

Protein-encoding ultraconserved elements provide a new phylogenomic perspective of Oestroidea flies (Diptera: Calyptratae)

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Abstract. The diverse superfamily Oestroidea with more than 15 000 known species includes among others blow flies, flesh flies, bot flies and the diverse tachinid flies. Oestroidea exhibit strikingly divergent morphological and ecological traits, but even with a variety of data sources and inferences there is no consensus on the relationships among major Oestroidea lineages. Phylogenomic inferences derived from targeted enrichment of ultraconserved elements or UCEs have emerged as a promising method for resolving difficult phylogenetic problems at varying timescales. To reconstruct phylogenetic relationships among families of Oestroidea, we obtained UCE loci exclusively derived from the transcribed portion of the genome, making them suitable for larger and more integrative phylogenomic studies using other genomic and transcriptomic resources. We analysed datasets containing 37–2077 UCE loci from 98 representatives of all oestroid families (except Ulurumyiidae and Mystacinobiidae) and seven calyptrate outgroups, with a total concatenated aligned length between 10 and 550 Mb. About 35% of the sampled taxa consisted of museum specimens (2–92 years old), of which 85% resulted in successful UCE enrichment. Our maximum likelihood and coalescent-based analyses produced well-resolved and highly supported topologies. With the exception of Calliphoridae and Oestridae all included families were recovered as monophyletic with the following conclusions: Oestroidea is monophyletic with Mesembrinellidae as sister to the remaining oestroid families; Oestridae is paraphyletic with respect to Sarcophagidae; Polleniidae is sister to Tachinidae; Rhinophoridae sister to (Luciliinae (Toxotarsinae (Melanomyiinae + Calliphorinae))); Phumosiinae is sister to Chrysomyinae and Bengaliinae is sister to Rhiniidae. These results support the ranking of most calliphorid subfamilies as separate families.

Introduction

Flies have had immense lasting effects on the earth and human civilization. They have been more than an annoying buzzing in the Earth's history, as they have colonized almost

all terrestrial ecosystems, living on dead or live tissues, spreading diseases and even become key plant pollinators. Of all insects, flies of the superfamily Oestroidea constitute dominant, explosive radiation of the order Diptera, including more than 15 000 species, which have greatly influenced Life on Earth. This superfamily includes well-known flies like blow flies (Calliphoridae), mesembrinellids (Mesembrinellidae), the New Zealand bat fly (Mystacinobiidae), bot flies (Oestridae), cluster flies (Polleniidae), nose flies (Rhiniidae), louse flies

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(Rhinophoridae), flesh flies (Sarcophagidae), tachinid flies (Tachinidae) and the enigmatic McAlpine's fly (Ulurumiidae) (Fig. 1). In urban environments, blow flies and flesh flies are mechanical vectors of important diseases, as they feed on garbage and corpses of animals and humans (Buenaventura *et al.*, 2009; Byrd & Castner, 2010). Whereas these flies are critical elements for public health, they are also useful as indicators of time and place of death in forensic investigations (Wells *et al.*, 2001; Mulieri *et al.*, 2012; Meiklejohn *et al.*, 2013; Vairo *et al.*, 2017). Carrion flies provide ecosystem services, such as nutrient recycling and pollination, which are essential for the sustainability and management of urban and wild ecosystems. In addition, some Oestroidea lineages develop in a close association with a broad range of hosts as parasitoids or kleptoparasitoids (Piwczyński *et al.*, 2017), which are hypothesized to have evolved independently across lineages. Polleniids develop on earthworms, ameniines and melanomyines (Calliphoridae) and some sarcophagids on terrestrial gastropods, rhinophorids on woodlice, tachinids on terrestrial arthropods (mostly insects) and miltogrammines (Sarcophagidae) as kleptoparasitoids on soil-nesting wasps, bees and ants (Eggleton & Belshaw, 1992; Feener Jr. & Brown, 1997). Tachinidae includes mostly parasitoids playing significant roles in regulating their host populations, and hence being relevant for biological control programmes (Stireman *et al.*, 2006). During the last decade, several studies have explored the phylogenetic relationships of Oestroidea (Marinho *et al.*, 2012; Singh & Wells, 2013; Zhang *et al.*, 2016a; Cerretti *et al.*, 2017; Michelsen & Pape, 2017; Buenaventura *et al.*, 2019; Cerretti *et al.*, 2019; Kutty *et al.*, 2019); however, our knowledge about their evolution and diversity patterns remains limited, especially with regard to Calliphoridae. The most limiting factor has been the identification and screening of orthologous loci across an evolutionarily distant set of taxa, which when compared in phylogenetic analyses, recover poorly supported relationships of the important major lineages. In addition, studies using molecular (Kutty *et al.*, 2010; Marinho *et al.*, 2012) or morphological data (Pape, 1992; Rognes, 1997; Cerretti *et al.*, 2017) have suffered from equivocal phylogenetic results due to limited taxon sampling, limited data or both.

Massively parallel DNA sequencing technologies are used to generate vast amounts of molecular data (Metzker, 2010; Shendure *et al.*, 2017). These technologies, also known as high-throughput sequencing or next-generation sequencing, outperform Sanger sequencing in efficiency to recover genomic data (Blaimer *et al.*, 2015; Matos-Maraví *et al.*, 2019) at a cost that is becoming more accessible. Approaches such as targeted enrichment (e.g. anchored hybrid enrichment, ultraconserved elements), random reduced-representation of the genome (e.g. transcriptomics, RAD-seq, MIG-seq) and whole-genome sequencing are being used to understand biological diversification in time and space.

Targeted enrichment has gained popularity in phylogenetic studies because it can recover DNA loci with a particular rate of evolution (fast and slow) or under different selective pressures (Lemmon & Lemmon, 2013). Targeted enrichment can also produce large amounts of molecular data from sub-optimally

preserved samples and/or highly fragmented DNA, such as those coming from museum specimens (McCormack *et al.*, 2016; Blaimer *et al.*, 2016b) whereas the use of transcriptomics and RAD-seq is generally limited to fresh or properly preserved tissue (e.g. tissues stored in liquid nitrogen or RNAlater). Given the practical limitations of collecting rare species, or species whose habitats are inaccessible or have completely disappeared, museum collections present an invaluable source of biological tissues for targeted-enrichment molecular studies. However, DNA derived from museum tissues and legacy preserved DNA aliquots from previous studies, is often highly degraded and low in concentration. DNA aliquots may face the same constraints of using suboptimally preserved samples like museum specimens. To date, only a few studies using anchored hybrid enrichment (AHE) or ultraconserved elements (UCE) have capitalized on museum specimens, but not on legacy DNA aliquots (Faircloth *et al.*, 2013; McCormack *et al.*, 2016; Blaimer *et al.*, 2016b; Baca *et al.*, 2017; Wood *et al.*, 2018; Derkarabetian *et al.*, 2019), although with few exceptions (Buenaventura *et al.*, 2019).

Ultraconserved elements and AHE methods target ultraconserved or highly conserved genome regions flanked by regions of greater nucleotide variability, which make them useful to reconstruct phylogenies at wide evolutionary scales (McCormack *et al.*, 2013b; da Fonseca *et al.*, 2016). Differences between these two methods seem to be related to targeting highly conserved noncoding and coding regions of the genome with UCE (Faircloth *et al.*, 2012) or coding regions with AHE (Lemmon *et al.*, 2012). However, studies on arthropods reported approximately 61% or more of the UCE loci overlapping with some coding genomic regions (Branstetter *et al.*, 2017; Hedin *et al.*, 2019; Kieran *et al.*, 2019). Even though invertebrate UCes have been characterized as predominantly coding sequences (Bossert & Danforth, 2018), UCE probes have usually been designed without much consideration on whether they capture loci from the transcribed portion of the genome and without refining them using available transcriptome resources. Thus, protein-coding portions are generally discovered during UCE data processing, but not at the probe design stage. Previous probe designs did not take into consideration the development of probes targeting loci only found within coding regions (McCormack *et al.*, 2012; McCormack *et al.*, 2013a; Smith *et al.*, 2014; Crawford *et al.*, 2015; Baca *et al.*, 2017; Branstetter *et al.*, 2017; Faircloth, 2017; Wood *et al.*, 2018; Oliveros *et al.*, 2019).

The design of taxon-specific UCE probes is a growing field, which is rapidly developing a body of knowledge (Branstetter *et al.*, 2017; Gustafson *et al.*, 2019a; Gustafson *et al.*, 2019b; Kulkarni *et al.*, 2020). Recent literature justified the use of tailored UCE probes as they frequently outperform general (i.e. universal) probes and should aid in locus recovery (Branstetter *et al.*, 2017; Gustafson *et al.*, 2019a; Gustafson *et al.*, 2019b; Kulkarni *et al.*, 2020). Previous molecular studies on Oestroidea (Kutty *et al.*, 2010; Buenaventura *et al.*, 2017; Buenaventura & Pape, 2017a; Buenaventura *et al.*, 2019) and similar radiations predict that reconstructing a strong phylogeny would require a large increase in molecular data (Dell'Ampio *et al.*, 2014;



Fig 1. Representative taxa of Oestroidea. (A) *Pachychoeromyia praegrands* Austen, Bengaliinae, Calliphoridae. (B) *Calliphora croceipalpis* Jaen-nicke, Calliphorinae, Calliphoridae. (C) *Comptosia verena* Walker, Chrysomyinae, Calliphoridae. (D) *Phormia regina* (Meigen), Chrysomyinae, Calliphoridae. (E) *Blepharicnema splendens* (Macquart), Luciliinae, Calliphoridae. (F) *Opsodexia bicolor* (Coquillett), Melanomyiinae, Calliphori-dae. (G) *Phumosa viridis* Kurahashi, Phumosiinae, Calliphoridae. (H) *Sarconesia chlorogaster* (Wiedemann), Toxotarsinae, Calliphoridae. (I) *Sar-conesiopsis magellanica* (Le Guillou), Toxotarsinae, Calliphoridae. (J) *Mesembrinella* sp., Mesembrinellidae. (K) *Cephemyia jellisoni* Townsend, Oestrinae, Oestridae. (L) *Pollenia pediculata* Macquart, Polleniidae. (M) *Rhinia apicalis* (Wiedemann), Rhiniinae, Rhiniidae. (N) *Rhinomyia cassotis* (Walker), Cosminiinae, Rhiniidae. (O) *Rhinomorinia* sp., Rhinophoridae. (P) *Metopia argyrocephala* (Meigen), Miltogramminae, Sarcophagidae. (Q) *Wohlfahrtia* sp., Paramacronychiinae, Sarcophagidae. (R) *Oxsarcodexia bakeri* (Aldrich), Sarcophaginae, Sarcophagidae. (S) *Lepidodexia aragua* (Dodge), Sarcophaginae, Sarcophagidae. (T) *Microchaetogyne* sp., Dexiinae, Tachinidae. (U) *Blondelia eufitchiae* (Townsend), Exoristinae, Tachinidae. (V) *Xanthomelanodes* sp., Phasiinae, Tachinidae. (W) *Cholomyia inaequipes* Bigot, Myiophasiini, Tachinidae, Tachinidae. (X) *Ruizella* sp., Tachin-inae, Tachinidae. Sex and body length are given for each specimen. Photos (A, B, M, N) by AT; (C–F, H–L, O–Q, T–X) by JMPL; (G) by Natural History Museum (UK); (R, S) by EB. [Colour figure can be viewed at wileyonlinelibrary.com].

Giarla & Esselstyn, 2015) and the combination of information from multiple genome regions to attempt to produce accurate species tree estimates (Degnan & Rosenberg, 2009). A unique tailored UCE probe set for Calypttratae and Oestroidea flies could increase locus recovery, whereas a generalized UCE probe set designed for the entire order like the Diptera-wide UCE probe set (Faircloth, 2017) would capture a smaller and perhaps an insufficient number of loci to reconstruct this rapid radiation. Comparative analyses on loci recovery between generalized and taxon-specific UCE probes for Diptera lineages should be considered in future studies.

Possibly due to the availability of the probe sets (Faircloth, 2017), lab protocols (see www.ultraconserved.org) and bioinformatics tools such as PHYLUCE (Faircloth, 2016), phylogenetic reconstructions using a UCE approach are becoming more frequent. It has been used to resolve the phylogeny of various vertebrate groups (Crawford *et al.*, 2012; McCormack *et al.*, 2012; Faircloth *et al.*, 2013; McCormack *et al.*, 2013a; Sun *et al.*, 2014; Crawford *et al.*, 2015) and arthropod taxa (Blaimer *et al.*, 2015; Faircloth *et al.*, 2015; Starrett *et al.*, 2016; Blaimer *et al.*, 2016a; Blaimer *et al.*, 2016b; Baca *et al.*, 2017; Branstetter *et al.*, 2017; Wood *et al.*, 2018; Forthman *et al.*, 2019; Kieran *et al.*, 2019; Gustafson *et al.*, 2019b). The use of UCEs to resolve relationships within dipterans has not been explored, and as flies are one of the four super-radiations of insects (along with beetles, wasps and moths) that account for the majority of animal life on Earth (Wiegmann *et al.*, 2011), they constitute an interesting taxon to be studied with the combined use of the UCE targeted enrichment method and massively parallel DNA sequencing technologies. Thus, we aimed to design a taxon-specific probe set to capture only protein-encoding UCEs to reconstruct the phylogenetic relationships of one of the largest biotic radiations on Earth: the Oestroidea flies.

Material and methods

UCE probe design

Our UCE probe design followed the standard UCE workflow (Faircloth *et al.*, 2012) to identify ultraconserved genomic regions and design enrichment probes targeting those regions. This workflow performs synteny-based genome-genome alignment to identify UCEs. Details of the workflow are found in Faircloth *et al.* (2012) and step-by-step procedures are also available online (<https://phyluce.readthedocs.io/en/latest/tutorial-four.html>) and unless otherwise noted scripts used below are contained within the PHYLUCE package (Faircloth *et al.*, 2012; Faircloth, 2016). Three FASTA format genome assemblies of *Glossina fuscipes* Newstead (Glossinidae, Hippoboscoidea) (GCA_000671735.1), *Lucilia cuprina* (Wiedemann) (Calliphoridae, Oestroidea) (GCA_000699065.2) and *Musca domestica* Linnaeus (Muscidae, Muscoidea) (GCA_000371365.1) were downloaded from GenBank and converted to 2bit format. The FASTA format genome and transcriptome assemblies of *Sarcophaga*

Table 1. Total number of merged, putatively conserved regions in each exemplar taxon that are shared with the base genome of *Sarcophaga (Liopygia) crassipalpis* Macquart after repetitive regions were removed

Family	Species	Genetic resource	# Shared regions
Glossinidae	<i>Glossina fuscipes</i>	Genome	107 624
Muscidae	<i>Musca domestica</i>	Genome	164 425
Calliphoridae	<i>Lucilia cuprina</i>	Genome	273 283
Sarcophagidae	<i>Sarcophaga crassipalpis</i>	Transcriptome	163 442

(*Liopygia*) *crassipalpis* Macquart (Sarcophagidae, Oestroidea), which were sequenced in a parallel project (Buenaventura *et al.* unpublished data), were also converted to 2bit format and included. We simulated reads (without sequencing error) from the genomes and the transcriptome, and aligned them to the base genome (= *S. crassipalpis*) using *art* (Huang *et al.*, 2012) with the built-in feature off. This genome assembly was used as the base genome due to our interest in having specific probes for Sarcophagidae, and also because of the contiguity, assembly quality and level of annotation of this genome (Buenaventura *et al.* unpublished data). We then aligned simulated reads to the base genome using *stampy* (Lunter & Goodson, 2011), which is efficient in aligning sequences to a divergent reference sequence. The resulting output was then converted to BAM format. Thus, by mapping simulated sequence data from exemplar genomes to the base genome sequence, we identified putatively orthologous loci shared between the exemplar taxa and the base taxon. These conserved regions had a sequence divergence of <5%. We then converted BAM files to BED format, order each line in the BED file by chromosome/scaffold/contig and merge together putative regions of conservation that are proximate using *bedtools* (Quinlan, 2014). This large number of conserved regions were filtered to remove repetitive regions using the script 'phyluce_probe_strip_masked_loci_from_set' (Faircloth *et al.*, 2012). The total number of merged, putatively conserved regions in each exemplar taxon that was shared with the base genome of *S. crassipalpis* after repetitive regions were removed, are found in Table 1. We then used the script 'phyluce_probe_get_multi_merge_table' to put the identified conserved loci across taxa into an SQLite database. The database was then queried to identify how many loci were shared between taxa using 'phyluce_probe_query_multi_merge_table'. We selected the total loci shared between our base taxon (genome of *S. crassipalpis*) and all other exemplar taxa (25 166 loci) to be sure these loci are found in most/all of our taxa to be enriched (Table 2).

The validation of conserved loci started with extracting FASTA sequences from the base genome using the script 'phyluce_probe_get_genome_sequences_from_bed'. We then designed a temporary probe set for the selected loci (25 166 loci) from the base taxon using the script 'phyluce_probe_get_tiled_probes', selecting two probes per locus with 3× tiling that overlap the middle of the targeted locus and removing potentially problematic probes with >25% repeat content and GC content outside of the range of 30–70%

Table 2. Number of loci shared between base taxon (genome of *Sarcophaga (Liopygia) crassipalpis* Macquart) and exemplar taxa

Shared between # of taxa	Loci shared between taxa count
Loci shared by base +1 taxa	494 396
Loci shared by base +2 taxa	150 697
Loci shared by base +3 taxa	67 767
Loci shared by base +4 taxa ^a	25 166

^a Design that was chosen for this probe set, 25166 loci shared between all taxa.

Table 3. Number of UCE loci shared between taxa

Shared between # of taxa	Loci shared between taxa count
Loci shared by 1 taxa	19 746
Loci shared by 2 taxa	19 106
Loci shared by 3 taxa	18 113
Loci shared by 4 taxa	15 705
Loci shared by 5 taxa ^a	9562
Loci shared by 6 taxa	1509

^a Design that was chosen for this probe set, 9562 loci shared between all taxa.

(30% > GC > 70%). We aligned the probes to themselves using the script 'phyluce_probe_easy_lastz', removed duplicates from the temporary probe set using the script 'phyluce_probe_remove_duplicate_hits_from_probes_using_lastz' and kept 25 004 loci and 38 755 probes. At this point, the genome assembly of *Drosophila melanogaster* Meigen (GCA_000001215.4) in FASTA and 2bit format, was used to represent an outgroup for Calyptratae and Oestroidea and to bridge the divergence between outgroups and ingroups. Then, we aligned the temporary probe set to each genome and the transcriptome using the script 'phyluce_probe_run_multiple_lastzs_sqlite' with 50% of the minimum sequence identity to accept a match. We extracted FASTA data from each of the exemplar sequences using the loci alignments created in the previous step and buffering each locus to 180 bp with the script 'phyluce_probe_slice_sequence_from_genomes' and used the script 'phyluce_probe_get_multi_fasta_table' to find, which loci we detect consistently (Table 2). Then, following a conservative approach, we only extracted 9562 conserved loci consistently detected in five out of six genetic resources (Table 3) used here (i.e. genomes of *G. fuscipes*, *L. cuprina*, *M. domestica*, *D. melanogaster* and the genome and transcriptome of *S. crassipalpis*) to design our temporary probes using the script 'phyluce_probe_query_multi_fasta_table'.

The design of the final probe set used all genetic resources and not only the base genome, which ensures a heterogeneous probe mix containing probes designed from each exemplar but targeting the same locus, which should produce a more universal probe set. Similar to the temporary probe set, we designed our final probe set for the selected loci (9562 loci) from all genetic resources using the script

'phyluce_probe_get_tiled_probe_from_multiple_inputs'. Thus, we selected two probes per locus with 3× tiling that overlap the middle of the targeted locus and removed potentially problematic probes with >25% repeat content and GC content outside of the range of 30–70%. We then aligned the probes to themselves using the script 'phyluce_probe_easy_lastz', removed duplicates from the temporary probe set using the script 'phyluce_probe_remove_duplicate_hits_from_probes_using_lastz' and kept 9562 shared loci and 95 231 probes. The number of total conserved loci or UCEs detected in each exemplar taxon were 9479 for *L. cuprina*, 9459 for *M. domestica*, 9323 for *G. fuscipes*, 9270 for the genome of *S. crassipalpis*, 9138 for *D. melanogaster* and 2650 for the transcriptome of *S. crassipalpis*, for a total of total 9562 shared loci.

The final probe set was parsed to produce the master probe set, which included only those probes matching the transcriptome of *S. crassipalpis*. Thus, our design of UCE probes used available transcriptome resources, produced in a parallel project (Buenaventura *et al.* unpublished data) as a strategy to refine our probe set and capture only protein-coding regions. This design resulted in 5117 probes targeting 2581 conserved loci or UCEs. These probes were titled 'calyps-v1-master-probe-list-SARCTRA' for clarity.

We ran in-silico tests on the master probe set. First, we aligned our master probe set to all genetic resources (i.e. genomes and transcriptome) plus some additional resources (genomes of Calliphoridae, *Calliphora vicina* Robineau-Desvoidy, GCA_001017275.1; *Cochliomyia hominivorax* (Coquerel), GCA_004302925.1; *Lucilia sericata* (Meigen), GCA_001014835.1; *Phormia regina* (Meigen), GCA_001735545.1; genomes of Muscidae, *Haematobia irritans* Linnaeus, GCA_003123925.1; *Stomoxys calcitrans* Linnaeus, GCA_001015335.1; genomes of Sarcophagidae, *Sarcophaga (Neobellieria) bullata* (Parker), GCA_001017455.1) using the script 'phyluce_probe_run_multiple_lastzs_sqlite' and then extracted FASTA data, including a flanking region (–flank 400) on each end for each loci using the script 'phyluce_probe_slice_sequence_from_genomes' and we matched FASTA contigs to probes with the script 'phyluce_assembly_match_contigs_to_probes' and extracted the FASTA data using the script 'phyluce_assembly_get_fastas_from_match_counts'. We aligned the conserved locus data using MAFFT (Katoh & Standley, 2013) through the script 'phyluce_align_seqcap_align'. We trimmed alignments with the script 'phyluce_align_get_gblocks_trimmed_alignments_from_untrimmed', removed the locus names from each alignment with the script 'phyluce_align_remove_locus_name_from_nexus_lines' and produced stats across aligned loci with the script 'phyluce_align_get_align_summary_data'. At this point, a 75% complete matrix with the script 'phyluce_align_get_only_loci_with_min_tax' was used to prepare a RAxML file for phylogenetic analysis with the script 'phyluce_align_format_nexus_files_for_raxml'. Our RAxML maximum likelihood analyses, using our in-silico captured UCEs, found phylogenetic relationships consistent with published phylogenies.

Sampling design and DNA extractions

Our taxa sampling included 98 ingroup species of the families Calliphoridae (22 spp., subfamilies Bengaliinae, Calliphorinae, Chrysomyinae, Luciliinae, Melanomyinae, Phumosiinae, Toxotarsinae), Mesembrinellidae (4 spp.), Oestridae (9 spp., subfamilies Cuterebrinae, Gasterophilinae, Hypodermatinae, Oestrinae), Polleniidae (1 sp.), Rhiniidae (17 spp., subfamilies Cosminiinae, Rhiniinae), Rhinophoridae (7 spp.), Sarcophagidae (27 spp., subfamilies Miltogramminae, Paramacronychiinae, Sarcophaginae), Tachinidae (11 spp., subfamilies Dexiinae, Exoristinae, Phasiinae, Tachininae) and seven outgroups representing the families Anthomyiidae (3 spp.), Fanniidae (3 spp.) and Scathophagidae (1 sp.). Two species were not included; these are the New Zealand bat fly (*Mystacinobia zelandica* Holloway) (Mystacinobiidae) and McAlpine's fly (*Ulurumyia macalpinei* Michelsen and Pape) (Ulurumyiidae). All specimens included in this study were collected in accordance with local regulations and all necessary permits were obtained. Voucher specimens have been deposited at the National Museum of Natural History (USNM collection), Museum für Naturkunde (ZMHB) and Wright State University (JOSC) (see Table S1 for specimen identifiers and collection data).

This study used eight existing DNA aliquots extracted during 2011 and 2012 for previous molecular studies (Zhang *et al.*, 2016b; Buenaventura *et al.*, 2017; Buenaventura & Pape, 2017a) and stored in a -20°C freezer. We extracted DNA from 37 pinned specimens from the USNM and JOSC collections, 47 specimens preserved in 96% Ethanol (some of which were stored at below freezing upon completion of the field expedition) and 13 specimens collected and placed directly in empty vials stored in Liquid Nitrogen in the NMNH Biorepository, as indicated in Supplementary Table S1 and Figure S1, where specimen identity, preservation method, targeted tissue for extraction, collection data and corresponding repositories at natural history museums of all specimens is provided. Sterilized forceps helped in manipulating and removing dust, pollen and other forms of accumulated debris on pinned specimens. Tissues from thorax, abdomen or legs were used for DNA extractions as specified in Table S1. DNA was nondestructively extracted from the thorax of pinned specimens, whereas it was destructively extracted from specimens preserved in 96% ethanol and liquid nitrogen by grinding the thoracic or leg tissue with a sterile pestle. We used a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, U.S.A.) and followed the manufacturer's protocol, but to maximize DNA yield the Proteinase K digestion ran for 48 h at 56°C and DNA was eluted in $100\text{ }\mu\text{L}$. To estimate the size of the genomic DNA, $10\text{ }\mu\text{L}$ of each extract were run for 40 min at 100 volts on 1.5% agarose SB (sodium borate) gels.

Library preparation, target enrichment and sequencing of UCEs

We quantified DNA for each sample using a Qubit fluorometer (High sensitivity kit, Life Technologies, Inc.) and sheared $0.5\text{--}81.3\text{ ng}$ (27.9 ng mean) DNA to a target size

of approximately $500\text{--}600\text{ bp}$ by sonication (Q800, Qsonica LLC.), and used this sheared DNA as input for a modified genomic DNA library preparation protocol following Blaimer *et al.* (2016a) and Faircloth *et al.* (2015). Each pool was enriched using our set of 5117 custom-designed probes (Arbor Biosciences, Inc.) targeting 2581 UCE loci in Calyptratae. Our library enrichment followed procedures for the MYcroarray MYBaits kit (Blumenstiel *et al.*, 2010), except we used a $0.1\times$ concentration of the standard MYBaits concentration, and added $0.7\text{ }\mu\text{L}$ of $500\text{ }\mu\text{L}$ custom blocking oligos designed against our custom sequence tags. We used the with-bead approach for PCR recovery of enriched libraries as described in Faircloth *et al.* (2015), with pool hybridization with UCE probes over a 24-h incubation period and 18 cycles in the post capture PCR reaction. Following post enrichment PCR, we purified resulting reactions using $1.0\times$ speedbeads and rehydrated the enriched pools in $22\text{ }\mu\text{L}$ TLE.

Post enrichment library concentration was quantified via qPCR using an SYBR[®] FAST qPCR kit (Kapa Biosystems) on a ViiA[™] 7 (Life Technologies), and based on the size-adjusted concentrations estimated by qPCR, we pooled libraries at equimolar concentrations and size-selected for $250\text{--}800$ with a BluePippin (SageScience) (1.5% agarose, $250\text{ bp} - 1.5\text{ kb}$), and the pool-of-pools was quality checked on an Agilent 2200 TapeStation. The pooled libraries were sequenced using two lanes of a 125-bp paired-end Illumina Hi-Seq 2500 run (University of Utah Genomics Core Facility).

Processing and alignment of UCE data

Illumiprocessor (Faircloth, 2013), based on the package Trimmomatic (Bolger *et al.*, 2014), was used to trim the demultiplexed FASTQ data output for adapter contamination and low-quality bases. All further data processing relied on the PHYLUCE package (Faircloth *et al.*, 2012; Faircloth, 2016) with Python scripts designed by the Smithsonian Institution Bioinformatics Group (available at www.github.com/SmithsonianWorkshops/Targeted_Enrichment/blob/master/phylyce.md). We computed summary statistics on the data and assembled the cleaned reads using Trinity (Grabherr *et al.*, 2011). To identify contigs representing enriched UCE loci from each species, species-specific contig assemblies were aligned to a FASTA file of all enrichment baits ($\text{min_coverage} = 70$, $\text{min_identity} = 80$), and sequence coverage statistics (avg, min, max) for contigs containing UCE loci were calculated. We created FASTA files for each UCE locus containing sequence data for taxa present at that particular locus and aligned these using MAFFT (Katoh & Standley, 2013) ($\text{min-length} = 20$, no-trim). Our alignments were trimmed using Gblocks (Castresana, 2000) with relaxed settings ($b1 = 0.5$, $b2 = 0.5$, $b3 = 12$, $b4 = 7$). We selected a subset of UCE alignments for phylogenetic analyses having varying taxon completeness ($10\text{--}100\%$) for each UCE locus (Table 4). These ten different matrixes were designed to evaluate the relative contribution of varying amounts of UCE loci to the construction of the phylogenetic tree.

Table 4. Datasets and data completeness

Dataset	Dataset completeness	UCE loci	Sequence length	Parsimony-informative sites	Parsimony-informative sites (%)	Global bootstrap support (GBS)
oest_10per-taxa	10%	2077	551 294	184 851	33.5	93.31
oest_20per-taxa	20%	1728	467 548	165 591	35.4	93.87
oest_30per-taxa	30%	1336	360 231	133 232	37	95
oest_40per-taxa	40%	999	267 507	101 297	37.9	94.67
oest_50per-taxa	50%	725	194 078	73 778	38	94.64
oest_60per-taxa	60%	470	128 399	48 876	38.1	93.13
oest_70per-taxa	70%	235	65 482	24 281	37.1	93.35
oest_80per-taxa	80%	37	10 820	3378	31.2	82.17
oest_90per-taxa	90%	2	735	179	24.4	0
oest_100per-taxa	100%	0	NA	NA	NA	NA

UCE loci refers to the number of UCE loci present in at least X percentage of the taxa. Datasets included 100 taxa. The percentage of data completeness corresponds to the percentage of taxa included having UCE loci. Since we captured a maximum of 80% of UCE loci per taxa, then datasets that were 90% and 100% data-complete for taxa were UCE data insufficient.

Phylogenetic inference

Each of the ten datasets of 37–2077 concatenated UCE loci having varying taxon completeness (10–80%) for each UCE locus (Table 4) was analysed with Maximum Likelihood (ML) best tree and bootstrap searches ($N = 100$) in RAxML v8.2.7 (Stamatakis, 2014). For the concatenated analyses, we calculated the global bootstrap support (GBS) by averaging all bootstrap support values on a given tree. We also partitioned the data by individual UCE loci using the Sliding-Window Site Characteristics approach and site characteristics, such as entropy implemented in the SWSC-EN algorithm, which generates partitions that account for heterogeneity in rates and patterns of molecular evolution within each UCE (Tagliacollo & Lanfear, 2018). We selected a partitioning scheme from the by-locus character sets with PartitionFinder2 (Lanfear *et al.*, 2017). Then, we sequentially ran an ML analysis for the best tree and 1000 replicates of ultrafast bootstrap on each locus for our gene trees estimations using IQ-TREE (Nguyen *et al.*, 2015). A multi-coalescent species tree analysis was carried out in ASTRAL-III (Zhang *et al.*, 2018) using gene trees (one tree search per gene) estimated by 100 ML searches conducted in RAxML v8.2.7 (Stamatakis, 2014). Statistical supports by ASTRAL-III are local posterior probabilities (LPP), which are branch support values that measure the support for a quadripartition, not a bipartition.

All of the above phylogenetic analyses were performed on the Smithsonian Institution High-Performance Cluster Hydra (SI/HPC) using Python scripts designed by MWL (some modified by Bonnie Blaimer and EB) and the Smithsonian Institution Bioinformatics Group (available at www.github.com/SmithsonianWorkshops/Targeted_Enrichment/blob/master/phylyce.md). Tips of final trees were renamed using a Perl script designed by MWL (available at www.github.com/MikeWLLoyd/Tree-Tip-Replacer).

Data availability

The data that support the findings of this study and the Calyptratae/Oestroidea-specific UCE probes are openly available in Dryad data at <https://doi.org/10.5061/dryad.3bk3j9kg1> (Buenaventura *et al.*, 2020).

Results

UCE probes and capture results

Summary results of empirically generated UCE data processed with *in silico* data are presented in Supplementary Table S1. Summary numbers for parsimony and invariant sites for each data matrix are reported in Supplementary Figure S3. We recovered more UCE loci than the average for two of the dried specimens used in this study (Table S1). All DNA extractions from specimens preserved in 96% ethanol ($= 50$) and liquid nitrogen ($= 12$) succeeded. Similarly, all existing DNA aliquots had ample well-preserved DNA (above $1 \text{ ng}/\mu\text{L}$) for the present study. Only two of 36 DNA extractions from pinned museum specimens failed, meaning there was too little DNA (less than $0.05 \text{ ng}/\mu\text{L}$) to be detected by the Qubit Fluorometer. The unsuccessful DNA extractions were museum specimens of *P. regina* (Calliphoridae: Chrysomyinae) collected in the year 2016 and *Rhinoestrus purpureus* (Brauer) (Oestridae: Oestrinae) collected in 1935, which, however, were included in the library preparation, enrichment and sequencing. 105 DNA extractions were enriched using the UCE probes and sequenced on an Illumina Hi-Seq lane. See Supplementary Table S1 and Figure S2 to compare specimen age *versus* total DNA extracted and UCEs captured coded for preservation method.

We obtained an average of 2 264 424 raw paired-end reads per sample. Trinity assembled reads into 334–75 722 contigs with an average of 9246 contigs assembled per sample (Table S1). These contigs had average lengths of 246.9–450.4 bp. From the total assembled contigs, we recovered a total of 2077 UCE loci across all taxa with an average of 836 UCE loci per sample and average lengths ranging from 221.5 to 619.6 bp. UCE loci were produced for all samples except for four pinned museum specimens: *Bengalia varicolor* (Fabricius) (Calliphoridae: Bengaliinae) collected in 1980, *Phumosiabdominalis* (Robineau-Desvoidy) (Calliphoridae: Phumosiinae) collected in 1986, *Gasterophilus intestinalis* (de Geer) (Oestridae: Gasterophilinae) collected in 1926 and *Hypoderma lineatum* (Viller) (Oestridae: Hypodermatinae) collected in 1976. These four specimens were included in the library preparation, enrichment

and sequencing, but were excluded from the phylogenetic analysis due to low UCE recovery. For two specimens, *P. regina* and *R. purpureus*, which did not have detectable levels of DNA in the extraction, we recovered 265 and 77 UCE loci, respectively. However, *R. purpureus* was excluded from the phylogenetic analysis because the recovered UCE loci for this sample were dropped in the PHYLUCE validation step where multiple contigs that map to a single locus are removed. Excluding the five species with low UCE recovery or no UCE recovery, the number of UCE loci recovered per sample ranged from 14 to 1713, with a total of 2077 UCE loci recovered out of 2581 UCE targets (Table 4). 35% of the sampled taxa consisted of pinned museum specimens (2–92 years old), of which 85% resulted in successful UCE enrichment. The age of the sampled taxa, excluding the five species with low UCE recovery or no UCE recovery, ranged from 2 to 54 years. The average capture efficiency was 42.22%.

The number of minimum and maximum UCE loci captured per family and subfamily varied considerably, but in general, the probes captured UCEs for all Oestroidea lineages, including the Calyptratae outgroups (Fig. 2). Broadly speaking, the three subfamilies of Sarcophagidae showed the highest minimum, maximum and average UCE loci capture, followed by Calliphoridae subfamilies Luciliinae, Chrysomyinae, Bengaliinae and the Tachinidae subfamily Dexiinae. The amount of input DNA did not follow a pattern, and it did not predict the number of UCE loci captured (Fig. 2A). However, when looking at the histogram of UCE loci captured per lineage, there was a trend with the tail of the histogram populated mostly with specimens of Gasterophilinae and Hypodermatinae (Oestridae), Melanomyinae and Toxotarsinae (Calliphoridae) (Fig. 2B), which were generally older than specimens of other lineages (Table S1). Unsurprisingly, the number of homologous UCEs dropped with increasing phylogenetic distance from Sarcophagidae, but even a species of Anthomyiidae had 945 homologous UCEs (Table S1).

Data completeness

All datasets were successfully analysed, except for datasets with 90% and 100% data completeness (= 90% and 100% of missing taxa), which were data insufficient (Table 4). In other words, since we captured a maximum of 80% of UCE loci per taxa, datasets with 90% and 100% data completeness for taxa were UCE data insufficient. We analysed datasets containing 37–2077 UCE loci from 98 representatives of the oestroid families and seven calyptrate outgroups with a total concatenated aligned length ranging between ten and 550 Kb (Table 4).

The relation between data completeness and percentage of parsimony-informative sites, and data completeness and GBS per dataset were visualized (Table 4, Figures. S3–S5). We found an average of 34.73% parsimony-informative sites between UCE alignments (10–90% data completeness). There was a slight increase in the percentage of parsimony-informative sites with a decrease of the maximum percentage of missing taxa at each UCE locus, which was observed for the datasets

having a maximum of 10–30% of missing taxa at each UCE locus (Figure S4). Datasets with a maximum of 40–60% of missing taxa at each UCE locus had a similar percentage of parsimony-informative sites, whereas a more drastic decrease in parsimony-informative sites was observed for more incomplete datasets (= 80–90% incomplete) (Figure S3). Higher values of GBS were not associated with datasets having a smaller maximum percentage of missing taxa at each UCE locus (Figure S5) since there were only slight fluctuations in GBS among datasets with varying levels of data completeness. A notably low value of GBS was only observed for the dataset having the largest maximum percentage of missing taxa at each UCE locus (Figure S5).

Phylogenetic results

Datasets having a maximum of 10–60% of missing taxa at each UCE locus produced identical topologies (topology #1, Figs. 3A, 4). Trees obtained from datasets having maximum 70% (topology #2, Fig. 3B) and 80% (topology #3, Fig. 3C) of missing taxa at each UCE locus received similar values of GBS (Figure S5), but their topologies were not equivalent to those recovered by other analyses. Our coalescent-based analysis used 725 UCE gene trees and produced a phylogeny (Fig. 5), in general, equivalent to topology #2 (Fig. 3B).

Major differences between topologies #1, #2 and #3 were the phylogenetic position of clades A (Rhinophoridae), B (Luciliinae (Toxotarsinae (Melanomyinae + Calliphoridae))), C (Phumosiinae + Chrysomyinae) and D (Bengaliinae + Rhiniidae) (Fig. 3). Topology #1 has (A + B) + (C + D) (Fig. 3A), whereas topology #2 has (C + ((A + B) + D)) (Fig. 3B) and topology #3 has (C + (A + (B + D))) (Fig. 3C). Important differences among these topologies are related to the sister grouping of clade D and clade C in topology #1 or with (A + B) in topologies #2 and #3. In our concatenated ML analyses, topology #1 was recovered in six out of eight phylogenetic analyses using more data-complete datasets (maximum 10–60% of missing taxa at each UCE locus) (Figure S5). Also, topology #1 had higher statistical branch support (highest GBS = 95, average of datasets with maximum 10–60% of missing taxa at each UCE locus GBS = 94.10, see values for each analysis in Figure S5), compared to topologies #2 (GBS = 93.35) and #3 (GBS = 82.17). Topology #1 was produced by datasets having higher percentages of parsimony-informative sites (Figure S5). Differences between topologies #1 and #2, especially regarding GBS values, are to be considered carefully as GBS is based on all nodes and not only on those concerning relationships of the most problematic lineages (i.e. clades A, B, C, D). Controversial relationships, especially regarding the sister-group of D were resolved with higher statistical support in topology #2 with D sister to (A + B) with BS = 68 while topology #1 had D sister to C with BS = 61 (Fig. 3). Interestingly, our coalescent-based analysis recovered topology #2 with D sister to (A + B) with LPP = 0.94 (Fig. 5).

For illustration purposes, we depicted our best trees from the ML searches on the concatenated UCE dataset in Fig. 4 (topology #1) and coalescent-based phylogeny in Fig. 5 (topology

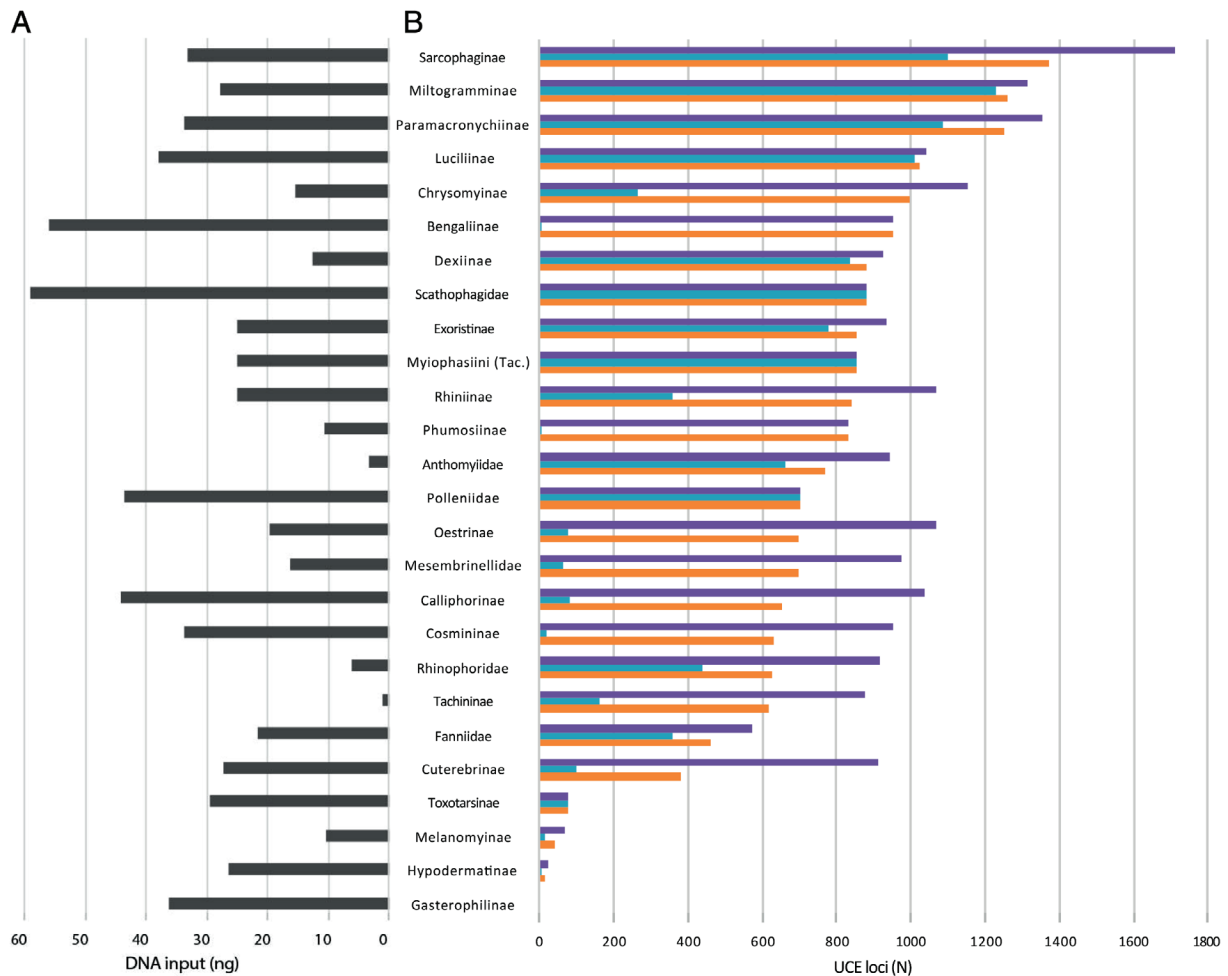


Fig 2. (A) DNA input per lineage. (B) UCE recovery per lineage. Maximum UCE loci (purple bars), minimum UCE loci (turquoise bars), average UCE loci (orange bars). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article). [Colour figure can be viewed at wileyonlinelibrary.com.]

#2). We recovered a highly resolved ML phylogeny with 99 nodes (Fig. 4), most of them displaying 100% bootstrap support (BS). Sixteen nodes had branch support values lower than BS = 95, 12 of them involving intraspecific relationships. These low-supported nodes involved five species for which less than 100 UCE loci were analysed (Table S1): *Melinda viridicyanea* (Robineau-Desvoidy) (Calliphoridae: Melanomyinae), *Villeneuveiella seguyi* (Grunin) (Rhiniidae: Cosmininae), *Hypoderma tarandi* Linnaeus (Oestridae: Hypodermatinae), *Melanomyia nana* (Meigen) (Calliphoridae: Melanomyinae) and *Bellardia viarum* (Robineau-Desvoidy) (Calliphoridae: Calliphorinae). Our coalescent-based phylogeny was better supported with only ten nodes having values lower than LPP = 0.70 (Fig. 5), five of them involved intraspecific relationships with the above-mentioned five species.

The superfamily Oestroidea was recovered as monophyletic in all our analyses. Most families were also recovered as monophyletic, the exceptions being Calliphoridae and Oestridae. At the subfamily level, the calliphorid subfamilies Calliphorinae

and Melanomyinae and the rhiniid subfamily Cosmininae were not recovered as monophyletic. Mesembrinellidae was recovered as sister to the remaining families with BS = 100 and LPP = 1.0. Oestridae was paraphyletic with respect to Sarcophagidae, with the subfamily Oestrinae as sister to all sarcophagids. Within Sarcophagidae, the subfamily Sarcophaginae was strongly supported as the sister-group to the clade (Miltogramminae + Paramacronychiinae). *Pollenia rudis* (Fabricius) (Polleniidae) was strongly supported as the sister-group to Tachinidae. Within the clade of the tachinids, *Cholomyia inaequipes* Bigot (Tachininae, Myiophasiini) received high BS and LPP as sister to the clade (Dexiinae + Phasiinae). The clade of the Tachinidae subfamilies (Dexiinae + Phasiinae) was strongly supported as sister to (Exoristinae + Tachininae). *Phumosi* sp. (Phumosiinae) was strongly supported as sister to Chrysomyinae (Calliphoridae), and strong support was also obtained for a clade consisting of the sister-group relationship between *Bengalia* sp. (Bengaliinae) and Rhiniidae. A weaker supported relationship (BS = 85, LPP = 0.72) was recovered between Rhinophoridae

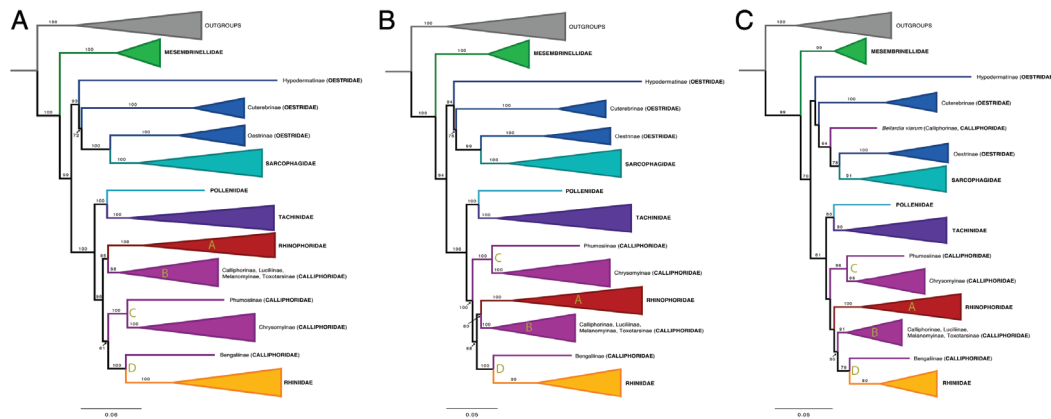


Fig 3. Phylogeny of Oestroidea flies. RAxML best tree estimated from the concatenated datasets having a maximum 10–60% (A, topology #1), 70% (B, topology #2) and 80% (C, topology #3) of missing taxa at each UCE locus. Support values from 100 RAxML bootstrap analyses are mapped on the respective nodes. Olive letters on nodes are discussed in the text. Species-level relationships have been collapsed to ease comparison. Scale bar depicts nucleotide substitution per site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article). [Colour figure can be viewed at wileyonlinelibrary.com].

and the clade (Luciliinae (Toxotarsinae (Melanomyiinae + Calliphorinae))). Similarly, some of the relationships within the clade (Melanomyiinae + Calliphorinae) were weakly supported and quite unstable throughout our analyses.

Discussion

Oestroid flies represent a massively diverse and taxonomically rich lineage. They play important ecological roles as nutrient recyclers, invertebrate parasitoids and as pollinators in various ecosystems. This group is also important to humans due to their vital roles in decomposing organic materials, as mechanical vectors of diseases and as indicators of time of death in forensic investigations. To explore the evolutionary history of this group of flies we designed UCE probes for Calyptratae flies, taking a subset of taxon-specific loci that are found in coding regions derived from a transcriptome. Our probe set resolved the clades (Sarcophagidae + Oestridae), (Polleniidae + Tachinidae), (Rhinophoridae + (Luciliinae (Toxotarsinae (Melanomyiinae + Calliphorinae)))), (Phumosiinae + Chrysomyinae) and (Bengaliinae + Rhiniidae), some of which were unresolved, inconclusively resolved or weakly supported in previously published studies (Pape, 1992; Kutty *et al.*, 2010; Marinho *et al.*, 2012; Singh & Wells, 2013; Junqueira *et al.*, 2016; Cerretti *et al.*, 2017; Cerretti *et al.*, 2019; Kutty *et al.*, 2019; Stireman *et al.*, 2019). In addition, we were successful in including traditional, pinned museum specimens in this study, which presents a significant resource for future phylogenetic studies in an age of museomics.

Age- and preservation-related factors of UCE sequence capture

Similar to most UCE phylogenetic studies using museum samples as a source of tissues (Blaimer *et al.*, 2016b; Van Dam *et al.*, 2017; Wood *et al.*, 2018; Hedin *et al.*, 2019; Kieran

et al., 2019), we successfully enriched several UCE loci from museum fly specimens. DNA extracted from these samples was generally low quality (i.e. highly fragmented, low in concentration), which in pinned museum specimens may be related to long initial drying time and causes DNA degradation due to enzymatic decomposition (Lindahl, 1993; Gilbert *et al.*, 2007; Van Dam *et al.*, 2017; Derkarabetian *et al.*, 2019). Specimen age is also usually an obstacle affecting DNA yielded after the extraction; however, we were able to recover enough DNA to enrich a relatively large number of loci from 50+ year old, pinned museum specimens. We observed a gradual decline in the UCE recovery rate with specimen age (Figure S2), as found in previous studies (Blaimer *et al.*, 2016b; Van Dam *et al.*, 2017). This suggests that obtaining enough DNA from older, pinned specimens is possible in studies using targeted enrichment in the future, but the maximum of 20 years should be considered (Blaimer *et al.*, 2016b). Successful UCE enrichment of our taxon sample was mostly from dry pinned specimens and samples preserved in 96% ethanol, which highlights this approach as advantageous compared with other approaches requiring specimens preserved in RNA-later or in liquid nitrogen. Several museums have large, generally poorly studied wet collections, including unsorted Malaise trap samples, which might be an important source of fragmented DNA to be enriched for UCEs. Our results also show that well-preserved DNA-rich samples, such as DNA aliquots are also suitable for UCE enrichment.

Calyptratae/Oestroidea-specific UCE probes

General UCE probe sets designed to work across larger taxonomic groups have proven successful at resolving phylogeny in various groups (Faircloth *et al.*, 2015; Starrett *et al.*, 2016; Van Dam *et al.*, 2017), but there is growing evidence for improved locus recovery through the use of probe sets tailored to focal taxa (Baca *et al.*, 2017; Branstetter *et al.*, 2017; Van Dam *et al.*, 2019; Gustafson *et al.*, 2019a;

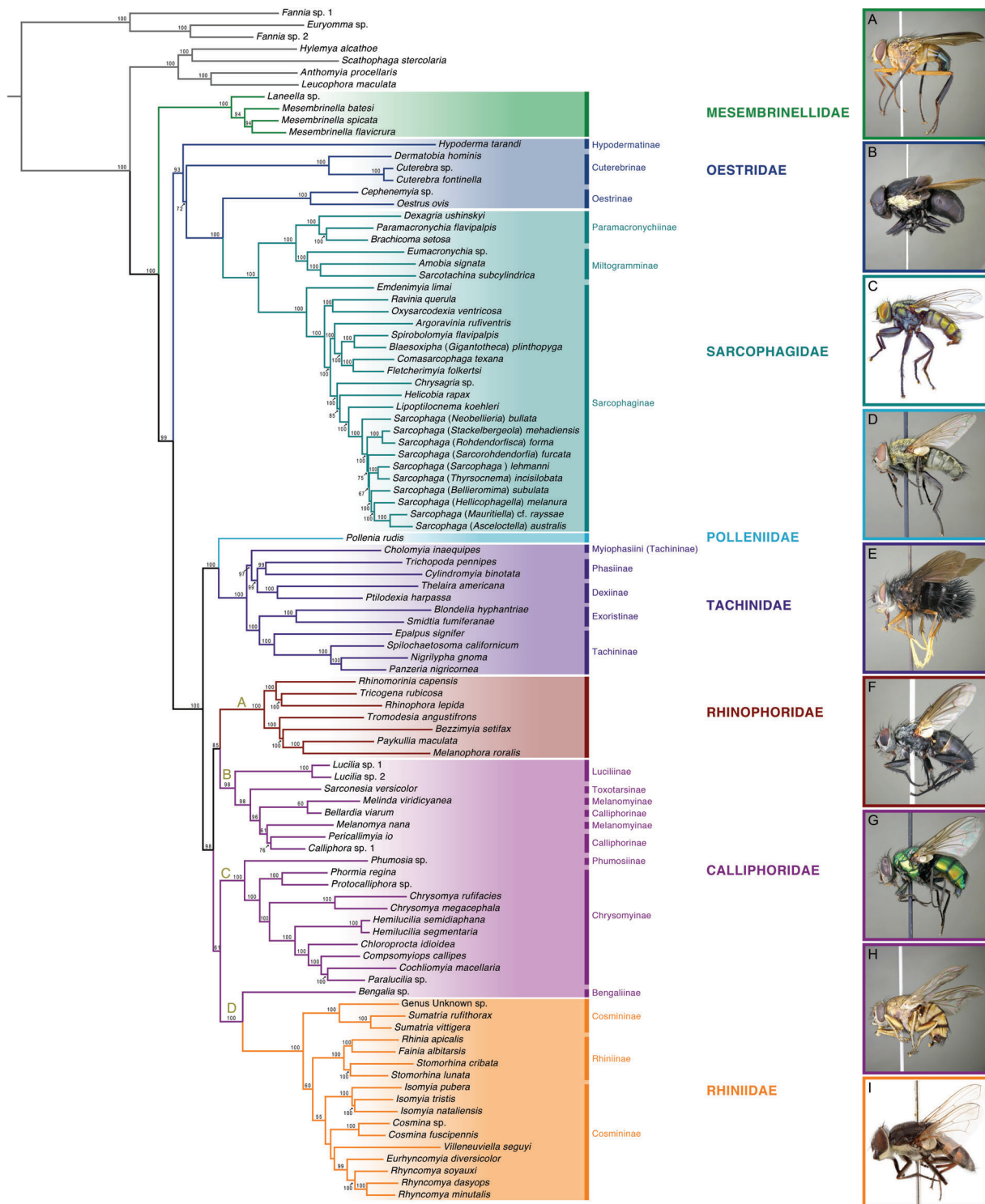


Fig 4. Phylogeny of Oestroidea flies. RAXML best tree estimated from the concatenated datasets having a maximum 50% of missing taxa at each UCE locus (topology #1), with support values from 100 RAXML bootstrap analyses mapped on the respective nodes. Olive letters on nodes are discussed in the text. Representative oestroid families are illustrated to the right: (A) *Mesembrinella* sp., Mesembrinellidae, (B) *Cuterebra* sp., Cuterebrinae, Oestridae, (C) *Emdenimyia* limai, Sarcophaginae, Sarcophagidae, (D) *Pollenia* angustigena, Polleniidae, (E) *Hystricia* sp., Tachininae, Tachinidae, (F) *Stevenia* deceptor, Rhinophoridae, (G) *Lucilia* coeruleiviridis, Lucilinae, Calliphoridae, (H) *Bengalia* sp., Bengaliinae, Calliphoridae, (I) *Stomorphina* lunata, Rhiniinae, Rhiniidae. Photos (A, B, D–H) by JMPL; (C) by EB; (I) by ATC. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article). [Colour figure can be viewed at wileyonlinelibrary.com].

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Gustafson *et al.*, 2019b; Kulkarni *et al.*, 2020). An increase in locus recovery is especially important in the study of rapid radiations (Dell'Ampio *et al.*, 2014; Giarla & Esselstyn, 2015), such as that of Oestroidea. The Diptera-wide UCE probe set by Faircloth (2017) was designed with a genome selection of fly families belonging to the early divergences within Diptera. This genome selection is most likely the result of the limited genomic resources available for Diptera at that time (Vicoso & Bachtrog, 2015; Dikow *et al.*, 2017), as it includes mostly the species of medical importance of Culicidae and model organisms of Drosophilidae. In fact, 37 out of 45 genomic resources used in the design by Faircloth (2017) belong to these two families. This Diptera-wide UCE probe set should be very useful to resolve phylogenetic relationships within Culicidae, Drosophilidae and among other closely related dipteran lineages. However, a ~200 Ma (Wiegmann *et al.*, 2011) evolutionary distance between Culicidae and Calyptratae (i.e. the clade were Oestroidea is nested) is possibly too large to capture enough UCEs to efficiently reconstruct the Oestroidea radiation. Studies on other arthropod lineages have shown that more generalized UCE probe sets capture decreasing numbers of loci with increasing phylogenetic distance from the focal taxon (Faircloth *et al.*, 2015; Gustafson *et al.*, 2019a). Also, this Diptera-wide UCE probe set used a member of Culicidae (*Aedes aegypti* (L.)) as the base genome. The base genome is a decisive taxon for the UCE probe design, which should be nested or related to the focal group, and it is against, which data from other sampled exemplar taxa within the focal group will be aligned during the probe design process (Faircloth, 2017). Our probe design used *S. crassipalpis* as the base genome, based on the high contiguity and level of annotation as measures of assembly quality of this genome, and also because this sarcophagid species is nested within the focal group. We observed that taxa for which we recovered the most UCE loci were frequently those with the closest relationship to the base genome and other genomes used for probe design, that is species of the families Sarcophagidae and Calliphoridae. It is notable that at least one taxon of each family/subfamily in our taxon sampling produced 800+ UCE loci, which indicates that these probes can efficiently recover UCE loci from all lineages within Oestroidea and that recovery rates would mostly depend on the DNA quality of each sample. Furthermore, our probe design also used the genetic resources of Muscidae and Glossinidae and could be used for phylogenetic studies of the entire Calyptratae clade. In addition, as we filtered our UCE probes with a transcriptome to capture only protein-encoding loci, our UCE dataset is suitable for larger and more integrative phylogenomic studies using both genomic and transcriptomic resources. It should be noted that most lineages within Calyptratae and the superfamily Oestroidea do not have available genomic resources (Vicoso & Bachtrog, 2015; Dikow *et al.*, 2017) to be used in phylogenomic studies in general, or in the design of UCE probe sets in particular. This highlights the need for a larger and more representative set of genomic resources for insect phylogenomics.

Oestroidea phylogeny

Our concatenated ML and coalescent-based analyses recovered a fully resolved and mostly well-supported phylogeny for Oestroidea flies. These analyses estimated generally identical topologies on the family, subfamily and species levels (Figs. 3–5). Uncertainty is confined only to a few nodes in the phylogeny with BS < 95 and LPP < 0.70. We found a few important differences, such as the position of the clade C or A + B as sister to D. These alternative resolutions of the tree are associated with relatively low branch support. One of our concatenated ML analyses has C + D with BS = 61 while coalescent-based analysis and another of our concatenated ML analyses have D + (A + B) with LPP = 94 and BS = 68. These differences in support and resolution are discussed below.

Oestroidea monophyly and radiation

The monophyly of Oestroidea has been corroborated by morphological (Griffiths, 1972; Pape, 1992; Lambkin *et al.*, 2013) and molecular characters (Kutty *et al.*, 2010; Wiegmann *et al.*, 2011; Marinho *et al.*, 2012; Caravas & Friedrich, 2013; Lambkin *et al.*, 2013; Cerretti *et al.*, 2017; Kutty *et al.*, 2019). Here we add support from UCE molecular data and test the monophyly of the oestroid families and subfamilies. The problem of deciphering large radiations is resolved here by a genome-scale alignment of UCE data, which yields a phylogenetic tree with high statistical support. Does this mean that with our genome-wide alignments, we now have reconstructed the true tree for Oestroidea? Answering this question requires a cross-validation experiment to assess whether the statistically significant inferences of two or more types of phylogenomic data are consistent. Indeed, the vast majority of our UCE-based phylogenetic relationships are largely consistent with transcriptome-based analyses (Kutty *et al.*, 2019). For example, the problematic nodes involving Rhinophoridae and Calliphoridae lineages Luciliinae, Toxotarsinae, Melanomyinae, Calliphorinae (i.e. clade A + B) and Chrysomyinae and Phumosiinae (i.e. clade C) were also problematic using transcriptomes (Kutty *et al.*, 2019; Fig. 1). Similarly, well-supported sister-group relationships of Bengaliinae and Rhiniidae, Chrysomyinae and Phumosiinae, Polleniidae and Tachinidae and Oestridae (nonmonophyletic) and Sarcophagidae are also supported by transcriptome-based analyses (Kutty *et al.*, 2019).

The Calyptratae radiation has been suggested as multiple episodes of rapid diversification in the early Tertiary (Kutty *et al.*, 2010) possibly associated with the opening of new niches following the Cretaceous-Palaeogene mass extinction event (Cerretti *et al.*, 2017). Our results are concordant with studies showing lineages within Sarcophagidae (Piwczyński *et al.*, 2014; Piwczyński *et al.*, 2017; Buenaventura & Pape, 2017a; Buenaventura *et al.*, 2019) and Tachinidae (Cerretti *et al.*, 2014b; Stireman *et al.*, 2019) as the dominant fast-evolving groups of Oestroidea. Furthermore,

our results support a super-radiation within the genus *Sarcophaga* Meigen (Sarcophagidae), as recent studies suggest (Piwczyński *et al.*, 2014; Buenaventura *et al.*, 2017; Buenaventura & Pape, 2017a; Buenaventura *et al.*, 2019). This super-radiation can be recognized by the presence of many short branches at the level of the subgeneric diversification, which is accompanied by lower BS and LPP in comparison with the rest of the tree nodes (Figs. 4, 5). Even larger radiations may have involved specific lineages within Tachinidae, which need further study using a larger taxon sampling within this family. The short internal branches within Sarcophagidae represent short intervals between speciation events, where it is likely that gene trees and species trees can conflict with the underlying species branching history (Degnan & Rosenberg, 2006, 2009), hampering phylogenetic inference. Similar gene tree discordance using UCEs in the study of rapid diversifications has been reported (Guillory *et al.*, 2020). However, combining many independently segregated loci sampled from multiple species per lineage and applying a coalescent-based approach should improve phylogenetic inference by reducing sampling error and allowing one to distinguish evolutionary signal from a methodological artefact (McCormack *et al.*, 2009; Kumar *et al.*, 2012; Giarla & Esselstyn, 2015). Then, in these recent super-radiations within Oestroidea, poor support for a series of splits within Sarcophagidae, and possibly Tachinidae, should be attributed to its explosive evolutionary history limiting phylogenetic signal, rather than by methodological artefacts.

Monophyly and relationships of Oestroidea families

Mesembrinellidae. Our results are consistent with morphological and molecular characters supporting Mesembrinellidae as a separate, monophyletic family and not as a subfamily of Calliphoridae (Guimarães, 1977; Kutty *et al.*, 2010; Marinho *et al.*, 2012; Singh & Wells, 2013; Cerretti *et al.*, 2017; Marinho *et al.*, 2017; Kutty *et al.*, 2019). Other molecular studies found Mesembrinellidae as nested within (Kutty *et al.*, 2010) or sister to Tachinidae (Marinho *et al.*, 2012; Junqueira *et al.*, 2016), or sister to Ulurumyiidae (Kutty *et al.*, 2019). We recovered the mesembrinellids as sister to the remaining Oestroidea, which is partially supported by transcriptome data (Kutty *et al.*, 2019), four nuclear loci (Stireman *et al.*, 2019) and by morphological characters (Michelsen & Pape, 2017). The monophyly of Mesembrinellidae is supported by characters, such as larvae hatch within the uterus and nourished by secretions of the female for an extended period (Guimarães, 1977; Ferrar, 1978; Rognes, 1986; Pape, 1992; Meier *et al.*, 1999). The phylogenetic relationships within Mesembrinellidae are still controversial. Marinho *et al.* (2017) treated *Eumesembrinella* Townsend, *Giovanella* Bonatto, *Huascaromusca* Townsend, *Laneella* Mello and *Mesembrinella* Giglio-Tos as separate genera and found support for the monophyly of *Eumesembrinella* and *Laneella* only. These authors also suggested *Eumesembrinella* as a junior synonym of *Mesembrinella* and *Giovanella* as a junior synonym of *Huascaromusca*. These suggestions were incorporated by Cerretti *et al.* (2017) who treated

all species as one single genus *Mesembrinella*. Whitworth & Yusseff-Vanegas (2019) revised the family and arranged species into genera *Laneella*, *Mesembrinella* and *Souzalopesiella* Guimarães, which was in agreement with Marinho *et al.* (2017). Our UCE data, although limited in taxon sampling, supports the monophyly of *Mesembrinella*, which is consistent with other molecular data (Cerretti *et al.*, 2017; Marinho *et al.*, 2017) and with the classification proposed by Whitworth & Yusseff-Vanegas (2019). Mesembrinellidae is limited to Neotropical rainforests (Marinho *et al.*, 2017) and its possible close phylogenetic relationship to the dung breeders of the Australian Ulurumyiidae (Kutty *et al.*, 2019); Michelsen & Pape (2017) suggest an interesting potential biogeographic connection through Antarctica.

Oestridae. The monophyly of the bot flies is strongly supported by morphological characters (Wood, 1986; Pape, 1992; Pape, 2001; Pape, 2006), but molecular characters produce different phylogenetic hypotheses for this lineage of mammal parasites. A well-supported, monophyletic Oestridae is supported by studies using COI (Otranto *et al.*, 2003), mitochondrial genomes (Junqueira *et al.*, 2016) and multi-locus datasets (Cerretti *et al.*, 2017). However, also using mitochondrial genomes, Zhang *et al.* (2016a) found that the monophyly of Oestridae depended on which outgroups are included. Our UCE phylogeny supports Oestridae rendered paraphyletic by Sarcophagidae, a close relationship that is consistent with other phylogenetic studies combining molecular and morphological data (Cerretti *et al.*, 2017) or using several molecular markers (Wiegmann *et al.*, 2011; Stireman *et al.*, 2019). However, other molecular datasets do not support the sister grouping of Oestridae with Sarcophagidae; mitochondrial genomes (Junqueira *et al.*, 2016) suggest Oestridae is sister to Tachinidae + Mesembrinellidae, whereas transcriptomes (Kutty *et al.*, 2019) point to Mystacobiidae as the sister lineage to Oestridae.

Within Oestridae, our ML concatenated analysis reconstructs the three subfamilies as paraphyletic with respect to Sarcophagidae, with Hypodermatinae sister to the clade (Cuterebrinae + (Oestrinae + Sarcophagidae)), whereas our coalescent-based analysis has Cuterebrinae sister to the clade (Hypodermatinae + (Oestrinae + Sarcophagidae)). These analyses additionally support the monophyly of the subfamilies Cuterebrinae and Oestrinae. Oestrinae emerges as the sister taxon to Sarcophagidae in all analyses. This sister-group relationship is also supported by the presence of a bilobed uterine pouch for embryonating eggs (Pape, 1992), although there seem to be morphological differences in shape and tracheation between the uterine pouches of Sarcophagidae and Oestridae, and further study of these characters is needed. The configuration of openings of posterior spiracles in second and third instar larvae also deserves attention in future studies. A configuration of these openings as a porous plate is shared by Hypodermatinae and Oestrinae (Zumpt, 1965; Ferrar, 1987; Pape, 1992), whereas these openings are composed of three slits in *Dermatobia* Brauer, *Ruttenia* Rodhain and *Neocuterebra* Grünberg (Ferrar, 1987); these were coded as such in Cuterebrinae and

Gasterophilinae in a morphology-based phylogenetic analysis of Oestroidea (Pape, 1992). Thus, morphological evidence would conflict with our ML concatenated UCE phylogeny, but supports our coalescent-based analysis. Finally, more DNA data is needed for subfamilies Gasterophilinae and Hypodermatinae to further test the monophyly and relationships of these lineages as well as the monophyly of Oestridae.

Sarcophagidae. Our results corroborate previous findings regarding the monophyly of sarcophagids (Pape, 1992; Song *et al.*, 2008; Kutty *et al.*, 2010; Marinho *et al.*, 2012; Piwczynski *et al.*, 2014; Zhang *et al.*, 2016a; Piwczynski *et al.*, 2017; Yan *et al.*, 2017; Kutty *et al.*, 2019) and its three subfamilies (Pape, 1996; Kutty *et al.*, 2010; Piwczynski *et al.*, 2017; Buenaventura & Pape, 2017a; Buenaventura *et al.*, 2019). Recent molecular studies have challenged the traditional classification and questioned subfamily level relationships. Paramacronychiinae was generally considered to be the sister-group of Sarcophaginae based on male genitalic traits (Pape, 1992; Pape, 1998; Giroux *et al.*, 2010). Analyses of molecular datasets have found contradictory evidence, some supporting the traditional classification with Paramacronychiinae as sister to Sarcophaginae (Kutty *et al.*, 2010), whereas more evidence is accumulating in support of Paramacronychiinae as sister to Miltogramminae (Piwczynski *et al.*, 2014; Piwczynski *et al.*, 2017; Buenaventura & Pape, 2017a; Buenaventura *et al.*, 2019). Buenaventura *et al.* (2019) also re-examined morphological structures and highlighted convergent male genitalic traits producing a conflicting phylogenetic signal, in contrast with molecular evidence.

Within the flesh flies, our results largely agree with phylogenetic relationships for Sarcophaginae based on morphological characters (Giroux *et al.*, 2010; Buenaventura & Pape, 2017b). The clade (*Oxysarcodexia* Townsend + *Ravinia* Robineau-Desvoidy) is an example of a sister-group relationship that is well corroborated by our UCE data and morphological characters. The 'Blaesoxipha clade' of Buenaventura & Pape (2017b) is represented here by *Blaesoxipha* Loew, *Comasarcophaga* Hall, *Fletcherimyia* Townsend and *Spirobolomyia* Townsend and are recovered as a well-supported clade. The internal relationships support those previously recovered by Giroux *et al.* (2010) with *Blaesoxipha* as sister to *Spirobolomyia* and *Comasarcophaga* sister to *Fletcherimyia*. Our results also support the 'Sarcophaga clade' of Buenaventura & Pape (2017b) with *Chrysagria* Townsend + (*Helicobia* Coquillett + (*Lipoptilocnema* Townsend + *Sarcophaga*)) (although *Peckia* Robineau-Desvoidy is not included here). Within the largest radiation of Sarcophaginae, phylogenetic relationships in the hyperdiverse genus *Sarcophaga* closely match those recovered in previous molecular phylogenies, with the Nearctic subgenus *Neobellieria* Blanchard as one of the earliest divergences (Buenaventura *et al.*, 2017; Buenaventura & Pape, 2017a) and the subgenus *Stackelbergeola* Rohdendorf as sister to subgenus *Rohdendorfsca* Grunin (Zhang *et al.*, 2016b).

Tachinidae. Tachinidae represents the largest radiation within Oestroidea (8592 spp., 1477 genera, 4 subfamilies) (O'Hara & Henderson, 2020). Their monophyly is well corroborated here and in previous molecular studies (Marinho *et al.*, 2012; Singh & Wells, 2013; Winkler *et al.*, 2015; Junqueira *et al.*, 2016; Zhang *et al.*, 2016a; Blaschke *et al.*, 2018; Stireman *et al.*, 2019; except Kutty *et al.*, 2010) as well as based on the morphology of both the larvae and adults, with their distinctive endoparasitoid larval life-history (Wood, 1987b; Pape, 1992; Stireman *et al.*, 2019). Our results support Poleniidae as sister to Tachinidae as found by previous studies (Nelson *et al.*, 2012; Singh & Wells, 2013; Winkler *et al.*, 2015; Cerretti *et al.*, 2017; Stireman *et al.*, 2019), but contrary to other studies that recovered Tachinidae as sister to Sarcophagidae (Pape, 1992), Oestridae (Zhao *et al.*, 2013), Mesembrinellidae (Marinho *et al.*, 2012; Junqueira *et al.*, 2016) or nested within Calliphoridae (Kutty *et al.*, 2010).

Within Tachinidae, our analyses recovered (Phasiinae + Dexiinae) as sister to (Exoristinae + Tachininae), which is in agreement with previous phylogenetic studies (Cerretti *et al.*, 2014b; Winkler *et al.*, 2015; Stireman *et al.*, 2019). Within the subfamily Tachininae, the sister-group relationship between the tribe Tachinini (represented by genus *Epalpus* Rondani) and the remaining Tachininae is congruent with a recent phylogeny for tachinids (Stireman *et al.*, 2019). However, genus *Panzezia* Robineau-Desvoidy (Ernestiini *s.l.*) was nested inside tribe Polideini, represented by the genera *Spilochaetosoma* Smith and *Nigrilypha* O'Hara. In addition, we recovered the enigmatic species *Cholomyia inaequipes* Bigot, as the sister taxon to the clade (Phasiinae + Dexiinae) (topology #1) or as sister to the rest of Tachinidae (topologies #2 and #3). *Cholomyia* Bigot was previously placed in the subfamily Dexiinae (Townsend, 1936; Guimarães, 1971) but later transferred to Myiophasiini (Tachininae) (O'Hara & Wood, 2004). Recent molecular analyses based on four nuclear loci recovered a clade of the tachinine tribes Macquartiini + Myiophasiini (including *C. inaequipes*) as a sister to the rest of the family (Stireman *et al.*, 2019), as reconstructed in some of our topologies (topologies #2 and #3). Thus, as suggested by O'Hara *et al.* (2019), some rearrangements may be necessary within the family with regard to the clade Macquartiini + Myiophasiini, which might constitute a separate subfamily (O'Hara & Henderson, 2020). Differences between phylogenetic reconstructions for *C. inaequipes* within Tachinidae found here and in a previous analysis (Stireman *et al.*, 2019) may be attributed to limited taxon sampling here.

The morphological diversity of Tachinidae has historically led to difficulties to define monophyletic groups and produce a stable classification scheme (e.g. Polideini, O'Hara, 2002). Only Dexiinae (excluding Eugherini) is supported by a hinged membranous dorsal connection between the basi- and distiphallus (Cerretti *et al.*, 2014b) and Phasiinae by the elongated medial plate of the hypandrium (Blaschke *et al.*, 2018). Other subfamilies are simply defined by the presence of combinations of characters, with several exceptions (Stireman *et al.*, 2019). At a higher taxonomic level, the eggs being laid embryonated is a potential character state uniting some Tachinidae and

Rhinophoridae, but the significance should be treated with caution. Also, some phylogenetic analyses of Tachinidae suggest multiple, independent shifts in reproductive strategy, including reversals (i.e. ovipary to ovolarvipary and/or vice versa), with different conclusions depending on the inference method used (e.g. Stireman *et al.*, 2019).

Rhinophoridae. This family includes 177 described species belonging to 33 genera (Cerretti *et al.*, 2020). Our data strongly support the monophyly of Rhinophoridae, which is consistent with the morphology of the first instar larvae (Pape, 1986; Pape & Arnaud, 2001) and phylogenetic estimations using molecular datasets (Singh & Wells, 2013; Winkler *et al.*, 2015; Kutty *et al.*, 2019, but see Kutty *et al.*, 2010). Based on morphological and biological similarities, phylogenetic affinities of the rhinophorids have generally pointed to Calliphoridae and Tachinidae as potential sister groups (Sabrosky & Arnaud, 1965; Wood, 1987a; McAlpine, 1989). Our phylogeny recovers Rhinophoridae as sister to clade B representing part of the traditional Calliphoridae (composed of subfamilies Calliphorinae, Luciliinae, Melanomyiinae and Toxotarsinae), which is consistent with morphological evidence (Tschorsnig, 1985; Rognes, 1986; Rognes, 1991). The early divergences within Rhinophoridae are composed by Old World taxa from the Palaearctic Region (*Rhinomorinia* Brauer and Bergenstamm, *Rhinophora* Robineau-Desvoidy, *Tricogena* Rondani, *Tromodesia* Rondani) whereas Neotropical taxa (*Bezzimyia* Townsend) form a sister-group relationship with a second clade of Palaearctic taxa (*Melanophora* Meigen, *Paykullia* Robineau-Desvoidy). Interestingly, the second Palaearctic clade is supported by a highly apomorphic morphological feature of the first instars of these parasitoids, which is intimately linked to the way in which they move. Thus, the first rhinophorid divergences include taxa (*Bezzimyia*, *Rhinomorinia*, *Rhinophora*, *Tricogena* [?], *Tromodesia*) that move in a leech-like fashion, whereas larvae of the second Palaearctic clade (*Melanophora*, *Paykullia*) move in a somersaulting fashion (Cerretti *et al.*, 2014a). Our phylogenetic estimation conflicts with a morphology-based analysis, including a larger sampling for Rhinophoridae (Cerretti *et al.*, 2014a).

Rhiniidae. This lineage includes almost 400 described species belonging to 30 genera and two subfamilies (Pape *et al.*, 2011; Rognes, 2013). Rhiniidae consistently emerges as a monophyletic group in our analyses, which is in agreement with published molecular (Kutty *et al.*, 2010; Marinho *et al.*, 2012; Singh & Wells, 2013; Cerretti *et al.*, 2017; Marinho *et al.*, 2017; Kutty *et al.*, 2019) and morphological evidence (Rognes, 1997). Until recently, rhiinids were considered a subfamily of Calliphoridae, but removed from it and raised to a family level based on the results of the above studies. Additional evidence that might support the monophyly of Rhiniidae is the shape of the paraphallus (i.e. heavily sclerotized, dorsal part of the phallic tube). A paraphallus dorsally complete with a latero-distal margin expanded ventrally is found in Rhiniidae, whereas the paraphallus has a dorso-medial insertion of variable

sizes with a latero-distal margin generally not expanded in other calliphorid lineages (Thomas-Cabianca, unpublished data).

The phylogenetic placement of Rhiniidae within Oestroidea has historically been unclear. Earlier studies placed Rhiniidae as sister to (Sarcophagidae + Calliphoridae) (Marinho *et al.*, 2012), all Oestroidea except Mystacinobiidae, Sarcophagidae and Ulurumyiidae (Kutty *et al.*, 2010), Rhinophoridae (Pape & Arnaud, 2001) or (Sarcophagidae + Tachinidae) (Rognes, 1997). Our phylogenetic analyses recover Rhiniidae as sister to Bengaliinae (clade D, part of traditional Calliphoridae), which is consistent with a growing body of evidence of recent molecular phylogenies (Singh & Wells, 2013; Cerretti *et al.*, 2017; Cerretti *et al.*, 2019; Kutty *et al.*, 2019). Interestingly, ecological data also point to a close relationship between Rhiniidae and Bengaliinae, as these are the only oestroid flies with a close, albeit poorly understood, ecological relationship with termites and ants (Peris, 1952; Ferrar, 1987; Kurahashi & Kirk-Spriggs, 2006; Singh & Rognes, 2014; Arce *et al.*, 2019).

Our concatenated ML analysis supports (Rhiniidae + Bengaliinae) (clade D) as sister to (Chrysomyiinae + Phumosiinae) (clade C), which agrees in part with Chrysomyiinae + (Rhiniidae + Bengaliinae) recovered in other molecular studies (Singh & Wells, 2013; Kutty *et al.*, 2019). However, these results had low branch support and they excluded Phumosiinae. Other molecular phylogenies recovered (Rhiniidae + Bengaliinae) as sister to Calliphoridae (excluding Chrysomyiinae) (Cerretti *et al.*, 2017) or to Luciliinae (Cerretti *et al.*, 2019). These other molecular phylogenies agree with our coalescent-based analysis. Consequently, there remains uncertainty regarding the cladogenesis of some Calliphoridae lineages, but they also confirm that these Calliphoridae lineages and (Rhiniidae + Bengaliinae) do share a common ancestor.

Our analyses support Cosmininae rendered paraphyletic by a monophyletic Rhiniinae. Additional evidence, which might support the monophyly of Rhiniinae is (i) desclerotization between the basi- and disti-phallus (these phallic sections are fused in Cosmininae), (ii) the absence of an epiphallus (present in Cosmininae) and (iii) a distal, prominent, globular paraphallus (less prominent in Cosmininae) (Thomas-Cabianca, unpublished data). Cosmininae exhibit extremely diverse morphological traits, which are still poorly studied. In addition, ecological associations and life cycles of Cosmininae species are mostly unknown (Cuthbertson, 1933; Cuthbertson, 1934; Peris, 1952; Zumpt, 1958; Kurahashi & Kirk-Spriggs, 2006). *Cosmina* Robineau-Desvoidy, *Isomyia* Walker, *Rhyncomya* Robineau-Desvoidy, *Stomorphina* Rondani and *Sumatria* Malloch emerged as monophyletic taxa, whereas the monophyly of *Eurhyncomya* Malloch, *Fainia* Zumpt and *Rhinia* Robineau-Desvoidy could not be tested due to limited taxon sampling. We also confirmed that the enigmatic genus *Villeneuveiella* Austen belongs to Rhiniidae. This is a large, yellow, night-flying species with rudimentary mouthparts that live in desert areas (Zumpt, 1957; Rognes, 2002). The larvae of *Villeneuveiella* have been observed attacking termite workers (Grunin, 1957), which supports the placement of this genus in Rhiniidae based on the aforementioned hypothesis

of an ant or termite association among rhiniids. *Villeneuveiella* was recovered as sister to (*Eurhyncomyia* + *Rhyncomyia*) in our ML concatenated analysis, but it was nested within *Cosmina* in our coalescent-based analysis. Both of these placements for *Villeneuveiella* are weakly supported and will need to be further studied in future research using a larger molecular dataset and broader taxon sampling for the entire lineage.

Calliphoridae. The blow flies are a key lineage for reaching an understanding of the evolution and phylogeny of the Oestroidea (McAlpine, 1989). Earlier authors highlighted the lack of morphological autapomorphies of calliphorids and considered this family to be nonmonophyletic (Griffiths, 1982; Rognes, 1997). This nonmonophyly is corroborated by this study and the previous phylogenomic analysis of Calyptratae by Kutty *et al.* (2019). Perhaps the most striking novel result regarding the calliphorids is the strong support for the sister-group relationships of Bengaliinae + Rhiniidae and Phumosiinae + Chrysomyinae. The sister grouping of Phumosiinae + Chrysomyinae is strongly supported by our data but in conflict with a clade proposed previously composed of Ameniinae, Mesembrinellidae and Phumosiinae and defined by unilarviparous reproduction (Guimarães, 1977; Ferrar, 1978; Meier *et al.*, 1999), elongated spermathecae and separated spermathecal ducts (Pape, 1992). Another remarkable result is the strong support for a sister-group relationship between the Andean lineage Toxotarsinae and the clade of Melanomyinae + Calliphorinae. Our analyses also find strong support for Luciliinae as sister to Toxotarsinae + (Melanomyinae + Calliphorinae), which agrees with earlier DNA analyses (Kutty *et al.*, 2010; Marinho *et al.*, 2012; Singh & Wells, 2013). Morphological characters support the sister grouping of Melanomyinae and Calliphorinae, and transferal of Melanomyinae species to Calliphorinae (Kurahashi, 1970; Downes, 1986; Schumann, 1986). Moreover, some Melanomyinae are snail predators/parasitoids (Shewell, 1987; Rognes, 1991) (not confirmed for genus *Melanomyia* Rondani), as are Helicoboscinae (not included here) and some Calliphorinae (e.g. *Onesia* Robineau-Desvoidy) (Rognes, 1991), with which they also share first instar larval characters (Shewell, 1987). Toxotarsinae has historically been considered a separate subfamily (Dear, 1985; Rognes, 1991; Pape, 1992), but it has also been included in or associated with, Chrysomyinae based on morphological characters, such as the stem-vein dorsally setose (Shewell, 1987). Our analyses support Chrysomyinae as a sister to Phumosiinae. However, the phylogenetic position of Chrysomyinae has historically been problematic. Chrysomyinae was recovered with the other carrion-eating blow flies like Luciliinae and Calliphorinae (Kutty *et al.*, 2010; Junqueira *et al.*, 2016; Cerretti *et al.*, 2017: Fig. 3), sister to (Bengaliinae + Rhiniidae) (Singh & Wells, 2013; Kutty *et al.*, 2019), clustered within a group consisting of Rhiniidae + Toxotarsinae (Pape, 1992), or sister to a clade consisting of the remaining blow-fly subfamilies plus the Rhinophoridae and Tachinidae (Cerretti *et al.*, 2017: Fig. 4).

No formal changes are proposed here until a more stable phylogenetic estimate can underpin any significant classification changes with regard to the calliphorids. Still, based on our phylogenetic analyses, two alternatives could be considered for achieving a more stable classification for Calliphoridae and related lineages in the future. These include (i) the monophyletic clades Bengaliinae, Chrysomyinae, Phumosiinae and clade B are raised to family-level status with clade B (composed of Calliphorinae, Luciliinae, Melanomyinae and Toxotarsinae) circumscribed as Calliphoridae, or, (ii) the families Rhiniidae and Rhinophoridae are synonymized under Calliphoridae to constitute subfamilies together with Bengaliinae, Calliphorinae, Chrysomyinae, Luciliinae, Melanomyinae, Phumosiinae and Toxotarsinae. Under any of these alternatives, Polleniidae would still be considered a separated family (Cerretti *et al.*, 2019). However, a stable and robust classification of all Calliphoridae lineages can only be achieved with the inclusion of other lineages not included here that is Ameniinae, Aphyssurinae, Auchmeromyinae and Helicoboscinae.

Polleniidae. The cluster flies or Polleniidae were represented in our study by the species *Pollenia rudis*. Our UCE data strongly supports Polleniidae as a sister to Tachinidae. The sister-group relationship between Polleniidae and Tachinidae is consistent with phylogenetic estimations using molecular datasets (Cerretti *et al.*, 2017; Cerretti *et al.*, 2019; Kutty *et al.*, 2019; Stireman *et al.*, 2019). The phylogenetic and taxonomic limits of the lineage of Polleniidae have recently been studied and clearly defined with regard to Tachinidae and to controversial taxa, such as *Alvamaja* Rognes and *Morinia* Robineau-Desvoidy (including the *carinata*-group), which were historically assigned to Rhinophoridae but recently reassigned to Polleniidae (Cerretti *et al.*, 2019). Pollenids are known as predators or parasitoids of earthworms (Rognes, 1991), but not all taxa seem to share these life strategies (Cerretti *et al.*, 2019).

Conclusions

Our phylogenomic approach combining taxon-specific, protein-encoding, UCE probes with a large taxon sampling obtained a well-supported phylogeny for Oestroidea. Our analyses also provided a strong phylogenetic hypothesis at the subfamily level, within the major clades of Oestroidea, including the traditionally challenging Calliphoridae. Future studies using UCEs should include and phylogenetically place enigmatic taxa, such as families Ulurumyiidae, Mystacinobiidae and subfamilies Ameniinae, Aphyssurinae, Auchmeromyinae and Helicoboscinae of Calliphoridae. We would also expect our taxon-specific UCE probes to be suitable for studying higher-level phylogenetic relationships within the whole Calyptratae clade. Our UCE probes designed to capture only protein-encoding UCEs make our dataset suitable for larger and more integrative phylogenomic studies using both genomic and transcriptomic resources. Thus, our taxon-specific probe design

provides a valuable toolset to address systematic questions, further our understanding of the timing of diversifications and help resolve long-standing controversial relationships within these fly radiations. Combining UCEs with other data sources (i.e. genome architecture, morphology, ecology and chemo-physiology) for an even broader taxon sampling could accelerate and advance the understanding of oestroid diverse morphologies, ecological roles in ecosystems and biogeography.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Specimen data. Specimen identity, preservation method, targeted tissue for extraction, collection data, specimen identifiers and repositories at Natural History Museums, DNA input data, contig data, UCE capture data.

Figure S1. Distribution of preservation methods in the specimens studied.

Figure S2. Specimen age *versus* DNA extracted and UCEs captured coded for preservation method. Specimen age *versus* DNA input obtained from (A) specimens preserved in 96% Ethanol, (C) old DNA aliquots, (E) specimens preserved in liquid Nitrogen and (G) dry pinned specimens. Specimen age *versus* UCE captured from: (B) specimens preserved in 96% Ethanol, (D) old DNA aliquots, (F) specimens preserved in liquid Nitrogen and (H) dry pinned specimens.

Figure S3. Percentage of parsimony-informative sites per matrix *versus* the maximum percentage of missing taxa at each UCE loci.

Figure S4. Global bootstrap support *versus* the maximum percentage of missing taxa at each UCE loci.

Figure S5. Heatmap of data completeness, global bootstrap support (GBS) and percentage of parsimony-informative sites per matrix. NA: does not apply.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study and the Calyptratae/Oestroidea-specific UCE probes are openly available in Dryad data at <https://doi.org/10.5061/dryad.3bk3j9kg1> (Buenaventura et al. 2020).

References

- Arce, B.J., Clout, S., Pat, D.L., Bharti, M., Pape, T. & Marshall, S.A. (2019) Viviparity and oviparity in termitophilous Rhiniidae (Diptera: Oestroidea) in the Western Ghats, India. *Oriental Insects*, **54**, 1–6. <https://doi.org/10.1080/00305316.2019.1618407>.
- Baca, S.M., Alexander, A., Gustafson, G.T. & Short, A.E.Z. (2017) Ultraconserved elements show utility in phylogenetic inference of Aedeptera (Coleoptera) and suggest paraphyly of 'Hydradephaga'. *Systematic Entomology*, **42**, 786–795. <https://doi.org/10.1111/syen.12244>.
- Blaimer, B.B., Brady, S.G., Schultz, T.R., Lloyd, M.W., Fisher, B.L. & Ward, P.S. (2015) Phylogenomic methods outperform traditional multi-locus approaches in resolving deep evolutionary history: a case study of formicine ants. *BMC Evolutionary Biology*, **15**, 271.
- Blaimer, B.B., LaPolla, J.S., Branstetter, M.G., Lloyd, M.W. & Brady, S.G. (2016a) Phylogenomics, biogeography and diversification of obligate mealybug-tending ants in the genus *Acropyga*. *Molecular Phylogenetics and Evolution*, **102**, 20–29. <https://doi.org/10.1016/j.ympev.2016.05.030>.
- Blaimer, B.B., Lloyd, M.W., Guillory, W.X. & Brady, S.G. (2016b) Sequence capture and phylogenetic utility of genomic ultraconserved elements obtained from pinned insect specimens. *PLoS One*, **11**, 1–20. <https://doi.org/10.1371/journal.pone.0161531>.
- Blaschke, J.D., Stireman, J.O., O'hara, J.E., Cerretti, P. & Moulton, J.K. (2018) Molecular phylogenetics and piercer evolution in the bug-killing flies (Diptera: Tachinidae: Phasiinae). *Systematic Entomology*, **43**, 218–238. <https://doi.org/10.1111/syen.12272>.
- Blumenstiel, B., Cibulskis, K., Fisher, S. et al. (2010) Targeted exon sequencing by in-solution hybrid selection. *Current Protocols in Human Genetics*, **66**, 18.4.1–18.4.24. <https://doi.org/10.1002/0471142905.hg1804s66>.
- Bolger, A.M., Usadel, B. & Lohse, M. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, **30**, 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Bossert, S. & Danforth, B.N. (2018) On the universality of target-enrichment baits for phylogenomic research. *Methods in Ecology and Evolution*, **9**, 1453–1460. <https://doi.org/10.1111/2041-210X.12988>.
- Branstetter, M.G., Longino, J.T., Ward, P.S. & Faircloth, B.C. (2017) Enriching the ant tree of life: enhanced UCE bait set for genome-scale phylogenetics of ants and other Hymenoptera. *Methods in Ecology and Evolution*, **8**, 768–776. <https://doi.org/10.1111/2041-210X.12742>.
- Buenaventura, E. & Pape, T. (2017a) Multilocus and multiregional phylogeny reconstruction of the genus *Sarcophaga* (Diptera, Sarcophagidae). *Molecular Phylogenetics and Evolution*, **107**, 619–629. <https://doi.org/10.1016/j.ympev.2016.12.028>.

- Buenaventura, E. & Pape, T. (2017b) Phylogeny, evolution and male genitalic functionality of Sarcophaginae (Diptera: Sarcophagidae). *Zoological Journal of the Linnean Society*, **183**, 808–906. <https://doi.org/10.1093/zoolinnean/zlx070>.
- Buenaventura, E., Camacho, G., Garcia, A. & Wolff, M. (2009) Sarcophagidae (Diptera) de importancia forense en Colombia: claves taxonómicas, notas sobre su biología y distribución. *Revista Colombiana de Entomología*, **35**, 189–196.
- Buenaventura, E., Whitmore, D. & Pape, T. (2017) 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>.
- Buenaventura, E., Szpila, K., Cassel, B.K., Wiegmann, B.M. & Pape, T. (2019) Anchored hybrid enrichment challenges the traditional classification of flesh flies (Diptera: Sarcophagidae). *Systematic Entomology*, **45**, 281–301. <https://doi.org/10.1111/syen.12395>.
- Buenaventura, E., Lloyd, M. W., Perilla-López, J. M., González, V. L., Thomas-Cabianca, A. & Dikow, T. (2020). *Protein-encoding ultra-conserved elements provide a new phylogenomic perspective of Oestroidea flies (Diptera, Calypttratae)*. *Dryad Digital Repository*. <https://doi.org/10.5061/dryad.3bk3j9kg1>.
- Byrd, J.H. & Castner, J.L. (2010) *Forensic Entomology: The Utility of Arthropods in Legal Investigations*, 2nd edn. Boca Raton: CRC Press.
- 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>.
- Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, **17**, 540–552.
- Cerretti, P., Lo Giudice, G. & Pape, T. (2014a) Remarkable Rhinophoridae in a growing generic genealogy (Diptera: Calypttratae, Oestroidea). *Systematic Entomology*, **39**, 660–690. <https://doi.org/10.1111/syen.12080>.
- Cerretti, P., O'Hara, J.E., Wood, D.M., Shima, H., Inclan, D.J. & Stireman, J.O. III (2014b) Signal through the noise? Phylogeny of the Tachinidae (Diptera) as inferred from morphological evidence. *Systematic Entomology*, **39**, 335–353.
- Cerretti, P., Stireman, J.O., Pape, T., O'Hara, J.E., Marinho, M.A.T., Rognes, K. & Grimaldi, D.A. (2017) First fossil of an oestroid fly (Diptera: Calypttratae: Oestroidea) and the dating of oestroid divergences. *PLoS One*, **12**, 1–24. <https://doi.org/10.1371/journal.pone.0182101>.
- Cerretti, P., Stireman, J.O., Badano, D., Gisondi, S., Rognes, K., Giudice, G.L. & Pape, T. (2019) Reclustering the cluster flies (Diptera: Oestroidea, Polleniidae). *Systematic Entomology*, **44**, 957–972. <https://doi.org/10.1111/syen.12369>.
- Cerretti, P., Badano, D., Gisondi, S., Giudice, G.L. & Pape, T. (2020) The world woodlouse flies (Diptera, rhinophoridae). *ZooKeys*, **2020**, 1–130. <https://doi.org/10.3897/zookeys.903.37775>.
- Crawford, N.G., Faircloth, B.C., McCormack, J.E., Brumfield, R.T., Winker, K. & Glenn, T.C. (2012) More than 1000 ultraconserved elements provide evidence that turtles are the sister group of archosaurs. *Biology Letters*, **8**, 783–786. <https://doi.org/10.1098/rsbl.2012.0331>.
- Crawford, N.G., Parham, J.F., Sellas, A.B. *et al.* (2015) A phylogenomic analysis of turtles. *Molecular Phylogenetics and Evolution*, **83**, 250–257. <https://doi.org/10.1016/j.ympev.2014.10.021>.
- Cuthbertson, A. (1933) The habits and life histories of some Diptera in Southern Rhodesia. *Proceedings of the Rhodesian Scientific Association*, **32**, 81–111.
- Cuthbertson, A. (1934) Biological notes on some Diptera in Southern Rhodesia. *Proceedings of the Rhodesian Scientific Association*, **33**, 32–50.
- Dear, J. (1985) A revision of the New World Chrysomyini (Diptera: Calliphoridae). *Revista Brasileira de Zoologia*, **3**, 109–169.
- Degnan, J.H. & Rosenberg, N.A. (2006) Discordance of species trees with their most likely gene trees. *PLoS Genetics*, **2**, 762–768. <https://doi.org/10.1371/journal.pgen.0020068>.
- Degnan, J.H. & Rosenberg, N.A. (2009) Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology & Evolution*, **24**, 332–340. <https://doi.org/10.1016/j.tree.2009.01.009>.
- Dell'Ampio, E., Meusemann, K., Szucsich, N.U. *et al.* (2014) Decisive data sets in phylogenomics: lessons from studies on the phylogenetic relationships of primarily wingless insects. *Molecular Biology and Evolution*, **31**, 239–249. <https://doi.org/10.1093/molbev/mst196>.
- Derkarabetian, S., Benavides, L.R. & Giribet, G. (2019) Sequence capture phylogenomics of historical ethanol-preserved museum specimens: unlocking the rest of the vault. *Molecular Ecology Resources*, **19**, 1531–1544. <https://doi.org/10.1111/1755-0998.13072>.
- Dikow, R.B., Frandsen, P.B., Turcatel, M. & Dikow, T. (2017) Genomic and transcriptomic resources for assassin flies including the complete genome sequence of *Proctacanthus coquillettii* (Insecta: Diptera: Asilidae) and 16 representative transcriptomes. *PeerJ*, **2017**, 1–20. <https://doi.org/10.7717/peerj.2951>.
- Downes, W. (1986) The Nearctic *Melanomya* and relatives (Diptera: Calliphoridae), a problem in Calypttrata classification. *Bulletin New York State Museum*, **460**, 1–35.
- Eggleston, P. & Belshaw, R. (1992) Insect parasitoids: and evolutionary overview. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **337**, 1–20.
- Faircloth, B. C. (2013). *Illumiprocessor: a trimmomatic wrapper for parallel adapter and quality trimming*. <https://doi.org/10.6079/J9ILL>
- Faircloth, B.C. (2016) PHYLUCE is a software package for the analysis of conserved genomic loci. *Bioinformatics*, **32**, 786–788.
- Faircloth, B.C. (2017) Identifying conserved genomic elements and designing universal bait sets to enrich them. *Methods in Ecology and Evolution*, **8**, 1103–1112. <https://doi.org/10.1111/2041-210X.12754>.
- Faircloth, B.C., McCormack, J.E., Crawford, N.G., Harvey, M.G., Brumfield, R.T. & Glenn, T.C. (2012) Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Systematic Biology*, **61**, 717–726. <https://doi.org/10.1093/sysbio/sys004>.
- Faircloth, B.C., Sorenson, L., Santini, F. & Alfaro, M.E. (2013) A Phylogenomic perspective on the radiation of ray-finned fishes based upon targeted sequencing of ultraconserved elements (UCEs). *PLoS One*, **8**, e65923. <https://doi.org/10.1371/journal.pone.0065923>.
- Faircloth, B.C., Branstetter, M.G., White, N.D. & Brady, S.G. (2015) Target enrichment of ultraconserved elements from arthropods provides a genomic perspective on relationships among Hymenoptera. *Molecular Ecology Resources*, **15**, 489–501. <https://doi.org/10.1111/1755-0998.12328>.
- Feener, D.H. Jr & Brown, B.V. (1997) Diptera as parasitoids. *Annual Review of Entomology*, **42**, 73–97.
- Ferrar, P. (1978) Macrolarviparous reproduction in *Euphumosia* (Diptera: Calliphoridae). *Journal of the Australian Entomological Society*, **17**, 13–17.
- Ferrar, P. (1987) *A guide to the breeding habits and immature stages of Diptera Cyclorrhapha*. *Entomograph* 8. E.J.Brill, Leiden and Copenhagen.
- da Fonseca, R.R., Albrechtsen, A., Themudo, G.E. *et al.* (2016) Next-generation biology: sequencing and data analysis approaches for non-model organisms. *Marine Genomics*, **30**, 3–13. <https://doi.org/10.1016/j.margen.2016.04.012>.
- Forthman, M., Miller, C.W. & Kimball, R.T. (2019) Phylogenomic analysis suggests Coreidae and Alydidae (Hemiptera: Heteroptera)

- are not monophyletic. *Zoologica Scripta*, **48**, 520–534. <https://doi.org/10.1111/zsc.12353>.
- Giarla, T.C. & Esselstyn, J.A. (2015) The challenges of resolving a rapid, recent radiation: empirical and simulated phylogenomics of Philippine shrews. *Systematic Biology*, **64**, 727–740. <https://doi.org/10.1093/sysbio/syv029>.
- Gilbert, M., Moore, W., Melchior, L. & Worobey, M. (2007) DNA extraction from dry museum beetles without conferring external morphological damage. *PLoS One*, **2**, e272.
- Giroux, M., Pape, T. & Wheeler, T.A. (2010) Towards a phylogeny of the flesh flies (Diptera: Sarcophagidae): morphology and phylogenetic implications of the acrophallus in the subfamily Sarcophaginae. *Zoological Journal of the Linnean Society*, **158**, 740–778. <https://doi.org/10.1111/j.1096-3642.2009.00561.x>.
- Grabherr, M.G., Haas, B.J., Yassour, M. *et al.* (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology*, **29**, 644–652.
- Griffiths, G.C.D. (1972) The phylogenetic classification of Diptera Cyclorrhapha, with special reference to the structure of the male postabdomen. *Series Entomologica*, **8**, 1–340.
- Griffiths, G.C.D. (1982) *On the systematic position of Mystacinobia (Diptera: Calliphoridae)*, Vol. **70–77**. *Memoirs of the Entomological Society*, Washington.
- Grunin, K.J. (1957) Neue arten der gattung *Villeneuveiella* Austen (Rhynchostrus Séguin) aus USSR und Iran (Diptera: Calliphoridae). *Entomologicheskoe Obozrenie*, **45**, 538–546.
- Guillory, W.X., French, C.M., Twomey, E.M. *et al.* (2020) Phylogenetic relationships and systematics of the Amazonian poison frog genus *Ameerega* using ultraconserved genomic elements. *Molecular Phylogenetics and Evolution*, **142**, 106638. <https://doi.org/10.1016/j.ympev.2019.106638>.
- Guimarães, J.H. (1971) Family Tachinidae. *A Catalogue of the Diptera of the Americas South of the United States* (ed. by N. Papavero), pp. 1–333. Departamento de Zoologia, Secretaria da Agricultura, São Paulo.
- Guimarães, J. H. (1977). A systematic revision of the Mesembrinellidae, stat. nov. (Diptera, Cyclorrhapha). *Arquivos de Zoologia do Estado de São Paulo*, 1–109.
- Gustafson, G.T., Alexander, A., Sproul, J.S., Pflug, J.M., Maddison, D.R. & Short, A.E.Z. (2019a) Ultraconserved element (UCE) probe set design: base genome and initial design parameters critical for optimization. *Ecology and Evolution*, **9**, 6933–6948. <https://doi.org/10.1002/ece3.5260>.
- Gustafson, G.T., Baca, S.M., Alexander, A.M. & Short, A.E.Z. (2019b) Phylogenomic analysis of the beetle suborder Adephaga with comparison of tailored and generalized ultraconserved element probe performance. *Systematic Entomology*, **45**, 552–570. <https://doi.org/10.1111/syen.12413>.
- Hedin, M., Derkarabetian, S., Alfaro, A., Ramírez, M.J. & Bond, J.E. (2019) Phylogenomic analysis and revised classification of atypoid mygalomorph spiders (Araneae, Mygalomorphae), with notes on arachnid ultraconserved element loci. *PeerJ*, **7**, e6864. <https://doi.org/10.7717/peerj.6864>.
- Huang, W., Li, L., Myers, J.R. & Marth, G.T. (2012) ART: a next-generation sequencing read simulator. *Bioinformatics*, **28**, 593–594.
- Junqueira, A.C.M., Azeredo-Espin, A.M.L., Paulo, D.F. *et al.* (2016) Large-scale mitogenomics enables insights into Schizophora (Diptera) radiation and population diversity. *Scientific Reports*, **6**, 1–13. <https://doi.org/10.1038/srep21762>.
- Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, **30**, 772–780.
- Kieran, T.J., Gordon, E.R.L., Forthman, M. *et al.* (2019) Insight from an ultraconserved element bait set designed for hemipteran phylogenetics integrated with genomic resources. *Molecular Phylogenetics and Evolution*, **130**, 297–303. <https://doi.org/10.1016/j.ympev.2018.10.026>.
- Kulkarni, S., Wood, H., Lloyd, M. & Hormiga, G. (2020) Spider-specific probe set for ultraconserved elements offers new perspectives on the evolutionary history of spiders (Arachnida, Araneae). *Molecular Ecology Resources*, **20**, 185–203. <https://doi.org/10.1111/1755-0998.13099>.
- Kumar, S., Filipski, A.J., Battistuzzi, F.U., Kosakovsky Pond, S.L. & Tamura, K. (2012) Statistics and truth in phylogenomics. *Molecular Biology and Evolution*, **29**, 457–472. <https://doi.org/10.1093/molbev/msr202>.
- Kurahashi, H. (1970) Tribe Calliphorini from Australian and Oriental regions, I. Melinda-group (Diptera: Calliphoridae). *Pacific Insects*, **12**, 519–542.
- Kurahashi, H. & Kirk-Spriggs, A.H. (2006) The Calliphoridae of Namibia (Diptera: Oestroidea). *Zootaxa*, **1322**, 131.
- Kutty, S.N., Pape, T., Wiegmann, B.M. & Meier, R. (2010) Molecular phylogeny of the Calyptratae (Diptera: Cyclorrhapha) with an emphasis on the superfamily Oestroidea and the position of Mystacinobiidae and McAlpine's fly. *Systematic Entomology*, **35**, 614–635. <https://doi.org/10.1111/j.1365-3113.2010.00536.x>.
- Kutty, S.N., Meusemann, K., Bayless, K.M. *et al.* (2019) Phylogenomic analysis of Calyptratae: resolving the phylogenetic relationships within a major radiation of Diptera. *Cladistics*, **35**, 605–622. <https://doi.org/10.1111/cla.12375>.
- Lambkin, C.L., Sinclair, B.J., Pape, T. *et al.* (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>.
- Lanfear, R., Frandsen, P., Wright, A.M., Senfeld, T. & Calcott, B. (2017) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, **34**, 772–773.
- Lemmon, E.M. & Lemmon, A.R. (2013) High-throughput genomic data in systematics and phylogenetics. *Annual Review of Ecology, Evolution, and Systematics*, **44**, 99–121. <https://doi.org/10.1146/annurev-ecolsys-110512-135822>.
- Lemmon, A.R., Emme, S.A. & Lemmon, E.M. (2012) Anchored hybrid enrichment for massively high-throughput phylogenomics. *Systematic Biology*, **61**, 727–744. <https://doi.org/10.1093/sysbio/sys049>.
- Lindahl, T. (1993) Instability and decay of the primary structure of DNA. *Nature*, **362**, 709–715.
- Lunter, G. & Goodson, M. (2011) Stampy: a statistical algorithm for sensitive and fast mapping of Illumina sequence reads. *Genome Research*, **21**, 936–939. <https://doi.org/10.1101/gr.111120.110>.
- Marinho, M.A.T., Junqueira, A.C.M., Paulo, D.F., Esposito, M.C., Villet, M.H. & Azeredo-Espin, A.M.L. (2012) Molecular phylogenetics of Oestroidea (Diptera: Calyptratae) with emphasis on Calliphoridae: insights into the inter-familial relationships and additional evidence for paraphyly among blowflies. *Molecular Phylogenetics and Evolution*, **65**, 840–854. <https://doi.org/10.1016/j.ympev.2012.08.007>.
- Marinho, M.A.T., Wolff, M., Ramos-Pastrana, Y., de Azeredo-Espin, A.M.L. & Amorim, D.d.S. (2017) The first phylogenetic study of Mesembrinellidae (Diptera: Oestroidea) based on molecular data: clades and congruence with morphological characters. *Cladistics*, **33**, 134–152. <https://doi.org/10.1111/cla.12157>.
- Matos-Maraví, P., Ritter, C.D., Barnes, C.J. *et al.* (2019) Biodiversity seen through the perspective of insects: 10 simple rules on 5 methodological choices and experimental design for genomic studies. *PeerJ*, **111**, 100–101.

- McAlpine, J.F. (1989) Phylogeny and classification of the Muscomorpha. *Manual of Nearctic Diptera* (ed. by J.F. McAlpine and D.M. Wood), pp. 1397–1518. Research Branch, Agriculture Canada, Canadian Government Publishing Centre, Ottawa, Ontario.
- McCormack, J.E., Huang, H. & Knowles, L.L. (2009) Maximum likelihood estimates of species trees: how accuracy of phylogenetic inference depends upon the divergence history and sampling design. *Systematic Biology*, **58**, 501–508. <https://doi.org/10.1093/sysbio/syp045>.
- McCormack, J.E., Faircloth, B.C., Crawford, N.G., Gowaty, P.A., Brumfield, R.T. & Glenn, T.C. (2012) Ultraconserved elements are novel phylogenomic markers that resolve placental mammal phylogeny when combined with species tree analysis. *Genome Research*, **22**, 746–754. <https://doi.org/10.1101/gr.125864.111>.
- McCormack, J.E., Harvey, M.G., Faircloth, B.C., Crawford, N.G., Glenn, T.C. & Brumfield, R.T. (2013a) A phylogeny of birds based on over 1,500 loci collected by target enrichment and high-throughput sequencing. *PLoS One*, **8**, e54848. <https://doi.org/10.1371/journal.pone.0054848>.
- McCormack, J.E., Hird, S.M., Zellmer, A.J., Carstens, B.C. & Brumfield, R.T. (2013b) Applications of next-generation sequencing to phylogeography and phylogenetics. *Molecular Phylogenetics and Evolution*, **66**, 526–538. <https://doi.org/10.1016/j.ympev.2011.12.007>.
- McCormack, J.E., Tsai, W.L.E. & Faircloth, B.C. (2016) Sequence capture of ultraconserved elements from bird museum specimens. *Molecular Ecology Resources*, **16**, 1189–1203. <https://doi.org/10.1111/1755-0998.12466>.
- Meier, R., Kotrba, M. & Ferrar, P. (1999) Ovoviviparity and viviparity in the Diptera. *Biological Reviews*, **74**, 199–258. <https://doi.org/10.1111/j.1469-185X.1999.tb00186.x>.
- Meiklejohn, K.A., Wallman, J.F. & Dowton, M. (2013) DNA barcoding identifies all immature life stages of a forensically important flesh fly (Diptera: Sarcophagidae). *Journal of Forensic Sciences*, **58**, 1–7. <https://doi.org/10.1111/j.1556-4029.2012.02220.x>.
- Metzker, M. (2010) Sequencing technologies - the next generation. *Nature Reviews Genetics*, **860**, 31–46.
- Michelsen, V. & Pape, T. (2017) Ulurumyiidae – a new family of calyptrate flies (Diptera). *Systematic Entomology*, **42**, 826–836. <https://doi.org/10.1111/syen.12252>.
- Mulieri, P.R., Mariluis, J.C. & Aballay, F.H. (2012) Two species of *Microcerella* (Diptera: Sarcophagidae) found in highland arid landscapes of Argentina, during forensic studies. *Journal of Medical Entomology*, **49**, 183–191.
- Nelson, L.A., Lambkin, C.L., Batterham, P. *et al.* (2012) Beyond barcoding: a mitochondrial genomics approach to molecular phylogenetics and diagnostics of blowflies (Diptera: Calliphoridae). *Gene*, **511**, 131–142. <https://doi.org/10.1016/j.gene.2012.09.103>.
- Nguyen, L.T., Schmidt, H.A., Von Haeseler, A. & Minh, B.Q. (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, **32**, 268–274. <https://doi.org/10.1093/molbev/msu300>.
- O'Hara, J.E. & Wood, D.M. (2004) *Catalogue of the Tachinidae (Diptera) of America North of Mexico*. Associated Publishers, Gainesville, Florida.
- O'Hara, J.E. (2002) Revision of the Polideini (Tachinidae) of America north of Mexico. *Studia Dipterologica*, **10**, 170.
- O'Hara, J. E. & Henderson, S. J. (2020). *World genera of the Tachinidae (Diptera) and their regional occurrence. Version 2.1*. North American Dipterists Society. URL <http://www.nadsdiptera.org/Tach/WorldTachs/Genera/Worldgenera.htm> [accessed on 9 March 2020].
- O'Hara, J.E., Henderson, S.J. & Wood, D.M. (2019) *Preliminary checklist of the Tachinidae of the world. Version 1.0*, p. 681.
- Oliveros, C.H., Field, D.J., Ksepka, D.T. *et al.* (2019) Earth history and the passerine superradiation. *Proceedings of the National Academy of Sciences of the United States of America*, **116**, 7916–7925. <https://doi.org/10.1073/pnas.1813206116>.
- Otranto, D., Traversa, D., Guida, B., Tarsitano, E., Fiorente, P. & Stevens, J. (2003) Molecular characterization of the mitochondrial cytochrome oxidase I gene of Oestridae species causing obligate myiasis. *Medical and Veterinary Entomology*, **17**, 307–315. <https://doi.org/10.1046/j.1365-2915.2003.00442.x>.
- Pape, T. (1986) A phylogenetic analysis of the woodlouseflies (Diptera, Rhinophoridae). *Tijdschrift voor Entomologie*, **129**, 15–34.
- Pape, T. (1992) Phylogeny of the Tachinidae family-group (Diptera: Calyptratae). *Tijdschrift voor Entomologie*, **135**, 43–86.
- Pape, T. (1996) Catalogue of the Sarcophagidae of the world (Insecta: Diptera). *Memoirs of Entomology International*, **8**, 1–558.
- Pape, T. (1998) A new genus of Paramacronychiinae (Diptera: Sarcophagidae), argued from a genus-level cladistic analysis. *Systematic Entomology*, **23**, 187–200.
- Pape, T. (2001) Phylogeny of Oestridae (Insecta: Diptera). *Systematic Entomology*, **26**, 133–171.
- Pape, T. (2006) Phylogeny and evolution of the bot flies. *The Oestrid Flies: Biology, Host-Parasite Relationships, Impact and Management* (ed. by D. Colwell, P. Scholl and M. Hall), pp. 20–50. CABI Publishers, Wallingford and Cambridge, MA.
- Pape, T. & Arnaud, P.H. (2001) *Bezzimyia* – a genus of native New World rhinophoridae (Insecta, Diptera). *Zoologica Scripta*, **30**, 257–297. <https://doi.org/10.1046/j.1463-6409.2001.00064.x>.
- Pape, T., Blagoderov, V. & Mostovski, M.B. (2011) Order DIPTERA Linnaeus, 1758. *Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness* (ed. by Z. Zhang Zootaxa: Magnolia Press, pp. 222–229.
- Peris, S. (1952) La subfamilia Rhiniinae (Dipt. Calliphoridae). *Annales Estación Experimental Aula Dei*, **3**, 1–224.
- Piwczyński, M., Szpila, K., Grzywacz, A. & Pape, T. (2014) A large-scale molecular phylogeny of flesh flies (Diptera: Sarcophagidae). *Systematic Entomology*, **39**, 783–799. <https://doi.org/10.1111/syen.12086>.
- Piwczyński, M., Pape, T., Deja-Sikora, E., Sikora, M., Akbarzadeh, K. & Szpila, K. (2017) Molecular phylogeny of Miltogramminae (Diptera: Sarcophagidae): implications for classification, systematics and evolution of larval feeding strategies. *Molecular Phylogenetics and Evolution*, **116**, 49–60. <https://doi.org/10.1016/j.ympev.2017.07.001>.
- Quinlan, A.R. (2014) BEDTools: the swiss-ermy tool for genome feature analysis. *Current Protocols in Bioinformatics*, **47**, 11.12.1–11.12.34. <https://doi.org/10.1002/0471250953.bi1112s47>.
- Rognes, K. (1986) The systematic position of *Helicobosca* Bezzi with a discussion of the monophyly of the calyptrate families Calliphoridae, Rhinophoridae, Sarcophagidae and Tachinidae (Diptera). *Entomologica Scandinavica*, **17**, 75–92.
- Rognes, K. (1991) *Blowflies (Diptera, Calliphoridae) of Fennoscandia and Denmark Ed Fauna Entomologica Scandinavica Vol. 24*. E. J. Brill/Scandinavian Science Press Ltd, Leiden.
- Rognes, K. (1997) The Calliphoridae (blowflies) (Diptera: Oestroidea) are not a monophyletic group. *Cladistics*, **13**, 27–66.
- Rognes, K. (2002) Blowflies (Diptera: Calliphoridae) of Israel and adjacent areas, including a new species from Tunisia. *Insect Systematics & Evolution*, **59**, 1–148.
- Rognes, K. (2013) A new species in the genus *Pseudorhynchomyia* Peris, 1952 and the identity of *P. deserticola* Zumpt and Argo, 1978 (Diptera, Rhiniidae). *Zootaxa*, **3736**, 249–264. <https://doi.org/10.11646/zootaxa.3736.3.3>.

- Sabrosky, C. W. & Arnaud, P. H. (1965). Family Tachinidae (Larvaevoridae). In *A Catalog of the Diptera of America North of Mexico, Agriculture Handbook 276*. (ed. by A. Stone, C. W. Sabrosky, W. W. Wirth, R. H. Foote & J. R. Coulson) pp. 961–1108. (United States Department of Agriculture, Agricultural Research Service: Washington, DC)
- Schumann, H. (1986) Family Calliphoridae. *Catalogue of Palaearctic Diptera. Calliphoridae – Sarcophagidae*, Vol. 12 (ed. by A. Soos and L. Papp), pp. 11–58. Elsevier, Amsterdam, The Netherlands.
- Shendure, J., Balasubramanian, S., Church, G.M., Gilbert, W., Rogers, J., Schloss, J.A. & Waterston, R.H. (2017) DNA sequencing at 40: past, present and future. *Nature*, **550**, 345–353. <https://doi.org/10.1038/nature24286>.
- Shewell, G.E. (1987) Calliphoridae. McAlpine, J.F., Peterson, B.V., Shewell, G.E., Teskey, H.J., Vockeroth, J.R. & Wood, D.M., *Manual of Nearctic Diptera*. Agriculture Canada Monograph No. 28, Vol. 2 Ottawa, Ontario: Canadian Government Publishing Centre, pp. 1133–1145.
- Singh, S. & Rognes, K. (2014) Taxonomy, biology and biocontrol potential of *Termitoemus marshalli* Baranov (Diptera: Calliphoridae, Bengaliinae) – a voracious predator of *Odontotermes* Holmgren termites (Isoptera: Termitidae) in India. *Studia dipterologica*, **21**, 267–282.
- Singh, B. & Wells, J.D. (2013) Molecular systematics of the Calliphoridae (Diptera: Oestroidea): evidence from one mitochondrial and three nuclear genes. *Journal of Medical Entomology*, **50**, 15–23. <https://doi.org/10.1603/ME11288>.
- Smith, B.T., Harvey, M.G., Faircloth, B.C., Glenn, T.C. & Brumfield, R.T. (2014) Target capture and massively parallel sequencing of ultra-conserved elements for comparative studies at shallow evolutionary time scales. *Systematic Biology*, **63**, 83–95. <https://doi.org/10.1093/sysbio/syt061>.
- Song, Z., Wang, X. & Liang, G. (2008) Molecular evolution and phylogenetic utility of the internal transcribed spacer 2 (ITS2) in Calyptratae (Diptera: Brachycera). *Journal of Molecular Evolution*, **67**, 448–464. <https://doi.org/10.1007/s00239-008-9144-y>.
- Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics (Oxford, England)*, **30**, 1312–1323. <https://doi.org/10.1093/bioinformatics/btu033>.
- Starrett, J., Derkarabetian, S., Hedin, M., Bryson, R., McCormack, J. & Faircloth, B.C. (2016) High phylogenetic utility of an ultraconserved element probe set designed for Arachnida. *Molecular Ecology Resources*, **17**, 812–823. <https://doi.org/10.1111/1755-0998.12621>.
- Stireman, J.O.I., O'Hara, J.E. & Wood, D.M. (2006) Tachinidae: evolution, behavior, and ecology. *Annual Review of Entomology*, **51**, 525–555.
- Stireman, J.O., Cerretti, P., O'Hara, J.E., Blaschke, J.D. & Moulton, J.K. (2019) Molecular phylogeny and evolution of world Tachinidae (Diptera). *Molecular Phylogenetics and Evolution*, **139**, 106358. <https://doi.org/10.1016/j.ympev.2018.12.002>.
- Sun, K., Meiklejohn, K.A., Faircloth, B.C., Glenn, T.C., Braun, E.L. & Kimball, R.T. (2014) The evolution of peafowl and other taxa with ocelli (eyespot): a phylogenomic approach. *Proceedings. Biological Sciences/The Royal Society*, **281**, 20140823. <https://doi.org/10.1098/rspb.2014.0823>.
- Tagliacollo, V.A. & Lanfear, R. (2018) Estimating improved partitioning schemes for ultraconserved elements. *Molecular Biology and Evolution*, **35**, 1798–1811.
- Townsend, C.H.T. (1936) *Manual of myology. Part IV*. Charles Townsend & Filhos, Itaquaquecetuba.
- Tschorsnig, H. (1985) Die strukur des mann lichen posrabdomens der Rhinophoridae (Diptera). *Stuttgarter Beiträge zur Naturkunde Serie. A*, **375**, 1–18.
- Vairo, K.P., Caneparo, M.F.d.C., Corrêa, R.C., Preti, D. & Moura, M.O. (2017) Can Sarcophagidae (Diptera) be the most important entomological evidence at a death scene? *Microcerella halli* as a forensic indicator. *Revista Brasileira de Entomologia*, **61**, 275–276. <https://doi.org/10.1016/j.rbe.2017.06.004>.
- Van Dam, M.H., Lam, A.W., Sagata, K. *et al.* (2017) Ultraconserved elements (UCEs) resolve the phylogeny of Australasian smurf-weevils. *PLoS One*, **12**, 1–21. <https://doi.org/10.1371/journal.pone.0188044>.
- Van Dam, M.H., Trautwein, M., Spicer, G.S. & Esposito, L. (2019) Advancing mite phylogenomics: designing ultraconserved elements for Acari phylogeny. *Molecular Ecology Resources*, **19**, 465–475. <https://doi.org/10.1111/1755-0998.12962>.
- Vicoso, B. & Bachtrog, D. (2015) Numerous transitions of sex chromosomes in Diptera. *PLoS Biology*, **13**, 1–22. <https://doi.org/10.1371/journal.pbio.1002078>.
- Wells, J.D., Pape, T. & Sperling, F.A. (2001) DNA-based identification and molecular systematics of forensically important Sarcophagidae (Diptera). *Journal of Forensic Sciences*, **46**, 1098–1102.
- Whitworth, T. & Yusseff-Vanegas, S. (2019) A revision of the genera and species of the Neotropical family Mesembrinellidae (Diptera: Oestroidea). *Zootaxa*, **4659**, 1–146. <https://doi.org/10.11646/zootaxa.4659.1.1>.
- Wiegmann, B.M., Trautwein, M.D., Winkler, I.S. *et al.* (2011) Episodic radiations in the fly tree of life. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 5690–5695.
- 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>.
- Wood, D.M. (1986) Are Cuterebridae, Gasterophilidae, Hypodermatidae and Oestridae a monophyletic group? *Abstracts of the First International Congress of Dipterology (Budapest, 17th–24th August, 1986)* (ed. by B. Darvas and L. Papp), p. 261. Hungarian Academy of Sciences, Budapest.
- Wood, D.M. (1987a) Rhinophoridae. *Manual of Nearctic Diptera Agriculture Canada, Volume 2, Monograph 28* (ed. by J.F. McAlpine, B.V. Peterson, G.E. Shewell, H.J. Teskey, J.R. Vockeroth and D.M. Wood), pp. 1187–1191. Research Branch, Agriculture Canada, Canadian Government Publishing Centre, Ottawa, Ontario.
- Wood, D.M. (1987b) Tachinidae. *Manual of Nearctic Diptera Agriculture Canada, Volume 2, Monograph 28* (ed. by J.F. McAlpine, B.V. Peterson, G.E. Shewell, H.J. Teskey, J.R. Vockeroth and D.M. Wood), pp. 1193–1269. Ottawa, Ontario: Research Branch, Agriculture Canada, Canadian Government Publishing Centre.
- Wood, H.M., González, V.L., Lloyd, M., Coddington, J. & Scharff, N. (2018) Next-generation museum genomics: phylogenetic relationships among palpimanoid spiders using sequence capture techniques (Araneae: Palpimanoidea). *Molecular Phylogenetics and Evolution*, **127**, 907–918. <https://doi.org/10.1016/j.ympev.2018.06.038>.
- Yan, L., Zhang, M., Gao, Y., Pape, T. & Zhang, D. (2017) First mitogenome for the subfamily Miltogramminae (Diptera: Sarcophagidae) and its phylogenetic implications. *European Journal of Entomology*, **114**, 422–429. <https://doi.org/10.14411/eje.2017.054>.
- Zhang, D., Yan, L., Zhang, M. *et al.* (2016a) Phylogenetic inference of Calyptrates, with the first mitogenomes for Gasterophilinae (Diptera: Oestridae) and Paramacronychiinae (Diptera: Sarcophagidae). *International Journal of Biological Sciences*, **12**, 489–504. <https://doi.org/10.7150/ijbs.12148>.

- Zhang, M., Buenaventura, E., Pape, T. & Zhang, D. (2016b) Molecules and morphology unite *Sarcophaga* (*Stackelbergeola*) Rohdendorf and *S. (Rohdendorfsca)* Grunin within megadiverse *Sarcophaga* Meigen (*sensu lato*) (Diptera, Sarcophagidae). *Invertebrate Systematics*, **30**, 463–478. <https://doi.org/10.1071/IS16007>.
- Zhang, C., Rabiee, M., Sayyari, E. & Mirarab, S. (2018) ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. *BMC Bioinformatics*, **19**, 15–30. <https://doi.org/10.1186/s12859-018-2129-y>.
- Zhao, Z., Su, T.J., Chesters, D. *et al.* (2013) The mitochondrial genome of *Elodia flavipalpis* Aldrich (Diptera: Tachinidae) and the evolutionary timescale of Tachinid flies. *PLoS One*, **8**, e61814. <https://doi.org/10.1371/journal.pone.0061814>.
- Zumt, F. (1957) Some remarks on the classification of the Oestridae s. lat (Diptera). *Journal of the Entomological Society of Southern Africa*, **20**, 154–161.
- Zumt, F. (1958). Calliphoridae (Diptera Cyclorrapha) part II: Rhiniini. *Exploration du Parc National Albert Mission G. F. de Witte (1933–1935)* **92**, 1–207.
- Zumt, F. (1965) *Myiasis in Man and Animals in the Old World*. London: Butterworths, London.

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