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Abstract. Members of the megadiverse insect order Diptera (flies) have successfully colonized all continents and nearly all habitats. There are more than 154 000 described fly species, representing 10–12% of animal species. Elucidating the phylogenetic relationships of such a large component of global biodiversity is challenging, but significant advances have been made in the last few decades. Since Hennig first discussed the monophyly of major groupings, Diptera has attracted much study, but most researchers have used non-numerical qualitative methods to assess morphological data. More recently, quantitative phylogenetic methods have been used on both morphological and molecular data. All previous quantitative morphological studies addressed narrower phylogenetic problems, often below the suborder or infraorder level. Here we present the first numerical analysis of phylogenetic relationships of the entire order using a comprehensive morphological character matrix. We scored 371 external and internal morphological characters from larvae, pupae and adults for 42 species, representing all infraorders selected from 42 families. Almost all characters were obtained from previous studies but required revision for this ordinal-level study, with homology assessed beyond their original formulation and across all infraorders. We found significant support for many major clades (including the Diptera, Culicomorpha, Bibionomorpha, Brachycera, Eremoneura, Cyclorrhapha, Schizophora, Calyptratae and Oestroidea) and we summarize the character evidence for these groups. We found low levels of support for relationships between the infraorders of lower Diptera, lower Brachycera and major lineages of lower Cyclorrhapha, and this is consistent with findings from molecular studies. These poorly supported areas of the tree may be due to periods of rapid radiation that left few synapomorphies in surviving lineages.

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Introduction

The four megadiverse insect orders, Diptera, Coleoptera, Lepidoptera and Hymenoptera, comprise approximately 50% of described animal species, and Diptera alone currently includes more than 10% of described species (Pape & Thompson, 2010). The Diptera (flies, mosquitoes and gnats) are remarkable in that the species display an extraordinary diversity of anatomical designs, ecological specializations and life history strategies, with numerous origins of phytophagy, predation and parasitism (Kitching *et al.*, 2005; Kutty *et al.*, 2007, 2010; Courtney *et al.*, 2009; Pape *et al.*, 2009; Wiegmann *et al.*, 2011). Diptera have successfully colonized all continents and nearly all habitats, including open oceans. Currently, the order is classified into approximately 10 000 genera, 150 families, 22–32 superfamilies, eight to 10 infraorders and two suborders (Yeates & Wiegmann, 1999). A revised subordinal classification was proposed for the 'Nematocera' (Amorim & Yeates, 2006), in the certain knowledge that this group is paraphyletic, although the composition of some major subordinal-level lineages at the base of the Diptera tree remain unclear (Bertone *et al.*, 2008). Diptera monophyly is well established on the basis of morphological synapomorphies, including the modification of the hind wings into halteres and specializations of the adult mouthparts and larval locomotory organs (Hennig, 1973).

Hennig was a dipterist and it comes as no surprise that the Diptera was the first megadiverse insect order that benefited from his precisely defined phylogenetic concepts and newly developed methods for tree reconstruction (Richter & Meier, 1994; Meier, 2005). In a series of papers, Hennig addressed the higher-level phylogenetic relationships of the lower Diptera and Cyclorrhapha (Hennig, 1954, 1958, 1965a,b, 1966, 1968, 1971) and his contributions culminated in a phylogenetic classification of the entire order (Hennig, 1973) (Fig. 1). Together with Griffiths' (1972) almost simultaneous work on the Cyclorrhapha, Hennig (1973) provided a platform for the analysis of Diptera relationships in the third volume of the *Manual of Nearctic Diptera* (MND). In the three chapters of this book (McAlpine, 1989; Wood & Borkent, 1989; Woodley, 1989), many new characters were introduced and older characters refined and redefined. The impact of the third volume of the MND on dipteran systematics cannot be overestimated (Yeates & Wiegmann, 2012); however, the phylogenetic analyses were qualitative in nature – no character matrices were published, and character transitions were assigned to nodes without parsimonious phylogenetic reconstruction.

Previous phylogenetic hypotheses

Numerous studies have tested the competing hypotheses in the publications by Hennig (1973), Griffiths (1972), and the MND, corroborating some and refuting others and proposing new higher-level taxa (Yeates & Wiegmann, 1999, 2005; Yeates *et al.*, 2007). Initial studies examined the homology of morphological character systems and their implications for phylogenetic relationships in the Diptera using qualitative phylogenetic methods as used in the MND, summarizing character distributions using hand-generated cladograms (e.g. Courtney, 1991; Oosterbroek & Theowald, 1991; Wood, 1991; Sinclair, 1992; Starý, 1992; Sinclair *et al.*, 1994, 2007). More recent morphological studies have tested the higher classification using quantitative, matrix-based phylogenetic methods. These studies have examined the relationships among the lower dipteran or nematocerous families (e.g. Oosterbroek & Courtney, 1995; Saether, 2000), lower Brachycera (e.g. Yeates, 2002), Empidoidea (e.g. Wiegmann *et al.*, 1993; Sinclair & Cumming, 2006), acalyptrates (e.g. Meier & Hilger, 2000; Buck, 2006), and oestroid calyptrates (e.g. Pape, 1992; Rognes, 1997). Molecular studies employing a range and variety of genes and an increased taxonomic range have complemented the morphology-based hypotheses, and all these studies have broadly supported phylogenetic conclusions based on morphological characters (e.g. Friedrich & Tautz, 1997; Bernasconi *et al.*, 2000; Wiegmann *et al.*, 2000, 2003; Meier & Baker, 2002; Moulton & Wiegmann, 2007; Petersen *et al.*, 2007; Bertone *et al.*, 2008; Kutty *et al.*, 2008, 2010; Gibson *et al.*, 2010; Trautwein *et al.*, 2010); however, only rarely are the results entirely congruent with previous studies (e.g. Wiegmann *et al.*, 2000). In some cases, gene regions have been analysed simultaneously with morphological data (Skevington & Yeates, 2000; Meier & Baker, 2002; Meier & Wiegmann, 2002; Remsen & O'Grady, 2002; Dikow, 2009). Quantitative studies have been designed to test competing hypotheses of dipteran relationships (Collins & Wiegmann, 2002; Meier & Baker, 2002; Moulton & Wiegmann, 2007; Winterton *et al.*, 2007). A supertree approach (Bininda-Emonds *et al.*, 2002) summarized nine phylogenetic hypotheses of the Diptera, all of which were based on morphological evidence (Yeates & Wiegmann, 2005), and produced results in agreement with previous studies at infraordinal level using non-quantitative methods (e.g. fig. 1, Yeates & Wiegmann, 1999).

The relationships of flies and the tempo of their evolution were analysed by Wiegmann *et al.* (2011), scoring species representing 95% (149 of 157) of recognized fly families and using 7–45 kb of mitochondrial and nuclear DNA per terminal. Diptera, Culicomorpha, Bibionomorpha, Neodiptera (Michelsen, 1996), Brachycera, Eremoneura, Cyclorrhapha, Schizophora and Calyptratae were recovered. In addition, the Deuterophlebiidae (a bizarre small family called mountain midges) were hypothesized to be sister to the remaining Diptera, the Perissommatidae were a sister group of the Bibionomorpha + Brachycera, and the Apystomyiidae were a sister group of the Cyclorrhapha. This last result agrees with another molecular study (Trautwein *et al.*, 2010). The lower Diptera ('Nematocera') and Aschiza were found to be paraphyletic, as expected (Yeates & Wiegmann, 1999). A significant point of difference between Wiegmann *et al.* (2011) and other studies is the monophyly of the lower Brachycera (Tabanomorpha, Stratiomyomorpha and Asiloidea; collectively Orthorrhapha), a traditionally paraphyletic group (e.g. Hennig, 1973) and whose paraphyly had been shown additionally by matrix-based numerical morphological studies (Yeates &
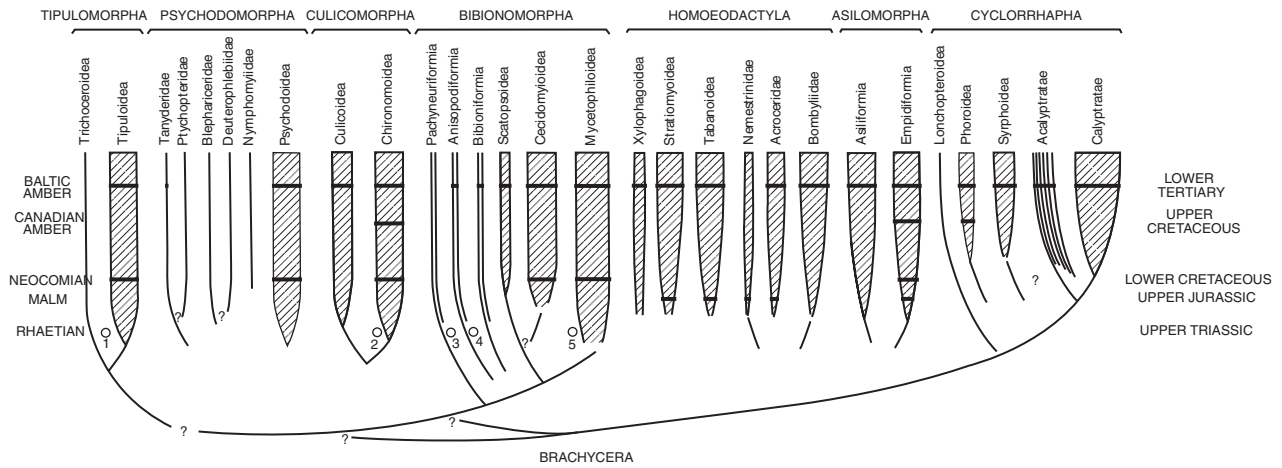
Fig. 1. Hennig's proposed classification (1973) was the first to discuss the monophyly of major groupings within and across Diptera.

Wiegmann, 1999; Yeates, 2002). Support for this node was moderately strong in molecular analysis (bootstrap support 86–95%; Table S1, Wiegmann *et al.*, 2011). The Neodiptera (Michelsen, 1996) also appeared as monophyletic in Wiegmann *et al.* (2011), a grouping proposed based on morphological characters but not recovered in earlier molecular analyses (Bertone *et al.*, 2008).

The signals from the mitochondrial and nuclear data used in Wiegmann *et al.* (2011) were compared by Caravas & Friedrich (2012), who found that both mitochondrial genomes and nuclear genes produce similar results when analysed separately, but that the nuclear genes reanalysed (6 kb) recovered a larger proportion of 17 well-supported 'benchmark' nodes in dipteran phylogeny than the mitochondrial genes alone (10 kb). All benchmark nodes were recovered when the mitochondrial and nuclear data were analysed together; however, separately, the mitochondrial data placed the tachinid *Exorista* outside the Muscomorpha (Asiloidea + Eremoneura sensu Caravas & Friedrich, 2012), a result not supported by any previous study. The Orthorrhapha, reintroduced by Wiegmann *et al.* (2011), was not recovered in any analysis, and Neodiptera received very weak support from combined analyses (Caravas & Friedrich, 2012).

Here we present the first ordinal-level phylogenetic hypothesis for all Diptera based on numerical analysis of an explicit, exemplar-based morphological character matrix. Although an earlier version of our morphological dataset was analysed in combination with molecular data and included in Wiegmann *et al.* (2011) and the results presented in supporting information, we consider it important to present the results of a separate analysis to document fully the characters, states and scorings used, and demonstrate the different signal that morphological data provides compared with the molecular data.

Our matrix of morphological characters for the order includes 371 external and internal characters from larvae, pupae and adults that span the dipteran phenotype. We document the history, definition and use of the characters used in the analysis in File S4. Our goals were: (i) to examine relationships

of the major infraordinal level groupings of Diptera, (ii) to examine relationships of superfamilies at phylogenetic suture zones, and (iii) to identify characters that support these clades. We scored 42 taxa chosen to represent an even and broad sample of family representatives across the order Diptera and four outgroups from other holometabolous orders. Many of these taxa were common and often available in culture, so that immature stages and fresh tissues could be obtained for a comprehensive coverage of the morphology and DNA markers. Here we present the results of the analyses of the morphological data and compare them with previous hypotheses, particularly those of Wiegmann *et al.* (2011).

Methods

A primary obstacle in coding characters across a megadiverse order of insects is determining homology between species that last shared a common ancestor 250 Ma and are as morphologically divergent as a midge and a blowfly. This is particularly so for larval head structures and male genitalia in the lower Diptera, Brachycera and Cyclorrhapha. For example, the head capsule of many lower Diptera larvae are more or less complete, whereas various components of the larval head of Cyclorrhapha are fused and invaginated into the thorax to form a special structure, the pseudocephalon (Courtney *et al.*, 2000). Our characters were drawn primarily from cuticular macrostructures, but soft anatomy, physiology, ultrastructure, etc. could not be fully assessed across all terminal taxa. In addition to the MND, several morphological studies of specific anatomical features were very instructive in the determination of homology, in particular for the antenna (Stuckenberg, 2001), male genitalia (Wood, 1991; Sinclair *et al.*, 1994; Cumming *et al.*, 1995), larval mouthparts (Courtney *et al.*, 2000), and wing venation (Wootton & Ennos, 1989; Saigusa, 2006; Starý, 2008).

Initially we established a list of 457 potentially informative morphological characters to cover the family-level diversity of

the order Diptera. More than 20 characters were novel, that is, additional to those used in previous phylogenetic studies of Diptera. Intensive evaluation determined that 86 were of poor phylogenetic utility, which was mostly due to ambiguous homology with the current taxon sample. Many characters were redefined to ensure states referred to homologous structures. The list was thus reduced to 371 external and internal (28%) morphological characters for larvae (93), pupae (11), and adults (267, including 55 head, 54 wing, 31 female genitalia, 49 male genitalia) (File S2). Note that this list contains characters considered of importance for defining families and for family-level relationships, even when these relationships concern families unsampled here. Rather than pruning these characters, we retain them for the convenience of future studies with a denser intra-familiar taxon sample. Specimens of 42 first-tier exemplar dipteran species and four holometabolous outgroups (File S1) were scored for the characters to produce a morphological matrix. Species were scored primarily through direct study of specimens and using literature for guidance only, with exceptions for Malpighian tubules, number of instars and adult abdominal ganglia. Although we initially included Strepsiptera as an outgroup, the recent literature indicates a consistent placement of Strepsiptera as sister group to the Coleoptera (Wiegmann *et al.*, 2009; Friedrich & Beutel, 2010; Beutel *et al.*, 2011), so this highly autapomorphic outgroup was removed from the final analysis in order to minimize the number of inapplicable character states in the matrix.

The morphological matrix is given in File S3 and is deposited along with phylogenetic results in Treebase (Tree-base.org; http://purl.org/phylo/treebase/phylows/study/TB2:S1 3373). Missing data are identified by '?' and inapplicable scores by '−'. Of 371 characters, 310 are binary and 61 multistate. As only 46 taxa (File S1) were scored for the tier 1 analyses, 17 characters were constant, 74 were parsimony-uninformative, and 280 were parsimony-informative. All characters were treated as unordered and with equal weight. Polymorphisms were interpreted as uncertainty. Character 105 (abdominal ganglia) is polymorphic for Tabanidae and Stratiomyidae, as the exemplar taxa could not be scored, and several states occur in these families (Yeates *et al.*, 2002; Merritt, 2005). The matrix was analysed with PAUP* V4.0b10 (Swofford, 2002) running 1000 heuristic random addition searches with TBR, as well as with TNT (Goloboff *et al.*, 2008) with an initial New Technology search set to 100 (using a driven search with sectorial search, ratchet, drift, and tree fusing; finding the minimum tree 10 times). An additional 'traditional search' based on 100 random addition sequences was used to confirm the results of the New Technology search. We used Bremer support (Bremer, 1992, 1994; Källersjö *et al.*, 1992) to measure the strength of evidence for nodes on a most parsimonious tree (MPT). Bremer support values were calculated with TREEROT v.2 (Sorenson, 1999) with 20 heuristic searches of the data. Branch support was calculated also using standard bootstrap in TNT with 500 replicates analysed with the same 'traditional search' settings as above.

Character states were mapped on a MPT using WIN-CLADA ver. 1.0 (Nixon, 2002), showing only unambiguous

changes rather than ambiguous reconstructions with accelerated transformation (ACCTRAN) or delayed transformation (DELTRAN) character-state optimization. As discussed in Agnarsson & Miller (2008), both ACCTRAN and DELTRAN behave inappropriately when confronted with missing data or inapplicable entries. Of the 17 066 character cells in our matrix, 1.5% is missing data and 15% inapplicable entries. Therefore, we chose to show only unambiguous synapomorphies on the MPT.

Parametric methods of phylogenetic inference generally have not been applied to morphology because of questionable stochastic models and computational complexity of the maximum likelihood approach. Development of Bayesian inference of phylogeny using Markov Chain Monte Carlo (MCMC) estimation of posterior probability distributions has made it easier to address complex, parameter-rich stochastic models within a statistical framework. Bayesian phylogenetic analysis on our dataset was carried out with MRBAYES v3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) and posterior probabilities were calculated using a MCMC sampling approach. These analyses used the standard model for morphological characters as implemented in MRBAYES v3.1.2 and proposed by Lewis (2001) and Nylander *et al.* (2004), with priors of all state frequencies (change rates) set equal, all topologies with equal probabilities, unconstrained branch lengths and temperature set to 0.30. By default MRBAYES v3.1.2 performs two independent runs: for each, starting trees were random and four simultaneous Markov chains (with a ratio of one cold to three hot chains) were completed for 10 M generations with trees sampled every 100 generations resulting in 100 000 saved trees for each run. Two separate analyses (each of 10 M generations) were completed so that four independent runs were performed, saving a total of 400 000 trees. Burn-in values for each run were set at 250 000 generations (25 000 trees) after the average standard deviation of split frequencies indicated that convergence of the MCMC chains had been reached. Initially MRBAYES v3.1.2 was used to discard the 25 000 trees as burn-in from each of the four runs, prior to outputting the Bayes tree with clade credibility (posterior probability) values for the entire Bayesian analysis. Secondly, a Bayes combined majority rule consensus tree (Margush & McMorris, 1981) from the four independent runs was generated in PAUP* V4.0b10 by importing sequentially the four MRBAYES tree files (*t files), excluding the first 25 000 trees of each tree file and retaining the remaining 75 000 trees, which were combined into one MRBAYES tree file of 300 000 trees. A Bayes combined majority rule consensus tree and each of the MPTs were loaded sequentially as constraints and used to filter the MRBAYES tree file of 300 000 trees using PAUP* V4.0b10 to investigate the composition of those MRBAYES trees.

Results

The maximum parsimony analysis found 17 (PAUP*) or 16 (TNT) most parsimonious trees. Tree statistics are as follows: tree length = 1081, consistency index (CI; Kluge &
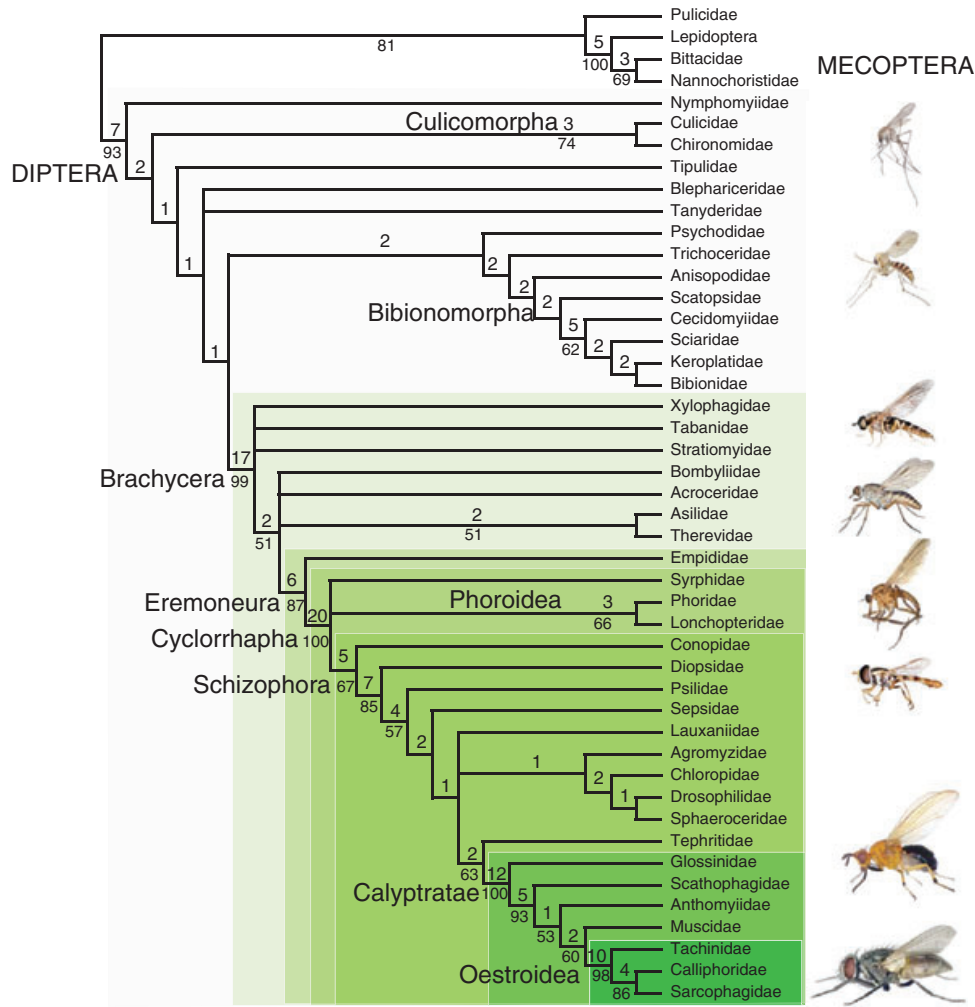
Fig. 2. Strict consensus tree of 16 most parsimonious trees (MPTs), with Bremer support values presented above the branches, and bootstrap values over 50% below the branches.

Farris, 1969) = 0.3969, CI excluding uninformative characters (Kluge & Farris, 1969) = 0.3480, retention index (RI; Farris, 1989) = 0.7389, and rescaled consistency index (RC; Farris, 1989) = 0.2932. The strict consensus (Schuh & Farris, 1981) is given in Fig. 2 with Bremer supports presented above the branches and bootstrap values below. The MPTs differ in the relationships inferred between the Tanyderidae and Blephariceridae in the lower Diptera; the Xylophagidae, Tabanidae, and Stratiomyidae and Acroceridae, Bombyliidae and Asilidae + Therevidae in the lower Brachycera; Syrphidae and Phoridae + Lonchopteridae in the lower Cyclorrhapha; and the Lauxaniidae and Agromyzidae + Chloropidae + Drosophilidae + Sphaeroceridae in the acalyptrates. One MPT was chosen with reference to the majority rule consensus tree, as the MPT included those nodes that were found most often in the remaining MPTs (Fig. 3). Unambiguous character state changes are mapped on this MPT with synapomorphies (shared derived states of informative characters with CI = 1) indicated with black squares.

Two separate Bayesian analyses yielded 400 000 trees. The average standard deviations of split frequencies at the completion of the two separate Bayesian analyses were 0.003 920 and 0.003 499, respectively. The convergence diagnostic potential scale reduction factor (PSRF; Gelman & Rubin, 1992) was 1 for both analyses, also indicating that convergence was met (Ronquist *et al.*, 2005, 2010). Following removal of the burn-in, the Bayesian clade credibility tree from MRBAYES was identical to the Bayes combined 50% majority rule consensus from PAUP* V4.0b10. The Bayes combined majority rule consensus tree showing all compatible nodes and clade credibility values (Fig. 4) is fully resolved. Filtering of the MRBAYES tree file of 300 000 trees showed that the Bayes combined majority rule consensus tree (length 1086 steps) was found 78 times in the Bayesian analyses.

Further investigation of the MRBAYES tree file showed that MPTs (i.e. trees of length 1081 steps) were found 42 times; however, that included duplicates of only nine of the 16 MPTs. The Bayes combined majority rule consensus
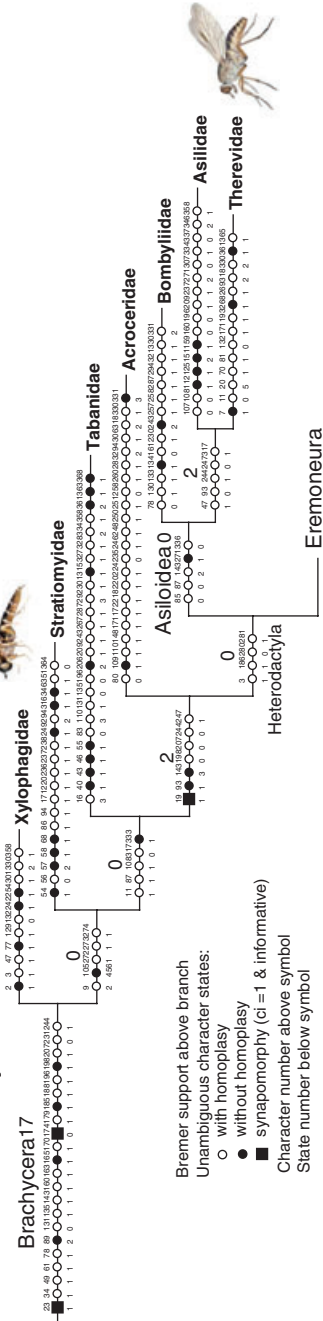
Fig. 3. Preferred most parsimonious tree with unambiguous character state changes mapped, and Bremer supports presented above the branches. CI, consistency index.
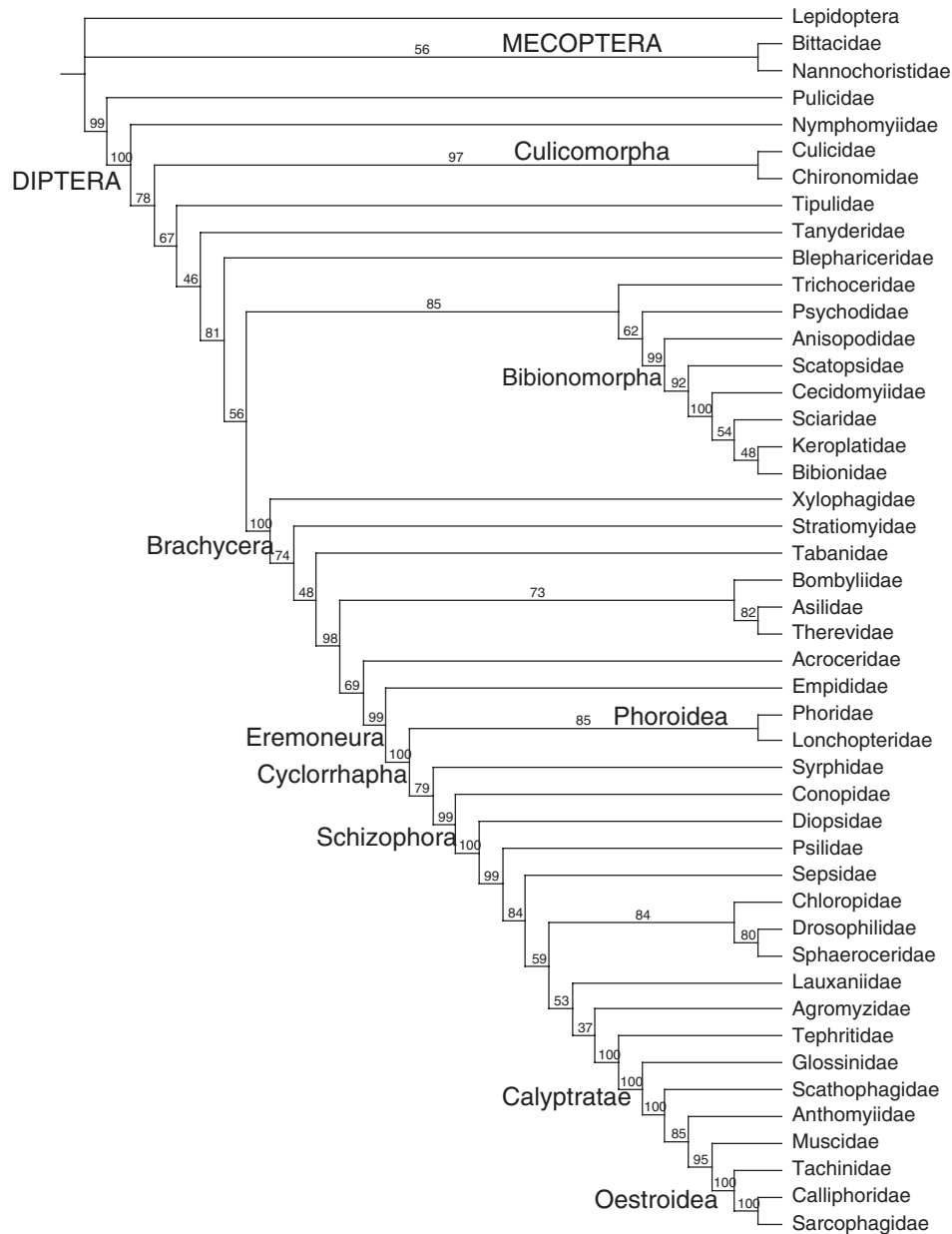
Part 3 Lower Eremoneura

Part 4 Higher Eremoneura

Fig. 3. Continued

Fig. 4. The Bayes combined majority rule consensus tree showing all compatible nodes and clade credibility (posterior probability) values above branches.

tree (Fig. 4) differs from all MPTs (e.g. Fig. 3) and the strict consensus of the MPTs (Fig. 2). This is because MRBAYES builds a maximum likelihood tree rather than searching for MPTs and only saves trees with distinct topologies. In the Bayes combined majority rule consensus tree (Fig. 4), Psychodidae are sister to Bibionomorpha and not to Trichoceridae + Bibionomorpha as in the strict consensus of the MPTs (Fig. 2). In the lower Brachycera, Acroceridae are sister to Eremoneura rather than to Asiloidea + Eremoneura. In the Schizophora, Lauxaniidae and Agromyzidae form a grade to Tephritidae + Calyptratae rather than to the clade of

Chloropidae (Drosophilidae + Sphaeroceridae). Because these differences are poorly supported in both analyses (Figs 3, 4), we discuss relationships as shown in the preferred MPT (Fig. 3).

Discussion

Here we discuss the first numerical analysis of morphological characters from a matrix spanning all Diptera, with scorings based on the examination of exemplar species. Our

results provide significant support for most currently recognized major clades (Figs 2–4). These include Diptera, Culicomorpha, Bibionomorpha, Brachycera, Eremoneura, Cyclorrhapha, Schizophora, Calyptratae and Oestroidea. Support for these clades and relationships within them are discussed below, with character numbers in parentheses (and state for multi-state characters in Fig. 3). We outline the character state distribution and evidence and plot the character states on the tree to identify synapomorphies and character conflict (Fig. 3). However, these synapomorphies are only applicable to the subset of taxa included in this analysis; some would be homoplasious with additional taxa included.

Diptera

The Diptera are well supported as monophyletic, with numerous undisputed synapomorphies (Hennig, 1973; Wood & Borkent, 1989). In the present analysis, the following synapomorphies support their monophyly: larval torma present (34, state 0); larval mandibular rotation is oblique or vertical (36, state 1); larval prementohypopharyngeal apparatus present (59, state 1); larval postmentum (hypostoma) with serrate anterior margin (62, state 1); scutum with transverse suture (172, state 0); hind wings developed as halteres (265, state 1); abdominal spiracle 8 in adult males absent (291, state 1); and male tergite 10 in adult males absent (364, state 1).

Lower Diptera

The lower Diptera – previously known as 'Nematocera' – is a convenient term for this paraphyletic assemblage of families (Yeates & Wiegmann, 1999). There has been little consensus with regard to the composition of, and relationships between, the infraorders of the lower Diptera. Molecular analysis of the early diversification of flies (Bertone *et al.*, 2008) established confidence in some clades, but many questions remain. Although the lower Diptera are almost fully resolved here, the clades are poorly supported (Fig. 3, part 1).

Our analyses suggest that the Nymphomyiidae are sister to the remaining Diptera. However, the morphological characters excluding the family from the remaining Diptera are highly homoplasious [e.g. presence of tibial spurs in the remaining Diptera (272, state 0; 273, state 0)] and may be due to losses resulting from miniaturization, a short non-feeding adult life [e.g. absence of labella (158, state 0)], and possibly neotenic retention of certain larval features (Courtney, 1994), a result also consistent with two molecular analyses (Bertone *et al.*, 2008; Wiegmann *et al.*, 2011).

The infraorder Culicomorpha are supported by a group of synapomorphies: metathoracic leg sheath bent in an S-shape (103, state 1); male pedicel enlarged and globular (142, state 1); antennal flagellum of male plumose (144, state 1); and aedeagus membranous (345, state 1). However, some of these synapomorphies would be homoplasious if additional taxa were included. For example, if Simuliidae were added

to the analysis, the antennal flagellum of male plumose (144, state 1) would be a synapomorphy for the Culicomorpha but reversed in Simuliidae. The Culicomorpha are found to be the sister taxon of all non-nymphomyiid Diptera combined, with the latter clade sharing several character states that in other classifications are considered part of the Diptera ground plan: discal cell present (248, state 0); three spermathecae (318, state 3); and ejaculatory apodeme developed (357, state 0). Only the latter of these is without homoplasy in our analysis. The Tipulidae (*sensu lato*) were found to be the sister taxon of the remaining Diptera. In contrast to previous morphological (e.g. Oosterbroek & Courtney, 1995) and molecular (Bertone *et al.*, 2008) analyses, the Trichoceridae were not part of this clade. Instead, trichocerids were part of a clade comprising (Blephariceridae + Tanyderidae) + ((Psychodidae + (Trichoceridae + Bibionomorpha)) + Brachycera). Unlike Bertone *et al.* (2008) and Wiegmann *et al.* (2011), the Tanyderidae, Blephariceridae, and Psychodidae do not form a clade, although Tanyderidae and Blephariceridae are sister taxa in some MPTs. The Bibionomorpha are resolved, but with low Bremer support and bootstrap support less than 50% (Fig. 3, part 1).

The absence of the clade, 'Neodiptera' (Michelsen, 1996), composed of Brachycera and Bibionomorpha (sensu Hennig, 1973), is particularly surprising, as this clade was erected based on morphology. Neodiptera, which finds support from molecular data (Wiegmann *et al.*, 2011), may not be recovered in our analyses because several putative synapomorphies reside in the skeletomuscular anatomy, which has made scorings unattainable for most taxa in our study.

Brachycera

The Brachycera are one of the best-supported lineages in Diptera and are certainly monophyletic. Numerous undisputed synapomorphies have been identified (Hennig, 1973; Woodley, 1989; Sinclair, 1992). The following apomorphies from our analyses support the monophyly of this clade: frontoclypeal apotome absent (23, state 1); three or more openings of each posterior tracheal trunk of last instar larva (89, state 2); postpronotal callus present (165, state 1); scutal transverse suture straight (174, state 0); notopleuron present (185, state 1); and subscutellum strongly convex (198, state 1) (Fig. 3, Part 2). The course of the transverse suture has usually been a key feature for diagnosing the families Tipulidae and Trichoceridae (V-shaped). All lower Diptera (except Nymphomyiidae, which has no visible suture) are scored here as V-shaped, awaiting a more detailed documentation and interpretation of this character across the lower Diptera. Scoring Tipulidae and Trichoceridae for a character state not found in the remaining Diptera does not change the topology of the consensus cladogram.

Lower Brachycera

The lower Brachycera emerge as paraphyletic in relation to the remaining Brachycera (Fig. 3, part 2), as has been accepted

since Hennig (1973) (but see Wiegmann *et al.*, 2011). Within the lower Brachycera, too few taxa are included to demonstrate the monophyly of the major infraorders (e.g. Tabanomorpha, Stratiomyomorpha). Acroceridae are assigned as sister group to Asiloidea + Eremoneura, supported by the presence of a reduced larval antenna (19, state 1); three larval instars (93, state 1) but with reversal in Therevidae + Asilidae; and one flagellar segment (not including the arista/stylus) (143, state 3). The Heterodactyla, a long-recognized lineage, traditionally defined by the presence of a setiform empodium (281, state 1) (see Griffiths, 1994), is also supported by the absence of the mediolobus (pad-like empodium) (280, state 0) (Fig. 3, part 2), but both characters are very homoplasious and the clade was only recovered in 88% of the MPTs. The Asiloidea represented by three of six families are monophyletic in 62% of the MPTs on the basis of the hind femur having one row of stout setae ventrally (271, state 1). State 2 (two rows of stout setae ventrally) is homoplasious, found in the Asilidae and Conopidae (Fig. 3, Part 2).

Eremoneura

The Eremoneura is a species-rich lineage comprising the Empidoidea and Cyclorrhapha. This is a well-supported clade (Griffiths, 1994; Cumming *et al.*, 1995; Yeates & Wiegmann, 1999) based on the following synapomorphies in our analysis: the fusion of the larval antennal and maxillary lobes (52, state 1); postgonites present (338, state 1); and subepandrial sclerite sclerotized, divided into bacilliform sclerites (processus longi) laterally (368, state 2). In addition, the following homoplasious character states provide further corroboration for the monophyly of the Eremoneura: female tergite 9 absent (303, state 1); posterior margin of epandrium deeply emarginate, U-shaped with basal connection (334, state 1); and lateral ejaculatory processes absent (360, state 1) (Fig. 3, part 3).

Cyclorrhapha

The Cyclorrhapha, the best-supported clade in our analyses, has been recovered in all previous morphological analyses (Griffiths, 1972; McAlpine, 1989). Support comes from synapomorphies, including absence of larval head capsule (1, state 0); presence of Bolwig's organ (21, state 1); larval mandible monocondylous (37, state 1); larval spiracles on anal division type III (83, state 2); pupation within a puparium formed from last larval cuticle (94, state 1, also present in Stratiomyidae); larval body tergites and sternites lightly sclerotized (97, state 2); adult clypeus widely separated from lower margin of face by membrane (128, state 1); antennae consisting of enlarged first flagellomere with apically elongated, one to three segmented arista (145, state 1); proepisternal depression (propleuron) present (167, state 1); abdominal tergites 1–2 fused into a syntergum (285, state 1); abdominal plaques absent (290, state 1, also absent

in Siphonaptera); male hypopygium circumverted, rotated permanently through 360° (331, state 4); phallus divided into basiphallus + distiphallus (352, state 1); phallapodeme present (355, state 1); and surstyli present (366, state 1) (Fig. 3, part 3). The strongly reduced and modified head (pseudocephalon), novel larval feeding structures and the development of the puparium are important innovations.

Lower Cyclorrhapha

Paraphyly of lower Cyclorrhapha is consistent with earlier studies (Griffiths, 1972; Cumming *et al.*, 1995). There is insufficient family-level coverage to discuss relationships within the lower Cyclorrhapha, although the distinct support for the Phoroidea (Lonchopteridae + Phoridae) is worthy of mention. Cumming *et al.* (1995) assigned Lonchopteridae as the sister group to the remaining Phoroidea and this relationship is supported here on the basis of the following homoplasious characters: anterior frontal bristles present (122, state 1); vibrissa present (126, state 1); abdominal spiracles in segments 1–5 all in tergite margin (283, state 1); and two spermathecae present (318, state 2) (Fig. 3, part 3). In a study of the larval head, Rotheray & Gilbert (2008) maintained the Lonchopteridae was the sister group to the remaining Cyclorrhapha, as suggested previously by Griffiths (1972) and Hennig (1973). The former study emphasized the importance of the open trough anterior to the larval mouth (12, state 1), absence of maxillary sheath for the larval mandible (not included in this study), and more than two larval mandibular sclerites (39, state 3) (Rotheray & Gilbert, 2008). Unfortunately, the sparse taxon sample for the lower Cyclorrhapha prevented us from testing rigorously the phylogenetic utility of these characters.

The Syrphoidea have been considered the sister clade of the Schizophora (Cumming *et al.*, 1995; Zatwarnicki, 1996; Yeates *et al.*, 2007). Although this hypothesis is compatible with our trees, our taxon sample did not include the family Pipunculidae and we could not test the suggestion that Syrphoidea are paraphyletic and that the Pipunculidae are the sister taxon of the Schizophora (Wiegmann *et al.*, 2011).

Schizophora

Schizophora comprise more than half the family-level diversity in Diptera (Yeates & Wiegmann, 1999), with some 80 recognized families, including all flies with a ptilinum and a full circumversion of the male genitalia completed within the puparium (Cumming *et al.*, 1995). Schizophora are supported here on the basis of the following apomorphic character states: puparium cleavage lines transverse dorsal (95, state 1); ptilinal fissure present (118, state 1); tip of pedicel with process into flagellum (141, state 1); and coxopleural streak present (202, state 0) (Fig. 3, Part 3). Homoplasy in most character systems has inhibited comprehensive quantitative phylogenetic analyses of the Schizophora (Yeates & Wiegmann, 1999; Yeates *et al.*, 2007).

Acalyptratae

Acalyptrates are paraphyletic, as often suggested previously, and as proposed by Wiegmann *et al.* (2011). No convincing synapomorphies uniting this lineage have ever been suggested. As many as ten acalyptrate superfamilies have been recognized (Griffiths, 1972; McAlpine, 1989; Yeates & Wiegmann, 1999; Yeates *et al.*, 2007; Woodley *et al.*, 2009), but different authors have proposed very different superfamily concepts. Unfortunately, our taxon sample for acalyptrates is sparse and neither superfamily monophyly nor competing superfamily concepts can be tested. Furthermore, the clade support for the relationships within the acalyptrates is generally low and it is premature to discuss the hypotheses in greater detail. However, a 'near-basal' position of the Psilidae and Diopsidae is not unexpected, although it is surprising that they are not sister groups on the MPTs. Perhaps surprisingly, but supporting the results of Gibson *et al.* (2010), the Conopidae are the sister group to the remaining Schizophora in our analyses based on the following synapomorphies for the non-conopid schizophoran clade: intermediate sclerite in larva fused with vertical plate (13, state 1); intermediate sclerite H-shaped (14, state 1); Cyclorrhaphan labral-like blade of final instar larva reduced, ending at base of mandible (26, state 1); larval posterior spiracles surrounded by tufts of cuticular outgrowths (88, state 1); and sclerites of female abdominal 7 fused (296, state 1).

Calyptratae

The Calyptratae are undoubtedly monophyletic (Hennig, 1971; Griffiths, 1972; McAlpine, 1989; Yeates & Wiegmann, 1999), finding much support in our analyses. Synapomorphies recognized in this study include: Cyclorrhaphan labral-like blade in first-instar larva present (25, state 0); orbital bristles proclinate (121, state 1); hyoid sclerite in adult mouthparts present (150, state 1); prestomal teeth present (162, state 1); and metathoracic spiracle with dense fringe composed of hair-like structures (204, state 1) (Fig. 3, part 4). Within the Calyptratae, the Oestroidea are supported by a 2 + 1 katepisternal setal pattern (182, state 2); anepimeral bristle present (183, state 1); vertical row of setae on meron (201, state 1); posterior fringe of metathoracic spiracle operculum-shaped (205, state 1); postgonal apodeme present (339, state 1); phallus with dorsal wall of distiphallic tube sclerotized and forming the dorsal plate (348, state 1); and mesohypophallic sclerotization present mid-ventrally along distiphallus (354, state 1) (Fig. 3, Part 4). The relationships within the calyptrates have been addressed with molecular data (Petersen *et al.*, 2007; Kutty *et al.*, 2008, 2010), who suggest a sister group relationship between the Hippoboscoidea and the remaining families and that the 'Muscoidea' were paraphyletic, with Muscidae being the sister group of the monophyletic Oestroidea (Kutty *et al.*, 2008). Our morphological analyses agree with regard to the earliest branching patterns within the calyptrates. The Oestroidea are monophyletic and the muscoids form a grade.

However, the relationships within the grade differ between the molecular and our morphological analyses.

Support levels and synapomorphies

Bremer support values are distributed unevenly through the tree (Fig. 3). Branches with high levels of support (defined as greater than six in this study) are the Diptera, Brachycera, Cyclorrhapha, Calyptratae and Oestroidea. The well-supported ingroup nodes are those that pertain to historically well-recognized and established clades that were already accepted before the 20th century (see Hennig, 1973). For most of these nodes, this is due to the many unreversed synapomorphies that occur on those branches.

Although the Brachycera and Calyptratae have high Bremer support values, they are supported by few unreversed synapomorphies. Across the tree, there are 35 unreversed synapomorphies on internal ingroup nodes for informative characters with a CI of 1 (black squares in Fig. 3). Excluding the Diptera, Cyclorrhapha and Oestroidea nodes, there are only 17 unreversed synapomorphies on the 38 internal ingroup nodes of the tree. There are another 37 synapomorphies on internal ingroup nodes for character states that have no homoplasy (black circles in Fig. 3) but where another state of that multistate character changes elsewhere on the tree. Most apomorphies are on terminal nodes (81 terminal vs 72 internal), representing uninformative autapomorphies for families (black circles). The remaining nodes throughout the tree are generally poorly supported, with the vast majority receiving a Bremer support of 1, and lacking bootstrap support greater than 50%.

Synapomorphies represented by a character state loss are considered to be less convincing, because, compared with presences, absences stand at a lower ontological level as observations (Nelson & Platnick, 1981; de Pinna, 1991; Agnarsson & Miller, 2008). This is not an issue in this study, as only four unambiguous synapomorphies on internal nodes for the ingroup are based on absence states – for Diptera (291, state 1; 364, state 1), Brachycera (23, state 1) and Cyclorrhapha (1, state 1) (Fig. 3) – and all affected clades are well supported based on presence states.

Homoplasy remains a major challenge for systematic studies. Homoplasy increases strongly with the addition of more taxa, but its relationship with number of characters is more subtle – most studies show a very slight decline in homoplasy levels with a larger number of characters (Sanderson & Donoghue, 1989; Meier *et al.*, 1991; Wiens, 2004). We tried to minimize homoplasy by carefully establishing primary homology statements through iterative testing, re-examination and, if necessary, redefinition or exclusion of characters. However, the vast majority of characters that were scored for this analysis remain homoplasious, which is probably due to the phylogenetic scope of this analysis, which included a wide variety of morphologically very divergent taxa. Similar results have been reported in other large-scale morphological analyses (Beutel *et al.*, 2011; Lawrence *et al.*, 2011). In our analyses, some characters that have been considered diagnostic apomorphies for families or groups of families become homoplasious

at the subordinal, ordinal or intraordinal level. For example, although the best-supported clade in this ordinal level analysis is the Cyclorrhapha, two apomorphies supporting that clade are homoplasious: pupation within a puparium formed from the last larval cuticle (94, state 1, present also in Stratiomyidae), and abdominal plaques absent (290, state 1, absent also in Siphonaptera). The latter, in particular, is due to the inclusion of non-Diptera taxa in this analysis.

The use of morphological characters is still strong in entomology (Bybee *et al.*, 2010) and the number of analyses is still increasing (Meier & Lim, 2009). Because of the challenges to character coding and homology assessment, Scotland *et al.* (2003) suggested that the optimal strategy for morphological data may be to use fewer, rigorously justified morphological characters. However, we disagree – much denser taxon sampling will increase the ability to discover homoplasy, and there is no evidence that the next character to be discovered will be any more or less informative, or more or less easy to interpret, than any previous one (Wiens, 2004).

Large-scale systematic analyses based on morphological characters are also likely to suffer from more missing data and inapplicable states. Beutel *et al.* (2011) reported analytical problems caused by taxa with numerous autapomorphies and inapplicable character states due to the loss of major structures (such as wings); however, they refrain from identifying their extent in their matrix. By contrast, Lawrence *et al.* (2011), in their ordinal study of Coleoptera, coded 516 characters for 366 taxa, presenting 188 856 character states with 3630 (1.9%) scored as unknown (?) and 12 194 (6%) inapplicable (−). In a superfamily study of the Hymenoptera, a poorly resolved consensus was reported from phylogenetic analysis of 111 terminals for 392 morphological characters (43 512 character states) with the suggestion that this resulted from the high percentage of missing data (41%, 17 840 character states), largely due to terminal mismatch (Sharkey *et al.*, 2011). Our matrix of 371 characters for 46 taxa has 17 066 character states of which 260 (1.5%) are scored as unknown (?) and 2625 (15%) inapplicable (−). This would appear to be a high percentage of inapplicable character states. Lawrence *et al.* (2011) noted also that a serious challenge in performing ordinal morphological analyses was the inapplicability of many characters in the outgroups. In our matrix, there are 1484 character states for the four outgroup taxa, of which 34 (2.3%) are scored as unknown (?) and 350 (24%) are inapplicable (−). Clearly a large proportion of the inapplicable states in our study are due to outgroups.

Conclusions

Rapid radiations in the Diptera

The lack of resolution within the lower Diptera, lower Brachycera and lower Cyclorrhapha may be due to periods of rapid radiation (Wiegmann *et al.*, 2011). Divergence times estimated for the three bursts of rapid diversification of dipteran lineages (220, 175 and 50 Ma; Wiegmann *et al.*, 2011)

correspond to recognized revolutions in terrestrial life on Earth, and are all in recovery periods following mass extinctions.

The first episodic radiation, the lower Diptera, is associated with the dramatic changes occurring on the Earth between the Permian of the Palaeozoic and the Mesozoic, and especially during the recovery through the Triassic (247–208 Ma) (Grimaldi & Engel, 2005). This Permian–Triassic event is the only known mass extinction event for insects, with eight or nine insect orders becoming extinct and ten more greatly reduced in diversity. The lower Diptera were part of the great radiation of modern insects that began 247 Ma in the early Triassic (Labandeira & Sepkoski, 1993).

Morphological studies are important for linking rapid radiations with key innovations that may explain them (Assis & de Carvalho, 2010), and some key innovations in Diptera may be related to major changes in environmental conditions through their history. Both the start and end of the Triassic geological period are marked by major extinction events. The radiation of the lower Brachycera is associated with the recovery following the Triassic–Jurassic extinction event. There was a gradual cooling and drying of the terrestrial ecosystems during the Jurassic (207 to 146 Ma) (Grimaldi & Engel, 2005), and as preferred aquatic and moist semi-aquatic terrestrial habitats dried, dipteran larvae may have evolved mechanisms to resist desiccation and thereby remained in the soil. The radiation of lower brachyceran lineages may have been fostered by the emergence of predatory and parasitic larvae better adapted to the more friable open soils.

The radiation of the Schizophora represents the largest insect radiation in the Tertiary (Grimaldi & Engel, 2005) and appears to be linked to the recovery following the K-T boundary impact (Wiegmann *et al.*, 2011). The prior evolution of the puparium may have allowed the rapid radiation of the Schizophora following the KT impact, as that adaptation provided resistance to desiccation in the drier terrestrial environments.

Prospects for the future

The present study is the first attempt of a matrix-based morphological phylogenetic analysis across Diptera. Although a significant part of the phylogenetic topology is consistent with recent molecular studies, some noteworthy conflicts point to areas of particular interest. Our study is a strong indication that we need to pay attention to a broader range of characters, but it is also an indication that some hypotheses of homology in our current dataset need to be revised. In addition, further taxon sampling is needed to provide better resolution and to provide the character distributions necessary for testing these homologies and building better evolutionary scenarios.

The much denser taxon sampling of the family-level molecular analysis in Wiegmann *et al.* (2011) may be partially responsible for some of the differences between the studies. For example, the Deuterophlebiidae are the sister taxon to all other Diptera in Wiegmann *et al.* (2011), but this family was not sampled here. The Apystomyiidae, a small family not sampled in this study, were found to be the sister taxon to the

Cyclorrhapha in Wiegmann *et al.* (2011). Nevertheless, results are broadly congruent: both propose the Diptera, Culicomorpha, Bibionomorpha, Brachycera, Eremoneura, Cyclorrhapha, Schizophora, Calyptratae and Oestroidea to be monophyletic. The monophyly of the Neodiptera and lower Brachycera found in Wiegmann *et al.* (2011) and discussed above, and the position of the Culicomorpha probably are the most significant incongruencies with respect to these studies.

This work summarizes accumulated morphological evidence used in Dipteran systematics. The character survey included synapomorphies for families not included in this analysis, making the character list a resource for dipteran systematic work beyond the scope of this study. We regard this study as a starting point for a new generation of systematists using morphological traits to study dipteran phylogeny.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/j.1365-3113.2012.00652.x

File S1. Morphological exemplar taxa.

File S2. Morphological character list.

File S3. Morphological data matrix.

File S4. Morphological character history and analysis.

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References

Agnarsson, I. & Miller, J.A. (2008) Is ACCTRAN better than DELTRAN? *Cladistics*, **24**, 1032–1038.

Amorim, D.S. & Yeates, D.K. (2006) Pesky gnats: ridding dipteran classification of the Nematocera. *Studia Dipterologica*, **13**, 3–9.

Assis, L.C.S. & de Carvalho, M.R. (2010) Key innovations: further remarks on the importance of morphology in elucidating systematic relationships and adaptive radiations. *Evolutionary Biology*, **37**, 247–254.

Bernasconi, J., Valsangiacomo, C., Piffaretti, J. & Ward, P. (2000) Phylogenetic relationships among Muscoidea (Diptera: Calyptratae) based on mitochondrial DNA sequences. *Insect Molecular Biology*, **9**, 67–74.

Bertone, M.A., Courtney, G.W. & Wiegmann, B.M. (2008) Phylogenetics and temporal diversification of the earliest true flies (Insecta: Diptera) based on multiple nuclear genes. *Systematic Entomology*, **33**, 668–687.

Beutel, R.G., Friedrich, F., Hörnschemeyer, T. *et al.* (2011) Morphological and molecular evidence converge upon a robust phylogeny of the megadiverse Holometabola. *Cladistics*, **27**, 341–355.

Bininda-Emonds, O.R.P., Gittleman, J.L. & Steel, M.A. (2002) The (super)tree of life: procedures, problems, and prospects. *Annual Review of Ecology and Systematics*, **33**, 265–289.

Bremer, K. (1992) Ancestral areas: a cladistic reinterpretation of the center of origin concept. *Systematic Biology*, **41**, 436–445.

Bremer, K. (1994) Branch support and tree stability. *Cladistics*, **10**, 295–304.

Buck, M. (2006) A new family and genus of acalyptrate flies from the Neotropical region, with a phylogenetic analysis of Carnoidea family relationships (Diptera, Schizophora). *Systematic Entomology*, **31**, 377–404.

Bybee, S.M., Zaspel, J.M., Beucke, K.A., Scott, C.H., Smith, B.W. & Branham, M.A. (2010) Are molecular data supplanting morphological data in modern phylogenetic studies? *Systematic Entomology*, **35**, 2–5.

Caravas, J. & Friedrich, M. (2012) Shaking the fly tree of life: performance analysis of nuclear and mitochondrial sequence data partitions. *Systematic Entomology*, in press.

Collins, K.P. & Wiegmann, B.M. (2002) Phylogenetic relationships and placement of the Empidoidea (Diptera: Brachycera) based on 28s rDNA and EF-1α sequences. *Insect Systematics and Evolution*, **33**, 421–444.

Courtney, G.W. (1991) Phylogenetic analysis of the Blephariceromorpha, with special reference to mountain midges (Diptera: Deuterophlebiidae). *Systematic Entomology*, **16**, 137–172.

Courtney, G.W. (1994) Biosystematics of the Nymphomyiidae (Insecta: Diptera): life history, morphology and phylogenetic relationships. *Smithsonian Contributions to Zoology*, **550**, 1–41.

Courtney, G.W., Sinclair, B.J. & Meier, R. (2000) Chapter 1.4 Morphology and terminology of Diptera larvae. *Contributions to a Manual of Palaearctic Diptera (With Special Reference to Flies of Economic Importance), Volume 1: General and Applied Dipterology* (ed. by L. Papp and B. Darvas), pp. 85–163. Science Herald, Budapest.

Courtney, G.W., Pape, T., Skevington, J.H. & Sinclair, B.J. (2009) Biodiversity of Diptera. *Insect Biodiversity: Science and Society* (ed. by P.H. Adler and R.G. Foottit), pp. 185–222. Blackwell Publishing, Oxford.

Cumming, J.M., Sinclair, B.J. & Wood, D.M. (1995) Homology and phylogenetic implications of male genitalia in Diptera – Eremoneura. *Entomologica Scandinavica*, **26**, 121–151.

Dikow, T. (2009) A phylogenetic hypothesis for Asilidae based on a total evidence analysis of morphological and DNA sequence data (Insecta: Diptera: Brachycera: Asiloidea). *Organisms, Diversity and Evolution*, **9**, 165–188.

Farris, J.S. (1989) The retention index and the rescaled consistency index. *Cladistics*, **5**, 417–419.

Friedrich, F. & Beutel, R.G. (2010) Good bye Halteria? The evolution of the thorax in Holometabola. *Cladistics*, **26**, 579–612.

Friedrich, M. & Tautz, D. (1997) Evolution and phylogeny of the Diptera: a molecular phylogenetic analysis using 28S rDNA sequences. *Systematic Biology*, **46**, 674–698.

Gelman, A. & Rubin, D. (1992) Inference from iterative simulation using multiple sequences. *Statistical Science*, **7**, 457–511.

Gibson, J.F., Skevington, J.H. & Kelso, S. (2010) Placement of the Conopidae (Diptera) within the Schizophora based on ten mtDNA

and nrDNA gene regions. *Molecular Phylogenetics and Evolution*, **56**, 91–103.

Goloboff, P.A., Farris, J.S. & Nixon, K.C. (2008) TNT, a free program for phylogenetic analysis. *Cladistics*, **24**, 774–786.

Griffiths, G.C.D. (1972) *The Phylogenetic Classification of the Diptera Cyclorrhapha, with Special Reference to the Structure of the Male Postabdomen*. Dr. W. Junk, The Hague.

Griffiths, G.C.D. (1994) Relationships among the major subgroups of Brachycera (Diptera): a critical review. *The Canadian Entomologist*, **126**, 861–880.

Grimaldi, D. & Engel, M.S. (2005) *Evolution of the Insects*. Cambridge University Press, Cambridge.

Hennig, W. (1954) Flügelgeäder und System der Dipteren unter Berücksichtigung der aus dem Mesozoikum beschriebenen Fossilien. *Beiträge zur Entomologie*, **4**, 245–388.

Hennig, W. (1958) Die Familien der Diptera Schizophora und ihre phylogenetischen Verwandtschaftsbeziehungen. *Beiträge zur Entomologie*, **8**, 505–688.

Hennig, W. (1965a) Phylogenetic systematics. *Annual Review of Entomology*, **10**, 97–116.

Hennig, W. (1965b) Die Acalyptratae des baltischen Bernsteins und ihre Bedeutung für die Erforschung der phylogenetischen Entwicklung dieser Dipteren-Gruppe. *Stuttgarter Beiträge zur Naturkunde*, **145**, 1–215.

Hennig, W. (1966) *Phylogenetic Systematics* (translated by D.D. Davis and R. Zangerl). University of Illinois Press, Urbana, Illinois.

Hennig, W. (1968) Kritische bemerkungen über den bau der flügelwurzel bei den Dipteren und die frage nach der monophylie der nematocera. *Stuttgarter Beiträge zur Naturkunde*, **193**, 1–23.

Hennig, W. (1971) Neue untersuchungen uber die familien der Diptera Schizophora (Diptera: Cyclorrhapha). *Stuttgarter Beiträge zur Naturkunde*, **226**, 1–76 (English translation by G.H. Harder, obtained from [WWW document]. URL http://www.canacoll.org/Diptera/Main/diptera.htm. [accessed on 13 February 2009]).

Hennig, W. (1973) Diptera (Zweiflügler). *Handbuch der zoologie*, IV Band, 2 Hälfte: Insecta. 2 Teil: Spezielles. 31 (ed. by J.-G. Helmcke, D. Starck and H. Wermuth), pp. 337. Walter De Gruyter, Berlin (English translation obtained from [WWW document]. URL http://www.canacoll.org/Diptera/Main/diptera.htm. [accessed on 13 February 2009]).

Huelsenbeck, J.P. & Ronquist, F. (2001) MrBayes: bayesian inference of phylogeny. *Bioinformatics*, **17**, 754–755.

Källersjö, M., Farris, J.S., Kluge, A.G. & Bult, C. (1992) Skewness and permutation. *Cladistics*, **8**, 275–287.

Kitching, R.L., Bickel, D. & Boulter, S. (2005) Guild analyses of Dipteran assemblages: a rationale and investigation of seasonality and stratification in selected rainforest faunas. *The Evolutionary Biology of Flies* (ed. by D.K. Yeates and B.M. Wiegmann), pp. 388–415. Columbia University Press, New York, New York.

Kluge, A.G. & Farris, J.S. (1969) Quantitative phyletics and the evolution of anurans. *Systematic Zoology*, **18**, 1–32.

Kutty, S.N., Bernasconi, M.V., Sifner, F. & Meier, R. (2007) Sensitivity analysis, molecular systematics and natural history evolution of Scathophagidae (Diptera: Cyclorrhapha : Calyptratae). *Cladistics*, **23**, 64–83.

Kutty, S.N., Pape, T., Pont, A.C., Wiegmann, B.M. & Meier, R. (2008) The Muscoidea (Diptera: Calyptratae) are paraphyletic: evidence from four mitochondrial and four nuclear genes. *Molecular Phylogeny and Evolution*, **49**, 639–652.

Kutty, S.N., Pape, T., Wiegmann, B.M. & Meier, R. (2010) Molecular phylogeny of the Calyptratae (Diptera: Cyclorrhapha) with an emphasis on the superfamily Oestroidea and the position of Mystacinobiidae and McAlpine's Fly. *Systematic Entomology*, **35**, 614–635.

Labandeira, C. & Sepkoski, J. (1993) Insect diversity in the fossil record. *Science*, **261**, 310–315.

Lawrence, J.F., Ślipiński, A., Seago, A.E., Thayer, M.K., Newton, A.F. & Marvaldi, A.E. (2011) Phylogeny of the Coleoptera based on morphological characters of adults and larvae. *Annales Zoologici (Warszawa)*, **61**, 1–217.

Lewis, P.O. (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology*, **50**, 913–925.

Margush, T. & McMorris, F. (1981) Consensus n-trees. *Bulletin of Mathematical Biology*, **43**, 239–244.

McAlpine, J.F. (1989) Phylogeny and classification of the Muscomorpha. *Manual of Nearctic Diptera, Vol. 3, Research Branch Agriculture Monograph, Vol. 32* (ed. by J.F. McAlpine), pp. 1397–1581. Canadian Government Publishing Centre, Hull.

Meier, R. (2005) Role of Dipterology in phylogenetic systematics: the insight of Willi Hennig. *The Evolutionary Biology of Flies* (ed. by D.K. Yeates and B.M. Wiegmann), pp. 45–62. Columbia University Press, New York, New York.

Meier, R. & Baker, R.H. (2002) A cladistic analysis of Diopsidae (Diptera) based on morphological and DNA sequence data. *Insect Systematics and Evolution*, **33**, 325–336.

Meier, R. & Hilger, S. (2000) On the egg morphology and phylogenetic relationships of Diopsidae (Diptera : Schizophora). *Journal of Zoological Systematics and Evolutionary Research*, **38**, 1–36.

Meier, R. & Lim, G.S. (2009) Conflict, convergent evolution, and the relative importance of immature and adult characters in endopterygote phylogenetics. *Annual Review of Entomology*, **54**, 85–104.

Meier, R. & Wiegmann, B.M. (2002) A phylogenetic analysis of Coelopidae (Diptera) based on morphological and DNA sequence data. *Molecular Phylogenetics and Evolution*, **25**, 393–407.

Meier, R., Kores, P. & Darwin, S. (1991) Homoplasy slope ratio: a better measurement of observed homoplasy in cladistic analyses. *Systematic Zoology*, **40**, 74–88.

Merritt, D.J. (2005) Evolution and development of the dipteran nervous system. *The Evolutionary Biology of Flies* (ed. by D.K. Yeates and B.M. Wiegmann), pp. 173–195. Columbia University Press, New York, New York.

Michelsen, V. (1996) Neodiptera – New insights into the adult morphology and higher level phylogeny of Diptera (Insecta). *Zoological Journal of the Linnean Society*, **117**, 71–102.

Moulton, J.K. & Wiegmann, B.M. (2007) The phylogenetic relationships of flies in the superfamily Empidoidea (Insecta: Diptera). *Molecular Phylogenetics and Evolution*, **43**, 701–713.

Nelson, G. & Platnick, N. (1981) *Systematics and Biogeography: Cladistics and Vicariance*. Columbia University Press, New York, New York.

Nixon, K.C. (2002) *WinClada Version 1.00.08*. L.H. Bailey Hortorium, Cornell University, Ithaca, New York [WWW document]. URL http://www.cladistics.com/about_winc.htm [accessed on 23 December 2010].

Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P. & Nieves-Aldrey, J.L. (2004) Bayesian phylogenetic analysis of combined data. *Systematic Biology*, **53**, 47–67.

Oosterbroek, P. & Courtney, G. (1995) Phylogeny of the nematocerous families of Diptera (Insecta). *Zoological Journal of the Linnean Society*, **115**, 267–311.

Oosterbroek, P. & Theowald, B. (1991) Phylogeny of the Tipuloidea based on characters of larvae and pupae (Diptera, Nematocera) with an index to the literature except Tipulidae. *Tijdschrift voor Entomologie*, **134**, 211–267.

Pape, T. (1992) Phylogeny of the Tachinidae family-group (Diptera: Calyptratae). *Tijdschrift voor Entomologie*, **135**, 43–86.

Pape, T. & Thompson, F.C. (2010) *Systema Dipterorum, Version 1.0.* [WWW document]. URL http://www.diptera.org/ [accessed on 25 April 2010].

Pape, T., Bickel, D. & Meier, R. (eds) (2009) *Diptera Diversity: Status, Challenges and Tools.* Brill, Leiden.

Petersen, F.T., Meier, R., Kutty, S.N. & Wiegmann, B.M. (2007) The phylogeny and evolution of host choice in the Hippoboscoidea (Diptera) as reconstructed using four molecular markers. *Molecular Phylogenetics and Evolution*, **45**, 111–122.

de Pinna, M. (1991) Concepts and tests of homology in the cladistic paradigm. *Cladistics*, **7**, 367–394.

Remsen, J. & O'Grady, P. (2002) Phylogeny of Drosophilinae (Diptera: Drosphilidae) with comments on combined analysis and character support. *Molecular Phylogenetics and Evolution*, **24**, 294–264.

Richter, S. & Meier, R. (1994) The development of phylogenetic concepts in Hennig's early theoretical publications (1947–1966). *Systematic Biology*, **43**, 212–221.

Rognes, K. (1997) The Calliphoridae (blowflies) (Diptera: Oestroidea) are not a monophyletic group. *Cladistics*, **13**, 27–66.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes Version 3.0: bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Ronquist, F., Huelsenbeck, J.P. & van der Mark, P. (2005) *MrBayes Version 3.1 Manual* [WWW document]. URL http://mrbayes.scs.fsu.edu/mb3.1_manual.pdf [accessed on 19 December 2006].

Ronquist, F., Huelsenbeck, J.P. & van der Mark, P. (2010) *MrBayes Version 3.2 Manual* [WWW document]. URL http://mrbayes.csit.fsu.edu/wiki/index.php/Manual_3.2 [accessed on 23 December 2010].

Rotheray, G.E. & Gilbert, F. (2008) Phylogenetic relationships and the larval head of the lower Cyclorrhapha (Diptera). *Zoological Journal of the Linnean Society*, **153**, 287–323.

Saether, O. (2000) Phylogeny of Culicomorpha (Diptera). *Systematic Entomology*, **25**, 223–234.

Saigusa, T. (2006) Homology of wing venation of Diptera. *Unpublished handout distributed at the, 6th International Congress of Dipterology*, Fukuoka, p. 26.

Sanderson, M. & Donoghue, M. (1989) Patterns of variation in levels of homoplasy. *Evolution*, **43**, 1781–1795.

Schuh, T. & Farris, J.S. (1981) Methods of investigating taxonomic congruence and their application in the Leptopodomorpha. *Systematic Zoology*, **30**, 331–350.

Scotland, R.W., Olmstead, R.G. & Bennett, J.R. (2003) Phylogeny reconstruction: The role of morphology. *Systematic Biology*, **52**, 539–548.

Sharkey, M.J., Carpenter, J.M., Vilhelmsen, L. *et al.* (2011) Phylogenetic relationships among superfamilies of Hymenoptera. *Cladistics*, **27**, 1–33.

Sinclair, B.J. (1992) A phylogenetic interpretation of the Brachycera (Diptera) based on the larval mandible and associated mouthpart structures. *Systematic Entomology*, **17**, 233–252.

Sinclair, B.J. & Cumming, J.M. (2006) The morphology, higher-level phylogeny and classification of the Empidoidea (Diptera). *Zootaxa*, **1180**, 1–172.

Sinclair, B.J., Cumming, J.M. & Wood, D.M. (1994) Homology and phylogenetic implications of male genitalia in Diptera – Lower Brachycera. *Entomologica Scandinavica,* **24**, 407–432.

Sinclair, B.J., Borkent, A. & Wood, D.M. (2007) The male genital tract and aedeagal components of the Diptera with a discussion of their phylogenetic significance. *Zoological Journal of the Linnean Society*, **150**, 711–742.

Skevington, J.H. & Yeates, D.K. (2000) Phylogeny of the Syrphoidea (Diptera) inferred from mtDNA sequences and morphology with particular reference to classification of the Pipunculidae (Diptera). *Molecular Phylogenetics and Evolution*, **16**, 212–224.

Sorenson, M.D. (1999) *TreeRot, Version 2.* Boston University, Boston, Massachusetts.

Starý, J. (1992) Phylogeny and classification of Tipulomorpha, with special emphasis on the family Limoniidae. *Acta Zoologica Cracoviensia*, **35**, 11–36.

Starý, J. (2008) The wing stalk in Diptera, with some notes on the higher-level phylogeny of the order. *European Journal of Entomology*, **105**, 27–33.

Stuckenberg, B.R. (2001) Pruning the tree: a critical review of classifications of the homeodactyla (Diptera, Brachycera), with new perspectives and an alternative classification. *Studia Dipterologica*, **8**, 3–42.

Swofford, D.L. (2002) *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b.10.* Sinauer Associates, Sunderland, Massachusetts.

Trautwein, M.D., Wiegmann, B.M. & Yeates, D.K. (2010) A multi-gene phylogeny of the fly superfamily Asiloidea (Insecta): taxon sampling and additional genes reveal the sister group to all higher flies (Cyclorrhapha). *Molecular Phylogenetics and Evolution*, **56**, 918–930.

Wiegmann, B.M., Mitter, C. & Thompson, F.C. (1993) Evolutionary origin of the Cyclorrhapha (Diptera): tests of alternative morphological hypotheses. *Cladistics*, **9**, 41–81.

Wiegmann, B.M., Tsaur, S.C., Webb, D.W. & Yeates, D.K. (2000) Monophyly and the relationships of the Tabanomorpha (Diptera: Brachycera) based on 28s ribosomal gene sequences. *Annals of the Entomological Society of America*, **93**, 1031–1038.

Wiegmann, B.M., Yeates, D.K., Thorne, J.L. & Kishino, H. (2003) Time flies, a new molecular time-scale for brachyceran fly evolution without a clock. *Systematic Biology*, **52**, 745–756.

Wiegmann, B.M., Kim, J. & Trautwein, M.D. (2009) Holometabolous insects (Holometabola). *The Timetree of Life* (ed. by B. Hedges and S. Kumar), pp. 260–263. Oxford University Press, New York.

Wiegmann, B.M., Trautwein, M.D., Winkler, I.S. *et al.* (2011) Episodic radiations in the fly tree of life. *Proceedings of the National Academy of Sciences*, **108**, 5690–5695.

Wiens, J.J. (2004) The role of morphological data in phylogeny reconstruction. *Systematic Biology*, **53**, 653–661.

Winterton, S.L., Wiegmann, B.M. & Schlinger, E.I. (2007) Phylogeny and Bayesian divergence time estimations of small-headed flies (Diptera: Acroceridae) using multiple molecular markers. *Molecular Phylogeny and Evolution*, **43**, 808–832.

Wood, D.M. (1991) Homology and phylogenetic implications of male genitalia in Diptera. The ground plan. *Proceedings of the Second International Congress of Dipterology, Bratislava, Czechoslavakia* (ed. by L. Weismann, I. Országh and A.C. Pont), pp. 255–284. SPC Academic Publishing, The Hague.

Wood, D.M. & Borkent, A. (1989) Phylogeny and classification of the Nematocera. *Manual of Nearctic Diptera, Vol. 3, Research Branch Agriculture Monograph, Vol. 32* (ed. by J. McAlpine), pp. 1333–1370. Canadian Government Publishing, Centre, Hull.

Woodley, N.E. (1989) Phylogeny and classification of the "Orthorrhaphous" Brachycera. *Manual of Nearctic Diptera, Vol. 3*, *Research Branch Agriculture Monograph, Vol. 32* (ed. by J.F. McAlpine), pp. 1371–1395. Canadian Government Publishing Centre, Hull.

Woodley, N.E., Borkent, A. & Wheeler, T.A. (2009) Phylogeny of the Diptera. *Manual of Central American Diptera*, Vol. 2 (ed. by B.V. Brown, A. Borkent, J.M. Cumming, D.M. Wood, N.E. Woodley and M.A. Zumbado), pp. 79–94. NRC Research Press, Ottawa.

Wootton, R.J. & Ennos, A.R. (1989) The implications of function on the origin and homologies of the dipterous wing. *Systematic Entomology*, **14**, 507–520.

Yeates, D.K. (2002) Relationships of the lower Brachycera (Diptera): a quantitative synthesis of morphological characters. *Zoologica Scripta*, **31**, 105–121.

Yeates, D.K. & Wiegmann, B.M. (1999) Congruence and controversy: toward a higher-level phylogeny of the Diptera. *Annual Review of Entomology*, **44**, 397–428.

Yeates, D.K. & Wiegmann, B.M. (2005) Phylogeny and evolution of Diptera: recent insights and new perspectives. *The Evolutionary Biology of Flies* (ed. by D.K. Yeates and B.M. Wiegmann), pp. 14–44. Columbia University Press, New York, New York.

Yeates, D.K. & Wiegmann, B.M. (2012) The impact of the Manual of Nearctic Diptera on phylogenetic Dipterology. In Ottawa Dipterology (1937–1989): festschrift commemorating the coordinators of the Manual of Nearctic Diptera and their contributions to building the Canadian National Collection of Insects. Part III. *The Canadian Entomologist*, **144**, 197–205.

Yeates, D.K., Merritt, D.J. & Baker, C.H. (2002) The adult ventral nerve cord as a phylogenetic character in brachyceran Diptera. *Organisms Diversity and Evolution*, **2**, 89–96.

Yeates, D.K., Wiegmann, B.M., Courtney, G.W., Meier, R., Lambkin, C. & Pape, T. (2007) Phylogeny and systematics of Diptera: two decades of progress and prospects. *Linnaeus Tercentenary: Progress in Invertebrate Taxonomy. Zootaxa*, **1668**, 565–590.

Zatwarnicki, T. (1996) A new reconstruction of the origin of the eremoneuran hypopygium and its implications for classification (Insecta: Diptera). *Genus*, **7**, 103–175.