic classification and evolutionary schemes of Wood (1987) and Herting (1984). Results from the various analyses are discussed briefly below.

Elongation factor 1-alpha

Maximum parsimony. The unweighted maximum parsimony (MP) analysis resulted in twelve equally parsimonious trees of length 1815 (four islands; recovered a minimum of twenty-four times out of 1000 replicates; Fig. 4). The differences in topology of these equally parsimonious trees are primarily involved with uncertainties in the relationships among clades within Blondeliini and Exoristini, and whether the *Gonia-Chaetogaedia* clade is allied with other microtype Goniini or with eryciine genera (Fig. 4). Each of these trees indicates monophyly of Blondeliini and Winthemiini and most support a monophyletic Exoristini. The latter two tribes are placed basally, which accords well with the hypothesis that the presence of unincubated eggs is the plesiomorphic condition (Wood, 1987). As in all

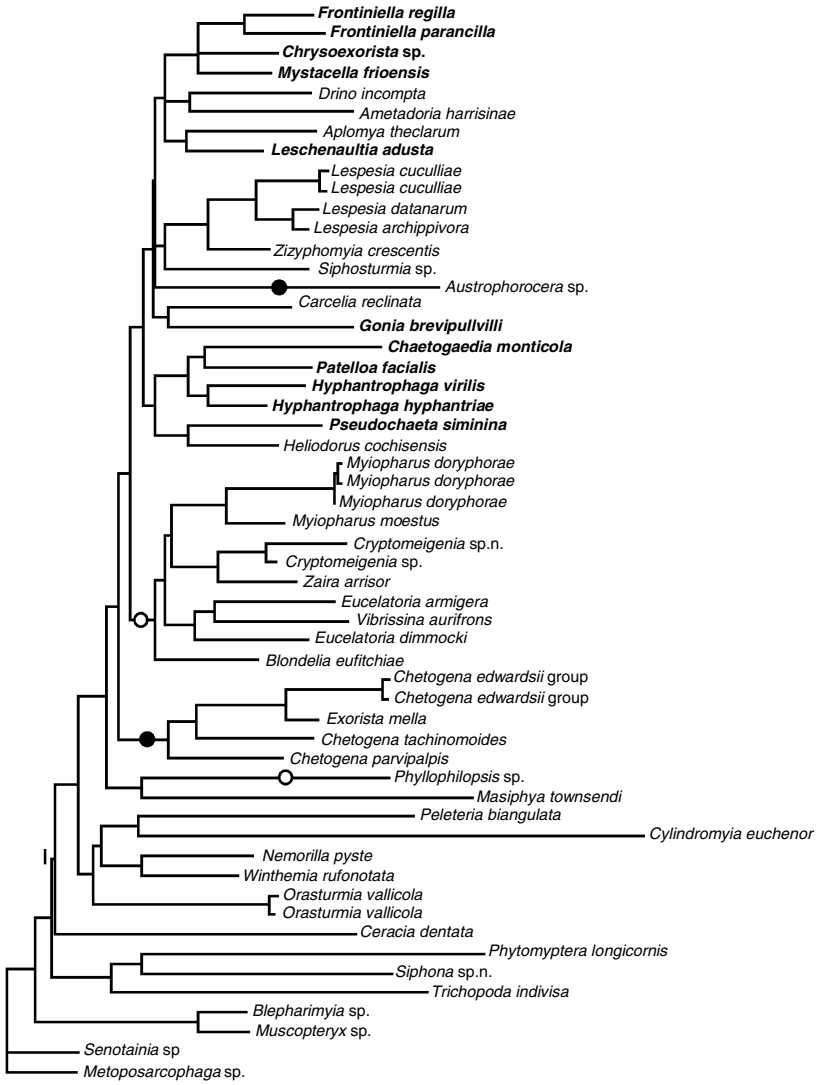

Fig. 6. Tree inferred by maximum likelihood analysis of the EF-1α dataset (TRN + I + Γ model, −lnL: 9143). Branch lengths are proportional to ML distances. See Fig. 4 for explanation of symbols.

analyses conducted, the microtype Goniini do not form a monophyletic group. The additional parsimony analysis with Goniini constrained to be monophyletic resulted in a MPT twelve steps longer (length = 1827). The basal position of genus *Drino* outside Exoristinae is problematic in all parsimony analyses, given its morphological resemblance to Eryciini. This placement is examined further in the discussion of taxa.

Additional parsimony analyses of the EF-1α dataset excluding third codon positions were almost entirely unresolved due to the small number of informative characters. Downweighting these positions with estimated rates of change derived from ML analysis produced a set of MPTs that were contradictory to any taxonomic classification or evolutionary scenario thus far proposed (Table 4, e.g. non-Exoristinae taxa interspersed within Exoristinae, Tachinidae not monophyletic, no recognized tribes monophyletic). The dramatic contradictions between the unweighted MP analysis and the ML-weighted analysis are consistent with a highly conserved amino acid sequence in this protein (Roger *et al.*, 1999). If the amino acid sequence of EF-1α is under strong stabilizing selection, then very few polymorphic characters should be located at first and second codon positions. Those that do occur are likely to represent convergent mutations due to the relatively few stable nucleotide changes allowable by selection. Thus, weighting these characters may result in an analysis dominated by convergent characters.

Parsimony analysis with the omission of the taxa *Drino*, *Peleteria* and *Phyllophilopsis* resulted in eight equally MPTs of length 1669 (Table 4). In these trees, the two phasiine taxa are reconstructed as sister groups, but

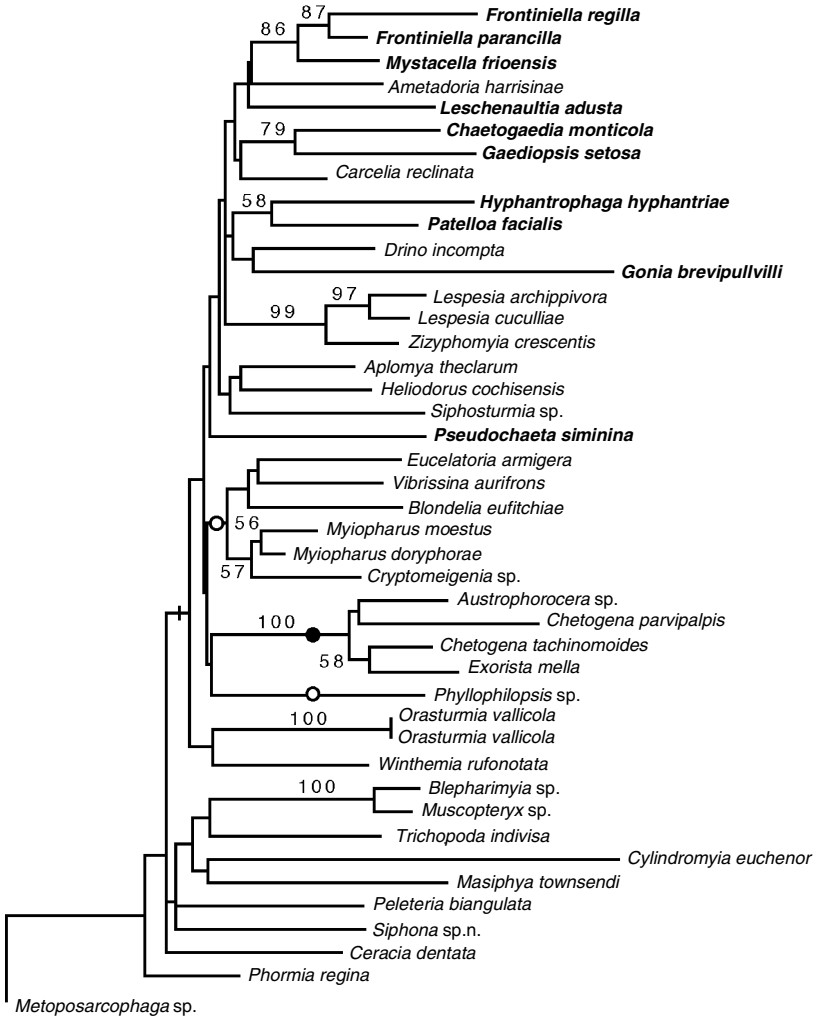

Fig. 7. Tree inferred by neighbour joining analysis of the 28S rDNA dataset employing uncorrected p distances (– 78 variable characters). Numbers above branches indicate bootstrap support. Branch lengths are proportional to distance. See Fig. 4 for explanation of symbols.

Winthemiini is reduced to a paraphyletic grade. Blondeliini and Exoristini are reconstructed as monophyletic tribes, and the G-E crown group is monophyletic with the exception of *Carcelia* and *Aplomya*, which are placed basal to Blondeliini and basal to Exoristinae-Winthemiini, respectively.

Neighbour joining. Despite the relatively simplistic nature of neighbour joining (NJ) methods, the analysis using uncorrected distances resulted in a tree that conforms very well to the taxonomic classification based on morphological characters (Fig. 5, e.g. Winthemiini, Blondeliini and Exoristini are each monophyletic groups). This is the only analysis in which the phasiine taxa *Cylindromyia* and *Trichopoda* were placed as sister taxa. The apparently good performance of this method supports the analyses of Takahashi & Nei (2000), which showed that for datasets consisting of many taxa of relatively short sequence length, simple models of inference like neighbour joining using p distances may actually outperform more complicated models of evolution. However, as for most analyses, only a few, rather terminal nodes are supported by high bootstrap values. More complex NJ analyses (e.g. Kimura 3-parameter model with rate heterogeneity) resulted in essentially the same tree, though the phasiines were not reconstructed as sister taxa.

Maximum likelihood. Fifteen trees of likelihood (–lnL) 9141.22 were recovered under the TRN + I + Γ model (Fig. 6). A single tree of slightly lower likelihood (–lnL = 9142.7) was recovered in a separate search using the same model parameters. The resulting trees are generally similar to that found in the MP analysis, but with a few important differences. First, the *Peleteria-Cylindromyia* clade joins Winthemiini and renders Exoristinae polyphyletic. This invasion of the *Peleteria-Cylindromyia* clade is also found in the ML analysis using codon-based rate heterogeneity, although in this latter case the clade appears

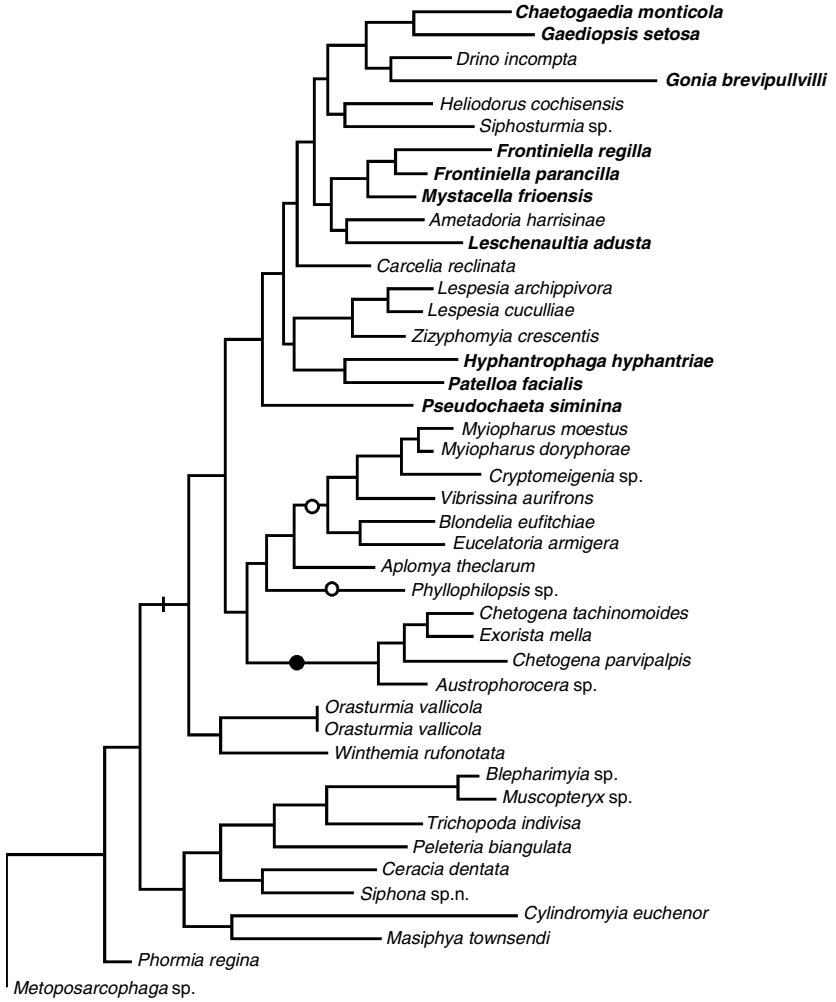

Fig. 8. One of two trees inferred by parsimony analysis of the Elision alignment of the 28S rDNA dataset (minus gaps of ten bases or greater (length = 7577). Branch lengths are proportional to changes (ACCTRAN default). See Fig. 4 for explanation of symbols.

at the base of the Goniini-Eryciini crown group (see below). Second, the mobile *Drino* resumes its position among the G-E crown group, sister to *Ametadoria*, as morphological taxonomy would suggest. Third, *Ceracia* is placed basal to the rest of Exoristinae. Somewhat disturbingly, this analysis groups *Aplomya* and *Carcelia* (macrotype Eryciini) as sister to *Leschenaultia* and *Gonia* (microtype Goniini), respectively. These are almost certainly incorrect reconstructions based on morphological considerations. The identical analysis with the microtype Goniini constrained to be monophyletic was aborted after 69 h. The tree resulting from this truncated search had a likelihood (−lnL) of 9147.04, which is quite close to the value obtained in the unconstrained search (Table 4). A comparison of these likelihood values using the χ^2 approximation suggests that this difference is significant assuming one degree of freedom (χ^2: 11.65, d.f. = 1, $P < 0.005$; again see criticisms of this test, Goldman, 1993; Huelsenbeck *et al.*, 1996a). Both KH (Kishino & Hasegawa, 1989) and SH (Shimodaira & Hasegawa, 1999) tests comparing these tree topologies resulted in no significant difference between the trees (KH: $P = 0.804$, SH: $P = 0.395$; although see discussion of *Heliodorus*).

The ML analysis performed on the EF-1α dataset using a GTR model with rate variation based on codon positions (see methods) resulted in one tree of likelihood (−lnL) 9472.078. This analysis resulted in a tree of significantly lower likelihood than that recovered with the TRN + I + Γ analysis above (based on the χ^2 approximation, $\chi^2 = 658.7$, d.f. = 4, $P < 0.001$), and several of the relationships indicated are probably erroneous. However, certain regions of the tree may illuminate some confusing relationships. For example, the G-E crown group itself is resolved into only two or three separate microtype clades (depending on *Heliodorus*), and the remaining eryciine clades include a sister-pairing of *Aplomya* and *Carcelia*. In addition, Winthemiini resume their monophyly and *Ceracia dentata* is placed as sister group to Exoristinae, reflecting the close

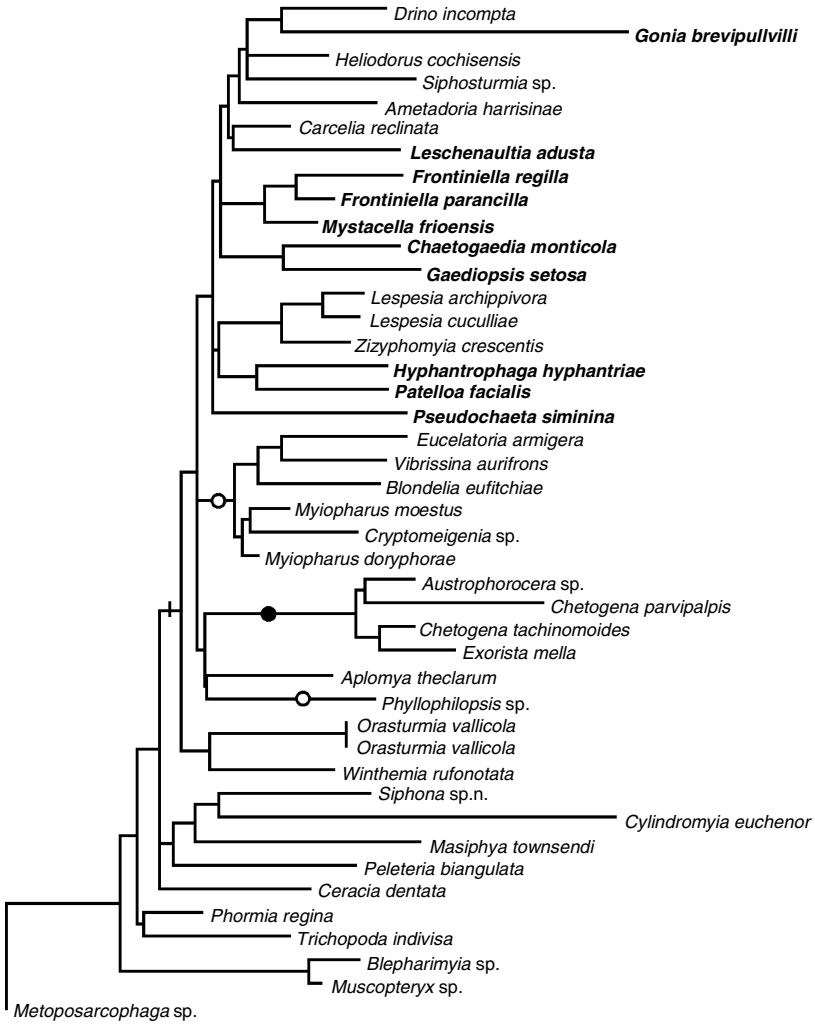

Fig. 9. Tree resulting from maximum likelihood analysis of the 28S rDNA dataset (minus seventy-eight variable characters under the TVM + I + Γ model (–lnL = 5203). Branch lengths are proportional to ML distances See Fig. 4 for explanation of symbols.

relationship suggested by Sabrosky & Arnaud (1965). Finally, as in the previous ML analysis, *Drino* is placed within the G-E crown group, but in this case it is joined by the non-exoristine *Peleteria-Cylindromyia* clade. The relationships that appear to be incorrectly constructed in this analysis (e.g. polyphyly of Blondeliini and Exoristini, *Peleteria-Cylindromyia* clade in G-E crown group) may be due to the strong influence of the few stable changes in first and second positions that are prone to convergence as discussed previously.

Simulated datasets. Trees inferred by the parametric simulation test generally contained the clades defined by the constraint tree, suggesting that some of the apparently aberrant relationships in trees based on the observed data may not be artefacts of incorrect inference. However, not a single tree was located in all the replicates that was entirely compatible with the original constraint tree. A few taxa were extremely mobile and were rarely reconstructed in positions consistent with the model tree. For example, *Peleteria* was joined with the other three tachinines in only 8% of replicates. The null hypothesis can be rejected, i.e. the relationships are unlikely to be explained purely by long branch attraction or other factors of incorrect inference, regarding the monophyly of Phasiinae with respect to Tachininae. Ninety-nine percent of simulations placed *Cylindromyia* and *Trichopoda* as sister taxa, and thus the separation observed in the trees inferred from the actual data is an unlikely outcome ($P = 0.01$). In addition, *Drino* was placed basally (in association with *Peleteria*) in none of the replicates, indicating that long branch attraction cannot easily explain this particular placement ($P < 0.01$). *Drino* was quite variable in its placement within the G-E crown group among replicates, e.g. 14% with *Aplomya*, 7% with *Siphostumia* + *Ametadoria*, 5% with *Gonia* and 3% with *Phyllophilopsis*, suggesting that its phylogenetic position may be difficult to reconstruct correctly. Fifty percent and 99% of simulations reconstructed the microtype Goniini

and Eryciini as poly- or paraphyletic, respectively. Thus, even if these tribes are monophyletic, they would only rarely be reconstructed as such with the data from this gene. For these clades, along with Blondeliini and Winthemiini, the model tree cannot be rejected. Furthermore, only 24% of trees inferred from the simulated datasets contained a monophyletic Exoristinae, indicating that there is a reasonable probability of reconstructing the subfamily as poly- or paraphyletic with the observed data even if it were monophyletic.

28S rDNA

Neighbour joining. The NJ tree inferred from the 28S dataset using simple uncorrected p distances indicates monophyly of Tachinidae, Exoristinae, Winthemiini, Exoristini and Blondeliini (Fig. 7, Table 4), although relationships within the G-E crown group are unstable especially with respect to microtype and non-microtype taxa. *Drino* is placed within the G-E crown group as sister to the microtype taxon *Gonia*.

The GTR + I + Γ NJ analysis produced essentially the same tree as the previous analysis, with Exoristini and Blondeliini as sister taxa. Again, microtype and nonmicrotype clades are interspersed, resulting in a reconstruction requiring at least six changes between microtype and nonmicrotype forms. Terminal branches in the G-E crown group are quite long relative to internal branches and bootstrap values are low for most clades, indicating that the reconstructed relationships must be taken with caution. Some additional relationships of interest include the placement by both NJ analyses of *Masiphya* outside of Exoristinae (as in many analyses of the EF-1α dataset), and a placement of *Phyllophilopsis* basal to Exoristini, with or without *Aplomya* as a sister (Fig. 7). Despite their simplicity, trees inferred by NJ with the 28S dataset are among the most consistent with recent tribal classifications, as found in NJ analyses of the EF-1α dataset.

Maximum parsimony. Several parsimony analyses were conducted with the 28S ribosomal dataset due to the uncertainties in the alignment of sequences. In general, most analyses recovered large numbers of equally parsimonious trees with little overall resolution (Table 4).

Basal relationships among tribes and certain genera were largely unresolved in the MP inference with extra-variable regions excluded (seventy-eight characters) and gaps coded as missing. Despite this uncertainty in basal relationships, each of the exoristine tribes aside from the G-E crown group were present in all 3064 MPTs (L = 711), and the G-E crown group was supported as a clade in 91% of trees (with the exception of *Aplomya* and *Pseudochaeta*). Analyses with gaps coded as a fifth base (– seventy-eight variable characters) resulted in a set of trees ($N = 314$, L = 803) very similar to those inferred with gaps coded as missing data. However, with this coding of gaps Blondeliini is only supported (with or without *Phyllophilopsis*) in 94% of MPTs and the G-E crown group (– *Aplomya*) is present in 89% of trees. The inclusion of gaps as characters resulted in better overall resolution of basal and terminal branches; all MPTs supported a monophyletic Tachinidae, Exoristinae and Exoristinae-Winthemiini (i.e. the winthemiines are basal). In addition, the analysis with gaps coded as fifth bases supported a sister relationship between the *Drino* and *Siphosturmia* (94% of trees), which share similar oviposition strategies, rather than pairing of *Drino* with the microtype goniine *Gonia* as in other analyses. The length of the branch subtending *Gonia* is the second longest over the entire tree suggesting that long branch attraction may be responsible for its association with *Drino* (Felsenstein, 1978). Parallel searches with the microtype Exoristinae constrained to be monophyletic were at least five or six steps longer (hsearch; start = stepwise; swap = TBR; 1000 replicates for gaps = missing and – seventy-eight or sixty-two variable characters, respectively).

Forty-five MPTs (L = 1372) resulted from an MP analysis with character weights based on ML estimates of rate variation among sites partitioned according to 28S rRNA secondary structural position. These trees were not consistent with the trees inferred in other parsimony or ML analyses, nor were they consistent with analyses of the EF-1α dataset or with morphological classification. Numerous presumably fallacious relationships abound in these reconstructions, including the placement of *Phormia regina* in the tachinid clade, *Mystacella* + *Peleteria* and *Blondelia* + *Ceracia* sister-pairings, and the placement of *Siphosturmia* in a clade of Blondeliini. However, even under this apparently improper weighting scheme Winthemiini, Exoristini and most Blondeliini are recovered as monophyletic clades. The apparent failure of this analysis may be due to incorrect translation of estimates of rate variation from a ML analysis to character weights in MP analyses. The appropriateness of translating rates of change directly into weights, as I have done, has not been thoroughly explored. In addition, the partitions that I defined to examine rate variation may not coincide with functional partitions in terms of the evolution of the 28S ribosomal gene.

The analysis of the Elision alignment resulted in two MPTs (one shown in Fig. 8). These MPTs differed only in the placement of the winthemiines (*Winthemia* and *Orasturmia*) either basal to Exoristinae vs basal to Tachinidae as a whole. Winthemiini, Exoristini, Blondeliini (– *Phyllophilopsis*) and Tachinidae are all reconstructed as clades. The G-E crown group is reconstructed (– *Aplomya*) and Exoristini is paired with Blondeliini (+ *Aplomya*) as in NJ analyses.

Maximum likelihood. The two ML analyses of the 28S data that utilized models with rate variation approximated by the gamma distribution (GTR + I + Γ and TVM + I + Γ) inferred phylogenetic trees with very similar topologies (Fig. 9). In both cases, *Phormia regina* was reconstructed as sister to the tachinid *Trichopoda*, rendering Tachinidae para- or polyphyletic. Aside from this, inferred relationships among Tachinidae are similar to other analyses. The

tree inferred by the GTR model is one of the few trees resulting from this study that places *Drino* with other macrotype eryciines (*Ametadoria* and *Siphosturmia*) rather than sister to a microtype Goniini (e.g. 28S NJ, MP, though see analysis of gaps = fifth base) or in some association with nonexoristine tachinids (e.g. EF-1α). A second ML analysis using the TVM + I + Γ model with the microtype Exoristinae constrained to be monophyletic had a likelihood score slightly lower than the unconstrained analysis ($\Delta\ln L = 14.05$). The six equally likely trees resulting from this constrained analysis were quite similar to the unconstrained trees aside from the specified constraint.

Incorporating rate variation among nucleotide positions based on the secondary structure of the 28S rRNA molecule into a ML analysis resulted in a reconstruction quite similar to those based on the GTR + I + Γ or TVM + I + Γ models, with a few important differences. First, Blondeliini is split into two clades, one of them forming a sister group to Exoristini and the other basal to this grouping. Second, the outgroup taxon *Phormia* is not reconstructed as sister to *Trichopoda* and remains outside Tachinidae. Third, relationships within the G-E crown group are somewhat different with *Carcelia* joining the *Chaetogaedia* + *Gaediopsis* clade, *Drino* and *Pseudochaeta* joining *Gonia*, and *Leschenaultia* joining the *Frontiniella* + *Mystacella* clade as in reconstructions based on the EF-1α data. In general, the additional complexity of the ML analysis utilizing empirically derived estimates of rate variation among sites does not appear to have increased resolution of problematic relationships. It may have actually introduced additional error by specifying a detailed but possibly incorrect model of evolution for this gene.

Combined analysis

A maximum parsimony analysis of the combined dataset resulted in three MPTs (L = 2644), the consensus of which is shown in Fig. 10. The only variation between these MPTs is related to how the outgroup taxa are related to one another, an issue that is not the concern of this study. The stronger influence of the EF-1α dataset in the combined analysis is apparent in the placement of *Drino* outside Exoristinae allied with the *Peleteria* + *Cylindromyia* clade and the inclusion of *Ceracia* and *Masiphya* in Exoristinae. In the combined analysis, Tachinidae, Exoristinae (– *Drino*), Winthemiini, Exoristini and Blondeliini (– *Phyllophilopsis*) form monophyletic clades. *Carcelia* and *Aplomya* are removed from an otherwise complete G-E crown group clade, taking up basal positions in association with the blondeliines and exoristines, respectively. In contrast to many other analyses, the microtype Goniini form only two clades, separated by the eryciines *Ametadoria* and *Siphosturmia*. A parallel MP analysis with the microtype Goniini constrained to be monophyletic resulted in a tree seven steps longer than the unconstrained tree (L = 2651). As a whole, the resulting trees from the combined MP analysis closely match those from the EF-1α dataset due to the larger number of informative sites.

The lack of support for many basal nodes in these analyses may be a consequence of homoplasy or noise present in the datasets. The EF-1α dataset was dominated by nucleotide changes in third positions that may have been near saturation, and strong selection for amino acid stability may have resulted in a convergence in the few changes located in first and second positions. The 28S rDNA dataset was difficult to align, and I may not have accurately modelled the evolution of this untranslated gene despite the many model variants examined. In addition to these methodological problems in recreating relationships within Exoristinae, there may be characteristics inherent in this clade that make robust phylogenetic estimation difficult or impossible. Several authorities on Tachinidae have indicated that the family is young and actively radiating (Crosskey, 1976; Wood, 1987). With over 8200 extant species in a family not known to exist before the Eocene/Oligocene (30–50 MY; Evenhuis, 1994), speciation and radiation must have been rapid. Exoristinae, containing over half of all described species, may represent the most recent radiation of the family. If speciation and phylogenetic radiation have indeed occurred rapidly in this group with little morphological or molecular evolution between divergence events, this may explain the difficulty that has plagued tachinid systematists in using morphological characters to delineate phylogenetic groups.

A modified summary tree of all analyses is presented in Fig. 11. Although somewhat more detailed than the initial provisional phylogeny depicted in Fig. 1, it generally supports most of the subfamily and tribal positions and definitions. Notable exceptions are the mixed Tachinine-Phasiine group and the lack of separation of Goniini and Eryciini. Certain relationships of interest are discussed further below.

Tachinidae. Tachinidae are reconstructed as a monophyletic group in the majority of analyses, and this is supported with reasonable bootstrap analysis in at least some analyses (EF-1α: NJ). Paraphyly is indicated in several ML analyses of the 28S dataset in which the calliphorid, *Phormia regina*, is situated basally among Tachinidae in association with *Trichopoda*. Vossbrinck & Friedman's (1989) phylogenetic analysis indicated a similar pairing of these two taxa using 28S rDNA sequence data. This study also placed *Gasterophilus* (Oestridae) as sister to the tachinid *Archytas marmoratus*. However, recent morphological analyses have not supported a close relationship between any calliphorid or oestrid clade and Tachinidae (Pape, 1992, 2001; Rognes, 1997). Few modern authorities question the monophyly of Tachinidae and the relationships indicated by the 28S sequence data in this and previous studies are most likely an example of evolutionary convergence in a hypervariable region of this gene.

The two phasiine taxa (Phasiinae) in this study, *Trichopoda* and *Cylindromyia*, were rarely reconstructed as sister taxa or as a paraphyletic grade despite the similarity in their morphology and shared use of hemipteran hosts. This

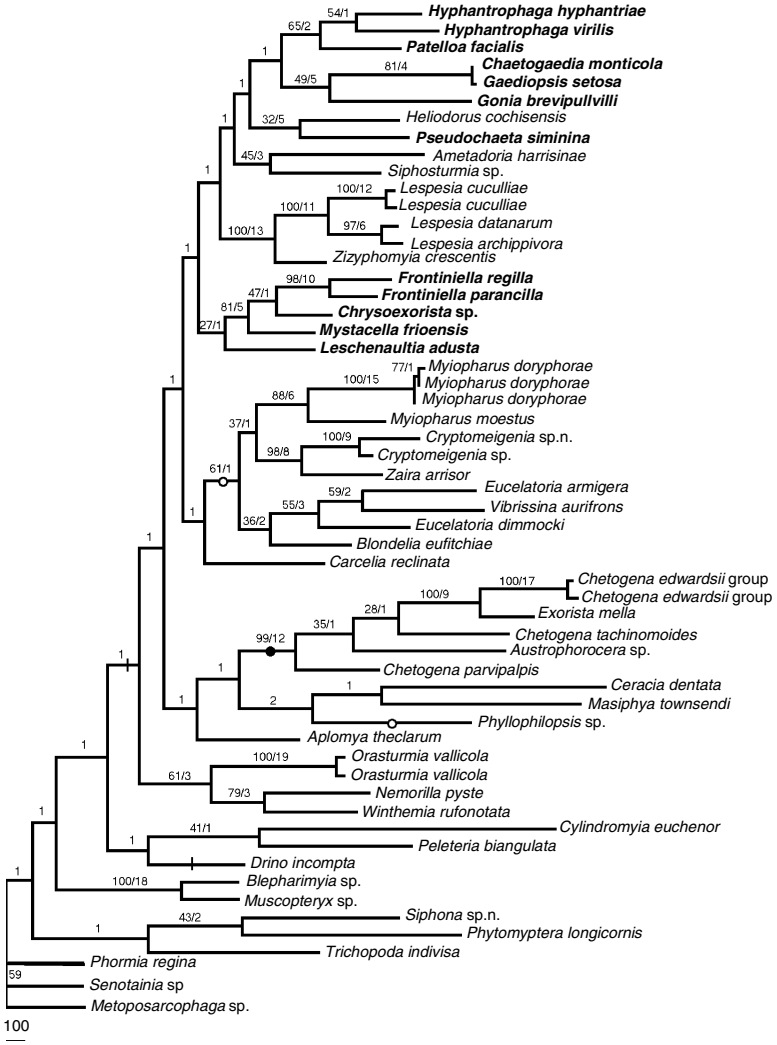

Fig. 10. Consensus of three trees inferred from maximum parsimony analysis of the combined EF-1α and 28S rDNA datasets (length = 2644). Numbers above branches indicate bootstrap support/decay indices. Bootstrap values are reported only for clades present in >50% of replicates. The reconstructed position of *Drino* is especially questionable. See Fig. 4 for explanation of symbols.

may be an artifact of the extremely long branch subtending *Cylindromyia*, which may cause this taxon to group with *Peleteria* or other taxa on long branches. In the combined parsimony analysis, *Cylindromyia* is subtended by the longest branch in the entire tree and *Trichopoda* is on the sixth longest. Alternatively, the splitting of the phasiines may reflect the true phylogeny. It is likely Phasiinae as a whole is paraphyletic with respect to certain tachinine taxa (J. K. Moulton, unpublished), and several authors have suggested that Tachininae are likely either para- or polyphyletic (e.g. Tschorsnig, 1985; Wood, 1987; Cantrell & Crosskey, 1989). Thus, the grouping of *Cylindromyia* among the tachinine taxa may reflect the origin of various tachinine clades within Phasiinae, or vice versa.

The tachinine taxa (Tachininae) included in this study (*Peleteria, Siphona*, *Phytomyptera* and perhaps *Ceracia*) are never reconstructed as a monophyletic group, although they all often cluster basally with Phasiinae. As stated previously, this may reflect true paraphyly or polyphyly of this taxonomic group. *Siphona* pairs with *Phytomyptera* in most reconstructions based on the EF-1α data, and they are always placed outside Exoristinae. This supports the view of several modern authors (Herting, 1984; Tschorsnig, 1985; O'Hara, 1989; Andersen, 1996) that these taxa are allied with Tachininae in contrast to their placement in Exoristinae by Sabrosky & Arnaud (1965) and Cantrell & Crosskey (1989).

The two representatives of Dexiinae in this study share the same tribe (Phyllomyini) and thus represent only a small portion of the diversity of this subfamily. They are reconstructed as sister taxa and are placed outside Exoristinae in all analyses, usually as one of several basal splits within Tachinidae (EF-1α) or as sister to *Trichopoda* (28S).

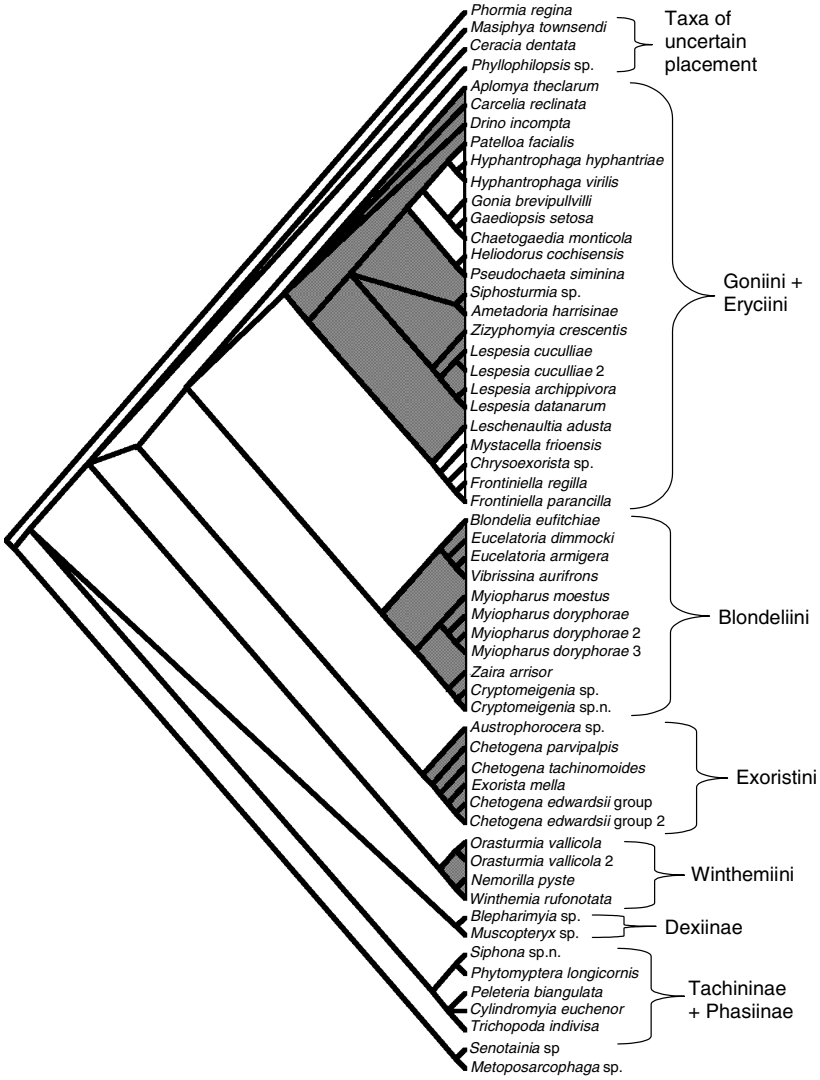

Fig. 11. A modified consensus tree of the taxa included in this study summarizing the results of the analyses and discussion.

Exoristinae. This subfamily is supported as a clade in nearly all analyses with the exception of a few taxa, including *Ceracia*, *Masiphya* and *Drino*, which are discussed individually below. In a few cases, ML analyses using the 28S data placed a *Peleteria-Cylindromyia* clade within Exoristinae, but this is almost certainly due to their long mobile branches and peculiar association with the problematic taxon *Drino*.

The tribe that *Ceracia dentata* is classified in, Acemyini, has been alternatively placed by various authors within Exoristinae (Sabrosky & Arnaud, 1965) or Tachininae (Cantrell & Crosskey, 1989). Unfortunately, trees inferred by the present analysis fail to completely resolve this issue. Most analyses (28S, ML of EF-1α) place *Ceracia* among the tachinine-phasiine outgroup, or as the sister to all other exoristines. Parsimony analyses of the EF-1α dataset group *Ceracia* with two other aberrant taxa, *Phyllophilopsis* and *Masiphya*, within Exoristinae in a sister-group position to Exoristini. An exploratory analysis of the EF-1α data with *Phyllophilopsis* removed reconstructed *Ceracia* with *Masiphya* outside Exoristinae as in the 28S analyses. The unusual use of orthopteran hosts by aceymyines (Wood, 1987) suggests that they may be quite divergent evolutionarily from other exoristines. In general, this study indicates that *Ceracia* either occupies a basal position within Exoristinae or is allied with Tachininae. Morphological analyses have also been faced with uncertainty in the placement of this taxon due to its possession of highly derived characters (Tschorsnig, 1985).

The uncertainty in the position of *Masiphya* parallels that of *Ceracia*. It tends to fall either outside Exoristinae in association with *Cylindromyia* (28S) or in a clade of aberrant taxa sister to Exoristini (as above, EF-1α). *Masiphya* and *Ceracia* are subtended by the second and third longest

branches in the combined analysis (out of 109 branches) and thus their placement may be heavily influenced by long branch attraction. Morphologically, *Masiphya* shows considerable similarity to the basal clades of Exoristinae (Winthemiini and Exoristini), although the unincubated eggs, piercing ovipositor and atypical male genitalia suggest that a relationship with Phasiinae is not out of the question (as suggested by Tschorsnig, 1985). In the EF-1α simulation analysis, *Masiphya* formed a clade with the winthemiines in only 7% of simulated trees despite the fact it was allied with these taxa in the tree upon which these simulations were based. This suggests that even if *Masiphya* belongs in Winthemiini, as Wood (1987) has proposed, it is unlikely that this relationship would be recovered due to the long branch associated with this taxon.

The positions of *Drino* inferred by the various analyses in this study are troublesome. MP analyses of the EF-1α data always place *Drino* outside Exoristinae (with *Cylindromyia* + *Peleteria*). However, NJ and ML analyses of the same data situate this taxon basally within the G-E crown group or in association with *Ametadoria*. Analyses of the 28S dataset always place *Drino* within the G-E crown group, but in most cases it is placed as a sister to the microtype taxon *Gonia*. *Drino* is clearly allied morphologically with other eryciine taxa (notably those classified in Sturmiini by Sabrosky & Arnaud, 1965), and possesses the membranous embryonated eggs that characterize this tribe (personal observation). A close association of *Drino* with the microtype taxon *Gonia* is very unlikely. Although *Drino* itself is not subtended by an exceptionally long branch, the taxa that it tends to associate with often do. *Gonia* is subtended by the second longest branch in the analyses of the 28S dataset (NJ, MP, ML), and, as stated previously, *Cylindromyia* and *Peleteria* both have very long branches. The reason behind this 'attraction' to long branch taxa is puzzling. Comparisons of the EF-1α and 28S rDNA sequences of *Drino* with other dipteran and non-dipteran taxa and multiple amplifications of the same species indicate that the problem cannot be explained by DNA contamination, because *Drino* clearly groups with other Tachinidae. The position of *Drino* was further explored by removing all non-exoristines and designating Winthemiini as the outgroup. In this case, *Drino* is placed at the base of the G-E crown group. In summary, *Drino* is almost certainly allied with the G-E crown group, most likely in association with other eryciines (as in the EF-1α ML analyses), or in a basal position. However, it appears that some peculiar, perhaps accelerated, molecular evolution has occurred in the *Drino* lineage making it difficult to ascertain its exact position. Additional sequences of other species in this genus may clarify this problem.

Winthemiini. Aside from *Masiphya*, the two (28S) or three (EF-1α) representative genera of this tribe form a clade in nearly all analyses of both genes, supporting Tschorsnig's (1985) view based on male genitalia that Winthemiini is monophyletic. This clade is positioned basally among Exoristinae in the vast majority of analyses, supporting the view of Herting (1960) and Wood (1987) that this tribe is 'primitive' among Tachinidae. In the analyses in which they are not reconstructed as monophyletic, they are joined by nongoniines subtended by very long branches (EF-1α, ML Trn + I + Γ).

Exoristini. As with Winthemiini, almost all analyses reconstruct Exoristini as a monophyletic clade sister to either Blondeliini or to the Blondeliini + G-E crown group (a basal lineage diverging subsequent to Winthemiini). The latter relationship is supported by the possession of unincubated macrotype eggs in this clade (Wood, 1987). *Austrophorocera* is somewhat mobile in EF-1α MP and ML analyses, joining the clade of long branch taxa adjacent to Exoristini (*Ceracia*, *Masiphya*, *Phyllophilopsis*) or the G-E crown group in some analysis. Overall, the current study strongly supports the monophyly of Exoristini.

Interestingly, all analyses indicate that *Chetogena* (of Wood, 1987; O'Hara & Wood, 1998) is paraphyletic with respect to *Exorista*, and perhaps *Austrophorocera*. This view is supported by characters of the male genitalia as well (Stireman, unpublished).

Blondeliini. Blondeliini is strongly supported as a clade in this study, with the possible exception of *Phyllophilopsis*. In a few analyses, polyphyly or paraphyly is indicated, but these analyses tend to be those in which potentially erroneous weights were applied to characters (EF-1α with ML weighting). In many analyses of the 28S data, the eryciine genus *Aplomya* is allied with Blondeliini, and in a few cases *Pseudochaeta* is as well (see discussion of these genera below).

In reconstructions based on EF-1α, *Blondelia* is either sister to a *Eucelatoria* + *Vibrissina* clade (NJ) or basal to the remaining Blondeliini (minus *Phyllophilopsis*). In 28S analyses, this genus is always sister to the former clade, a relationship that is supported by several morphological characters including sternite 7 modified into hooklike piercer (Wood, 1985, 1987). The MP, NJ and ML analyses of the EF-1α dataset indicate that *Eucelatoria* is paraphyletic with respect to *Vibrissina*, corroborating Wood's (1985) view that there is really no justification for maintaining these taxa as separate genera. The relationship between *Eucelatoria, Myiopharus* and the well supported *Zaira* + *Cryptomeigenia* clade is split in the EF-1α analyses, with some inferred trees uniting the former two taxa (e.g. MP), some trees uniting the latter two (e.g. NJ, ML) and one tree uniting *Eucelatoria* with the *Cryptomeigenia* clade (MP). All 28S analyses support a clade composed of *Myiopharus* and *Cryptomeigenia*, with the former often being paraphyletic with respect to the latter. In the combined dataset, *Myiopharus* is placed as sister to the *Cryptomeigenia* clade, and this appears to be the most likely reconstruction of relationships.

The phylogenetic position of *Phyllophilopsis* among Tachinidae is difficult to infer from the analyses in this study. All analyses of the EF-1α dataset place *Phyllophilopsis* in a trio of long branch taxa with *Masiphya* and *Ceracia*, that

forms the sister group to Exoristini. Analyses of the 28S data support a basal position for this taxon among Blondeliini (if *Aplomya* is included; MP, ML), or a basal position with respect to Exoristini (MP Elision, NJ, ML). *Phyllophilopsis* is subtended by a rather long branch (seventh longest in the combined dataset). The parsimony simulation analysis placed this genus with Exoristini in 11% of replicates, and *Masiphya* in 7% of replicates, despite the fact that the constraint tree upon which the simulation was based placed *Phyllophilopsis* within Blondeliini. The most frequent placement of *Phyllophilopsis* among replicates (22%) is as sister to Blondeliini + G-E crown group. These results suggest that the relatively long branch of *Phyllophilopsis* may be obscuring its true phylogenetic position, but its inclusion in Blondeliini is questionable.

Eryciini. Eryciini of Herting (1984) and Wood (1987) includes essentially all the remaining Exoristinae that have not already been accounted for (in Winthemiini, Exoristini and Blondeliini) and that do not possess microtype eggs. The tribe most likely does not reflect a monophyletic clade (Wood, 1987), and serves primarily as temporary placement for the included taxa until their interrelationships are better understood. There are no synapomorphies that all members of the tribe share, though there is a trend towards ovolarvipary. Previous authors (e.g. Townsend, 1936–1941; Mesnil, 1965; Sabrosky & Arnaud, 1965) separated Eryciini into a series of tribes, but many if not most of these tribes are quite likely para- or polyphyletic themselves (Wood, 1987). The current analyses tend to place Eryciini in two to five separate clades interspersed with variable numbers of microtype goniine clades, which together form the 'G-E crown group'.

The species of *Lespesia* and *Zizyphomyia* always form a strongly supported clade. *Ametadoria* and *Siphosturmia* are also placed together in a clade in most analyses, sometimes joined by *Drino* (28S ML, MP) or *Heliodorus* (28S ML), or both. The other taxa included in this study that were formerly placed in Sturmiini by Sabrosky & Arnaud (1965), *Mystacella* (with microtype eggs) and *Zizyphomyia* (closely allied with *Lespesia*), do not appear to be closely related to the aforementioned three taxa, although *Siphosturmia* joins the *Zizyphomyia* + *Lespesia* clade in at least one analysis (EF-1α ML).

Carcelia is somewhat derived morphologically, possessing a unique pedicellate egg (Clausen, 1940). Its reconstructed position within Exoristinae varies considerably between analyses in this study. Analyses place *Carcelia* alternatively as a basal lineage within the G-E crown group (EF-1α NJ, ML; 28S Elision, ML), basal to Blondeliini (combined) or in various positions among the G-E crown group (28S MP, NJ). There is no hint of a relationship between *Carcelia* and the microtype *Hyphantrophaga* as is suggested by Sabrosky & Arnaud's (1965) Carceliini. Given its affinities with other eryciine taxa, as well as the relatively wide support from both datasets, a basal position within the G-E crown group is the most likely reconstruction. However, this conclusion is far from certain.

Aplomya represents one of the few eryciine clades that possesses thick-shelled, unincubated eggs characteristic of Exoristini and Winthemiini (Wood, 1987). Its reconstructed position varies in much the same way as *Carcelia* between analyses, though the position of *Aplomya* is even more uncertain. *Aplomya* is placed either in association with Winthemiini (EF-1α MP), Exoristini (EF-1α, 28S ML), Blondeliini (28S MP, Elision) or the G-E crown group (EF-1α, 28S NJ). All except a derived position within the G-E crown group are potentially consistent with *Aplomya*'s possession of the presumably 'primitive' egg type among tachinids (some Blondeliini also have unincubated eggs, Wood, 1987). Whatever the relationship, *Aplomya* does not appear to clearly belong to any of the recognized tribes and it may merit its own tribal designation. The same may be true of *Carcelia* and relatives (e.g. *Gymnocarcelia*), as has been indicated by Sabrosky & Arnaud (1965) and Cantrell & Crosskey (1989).

Heliodorus was described rather recently (Reinhard, 1964), and though it has been considered an eryciine by D. M. Wood (unpublished), its placement remains uncertain. The present analyses do little to resolve this issue. A close relationship with *Pseudochaeta* (Goniini) is indicated by analyses of the EF-1α data, whereas reconstructions based on 28S rDNA invariably group *Heliodorus* with *Siphosturmia* or other macrotype eryciine genera. It is unknown whether it possesses macrotype or microtype eggs, and only male specimens were available for examination. Given this uncertainty, I have attempted to be conservative in analyses (EF-1α) that compare trees in which Goniini are constrained to be monophyletic to unconstrained trees by including *Heliodorus* in that clade.

Goniini (and microtype eggs). In the majority of trees, a clade is present that consists of *Leschenaultia*, *Mystacella*, *Chrysoexorista* and the two *Frontiniella* species (EF-1α ML, NJ, MP; 28S Elision, NJ), with or without the addition of an eryciine near *Leschenaultia*. The remaining microtype taxa are more variable in composition and arrangement, although the combined analysis reconstructs them as a clade. The two representatives of the former Chaetogaediini, *Chaetogaedia* and *Gaediopsis*, are always reconstructed as sister groups when they are both present (28S), and they are often united with *Gonia* (28S, EF-1α NJ; combined). *Hyphantrophaga* (two species) and *Patelloa* usually form a sister-group association, as well. The relationships between these various doublets are, however, quite variable and they often involve members of Eryciini as well. In several analyses, *Pseudochaeta* leaves the G-E group all together and becomes allied with Blondeliini (28S MP, NJ, some ML). This taxon's mobility, often-basal position within Goniini, and association with the presumed eryciine *Heliodorus*, may reflect the divergence of this taxon and other members of Sabrosky & Arnaud's (1965) Eumasicerini from the more typical microoviparous forms.

Prior to Herting's (1960, 1984) aggregation of the microtype (or microoviparous) Exoristinae into a single tribe (Goniini), they were widely distributed among several

tribes, some of which contained both macro- and microoviparous taxa (e.g. Sturmiini, Eryciini, Carceliini; Sabrosky & Arnaud, 1965). Wood (1987) and O'Hara & Wood (unpublished classification) have more or less adopted Herting's scheme for the Nearctic Tachinidae. Despite the reliance on the presence of microtype eggs as a uniting synapomorphy of Goniini in the recent classificatory schemes of Herting (1960, 1984) and Wood (1987), all analyses in the current study indicate at least three changes in this character complex (Table 4). If *Heliodorus* is found to possess microtype eggs, a minimum of two changes are still required on any tree recovered from the current analyses.

Undoubtedly, this oviposition strategy requires extreme alterations in the morphology and physiology of eggs (see Clausen, 1940), and behaviour of the female. Thus, it would seem difficult for this complex character to evolve independently in multiple lineages. However, several genera of Blondeliini (e.g. *Anisia*) parasitize hosts by depositing minute eggs on the food of the host that are ingested (Wood, 1985), indicating that the general strategy has most likely evolved at least twice. It is also possible that the character may have been lost in certain taxa. Presumably, the microoviparous habit evolved as a strategy for attacking nocturnal or otherwise inaccessible hosts. Many taxa with microtype eggs are currently known to attack diurnal exposed hosts, and perhaps reversions to ancestral egg morphology have occurred in these lineages. Further phylogenetic analysis with denser taxon sampling of the G-E crown group, and a comparative analysis of egg morphology is needed to fully resolve this issue.

Conclusions

The current study represents the first modern phylogenetic analysis of Exoristinae, as well as Tachinidae as a whole. Although many nodes are only weakly supported, analyses of the two molecular datasets generally support the recent classification systems of Herting (1984) and Wood (1987) that are based in part on reproductive characters of females (Fig. 11). However, there are some important exceptions to this generality. These include the failure of the outgroup relationships to support a monophyletic Phasiinae or Tachininae, the placement of *Masiphya* either outside Exoristinae or in a clade sister to Exoristini, and the reconstruction of *Aplomya* and *Phyllophilopsis* outside the tribes that they have been placed in by Herting (1984), Tschorsnig (1985) and O'Hara & Wood (unpublished classification). Perhaps the most important difference between the present phylogenetic study and the classification proposed by Herting (1984) is the lack of monophyly for those taxa possessing microtype eggs, and the suggestion that this character complex may have arisen multiply or been lost in some taxa. In addition, this study has presented new hypotheses concerning the relationships among many taxa, including the close relationship between *Myiopharus* and *Zaira*-group blondeliines, and the paraphyly of *Chetogena* with respect to *Exorista*. Hopefully, the hypotheses of relationships presented here will stimulate further investigation of the higher level relationships among Tachinidae and the evolution of their remarkable oviposition strategies.

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