Zootaxa 4374 (1): 107–143
http://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.4374.1.7
http://zoobank.org/urn:lsid:zoobank.org:pub:13A1A730-B5A9-4305-9AF5-409FD36F0384

The State of Phylogenetic Analysis: Narrow Visions and Simple Answers —Examples from the Diptera (flies)

ART BORKENT
Research Associate, Royal British Columbia Museum and the American Museum of Natural History, 691-8th Ave. SE, Salmon Arm, British Columbia, V1E 2C2, Canada. E-mail: artborkent@telus.net

Abstract

The order Diptera is remarkably diverse, not only in species but in morphological variation in every life stage, making them excellent candidates for phylogenetic analysis. Such analysis has been hampered by methods that have severely restricted character state interpretation. Morphological-based phylogenies should be based on a deep understanding of the morphology, development and function of character states, and have extensive outgroup comparisons made to determine their polarity. Character states clearly vary in their value for determining phylogenetic relationships and this needs to be studied and utilized. Characters themselves need more explicit discussion, including how some may be developmentally or functionally related to other characters (and potentially not independent indicators of genealogical relationship). The current practice by many, of filling a matrix with poorly understood character states and highly limited outgroup comparisons, is unacceptable if the results are to be a valid reflection of the actual history of the group.

Parsimony analysis is not an objective interpretation of phylogenetic relationships when all characters are treated as equal in value. Exact mathematical values applied to characters are entirely arbitrary and are generally used to produce a phylogeny that the author considers as reasonable. Mathematical appraisal of a given node is similarly inconsequential because characters do not have an intrinsic mathematical value. Bremer support, for example, provides values that have no biological reality but provide the pretence of objectivity. Cladists need to focus their attention on testing the validity of each synapomorphy proposed, as the basis for all further phylogenetic interpretation, rather than the testing of differing phylogenies through various comparative programs.

Current phylogenetic analyses have come to increasingly depend on DNA sequence-based characters, in spite of their tumultuous history of inconsistent results. Until such time as sequences can be shown to produce predictive phylogenies (i.e., using Hennigian logic), independent of morphological analysis, they should be viewed with caution and certainly not as a panacea as they are commonly portrayed.

The purported comprehensive analyses of phylogenetic relationships between families of Diptera by Wiegmann *et al.* (2011) and Lambkin *et al.* (2013) have serious flaws and cannot be considered as the "Periodic Table" of such relationships as originally heralded.

Systematists working on Diptera have a plethora of complex and informative morphological synapomorphies in every life stage, either described or awaiting study. Many lineages have the potential of providing a wealth of evolutionary stories to share with other biologists if we produce stable phylogenies based on weighted synapomorphies and interpreted to elucidate the zoogeographic and bionomic divergence of the group. Some lineages are devoid of convincing synapomorphies and, in spite of our desires, should be recognized as being largely uninterpretable.

Key words: Cladistics, parsimony analysis, outgroup, character state, character weighing, genomics, molecular, sequence

> "Computers are useless. All they give you are answers." Pablo Picasso.
>
> "At the same time a pseudoscience based on quantitative manipulations of questionable phenetic data has focused on cladograms at the exclusion of character analysis. Reversing this trend will require a return to the refinement of Hennig's theories and methods and the integration of diverse available data." Wheeler (2008a).

Accepted by D. Bickel: 3 Nov. 2017; published: 17 Jan. 2018

Introduction

When one reads the current literature in Diptera systematics it appears that we are living in a golden age of phylogenetics. Group after group is being sequenced and the resultant phylogenies abound (e.g., Buenaventura *et al.*, 2016; Caravas & Friedrich, 2013; Chapman *et al.*, 2012; Ding *et al.*, 2015; Haseyama *et al.*, 2015; Semelbauer, 2016; Ševčík, 2016; Wang *et al.*, 2016; Winkler *et al.*, 2015; Winterton *et al.*, 2016; Young *et al.*, 2016a; Zhang *et al.*, 2016). Molecular analyses are promising to give final answers (Trautwein *et al.*, 2017; Yeates *et al.*, 2016) and morphological analyses are often as straightforward as scoring variable character states in a matrix, choosing a few taxa as outgroups and determining the most parsimonious tree with a program (and options) of choice (usually PAUP or Mesquite + TNT). Additionally, some morphologists appear inclined to entirely hand over phylogenetic questions to molecular analysis. A recent book on the morphology of insects by Beutel *et al.* (2014: viii), in reflecting on molecular analyses in their introduction, state "… it is probably not over optimistic to assume that a more or less completely resolved hexapod phylogeny (on the interordinal level) will be available in the very near future". As such, a recent book *Next Generation Systematics* (Olson *et al.*, 2016) appears to have redefined the concept of systematics as equivalent to phylogenomics and the study of biodiversity to the exclusion of morphological study. Increasingly, groups of scientists without any, or very little taxonomic/systematic training are pursuing molecular sequences (sometimes just the *CO1* gene) apparently in the belief that simply plugging the sequence data into a computer program will give a reasonable (or at least publishable) phylogeny (e.g., Ander *et al.*, 2013; Chu *et al.*, 2016; Grace-Lema *et al.*, 2015; Karimian *et al.*, 2014; Muñoz-Muñoz *et al.*, 2014; Norris & Norris, 2015; Pagès *et al.*, 2009; Sum *et al.*, 2014; Talavera *et al.*, 2017; Tay *et al.*, 2016; Wang *et al.*, 2014). There is a common conception that genomes will provide solid phylogenetic answers and that the characterization of species by morphological systematists, if not entirely replaced with barcodes, may even be largely superseded by phenomics, the scanning and computer analysis of morphological structures (Deans *et al.*, 2015; La Salle *et al.*, 2009). There is a remarkable level of faith in techniques, in spite of fundamental problems regarding the nature of morphological characters, how they are interpreted and a very questionable history of molecular sequences in providing accurate portrayals of cladistic relationships.

In this paper I suggest that we have gone off the tracks in how we are approaching phylogenetic questions and that systematics has become largely reduced to a set of inappropriate techniques that fail to address the most fundamental question of the validity of a given character's polarity. Increasingly studies are avoiding broader evolutionary questions which interpret the likely meaning of the information gathered. Students have come to accept that phylogenetic hypotheses based on morphology are primarily composed of comparing various phylogenetic patterns derived from a matrix of characters, instead of the careful determination of the polarity of each character state. Somewhere we lost the idea that Hennig's (1950, 1965, 1966) fundamental contribution to systematic thought was the logic of how to interpret whether a given character state was plesiomorphic or apomorphic and that such interpretation was a testable hypothesis. It is through interpretation of the polarity of each character state that the entire phylogenetic construct depends and it is in that arena that systematists should be producing the most sweat.

In this paper, I focus on issues present in phylogenetic analyses in the Diptera because I am most familiar with this order. However, virtually all the problems discussed here appear to be prevalent throughout at least zoology.

The Diptera (true flies) are, for several reasons, an excellent group for the study of phylogenetic relationships and the techniques that are best suited to interpret these (Pape *et al.*, 2009; Yeates & Wiegmann, 2005). First, the order has diverged morphologically in every stage. Larvae are often so different from one another that debates have ensued over the homology of different mouthparts, even within a given family. Pupae and adults are also highly divergent and as such provide a further wealth of features to interpret. Second, and as a corollary of this variation, the divergence of each life stage provides an outstanding platform to corroborate whether select synapomorphies (high-weight) from different stages provide congruent phylogenies. Third, the Diptera are remarkably species rich with over 159,000 species named (Borkent *et al.*, in press). Although presently considered to represent an impressive 12% of all eukaryotic life, this estimate is likely far too low and there are a huge number of taxa awaiting description. As such, dipterists have a vast array of morphological variation available for study that is helpful in determining homologies and homologous character states. Fourth, due to their abundance in the plethora of amber deposits around the world, the Diptera are particularly well represented by a very rich fossil record, allowing for the most detailed of morphological study. Often, even Lebanese amber (125-129 million years old),

the oldest deposit bearing abundant insect inclusions, has specimens that look largely as if they had been recently placed in resin. Some of these have been partially cleared during preservation, allowing details of mouthparts and genitalia, for example, to be studied. Such fossils provide additional information regarding character states and, especially whether two or more character states are truly representative of a given phylogenetic node. So too, we should expect accurate phylogenetic patterns based on extant taxa to be reflected in a fossil record in which successively older lineages are represented by successively older fossils. It seems likely that Hennig, who was intimately familiar with every life stage and the more limited fossil record (then), was attracted to the study of Diptera for these very reasons, all lending themselves to cladistic analysis. He hypothesized the first detailed familial phylogeny of the order and provided a wealth of features supporting it. Subsequent study has confirmed much of Hennig's conclusions and provided further resolution of various groups, while others are still contentious.

Current study, however, of morphologically-based phylogenetic relationships among families of Diptera is largely at a standstill, in spite of the public appearance of having been largely resolved. In fact, there are a large number of fundamental questions yet remaining. An analysis of the interfamilial relationships within the Diptera, based on molecular and morphological data was published with great fanfare by Wiegmann *et al.* (2011), the result of a cooperative effort among 27 authors in 17 institutions. Declared to be the "new Periodic Table for flies" it was stated to be based on the "most complete set of fly genetic and structural anatomy data ever collected" (Kulikowski, 2011). A companion paper by Lambkin *et al.* (2013), based on far fewer taxa, described in more detail the morphological component of the study. Readers might have been led to believe that the interfamilial relationships are completely understood by these analyses and numbers of subsequent authors treat it as such. In fact, much is uncertain, including the approach taken. More on this below.

SUMMARY: Diptera are an excellent group for phylogenetic analysis but practical and theoretical approaches have hampered their interpretation. Both morphological and molecular analyses require scrutiny.

There are, in addition, a plethora of intrafamilial studies by the community of dipteran systematists and, worldwide, most of the approximately 160 families of flies have one or more taxonomic experts. Most modern morphological phylogenetic analyses of various groups, however, generally are as straightforward as inserting a variety of morphological features into a matrix that is subsequently analyzed by one or more programs (with various options), using rather limited outgroup comparisons to produce the most parsimonious and/ or maximum likelihood tree(s). Character states are most often not discussed; nor is it generally clear which character states support which nodes. Molecular analyses, sometimes in conjunction with morphological data, are generally so technique-bound for those not intimately familiar with molecular analysis as to be virtually opaque. Such phylogenies are taken on faith that the techniques must be valid. The mix of morphological and molecular data, the so-called "whole evidence" approach is also often of uncertain value because the elements cannot (or only with great difficulty) be identified in some studies. In short, my repeated question of "What is the evidence supporting that relationship?" is often either unavailable or can only be extracted with substantial effort (including rerunning the analyses to determine this, if all options are stated). Such methodological inaccessibility, along with a shift away from detailed morphological explanation, is hampering our ability to assess phylogenetic relationships with regard to the fundamental tenants of cladistic analysis.

Diptera systematics is in a crisis that needs to be addressed. The further we depart from the study of nature (here character states and the organisms that bear them), the more we will be immersed in a caricature of nature of our own construction. Complex mathematical models, often intimidating but bearing an aura of authenticity and authority, have become the *modus operandi*. A serious consequence is that many students are skilled in methods (at an operational level) and comparatively ignorant of the organisms at hand. Below, I describe some of the issues that I believe need addressing. My desire is to encourage a broader perspective in our studies and far greater clarity in our analyses, especially regarding the characters studied. Diptera could be more fully utilized as an exemplary group for portraying evolutionary patterns and how these pattern should be best presented within the broader scientific community.

The problems

1. Approach of Many Systematists Toward Morphological Phylogeny Construction

"It is foolish to make confident statements about these matters if one does not devote a lot of time to them. It is useful practice to question every detail." Aristotle.

Every systematist begins, or should begin, their study with examination of specimens and an understanding of the literature (at least some publications). For too many the study of the specimens is short-lived, lasting only as long as a level of certainty is attained regarding how to recognize the different taxa. Combined with a scan of the different species for features shared by only some, a matrix of character states, determined first-hand or with literature, is often rapidly filled in and an outgroup of one or a few taxa provides the tool to polarize the ingroup variation. For a variety of reasons, discussed below, the resultant phylogenies vary in their mathematically determined parsimony and a restricted number of these provided in the publication. Rarely are the details, function or development of the characters discussed, nor the relationship of a given character to a greater whole of the organism portrayed. With a claim of objectivity, all characters are treated equally, unless a crazy (i.e., obviously ridiculous) phylogeny is produced, in which case various characters might be more strongly weighted to produce more "acceptable" results. Why these features might be more important or how one can determine the relative weight (e.g., 10 vs. 1) is unclear. For many, this is the end of the process and readers are left wondering what the significance of the phylogeny might be in interpreting behaviour, habitats, distribution and other interesting aspects of diversification of the group at hand. So too, many workers have limited knowledge of the bionomics of the group and restricted interest in other life stages. This appears to me to be a rather boring, simplistic and likely inaccurate understanding of the group at hand. If treated in such a mechanical and superficial manner, there is every reason to believe that much of what is accomplished could be duplicated by a combination of electronic specimen scans, computer generated phenomics, input and analysis. Sokal & Rohlf (1970), as statistician/ biologists, suggested that if phylogenetic information was a question of processing complex information, there was every reason to believe that this could be ultimately mechanized. A distinct possibility of *Deus ex machina*. But this is a blind machine, divorced from the complexity of character states, their cladistic interpretation, the actions and ecological context of the organism, and other clues to its evolutionary history.

Further to this, a dependency on literature as a source of character states is often fatal (but common). There is no replacement for first-hand examination of taxa (and therefore of well curated collections). Many years ago when I arrived in Ottawa, Canada to study with Monty Wood, I was confused by many conflicting publications describing characters and purported homologies among different families of nematocerous Diptera (e.g., Saether, 1977). In discussing any of these, Monty shortly directed me to the collection and specimens to study first-hand. It was an early lesson that much in the literature is flawed (or limited) and that there was no replacement for first-hand examination of specimens if we are to improve our science at this level.

SUMMARY: Many modern phylogenies have limited input of poorly understood characters, examine few taxa as outgroups and treat character states as equal in weight, here considered to be serious problems.

I realize that this is somewhat of a caricature and that some dipterists are doing more thorough and excellent work. However, it remains that the debate between different conclusions most often deals primarily with methods and not data. Overall there is a satisfaction with a maximum parsimony tree, rather than gathering more data, more opportunities to refine or complicate our perspectives. Students are learning how to manage the data rather than having a background in insect morphology, development, and behaviour that would provide the tools necessary to think about what character states might mean and their relative worth in indicating relationships. Because of a current virtual vacuum in the discussion of character weighing or what features may be functionally related (and more likely to be evolutionarily connected), there appears to be no need to go back to the data that was input in the first place when confronted with character states indicating conflicting phylogenetic conclusions. The question appears not to be "Which of my character states may have been misinterpreted?" but "How might I manipulate the data to obtain a more parsimonious tree?" For many this means tweaking the data (providing some character

weighing, eliminating some character states, or other modifications) that will then provide something that seems "more reasonable" or intuitively more accurate. Students are given the understanding that phylogeny construction is fundamentally a computer game that doesn't question the value of the character states initially inputted and promotes a rapid appraisal of character states and then subsequent analysis, with emphasis placed on learning the various programs (and their options) to manipulate the data. Such limitations are often apparent in their publications.

2. Polarizing Character States

The interpretation of each character state is the most important and fundamental aspect of phylogenetic construction (Brower, 2000; Eldredge & Cracraft, 1980; Hennig, 1950, 1965, 1966; Hennig & Schlee, 1978; Schlee, 1978) and should demand the greatest attention and discussion by systematists. It is entirely on the basis of the validity of each separate character state that phylogenetic conclusions rest.

As an undergraduate and graduate student at the University of Alberta in the 1970s cladistics was taught as follows. One picked a group (say a genus) to work on, somewhat diverse and hopefully with some preliminary evidence that it was monophyletic (e.g., one or more features unique within the family and hopefully even more broadly distinctive). As one determined the variation in character states in the genus, the question arose as to what character state was apomorphic and which was plesiomorphic. As such, a search of the outgroup began. The outgroup was not one or two exemplars but consisted of an increasing range of taxa, initially including a taxon one thought of as the closest relative (or at least similar) and moving successively outward to encompass a larger and larger array of taxa. A good start would be able to say that a feature of one or more of the species in the genus was unique when compared to members of another genus in the family (hopefully all members of that genus), suggesting that the feature was apomorphic within the genus under study. However, a logical worry would be that the genus being studied was a basal lineage within the subfamily or family, so that the "unique" feature was actually plesiomorphic and the genus possibly paraphyletic. Therefore, in the face of such a possibility, it would be advisable to expand the outgroup to at least two monophyletic nodes below the group at hand. Although it is true that logically only one taxon is needed to polarize a feature, it would have been considered poor science (at that time unacceptable) to make such a limited comparison. It would be all the more informative and convincing if that one character state was unique within the subfamily, family, superfamily or even the entire order. For most studies today, one or a few outgroups are considered ample and these are most often exemplars, not as many species as possible in whole genera or families. I consider this to be a highly minimalistic and certainly a limited approach to determining whether a feature is derived or not. It is a mystery to me how many systematists have found themselves content with examining just a few taxa for outgroup comparisons, when it is clear that examining as many taxa as possible can provide far stronger evidence that a feature has evolved *de novo*. This is especially relevant when a major concern is whether homoplasy has occurred, a feature of virtually every group with more than a few species and/or characters.

As currently practiced, a limited outgroup allows for the polarization of each and any character a researcher decides to put into her/his matrix. This fail-proof method of determining synapomorphies (and often lots of them) allows for only a superficial understanding of characters. Broad outgroup comparisons are necessary to learn which features are truly unique and which are highly homoplastic, with of course, others being intermediate and more difficult to interpret. Hennig (1966) noted the importance of extensive comparisons among taxa to fully understand homology and hence synapomorphies (Kavanaugh, 1972; Schlee, 1978). The limited outgroup approach invalidates and is antithetical to building phylogenies that reflect actual genealogical relationships and that are predictive regarding further characters and species.

There is one circumstance, however, when it is appropriate to use a limited number of exemplars in morphological / phylogenetic studies. When a character state is newly discovered (i.e., no or little literature available) and is complex and/or difficult to investigate, there are inherent limits to the number of taxa in the outgroup that can be studied if the author hopes to publish the results in a reasonable time frame. In such instances of limited knowledge it is best to select early lineages within each of the available outgroups for study. In my analysis of the pupal structures of the families of Culicomorpha (Borkent, 2012), for example, I recognized that I could not study all available pupal material of this group of 18,103 species if I was to make fresh comparisons and

interpret, as would be expected, many new features of phylogenetic importance not previously reported for most taxa. Even for features that were reported in some families (e.g., Culicidae, Chironomidae), the literature was so large that I didn't have the time to consult all publications (although I did study much of that literature). I had a collection of many Ceratopogonidae available (Borkent, 2014) but lacked broad first-hand material of most other families. As such, I made a special effort to obtain specimens through loans and primarily studied the early lineages of each of the seven other families of Culicomorpha. For example, within the Simuliidae, with 2,132 species and 26 genera worldwide (Adler & Crosskey, 2012), the genus *Parasimulium* is confidently considered the sister group of all other members. In looking for homologous character states among the families, it was logical to focus study on the members of the earliest lineage(s) of each. Even so, there is a serious risk that the earliest lineage(s) have many apomorphic features (think platypus within the Mammalia) and so adding some further early taxa (e.g., *Prosimulium*) enhances the likelihood that homologous character states are correctly identified. For some families like Thaumaleidae, no intrafamilial phylogeny is known and so, for this small family I studied a variety of taxa. For each of the families, I asked available experts to appraise my characterization of their family, adding significant insight to the phylogenetic interpretation of given features. As such, this exemplar approach (comparing early lineages in each family) allowed me to maximize the likelihood of detecting homologous character states among the families of Culicomorpha.

Today the use of limited exemplars has become the *modus operandi* and is nearly universal in studies using an approach combining and/or comparing molecular and morphological data (e.g., Kitching *et al.*, 2015; Petersen *et al.*, 2010; Tkoč *et al.*, 2016; Winterton & Ware, 2015). Such limited examination is inherent to current methods utilized in sequence analyses but is unacceptably reductionist for studies including morphological features. An exemplar approach is used even when morphological character states are known for far more taxa (e.g., Cerretti & Pape, 2012; Forster *et al.*, 2016; Kits *et al.*, 2013; Koch *et al.*, 2015; Schneeberg & Beutel, 2014; Wagner & Stuckenberg, 2016; Williams *et al.*, 2016; Yassin, 2013) and cannot be considered as thorough science, and especially not when it ignores previously published information. Such limited comparisons strongly reduce the possibility of understanding character state distributions in any sort of breadth, and increases the odds of misinterpreting the results.

SUMMARY: Limited outgroup comparisons allow nearly every ingroup character state to be polarized and present a minimalistic approach. Broad comparisons allow for identification of high-weight synapomorphies, if present, and those which are homoplastic.

For many features, character states are discovered to vary in the outgroup (are homoplastic) and two questions come into play. The first quandary should always be whether the homoplasy has been accurately determined in the first place. One should always go back to the specimens and put two examples of the homoplastic features under a microscope (preferably side by side) and look again. Often, details not previously detected become apparent with further scrutiny. A characterization of "thorax with long setae" as initially scored, may be actually quite different between two species when re-examined and studied in more detail. This is a process of "getting it right" and should be an important and first line of investigation in dealing with homoplasy.

The second question concerns the distribution of the character state. If present in close relatives (i.e., within several well established nodes), the character state should be considered to be of low value and often disregarded as an indicator of relationship. If the homoplasy is present in only distant taxa, it would likely have greater value as a synapomorphy in the group at hand. For example, there is a group of species within the genus *Stenochironomus* (Chironomidae, Chironominae, Chironomini) that have brilliantly shiny eyes as adults (Borkent, 1984). Within the nematocerous Diptera, the only other known instances of this are in a few species in each of the following families: Tanypodinae (Chironomidae), Simuliidae, Tipulidae and Cecidomyiidae. There are so many branching points between these groups that it is likely that it evolved independently in each and therefore is a good indicator of relationship at least within *Stenochironomus*.

There is ample evidence that some features of organisms have evolved more than once and often these are tied to common environmental conditions. Many desert mammals have longer legs (to elevate them above the hot substrate), wing pigmentation in Diptera has likely evolved hundreds of times, the lengths of setae at the apex of the larval abdomen of some species of *Culicoides* are related to substrate type (Kettle & Elson, 1976), and it would

certainly be important to avoid using "foreleg yellow" as a synapomorphy, when it occurred sporadically in nearly every genus in a family.

The above discussion is not meant to denigrate the potential value of a matrix in determining character state polarity. Clearly, matrices have made character state analysis more explicit in portraying their distribution within the taxon under study. Every species is scored and the reader can quickly understand the presence or absence of character states. Matrices are capable of showing character state distributions in a broad outgroup (although rarely including more than a few taxa). The use of a groundplan value can be used to indicate truly unique features, or a conclusion from broad comparisons. However, a general lack of character state discussion eliminates the possibility that the reader can evaluate the character state distribution in the outgroup in any detail. I always want to know if the author has studied 5, 10, or 200 species in the outgroup and what they were. Statements such as "unique within the Ceratopogonidae and absent in all other Culicomorpha other than a few derived Chironomidae" are informative and a testing ground for the next student of the group. The outgroup taxa studied should be listed in materials and methods and noted in conjunction with the discussion of a given feature. The exact comparisons made for each character (which often vary according to published or current research) should be explicitly stated when varying from the list in materials and methods. In addition, further morphology and function of a character and character state should be discussed (more on this below). When study is limited and comparisons of a given structure restricted to a small outgroup, it is reasonable for readers to be strongly suspicious of the resultant phylogenetic results.

It is interesting that Hennig's (1973) massive publication on Diptera and Brundin's (1966) famous book on transantarctic relationships of Chironomidae did not provide outgroup comparisons for their proposed synapomorphies. They probably assumed that their audiences had a broad knowledge of character states, a level of expertise that no longer exists. Even within such large families as Culicidae and Chironomidae there are few who have a broad understanding of the morphology of an entire family, including larvae, pupae and adults. In the face of continuing specialization, it behoves every author to explicitly discuss the basis for their decisions regarding homology and polarity if they are to be truly transparent. A vital part of doing good science is knowing what comparisons have been made, allowing subsequent researchers to access the proposed synapomorphy with greater rigour and clarity.

Such discussion regarding character states and their polarity should not only address the outgroup distribution, detailed morphology and development, but also point out any problems of interpretation. For any larger group with a number of features, it is misleading to portray each character state as if there were no questions or remaining challenges to its interpretation. To simply provide data as 0s and 1s is a reductionist presentation that introduces an opacity that others cannot penetrate—hardly the characterization of progressive science. As it stands, most recent morphologically-based phylogenies simply, at best, itemize their characters, with the plesiomorphic and apomorphic states merely listed (often relegated to supplemental files) (e.g., Fu *et al.*, 2016; Gibson & Skevington, 2013; Inclán *et al.*, 2016; Kits *et al.*, 2013; Meier & Wiegmann, 2002; Morgulis *et al.*, 2016; Wagner & Stuckenberg, 2016; Williams *et al.*, 2016; Young *et al.*, 2016b). Such presentations limit scientific dialogue, criticism, and development.

3. What is a Character?

Imbedded in current phylogenetic analyses are mathematical gauges of the validity or relative strength of each node on a cladogram. Bremer support for example, provides a number indicating the level of evidence present for the monophyly of a particular node. Fundamental to the question of the importance of the numerical portrayal of evidence is the nature of the characters utilized (Stevens, 2000). On the face of it, it is as simple as the more synapomorphic states we can discover for a node, the more likely the validity of that node.

Presumably, morphologically-based systematists search for independent indicators of relationship for a given group of species. It is vital, therefore, that evidence for such independence (or an expression of ignorance) be stated regarding the various characters and, as noted above, calls for an explicit discussion of the characters utilized in a study. Clearly, when one is proposing three synapomorphies as evidence for a specific node, all of which are related to the relative thickness of three different wing veins, there is a strong possibility that there is actually only one feature "thicker wing veins". The opposite is also likely to be true – three congruent synapomorphies from each of

three different life stages and functionally unrelated, such as features of larval mouthparts, pupal respiratory organ and adult male parameres, are particularly strong evidence that a particular node reflects the true ancestry of the included taxa. In many situations it is not understood how features may be functionally and/or developmentally related but this is no excuse for lack of discussion. It is up to authors to either convince the reader of a level of independence, or admit uncertainty, providing clarity and analytical opportunities for the next student of the group.

SUMMARY: Characters require detailed study. Overly split characters can bias phylogenetic conclusions. Strong support for a given node is enhanced when two or more independent synapomorphies are present.

Understanding the development of a feature is often of critical importance for indicating independent features from different semaphoronts (Heming, 2003). For example, the pupal mouthparts of Culicidae are extremely elongate and extend to the apex of the wings and then curve dorsally, following the rounded margin of the wing. Outgroup comparisons indicate this condition is unique within the Diptera and a synapomorphy of the family. Clearly, this pupal feature is directly related to the presence of elongate mouthparts of the adults and therefore the two are not independent indicators of relationship. This example is obvious but there are many that are not and require detailed study and interpretation. The pupae of Simuliidae have an elongate posterolateral extension of tergite 1 that is unique within the Diptera. Further study, however, indicated that it contains the developing elongate setae of adult tergite 1, a feature of the adult previously recognized as a synapomorphy of the family. These features in the two life stages are directly related developmentally (Borkent, 2012). Many characters are interconnected structurally and developmentally and such information is critical to a valid interpretation of the significance of proposed synapomorphies.

Finally, limiting character state information to mere lists and matrices often makes characters seem simpler than they are, which produces a loss of information. Character states need to be discussed because many (all?) are more complex than just a short descriptor and a numeral. For too many authors it appears that the character states described in their species descriptions for two or more species are those to be plugged into their matrix for phylogenetic study. This approach does an end run around the problem of what a character is. Further to this, it is far more interesting and valuable to us to understand an author's presentation of the actual character states utilized in a given phylogeny (e.g., Amorim & Rindal, 2007; Borkent, 2014; Brown *et al.*, 2015; Courtney, 1991, 1994b; Harbach & Kitching, 1998; Sinclair & Cumming, 2006), than solely a numerical value representing the features present on a node.

4. Character Weighing

"Have no fear of perfection - you'll never reach it" Salvador Dali

Probably in common with all systematists, I regularly find myself explaining to non-specialists what it is that I do. I explain my role in finding and describing new species and revising our understanding of the old. I describe the thrill of discovery and the intimacy of characterizing and naming a species for the first time and just how many species there are that yet need names. I often relate the excitement of knowing that every part of the specimens I examine has a function and an evolutionary history. As evolutionary biologists we have come to understand that, with sufficient study, the peculiar features we see most often have a peculiar and significant story that is embedded in the evolution and natural history of that species. The weird hind wings of Diptera function as gyroscopes, allowing magnificent feats of flight, especially by higher lineages. The long, piercing mouthparts of mosquitoes allow each female to explore and find subcutaneous capillaries. The list of such adaptations is long and tells us that when we discover new features of unknown significance, it is only a question of lack of study. It is a provocation to discover what their function and evolutionary history might be.

This reality reflects a fundamental aspect of character interpretation and analysis that concerns the relative value of a character state in indicating phylogenetic relationships (Wheeler, 2007). It is already clear to dipterists that the length and colour of setae are in general relatively poor indicators of relationships, while the presence of a puparium is a more secure and better predictor of additional synapomorphies and the phylogenetic position of

further taxa. As noted above, thorough and extensive outgroup comparisons often allows for the identification of which features are homoplastic and which may be strong synapomorphies. This, of course, does not include all features and there are many of less certain interpretation (e.g., some homoplasy in some related taxa or ingroup conflict). What about other criteria to determine relative worth? Although rarely discussed today, earlier years saw numbers of authors discussing and analyzing the problem (Hecht, 1976; Hennig & Schlee, 1978; Neff, 1986; Sharkey, 1989; Schlee, 1976; Wheeler, 1986). In summary, they described at least the following important factors as evidence for considering the relative weight of a given character.

1. *Character state distribution (consistency)*. Broad and extensive comparisons with a maximal number of taxa increase the likelihood of correct interpretation of character state polarity.
2. *Morphological complexity.* Increased complexity increases confidence of interpretation of homology and stronger evidence of polarity.
3. *Functional complexity.* Shared complex function of a morphological character increases the likelihood of the valid interpretation of apomorphic states. Those features affected by common ecological parameters are lower weight.
4. *Developmental pathways*. Complex development suggests increased confidence of character states as apomorphic.
5. *Distinctive behaviours*. When tied to apparently derived morphological features, distinctive behaviours may provide additional evidence for higher or lower weight of that feature.
6. *Correlation of characters*. Greater number of synapomorphies at a particular node increases confidence in interpretation of each of these as synapomorphic. Although not generally discussed, this criterion requires serious consideration of whether the character states considered are truly independent.

Some authors have complained that such weighing is not objective but depends on a subjective appraisal by the researcher. This objection is likely true, but only in a mathematical sense. The problem, however, lies in the nature of characters themselves. Enough groups of organisms have been studied since Linnaeus (1758) that it is clear that certain features are better indicators of genealogical relationship than others in every well-understood group (e.g., bones and teeth of mammals). Identification of such high-weight character states result in stable nested sets of such characters and corroborate their interpretation as high-weight. Such a broad pattern of agreement shows we can expect the same from more poorly understood groups. Nelson & Platnick (1981:304) pointed out some time ago that "systematics in general consists of the search for defining characters of groups". Simply ignoring the problem and treating all characters as equal may have the pretence of mathematical objectivity but that approach certainly provides impoverished results that are out of touch with reality (Williams *et al.*, 2010).

When some characters are treated as being of higher weight (and especially if this is discussed), the claim of lack of objectivity by some suggests that we are left with an arbitrary system in which the next researcher can simply reweigh the characters or treat other features as more significant. Not so. If the arguments for considering certain character states as superior indicators of phylogenetic relationship are presented explicitly, the next student of the group can understand the reasons for the previous decisions and further test the concepts. Other hypotheses might be presented and that is how science progresses. There may be some standoffs but in most groups pursued in this way, a consensus appears as to which character states are the better indicators of relationship within a broader context of a growing system of nested sets. These are the characters which provide stable and predictive phylogenies.

SUMMARY: Some synapomorphies are better indicators of phylogenetic relationships than others. A goal of systematics is to identify these (high-weight character states) to produce predictive and stable phylogenies.

An obvious problem of using weighing criteria in providing a non-numerical estimate of the merit of a given synapomorphy is that they cannot be used as such in current practice. While numerous programs do have a mechanism to designate higher weight to a given character state, it is unclear what weight should be given. My understanding, based on my own experience and the confessions of others, is that a feature thought to be a unique, distinctive, developmentally and morphologically complex synapomorphy is given incremental weight within a

study until the resultant phylogeny provides the relationship desired. Although having the appearance of objectivity (because it is mathematically presented), actually the process and reasoning of the author is generally opaque. It is clearer and would be better understood if authors discussed what they have done and why and it is that information that can help the next researcher pursue the study of that feature (perhaps it was not as distinct, complex or unique as previously thought) in relation to other character states (e.g., Amorim & Rindal, 2007; Jaschhof, 2011).

Every systematist picks and chooses the characters that will provide evidence of species differences and character states that may provide evidence of phylogenetic relationship. It is one of the reasons that we know more about the nature of the mouthparts of adult biting Diptera and the details of male genitalia than we do about the states and distribution of many thoracic sclerites. Under the guise of objectivity, some systematists imagine that character selection is a value-neutral process (or at least they wish it was), as if the totality of the organisms have been thoroughly scrutinized and included in analyses (the dream of phenomics). Aside from philosophical objections to phenetics and its descendant algorithms (Williams *et al.*, 2010), in fact it should be a primary goal of every phylogenetic study to search for those particular synapomorphies that are high-weight (Schlee, 1969). It is a sad state of affairs when the only features a group seems to provide are those of wing pigmentation and number of setae; those are taxa which cannot be confidently interpreted. However, the Diptera are rich in strange and weird adaptations that can provide solid evidence for many (but not all) nodes. The halter of Diptera, cyclorrhaphan larval mouthparts, and the articulated pupal terminal processes of the pupae of Chaoboridae and Culicidae are all so distinctive, unique, and morphologically complex that they each certainly evolved only once. When other, more simple, features conflict it should provide us with insight as to the evolutionary flexibility of those more simple character states (i.e., their susceptibility to homoplasy). Otherwise, we are caught in an endless cycle of introducing further minor features that will continuously change our phylogenetic conclusions (Jaschhof, 2011). This should be embarrassing to us as scientists. Of course, 'good' taxonomists have always recognized (and looked for) such features (e.g., Yassin, 2013) and it is one of the reasons that our system of families and most genera have survived from pre-Hennigian times. The more refined logic of cladistics has supported most of these earlier understandings of relationships, dependent as they were on one or more 'strong' features. It has only been in recent years, with the addition of often poorly understood features analyzed with the ‘objectivity’ of parsimony programs that some significantly and often wildly varying phylogenies have appeared in some groups (more on this below). Without proper weighting, confusion reigns.

In this regard it is interesting that in fossil work, many systematists do not complete a matrix of equally valued character states but zero in on those features that group them with other taxa known to them (i.e., higher weight synapomorphies) (e.g., Dikow & Grimaldi, 2014; Grimaldi, 2016; Oberprieler & Yeates, 2012). Even when a matrix is used to elucidate the position of fossils, discussion in the text most often refers to specific synapomorphies as strongest evidence of relationships (e.g., Arillo *et al.*, 2015; Grimaldi & Barden, 2016). There is a general recognition that measuring setal lengths and antennal/head width ratios will not likely indicate their proper (i.e., correct) position within the cladogram of extant taxa.

Just as we have discovered that earlier studies, highly selective of features (e.g., Edwards, 1926), were predictive in the discovery of further synapomorphies and the phylogenetic position of new taxa (extant and fossil), there is every reason to believe that a similar approach would continue to be predictive (Schlee, 1969). Aside from this evidence from well-understood groups (e.g., stable for decades), the congruence in many groups of high-weight features in different life stages is strong evidence that the selection of such features should be the first line of investigation and the center of argumentation to resolve phylogenetic problems.

5. Parsimony

> "The real purpose of the scientific method is to make sure nature hasn't mislead you into thinking you know something you actually don't know." R. Prsig, *Zen and the Art of Motorcycle Maintenance*

Occam’s Razor, stating that “among competing hypotheses, the one with the fewest assumptions should be selected” is fundamental to scientific thought. For evolutionary biologists this means that the most likely phylogeny is the one that makes the least assumptions and provides the simplest interpretation of relationships as indicated by the data. The standard for the portrayal of the distribution of character states within a given group is a

matrix. This useful format provides the basis for a variety of software analyses to produce one or more phylogenies. It provides an explicit and valuable presentation of data and allows for the simultaneous analysis of large datasets. How the data are interpreted varies between authors and programs utilized and presents challenges as to whether they deduce authentic historical, genealogical relationships.

As developed by numerous mathematically inclined evolutionary biologists over the past 30 years, we are presently in an age where the phylogeny with the least assumptions is one in which all character states are treated *a priori* as equal in their value. Aside from questions regarding the extent of the outgroup and the problem of defining a given character and its weight discussed above, there is the question of what is the simplest answer to a pattern of synapomorphies. If a systematist has no idea as to how distinctive a synapomorphy is (because of limited outgroup comparisons) nor the function or development of any of the character states in the analysis, it may make sense to let a program decide on the simplest pattern, treating them all as equal. But this is an unfortunate state of affairs, reflecting the limitations of the study and we hope as scientists that with further investigation, certain characters will be understood as more complex than others and that further outgroup comparisons may indicate that some are more susceptible to homoplasy than others (Hennig, 1972; Schlee, 1978). A maximum parsimony tree based on character states of equal value should be understood as a group that is poorly understood and its phylogenetic pattern should be viewed with high levels of scepticism.

A few years ago at a Diptera congress I noted to a colleague that I did not believe the current presentation of the phylogenetic relationships for a group of Diptera families. The self-assured response was "science isn't about belief but about the objective analysis of the data". Yes, of course science is about objective analysis, but if the data input and analysis is restricted to certain models, we will only get the limited answers that are predicated by those models. My statement of belief was based on the first-hand comparison of many of the taxa in the problem area, convincing me of certain homologous character states, of characters I considered to be highly unlikely to have evolved more than once.

In my experience, those who are immersed in mathematical models of phylogenetic analysis challenge those who are not by asking "Who are you to determine the weight of a character state?" and indicate that such decisions are subjective and antithetical to parsimonious analysis. The answer to that question, which may intimidate a student new to the group, is rather simple: I am a person who has examined thousands of species and their morphology, in various life stages, and have spent significant time with living organisms watching their behaviour. As such I have gained a measure of confidence as to which character states are homologous and are particularly important to the diversification of my group. Just as the placenta, shape of the uterus and the form of teeth are fundamental to understanding the monophyly and diversification of the mammals, an expert understanding of a large group certainly provides a person with a better understanding of the various characters fundamental to the diversification of its included taxa. Under the guise of the current model of parsimony, we have institutionalized a level of absurdity. We have forgotten that characters DO vary in their phylogenetic importance, that features are often interconnected structurally and developmentally and that they have a function (which can provide strong evidence of unique adaptation).

An important point regarding the use of high-weight synapomorphies in well-established groups is that they result in predictive phylogenies. Such character states result in stable and often complex series of nested sets. When strong synapomorphies are present in different semaphoronts such as larvae, pupae and adults, the confidence that these reflect true historical relationships is high.

It is clear that our understanding of most of the character states of most insect groups is elemental and that we do not have a clue as to what most character states might be for, their development, nor the internal components (e.g., muscles) of those character states. This is only because our systematics is yet young, our groups often have a huge number of species, and most of us are the first explorers of the taxa at hand. Such ignorance, however, is not an excuse for avoidance. We should look to well-understood groups as models of what to look for in the taxa we study and recognize that parsimony is NOT treating every character equally but is actually an active and hopefully successful search for those features most indicative of phylogenetic relationships.

Parsimony within phylogenetic systematics is therefore not a minimalistic crunching of numbers of character states but the simplest explanation of a more complex and rich pattern in which some character states are clearly better indicators of relationship than others. For example, there can be little doubt that the articulated, membranous, three-ribbed, articulated terminal processes at the apex of the abdomen of pupae of Chaoboridae and Culicidae (Borkent, 2012, figs 26D, E) are not only very similar in detail (e.g., chaetotaxy) but are complex and unique within the Holometabola (other nematocerous Diptera and orthorrhaphous Brachycera basically have unarticulated,

V-shaped, solid (not membranous) terminal processes). They are of fundamental importance to a unique mode of swimming (a somersaulting movement that flips the pupa through the water). A conflicting "synapomorphy" interpreted as "predaceous larvae with prehensile antennae" is present in Chaoboridae and Corethrellidae (Forster *et al.*, 2016) but the details and mode of action appear to differ markedly in these two families (Borkent, 2008:206). As such, the most parsimonious explanation is that the pupal terminal processes are certainly the better indicator of relationship. Such an interpretation demands detailed explanation in text and is beyond the capabilities of a matrix and maximum parsimony tree. Comprehensive explanations should be a hallmark of our work as systematists.

SUMMARY: Arguments about differing phylogenies should concern the character states themselves, rather than various levels of mathematically determined parsimony. There is no biological basis for mathematical values to be applied to various nodes of a phylogeny.

The popular portrayal of tree length, consistency indices, retention indices and Bremer support values, amongst others, portray the facade of objective analysis in part because many other areas of science have advanced in concert with mathematical applications, not only in the 'hard' sciences of physics, chemistry and astronomy, but also in ecology, physiology and other areas of biology. However, it is my contention that the mathematical evaluation of a set of characters in phylogenetic analysis is meaningless in the face of the impossibility of providing an objective value to a given character's relative value in indicating phylogenetic relationships. Such is the nature of our studies. Characters, although treated mathematically are not really 'equal' in any biological sense of the word. Additional problems with defining a given character means that morphological phylogenetists will always need to make somewhat subjective decisions, that will, if they are on the correct path, lead to a parsimonious conclusion, one that will eventually most simply explain most of the facts. If our work is defined by the need to express ourselves entirely in mathematical models, we will eventually be restricted to a distressingly simplified and inaccurate version of what evolution has done in our groups.

If character states do vary in their value as indicators of relationship (as they surely do), various measures of the mathematical values of these differences are arbitrary (i.e., subjective) and give a false sense of validity to a given cladogram. In my opinion, this is one of the reasons that so many systematists today avoid discussion of the synapomorphies utilized, character weighing and the definition of what a character actually is. To do so is to admit that the mathematical values associated with presented trees are artificial and obscure the real issue at hand, that being the quality (or weight) of each of the synapomorphies discovered.

A particularly distressing phenomenon produced by the mathematical analysis of phylogenetic trees has been the proclivity for systematists to place high accent in terms of time and concern on the appraisal of conflicting trees (Wheeler, 2008b). In contrast to this exercise, when conflicts in character states occur, the first and most important step is to go back to the specimens and examine once again the character states to reappraise the hypothesis of homology, checking the details to confirm whether the state is truly homologous or not (Hawkins, 2000; Schlee, 1978). It is my strong experience that a second (and sometimes third and fourth) appraisal provides further evidence for or against homology and a clearer resolution of the phylogeny. When characters truly conflict it is clearer and therefore more amenable to further study to describe the conflict in text (with the character states involved) than to bury it in the presentation of mathematically defined phylogenies as is current practice. The question of the most likely phylogeny hangs on the valid interpretation of the character states involved (including their relative merit).

The amount of time and effort to understand the characters themselves is reflected in the quality of the ultimate phylogeny produced. It is hard work to study character states in depth. The current simplicity of plugging in any set of features into a matrix and generating a phylogeny is fast, easy, fun, and most likely nearly always wrong. There is no replacement for really understanding the morphology, development and function of a feature, within one's group and in a broad outgroup. In my experience a single feature often takes several days (or more) before I am confident of homology and its polarization. This process is rarely taught to students anymore. The thrill of the conclusions surpasses the grunt of careful study. It is as if we have taxonomic ADHD, unable to spend the time to really get to know our groups before leaping to the conclusion (and/or accepting this in others).

There is another serious problem with the current presentation of parsimony trees (and its permutations), especially when character states are not discussed. Each program as currently used has numbers of options, with

many having variable settings. For example, TNT (Goloboff *et al.*, 2003) has sectorial searches, ratchet (up/downweighting probabilities set to variable percentages, with varying numbers of replicates), tree drifting and tree fusing, various search parameters, number of most parsimonious cladogram(s) to be found, the maximum number of trees to be held, how multistate should be treated and GC (Groups present/Contradicted) settings, amongst others. Many of these options are poorly understood by most (in my experience of asking for further explanations) and are largely based on blackbox computer algorithms. There are two serious issues with these options. First is that most users don't understand exactly what is happening with their data. Second, embedded in such analyses is the strong possibility that future researchers will have no clue as to how the data were actually managed (i.e., what the resultant phylogeny actually portrays). For example, Saether (2000) in his analysis of the families of Culicomorpha, noted that "Parsimony analysis was performing using PAUP 3.1.1 and MacClade 3.06 on a Power Macintosh 8200/120. All searches with only family data were performed using the branch and bound procedure. When Chironomidae was divided into subfamilies, heuristic searches with 1000 replications were performed under different options. The cladograms were compared using MacClade 3.06." This procedure likely cannot be repeated by current scientists and the details of the analysis are therefore uncertain. In science, the ability to replicate analyses is a vital and important aspect of verification and revision. Future systematists will wonder how we handled our data and what it means, especially when character states are merely listed simplistically. Systematists are not like astrophysicists who continuously refine their methods to update what is known about the universe. Revisions of taxa often stand alone for decades before another person works on the group. Surely we are motivated to have our readers, years down the road, understand what it was that we did.

SUMMARY: Computer programs for analyzing matrix data are complex, with many options, many of which are poorly understood by most systematists. Many of these programs are quickly outdated, making repetition of older analyses difficult.

Because of the present focus on the resultant tree, whether it is maximum parsimony, strict consensus, or majority rule, most papers indicate only values on the resultant tree(s) that appraise the distribution of the character states. Too few authors show the actual character states supporting a given node and thereby produce another level of opacity, leaving the interested reader wondering about what the actual synapomorphies might be for a given node. This makes it very difficult to appraise a given phylogenetic presentation if one wants to know the actual synapomorphies supporting the conclusion. To those who know nothing or little of the group, knowing what character state supports what node may not be very important because they don't have the knowledge to evaluate the character states utilized (nor do they have any hope of learning more in this regard). As it stands, Yeates' *et al.* (2007) statement that "Dipteran systematists are today much better able to determine the relative support for competing hypotheses of relationship" cannot be considered realistic – in fact we are in an increasing fog of uncertainty regarding those competing hypotheses.

6. Genomes vs. Phenotype

"The answers you get depend upon the questions you ask" Thomas Kuhn

Genomic studies often give the pretence of providing final answers to phylogenetic relationships and faith in the model is often in the realm of religious conviction. The past 35 or so years have seen a marked advance in the influence of gene-based phylogenies. Beginning with sequences of a single gene for a taxonomic group, which "proved" various relationships that were often vehemently denied by morphologists (e.g., bats evolving at least twice, gorillas being the sister species of humans, etc.) to the debates among geneticists when second genes provided conflicting patterns, to an increase in the number of genes utilized, always indicating accurate results and always providing new and fresh phylogenies (Kjer *et al.*, 2016). Today we are still on the border of the Promised Land with the assurance that transcriptomes, representing complete sequences of a group of mRNA molecules, will provide access to the final answers (Jiménez-Guri *et al.*, 2013). For many this has been applauded and accepted, in spite of the often strongly fluctuating conclusions from one study to the next. Now that total transcriptomes in at

least some groups are providing some irregularities (e.g., Fernández *et al.*, 2016), there is the ever-present caveat that this will all soon be sorted out (although Kjer *et al.*, 2016 invoke a fundamental scientific premise and warn that merely more data may not provide better answers). Yeates *et al.* (2016) for example, state "We predict that insect phylogenomic analyses will become much more sophisticated, and produce more reliable results, in the near future". It appears however, that nature is more complex than the sequences might suggest and there are obvious and often confusing complications (Nelson & Buggs, 2016). Although the idea that nested sets of mutations has merit (if properly polarized) (Sperling & Roe, 2009; Kück & Wägele, 2016), this is not the process that is currently implemented by molecular systematists (more on this below).

This pattern of changing and unreliable analyses alone should give us pause. Earlier concerns regarding the fluctuations in phylogenetic conclusions based on sequences resulted in a "whole evidence" approach that incorporates both morphological and molecular data (Yeates *et al.*, 2007), a development that was largely a result of too many earlier molecular publications giving poor, but proudly proclaimed, results. It reflects a time when the protestations of morphologically-based cladists were scorned and ignored as dated and irrelevant (Crisci, 2006). Some of that elitist attitude is yet prevalent among many in the molecular arena, often accompanied by a poor understanding of the morphology of the organisms being studied. A number of years ago the National Science Foundation in the United States began to require molecular phylogenetic studies to include a systematist who knew the morphology of the group, presumably in the hopes of providing better phylogenetic results. However, this was sometimes seen as a reluctant 'add-on' to the otherwise purported more rigorous and definitive molecular portion of the study. Published results testify to this perspective: many 'whole evidence' papers combine a limited set of morphological features with a much higher number of molecular characters, effectively excluding any significant impact of morphological evidence, or present complete sequence analyses with commentaries on selected morphological features.

SUMMARY: Molecular sequence analyses have been volatile, producing inconsistent phylogenetic results over the years.

Molecular sequences have the appeal of simple character state values (A,C,G,T and/or A,C, G, U) and bulk of data (hundreds, thousands and more base pairs). This simple data form lends itself to mathematical analysis (rightly so) and thereby (wrongly so) suggesting a high level of objective confidence. Morphological characters are often difficult to define, complex in their development and present challenges in interpretation (and take more time to explore). However, the morphological characters we study are emergent properties (Brower, 2016; Stevens, 2000), the product of genes and development in a continuously changing environment, including within the cell, surrounding cells and tissues, and the environment outside the organism. For example, the human genome has only about 21,000-23,000 genes while the human brain holds at least 100,000 different organic chemicals (Rose, 2005). It has been clear for decades that there is a marked discrepancy in complexity between a tabulation of numbers (and sequences) of genes and the phenotype of the resultant organism. Part of the reason, of course, is that genes can work in different combinations as well as at different times during the development of an organism. A molecular sequence is, as such, a comparatively simple portrayal of what is actually being performed by those genes. It is more than reasonable to expect, therefore, that the complexity of morphology, with its underlying intricate and complex gene action, would provide a surer basis for accurately understanding complex synapomorphies that would result in more accurate phylogenetic relationships (Wagner, 2014; Wheeler, 2008b). The evolution of the larval form of the Holometabola, with its underlying phenomena of setting aside embryonic cells by the embryo for later development of adult structures is so complex that there can be no doubt that it indicates the monophyly of this group. The molecular sequences that ultimately determine this phenotypic expression are much simpler by virtue of their expression as part of a DNA and/or RNA molecule. The action of the genes involved in this regard can be expected to be complex and will be a fascinating source for understanding the development of such a synapomorphy.

There is another problem of fundamental importance. It appears that molecular studies have lost the fundamental cladistic tools of Hennigian logic to determine their results; certainly there is no evidence that the results are cladistic in any traditional sense of the word. As Mooi & Gill (2010) and Williams & Ebach (2010) strongly argue, there is an absence of evidence of synapomorphies when molecular results are presented. Indeed,

the use of the terms "synapomorphy" and "plesiomorphy" are virtually absent from the literature on molecular phylogenies (but see Nelson & Buggs, 2016). Kück & Wägele (2016) have recently shown how nearly all published molecular sequence analyses are fundamentally flawed because of a lack of consideration of plesiomorphic/apomorphic states. Likelihood analysis, the current method of choice for sequence analysis, is a complex mathematical construct that utilizes one or more of a plethora of available programs to organize the information. Those using such complex programs as, for example, Modeltest 3.7 (Posada & Crandall, 1998) feed sequences into the program which then determines which of the 56 different models are "best" for the data at hand, using neighbour joining at least in part. This process, with at least some underlying phenetic components, is complex and how data are handled in detail is largely opaque to most phylogenetists.

SUMMARY: Most molecular sequence analyses are phenetic, not cladistic and lack any identification of synapomorphies.

Parsimony analysis of sequence data is being used in some molecular studies but the method has been rejected by many molecularly-based systematists because it is less reliable in various simulation studies (e.g., Anderson & Swofford, 2004; Hall, 2005).

When Hennig (1950, 1965, 1966) showed the importance of distinguishing plesiomorphic and apomorphic character states, he provided the means to determine whether a grouping of taxa was based on plesiomorphic or apomorphic features, and therefore allowing the recognition of those autapomorphic taxa that belong to a group of less derived taxa and those based on symplesiomorphy. He indicated that this was the only rational basis for constructing phylogenies. In short, he showed that phenetically-based phylogenies were logically flawed (Williams *et al.*, 2010). In my experience, however, most molecularly-focused colleagues cannot identify lineages based on molecularly-based synapomorphies, seem to be puzzled by the question, and have left concern for synapomorphic character states behind.

Some groups of organisms (and/or some included lineages) appear to have a paucity of morphological synapomorphies that might indicate their relationships, leading to increased emphasis on molecular methods. However, when a rigorous method such as Hennigian cladistics fails to uncover logically based synapomorphies, it is misleading to turn to another method of questionable reliability and logic to propose phylogenetic relationships (if these cannot hypothesize synapomorphies) (Cruickshank, 2011; Kück & Wägele, 2016). The correct response is to confess that there is not sufficient evidence to resolve such a group (or particular nodes). It should be kept in mind that for the vast majority of Diptera, our knowledge of morphology (external and internal) is extremely limited (Wheeler, 2008b) and there are huge research opportunities for detailed study to provide more (and better understood) characters for further phylogenetic analysis (e.g., Brown *et al.*, 2015; Sinclair, 2013; Tachi, 2014; Wipfler *et al.*, 2012). So too, some distinctive genes or characters within them can be understood as synapomorphies.

Molecular sequence analyses have become increasingly complex, to the point that it is highly doubtful that morphologically-based, and likely many molecularly-based systematists can understand what the methodologies, let alone the results actually mean in any sort of detail. As an example, Caravas & Friedrich (2013), in their materials and methods state that for tree analysis "Custom Perl scripts (available upon request) using BIOPERL (Stajich *et al.*, 2002) and BIO::PHYLO (Vos *et al.*, 2011) were written to parse tree data and generate summaries" and for tree congruence testing "Tree topologies were evaluated using the Kisihino–Hasegawa (KH) test (Kishino & Hasegawa, 1989) and the Shimodaira–Hasegawa (SH) test (Shimodaira & Hasegawa, 1999) with RELL multiscale bootstrap resampling (Kishino *et al.*, 1990) as implemented in PAUP 4b10 (Swofford, 2003). All sites included in each dataset were combined into a single heterogeneous partition and assigned a GTR + I + Γ model". These methods are largely incomprehensible except to a very few. Protocols are regularly changing, often fundamentally, and their significance is abstruse. As such, the level of communication among systematists in this arena has decreased to the point that most colleagues are excluded from the discussion (and therefore from the scientific debate). Morphologically-based systematists simply go back to their microscopes to look at specimens or need to cooperate with a molecularly-based colleague in whom they blindly place their phylogenetic trust. There is a critical disciplinary need here for those doing genetic research to clearly communicate their methodology in a way that translates to the wider literature, rather than relying on the faith of colleagues.

There is another pattern in published works that suggests sequence analyses may be an unreliable basis for determining phylogenetic relationships. For studies where authors have little or no first-hand knowledge of the morphology of taxa being sequenced, the results are often highly irregular and often widely inconsistent with morphologically-based interpretation. Virtually every morphologically-based systematist I know has examples of such stand-alone studies of which they think poorly (i.e., spectacularly off-base). On the other hand, there are numbers of publications in which both sequences and morphology (at least partially) are examined. Some are combined studies that partition morphological and molecular characters at least as an initial approach to examine levels of congruence (e.g., Germann *et al.*, 2010; Hash *et al.*, 2017; Petersen *et al.*, 2010; Roháček & Tóthová, 2014; Skevington & Yeates, 2000; Su *et al.*, 2008; Williams *et al.*, 2016). Some others combine the characters, appearing to effectively swamp the morphological characters with the molecular characters (e.g., Fu *et al.*, 2016; Gibson *et al.*, 2013; Kirk-Spriggs & Wiegmann, 2013; Tóthová *et al.*, 2013; Wiegmann *et al.*, 2011). Yet others complete a molecular analysis and then discuss the level of congruence with known (or a chosen few) morphological features or merely compare their results to the current classification of the group (e.g., Cranston *et al.*, 2012; Curler & Moulton, 2012; Demari-Silva *et al.*, 2011; Grace-Lema *et al.*, 2015; Haseyama *et al.*, 2015; Hash *et al.*, 2013; Kang *et al.*, 2017; Mohanty *et al.*, 2009; Morita *et al.*, 2016; Pu *et al.*, 2017; Semelbauer, 2016; Tachi, 2013; Tkoč *et al.*, 2016; Wang *et al.*, 2014; Watts *et al.*, 2016). Clearly, for the latter two groups of authors, molecular analyses were considered *a priori* as superior to morphological analysis.

SUMMARY: Molecularly-based phylogenies do not appear to be independently derived when strong morphological synapomorphies are known.

For those authors who partition morphological and molecular data and indicate high levels of congruence, it is unclear that the results are truly independently derived. Considering the complexity and plethora of options for analysis of both molecular and morphological datasets, there is a distinct possibility that there are biased choices being made by authors to make molecular and morphological results appear more congruent than might otherwise be the case. This possibility is not just conjecture. Behind the scenes, I have noted a disturbing and serious bias in the approach taken in at least some molecular studies that is particularly evident to me working on certain Diptera but which is likely more broadly distributed (in talking to some colleagues). For at least some of those with a strong molecular perspective, tentative results are regularly run by systematists who are doing morphological work to see if the phylogenetic relationships are "reasonable". If so, the results are published as such but with the intimation that the pattern is solely or primarily the result of molecular analysis. If molecular patterns are not more or less blessed by a morphologically-based cladist, the results appear to be tweaked, using different models of analysis until patterns align more or less with morphological understanding. These appear to be published without reporting any reanalyses that may have been done. This suggests to me a strong uncertainty regarding molecular analysis in such groups in which there are a significant number of high-weight synapomorphies and a solid morphologically-based phylogeny (at least known to the systematist, even if not published). It is similarly striking that the nodes in insect phylogeny that are morphologically well supported are also those well supported by published molecular results, while those that are difficult to interpret morphologically (e.g., Ephemeroptera, hemimetabolous insects) have also produced contentious molecular results (Kjer *et al.*, 2016; Yeates *et al.*, 2016). In my opinion, it would be valuable and interesting to test molecular and morphological approaches with a number of double-blind studies to test their level of congruence.

At the present time, in groups where there is a paucity of morphological divergence (and hence few or no synapomorphies), such as many plant groups, microbiota and many sibling species groups in most larger taxa, molecular results are currently presented in such conclusive terms that they are strongly believed by most readers to be the only “reasonable” phylogenies available and cannot be refuted by morphological evidence. They become the only source of supposedly accurate phylogenetic information for those working in these arenas. Yet considering the conflict in many groups where morphological synapomorphies are available, this confidence is likely mislaid.
Phylogenies based on *CO1* sequences are particularly popular with fellow biologists who are not systematists (and often have never examined the taxa they are sequencing). These phylogenies may appear to reflect divergence in recently evolved lineages, where overall similarity may strongly reflect phylogenetic relationships. In such groups where there are very small morphological differences, it may give the appearance of accuracy. Similarity can often

reflect phylogenetic relationship. However, it is important to remember that a central point of cladistic analysis is its unique capacity to identify groups based on symplesiomorphy and those that are confounded by autapomorphic members within a monophyletic group. If such molecular analyses are acceptable to the community of systematists, we should also be willing to mistakenly entertain all phenetic studies as valid indicators of genealogical relationships (and forget about cladistics altogether).

One difficulty as presented by some studies where *CO1* sequencing and, perhaps, that of another gene or two is used as a phylogenetic tool is that they often concern relatively small groups of morphologically similar species such as species groups of *Culicoides* (Ander *et al.*, 2013; Bakhoum *et al.*, 2013; Bellis *et al.*, 2014; Harrup *et al.*, 2016; Muñoz-Muñoz *et al.*, 2014; Pagès *et al.*, 2009; Sarvašová, *et al.*, 2017; Talavera *et al.*, 2017; Tay *et al.*, 2016). Morphologically-based synapomorphies are less likely to be evident (or present) in such similar species groups. If *CO1* sequences were interpreted cladistically, there may be an interesting test of their reliability. In a few families of Diptera such as Simuliidae and Chironomidae, polytene chromosomes can be used to identify monophyletic groups, based on unique rearrangements that can be interpreted in some of these groups as synapomorphies (Adler *et al.*, 2004; Adler *et al.*, 2016a, 2016b; Senatore *et al.*, 2014). It would be informative to compare the two methods to determine the degree of congruence and especially so in a group that has been recognized on the basis of symplesiomorphy or includes an autapomorphic lineage (to see if the *CO1* sequences can identify these).

SUMMARY: Phylogenies based on one or two genes such as *CO1* are likely phenetic and therefore only appear to provide reasonable results. There is no apparent evidence they are cladistic analyses.

At least at a broad level, it seems that some gene sequences may be more indicative of relationships (at least of a certain age) than others (if interpreted cladistically), reflecting a similar state to morphological features that also vary in their value. With morphological features, there are clues how to at least partially rank the importance (i.e., weight) of different features. It is unclear how to determine, *a priori*, which genes are better indicators than other genes, other than a general recognition that mitochondrial genes produce more consistent results for recently evolved lineages than nuclear genes, which are more applicable to older lineages (but see Caravas & Friedrich, 2013). There is, however, even in this, a problem of determining what a recently evolved versus an older lineage actually is, without knowing what the morphology and fossil record provide.

At present, there is a wonderfully insulated environment for much molecular work allowing for poor results to be blamed on the use of inappropriate genes, misapplied options, or insufficient sampling, while 'good' results are the product of proper genes, sufficient taxon sampling and proper programs (models). With the recognition that some groups change more rapidly than others (and some genes vary in their rate of change during time), there are no standards to be applied to the unknown group or at least this is done by extension from “successfully” interpreted groups. This provides a perfect arena for providing perpetual excuses for poor results and laudatory claims for those that are close to morphological results, or for which no morphological data are available. It is well past time to provide some independent study to examine whether molecular sequences actually provide testable and predictive phylogenetic results and that there are actual synapomorphies amongst the sequences. The Diptera, with their wealth of morphological divergence, are a premier group to compare morphological characters against sequence data and provide a more rigorous methodology for interpreting phylogenetic results.

In the meantime, we generally continue to confront morphological and genetic differences in results by burying them in “whole evidence” studies or by the prepublication tweaking of sequence studies. The lack of clarity in sequence analyses appears to have become standard and the primary hope now has become that the addition of more data and more taxa will provide “true” phylogenies, all in the absence of the Hennigian logic of synapomorphies.

Of course, study of sequences is important but depends, in part, on the questions being asked. The many inconsistencies between sequence and morphologically-based phylogenies could lead to interesting biological questions regarding the basis for such incongruence (Wagner, 2014). One question, for example, might be “Why do many sequences indicate unusual (= morphologically unacceptable) phylogenetic relationships?” We have some answers regarding the problems with earlier results that reflect an earlier lack of knowledge regarding rates of change of certain genes, fluctuations in rate of change, inclusion of third codon positions, and other phenomena.

The pattern of phylogenetic incongruence, based on different genes (or combinations) and resulting in trees with different topologies, has become more and more obvious over the years (Morrison, 2017). It has become clear that sequences do not provide a straight-forward arrangement of character states that can be easily interpreted. Rather, the sequences are complex mosaics composed of portions with different phylogenetic histories, producing phylogenetic incongruence for the different elements of that mosaic. How the position and arrangement of gene sequences behave is clearly complex and there is an obvious underlying message from geneticists – there is yet much to learn about sequence arrangements and we may be a long way (and perhaps unattainably so) from predictably understanding their variation (i.e., being able to use consistent models of interpretation to provide accurate, testable phylogenies independent of morphological oversight).

SUMMARY: Sequence data may be inappropriate for phylogenetic analysis but distinctive genes can be interpreted cladistically.

In spite of the present inability of sequence data to give consistent results, there remains a rich opportunity to use the genes themselves as evidence for phylogenetic relationships (Nelson & Buggs, 2016). For example, primates have non-functional genes for generating vitamin C, genes that are functional in other mammals (Drouin *et al.*, 2011; Mukherjee, 2016). As such, the non-functional gene is an additional synapomorphy for these primates. There remains the distinct possibility that genomic information may give important phylogenetic information when interpreted cladistically. Nelson & Buggs (2016) point out that the identification of taxonomically restricted genes allows for the logical identification of such genes as synapomorphic and is amenable to broad outgroup comparisons. They also discuss how research of such genes has been highly constricted and therefore markedly underutilized.

The Phylogeny of the Diptera – the analyses by Wiegmann *et al.* (2011) and Lambkin *et al.* (2013)

"Who are you going to believe, me or your own eyes?" Groucho Marx

The study of the phylogenetic relationships among the families of Diptera has a rich tradition, in which early workers proposed a number of arrangements (Crampton, 1924; de Meijere, 1916; Edwards, 1926; Malloch, 1917). Hennig's (1973, 1981) landmark overview of the Diptera laid out the cladistic basis for the interfamilial (and often subfamilial) relationships, including data from immature stages and fossils, and it seems likely that the group, with its wealth of morphological divergence was a strong influence supporting and confirming his theoretical work. In short, the Diptera make sense phylogenetically when synapomorphies are recognized. Features of adults, pupae and larvae become congruent at certain nodes when apomorphic and plesiomorphic features are distinguished. Subsequent morphologically-based work largely supported Hennig's conclusions, although there was significant further resolution and obviously plenty of questions remaining for further study (e.g., Borkent, 2012; Buck, 2006; Courtney, 1990, 1991, 1994a; Cumming *et al.*, 1995; McAlpine, 1989; Michelsen, 1996; Oosterbroek & Courtney, 1995; Pape, 1992; Sinclair, 1992; Sinclair & Cumming, 2006; Sinclair *et al.*, 1994, 2007; Starý, 2008; Wood & Borkent, 1989; Woodley, 1989; Yeates, 2002).

Wiegmann *et al.* (2011) and Lambkin *et al.* (2013) recently published partially complementary results of a six-year (2004-2009) cooperative effort between 17 different laboratories studying Diptera phylogeny and supported by an NSF grant of $2.4 million (US). Wiegmann *et al.* (2011) interpreted the results of a mix of sequence and morphological data while Lambkin *et al.* (2013) provided just the morphological analysis. The ambitious goal of this project was to fashion a phylogenetic synthesis that would incorporate both molecular and morphological data, building on previous work (especially morphological) and establishing a solid basis to advance further work on the phylogenetic relationships within the order.

Wiegmann *et al.* (2011) claimed to provide a comprehensive approach by incorporating both molecular and morphological data, to produce, as stated by Wiegmann (Kulikowski, 2011), a "Periodic Table" of Diptera phylogeny. The initial goal of this project was laudable and in the interest of full disclosure, I note that I was initially pleased to join in this cooperative venture as a dipteran morphologist working on nematocerous Diptera

when it began in 2004. Most of the work done, however, was molecular, with only three participants working on the morphological aspect of the nematocerous portion, and with very limited discussion. Indeed, it appeared that there was little new input on the morphological portion, being largely restricted to previously studied character states (there was refinement to some of the characters, Lambkin *et al.* 2013-S4). I also admit that part of my role was to input more original pupal data, and this was limited to the Culicomorpha (Borkent, 2012).

Once the results came in during the last months of the project, however, I withdrew my name as co-author on both manuscripts (in May, 2010). Below are some of the reasons for my lack of confidence regarding the conclusions derived from the studies. This section explains some thoughts behind my dissension for two reasons. One is to encourage further discussion concerning the phylogenetic relationships of Diptera, one of the truly outstanding groups of organisms amenable to phylogenetic analysis. The other is to ensure that the broader community is aware that not all studying dipteran phylogenetics consider the Wiegmann *et al.* (2011) results as anything remotely close to a "Periodic Table" of Diptera relationships. These results certainly are not authoritative and should not to be used as a template for further study, as has been done by many, with almost 400 citations as of this writing (e.g., Chapman *et al.*, 2012; Dijkstra *et al.*, 2014; Espíndola *et al.*, 2012; Friedemann *et al.*, 2014; Tkoč *et al.*, 2016; Mitterboeck *et al.*, 2016; Nagler & Haug, 2015; Pape *et al.*, 2011; Rotheray & Lyszkowski, 2015; Schneeberg & Beutel, 2014; Schneeberg *et al.*, 2013; Vicoso & Bachtrog, 2015).

SUMMARY: The phylogenetic relationships between the families of Diptera as presented by Wiegmann *et al.* (2011) and Lambkin *et al.* (2013) are seriously flawed and should not be used as foundations for further interpretation.

So what actually is flawed in the Wiegmann *et al.* (2011) and Lambkin *et al.* (2013) papers? First, both studies used an exemplar approach to study the interfamilial relationships. This was obviously based on the availability of material to be sequenced. This makes partial sense when working on genetic features, considering the restrictions on the number of species with fresh material available and the amount of sequencing to be undertaken. It makes no sense for the morphological portion. Of the approximately 160 families of Diptera and about 160,000 species already described, why limit morphological information to 42 species, with each representing one of 42 families? To exacerbate this, many of the taxa were highly derived members of their prospective families. For example, the Chironomidae were represented by *Chironomus tepperi*, a member of one of the most advanced lineages within the family (Cranston *et al.*, 2012) and Cecidomyiidae by *Mayetiola destructor,* also belonging to a highly advanced lineage (Gagné, 1989; Jaschhof & Jaschhof, 2009). With the morphological portion restricted to just these exemplars, variation known and present within a given family was ignored, making the analysis easier but the results strongly suspect. Such is the case, for example, with the treatment of the number of spermathecae present. The feature is of phylogenetic significance in higher Diptera but in some nematocerous families varies widely and does not appear informative within this group of families (see below). There is no excuse for not considering the variation of this and other features within the analysis other than ease of numerical analysis.

SUMMARY: The morphological analysis was based on 42 species, each representing only 42 of about 160 families of Diptera. Variation within a given family was ignored.

Aside from the highly restrained survey of taxa, the breadth of the taxa included (spanning the order) also presented challenges inherent in any matrix with increasing coverage: some synapomorphies are valid for certain groups and are highly homoplastic within others.

Any problems with the morphological portion of the study are insignificant, however, in the Wiegmann *et al.* (2011) paper because the matrix of morphological character states was not presented. Nor was it clear how the molecular and morphological data, portrayed as combined, were considered. If each character was considered separately, the molecular data would have swamped any influence by the 371 morphological features. Aside from reflecting the distain for the morphological data, the obscurity of the treatment of the data in combination makes the conclusions suspect.

Two years later, Lambkin *et al.* (2013) presented the morphological data for this "first tier" group of 42 families. They also provided the 371 characters alluded to but never presented by Wiegmann *et al.* (2011). Wiegmann *et al.* (2011) intimate in the abstract of their paper that the 371 morphological features were scored for all the families presented (i.e., 149 out of 157 families of Diptera). Their text, however, indicates that only the 42 "first tier" families were scored for morphology. It should be noted that 76 of the characters were autapomorphic for a single family at this level and therefore not informative for either of the analyses.

Although Lambkin *et al.* (2013) state "Here we present the first numerical analysis of phylogenetic relationships of the entire order using a comprehensive morphological character matrix" they actually ignored 73% of the families of Diptera, nearly all of which have been incorporated in previous phylogenetic literature. Combined with the exemplar approach of a single species per family, the result is actually a superficial analysis of the phylogenetic relationships among those remaining 42 families. It is incomprehensible why such a limited approach was taken when so much more is known for Diptera. One then wonders how the highly constrained analysis of the morphological features in Lambkin *et al.* (2013) affected the "combined" analysis of Wiegmann *et al.* (2011). Further to this, many of the most controversial hypotheses on dipteran relationships presented in Wiegmann *et al.* (2011) (e.g., position of Deuterophlebiidae, Apystomyiidae and Pipunculidae) were not tested by Lambkin *et al.* (2013) because these groups were not represented in their morphological analysis.

During the development of this project, various phylogenetic conclusions were presented based on the data available so far. Such modifications should be expected during the course of any study. However, this was not a case of better and better refinement but of often wildly fluctuating conclusions. Such presentations were initially devoid of the actual character states on the cladogram, making them nearly useless for determining the actual morphological evidence supporting a given node. In the last few months before the deadline for submission, requests to receive phylogenies with character states indicated at each node clearly revealed the reason, for example, why Nymphomyiidae was considered at that point to be the sister group of the Brachycera. This peculiar conclusion was based on five character states, namely the presence of an adult hypostomal bridge, the loss of foretibial spurs, the loss of midtibial spurs and the loss of hind tibial spurs and finally, the loss of the maxillary palpus (the later bringing the Nymphomyiidae close to the base of the Brachycera because the exemplar for the Acroceridae (*Ogcodes basalis*) was also missing its mouthparts (but present in some other Acroceridae)). The hypostomal bridge is poorly understood in nematocerous Diptera (Schneeberg & Beutel, 2011) and is present in Nannochoristidae; its validity needs new and comparative observations including re-evaluation of the scoring for this feature by Lambkin *et al.* (2013) (it is present, for example, in Tipulidae and Culicidae). The remaining four character states (all losses) were, first of all quite likely related between the three leg characters (evolutionarily linked and not independent indicators of relationship) and secondly are susceptible to known homoplasy (such losses occur in a number of other families). Pointing this out resulted in a new placement for the Nymphomyiidae near the very base of the phylogeny where it was presented as such by Wiegmann *et al.* (2011) and subsequently by Lambkin *et al.* (2013). Such changes (there were others) are indicative of a highly vulnerable dataset and hardly the basis for a "Periodic Table".

It is interesting to examine the concluding morphological evidence for Nymphomyiidae as the sister group of all remaining Diptera as presented in Lambkin *et al.* (2013). Nymphomyiidae are very small, highly modified flies that are markedly reduced in numerous features in each life stage (Courtney, 1994a). Five synapomorphies were presented as indicative of the monophyly of all remaining Diptera, four of which were noted as homoplastic and one as unique as follows (numbers indicating their synapomorphies; apomorphic state given here in bold):

105 (homoplastic), less than 6 adult abdominal ganglia. More than 6 adult abdominal ganglia are present in Pulicidae, Nymphomyiidae and Trichoceridae (see Lambkin *et al.*, 2013, file S3, showing missing observations for 6 of 14 families of nematocerous Diptera).

214 (homoplastic but shown as unique), upper calypter developed. An upper calypter is not developed in the outgroups, Scatopsidae, Cecidomyiidae, Nymphomyiidae, Phoridae, Lonchopteridae, Diopsidae, Sphaeroceridae, and Scathophagidae.

272, 273 (both homoplastic), presence of tibial spurs on the foreleg and midleg, respectively. This feature is homoplastic in many nematocerous Diptera.

306 (homoplastic), female tergite 10 a separate sclerite from sternite 10. Female T10 is not a separate sclerite in the outgroups Lepidoptera and Pulicidae (but separate in Bittacidae) and the following families of Diptera:

Nymphomyiidae, Bibionidae, Psychodidae, Scatopsidae, Cecidomyiidae, Anisopodidae, Acroceridae, and Schizophora except Diopsidae.

Character 105 is known for few nematocerous Diptera and needs more study. Character 214 is questionably homologous between lower and more derived Diptera. Certainly the absence of the upper calypter cannot be seriously considered as a synapomorphy– the markedly reduced wing of Nymphomyiidae is missing most of the wing features present in other Diptera and nearly all other holometabolous insects. Characters 272 and 273 are significantly homoplastic within a number of nematocerous Diptera families. For example, the foretibial and midtibial spurs are present or absent within Ceratopogonidae and Blephariceridae. It additionally appears likely that the tibial spurs of Diptera are homologous to those present in other orders (e.g., Mecoptera). Sternite and tergite 10 (character 306) are questionably present in many nematocerous Diptera, let alone whether they are separate or not. Courtney (1994a) did not recognize segment 10 in his detailed study of Nymphomyiidae. Saether (1977) indicates the presence of a segment 10 in only some nematocerous Diptera. As such, the position of Nymphomyiidae as sister group to all remaining Diptera is highly suspect, with 3 out of 5 characters due to loss and the remaining characters 105 and 306 regarding number of abdominal ganglia (possibly neotenic in Nymphomyiidae; Wood & Borkent, 1989:1345-1346; Courtney, 1994a) and the state of tergite and sternite 10 of females poorly understood. Further to this, although not indicated on the cladogram, Lambkin *et al.*, (2013; S4) described the presence of the adult labellum (their character 158) as a synapomorphy of Diptera other than Nymphomyiidae. Regardless, the absence of the labellum in Nymphomyiidae, with its reduced head and mouthparts cannot be understood as even weak evidence of their sister group relationship to remaining Diptera. Conflicting evidence indicating a different relationship within the Blephariceromorpha is noted below.

SUMMARY: Examination of character states indicating Nymphomyiidae as sister group to all remaining Diptera indicates these are tenuous at best.

Because of the combination of genomic data for most of the families and the morphological data for only 42 families, some results in Wiegmann *et al.* (2011) circumvent previously published interfamilial phylogenetic hypotheses. For example the Chaoboridae and Corethrellidae are shown as sister groups, based solely on sequence data, as neither were included in the morphological analysis. The systematics and relationships between three families of Culicoidea, namely Corethrellidae (frog biting midges), Chaoboridae (phantom midges) and Culicidae (mosquitoes) have been studied in some detail. All three were once considered to be members of Culicidae, then with Chaoboridae separated and including Corethrellidae and then finally with all three distinguished. The recognition of Corethrellidae as a distinctive family was based on two synapomorphies which grouped Chaoboridae and Culicidae but excluded Corethrellidae (Wood & Borkent, 1989). These were the shared presence of the markedly derived pupal terminal processes (Borkent, 2012: figs 26D, E) and precocious adult eye development in the larvae of these two families, each of these with a complex morphology that is unique within the Diptera. I consider both of these features to be high-weight features and it would be entirely untenable to have, especially, the pupal terminal processes evolve twice. The interesting question is, therefore, why didn't the gene sequences indicate the sister group relationship between Chaoboridae and Culicidae? And by extension, what other relationships were mis-portrayed, in conflict with strong morphological evidence?

SUMMARY: Previously proposed relationships and their supporting synapomorphies were not discussed.

These, and other conflicts between sequence and morphological data were not discussed by Wiegmann *et al.* (2011). It will be difficult to progress in our understanding of phylogenetic relationships between the families if previous morphological evidence of relationships is not discussed (and either confirmed or reinterpreted). The Nymphomyiidae, noted above, have been placed with the Deuterophlebiidae and Blephariceridae in the infraorder Blephariceromorpha by earlier work (Wood & Borkent, 1989; Oosterbroek & Courtney, 1995; Courtney, 1990, 1991, 1994a, b). It is important to understand the fate of the synapomorphies proposed in those works, when each

of the three families is now placed separately. Again, it is each of those synapomorphies that is the morphological evidence for or against the placement of these three families. Of course, the main point of Wiegmann *et al.* (2011) was the importance of molecular sequences, where the bulk of the paper and its conclusions rested. That the publication was primarily a molecular study was also understood by such subsequent work as Caravas & Friedrich (2013), who considered the results of Wiegmann *et al.* (2011) as entirely molecular, completely ignoring what impact the morphology might have had.

This project and many others like it (combining sequence and morphological data) often fail to put enough effort into the interpretation of the morphological characters. There is a propensity to consider morphological data input into a matrix as rather straightforward and quick. In fact, the opposite is the case. My experience of working on a given character state is that interpretation of its polarity requires at least two elements. One is the presence of a good representative collection, so character states can be compared directly and the other is a lot of time, often several days, to be confident of homology and to determine polarity of one character. Monty Wood and I (Wood & Borkent, 1989) spent over eight years examining each of the 83 characters we determined and/or confirmed as synapomorphic within the nematocerous Diptera. Most of these required days of making careful, side by side comparisons and a great deal of discussion. At the end of many such efforts, a character state was often rejected because of uncertain homology, or lack of appropriate material (especially of immatures). In short, a major effort to discover fresh synapomorphies among families of Diptera requires the input from many more dipteran systematists who know their group(s) than were involved with the Wiegmann *et al.* (2011) and Lambkin *et al.* (2013) project. Regardless, in this case, a few more character states would not have made any difference because of the construct of the analysis.

Lambkin *et al.* (2013), to their credit, provide a discussion of the recent history of each of the characters used and explain in part some differing interpretation of those characters. They do not, however compare the evidence at a particular node against previously published conclusions. As such, it is informative to examine the actual synapomorphies at a given node presented in their study and the results of their parsimony analysis. Aside from the Nymphomyiidae discussed above as the hypothesized sister group to all remaining Diptera, the next sister group relationship indicated the monophyly of the Diptera to the exclusion of Nymphomyiidae and Culicomorpha. This was supported by three synapomorphies, two of which were homoplastic (numbers from their study) as follows:

248 (homoplastic) Discal or discal medial cell absent (plesiomorphic). Of the 14 nematocerous families considered, only Tipulidae, Trichoceridae, Tanyderidae, and Anisopodidae were stated to have the derived condition (cell present). Lepidoptera, where the cell is absent, was stated to have the primitive condition. As presented, the discal cell therefore evolved independently in Mecoptera (Bittacidae and Nannochoristidae) and in Diptera other than Culicomorpha and Nymphomyiidae (with subsequent losses). Wood & Borkent (1989, their character 80) considered the loss of the cell to be derived but pointed out that there have been many independent losses; they considered it a weak synapomorphy of the Culicomorpha. They understood the feature to be homologous between Mecoptera and Diptera.

The interpretation by Lambkin *et al.* (2013), with *de novo* evolution of the medial cell in the Mecoptera, is highly unlikely and its interpretation as a synapomorphy of Diptera other than Culicomorpha and Nymphomyiidae unacceptable. Further to this, the lack of a discal cell in Nymphomyiidae is highly suspicious. The wing venation of this family is so reduced that the medial vein can only be identified at its base (Courtney, 1994a) and to consider the absence of the discal cell as plesiomorphic in this family is more than questionable.

318 (homoplastic) Three spermathecae (plesiomorphic). Lambkin *et al.* (2013) describe the outgroup distribution of the number of spermathecae as "Trichoptera, Lepidoptera and Mecoptera have one spermatheca and Siphonaptera one or two (Oosterbroek & Courtney, 1995). The plesiomorphic number of spermathecae in Diptera is hypothesized to be three, and this number is found in most lower Brachycera and Cyclorrhapha". It is illogical to consider the outgroup as having 1 or 2 spermathecae and the plesiomorphic condition in Diptera to be 3. This is not a cladistic argument.

As presented, 3 spermathecae are present in Bibionidae, Blephariceridae, Trichoceridae, Tanyderidae, and Tipulidae. The smaller numbers of spermathecae found in other Diptera were further included as synapomorphies with each scored on the basis of the single exemplar species. The real world is more complex. The number of spermathecae varies strongly within the nematocerous Diptera. For example, there are actually 1 or 3 in

Trichoceridae, 2 or 3 in Tipulidae, 2 or 3 in Tanyderidae, 0-2 in Cecidomyiidae and Psychodidae, 1 in Corethrellidae as sister group of Chaoboridae (with 3) + Culicidae (with 1 or 3) and 2 or 3 in Chironomidae, making this presented synapomorphy, when all families are considered, presently uninterpretable and for the families considered by Lambkin *et al.* (2013), unacceptably interpreted.

Deuterophlebiidae and Nymphomyiidae, as the purported (Lambkin *et al.*, 2013; Wiegmann *et al.*, 2011) earliest and second earliest lineages of Diptera, respectively, do not have spermathecae at all (Courtney, 1994a, b).

357 Ejaculatory apodeme absent (plesiomorphic). The character state distribution of this feature by Lambkin *et al.* (2013) shows that it is absent in non-Diptera and present in all nematocerous Diptera other than Culicomorpha and Nymphomyiidae, indicating that it is correctly identified as synapomorphic for this lineage. However, it is important to know that an ejaculatory apodeme has been previously identified in Mecoptera but there are questions of homology with that found in Diptera (Sinclair *et al.*, 2007). Further to this, the absence of an ejaculatory apodeme in Culicomorpha is almost certainly related to the presence of a spermatophore, a different mechanism to deliver sperm, which is considered a derived condition within the Diptera and quite possibly derived from a lineage with an ejaculatory apodeme.

This pattern of questionable synapomorphies is scattered throughout the paper. There are therefore, numerous questions regarding the actual synapomorphies supporting various lineages in Lambkin *et al.* (2013), accenting the need to make broad comparisons and to better understand the actual synapomorphies proposed.

TEXT BOX: The monophyly of all Diptera other than Nymphomyiidae and Culicomorpha was based on three questionably interpreted synapomorphies.

The problem of character delimitation discussed above also impacted the morphologically-based study by Lambkin *et al.* (2013). For example, Keroplatidae and Bibionidae are treated as sister groups based on the following five character states, all of which are reversals to the plesiomorphic outgroup (to Diptera) state (numbers = their synapomorphies):

230 - sc-r present
250 - basal medial cell present
251 - bm-cu present
264 – posterior veins of wings well-developed
364 - male tergite 10 present

All of these are homoplastic, even within some families, and obviously the first four are wing characters that could easily be related to each other and therefore likely not to be independent indicators of relationship. This, therefore, impacts Bremer support values, as well as the analysis itself. The presence of tergite 10 in males is true of a number of other families (see their matrix). There are too many other instances of such presented evidence (as with the loss of fore- and midtibial spurs of Nymphomyiidae noted above) and these need to be scrutinized and reinterpreted.

Some readers will note that nine characters included in the text of supplement S4 discuss conditions in Strepsiptera. This was a relict of a time when this order was considered as close to Diptera, but was excluded in the last months of the project due to widespread agreement that the group is related to Coleoptera. It was eliminated from the matrix (supplement S3) but not from the text, where it is largely unimportant to the discussion of the character states.

The limits of genomic studies discussed above also apply to the results of Wiegmann *et al.* (2011). Synapomorphies based on gene sequences are not presented (if they were present), the programs used are opaque to a large majority of dipteran systematists, and the results presented with fervour but without data (other than the publicly stored sequences).

This overview indicates that there are serious flaws in both Wiegmann *et al.* (2011) and Lambkin *et al.* (2013)

and that there is a great need to go back to the characters themselves, to check their homology, read the pertinent literature, and to search for further morphological features which will provide more information on phylogenetic relationships between all the families of Diptera. The Wiegmann *et al.* (2011) paper may indeed have been a "Periodic Table" but only in the sense that if this approach continues, we will indeed see new and questionable phylogenetic arrangements on a very periodic basis.

How are We Serving the Next Generation?

Peter: "You see, I don't know any stories. None of the lost boys know any stories."
Wendy: "How perfectly awful". *Peter Pan*, by J.M. Barrie.

In March, 2004, I visited La Selva Biological field station in north-eastern Costa Rica. The station is hosted by a consortium of American universities and provides a number of field courses. While collecting there, I had the opportunity to meet with a group of 14 fourth-year biology undergraduate and graduate students who were tremendously excited by the diversity they were experiencing in the surrounding lowland rainforest. I wanted to discuss broader patterns of diversity and to make reference to, for example, our knowledge of the insects of British Columbia, where there are about 15,000 named and an estimated 13,000 unnamed species. There are similar levels of ignorance in much of North America, providing a wealth of research opportunities. By way of introduction I asked one of the students where he was from, but when I began "When you are in the woods in Pennsylvania ….." he shook his head, I stopped, and he stated he'd never been in the woods before, nor in any local park in his life. Being an evolutionary biologist means being familiar with the idea that there is also variation in the human family but this was rather peculiar. As it turned out, nearly half of the students had never been in nature before. Admittedly, the sample size was small, but it appears that students increasingly are learning a rather reductionist perspective of biology and have very limited first-hand experience of fauna and/or flora. This also pertains to first-hand experience with organisms under the microscope. Surely we cannot expect students, smart as they are, to develop a broad understanding of nature, and the challenges of interpreting it, without profound and detailed experiences of nature itself.

The same pertains to systematics. If character states vary in their value in indicating evolutionary relationships and judging such value depends on understanding their morphology, development, function, and history, there are many students in systematics today who are ill prepared to interpret observations of the specimens they study (Crisci, 2006). It is rare to meet a taxonomy graduate today who has had a course in each of the following, all of which are vital for understanding systematic information: insect morphology, insect systematics, insect development (including embryology), insect physiology, insect ecology, palaeontology (including Mesozoic and Tertiary eras), and Quaternary and Holocene history. The majority appear to have had a course in insect systematics or at least a general entomology course, if they are lucky enough to have attended a university that yet offers such courses. As such, many students who are entering graduate work in systematics appear to be limited to the study of one stage of the limited group they (or their professor) has chosen, have little understanding of the features they are examining, nearly all of which have a broader evolutionary context of modification, have trouble understanding the broader environment in which their species live, and cannot distinguish the Paleocene from the Pleistocene. This dearth of knowledge markedly decreases the odds of them being able to produce the sort of integrated and interesting studies that portray a far more encompassing evolutionary understanding of their groups.

TEXT BOX: The training of young systematists should include a broad understanding of morphology, development, function and history of insects. Earth history should also be part of every systematist's background.

A reductionist focus on producing maximum parsimony trees and molecular sequences discussed above leads many to believe that scoring characters is straightforward and that sequences do indeed provide the best answers to questions regarding genealogical relationships. There is little emphasis on the need to study a broad outgroup to enhance the likelihood of correctly identifying strong synapomorphies. This most fundamental of cladistic principles seems to bypass some students entirely (and perhaps their professors). In conjunction with this, the

attention span required to spend time really studying specimens (both preserved and in nature) seems to be increasingly diminished, hand in hand, of course, with a general societal love affair with i-technology. There is a great need to encourage students to once again spend time with the organisms they wish to interpret before leaping to programs that can arrange the 0s and 1s from their matrices and then to quickly push these through programs to produce phylogenies.

Further to this, as teachers, it has been challenging to portray what it means for us as systematists to study our families. While there is a strong need for much broader education, more time spent studying specimens and much more field work, there are other aspects of doing good science that are also important. Creativity and a creative environment are vital to produce science that truly tests previous ideas and comes up with new and fresh perspectives. Each of our groups of Diptera, some more than others, has the potential to uncover valuable and new insights. The old adage that chance favours the prepared mind has always been true but there is an additional component that really helps. Immersion in a particular group provides the opportunity for creativity that in turn can result in falling in love with the beasts we study. It is when students attain this level of involvement, of having a consuming desire to uncover meaning, that we can expect innovative science.

I am more than aware that there are huge pressures at our universities for professors, teaching large classes, persistently seeking financial support, accessing ever diminishing institutional resources (especially for field work), competing with i-technology for the attention of students, and lacking in sufficient staff to teach an array of courses and more. So too, there is high pressure to publish and provide the evidence of productivity (hence professor names as coauthors on all published graduate work, regardless of their contribution). There has been a distressing decline in entomology courses offered at nearly all universities and it appears fewer and fewer students are graduating and taking up the gauntlet for many of our families. All of these factors limit the essential training of upcoming taxonomists. Whatever constraints are in place, it is vital that we direct our focus as systematists and educators on the central questions at hand, the study of actual specimens, their features and phylogenetic relationships, and the biological significance (present and historical) of those phylogenies. Only then can we continue to produce comprehensive and meaningful science.

Conclusion

> When the people applauded him wildly, he [Phocion] turned to one of his friends and said, "Have I said something foolish?"—Diogenes Laertius (150 BC).

The systematics of Diptera has seen, over the past few decades, tremendous advance in our understanding of the diversity and diversification of many families. Numerous species have been described, new phylogenetic relationships have been proposed and there is a renaissance in the discovery and description of fossils.

However, it is my contention that cladistics has largely become reduced to a computer game that is justified with conviction because it is simple, self-feeding and nearly immune to outside criticism. It discourages systematists from making broad outgroup comparisons and from studying the nature of character states (e.g., function and development). It further allows for the easy introduction of additional, often superficial, characters to the analysis to produce yet another phylogeny.

There is a considerable need, therefore, for a re-examination of the central procedures and tenets of analysis. Characters need to be studied in detail and carefully defined so that they are not overly split into component elements, biasing subsequent phylogenetic interpretation. Character states vary in their importance in indicating phylogenetic relationships and can be partially appraised by their complexity, development, function and, particularly, their distribution in the outgroup. Character state distributions require broad outgroup comparisons to distinguish those distinctive synapomorphies that are unique (or nearly unique) from those which are repeatedly homoplastic. Unfortunately, many no longer view these working principles as fundamental to understanding the evolutionary relationships between taxa, although these were clearly the essential working principles for Hennig (1950, 1965, 1966) and other outstanding cladists.

Parsimony analysis, as currently practiced, places emphasis on the treatment of character states as equal in value in determining phylogenetic relationships. Such analysis, however, is minimalist and should emphasize instead certain synapomorphies that are superior indicators of relationships than others. In spite of years of

mathematically inclined biologists emphasizing mathematical analysis of data, in reality, there is no biological basis for assigning a mathematical value to a given synapomorphy. Deduced values such as Bremer support, and others, provide an apparent means of determining the relative confidence in a given node in a cladogram. However, it is the character states themselves which need to be appraised as evidence for a given cladistic hypothesis (Jaschhof, 2011). Congruent character states which are likely to be evolutionarily independent (e.g., a genitalic and antennal synapomorphy) are better evidence for a given node than those intimately related in structure and function (e.g., two wing veins). Parsimony analysis needs to be thought of as competing hypotheses involving explicit discussion of character states and thereby providing the most parsimonious interpretation of a given set of character states.

There is another barrier to transparency in the analysis of phylogenetic data. The theoretical work done in phylogenetics over the past few decades by biologists who are mathematically predisposed has produced new statistical methods and associated computer programs. Both morphologically-based and molecularly-based data analyses currently depend on complex algorithms which are likely to be mostly a blackbox of options and data manipulation that is poorly understood by most. The current accent on manipulating various resultant cladograms to determine those which are "most parsimonious" should be largely replaced with a reexamination of the character states themselves, in order to clarify which synapomorphies conflict. Without addressing both the nature of characters and how best to interpret them, nature, in this case phylogenies, can become caricatures made in our own image and will produce the spectacularly bleak future in which our proposed phylogenetic hypotheses are mere fabrications of algorithms.

Authors are therefore encouraged to be more explicit regarding their description of the character states being presented and provide broad outgroup distributions for each of these. Using various programs to analyze data, the concluding cladograms should clearly indicate what character states support which nodes. Text should discuss each character state and particular nodes, indicating which appear to be weak (one or more homoplastic features) and those with high-weight, independently derived synapomorphies. Readers and subsequent students of the group can then more critically examine the evidence supporting various phylogenetic conclusions.

Molecular sequence work has a long history of providing questionable phylogenetic conclusions, often changing radically with the subsequent addition of more genes and/or more sequences. In spite of this, there is the continued, financially well-supported, impetus to continue to add more data to this form of phylogenetic interpretation. As currently practiced, most molecular studies are either phenetic (distance-based trees) or are model driven (maximum likelihood analysis) and cannot be considered cladistic (Cruickshank, 2011; Stevens, 2000; Kück & Wägele, 2016). Because most are not based on synapomorphies, they are, as shown by Hennig (1950, 1965, 1966), logically flawed. They cannot adequately identify autapomorphic lineages originating from within a more homogeneous group of taxa or adequately interpret groups based on symplesiomorphy. Further to this, virtually all sequence studies are clouded by a highly opaque methodology, which makes it nearly impossible for virtually all morphologically-based systematists, and even many who, at least partially blinded, do use the techniques, to examine the basis for the conclusions presented.

In spite of continuing uncertainties, faith that molecular techniques will produce accurate phylogenies has produced two strong trends in phylogenetic studies. The first is the increasing dependency on molecular sequencing to provide "final" phylogenies. As such, I am uncertain whether others noticed that the "Periodic Table" of Diptera phylogeny by Wiegmann *et al.* (2011) did not provide the morphological data they claimed to have included, in some manner, in their analysis. Those authors do not stand alone as an example of 'total evidence' papers that are clearly biased toward the molecular portion of the paper (e.g., Gibson *et al.*, 2013; Petersen *et al.*, 2010; Su *et al.*, 2008; Tóthová *et al.*, 2013; Winterton & Ware, 2015). The second strong trend is the unfortunate belief by many non-systematists that phylogenies are as easy to produce as determining a barcode or another limited set of sequences for each species and popping the results into a program, whether they do it themselves or farm the process out to someone who knows the techniques and is awarded coauthorship. By way of example, as far as I can determine, the following publications on Ceratopogonidae, Culicidae and Psychodidae did not include a systematist who knew the morphology of the taxa at hand: Ander *et al.* (2013), Chu *et al.* (2016), Dixit *et al.* (2010), Grace-Lema *et al.* (2015), Karimian *et al.* (2014), Mohanty *et al.* (2009), Muñoz-Muñoz *et al.* (2014), Norris & Norris (2015), Pagès *et al.* (2009), Sum *et al.* (2014), Tay *et al.* (2016), Wang *et al.* (2014). One of these, at least, based their study entirely on downloaded sequences from databases to generate their phylogenetic results (Wang *et al.*, 2014). Unfortunately, this belief in the technique has become widespread, especially in certain

groups of economic importance (e.g., biting flies), and has become divorced from specialized knowledge of the groups themselves.

All these issues are evident in the purported landmark analyses of relationships between the families of Diptera by Wiegmann *et al.* (2011) and Lambkin *et al.* (2013). The morphological component, which had a highly questionable impact on the Wiegmann *et al.* (2011) study and was superficially treated by Lambkin *et al.* (2013), requires a fresh approach incorporating the known diversity of Diptera (all families and including intrafamilial variation). Molecular work has the potential to add another rich layer of knowledge to our understanding of the Diptera, but only if it is combined with a deep understanding of character morphology, development and function and broad outgroup comparisons of each character state. If this is pursued, there is every reason to believe that Diptera will become one of the outstanding groups exemplifying evolutionary patterns and processes.

Another major concern regarding the current presentation of most phylogenetic results is the ignoring of previous hypotheses. The Wiegmann *et al.* (2011) and Lambkin *et al.* (2013) papers produced a new phylogeny of the Diptera but did not discuss the evidence for previous, conflicting relationships. This is a characteristic of virtually all molecularly-based studies and a significant number of morphologically-based works. For example, in their reassessment of the Wiegmann *et al.* (2011) sequences, Caravas & Friedrich (2013) state, "Thus, the organization of the major brachyceran clades should be considered an open question in dipteran systematics…" and "we conclude that noncalyptrate fly relationships remain tentative and an important challenge for future studies". This is simply ridiculous and ignores the important work by, among others, Woodley (1989), Woodley *et al.* (2009), and Yeates (2002). If we are to progress in our science of phylogenetic interpretation, it is vital that we discuss, criticize and reinterpret previous results that are now conflicting. Instead of claims to truth and ignoring earlier publications, it is critically important that we point out conflicting results regarding morphological divergence. Ignorance of synapomorphies in previously published literature is no excuse for proposing new relationships based on either (or both) new matrices and sequences as if they are *de novo*. Science never operates in a vacuum, but is an ongoing refinement of hypotheses.

The issues discussed here often result in highly varying phylogenetic conclusions. At the International Congress of Dipterology in Potsdam in 2014, Dalton de Souza Amorim noted during a panel discussion, "If I were to come every year to these congresses and present phylogenies of the same groups, denying the ones I published the year before, I would blush." Indeed, we undermine our status as systematists if we produce specious phylogenies based on poorly understood morphological features or yet more DNA sequence data that allow for constant and radical revision of our phylogenetic conclusions. It won't be long before others will recognize that our results are so endlessly flexible as to be inconsequential to their understanding of nature. Our phylogenetic results are only as good as how thoroughly we have studied and understood the character states making up a given phylogeny.

TEXT BOX: The future of phylogenetic analysis requires detailed understanding of characters and how they are treated. Parsimony generally requires the weighing of synapomorphies, generating stable phylogenies and the basis for interpretation of bionomic features and historical patterns of distribution.

This paper is not the first to lament the lack of more rigorous study of morphological synapomorphies and the misleading results of so many sequence studies. Wheeler (2007, 2008a, 2008b, 2009) has repeatedly called for a return to the careful and rigorous study of morphological character states and has warned of the dangers of sequence analysis as presently interpreted. Mooi & Gill (2010) elegantly discussed the situation in fish systematics, where the focus on trees was to the detriment of the study (and testing) of morphological synapomorphies and the propensity to increasingly depend on sequences was based on models (and not synapomorphies). Mooi & Gill's paper produced a vigorous debate (17 papers in *Zootaxa* 2946) that is informative and provided examples of the consequences of not paying sufficient attention to morphologically-based synapomorphies (Britz & Conway, 2011a, 2011b). Another example is a recent morphological study of a group of 9,000 scaly reptile species incorporating 610 morphological characters indicating that the historical placement of iguanas, chameleons and their close relatives in the lower branches of the phylogenetic tree is correct and refutes over 10 years of sequence data indicating that they are among the more derived lineages (Gauthier *et al.*, 2012). Although the current paper was largely limited to examples from studies of Diptera, it is clear that the methodologies and their associated problems extend more broadly in at least zoology.

There is a final reason to re-examine our current phylogenetic practices. Well established phylogenies can provide a powerful tool for understanding the huge diversity of species present in the order Diptera and how this has come about. During part of my training as a systematist, my supervisor George Ball would regularly stand up during the question period after a presentation and ask a poignant question "What is the biological significance of your work?" –often resulting in a stammering and inadequate reply. It really is an excellent question, which we should all ask ourselves regularly. The phylogenies we produce call for their use in interpreting the bionomic features of our taxa, as well as their historical zoogeography. It is the most powerful item in a systematist's toolbox and certainly one of the most important contributions we can make to the broader community of biologists. When Theo Dobzhansky (1973) famously wrote "nothing in biology makes sense except in the light of evolution" he could have been making the statement directly to our community of systematists. In a time when reductionist science has come to dominate biology and where modelling often replaces actual study of nature, systematists have the opportunity to present comprehensive syntheses of biodiversity that transcends any other area of biology. To generate such insights, we need to renew our understanding of homologous character states, increase our confidence in resultant phylogenies, and have the skills and drive to interpret what those phylogenies might be telling us. One of the great opportunities that continues to be present for those studying Diptera is to provide new and exciting insights into evolutionary patterns and processes. We can only do this if we question emerging methodologies and build rigorously from the best practices discussed here.

Acknowledgments

This paper was written while on a three-month expedition/ sabbatical to Bolivia with my wife, Annette Borkent. As always, she supports my work with ideas, love and finances and I am hugely grateful. My thoughts and concern for the future of our science were strongly influenced by my training in Edmonton, Alberta and Ottawa, Ontario, and over the years by many valued colleagues. Especially important have been D. Monty Wood, George E. Ball, Jeffrey M. Cumming, Douglas A. Craig, Mathias Jaschhof, Christopher J. Borkent, Bradley J. Sinclair, Gregory R. Curler, Dalton de Souza Amorim, and Brian V. Brown. They may not agree with all written here but I thank each of them for many hours of discussion regarding Diptera, characters, and how best to present our science. Ralph E. Harbach kindly put me in touch with a number of pertinent publications on Culicidae.

I express my thanks to Annette Borkent, Mike Borkent, Brian V. Brown, Jeffrey M. Cumming, Ralph E. Harbach, Mathias Jaschhof, Felix Sperling and Norman Woodley for critical reviews of this paper. I also appreciate the critique and advice by one reviewer who preferred to remain anonymous.

References

Adler, P.H. & Crosskey, R.W. (2012) World Blackflies (Diptera: Simuliidae): a comprehensive revision of the taxonomic and geographical inventory. Available from: https://blackflies.info/sites/blackflies.info/files/u13/blackflyinventory_web_2012_0.pdf (accessed 16 Febuary 2017)

Adler, P.H., Currie, D.C. & Wood, D.M. (2004) *The black flies (Simuliidae) of North America.* Cornell University Press, Ithaca, 941 pp.

Adler, P.H., Kúdelová, T., Kúdela, M., Seitz, G. & Ignjatović-Ćupina, A. (2016a) Cryptic biodiversity and the origins of pest status revealed in the macrogenome of (Diptera: Simuliidae), history's most destructive Black Fly. *PLoS ONE*, 11 (1), e0147673.
https://doi.org/10.1371/journal.pone.0147673

Adler, P.H., Yadamsuren, O. & Procunier, W.S. (2016) Chromosomal translocations in Black Flies (Diptera: Simuliidae) — facilitators of adaptive radiation? *PLoS ONE*, 11 (6), e0158272.
https://doi.org/10.1371/journal.pone.0158272

Ander, M., Troell, K. & Chirico, J. (2013) Barcoding of biting midges in the genus *Culicoides*: a tool for species determination. *Medical and Veterinary Entomology*, 27, 323–331.
https://doi.org/10.1111/j.1365-2915.2012.01050.x

Anderson, F.E. & Swofford, L. (2004) Should we be worried about long-branch attraction in real data sets? Investigations using metazoan 18S rDNA Mol. *Molecular Phylogenetics and Evolution*, 33, 440–451.
https://doi.org/10.1016/j.ympev.2004.06.015

Arillo, A., Peñalver, E., Pérez de la Fuente, R., Delclòs, X., Criscione, J., Barden, P.M., Riccio, M.L. & Grimaldi, D.A. (2015) Long–proboscid brachyceran flies in Cretaceous amber Diptera, Stratiomyomorpha: Zhangsolvidae). *Systematic*

Entomology, 40, 242–267.
https://doi.org/10.1111/syen.12106
Amorim, D.S. & Rindal, E. (2007) Phylogeny of the Mycetophiliformia, with proposal of the subfamilies Heterotrichinae, Ohakuneinae, and Chiletrichinae for the Rangomaramidae (Diptera, Bibionomorpha). *Zootaxa*, 1535, 1–92.
Bakhoum, M.T., Fall, M., Fall, A.G., Bellis, G.A., Gottlieb, Y., Labuschagne, K., Venter, G.J., Diop, M., Mall, I., Seck, M.T., Allene, X., Diarra, M., Gardes, L., Bouyer, J., Delécolle, J.-C., Balenghien, T. & Garros, C. (2013) First record of *Culicoides oxystoma* Kieffer and diversity of species within the Schultzei Group of *Culicoides* Latreille (Diptera: Ceratopogonidae) Biting Midges in Senegal. *PLoS ONE*, 8 (12), e84316.
https://doi.org/10.1371/journal.pone.0084316
Bellis, G., Dyce, A., Gopurenko, D., Yanase, T., Garros, C., Labuschagne, K. & Mitchell, A. (2014) Revision of the *Culicoides* (*Avaritia*) Imicola complex Khamala & Kettle (Diptera: Ceratopogonidae) from the Australasian region. *Zootaxa*, 3768 (4), 401–427.
https://doi.org/10.11646/zootaxa.3768.4.1
Beutel, R.G., Friedrich F., Ge, S-Q. & Yang, X-K. (2014) *Insect Morphology and Phylogeny: A textbook for students of entomology*. De Gruyter, Berlin/Boston, 516 pp.
Borkent, A. (1984) The systematics and phylogeny of the *Stenochironomus* complex (*Xestochironomus*, *Harrisius*, and *Stenochironomus*) (Diptera: Chironomidae). *Memoirs of the Entomological Society of Canada*, 128, 1–269.
Borkent, A. (2008) The Frog-Biting Midges of the world (Corethrellidae: Diptera). *Zootaxa*, 1804, 1–456.
Borkent, A. (2012) The pupae of Culicomorpha—morphology and a new phylogenetic tree. *Zootaxa*, 3396, 1–98.
Borkent, A. (2014) The pupae of the Biting Midges of the World (Diptera: Ceratopogonidae), with a generic key and analysis of the phylogenetic relationships between genera. *Zootaxa*, 3879 (1), 1–327.
https://doi.org/10.11646/zootaxa.3879.1.1
Borkent, A., Brown, B.V., Adler, P.H., Amorim, D.S., Barber, K., Bickel, D., Boucher, S., Brooks, S.E., Burger, J., Burington, Z.L., Capellari, R.S., Costa, D.N.R., Cumming, J.M., Curler, G., Dick, C.W., Epler, J.H., Fisher, E., Gaimari, S.D., Gelhaus, J., Grimaldi, D.A., Hash, J., Hauser, M., Hippa, H., Ibáñez-Bernal, A., Jaschhof, M., Kameneva, E.P., Kerr, P.H., Korneyev, V., Korytkowski, C.A., Kung, G.-A., Kvifte, G.M., Lonsdale, O., Marshall, S.A., Mathis, W., Michelsen, V., Naglis, S., Norrbom, A.L., Paiero, S., Pape, T., Pereira-Colavite, A., Pollet, M., Rochefort, S., Rung, A., Runyon, J.B., Savage, J., Silva, V.C., Sinclair, B.J., Skevington, J.H., Stireman III, J.O., Swann, J., Vilkamaa, P., Wheeler, T., Whitworth, T., Wong, M., Wood, D.M., Woodley, N., Yau, T., Zavortink, T.J. & Zumbado, M.A. (in press) Remarkable fly (Diptera) diversity in a patch of Costa Rican cloud forest: Why inventory is a vital science. *Zootaxa*.
Britz, R. & Conway, K.W. (2011a) The Cypriniformes tree of confusion. *Zootaxa*, 2946, 73–78.
Britz, R. &. Conway, K.W (2011b) Additions to "The Cypriniformes tree of confusion". *Zootaxa*, 2946, 142–142.
Brower, A.V.Z. (2000) Homology and the inference of systematic relationships: some historical and philosophical perspectives. *In*: Scotland, R. & Pennington, R.T. (Eds.), *Homology and systematics. Coding characters for phylogenetic analysis.* Taylor & Francis, London and New York, pp. 10–21.
Brower, A.V.Z. (2016) Emergent properties. *Cladistics*, 32, 577–579.
https://doi.org/10.1111/cla.12152
Brown, B.V., Amorim, D.S. & Kung, G.-A. (2015) New morphological characters for classifying Phoridae (Diptera) from the structure of the thorax. *Zoological Journal of the Linnean Society*, 173, 424–485.
https://doi.org/10.1111/zoj.12208
Brundin, L. (1966) Transantarctic relationships and their significance, as evidenced by chironomid midges, with a monograph of the subfamilies Podonominae and Aphroteniinae and the austral Heptagyiae. *Kungliga Svenska Vetenskapsakademiens Handlingar*, Fjarde Serien, 11 (1), 1–472, 30 pls.
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.
https://doi.org/10.1111/j.1365-3113.2006.00328.x
Buenaventura, E., Whitmore, D. & Pape, T. (2016) Molecular phylogeny of the hyperdiverse genus *Sarcophaga* (Diptera: Sarcophagidae), and comparison between algorithms for identification of rogue taxa. *Cladistics*, 33, 109–133.
https://doi.org/10.1111/cla.12161
Caravas, J. & Friedrich, M. (2013) Shaking the Diptera tree of life: performance analysis of nuclear and mitochondrial sequence data partitions. *Systematic Entomology*, 38, 93–103.
https://doi.org/10.1111/j.1365-3113.2012.00657.x
Cerretti, P. & Pape, T. (2012) Phylogenetics and taxonomy of *Ventrops* - the largest genus of Afrotropical Rhinophoridae (Diptera). *Invertebrate systematics*, 26, 274–292.
https://doi.org/10.1071/IS12001
Chapman, E.G.,, Przhiboro, A.A., Harwood, J.D., Foote, B.A. & Hoeh,W.R. (2012) Widespread and persistent invasions of terrestrial habitats coincident with larval feeding behavior transitions during snail-killing fly evolution (Diptera: Sciomyzidae). *BMC Evolutionary Biology*, 12 (175), 1–22.
https://doi.org/10.1186/1471-2148-12-175
Chu, H., Li, C., Guo, X., Zhang, H., Luo, P., Wu, Z., Wang, G. & Zhao, T. (2016) The phylogenetic relationships of known mosquito (Diptera: Culicidae) mitogenomes. *Mitochondrial DNA Part A*, 1–5. [published online]
https://doi.org/10.1080/24701394.2016.1233533

Courtney, G.W. (1990) Cuticular morphology of larval mountain midges (Diptera: Deuterophlebiidae): implications for the phylogenetic relationships of Nematocera. *Canadian Journal of Zoology*, 68, 556–578.
https://doi.org/10.1139/z90-081

Courtney, G.W. (1991) Phylogenetic analysis of the Blephariceromorpha, with special reference to mountain midges (Diptera: Deuterophlebiidae). *Systematic Entomology*, 16, 137–172.
https://doi.org/10.1111/j.1365-3113.1991.tb00683.x

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

Courtney, G.W. (1994b) Revision of Palaearctic mountain midges (Diptera: Deuterophlebiidae), with phylogenetic and biogeographic analyses of world species. *Systematic Entomology*, 19, 1–24.
https://doi.org/10.1111/j.1365-3113.1994.tb00576.x

Crampton, G.C. (1924) Remarks on the phylogeny and interrelationships of nematocerous Diptera. *Psyche (Camb.)*, 31, 238–242.
https://doi.org/10.1155/1924/60102

Cranston, P.S., Hardy, N.B. & Morse, G.E. (2012) A dated molecular phylogeny for the Chironomidae (Diptera). *Systematic Entomology*, 37, 172–188.
https://doi.org/10.1111/j.1365-3113.2011.00603.x

Crisci, J.V. (2006) One-dimensional systematist: perils in a time of steady progress. *Systematic Botany*, 31, 217–221.
https://doi.org/10.1600/036364406775971859

Cruickshank, R.H. (2011) Exploring character conflict in molecular data. *Zootaxa*, 2946, 45–51.

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.
https://doi.org/10.1163/187631295X00143

Curler, G.R. & Moulton, J.K. (2012) Phylogeny of psychodid subfamilies (Diptera: Psychodidae) inferred from nuclear DNA sequences with a review of morphological evidence for relationships. *Systematic Entomology*, 37, 603–616.
https://doi.org/10.1111/j.1365-3113.2012.00634.x

Deans, A.R., Lewis, S.E., Huala, E., Anzaldo, S.S., Ashburner, M., Balhoff, J.P., Blackburn, D.C., Blake, J.A., Burleigh, J.G., Chanet, B., Cooper, L.D., Courtot, M., Csösz, S., Cui, H., Dahdul, W., Das, S., Dececchi, T.A, Dettai, A., Diogo, R., Druzinsky, R.E., Dumontier, M., Franz, N.M., Friedrich, F., Gkoutos, G.V., Haendel, M., Harmon, L.J., Hayamizu, T.F., He, Y., Hines, H.M., Ibrahim, N., Jackson, L.M., Jaiswal, P., James-Zorn, C., Köhler, S., Lecointre, G., Lapp, H., Lawrence, C.J., Novère, N., Lundberg, J.G., Macklin, J., Mast, A.R., Midford, P.E., Mikó, I., Mungall, C.J., Oellrich, A., Osumi-Sutherland, D., Parkinson, H., Ramírez, M.J., Richter, S., Robinson, P.N., Ruttenberg, A., Schulz, K.S., Segerdell, E., Seltmann, K.C., Sharkey, M.J., Smith, A.D., Smith, B., Specht, C.D., Squires, R.B., Thacker, R.W., Thessen, A., Fernandez-Triana, J., Vihinen, M., Vize, P.D., Vogt, L., Wall, C.E., Walls, R.L., Westerfeld, M., Wharton, R.A., Wirkner, C.S., Woolley, J.B., Yoder, M.J., Zorn, A.M. & Mabee, P.M. (2015) Finding our way through phenotypes. *PLoS Biology*, 13, e1002033.
https://doi.org/10.1371/journal.pbio.1002033

Demari-Silva, B., Vesgueiro, F.T., Sallum, M.A.M. & Marrelli, M.T. (2011) Taxonomic and phylogenetic relationships between species of the genus *Culex* (Diptera: Culicidae) from Brazil inferred from the cytochrome *c* Oxidase I mitochondrial gene. *Journal of Medical Entomology*, 48, 272–279.
https://doi.org/10.1603/ME09293

de Meijere, J.C.H. (1916) Beitrage zur Kenntnis der Dipterenlarven und puppen. *Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere*, 40, 177–322.

Dikow, T. & Grimaldi, D.A. (2014) Robber flies in Cretaceous ambers (Insecta: Diptera: Asilidae). *American Museum Novitates*, 3799, 1–19.
https://doi.org/10.1206/3799.1

Dijkstra, K.D.B., Monaghan, M.T. & Pauls, S.U. (2014) Freshwater biodiversity and aquatic insect diversification. *Annual Review of Entomology*, 59, 143–163.
https://doi.org/10.1146/annurev-ento-011613-161958

Ding, S., Li, X., Wang, N., Cameron, S.L., Mao, M., Wang, Y., Xi, Y. & Yang, D. (2015) The phylogeny and evolutionary timescale of Muscoidea (Diptera: Brachycera: Calyptratae) inferred from mitochondrial genomes. *PLoS ONE*, 10, e0134170.
https://doi.org/10.1371/journal.pone.0134170

Dixit, J., Srivastava, H., Sharma, M., Das, M.K., Singh, O.P., Raghavendra, K., Nanda, N., Dash, A.P., Saksena, D.N. & Das, A. (2010) Phylogenetic inference of Indian malaria vectors from multilocus DNA sequences. *Infection, Genetics and Evolution*, 10, 755–763.
https://doi.org/10.1016/j.meegid.2010.04.008

Dobzhansky, T. (1973) Nothing in biology makes sense except in the light of evolution. *American Biology Teacher*, 35 (3), 125–129.
https://doi.org/10.2307/4444260

Drouin, G., Godin, J.-R. & Pagé, B. (2011) The genetics of vitamin C loss in vertebrates. *Current Genomics*, 12, 371–378.
https://doi.org/10.2174/138920211796429736

Edwards, F.W. (1926) The phylogeny of nematocerous Diptera: a critical review of some recent suggestions. *Verhandlungen des III. Internationalen Entomologen-Kongresses*, Zürich, 19–25 Juli, 1925, 1, 111–130.
Eldredge, N. & Cracraft, J. (1980) *Phylogenetic patterns and the evolutionary proces*s. Columbia University Press, New York, viii + 349 pp.
Espíndola, A., Buerki, S., Jacquier, A., Ježek, J. & Alvarez, N. (2012) Phylogenetic relationships in the subfamily Psychodinae (Diptera, Psychodidae). *Zoologica Scripta*, 41, 489–498.
https://doi.org/10.1111/j.1463-6409.2012.00544
Fernández, R., Edgecombe, G.D. & Giribet, G. (2016) Exploring phylogenetic relationships within Myriapoda and the effects of matrix composition and occupancy on phylogenomic reconstruction. *Systematic Biology*, 65, 871–889.
https://doi.org/10.1093/sysbio/syw041
Forster, M., Beutel, R.G. & Schneeberg, K. (2016) Catching prey with the antennae - the larval head of *Corethrella appendiculata* (Diptera: Corethrellidae). *Arthropod Structure & Development*, 45, 594–610.
https://doi.org/10.1016/j.asd.2016.09.003
Friedemann, K., Schneeberg, K. & Beutel, R.G. (2014) Fly on the wall - attachment structures in lower Diptera. *Systematic Entomology*, 39, 460–473.
https://doi.org/10.1111/syen.12064
Fu, Z., Toda, M.J., Li, N.N., Zhang, Y.P. & Gao, J.J. (2016) A new genus of anthophilous drosophilids, Impatiophila (Diptera, Drosophilidae): morphology, DNA barcoding and molecular phylogeny, with descriptions of thirty-nine new species. *Zootaxa*, 4120 (1), 1–100.
https://doi.org/10.11646/zootaxa.4120.1.1
Gagné, R.J. (1989) *The plant-feeding gall midges of North America.* Cornell University Press, Ithaca & London, xi + 356 pp.
Gauthier, J.A., Kearney, M., Maisano, J.A., Rieppel, O. & Behlke, A.D.B. (2012) Assembling the squamate tree of life: perspectives from the phenotype and the fossil record. *Bulletin of the Peabody Museum of Natural History*, 53, 3–308.
https://doi.org/10.3374/014.053.0101
Germann, C., Pollet, M., Tanner, S., Backeljau, T. & Bernasconi, M.V. (2010) Legs of deception: disagreement between molecular markers and morphology of long-legged flies (Diptera, Dolichopodidae). *Journal of Zoological Systematics and Evolutionary Research*, 48, 238–247.
https://doi.org/10.1111/j.1439-0469.2009.00549.x
Gibson, J.F. & Skevington, J.H (2013) Phylogeny and taxonomic revision of all genera of Conopidae (Diptera) based on morphological data. *Zoological Journal of the Linnean Society*, 167, 43–81.
https://doi.org/10.1111/j.1096-3642.2012.00873.x
Gibson, J.F., Skevington, J.H. & Kelso, S. (2013) A phylogenetic analysis of relationships among genera of Conopidae (Diptera) based on molecular and morphological data. *Cladistics*, 29, 193–226.
https://doi.org/10.1111/j.1096-0031.2012.00422.x
Goloboff, P., Farris, J. & Nixon, K. (2003) T.N.T.: Tree Analysis using New Technology. Program and documentation. Available from: https://www.lillo.org.ar/phylogeny/tnt/ (accessed 12 December 2017)
Grace-Lema, D.M., Yared, S., Quitadamo, A., Janies, D.A., Wheeler, W.C., Balkew, M., Hailu, A., Warburg, A. & Clouse, R.M. (2015) A phylogeny of sand flies (Diptera: Psychodidae: Phlebotominae), using recent Ethiopian collections and a broad selection of publicly available DNA sequence data. *Systematic Entomology*, 40, 733–744.
https://doi.org/10.1111/syen.12135
Grimaldi, D.A. (2016) Diverse orthorrhaphan flies (Insecta: Diptera: Brachycera) in amber from the cretaceous of Myanmar: Brachycera in Cretaceous amber, Part VII. *Bulletin of the American Museum Of Natural History*, 408, 1–131.
https://doi.org/10.1206/0003-0090-408.1.1
Grimaldi, D.A. & Barden, P. (2016) The Mesozoic Family Eremochaetidae (Diptera: Brachycera) in Burmese amber and relationships of Archisargoidea: Brachycera in Cretaceous amber, Part VIII. *American Museum Novitates*, 3865, 1–29.
https://doi.org/10.1206/3865.1
Hall, B.G. (2005) Comparison of the accuracies of several phylogenetic methods using protein and DNA sequences. *Molecular Phylogenetics and Evolution*, 22, 792–802.
https://doi.org/10.1093/molbev/msi066
Harbach, R.E. & Kitching, I.J. (1998) Phylogeny and classification of the Culicidae (Diptera). *Systematic Entomology* 23, 327–370.
https://doi.org/10.1046/j.1365-3113.1998.00072.x
Harrup, L.E., Laban, S., Purse, B.V., Reddy, Y.K., Reddy, Y.N., Byregowda, S.M., Kumar, N., Purushotham, K.M., Kowalli, S., Prasad, M., Prasad, G., Bettis, A.A., De Keyser, R., Logan, J., Garros, C., Gopurenko, D., Bellis, G., Labuschagne, K., Mathieu, B. & Carpenter, S. (2016) DNA barcoding and surveillance sampling strategies for *Culicoides* biting midges (Diptera: Ceratopogonidae) in southern India. *Parasites & Vectors*, 9, 461.
https://doi.org/10.1186/s13071-016-1722-z
Haseyama, K.L.F., Wiegmann, B.M., Almeida, E.A.B. & de Carvalho, C.J.B. (2015) Say goodbye to tribes in the new house fly classification: a new molecular phylogenetic analysis and an updated biogeographical narrative for the Muscidae (Diptera). *Molecular Phylogenetics and Evolution*, 89, 1–12.
https://doi.org/10.1016/j.ympev.2015.04.006
Hash, J.M., Brown, B.V., Smith, P.T. & Kanao, T. (2013) A molecular phylogenetic analysis of the genus *Dohrniphora*

(Diptera: Phoridae). *Annals of the Entomological Society of America*, 106, 401–409. https://doi.org/10.1603/AN12053
Hash, J.M., Heraty, J.M. & Brown, B.V. (2017) Phylogeny, host association and biogeographical patterns in the diverse millipede-parasitoid genus *Myriophora* Brown (Diptera: Phoridae). *Cladistics*, 1–20. [published online] https://doi.org/10.1111/cla.12189
Hawkins, J.A. (2000) A survey of primary homology assessment. *In*: Scotland, R.W. & Pennington, R.T. (Eds.), *Homology and systematics: coding characters for phylogenetic analysis.* Taylor and Francis, New York, pp. 22–53.
Hecht, M.K. (1976) Phylogenetic inference and methodology as applied to the vertebrate record. *In*: Hecht, M.K. (Ed.), *Evolutionary Biology, Volume 9.* Plenum Press, New York, pp. 335–363. https://doi.org/10.1007/978-1-4615-6950-3_7
Heming, B.S. (2003) *Insect Development and Evolution*. Cornell University Press, Ithaca, 464 pp.
Hennig, W. (1950) *Grundzüge einer Theorie der phylogenetischen Systematik.* Deutscher Zentralverlag, Berlin, 370 pp.
Hennig, W. (1965) Phylogenetic systematics. *Annual Review of Entomology*, 10, 97–116. https://doi.org/10.1146/annurev.en.10.010165.000525
Hennig, W. (1966) *Phylogenetic systematics.* Translated by Davis, D.D. & Zangerl, R., University of Illinois Press, Urbana, 263 pp.
Hennig W. (1972) Insektenfossilien aus der unteren Kreide. IV. Psychodidae (Phlebotominae), mit einer kritischen Übersicht über das phylogenetische System der familie und die bisher beschriebenen Fossilien (Diptera). *Stuttgarter Beiträge zur Naturkunde*, Serie (B), 241, 1–69.
Hennig, W. (1973) Ordnung Diptera (Zweiflügler). *Handbuch der Zoologie*, 4 (2), 2, 31, 1–337.
Hennig, W. (1981) *Insect phylogeny.* John Wiley & Sons, England. xi + 514 pp. [Pont, A.C. (transl. & Ed.)]
Hennig, W. & Schlee, D. (1978) Abriß der phylogenetischen Systematik. *Stuttgarter Beiträge zur Naturkunde*, Serie A (Biologie), 319, 1–11.
Inclán, D.J., Stireman, J.O. & Cerretti., P. (2016) Redefining the generic limits of *Winthemia* (Diptera : Tachinidae). *Invertebrate Systematics*, 30, 274–289. https://doi.org/10.1071/IS15037
Jaschhof, M. (2011) Phylogeny and classification of the Sciaroidea (Diptera: Bibionomorpha): Where do we stand after AMORIM & RINDAL (2007)? *Beiträge zur Entomologie*, 61, 455–463.
Jaschhof, M. & Jaschhof, C. (2009) The wood midges (Diptera: Cecidomyiidae: Lestremiinae) of Fennoscandia and Denmark. *Studia Dipterologica Supplement*, 18, 1–333.
Jiménez-Guri, E., Huerta-Cepas, J., Cozzuto, L., Wotton, K.R., Kang, H., Himmelbauer, H., Roma, G., Gabaldón, T. & Jaeger, J. (2013) Comparative transcriptomics of early dipteran development. *BMC Genomics*, 14,123. https://doi.org/10.1186/1471-2164-14-123
Kang, Z., Zhang X., Ding, S., Tang, C., Wang, Y., Jong, H., Cameron, S.L., Wang, M. & Yang, D. (2017) Transcriptomes of three species of Tipuloidea (Diptera, Tipulomorpha) and implications for phylogeny of Tipulomorpha. *PLoS ONE*, 12 (3), e0173207. https://doi.org/10.1371/journal. pone.0173207
Karimian, F., Oshaghi, M.A., Sedaghat, M.M., Waterhouse, R.M., Vatandoost, H., Hanafi-Bojd, A.A., Ravasan, N.M. & Chavshin, A.R. (2014) Phylogenetic analysis of the Oriental-Palearctic-Afrotropical members of *Anopheles* (Culicidae: Diptera) based on nuclear rDNA and mitochondrial DNA characteristics. *Japanese Journal of Infectious Diseases*, 67 (5), 361–367. https://doi.org/10.7883/yoken.67.361
Kavanaugh, D.H. (1972) Hennig's principles and methods of phylogenetic systematics. *The Biologist*, 54, 1125–127.
Kettle, D.S. & Elson., M.M. (1976) The immature stages of some Australian *Culicoides* Latreille (Diptera: Ceratopogonidae). *Journal of the Australian Entomological Society*, 15, 303–332. https://doi.org/10.1111/j.1440-6055.1976.tb01711.x
Kirk-Spriggs, A.H. & Wiegmann, B.M. (2013) A revision of Afrotropical Quasimodo flies (Diptera: Schizophora; Curtonotidae). Part IV—the continental Afrotropical species of *Curtonotum* Macquart, with descriptions of thirteen new species and a combined phylogenetic analysis of the Curtonotidae. *Zootaxa*, 3684 (1), 1–165. https://doi.org/10.11646/zootaxa.3684.1.1
Kitching, I.J., Culverwell, C.L. & Harbach, R.E. (2015) The phylogenetic conundrum of *Lutzia* (Diptera: Culicidae: Culicini): a cautionary account of conflict and support. *Insect Systematics & Evolution*, 46, 269–290. https://doi.org/10.1163/1876312X-45032119
Kits, J.H., Marshall, S.A. & Skevington, J.H. (2013) Phylogeny of the Archiborborinae (Diptera: Sphaeroceridae) based on combined morphological and molecular analysis. *PLoS One*, 8 (1), e51190. https://doi.org/10.1371/journal.pone.0051190
Kjer, K.M., Simon, C., Yavorskaya, M. & Beutel, R.G. (2016) Progress, pitfalls and parallel universes: a history of insect phylogenetics. *Journal of the Royal Society Interface*, 13, 20160363. https://doi.org/10.1098/rsif.2016.0363
Koch, N.M., Soto, I.M. & Ramírez, M.J. (2015) First phylogenetic analysis of the family Neriidae (Diptera), with a study on the issue of scaling continuous characters. *Cladistics*, 31, 142–165. https://doi.org/10.1111/cla.12084

Kück, P. & Wägele, J.W. (2016) Plesiomorphic character states cause systematic errors in molecular phylogenetic analyses: a simulation study. *Cladistics*, 32, 461–478.
https://doi.org/10.1111/cla.12132

Kulikowski, M. (2011) Fly tree of life mapped, adds big branch of evolutionary knowledge. North Carolina State University press release, March 14, 2011. Available from: https://news.ncsu.edu/2011/03/038mkwiegmannpnas/ (accessed 1 February 2017)

Lambkin, C.L., Sinclair, B.J., Pape, T, Courtney, G.W., Skevington, J.H., Meier, R., Yeates, D.K., Blagoderov, V. & Wiegmann, B.W. (2013) The phylogenetic relationships among infraorders and superfamilies of Diptera based on morphological evidence. *Systematic Entomology*, 38, 164–179.
https://doi.org/10.1111/j.1365-3113.2012.00652.x

La Salle, J., Wheeler, Q., Jackway, P., Winterton, S., Hobern, D. & Lovell, D. (2009) Accelerating taxonomic discovery through automated character extraction. *Zootaxa*, 2217, 43–55.

Linnaeus, C. (1758) *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis.* Tomus I. Editio decima, reformata. L. Salvii, Homiae (Stockholm), iv + 824 pp.

Malloch, J.R. (1917) A preliminary classification of Diptera, exclusive of Pupipara, based upon larval and pupal characters, with keys to imagines in certain families. Part 1. *Bulletin of the Illinois State Laboratory of Natural History*, 12, 161–409, pls. 28–57. [1918]
https://doi.org/10.5962/bhl.title.9383

McAlpine, J.F. (1989) Phylogeny and classification of the Muscomorpha. *In:* McAlpine, J.F. & Wood, D.M. (Coords.), *Manual of Nearctic Diptera. Volume 3.* Agriculture Canada Monograph 32, pp. 1397–1518.

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.
https://doi.org/10.1016/S1055-7903(02)00276-2

Michelson, 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.
https://doi.org/10.1111/j.1096-3642.1996.tb02149.x

Mitterboeck, T.F., Fu, J.Z., Adamowicz, S.J. (2016) Rates and patterns of molecular evolution in freshwater versus terrestrial insects. *Genome*, 59, 968–980.
https://doi.org/10.1139/gen-2016-0030

Mohanty, A., Swain, S., Kar, S.K. & Hazra, R.K. (2009) Analysis of the phylogenetic relationship of *Anopheles* species, subgenus *Cellia* (Diptera: Culicidae) and using it to define the relationship of morphologically similar species. *Infection, Genetics and Evolution*, 9, 1204–1224.
https://doi.org/10.1016/j.meegid.2009.06.021

Mooi, R.D. & Gill, A.C. (2010) Phylogenies without synapomorphies — a crisis in fish systematics: time to show some character. *Zootaxa*, 2450, 26–40.

Morgulis, E., Freidberg, A. & Dorchin, N. (2016) Phylogenetic revision of *Tephritomyia* Hendel (Diptera: Tephritidae), with description of 14 new species. *Annals of the Entomological Society of America*, 109, 595–628.
https://doi.org/10.1093/aesa/saw026

Morita, S.I., Bayless, K.M., Yeates, D.K & Wiegmann, B.M. (2016) Molecular phylogeny of the horse flies: a framework for renewing tabanid taxonomy. *Systematic Entomology*, 41, 56–72.
https://doi.org/10.1111/syen.12145

Morrison, D.A. (2017) Book review: Next Generation Systematics *In*: Olson, P.D., Hughes, J. & Cotton, J.A. (Eds.), *Systematic Biology*, 66 (1), pp. 121–123.
https://doi.org/10.1093/sysbio/syw081

Mukherjee, S. (2016) *The gene: an intimate history.* Simon and Schuster, New York, London, Toronto, Sydney, New Delhi, 608 pp.

Muñoz-Muñoz, F., Talavera, S., Carpenter, S., Nielsen, S.A., Werner, D. & Pagès, N. (2014) Phenotypic differentiation and phylogenetic signal of wing shape in western European biting midges, *Culicoides* spp., of the subgenus *Avaritia. Medical and Veterinary Entomology*, 28, 319–329.
https://doi.org/10.1111/mve.12042

Nagler, C. & Haug, J.T. (2015) From fossil parasitoids to vectors: insects as parasites and hosts. *Fossil Parasites*, 90, 137–200.
https://doi.org/10.1016/bs.apar.2015.09.003

Neff, N.A. (1986) A rational basis for a priori character weighting. *Systematic Zoology*, 35, 110–123.
https://doi.org/10.2307/2413295

Nelson, G.J. & Platnick, N.I (1981) *Systematics and biogeography: cladistics and vicariance.* Columbia University Press, New York, 567 pp.

Nelson, P.A & Buggs, R.J.A. (2016) Next-generation apomorphy: the ubiquity of taxonomically restricted genes. *In:* Olson, P.D., Hughes J. & Cotton, J.A. (Eds.), *Next generation systematics.* Cambridge University Press, pp. 237–264.
https://doi.org/10.1017/CBO9781139236355.013

Norris, L.C. & Norris, D.E. (2015) Phylogeny of anopheline (Diptera: Culicidae) species in southern Africa, based on nuclear and mitochondrial genes. *Journal of Vector Ecology*, 40, 16–27.
https://doi.org/10.1111/jvec.12128

Oberprieler, S.K. & Yeates, D.K. (2012) *Calosargus talbragarensis* new species: the first brachyceran fly from the Jurassic of Australia (Diptera, Archisargidae). *Journal of Paleontology*, 86, 641–645. https://doi.org/10.1666/11-126R.1

Olson, P.D., Hughes, J. & Cotton, J.A. (Eds.) (2016) *Next generation system*atics. Cambridge University Press, Cambridge, x + 347 pp.

Oosterbroek, P. & Courtney, G. (1995) Phylogeny of the nematocerous families of Diptera (Insecta). *Zoological Journal of the Linnean Society*, 115, 267–311. https://doi.org/10.1111/j.1096-3642.1995.tb02462.x

Pagès, N., Munñoz-Muñoz, F., Talavera, S., Sarto, V., Lorca, C. & Núñez, J.I. (2009) Identification of cryptic species of *Culicoides* (Diptera: Ceratopogonidae) in the subgenus *Culicoides* and development of species-specific PCR assays based on barcode regions. *Veterinary Parasitiology*, 165, 298–310. https://doi.org/10.1016/j.vetpar.2009.07.020

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

Pape, T., Bickel, D. & Meier, R. (Eds.) (2009) *Diptera diversity: status, challenges and tools.* Brill, Leiden, Boston, xix + 459 pp.

Pape, T., Blagoderov, V. & Mostovski, M.B. (2011) Order Diptera Linnaeus, 1758. *In*: Zhang, Z.-Q. (Ed.), Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. *Zootaxa*, 3148, 222–229.

Petersen, M.J., Bertone, M.A., Wiegmann, B.M. & Courtney, G.W. (2010) Phylogenetic synthesis of morphological and molecular data reveals new insights into the higher-level classification of Tipuloidea (Diptera). *Systematic Entomology*, 35, 526–545. https://doi.org/10.1111/j.1365-3113.2010.00524.x

Posada, D. & Crandall, K.A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, 14, 817–818. https://doi.org/10.1093/bioinformatics/14.9.817

Pu, D.Q., Liu, H.L., Gong, Y.Y., Ji, P.C., Li, Y.J., Mou, F.S. & Wei, S.J. (2017) Mitochondrial genomes of the hoverflies *Episyrphus balteatus* and *Eupeodes corollae* (Diptera: Syrphidae), with a phylogenetic analysis of Muscomorpha. *Scientific Reports*, 7, 10. https://doi.org/10.1038/srep44300

Roháček, J. & Tóthová, A. (2014) Morphology versus DNA - what will bring clarity to the relationships of phylogenetically unclear genera of Anthomyzidae (Diptera)? *Arthropod Systematics & Phylogeny*, 72, 165–176.

Rose, S. (2005) *The Future of the Brain: The Promise and Perils of Tomorrow's Neuroscience.* Oxford University Press, 352 pp.

Rotheray, G. & Lyszkowski, R. (2015) Diverse mechanisms of feeding and movement in Cyclorrhaphan larvae (Diptera). *Journal of Natural History*, 49, 2139–2211. https://doi.org/10.1080/00222933.2015.1010314

Saether, O.A. (1977) Female genitalia in Chironomidae and other Nematocera: morphology, phylogenies, keys. *Bulletin of the Fisheries Research Board of Canada*, 197, 1–209.

Saether, O.A. (2000) Phylogeny of the Culicomorpha (Diptera). *Systematic Entomology*, 25, 223–234. https://doi.org/10.1046/j.1365-3113.2000.00101.x

Sarvašová, A., Kočišová, A., Candolfi, E. & Mathieu, B. (2017) Description of *Culicoides* (*Culicoides*) *bysta* n. sp., a new member of the Pulicaris group (Diptera: Ceratopogonidae) from Slovakia. *Parasites & Vectors*, 10, 279. https://doi.org/10.1186/s13071-017-2195-4

Schlee, D. (1969) Hennig's principle of phylogenetic systematics, an "Intuitive, Statistico-phenetic taxonomy"? *Systematic Zoology*, 18, 127–134. https://doi.org/10.2307/2412420

Schlee, D. (1976) Structures and functions, their general significance for phylogenetic reconstruction in recent and fossil taxa. *Zoologica scripta*, 5, 181–184. https://doi.org/10.1111/j.1463-6409.1976.tb00697.x

Schlee, D. (1978) Anmerkungen zu phylogenetischen Systematik: Stellungnahme zu einigen Mißverständnissen. *Stuttgarter Beiträge zur Naturkunde. Serie A (Biologie)*, 320, 1–14.

Schneeberg, K. & Beutel, R.G. (2011) The adult head structures of Tipulomorpha (Diptera, Insecta) and their phylogenetic implications. *Acta Zoologica*, 92, 316–343. https://doi.org/10.1111/j.1463-6395.2010.00463.x

Schneeberg, K. & Beutel, R.G. (2014) The evolution of head structures in lower Diptera. *Science Open Research*. [published online] https://doi.org/10.14293/S2199-1006.1.SOR-LIFE.ALTCE1.v2

Schneeberg, K., Krause, K. & Beutel, R.G. (2013) The adult head of *Axymyia furcata* (Insecta: Diptera: Axymyiidae). *Arthropod Systematics & Phylogeny*, 71, 91–102.

Semelbauer, M. (2016) Molecular phylogeny of lauxaniid flies (Diptera, Cyclorrhapha) confirms non-monophyly of *Sapromyza* Fallén 1810. *Insect Systematics & Evolution*, 47, 389. https://doi.org/10.1163/1876312X-47032148

Senatore, G.L., Alexander, E.A., Adler, P.H. & Moulton, J.K. (2014) Molecular systematics of the *Simulium jenningsi* species group (Diptera: Simuliidae), with three new fast-evolving nuclear genes for phylogenetic inference. *Molecular Phylogenetics and Evolution*, 75, 138–148.

https://doi.org/10.1016/j.ympev.2014.02.018
Ševčík, J., Kasprák, D., Mantič, M., Fitzgerald, S., Ševčíková, T., Tóthová A. & Jaschhof, M. (2016) Molecular phylogeny of the megadiverse insect infraorder Bibionomorpha sensu lato (Diptera). *PeerJ*, 4, e2563.
https://doi.org/0.7717/peerj.2563
Sharkey, M.J. (1989) A hypothesis-independent method of characters weighting for cladistic analysis. *Cladistics*, 5, 63–86.
https://doi.org/10.1111/j.1096-0031.1989.tb00483.x
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.
https://doi.org/10.1111/j.1365-3113.1992.tb00335.x
Sinclair, B.J. (2013) Rediscovered at last: a new enigmatic genus of Axymyiidae (Diptera) from western North America. *Zootaxa*, 3682 (1), 143–150.
https://doi.org/10.11646/zootaxa.3682.1.7
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.
https://doi.org/10.1163/187631293X00190
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.
https://doi.org/10.1111/j.1096-3642.2007.00314.x
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.
https://doi.org/10.1006/mpev.2000.0787
Sokal, R.R. & Rohlf, F.J. (1970) The *intelligent ignoramus*, an experiment in numerical taxonomy. *Taxon*, 19, 305–319.
https://doi.org/10.2307/1219053
Sperling, F.A.H & Roe, A.D. (2009) Chapter 16. Molecular dimensions of insect taxonomy. *In*: Foottit, R. & Adler, P. (Eds.), *Insect Biodiversity: Science and Society*, 1st edition. Blackwell Publishing, pp. 397–415.
https://doi.org/10.1002/9781444308211.ch16
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.
https://doi.org/10.14411/eje.2008.003
Stevens, P.F. (2000) On characters and character states: do overlapping and non-overlapping variation, morphology and molecules all yield data of the same value *In*: Scotland, R. & Pennington, R.T. (Eds.), *Homology and systematics. Coding characters for phylogenetic analysis.* Taylor & Francis, London & New York, pp. 81–105.
https://doi.org/10.1111/j.1096-0031.2008.00222
Su, K.F.Y., Kutty, S.N. & Meier, R. (2008) Morphology versus molecules: the phylogenetic relationships of Sepsidae (Diptera: Cyclorrhapha) based on morphology and DNA sequence data from ten genes. *Cladistics*, 24, 902–916.
https://doi.org/10.1111/j.1096-0031.2008.00222
Sum, J.-S., Lee, W.-C., Amir, A., Braima, K.A., Jeffery, J., Abdul-Aziz, N.M., Fong, M.-Y. & Lau, Y.-L. (2014) Phylogenetic study of six species of *Anopheles* mosquitoes in Peninsular Malaysia based on inter-transcribed spacer region 2 (ITS2) of ribosomal DNA. *Parasites & Vectors*, 7, 309.
https://doi.org/10.1186/1756-3305-7-309
Tachi, T. (2013) Molecular phylogeny and host use evolution of the genus *Exorista* Meigen (Diptera: Tachinidae). *Molecular Phylogenetics and Evolution*, 66, 401–411.
https://doi.org/10.1016/j.ympev.2012.10.017
Tachi, T. (2014) Homology of the metapleuron of Cyclorrhapha, with discussion of the paraphyly of Syrphoidea (Diptera: Aschiza). *Insect Systematics & Evolution*, 45, 395–414.
https://doi.org/10.1163/1876312X-45012112
Talavera, S., Muñoz-Muñoz, F., Verdún, M. & Pagés, N. (2017) Morphology and DNA barcoding reveal three species in one: description of *Culicoides cryptipulicaris* sp. nov. and *Culicoides quasipulicaris* sp. nov. in the subgenus *Culicoides*. *Medical And Veterinary Entomology*, 3, 178–191.
https://doi.org/10.1111/mve.12228
Tay, W.T., Kerr, P.J. & Jermiin, L.S. (2016) Population genetic structure and potential incursion pathways of the Bluetongue Virus vector *Culicoides brevitarsis* (Diptera: Ceratopogonidae) in Australia. *PLoS ONE*, 11 (1), e0146699.
https://doi.org/10.1371/journal.pone.0146699
Tkoč, M., Tóthová, A., Ståhls, G., Chandler, P.J. & Vaňhara, J. (2016) Molecular phylogeny of flat-footed flies (Diptera: Platypezidae): main clades supported by new morphological evidence. *Zoologica Scripta*, 46, 429–444.
https://doi.org/10.1111/zsc.12222
Tóthová, A., Rozkošný, R., Knutson, L., Kutty, S.N., Wiegmann, B.M. & Meier, R. (2013) A phylogenetic analysis of Sciomyzidae (Diptera) and some related genera. *Cladistics*, 29, 404–415.
https://doi.org/10.1111/cla.12002

Trautwein, M.D., Wiegmann, B.M., Beutel, R., Kjer, K.M. & Yeates, D.K. (2017) Advances in insect phylogeny at the dawn of the postgenomic era. *Annual Review of Entomology*, 57, 449–68.
https://doi.org/10.1146/annurev-ento-120710-100538

Vicoso, B. & Bachtrog, D. (2015) Numerous Transitions of Sex Chromosomes in Diptera. *PLoS Biol*, 13 (4), e1002078.
https://doi.org/10.1371/journal. pbio.1002078

Wagner, G.P. (2014) *Homology, genes and evolutionary innovation*. Princeton University Press, Princeton, 478 pp.

Wagner, R. & Stuckenberg, B. (2016) Cladistic analysis of subfamily Bruchomyiinae (Diptera: Psychodidae). *Zootaxa*, 4092, 151–174.
https://doi.org/10.1515/9781400851461

Wang, G., Li, C., Guo, X., Xing, D., Dong, Y. & Zhao, T. (2014) Molecular phylogenetic analysis of the subgenera *Anopheles* and *Cellia* (Diptera: Culicidae) based on nuclear ribosomal sequences. *African Entomology*, 22, 660–669.
https://doi.org/10.4001/003.022.0323

Wang, K., Li, X., Ding, S., Wang, N., Mao, M., Wang, M. & Yang, D. (2016) The complete mitochondrial genome of the *Atylotus* miser (Diptera: Tabanomorpha: Tabanidae), with mitochondrial genome phylogeny of lower Brachycera (Orthorrhapha). *Gene*, 586, 84–196.
https://doi.org/10.1016/j.gene.2016.04.013

Watts, M., Winkler, I.S., Daugeron, C., de Carvalho, C.J.B., Turner, S.P. & Wiegmann, B.M. (2016) Where do the Neotropical Empidini lineages (Diptera: Empididae: Empidinae) fit in a worldwide context? *Molecular Phylogenetics and Evolution*, 95, 67.
https://doi.org/10.1016/j.ympev.2015.10.019

Wheeler, Q.D. (1986) Character weighting and cladistic analysis. *Systematic Biology*, 35, 102–109.
https://doi.org/org/10.1093/sysbio/35.1.102

Wheeler, Q.D. (2007) Invertebrate systematics or spineless taxonomy? *Zootaxa*, 1668, 11 –18.

Wheeler, Q.D. (2008a) Ch. 1 Introductory. Toward the new taxonomy. *In:* Wheeler, Q.D. (Ed.), *The new taxonomy. The systematics association special volumes series 76*. CRC Press, Boca Raton, pp. 1–17.

Wheeler, Q.D. (2008b) Undisciplined thinking: morphology and Hennig's unfinished revolution. *Systematic Entomology*, 32, 2–7.
https://doi.org/10.1111/j.1365-3113.2007.00411.x

Wheeler, Q.D. (2009) Chapter 14. The science of insect taxonomy: prospects and needs. *In*: Foottit, R.G. & Adler, P.H. (Eds.), *Insect Biodiversity: Science and Society.* Blackwell Publishing Ltd., pp. 359–380.
https://doi.org/10.1002/9781444308211.ch14

Wiegmann, B.M., Trautwein, M.D., Winkler, I.S., Barr, N.B., Kim, J.-W., Lambkin, C., Bertone, M.A., Cassel, B.K., Bayless, K.M., Heimberge, A.M., Wheeler, B.M., Peterson, K.J., Pape, T., Sinclair, B.J., Skevington, J.H., Blagoderov, V., Caravas, J., Kutty, S.N., Schmidt-Ott, U., Kampmeier, G.E., Thompson, F.C., Grimaldi, D.A., Beckenbach, A.T., Courtney, G.W., Friedrich, M., Meier, R. & Yeates, D.K. (2011) Episodic radiations in the fly tree of life. *Proceedings of the National Academy of Sciences*, 108, 5690–5695.
https://doi.org/10.1073/pnas.1012675108

Williams, D.M. & Ebach, M.C. (2010) Molecular systematics and the 'blender of optimization': is there a crisis in systematics? *Systematics and Biodiversity*, 8, 481–484.
https://doi.org/10.1080/14772000.2010.530303

Williams, D.M., Ebach, M.C. & Wheeler, Q.D. (2010). Beyond belief, the steady resurrection of phenetics. *In:* Williams, D.M. & Knapp, S. (Eds.), *Beyond cladistics: the branching of a paradigm*. University of California Press, Berkeley/Los Angeles/London, pp. 169–195.
https://doi.org/10.1525/california/9780520267725.003.0010

Williams, K.A., Lamb, J. & Villet, M.H. (2016) Phylogenetic radiation of the greenbottle flies (Diptera, Calliphoridae, Luciliinae). *Zookeys*, 568, 59–86.
https://doi.org/10.3897/zookeys.568.6696

Winkler, I.S., Blaschke, J.D., Davis, D.J., Stireman, J.O., O'Hara, J.E., Cerretti, P. & Moulton, J.K. (2015) Explosive radiation or uninformative genes? Origin and early diversification of tachinid flies (Diptera: Tachinidae). *Molecular Phylogenetics and Evolution*, 88, 38–54.
https://doi.org/10.1016/j.ympev.2015.03.021

Winterton, S.L. & Ware, J.L. (2015) Phylogeny, divergence times and biogeography of window flies (Scenopinidae) and the therevoid clade (Diptera: Asiloidea). *Systematic Entomology*, 40, 491–519.
https://doi.org/10.1111/syen.12117

Winterton, S.L., Hardy, N.B., Gaimari, S.D., Hauser, M., Hill, H.N., Holston, K.C., Irwin, M.E., Lambkin, C.L., Metz, M.A., Turco, F., Webb, D., Yang, L., Yeates, D.K. & Wiegmann, B.M. (2016) The phylogeny of stiletto flies (Diptera: Therevidae). *Systematic Entomology*, 41, 144–161.
https://doi.org/10.1111/syen.12147

Wipfler B., Courtney, G.W., Craig, D.A. & Beutel, R.G. (2012) First l-CT-based 3D reconstruction of a dipteran larva—the head morphology of *Protanyderus* (Tanyderidae) and its phylogenetic implications. *Journal of Morphology*, 273, 968–980.
https://doi.org/10.1002/jmor.20035

Wood, D.M. & Borkent, A. (1989) Phylogeny and classification of the Nematocera. *In:* McAlpine, J.F. & Wood, D.M.

(Coords.), Manual of Nearctic Diptera. Vol. 3. *Agriculture Canada Monograph*, 32, pp. 1333–1370.
Woodley, N.E. (1989) Phylogeny and classification of the "orthorrhaphous" Brachycera. *In:* McAlpine, J.F. & Wood, D.M. (Coords.), Manual of Nearctic Diptera. Vol. 3. *Agriculture Canada Monograph*, 32, pp. 1371–1395.
Woodley, N.E., Borkent, A & Wheeler, T.A. (2009) 5. Phylogeny. *In:* Brown, B.V., Borkent, A., Cumming, J.M., Wood, D.M., Woodley, N.E. & Zumbado, M.A. (Eds.), *Manual of Central American Diptera. Vol. 1*. NRC Research Press, Ottawa, pp. 79–94.
Yassin, A. (2013) Phylogenetic classification of the Drosophilidae Rondani (Diptera): the role of morphology in the postgenomic era. *Systematic Entomology*, 38, 349–364.
https://doi.org/10.1111/j.1365-3113.2012.00665.x
Yeates, D.K. (2002) Relationships of the lower Brachycera (Diptera): a quantitative synthesis of morphological characters. *Zoologica Scripta*, 31, 105–121.
https://doi.org/10.1046/j.0300-3256.2001.00077.x
Yeates, D.K. & Wiegmann, B.M. (Eds.) (2005) *The evolutionary history of flies.* Columbia University Press, New York, Chichester, West Sussex, ix + 430 pp.
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. *Zootaxa*, 1668, 565–590.
Yeates, D.K., Meusemann, K., Trautwein, M., Wiegmann, B. & Zwick, A. (2016) Power, resolution and bias: recent advances in insect phylogeny driven by the genomic revolution. *Current Opinion in Insect Science*, 13, 16–23.
https://doi.org/10.1016/j.cois.2015.10.007
Young, A.D., Lemmon, A.R., Skevington, J.H., Mengual, X., Ståhls, G., Reemer, M., Jordaens, K., Kelso, S., Lemmon, E.M., Hauser, M., De Meyer, M., Misof, B. & Wiegmann, B.M. (2016a) Anchored enrichment dataset for true flies (order Diptera) reveals insights into the phylogeny of flower flies (family Syrphidae). *BMC Evolutionary Biology*, 16, 143. https://doi.org/10.1186/s12862-016-0714-0
Young, A.D., Marshall, S.A. & Skevington, J.H. (2016b) Revision of *Platycheirus* Lepeletier and Serville (Diptera: Syrphidae) in the Nearctic north of Mexico. *Zootaxa*, 4082 (1), 1–317.
https://doi.org/10.11646/zootaxa.4082.1.1
Zhang, X., Kang, Z., Mao, M., Li, X., Cameron, S.L., de Jong, H., Wang, M. & Yang, D. (2016) Comparative Mt genomics of the Tipuloidea (Diptera: Nematocera: Tipulomorpha) and its implications for the phylogeny of the Tipulomorpha. *PLoS ONE*, 11 (6), e0158167.
https://doi.org/10.1371/journal.pone.0158167