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Placement of Conopidae (Diptera) within Schizophora based on mtDNA and nrDNA gene regions

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ABSTRACT

The first attempt to phylogenetically place Conopidae using molecular characters, as well as the largest molecular analysis of relationships within Schizophora (Diptera) to date, is presented. Twenty-eight taxa from 11 acalyptrate families and seven acalyptrate superfamilies are represented. Nearly 12,800 bp of sequence data from 10 genes representing both mitochondrial (cytochrome oxidase I (COI), cytochrome *b* (cytB), and 12S) and nuclear genes (28S, the carbamoyl phosphate synthetase region of CAD (CAD), elongation factor-1 α (EF-1 α), *white*, alanyl-tRNA synthetase (AATS), triose phosphate isomerase (TPI), and phosphogluconate dehydrogenase (PGD)) are analysed. Parsimony and Bayesian analyses strongly support the monophyly of both Conopidae and Schizophora. While in the parsimony analysis, Conopidae are placed as sister to the remaining Schizophora, the Bayesian analysis recovers a Conopidae + Lauxaniidae clade. The value of nuclear, mitochondrial, ribosomal, and protein-coding gene sequence data for answering phylogenetic questions at different levels of divergence is evaluated.

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1. Introduction

Conopidae are a fascinating family commonly referred to as thick-headed flies. They are found in every part of the world excluding Pacific Islands and Antarctica. While the biology of adults is poorly known, larvae are obligatory endoparasitoids of other insects. Host records have been reported for approximately 5% of described species, most of which are parasitoids of bees and wasps (Freeman, 1966; Smith, 1966). Members of one subfamily, Stylogasterinae, attack and oviposit on cockroaches and crickets, which, in some recorded cases, are flushed out of underbrush by advancing ant armies (Aldrich, 1930; Woodley and Judd, 1998). As the impact of their role as pollinators is suspected to be minimal (Maeta and Macfarlane, 1993), the largest economic impact of Conopidae is likely to be their role as parasitoids of honeybees (*Apis mellifera* Linnaeus, 1758) and other hymenopteran pollinators (Freeman, 1966; Mei, 1999; Severin, 1937; Smith, 1966).

Conopidae are typically organised into four subfamilies: Conopinae, Dalmanniinae, Myopinae, and Stylogasterinae (e.g., Chvála and Smith, 1988; Smith, 1989). The subfamily Stylogasterinae has been proposed as a separate family (Rohdendorf, 1964; Smith and

Cunningham-Van Someren, 1985) based on its unusual biology and distinct morphology. Within the subfamily Myopinae, the tribe Zodionini has been proposed as a separate subfamily based on its singly geniculate proboscis (Hendel, 1936). These proposed changes to the classification of Conopidae have been neither widely accepted nor tested phylogenetically.

Early classifications placed Conopidae within Syrphoidea based on adult morphological similarities (reviewed in De Meijere, 1904). The presence of a ptilinum in Conopidae, however, refutes this hypothesis, as two independent origins of this structure seem unlikely. The ptilinum is a complex facial structure that helps with eclosion from the puparium (Hennig, 1973). A single lineage with a synapomorphic ptilinum (“Schizophora”) is a more likely explanation (McAlpine, 1989). The question of how Conopidae is related to the remaining schizophoran families, however, has been the source of some debate.

Enderlein (1936) proposed Conopidae as sister to the remaining schizophoran flies (“Muscaria”). Hennig (1958, 1966, 1971) supported this conclusion but noted a lack of unequivocal synapomorphies for “Muscaria”. A recent hypothesis based on morphological analysis (Korneyev, 2000; McAlpine, 1989) is that the sister group to Conopidae is within one of two schizophoran superfamilies: Tephritoidea or Diopsoidea (Fig. 1). Two recent studies (Han et al., 2002; Han and Ro, 2005) include representatives of Conopidae in molecular phylogenetic analyses of Tephritoidea. Both of these studies place Conopidae + Diopsoidea as sister to remaining schizophoran exemplars (Fig. 1). Thus, at least two competing

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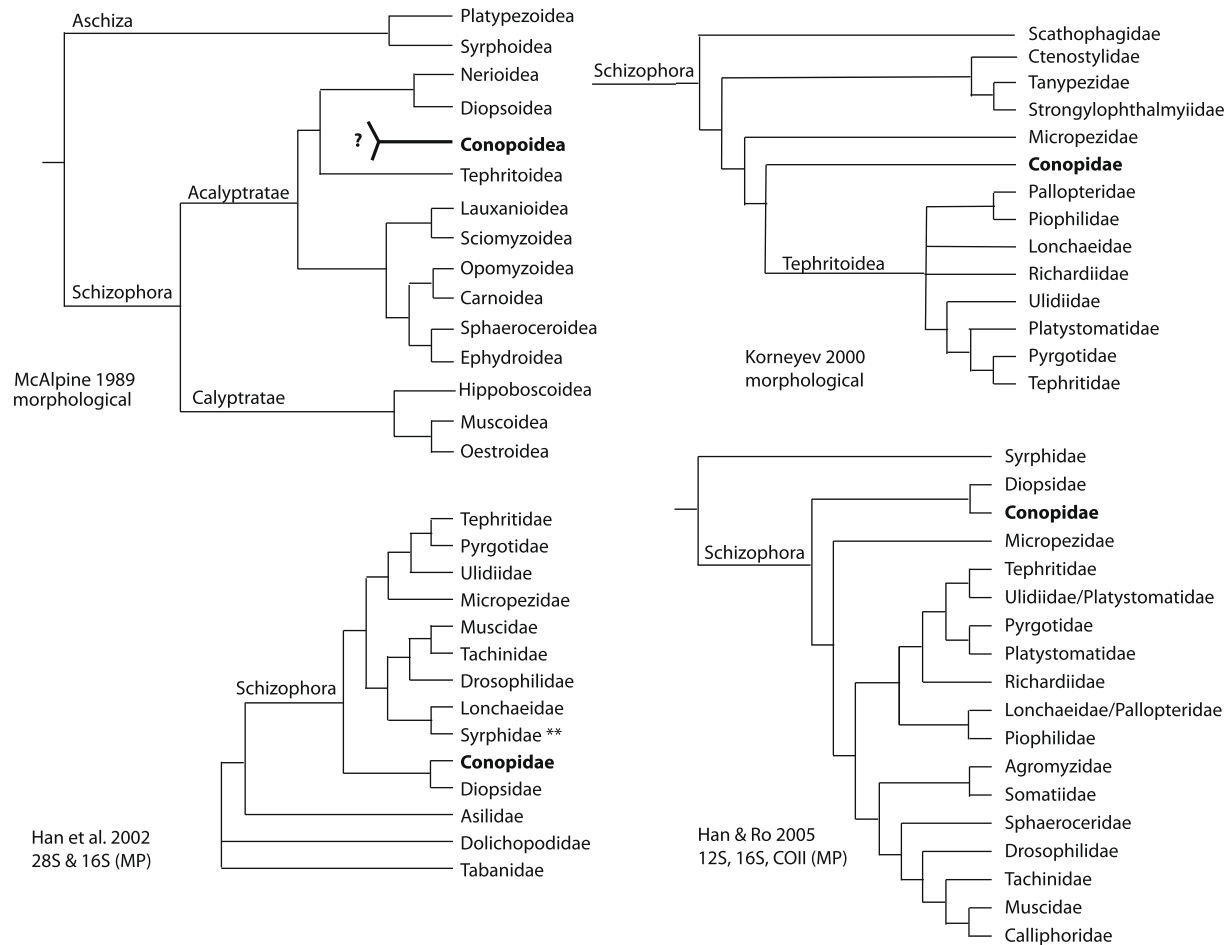


Fig. 1. Phylogenetic hypotheses of schizophoran relationships including examples of Conopidae. **Syrphidae is not classified under Schizophora although it was recovered as such in Han et al., 2002.

hypotheses are present for the placement of Conopidae within Schizophora. Determining support for one of these hypotheses, or an alternate hypothesis, is necessary for future phylogenetic work within schizophoran groups. Whatever the placement of Conopidae may be it will help to establish a ground plan for characters within Schizophora. In Hennig's (1971) approximation, the placement of Conopidae, represented one of the two most pressing issues in Schizophoran systematics, the other being the uncertain monophyly of the two schizophoran subgroupings, Acalyptratae and Calyptratae.

A recent review of dipteran phylogenetics (Yeates and Wiegmann, 1999) emphasises the need for superfamily-level relationships within Schizophora and Acalyptratae to be clarified through the use of new approaches. In both previous molecular phylogenetic studies including Conopidae specimens (Han et al., 2002; Han and Ro, 2005), the authors admit a lack of sufficient ingroup and outgroup sampling and conclude that the sequencing of further genes, including nuclear protein-coding genes, is necessary for future dipteran molecular phylogenetic studies. Other researchers (Collins and Wiegmann, 2002a,b; Kutty et al., 2008; Moulton and Wiegmann, 2004, 2007; Petersen et al., 2007; Wiegmann et al., 2000; Winterton et al., 2007) have utilised DNA sequences as characters in phylogenetic analyses of Diptera at the family or superfamily level. It should be noted, however, that each of these studies focused on a single family or superfamily ingroup and were not intended as comprehensive analyses of dipteran, schizophoran, or acalyptrate relationships as a whole. There has been a trend of both increasing number of nucleotide characters and increasing

percentage of phylogenetically informative characters. There exists, however, a lack of overlap in genes of choice that significantly hampers the future possibility of data combination. The nuclear ribosomal gene, 28S, appears to be the only gene approaching universal usage in dipteran phylogenetic studies.

Two previous studies (Petersen et al., 2007; Winterton et al., 2007) compared the phylogenetic information contributed by the genes used in their studies using partitioned Bremer support (PBS) values. Cameron et al. (2007) used complete mitochondrial genomes of a number of Diptera to assess the relative informative value of all mitochondrial gene sequences. There is, however, no consensus as to which genes, either mitochondrial or nuclear, provide the highest phylogenetic signal at a given level of divergence within the Diptera. Especially lacking has been the determination of which gene(s) have evolved on a timescale that comprise sufficient levels of phylogenetically informative changes to adequately resolve the radiation of Schizophora. While comparisons of the phylogenetic utility of mitochondrial versus nuclear gene sequence characters (e.g., Baker et al., 2001; Light and Reed, 2009; Lin and Danforth, 2004) and ribosomal versus protein-coding gene sequence characters (e.g., Almeida and Danforth, 2009; Danforth et al., 2005; Mueller, 2006) have been completed for other animal groups, such a comparison using dipteran examples is necessary.

The primary purposes of this study are to test the hypothesis of a monophyletic Conopidae and to test past hypotheses for the placement of Conopidae within Schizophora. To this end, a selection of taxa representing all five proposed conopid subfamilies are presently examined using molecular phylogenetic techniques.

Table 1
List of taxa included in present study with GenBank accession numbers. Superfamily classifications follow [McAlpine, 1989](#).

Taxa	Species	Author	Geographic origin	Voucher #	COI	CytB	12S	28S	AATS	CAD	EF1 α	PGD	tpi	White
Empidoidea														
Brachystomatidae	<i>Heterophlebus versabilis</i>	Collin (1933)	Chile	2740	HM062531	N/A	HM062581	HM062609	HM062635	HM062728	HM062658	HM062752	HM062682	HM062718
Aschiza														
Platypezoidea														
Platypezidae	<i>Platypeza</i> sp.		USA (NC)	2753	HM062540	HM062563	HM062590	HM062618	HM062644	HM062736	HM062666	HM062759	HM062691	N/A
Lonchopteridae	<i>Lonchoptera tristis</i>	Meigen (1824)	Denmark	2747	HM062534	HM062558	HM062584	HM062612	HM062638	HM062731	HM062661	HM062754	HM062687	HM062704
Phoridae	<i>Conicera (Conicera) dauci</i>	Meigen (1830)	Canada (QC)	2748	HM062538	HM062562	HM062588	HM062616	HM062642	HM062735	HM062664	HM062757	HM062688	N/A
Syrphoidea														
Syrphidae	<i>Toxomerus marginata</i>	Meigen (1822)	Canada (ON)	2752	HM062546	HM062568	HM062596	HM062624	HM062649	HM062742	HM062672	HM062762	HM062690	HM062701
Pipunculidae	<i>Pipunculus</i> sp. nov.		USA (NM)	2744	HM062539	N/A	HM062589	HM062617	HM062643	N/A	HM062665	HM062758	HM062686	HM062699
Schizophora														
Acalypratae														
Conopoidea														
Conopidae														
Conopinae	<i>Physocephala marginata</i>	Say (1823)	Canada (QC)	1680	N/A	N/A	HM062570	HM062599	HM062626	HM062719	HM062651	HM062744	HM062674	HM062716
	<i>Leopoldius coronatus</i>	Rondani (1857)	Germany	2481	HM062522	HM062548	HM062571	HM062598	HM062627	HM062720	N/A	HM062745	HM062679	HM062707
Dalmaniinae	<i>Dalmannia nigriceps</i>	Loew (1866)	Canada (ON)	2259	HM062526	HM062551	HM062576	HM062604	HM062630	HM062725	HM062656	N/A	HM062676	HM062702
	<i>Dalmannia vitiosa</i>	Coquillett (1892)	Canada (QC)	2469	HM062527	HM062552	HM062577	HM062605	HM062631	HM062726	N/A	N/A	HM062678	HM062697
Myopinae	<i>Myopa vesiculosa</i>	Say (1823)	Canada (QC)	2460	HM062523	HM062549	HM062572	HM062600	N/A	HM062721	HM062652	HM062746	HM062677	HM062714
	<i>Thecophora occidentis</i>	Walker (1849)	Canada (ON)	2731	HM062524	HM062550	HM062573	HM062601	HM062628	HM062722	HM062653	HM062747	N/A	N/A
Stylogasterinae	<i>Stylogaster neglecta</i>	Williston (1883)	USA (NM)	2202	HM062528	HM062553	HM062578	HM062606	HM062632	N/A	N/A	HM062749	HM062675	HM062713
	<i>Stylogaster stylata</i>	Fabricius (1805)	French Guyana	2606	HM062529	HM062554	HM062579	HM062607	HM062633	N/A	N/A	HM062750	HM062681	HM062705
Zodioninae	<i>Zodion fulvifrons</i>	Say (1823)	Canada (QC)	2457	N/A	N/A	HM062574	HM062603	N/A	HM062723	HM062654	N/A	N/A	HM062710
	<i>Zodion cinereum</i>	Fabricius (1794)	Italy	2492	HM062525	N/A	HM062575	HM062602	HM062629	HM062724	HM062655	HM062748	HM062680	HM062703
Nerioidea														
Micropezidae	<i>Taeniptera trivittata</i>	Macquart (1835)	USA (SC)	2758	HM062535	HM062559	HM062585	HM062613	HM062639	HM062732	HM062662	HM062755	HM062693	HM062709
Diopsoidea														
Psilidae	<i>Chyliza scrobiculata</i>	Melander (1920)	Canada (BC)	2719	HM062542	N/A	HM062592	HM062620	HM062646	HM062738	HM062668	N/A	N/A	HM062711
Strongylophthalmyiidae	<i>Strongylophthalmyia angustipennis</i>	Melander (1920)	Canada (ON)	2741	HM062545	HM062567	HM062595	HM062623	HM062648	HM062741	HM062671	N/A	HM062683	HM062698
Tephritoidea														
Platystomatidae	<i>Lamprogaster nigripes</i>	Macquart (1851)	Australia (QD)	2743	HM062541	HM062564	HM062591	HM062619	HM062645	HM062737	HM062667	HM062760	HM062685	HM062715
Tephritidae	<i>Campiglossa pygmaea</i>	Novak (1974)	USA (OR)	2745	HM062547	HM062569	HM062597	HM062625	HM062650	HM062743	HM062673	HM062763	N/A	N/A
Pyrgotidae	<i>Pyrgota undata</i>	Wiedemann (1830)	Canada (ON)	1527	HM062543	HM062565	HM062593	HM062621	N/A	HM062739	HM062669	HM062761	N/A	HM062696

(continued on next page)

Table 1 (continued)

Taxa	Species	Author	Geographic origin	Voucher #	COI	CytB	12S	28S	AATS	CAD	EF1 α	PGD	tpi	White
Palloppteridae	<i>Toxoneura superba</i>	Loew (1861)	Canada (ON)	2751	HM062537	HM062561	HM062587	HM062615	HM062641	HM062734	HM062663	HM062756	N/A	HM062712
Lauxaniioidea Lauxaniidae	<i>Minettia lupulina</i>	Fabricius (1787)	USA (OR)	2746	HM062532	HM062556	HM062582	HM062610	HM062636	HM062729	HM062659	HM062753	N/A	HM062706
Lauxaniidae	<i>Melanina</i> sp.		Australia (QD)	495	HM062533	HM062557	HM062583	HM062611	HM062637	HM062730	HM062660	N/A	HM062694	HM062708
Sphaerocerioidea Sphaeroceridae	<i>Rachispoda</i> sp.		Canada (ON)	2750	HM062544	HM062566	HM062594	HM062622	HM062647	HM062740	HM062670	N/A	HM062689	HM062695
Ephydroidea Drosophilidae	<i>Drosophila</i> sp.		Chile	2742	HM062530	HM062555	HM062580	HM062608	HM062634	HM062727	HM062657	HM062751	HM062684	HM062700
Calyptratae Muscoidea Muscidae	<i>Spilogona</i> sp.		USA (OR)	2756	HM062536	HM062560	HM062586	HM062614	HM062640	HM062733	N/A	N/A	HM062692	HM062717

In addition, exemplar specimens of 17 dipteran families representing eight schizophoran lineages and three non-schizophoran lineages (*sensu* Woodley et al., 2009) are included to determine the sister group to Conopidae (Table 1). The determination of this sister group will facilitate future morphological and molecular research on the phylogenetic relationships within Conopidae as well as within Schizophora and Acalyptratae.

Another purpose of this study is to determine the comparative utility of currently-used and newly-developed mitochondrial and nuclear gene sequences as molecular characters for phylogenetic studies of dipteran families and of Conopidae in particular. To this end, DNA sequences from a total of 11 genes, including mitochondrial genes, nuclear genes, ribosomal genes, and protein-coding genes are examined: 12S ribosomal DNA, cytochrome *c* oxidase subunit I (COI), cytochrome *b* (CytB), 28S ribosomal DNA, the carbamoyl phosphate synthetase region of CAD (CAD), elongation factor-1 α (EF-1 α), *white*, *wingless*, alanyl-tRNA synthetase (AATS), triose phosphate isomerase (TPI), and phosphogluconate dehydrogenase (PGD).

2. Materials and methods

2.1. Taxon sampling

Exemplars of 10 species of Conopidae, representing all five putative subfamilies of Conopidae, (Conopinae, Dalmanniinae, Myopinae, Stylogasterinae, and Zodioninae) are included in the present study (Table 1). Also included are 18 specimens from 17 other dipteran families. These families serve as representatives of 10 superfamilies. *Heterophlebus versabilis* (Collin, 1933) (Brachysomatidae) is used to root all trees.

2.2. DNA extraction and amplification

Total genomic DNA was extracted from whole specimens using a DNeasy[®] Tissue kit (Qiagen Inc., Santa Clara, CA, USA). Following extraction, specimens were critical-point dried and deposited as vouchers in the Canadian National Collection of Insects, Arachnids, and Nematodes (CNC).

For genes amplified using *Taq* polymerase (Table 2), DNA amplifications were carried out in 25 μ L reactions with 17 μ L ddH₂O, 2.5 μ L 10 \times PCR buffer, 2.5 μ L 25 mM MgCl₂, 0.5 μ L of each 10 μ M primer, 0.5 μ L 10 μ M dNTPs, 0.5 μ L *Taq* DNA polymerase (Promega Corp., Madison, WI, USA), and 1 μ L genomic DNA template. For genes amplified using ExTaq polymerase, DNA amplifications were carried out in 25 μ L reactions with 16.75 μ L ddH₂O, 2.5 μ L 10 \times ExTaq PCR buffer (containing 20 mM MgCl₂), 0.625 μ L 25 mM MgCl₂, 1 μ L of each 10 μ M primer, 2 μ L 10 μ M dNTPs, 0.125 μ L ExTaq HS DNA polymerase (Takara Bio USA, Madison, WI, USA), and 1 μ L genomic DNA template. Amplification cycles were performed on an Eppendorf ep Gradient S Mastercycler (Eppendorf AG, Hamburg, Germany). PCR amplification cycles for each gene segment are listed in the Supplementary material Table SM-1.

2.3. DNA sequencing and editing

Amplification products and negative controls were visualised on 1% agarose electrophoresis gels and purified for sequencing using either a QIAquick Gel Extraction kit[®] (Qiagen Inc., Santa Clara, CA, USA) or an ExoSAP-IT[®] protocol (USB Corp., Cleveland, OH, USA). Sequencing of purified products was performed at the Agriculture & Agri-Food Canada, Eastern Cereal and Oilseed Research Centre Core Sequencing Facility (Ottawa, ON, Canada). Sequencing reactions were carried out in a volume of 10 μ L and used an ABI BigDye[®] Terminator v3.1 Cycle Sequencing kit

Table 2

Primer oligonucleotides used for PCR amplification of selected gene segments, including original reference for primers and type of polymerase used.

Gene locus	Primer	Sequence	Reference	Polymerase
12S	12Sai	AAACTAGGATTAGATACCCTATTAT	Simon et al. (1994)	Taq
	12Sbi	AAGAGCGACGGGCGATGTGT	Simon et al. (1994)	Taq
COI	Pat	TCCAATGCACTAATCTGCCATATTA	Simon et al. (1994)	Taq
	Jerry	CAACATTATTTTGATTTTTTGG	Simon et al. (1994)	Taq
	hebF (LCO1490)	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)	Taq
	hebR (HCO2198)	TAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)	Taq
	MLepF	GCTTCCACGAATAAATAATA	Hajibabaei et al. (2006)	Taq
	MLepR	CCTGTCCAGCTCCATTTTC	Hajibabaei et al. (2006)	Taq
CytB	10933F	TATGTTTTACCTTGAGGACAAATATC	Simon et al. (1994)	ExTaq
	11683R	AAATTCATCTTATGTTTTCAAAC	Simon et al. (1994)	ExTaq
AATS	A1x40F	GNATGAAYCARITTYAARCCNAT	B. Wiegmann (pers. comm.)	ExTaq
	rA1x244	CATNCCRCARTCNATRTGYTT	B. Wiegmann (pers. comm.)	ExTaq
EF1 α	M44-1	GCTGAGCGYGARCGTGGTATCAC	Cho et al. (1995)	ExTaq
	rcM4	ACAGCVACKGYTYGYCTCATRTC	Cho et al. (1995)	ExTaq
	2477–2495 (S)	CTTGCTTTCACHTTGGGTG	Baker et al. (2001)	ExTaq
	2934–2954 (A)	CTTCGTGATGCATTTCACCGG	Baker et al. (2001)	ExTaq
white	11404–11426 (S)	TGYCNTATGTNRCARCARGAYGA	Baker et al. (2001)	ExTaq
	11975–11997 (A)	ACYTGNACRTAAAARTCNGCNGG	Baker et al. (2001)	ExTaq
wingless	LepWG1F	GARTGYAARTGYCAYGGYATGTCTGG	Brower and DeSalle (1998)	ExTaq
	PompWG2R	ACTGCGCAGCACCAAGTGAATGTGCA	Pilgrim et al. (2008)	ExTaq
TPI	tpi111FBb	GGNAAYTGAAKATGAAYGG	Bertone et al. (2008)	ExTaq
	tpi275R	GCCANACNGGYTCRTANGC	Bertone et al. (2008)	ExTaq
PGD	pgd2F	GATATHGARTAYGGNGAYATG	Bertone et al. (2008)	ExTaq
	pgd3R	GTRTGNGCNCRAARTARTC	Bertone et al. (2008)	ExTaq
	pgd4R	CCNGTCCARTTNGTRTG	Bertone et al. (2008)	ExTaq
28S	rc28AB	ACTACCCCTGAATTTAAGCA	Bertone et al. (2008)	Taq
	28C	GCTATCTGAGGAAACTTCGG	Bertone et al. (2008)	Taq
	rc28B	CCCGTCTGAAACACGGACC	Bertone et al. (2008)	Taq
	28E	CCTTATCCCGAAGTTACG	Wiegmann et al. (2000)	Taq
	rc28D	CCGCAGCTGGTCTCCAAG	Wiegmann et al. (2000)	Taq
	28I	GGGTCTTCTTCCCGCT	B. Wiegmann (pers. comm.)	Taq
	rc28F	GTGATTTCTGCCAAGTCTCTG	B. Wiegmann (pers. comm.)	Taq
	28Q	AACTCCCTACTGGCAAT	Collins and Wiegmann (2002a)	Taq
	28 K	GAAGAGCCGACATCGAAG	Wiegmann et al. (2000)	Taq
	rc28Q	GGACATTGCCAGGTAGGGAGTT	Wiegmann et al. (2000)	Taq
	28ZC	TGGATCGCAGTATGCCAGCT	Bertone et al. (2008)	Taq
	CAD	54F	GTNGTNTTYCARACNGGNATGGT	Moulton and Wiegmann (2004)
364R		TCNCANGCRAANCCRTGRTTYTG	Moulton and Wiegmann (2004)	ExTaq
405R		GCNGTRTGYTCNGGRTGRAAYTG	Moulton and Wiegmann (2004)	ExTaq
338F		ATGAARTAYGGYAATCGTGGHAYAA	Moulton and Wiegmann (2004)	ExTaq
680R		AANGCRTCNCCNACMACYTCRTAYTC	Moulton and Wiegmann (2004)	ExTaq
581F2		GGWGGWCAAACWGCWYTMAYTYGGG	Moulton and Wiegmann (2004)	ExTaq
843R		GCYTTYGRAANGCYTYCRAA	Moulton and Wiegmann (2004)	ExTaq
787F		GGDGTNACNACNGCNTGYTTYGARCC	Moulton and Wiegmann (2004)	ExTaq
1098R		TTNGGNAGYTGNCNCCCAT	Moulton and Wiegmann (2004)	ExTaq
1057F		GTNACNACNGAYTAYGAYATGTG	Moulton and Wiegmann (2004)	ExTaq
1278R		TCRTTNTTYTWWGCRATYAAYTGCAT	Moulton and Wiegmann (2004)	ExTaq
1436R		CCRTGYTCNGCRTARAARTC	Moulton and Wiegmann (2004)	ExTaq

(PE Applied Biosystems, Foster City, CA, USA). Sequencing reactions were purified using the ABI ethanol/EDTA/sodium acetate precipitation protocol and analysed on an ABI 3130xl Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA). All sequence chromatograms were edited and contigs formed using Sequencher 4.7 (Gene Codes Corp., Ann Arbor, MI, USA).

2.4. Sequence alignment

2.4.1. Ribosomal genes

ClustalX (Thompson et al., 1997) with default settings was used to produce an alignment for 12S. 28S was aligned by hand according to the secondary structure model proposed by Kjer et al. (1994) for *Aedes albopictus* (Skuse, 1894) and by Hancock et al. (1988) for *Drosophila melanogaster* Meigen, 1830. The region amplified corresponds to almost the entire length of the 28S gene. Expansion segments that matched the location of those listed by Kjer et al. (1994)

and Hancock et al. (1988) were identified for the 28S matrix. While 10 of these expansion segments could be aligned across all taxa, the three largest segments (D2, D8, and D10 – 946 base pairs (bp) in total) could not be unambiguously aligned and were excised from the matrix.

2.4.2. Protein-coding genes

The remaining genes are protein-coding and were aligned manually according to translated amino acid codons with the following notes. For CAD, the region amplified corresponds to the entire carbamoyl phosphate synthetase region (CPS) of the CAD gene (Moulton and Wiegmann, 2004). Three introns were identified, occupying positions 871–1041, 1147–1245, and 4042–4112 in our alignment. They were excluded from analysis. For PGD, one intron corresponding to position 538–610 in our alignment was identified and excised from the matrix prior to analysis. For TPI, a 50 bp intron located at position 179–229 of our matrix was

identified for only one taxon, *Heterophlebus versabilis* (Brachystomataidae). It was excluded from the analysis. The *white* sequence amplified corresponds with the 3' end of exon 3 (Bennett and Frommer, 1997). The final ~150 bp of all sequences were hyper-variable and unalignable. This segment corresponds to the 5' end of intron 3 and was excluded from analysis.

2.5. Parsimony analyses

Parsimony analyses were conducted using PAUP* 4.0 (Swofford, 2003). All characters were treated as unordered. An heuristic search with tree bisection-reconnection (TBR) branch swapping in a random stepwise addition of taxa was repeated 100 times. Two weighting schemes, the first with equal weighting and the second with the third codon position (nt3) weighted to zero were explored for the combined data set. Likewise, gaps were treated as either missing or as a fifth state for the combined dataset. For all individual genes and concatenation subsets, nt3 were weighted to zero and gaps were treated as a fifth state. Node support for individual genes and all concatenations was determined by jackknife resampling with 36% of characters excluded and 100 random replicates. PBS values for the combined dataset (nt3 weighted to zero, gaps as fifth state) were calculated for each gene partition using TreeRot v3 (Sorenson and Franzosa, 2007) and PAUP* 4.0 (Swofford, 2003) using an heuristic search and 100 random replicates. Topological congruence between most parsimonious tree(s) of concatenated subsets and the most parsimonious tree of the total evidence dataset was calculated with the Congruent Topological Information (CTI) statistic of Almeida and Danforth (2009). This statistic gives the proportion of nodes in the most parsimonious total evidence tree recovered by the most parsimonious tree(s) of a given subset.

2.6. Bayesian analysis

Models of evolution were determined based on the Akaike Information Criterion (AIC) for each gene partition separately using ModelTest 3.7 (Posada and Crandall, 1998). Bayesian analyses on the total evidence dataset were conducted using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) with a Markov Chain Monte Carlo (MCMC) method. The dataset was partitioned into 10 genes and parameter estimation was unlinked for each gene partition. Four chains (three hot, one cold) were run simultaneously for 20,000,000 generations. Trees were sampled every 1000 generations and each simulation was run twice. The MCMC chains achieved stationarity (standard deviation of split frequencies <0.01; all parameter estimates asymptotic) at 316,000 generations. Following the discard of the first 316 samples as burn-in, 19,685 samples were used for each simulation to generate a majority-rule consensus tree, posterior probabilities for each node, and branch length estimates.

3. Results

3.1. *wingless*

The *wingless* gene has been used successfully in past phylogenetic analyses of schizophoran flies (e.g., Baker et al., 2001; Kotrba and Balke, 2006; Marshall et al., 2009). A pair of primers used in an analysis of Vespoidea (Hymenoptera) (Pilgrim et al., 2008) was chosen to amplify the gene for the present taxa (Table 2). In total, ~130 bp at the 5' terminus and ~200 bp at the 3' terminus were alignable for all taxa. The central 800 bp of sequence was unalignable across all taxa. Some individual taxa included multiple ~100 bp introns. Furthermore, a BLAST search of the recovered *wingless* se-

quences revealed similarities to widely disparate taxa from Orthoptera to Lepidoptera to Hymenoptera for most sequences. Also, four sequences revealed a similarity to the *wnt-4* gene. This result speaks to the established orthologous nature of the *wingless* gene. With up to five different orthologs likely present in the dipteran genome (Sidow, 1992), homology cannot be established with any confidence and all data from *wingless* were excluded from further analysis.

3.2. Individual gene sequence characteristics and parsimony analyses

Gene segments were successfully amplified and sequenced for between 20 (PGD) and 28 (12S and 28S) taxa for each gene (Tables 1 and 3). The lengths of the sequences included in analyses range from 374 bp (12S) to 3824 bp (CAD) (Table 3). The percentages of characters that are constant range from 30.4% (PGD) to 69.9% (28S). The percentages of characters that are phylogenetically informative range from 17.4% (28S) to 62.9% (PGD). Base frequencies reveal an A/T bias in all genes, ranging from 52% (*white*) to 76.9% (12S). Base frequencies are heterogeneous across all taxa for each of the eight protein-coding genes ($\chi^2 > 88.8$, $p < 0.05$). Base frequencies are homogeneous across all taxa for each of the two ribosomal genes ($\chi^2 < 55$, $p > 0.05$). The shape parameter of the gamma distribution (alpha), as calculated by ModelTest 3.7, ranges from 0.233 (COI) to 0.858 (CAD).

Parsimony analysis recovered between one (28S, AATS, *white*) and 56 (EF-1 α) most parsimonious trees for each gene (all trees not shown). Lengths of shortest trees range from 213 (EF-1 α) to 3158 (28S) (Table 3). Consistency Index (CI) values for most parsimonious trees range from 0.414 (COI) to 0.563 (EF-1 α). Retention Index (RI) values for most parsimonious trees range from 0.369 (COI) to 0.581 (EF-1 α). Also included in Table 3 are PBS values and jackknife support (JKS) values for each gene and for each node concordant with a node in the most parsimonious total evidence tree (Fig. 2: nt3 weighted to zero; gaps coded as fifth base).

3.3. Concatenated sequence subset characteristics and parsimony analyses

Concatenated subsets were analysed for mitochondrial DNA (mtDNA – COI, cytB, and 12S), nuclear DNA (nrDNA – 28S, AATS, CAD, EF1 α , PGD, TPI, and *white*), ribosomal DNA (rDNA – 12S and 28S), and protein-coding DNA (PCG – COI, cytB, AATS, CAD, EF1 α , PGD, TPI, and *white*). For each subset analysed all taxa were included and between 2587 (mtDNA) and 10211 (nrDNA) base pairs were included in analysis (Table 3). The percentage of characters that are constant range from 47% (PCG) to 66.8% (rDNA). The percentage of characters that are phylogenetically informative range from 20% (rDNA) to 45.4% (PCG). Base frequencies reveal an A/T bias in all subsets, ranging from 56.3% (nrDNA) to 64.2% (mtDNA). Base frequencies are heterogeneous across all taxa for mtDNA, nrDNA, and PCG ($\chi^2 > 645$, $p < 0.01$). Base frequencies are homogeneous across all taxa for rDNA ($\chi^2 = 52.2$, $p = 0.995$). The shape parameter of the gamma distribution (alpha), as calculated by ModelTest 3.7, ranges from 0.524 (rDNA) to 0.557 (mtDNA).

Parsimony analysis recovered either one (mtDNA, rDNA, PCG) or three (nrDNA) most parsimonious trees (all trees not shown). Lengths of shortest trees range from 2436 (mtDNA) to 7713 (nrDNA) (Table 3). Consistency Index (CI) values for most parsimonious trees range from 0.408 (mtDNA) to 0.485 (nrDNA). Retention Index (RI) values for most parsimonious trees range from 0.334 (mtDNA) to 0.394 (nrDNA). Also included in Table 3 are PBS and JKS values for each subset and for each node concordant with a node in the most parsimonious total evidence tree (Fig. 2: nt3 weighted to zero; gaps coded as fifth base).

Table 3
Summary of results for individual and concatenated gene partitions.

	Individual Genes								Concatenations							Total Evidence			
	COI	cytB	12S	28S	AATS	CAD	EF1 α	PGD	TPI	White	mtDNA	nrDNA	rDNA	PCG	Equal/5th	Equal/missing	nt3=0/missing	nt3=0/5th	
# taxa included	26	22	28	28	25	25	23	20	21	24	28	28	28	28	28	28	28	28	
# characters analysed	1495	718	374	3361	553	3824	827	731	453	462	2587	10211	3735	9063	12798	12798	12798	12798	
% characters constant	50.9	44.2	39.5	69.9	46.1	48.0	58.6	30.4	33.8	49.1	47.4	54.1	66.8	47.0	52.8	54.5	54.5	52.8	
% characters informative	40.7	46.8	42.7	17.4	47.7	44.6	33.4	62.9	57.6	44.9	42.7	36.8	20.0	45.4	38.0	37.1	37.1	38.0	
Average nucleotide frequencies																			
A	29.5	32.0	37.4	30.8	28.3	29.6	29.4	28.0	29.4	24.8	27.1	30.0	31.6	28.1	29.4	29.4	29.4	29.4	
C	16.6	16.4	8.0	17.5	16.1	20.2	21.3	21.1	18.7	24.7	17.3	18.8	16.4	19.8	18.5	18.5	18.5	18.5	
G	15.3	11.6	15.1	23.6	25.7	25.6	27.0	25.1	29.2	23.3	18.5	24.8	22.6	22.0	23.5	23.5	23.5	23.5	
T	38.5	40.0	39.5	28.1	29.9	24.6	22.2	25.8	22.7	27.2	37.1	26.3	29.4	30.1	28.6	28.6	28.6	28.6	
Alpha	0.233	0.839	0.601	0.447	0.253	0.858	0.443	0.436	0.579	0.750	0.557	0.538	0.524	0.549					
# most parsimonious trees	18	16	10	2	1	1	56	7	4	1	1	3	1	1	1	1	2	1	
Length of shortest tree(s)	908	637	797	3158	382	2170	213	784	549	238	2436	7713	4009	6136	27194	26213	9262	10204	
Consistency Index (CI)	0.414	0.440	0.425	0.493	0.487	0.504	0.563	0.533	0.463	0.475	0.408	0.485	0.473	0.463	0.388	0.385	0.460	0.464	
Retention Index (RI)	0.369	0.380	0.384	0.385	0.556	0.430	0.581	0.468	0.419	0.481	0.334	0.394	0.369	0.386	0.317	0.316	0.372	0.373	
Support for nodes (PBS/JKS)																			
1	–3/–	3/–	8/100	25/100	5/84	0/68	0/–	8/100	11/100	1/68	8/100	50/100	33/100	25/100	100	100	100	58/100	
2	16/100	14/100	9/97	66/100	18/100	0/50	0/–	–1/–	12/100	9/97	39/99	84/100	75/100	68/100	100	100	100	143/100	
3	4/50	3/–	0/–	–3/–	–1/–	2/50	1/–	0/–	2/–	1/–	7/50	0/–	–3/–	10/89	100	99	61	7/57	
4	0/–	0/–	7/89	13/87	0/–	6/77	2/–	3/89	0/–	0/–	7/88	24/100	20/100	11/95	100	100	100	31/100	
5	0/–	–5/–	0/74	51/100	0/–	0/100	0/93	4/–	–6/–	4/–	–5/64	53/100	51/100	–3/98	100	100	100	48/100	
6	–2/61*	1/–	1/–	12/–	0/–	–2/63	4/–	3/66*	–8/–	0/–	0/–	9/74	13/–	–4/–	68	–	73	9/82	
7	0/50	–2/–	0/–	8/67	1/67*	0/56	0/52*	0/–	1/–	1/–	–2/–	11/93	8/57	1/–	100	94	63	9/68	
8	6/100	0/–	21/98	23/100	17/98	0/–	3/–	10/98	15/99	1/99	27/100	69/100	44/100	52/100	100	100	100	96/100	
9	–5/–	12/59*	2/50	5/50	8/–	0/56*	0/52*	–2/–	–5/–	4/–	9/73	10/56	7/67	12/100	65	53	94	19/95	
10	11/–	2/–	2/–	2/–	0/–	1/–	2/–	–2/–	–8/–	0/–	15/–	–5/–	4/–	6/50	–	–	73	10/74	
11	–4/–	2/–	–1/–	19/–	–7/–	2/–	–3/–	0/–	3/–	–4/–	–3/–	10/–	18/–	–11/–	–	–	–	7/50	
12	6/78	4/53	6/50	25/100	–5/–	11/100	–1/76	4/–	0/–	10/81	16/87	44/100	31/100	29/100	100	100	100	60/100	
13	–4/–	2/–	–1/–	19/50	–7/–	2/50	–3/–	0/–	3/–	–4/–	–3/–	10/50	18/55	–11/–	63	–	–	7/50	
14	1/–	1/–	1/–	2/–	–2/–	–3/–	2/–	2/–	–2/–	0/–	3/–	–1/–	3/–	–1/–	–	–	–	2/50	
15	2/–	–3/–	–5/–	19/71	–6/–	6/50	0/–	–2/–	0/–	1/–	–6/–	18/88	14/85	–2/–	–	–	<50	12/87	
16	–4/–	2/–	–1/–	19/–	–7/–	2/50	–3/–	0/–	3/–	–4/–	–3/–	10/–	18/–	–11/–	<50	–	–	7/50	
17	5/–	8/79	3/–	11/–	–5/–	13/58	1/–	0/100	–5/–	1/–	16/–	16/100	14/–	18/99	100	99	99	32/100	
18	4/–	–3/–	0/–	5/93	–2/–	7/50	1/–	–2/–	0/–	–1/–	1/–	8/80	5/89	4/–	58	<50	57	9/72	
19	–4/–	2/–	–1/–	19/–	–7/–	2/50	–3/–	0/–	3/–	–4/–	–3/–	10/–	18/–	–11/–	<50	–	–	7/54	
20	1/–	5/–	3/–	5/50	0/–	–6/–	–2/50	5/–	0/–	–2/–	9/–	0/50	8/50	1/55	–	–	<50	9/65	
21	–3/–	0/–	6/–	6/50	6/–	0/–	3/50	0/50	6/77*	–4/–	3/–	17/98	12/75	8/81	65	<50	73	20/99	
22	0/–	0/–	2/–	10/62	0/–	0/–	1/–	–1/–	1/–	–3/–	2/–	8/70	12/80	–2/–	<50	–	–	10/83	
23	2/–	0/50*	4/–	10/80	0/–	0/–	–1/–	–2/–	–3/–	1/–	6/–	5/83	14/79	–3/–	–	–	–	11/85	
24	2/–	2/–	–7/–	29/94	2/–	1/91	2/50	–9/–	–2/–	0/–	–3/–	23/98	22/99	–2/54	<50	–	73	20/93	
25	–2/–	0/–	0/–	17/81	4/–	–7/–	–1/–	–4/–	6/50	3/–	–2/–	18/91	17/79	–1/–	54	–	–	16/79	
TBS (overall/within Conopidae)	29/16	50/26	59/48	417/200	12/48	37/6	3/8	14/25	27/22	11/21	138/90	501/310	476/248	183/172					

Equal – equal weighting; nt3 = 0 – nt3 weighted to 0; missing – gaps treated as missing; 5th – gaps treated as a fifth state; alpha – shape parameter of the gamma distribution (as calculated by ModelTest 3.7); PBS – partitioned Bremer support; TBS – total Bremer support; JKS – jackknife support values (36% exclusion); nodes not recovered indicated as – nodes depicting relationships within Conopidae highlighted.

* Nodes for which jackknife supports are above 50%, but for which all taxa included in that node for the full dataset are not available for that gene.

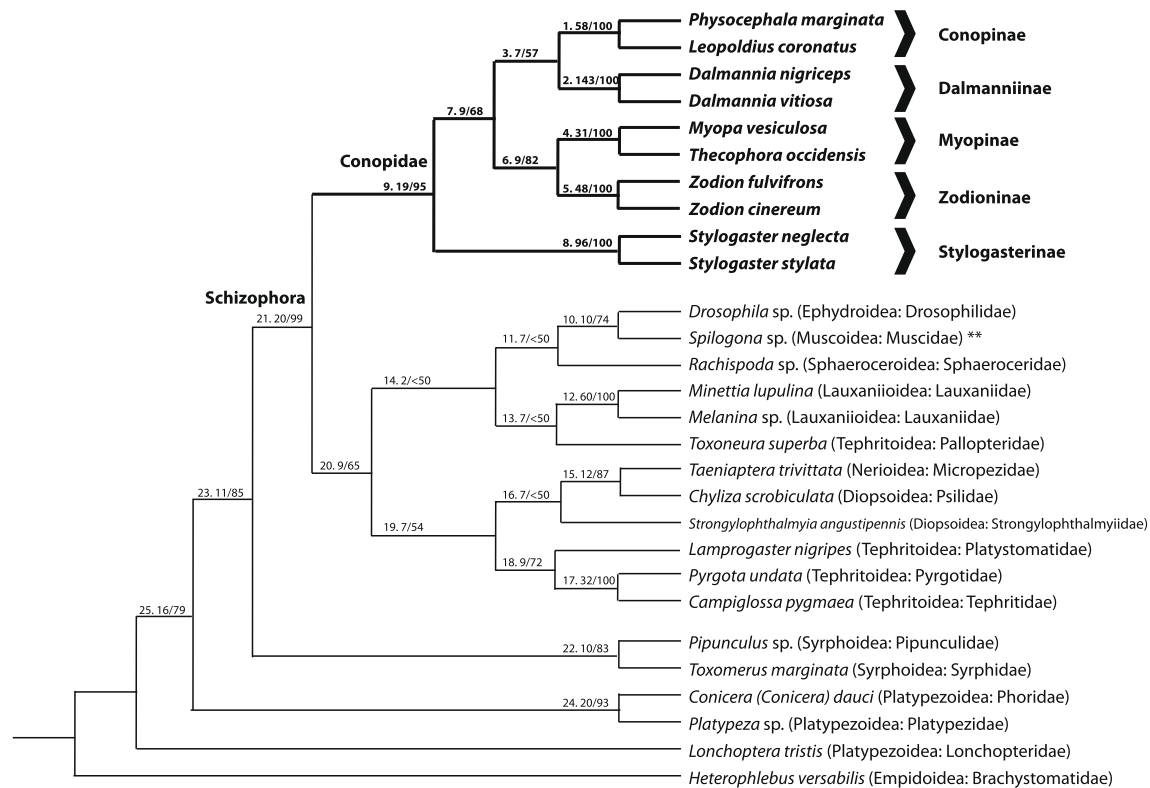


Fig. 2. Single most parsimonious cladogram generated from combined COI, cytB, 12S, 28S, AATS, CAD, EF1 α , PGD, TPI, and white DNA sequence data (treelength = 10,204, CI = 0.464, RI = 0.373). nt3 weighted to zero and gaps coded as a fifth base. Numbers above nodes represent – number of node. Total Bremer support/jackknife support value. Taxon labels include superfamily:family classifications according to McAlpine, 1989. **Indicates the sole representative of Calypttratae included, remaining schizophoran taxa are classified under Acalypttratae (McAlpine, 1989).

3.4. Total evidence parsimony analysis

The complete dataset with all 10 genes included comprises 28 taxa and 12,798 characters (Table 3). Of these characters, 6753 (52.8%) are constant and 4861 (38.0%) are phylogenetically informative. Nucleotide frequencies (A = 29.4%, C = 18.5%, G = 23.5%, and T = 28.6%) reveal an A/T bias common to insect genomes. Base frequencies are heterogeneous across all taxa ($\chi^2 = 140.891$, d.f. = 81, $p < 0.000$). With gaps coded as a fifth base and nt3 weighted to zero, a single most parsimonious tree was found with a length of 10,204 (CI = 0.464, RI = 0.373) (Fig. 2). This tree is well supported with JKS values above 50% for all but two nodes and values of 100% for 10 nodes. Total Bremer support values are likewise high (>5) for all nodes but one. Conopidae is recovered as monophyletic (Fig. 2; node 9) with high support values (PBS = 19; JKS = 95). Three pairs of congeners within Conopidae (*Dalmannia* node 2; *Zodion* node 5; *Stylogaster* node 8) as well as each of the other two conopid subfamilies (Conopinae node 1; Myopinae node 4) are all recovered as monophyletic with high levels of support (PBS > 30; JKS = 100). Schizophora, likewise, is recovered as monophyletic (node 21) with high support values (PBS = 20; JKS = 99). Schizophora excluding Conopidae is recovered as monophyletic (node 20) with weak support values (PBS = 9; JKS = 65). Two representatives of Lauxaniidae are recovered as monophyletic (node 12) with high support values (PBS = 60; JKS = 100). Acalypttratae is recovered as paraphyletic with respect to the sole representative of Calypttratae (Muscidae: *Spilogona* sp.). Of superfamilies proposed by McAlpine (1989) for which multiple exemplars are included, only Syrphoidea (node 22) is recovered as monophyletic (PBS = 10; JKS = 83).

A plot of uncorrected pairwise divergence at nt1 and nt2 versus uncorrected pairwise divergence at nt3 for the entire dataset (not

shown) shows a plateau in sequence divergence in nt3 corresponding to approximately 6% sequence divergence in nt1 and nt2. This plateau corresponds to the divergence within subfamilies of Conopidae and suggests saturation of substitutions in nt3 beyond this level.

Parsimony analysis with gaps coded as missing recovered two most parsimonious trees (treelength = 9262, CI = 0.460, RI = 0.372), but with reduced JKS values at all nodes. Analysis with equal weights for all characters and gaps coded as a fifth base recovered a single most parsimonious tree (treelength = 27,194, CI = 0.388, RI = 0.317), also with JKS values reduced for most nodes. Analysis with equal weights for all characters and gaps coded as missing recovered a single most parsimonious tree (treelength = 26,213, CI = 0.385, RI = 0.316), also with JKS values reduced for most nodes (Table 3). Topologies for these alternate analyses (not shown) differed from the tree shown in Fig. 2. All key clades (i.e., Conopidae, Conopinae, *Dalmannia*, Myopinae, *Zodion*, *Stylogaster*, Lauxaniidae, Schizophora) were recovered as monophyletic in each alternate analysis.

3.5. Subset congruence analysis

Most parsimonious trees of concatenated subsets based on gene type (mtDNA, nrDNA, rDNA, and PCG) recovered varying degrees of congruence with the total evidence most parsimonious tree (Fig. 4). mtDNA recovered 32% of nodes overall, but this was divided between 78% of nodes within Conopidae and just a single node (6%) outside of Conopidae. nrDNA recovered 64% of nodes overall, 67% of nodes within Conopidae, and 63% of nodes outside of Conopidae. rDNA recovered 64% of nodes overall, 67% of nodes within Conopidae, and 63% of nodes outside of Conopidae. PCG

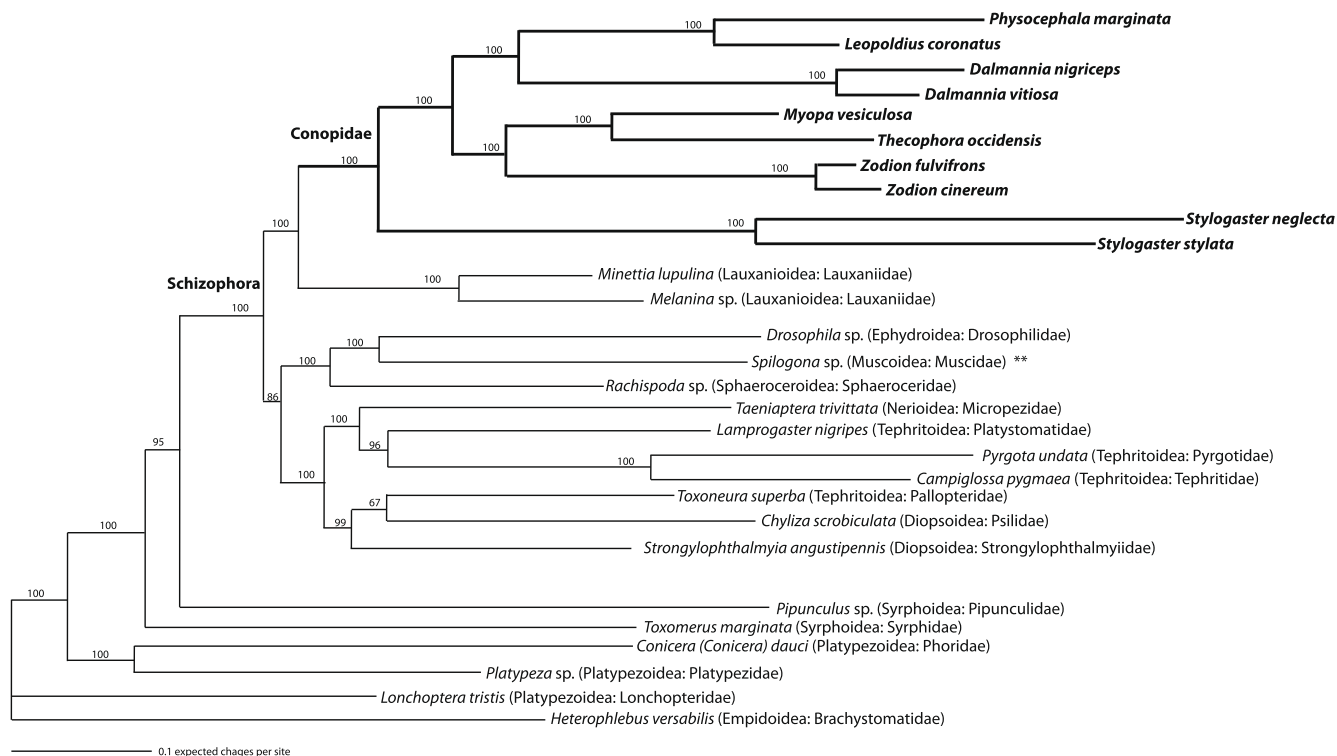


Fig. 3. Majority rule consensus cladogram of Bayesian Markov Chain Monte Carlo analysis (20,000,000 generations) of combined COI, cytB, 12S, 28S, AATS, CAD, EF1 α , PGD, TPI, and white DNA sequence data, including branch lengths. Numbers at each node represent posterior probabilities. Taxon labels include superfamily:family classifications according to McAlpine, 1989. **Indicates the sole representative of Calyptratae included; remaining schizophoran taxa are classified under Acalyptratae (McAlpine, 1989).

recovered 52% of nodes overall, 78% of nodes within Conopidae, and only 38% of nodes outside of Conopidae.

3.6. Bayesian analysis

The tree recovered by Bayesian analysis differs from that of the parsimony analysis and has 100% posterior probabilities (PP) at 20 of 25 nodes (Fig. 3). Conopidae are recovered as monophyletic (PP = 100). Congeners within Conopidae as well as each of the other two conopid subfamilies are all recovered as monophyletic, all with 100% posterior probabilities. Lauxaniidae and Schizophora are both recovered as monophyletic (PP = 100). Schizophora excluding Conopidae is not recovered as monophyletic. Instead, Conopidae + Lauxaniidae is recovered (PP = 100). Acalyptratae is recovered as paraphyletic with respect to the sole representative of Calyptratae (Muscidae: *Spilogona* sp.). None of the superfamilies proposed by McAlpine (1989) for which multiple exemplars are included are recovered as monophyletic.

4. Discussion

4.1. Total evidence and the preferred trees

Recovery and strong support for the monophyly of previously defined taxonomic groups has been suggested as a measure of the strength of a dataset (Wild and Maddison, 2008; Winterton et al., 2007; Yoder et al., 2001), and this method is employed here. Specifically, recovery of congeneric and confamilial nodes with high support values is taken as evidence of a well-resolved tree. The parsimony tree based on the total evidence dataset, with nt3 weighted to zero and gaps coded as a fifth base (Fig. 2) recovers and strongly supports genera within Conopidae (*Dalmannia*, *Zodion*, and *Stylogaster*) and a family within Schizophora (Lauxaniidae).

The Bayesian analysis tree based on the total evidence dataset, with model parameters for each gene estimated individually (Fig. 3) recovers all of the same nodes with high support. No individual gene, data subset, or alternate analysis recovers these key nodes with the same level of support (Table 3). This result supports previous research (e.g., Cameron et al., 2007; Mitchell et al., 2000) indicating that combined molecular datasets will produce more accurate and better-supported trees than individual gene trees. As such, all genes analysed are included in the final total evidence analysis.

4.2. Phylogeny of Schizophora

The monophyly of Schizophora has been established by a number of morphological analyses (Cumming et al., 1995; Griffiths, 1972; McAlpine, 1989) and synapomorphies for the group have been proposed, including the presence of the ptilinum and complete rotation of the male genital capsule within the puparium. Our data supports monophyly of Schizophora with high PBS, JKS, and PP values (Figs. 2 and 3).

Schizophora has been historically divided into two subgroups, Calyptratae and Acalyptratae. While Calyptratae have a number of generally-accepted synapomorphies (McAlpine, 1989), the monophyly of Acalyptratae has been a source of controversy. Morphological analyses (Griffiths, 1972; McAlpine, 1989) suggest little or no support for a monophyletic Acalyptratae. Some molecular analyses (Cameron et al., 2007; Collins and Wiegmann, 2002b; Han and Ro, 2005; Han et al., 2002) have recovered Acalyptratae as paraphyletic with respect to Calyptratae. Other studies (Junqueira et al., 2004; Moulton and Wiegmann, 2004) have recovered Acalyptratae as monophyletic. The focus of all of these studies, however, was on resolving family-level or lower relationships and not the relationships of Acalyptratae overall. As such, they did not include exemplars from across all acalyptrate superfamilies.

Considering the broad family-level diversity of Acalyptratae, these studies should not be considered comprehensive molecular analyses of acalyptrate phylogeny (Woodley et al., 2009). The present study, including 11 of 65 acalyptrate families and seven of 10 acalyptrate superfamilies (*sensu* McAlpine, 1989), represents the largest single sampling of acalyptrate diversity to date, but is still far from comprehensive. Further sampling from still more acalyptrate families will be necessary to resolve the question of acalyptrate monophyly.

Enderlein (1936) first proposed a sister group relationship between “Archischiza” (Conopidae) and “Muscaria” (the remaining Schizophora). Hennig (1958) elaborated on Enderlein’s classification. In his opinion, autapomorphies of Conopidae include a parasitoid lifestyle, peculiar posterior spiracles in the larva, a specialised proboscis, and the development of the female fifth sternite into a theca. Hennig is not clear as to the placement of Stylogasterinae as the females of this subfamily do not possess a theca. Hennig could propose only two vague synapomorphies for Muscaria: a relatively longer anal cell and relatively longer femora of the ptilinal suture. In addition, the number of synapomorphic characters of the Conopidae that actually represent plesiomorphies of the Schizophora was not speculated. Subsequent research placed Conopidae as near to Tephritoidea (Korneyev, 2000) or Diopsidae (Han and Ro, 2005), although placement of the Conopidae was not the goal of these studies.

The sister group relationship between Conopidae and Diopsidae as proposed by Han et al. (2002) and Han and Ro (2005) cannot be directly tested with the data presented here. Rejection of this proposed relationship is possible based on the inclusion of two other members of Diopsoidea (Psilidae: *Chyliza scrobiculata* and Strongylophthalmyiidae: *Strongylophthalmyia angustipennis*). These taxa cluster together and are well-removed from Conopidae in both parsimony and Bayesian analyses (Figs. 2 and 3). Likewise, no members of Tephritoidea are recovered as possible sister to Conopidae.

The present data are equivocal on the nearest sister group to Conopidae. The preferred parsimony tree (Fig. 2) supports the original conclusions of Enderlein and Hennig, with Conopidae as sister to the remaining Schizophora (“Muscaria”). Support for the “Muscaria” clade, however, is weak. This clade is not recovered with high support in any other parsimony analysis. Bayesian analysis recovers a sister group relationship between Conopidae and Lauxaniidae with high support (PP = 100). This is a novel hypothesis for the placement of the Conopidae. The topology of the preferred parsimony tree, while not including a Conopidae + Lauxaniidae clade, does entertain the possibility of such a relationship. If three of the most weakly supported nodes (13, 14, and 20) were to be collapsed, a polytomy would be formed that would include Lauxaniidae as one of four possible sister clades to Conopidae. The present study is the first molecular analysis, and the only one of any kind besides McAlpine (1989), to include representatives of both Lauxaniidae and Conopidae. This could explain why a relationship between the two has not yet been proposed. It is important to note that in McAlpine’s (1989) hypothesis, the closest superfamilies to Lauxaniidae are Sciomyzoidea, Carnoidea, and Opomyzoidea (Fig. 1), none of which are included in the present study. Further sampling of these groups will help to further elucidate the relationships within this branch of Schizophora.

4.3. Monophyly of Conopidae

Working only with Neotropical species, Rohdendorf (1964) proposed *Stylogaster* as a separate family. Smith and Cunningham-Van Someren’s (1985) research, including Nearctic, Oriental, Afrotropical, and Australasian species, agreed with the family status of *Stylogasteridae*. Non-aculeate host biology and unique larval

structure are cited as support for separate family status. It is noted, however, that complete data on these features for all Conopidae s.s. (especially Dalmanniinae) is lacking. Hennig’s (1966) analysis of the fossil specimens of Conopidae concluded that, based on their likely age of divergence, *Stylogasteridae* and Conopidae s.s. are as valid as family classifications as many other recognised acalyptrate families. Smith and Peterson (1987) considered similarities between *Stylogaster* and Dalmanniinae strong enough to warrant the maintenance of *Stylogasterinae* as a subfamily within Conopidae. All of these past studies have suggested a sister group relationship between *Stylogaster* (either as family or subfamily) and the remaining Conopidae.

In our analysis, Conopidae (including *Stylogaster*) are recovered as monophyletic (Figs. 2 and 3), with high PBS, JKS, and PP values. It is also noted that all alternate analyses and data subsets recover the Conopidae as monophyletic. Each of the five proposed subfamilies within Conopidae are recovered as monophyletic with high PBS, JKS, and PP values in all analyses. *Stylogaster* could clearly be elevated to family status as a monophyletic sister to the Conopidae s.s. However, we do not support the proliferation of family group names within Schizophora and suggest maintaining *Stylogaster* as a subfamily within Conopidae. This decision maintains a clear link between these lineages and highlights the relationship between *Stylogaster* and other conopids. Relationships between conopid subfamilies show weak support in the preferred parsimony tree, but high PP values in the Bayesian tree. These relationships vary in alternate analyses (not shown). A revision of classification within Conopidae awaits a larger analysis including representatives of as many genera as possible (Gibson et al., in preparation).

4.4. Mitochondrial DNA vs. nuclear DNA

Past research has drawn direct comparisons between the phylogenetic utility of mitochondrial and nuclear DNA sequence data (Baker et al., 2001; Light and Reed, 2009; Lin and Danforth, 2004). In these studies, mtDNA and nrDNA were compared based on levels of support for nodes on total evidence trees, CI values, and degree of heterogeneity in among-site rate variation (“alpha”). Specifically, recovery of congruent nodes with large support values, large CI values, and large “alpha” values are considered signs of an informative gene or gene subset. The general conclusion has been that mtDNA is of less phylogenetic value than nrDNA for determining family-level relationships. At deep nodes it provides little or contradictory information and is suited only to shallow (i.e., species- and genus-level) analysis.

Our data subset of seven nrDNA genes recovers all key nodes (i.e., nodes 2, 5, 8, 12) with high PBS and JKS values (Table 3). The data subset of three mtDNA genes offers weak support for the monophyly of *Zodion* (node 5). Likewise, the nrDNA dataset produces a higher Consistency Index (CI) than the mtDNA. Furthermore, the lowest CI of any of the nuclear genes (TPI) is higher than the highest CI of the mitochondrial genes (cytB). Both mtDNA and nrDNA exhibit heterogeneous base composition across taxa ($\chi^2 > 273$, $p < 0.01$ and $\chi^2 > 646$, $p < 0.01$, respectively). Unexpectedly, the mtDNA dataset has a larger mean “alpha” value (0.557) than does the nrDNA dataset (0.538). This contradictory result can be explained by a closer examination of the mitochondrial genes. One gene, COI, has an exceptionally low “alpha” value. The other two mtDNA genes perform at average or above average levels in this and other measures, with the exception of support for nodes outside of Conopidae. Calculated CTI values (Fig. 4) confirm these results, with mtDNA recovering more congruent nodes within Conopidae and nrDNA recovering more congruent nodes overall and outside of Conopidae. Our data suggests that the mitochondrial genes tested are of lesser value for family-level and higher prob-

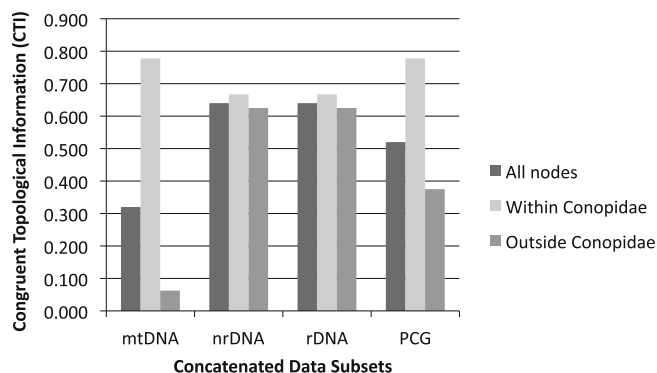


Fig. 4. Congruent Topological Information (CTI) of data subset most parsimonious tree compared to total evidence most parsimonious tree (see Section 2.5 for CTI calculation). CTI calculated for: “All nodes” – all nodes in total evidence tree (nodes 1–25). “Within Conopidae” – nodes containing only Conopidae (nodes 1–9). “Outside Conopidae” – nodes not containing only Conopidae (nodes 10–25).

lems, yet may be quite useful within a more closely related taxon set (i.e., genus and species level).

4.5. Ribosomal DNA vs. protein-coding DNA

Previous attempts to compare the phylogenetic utility of ribosomal genes to that of protein-coding genes (Almeida and Danforth, 2009; Cameron et al., 2007; Danforth et al., 2005; Mueller, 2006) found that there was little difference in the value of the two sets of genes. Ease of alignment (coding genes) and presence of universal primers (rDNA) were found to be the main means of preferring one set of genes to the other.

Our data subset of two rDNA genes recovers all key nodes with high PBS and JKS values (Table 3). The data subset of eight PCG offers contradictory Bremer support for the monophyly of *Zodion*. At the deepest nodes (i.e., 23 and 25), only the rDNA subset provides any support. The rDNA dataset produces a most parsimonious tree with a nearly equal CI to that of the PCG dataset. The PCG dataset has a mean “alpha” value (0.549), higher than that of the rDNA dataset (0.524), but with a much wider range of values within the included genes (0.253–0.858). These values suggest that neither dataset subset is clearly superior. Calculated CTI values (Fig. 4) confirm these results, with PCG recovering slightly more congruent nodes within Conopidae and rDNA recovering more congruent nodes overall and outside of Conopidae.

Other values to be noted are the proportion of constant and informative sites and the degree of heterogeneity of base frequencies among taxa. The rDNA dataset includes nearly 70% constant characters and only 20% of characters phylogenetically informative. This is in stark contrast to the PCG dataset (47% and 45%, respectively). One gene seems to explain this discrepancy. The 28S ribosomal DNA sequence provides a large proportion of the Bremer support at nearly every node yet includes only 17% informative characters. Likewise, combined and individually, the ribosomal genes exhibit homogeneous base frequencies among taxa. This would suggest that 28S and 12S are of equal value to protein-coding genes for dipteran phylogenetic research. Further analysis will reveal the regions that are most phylogenetically informative and the regions that are identically conserved across diverse dipteran lineages.

5. Conclusions

Analysis of the current dataset allows a number of phylogenetic conclusions to be drawn. The monophyly of Schizophora, Conopidae (including *Stylogaster*), and each of the five hypothesised sub-

families of Conopidae are strongly supported. Paraphyly of Acalyptratae with respect to Calyptratae is supported, although sampling of more calyptrate families and of the remaining acalyptrate superfamilies is necessary. A sister group relationship between Conopidae and the remaining Schizophora (“Muscaria” *sensu* Enderlein, 1936) is supported in parsimony analyses. Bayesian analysis suggests that Conopidae + Lauxaniidae is sister to the remaining Schizophora. Closer examination of both analyses reveals that this latter relationship, though surprising, is supported by our data. Lauxaniidae has not yet been offered as a possible sister to Conopidae, nor has it been directly tested in any molecular analysis to date. Future sampling of still more acalyptrate families will be necessary to shed more light on schizophoran relationships. Relationships between subfamilies and genera of Conopidae, likewise, will require further examination with greater sampling of genera.

With regard to comparisons of gene sequences based on phylogenetic information, the nuclear genes tested provide more value, but the mitochondrial genes tested are still useful, especially when investigating more recent divergences. The two most commonly sequenced genes in dipteran molecular phylogenetics are COI and 28S. Of these, COI appears to be a poor choice in terms of phylogenetic information provided outside of species and genus-level relationships. The other popular gene, 28S appears to be highly useful for the study of schizophoran relationships, but either the entire gene must be sequenced or further refinement to target only variable subsections of the gene is necessary. In general, the greatest resolution can be provided by including sequence data from all four gene categories: mtDNA, nrDNA, rDNA, and PCG.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympcv.2010.03.026.

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