Departamento de Zoologia
Instituto de Biociências
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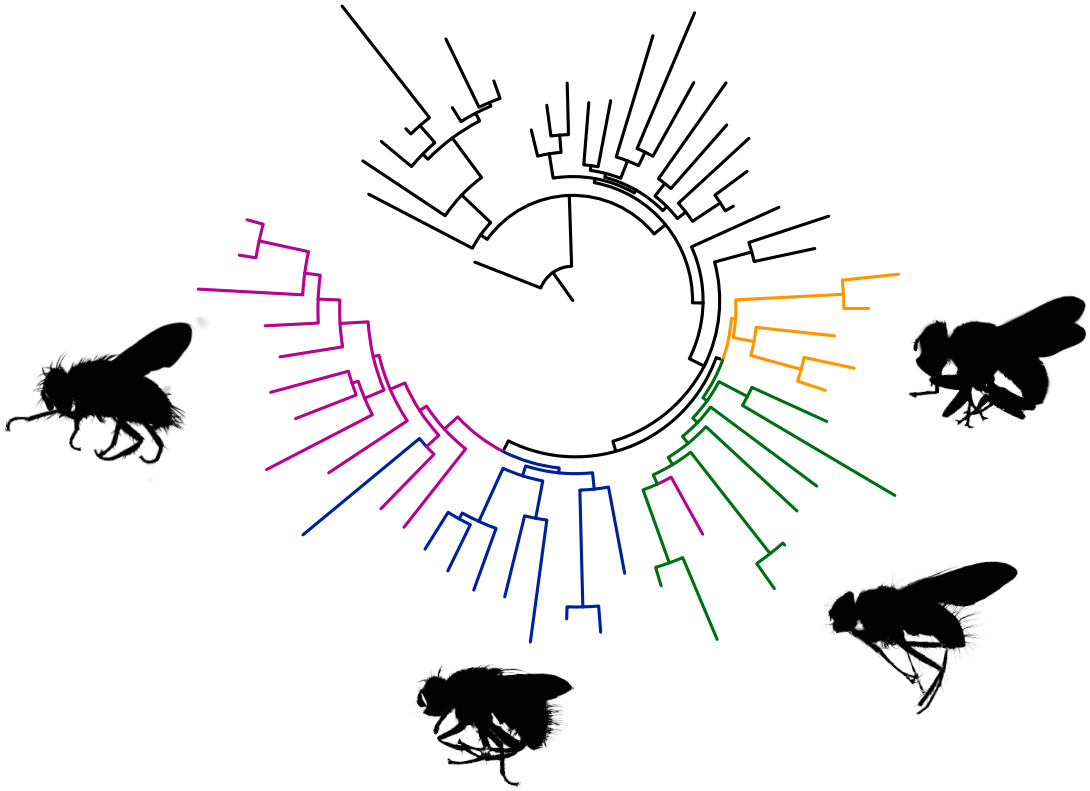
Filogenia molecular de Tachinidae (Diptera, Brachycera, Calyptratae) baseado em sequenciamento de nova geração, com enfoque nos limites e relações subfamiliares

Molecular Phylogeny of Tachinidae (Diptera, Brachycera, Calyptratae) based on next-generation sequencing, focusing on subfamilies boundaries and relationships



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À minha amada família
e ao Lucas. Que me
apoiaram incondicionalmente

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Resumo

Tachinidae é uma das maiores famílias da ordem Diptera. Elas são encontradas em praticamente todos os ambientes terrestres pelo mundo, incluindo desertos, florestas, pastagens, montanhas e tundra. Apresentamos aqui a primeira análise filogenômica dessa família utilizando dados transcriptômicos. A análise é baseada em 36 espécies do grupo interno que compõem os taquinídeos, e 23 espécies do grupo externo, representando as outras famílias de Oestroidea. Quatro matrizes foram montadas: três matrizes de aminoácidos com diferentes coberturas, 100% de cobertura (92 genes codificadores de proteínas de cópia única), 75% de cobertura (1304 genes codificadores de proteínas de cópia única) e 50% de cobertura (1890 genes codificadores de proteínas de cópia única); e uma de nucleotídeos com 75% de cobertura (1304 genes codificadores de proteínas de cópia única). De modo geral, as topologias são bem resolvidas, com alto suporte nos nós e com poucas alterações entre as três árvores obtidas com variação de cobertura, com diferentes métodos de análise (máxima verossimilhança, máxima parcimônia), e com as matrizes de aminoácido e nucleotídeo. A maioria dos nós na análise de Máxima Verossimilhança possui 100% de suporte de *ultrafast bootstrap*. As análises de Máxima Parcimônia também possuem estabilidade de 100% de jackknife ou bootstrap em quase todos os nós. Entretanto, a árvore de máxima parcimônia também apresenta mais variação em suas análises de suporte por Bremer, variando entre 125 e 18442. Esse estudo recuperou as quatro subfamílias de Tachinidae com o seguinte relacionamento: Phasiinae + Dexiinae e Tachininae + Exoristinae. Importante ressaltar alguns resultados interessantes obtidos no estudo. A tribo Myiophasiini (Tachininae) formou uma linhagem separada, aparecendo como grupo-irmão de todos os outros Tachinidae. A tribo Neotropical Iceliini (previamente em Tachininae) é recuperada dentro de Exoristinae, irmão de *Winthemia.* Por fim, Masyphyini (previamente em Exoristinae) é recuperado dentro de Dexiinae, próximo de Dexiini. De modo geral, nossos resultados são congruentes com estudos recentes envolvendo a família Tachinidae. Espera-se que esses resultados possam formar uma base para estudos futuros sobre a filogenia de Tachinidae.

Abstract

The Tachinidae is one of the largest families of Diptera. They are found in nearly all terrestrial environments throughout the world, including deserts, forests, grasslands, mountains, and tundra. The first phylogenomic analyses of Tachinidae using transcriptomic data is presented here. The analyses are based on 36 species for the ingroup, composing the tachinids, and 23 species for the outgroup, representing the other Oestroidea families. Four datasets were constructed: three for amino acid data with different coverages, 100% coverage (92 single-copy protein-coding genes), 75% coverage (1304 single-copy protein-coding genes), and 50% coverage (1890 single-copy protein-coding genes); and one for nucleotide data with 75% coverage (1304 single-copy protein-coding genes). Overall, the obtained topologies are well resolved, with strong node support and small changes among the trees obtained with variation of coverage and different approaches (maximum likelihood, maximum parsimony), and with amino acid and nucleotide matrices. Most nodes in Maximum Likelihood analyses have 100% ultrafast bootstrap support. The Maximum Parsimony analyses also show stability with 100% support with jackknife and bootstrap in almost every node. However, the maximum parsimony tree presents more variation with Bremer support ranging from 125 to 18442. The analyses recovered the four tachinid subfamilies with the following relationship: Phasiinae + Dexiinae and Tachininae + Exoristinae. Interestingly, the tribe Myiophasiini (Tachininae) forms a different lineage, a clade sister to all the remaining Tachinidae. The Neotropical tribe Iceliini (former Tachininae) is recovered within Exoristinae, sister to *Winthemia*. Also, Masyphyini (former Exoristinae) is recovered within Dexiinae, close to Dexiini. In general, results presented herein are congruent with recent studies that include tachinids. Hopefully, these results can be a scaffold for future studies with Tachinidae phylogeny.

Introdução Geral

Tachinidae é uma das maiores famílias em número de espécies da ordem Diptera, com aproximadamente 8.500 espécies descritas (O'Hara, 2013). A diversidade da família é muito grande, principalmente na região neotropical, que possui 3.032 espécies em 775 gêneros e 57 tribos (O'Hara & Henderson, 2020). Tachinidae é apresentada como um grupo verdadeiro a partir de oito apomorfias não homoplásicas: plastrão dorsal do ovo ausente, membrana do ovo sem coloração, ovolarviparidade, mandíbulas reduzidas no primeiro instar larval, labro fortemente desenvolvido no primeiro instar, subescutelo fortemente convexo, ovissaco da fêmea muito longo e geralmente espiralado quando cheio, e ovissaco da fêmea envelopado por uma rede traqueal bem desenvolvida (Cerretti *et al.,* 2014). É caracterizado por suas larvas serem endoparasitóides de artrópodes, em sua maioria insetos. Dessa forma, atuam como reguladores de populações de grandes grupos de Coleoptera, Hemiptera e Lepidoptera, e, por isso, são extremamente importantes para controle biológico de pragas agrícolas (Stireman *et al.,* 2006).

As subfamílias de Tachinidae tem um longo histórico de mudanças e incertezas nas classificações. Crosskey (1976) propôs cinco subfamílias, baseando-se nas características de quetotaxia e pruinosidade: Phasiinae, Dufouriinae, Proseninae, Tachininae e Goniinae. Herting (1984), por sua vez, propôs quatro subfamílias utilizando prioritariamente aspectos biológicos e adaptações como critério: Exoristinae, Tachininae, Phasiinae e Dexiinae. Wood (1987), baseado em caracteres morfológicos como quetotaxia, morfologia dos ovos, além de comportamento de oviposição e relação parasita-hospedeiro, sugeriu as mesmas quatro subfamílias. Atualmente, essa classificação em quatro subfamílias é a mais amplamente aceita (Herting, 1984; Wood, 1987; O'Hara & Wood, 2004; Cerretti *et al.,* 2014), com aproximadamente 50 tribos (O'Hara & Wood, 2004), porém um estudo recente (Santis, 2016) demonstra que talvez uma quinta subfamília possa ser reconhecida.

Apesar da monofilia e composição de Tachinidae estar relativamente estável, questões sobre sua origem e relações dentro de Oestroidea não estão. Em particular a identidade do grupo-irmão de Tachinidae permanece como uma incerteza, incluindo Sarcophagidae (Pape, 1992; Rognes, 1997), "Calliphoridae" (Wiegmann *et al.,* 2011), Mesembrinellidae (Marinho *et al.,* 2012), Oestridae (Zhang *et al.,* 2016), e Rhinophoridae + Polleniinae (Kutty *et al.,* 2010) e Polleniinae (Nelson *et al.,* 2012; Singh & Wells, 2013; Winkler *et al.,* 2015; Cerretti *et al.,* 2017; Blaschke *et al.,* 2018; Cerretti *et al.,* 2019; Kutty *et al.,* 2019; Stireman *et al.,* 2019; Buenaventura *et al.,* 2020).

Devido à grande quantidade de homoplasias e reversões morfológicas, a classificação de subgrupos de Tachinidae mostrou-se muito complexa. Assim, alguns pesquisadores começaram a utilizar dados moleculares para tentar definir e detalhar melhor essas relações. Stireman (2002) e Tachi & Shima (2010) apresentaram os primeiros estudos moleculares sobre Tachinidae, com enfoque na subfamília Exoristinae. Stireman (2002) utilizou dois genes (28S rDNA e EFI) recuperando somente Exoristinae como monifilético. A classificação encontrada das subfamílias foi "Tachinidae" + "Phasiinae" e "Dexiinae" + Exoristinae. Por outro lado, Tachi & Shima (2010) usaram quatro genes (16S, 18S, 28S rDNA e *White*). Eles recuperaram Tachinidae e Exoristinae como monofiléticos e o relacionamento das subfamílias como a seguinte: "Dexiinae" + ("Phasiinae" + ("Tachininae" + Exoristinae)). Ambos os estudos encontraram Sarcophagidae como grupo irmão de Tachinidae. Tanto Stireman (2002), quanto Tachi & Shima (2010) obtiveram uma amostragem taxonômica muito baixa para as outras subfamílias que não eram o enfoque do estudo.

Cerretti *et al*. (2014) apresentaram a primeira análise morfológica da família Tachinidae, com amostragem de 492 espécies. Esse estudo mostrou algumas relações diferentes do que previamente obtido. Phasiinae e Exoristinae foram recuperados como monofiléticos. Em contrapartida, Tachininae foi recuperado como polifilético com a linhagem Myiophasiini + Palpostomatini como grupo-irmão de todos os tachinídeos restantes. Dexiinae também foi encontrado como polifilético com Eutherini dentro de Exoristinae. A relação das subfamílias é recuperada como "Dexiinae" + Phasiinae e Exoristinae + "Tachininae".

Blaschke *et al*. (2018) apresentaram uma análise filogenética detalhada focada na subfamília Phasiinae utilizando quatro genes nucleares codificadores de proteína. Eles confirmaram as relações subfamiliares de Cerretti *et al*. (2014), apresentando Phasiinae + Dexiinae e Exoristinae + Tachininae, com todos monofiléticos. Blaschke *et al*. (2018) recuperaram Polleniidae como grupo-irmão de Tachinidae.

Stireman *et al*. (2019) conduziram a análise molecular mais detalhada da família até o momento. Eles usaram quatro genes (28S rDNA, CAD, MAC, MCS) com uma amostragem de 504 terminais. A família e as subfamílias Phasiinae e Exoristinae foram recuperados como monofiléticas. Tachininae mais uma vez é recuperada como polifilética com as linhagens Myiophasiini + Macquartini como grupo-irmão de todos os outros tachinídeos. Polleniidae também é recuperado como grupo-irmão de Tachinidae.

Recentemente, Kutty *et al*. (2019) apresentaram a primeira hipótese filogenômica para Calyptratae baseado em dados de transcriptomas. Amostraram 40 espécies de Calyptratae de 14 diferentes famílias e 1 espécie de *Drosophila melanogaster* como grupo externo. Tachinidae foi obtida como monofilética e o seguinte relacionamento entre subfamílias ("Dexiinae" + Phasiinae) + ("Tachininae" + Exoristinae). O grupo-irmão de Tachinidae também foi recuperado como Polleniidae. Kutty *et al*. (2019) afirmaram também que a relação entre as subfamílias de Tachinidae é ainda controversa, principalmente, devido ao fato de Dexiinae e Tachininae possivelmente não serem monofiléticas. Até o momento, não há nenhum estudo publicado com sequenciamento de nova geração para a família Tachinidae.

Os estudos com transcriptomas abriram caminho para estudar informações genéticas e funcionais armazenadas em qualquer organismo em escala e velocidade sem precedentes (Haas *et al*., 2013). Sendo o transcriptoma somente uma fração do genoma, os dados de RNA-Seq podem prover um caminho muito mais rápido e relativamente barato, ao alcance de qualquer laboratório com recursos razoáveis (Haas *et al*., 2013).

De acordo com Hittinger *et al.* (2010), transcriptomas oferecem fontes alternativas de sequência de ortólogos que são mais fáceis de amostrar do que genomas completos por três motivos. Primeiro, depois do processamento de RNA, transcriptomas são tipicamente muito menores do que um genoma. Segundo, transcriptomas contêm poucas regiões de sequências simples e elementos repetitivos. E terceiro, a grande abundância e desigualdade de transcritos (que variam em mais de cinco ordens de magnitude) significa que mesmo sequências de baixa cobertura devem fornecer uma profundidade de algumas centenas de loci simplesmente por sequenciar transcritos em proporção à sua representação na biblioteca.

Apesar da recente disseminação de estudos filogenéticos moleculares em Diptera, em sua maioria com sequenciamento tradicional ou genoma mitocondrial, ainda não há trabalhos com NGS especificamente para Tachinidae. O enfoque principal desse projeto é analisar sequências de transcriptomas e genes ortólogos para compreender as relações entre as subfamílias de Tachinidae.

Essa dissertação possui um capítulo em formato de artigo científico. Já está escrito na língua inglesa, porém a revista científica para submissão ainda não foi escolhida.

Referências

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): Phasiine phylogenetics and piercer evolution. Systematic Entomology. 43:218–238.

Buenaventura E., Lloyd M.W., Perilla López J.M., González V.L., Thomas-Cabianca A., Dikow T. 2020. Protein-encoding ultraconserved elements provide a new phylogenomic perspective of Oestroidea flies (Diptera: Calyptratae). Syst Entomol. https://doi.org/10.1111/syen.12443.

Cerretti P., O'Hara J.E., Wood D.M., Shima H., Inclan D.J., Stireman J.O. 2014. Signal through the noise? Phylogeny of the Tachinidae (Diptera) as inferred from morphological evidence: Phylogeny of Tachinidae. Syst Entomol. 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: Calyptratae: Oestroidea) and the dating of oestroid divergences. PLoS ONE. 12: e0182101.

Cerretti P., Stireman J.O., Badano D., Gisondi S., Rognes K., Giudice G.L., Pape T. 2019. Reclustering the cluster flies (Diptera: Oestroidea, Polleniidae). Syst Entomol. 44:957–972.

Crosskey R.W. 1976. A taxonomic conspectus of the Tachinidae (Diptera) of the Oriental Region. Bulletin of the British Museum (Natural History), Entomology. 26:357.

Haas B.J., Papanicolaou A., Yassour M., Grabherr M., Blood P.D., Bowden J., Couger M.B., Eccles D., Li B., Lieber M., MacManes M.D., Ott M., Orvis J., Pochet N., Strozzi F., Weeks N., Westerman R., William T., Dewey C.N., Henschel R., LeDuc R.D., Friedman N., Regev A. 2013. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat Protoc. 8:1494–1512.

Herting B. 1984. Catalogue of Paleartic Tachinidae (Diptera). Stuttgarter Beiträge zur Naturkunde. 369:228.

Hittinger C.T., Johnston M., Tossberg J.T., Rokas A. 2010. Leveraging skewed transcript abundance by RNA-Seq to increase the genomic depth of the tree of life. Proceedings of the National Academy of Sciences. 107:1476–1481.

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.

Kutty S.N., Meusemann K., Bayless K.M., Marinho M.A.T., Pont A.C., Zhou X., Misof B., Wiegmann B.M., Yeates D., Cerretti P., Meier R., Pape T. 2019. Phylogenomic analysis of Calyptratae: resolving the phylogenetic relationships within a major radiation of Diptera. Cladistics. 35, 605-622.

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.

Nelson L.A., Lambkin C.L., Batterham P., Wallman J.F., Dowton M., Whiting M.F., Yeates D.K., Cameron S.L. 2012. Beyond barcoding: A mitochondrial genomics approach to molecular phylogenetics and diagnostics of blowflies (Diptera: Calliphoridae). Gene. 511:131–142.

O Hara J. 2013. History of tachinid classification (Diptera, Tachinidae). ZK. 316:1–34.

O'Hara J.E., Henderson S.J. 2020. World Genera of the Tachinidae (Diptera) and their regional occurrence. Version 11.0. PDF document, 90pp. Available from: http://www.nadsdiptera.org/Tach/WorldTachs/Genera/Gentach_ver11.pdf (accessed on 9 March 2021).

O'Hara J.E., Wood D.M. 2004. Catalogue of the Tachinidae (Diptera) of America north of Mexico. Gainesville, Fla: Associated Publishers.

Pape T. 1992. Phylogeny of the Tachinidae Family-Group (Diptera: Calyptratae). Tijdschrift voor Entomologie. 135:43–86.

Rognes K. 1997. The Calliphoridae (Blowflies) (Diptera: Oestroidea) are Not a Monophyletic Group1. Cladistics. 13:27–66.

Santis M.D. 2016. Análise cladística da tribo Dufouriini (Diptera, Tachinidae). Dissertação de Mestrado. Programa de Pós-Graduação em Zoologia, Instituto de Biociências, Universidade de São Paulo.

Singh B., Wells J.D. 2013. Molecular Systematics of the Calliphoridae (Diptera: Oestroidea): Evidence from One Mitochondrial and Three Nuclear Genes. J Med Entomol. 50:15–23.

Stireman J.O. 2002. Phylogenetic relationships of tachinid flies in subfamily Exoristinae (Tachinidae: Diptera) based on 28S rDNA and elongation factor-1α: Phylogeny of Exoristinae (Tachinidae). Systematic Entomology. 27:409–435.

Stireman J.O., O'Hara J.E., Wood D.M. 2006. TACHINIDAE: Evolution, Behavior, and Ecology. Annu. Rev. Entomol. 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.

Tachi T., Shima H. 2010. Molecular phylogeny of the subfamily Exoristinae (Diptera, Tachinidae), with discussions on the evolutionary history of female oviposition strategy. Systematic Entomology. 35:148–163.

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

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.

Wood D.M. 1987. Tachinidae. In: McAlpine J.F., editor. Manual of Neartic Diptera. Biosystematics Research Centre, Ottawa, Ontario. p. 1193–1269.

Zhang D., Yan L., Zhang M., Chu H., Cao J., Li K., Hu D., Pape T. 2016. Phylogenetic inference of calyptrates, with the first mitogenomes for Gasterophilinae (Diptera: Oestridae) and Paramacronychiinae (Diptera: Sarcophagidae). Int. J. Biol. Sci. 12:489–504.

Objetivos

O objetivo desse trabalho foi reconstruir uma filogenia molecular confiável da família Tachinidae usando dados transcriptômicos e genes ortólogos com enfoque nas relações entre as quatro subfamílias de Tachinidae. Além disso, comparar os resultados obtidos com outros estudos morfológicos e moleculares do grupo. Os objetivos específicos são os seguintes:

1. Atestar a monofilia de Tachinidae;
2. Verificar se as quatro subfamílias (Phasiinae, Dexiinae, Tachininae e Exoristinae) são monofiléticas;
3. Estabelecer a relação entre as subfamílias;
4. Definir o grupo-irmão de Tachinidae;
5. Analisar e discutir possíveis diferenças entre as análises filogenéticas realizadas (matrizes de aminoácido e nucleotídeos; análise de Máxima Verossimilhança e Máxima Parcimônia);
6. Atestar se os resultados obtidos com sequenciamento de nova geração são congruentes com estudos prévios com dados morfológicos e moleculares.

Chapter 1

Molecular Phylogeny of Tachinidae (Diptera, Brachycera, Calyptratae) based on next-generation sequencing, focusing on subfamilies boundaries and relationships

Abstract

The Tachinidae is one of the largest families of Diptera. They are found in nearly all terrestrial environments throughout the world, including deserts, forests, grasslands, mountains, and tundra. We present a phylogenomic analysis of Tachinidae using transcriptomic data. The analyses are based on 36 species for the ingroup, composing the tachinids, and 23 species for the outgroup, representing the other Oestroidea families. We constructed four datasets: three out of four with amino acid data, 100% coverage (92 single-copy protein-coding genes), 75% coverage (1304 single-copy protein-coding genes), and 50% coverage (1890 single-copy protein-coding genes); and one with nucleotide data, 75% coverage (1304 single-copy protein-coding genes). Overall, our topologies are well resolved, with strong node support. We obtained the trees analyzing three matrices with different coverage, and only small changes were found among them. In general, our results are congruent with recent studies that include tachinids, with some important changes. Our analyses recovered Polleniidae as sister to Tachinidae and the four tachinid subfamilies with the following relationship: Phasiinae + Dexiinae and Tachininae + Exoristinae. Phasiinae is recovered as monophyletic, Dexiinae as paraphyletic, and Exoristinae and Tachininae are recovered as polyphyletic. Interestingly, the tribe Myiophasiini (Tachininae) forms a different lineage, a clade sister to all the remaining Tachinidae. The neotropical tribe Iceliini (former Tachininae) is recovered within Exoristinae, sister to Winthemia. Also, Masyphyini (former Exoristinae) is recovered within Dexiinae, close to Dexiini.

Key words: Tachinidae, subfamilies, transcriptome, phylogenomic.

1. Introduction

Transcriptomic data became very used for phylogenetic analyses recently, since increasing the number of genetic markers has successfully resolved many higher-level relationship in various arthropod lineages (Rehm *et al.* 2011; Dell'Ampio *et al.* 2014; Misof *et al.* 2014; Peters *et al.* 2014; Dikow *et al.* 2017; Kutty *et al.* 2018; Pauli *et al.* 2018; Bossert *et al.* 2019; Kutty *et al.* 2019; Yan *et al.* 2020). Considering that high quality phylogenomic data matrices can be constructed with a very small number of short sequence reads from transcriptome, difficulty for obtaining samples should no longer prohibitively limit taxon selection (Hittinger *et al.* 2010). Thus, with the transcriptomic approach a phylogenetic analysis can be done with a relatively low taxa sampling (Rehm *et al.* 2011; Dell'Ampio *et al.* 2014; Misof *et al.* 2014; Peters *et al.* 2014; Dikow *et al.* 2017; Kutty *et al.* 2018; Pauli *et al.* 2018; Bossert *et al.* 2019; Kutty *et al.* 2019; Yan *et al.* 2020) in comparison with other molecular approaches (Blaschke *et al.* 2018; Cerretti *et al.* 2017; Stireman *et al.* 2019, 2021).

Tachinidae is one of the largest families of Diptera, with approximately 8,592 described species worldwide (O'Hara & Henderson 2020). Within Diptera, they rank second in number of described species only to the Tipulidae family (Pape *et al.* 2011; Stireman *et al.* 2019). Tachinids are found in nearly all terrestrial environments worldwide, with the Neotropical region being the most diverse (Stireman *et al.,* 2006). The Tachinidae is commonly considered a family that is in the beginning of its diversity (Stireman *et al.* 2021). Studies with molecular clock suggests a phylogenetic origin of the family from 24-34 Mya (Wiegmann *et al.* 2011; Zhao *et al.* 2013; Junqueira *et al.* 2016; Cerretti *et al.* 2017; Stireman *et al.* 2021).

The remarkable diversification of Tachinidae can perhaps be related to their parasitoid habit and the association with extensively host transitions (Stireman *et al.* 2019). All known tachinids are endoparasitoids of insects or other arthropods (Stireman *et al.* 2021). Tachinids generally function as regulators of major groups of herbivorous insects, controlling herbivore populations and structuring ecological communities (Stireman *et al.* 2006). Due to their exceptional diversity, broad distribution and ecological role as controllers of primarily herbivorous insects, basic and applied researchers have studied the tachinids, as well as taxonomists (Stireman *et al.* 2006; O Hara 2013; Cerretti *et al.* 2014). However, the understanding of tachinids classification

and identification is extremely challenging, and these difficulties have only obstructed the understanding of their biology, ecology and potential for major groups of herbivore insects (Cerretti *et al.* 2014).

Tachinidae is a very large and relatively young group. (Wiegmann *et al.* 2011; Zhao *et al.* 2013; Junqueira *et al.* 2016; Cerretti *et al.* 2017; Stireman *et al.* 2021). Due to these, Tachinidae remain relatively poorly know, despite their ecological importance and notable diversity (Stireman *et al.* 2019). Only recently studies have been applied to understand the relationship within Tachinidae, using morphological (Cerretti *et al.* 2014) or molecular data (Stireman 2002; Tachi & Shima 2010; Winkler *et al.* 2015; Blaschke *et al.* 2018; Stireman *et al.* 2019, 2021).

1.1. *A brief history of the classification of Tachinidae subfamilies*

Monophyly of Tachinidae is supported by their endoparasitism of insects, modifications of the mouth parts in the first instar (labrum strongly developed), and swollen adult subscutellum (Wood 1987; Pape 1992; Cerretti *et al.* 2014; Stireman *et al.* 2019). In addition to morphological characters, the monophyly of Tachinidae is also corroborated by strong molecular support (Tachi & Shima, 2010; Winkler *et al.,* 2015; Blaschke *et al.,* 2018; Kutty *et al.,* 2019; Stireman *et al.,* 2019; Buenaventura *et al.,* 2020).

The classification of Tachinidae and its subfamilies has a long history of changes and uncertainties. Five subfamilies of the Tachinidae were recognized by (Herting 1957): Echinomyiinae, Dexiinae, Phasiinae, Ocypterinae, and Eutachininae (O Hara 2013). Later, (Herting 1960) proposed a restructuring of Tachinidae, changing the subfamilies to four: Exoristinae, Echinomyiinae, Phasiinae, and Dexiinae (O Hara 2013). (Verbeke 1962), using mostly male genitalia structures, concluded that the Tachinidae were best divided into six subfamilies: Phasiinae, Echinomyiinae, Eutachininae, Dexiinae, Voriinae, and Dufouriinae (O Hara 2013). (Mesnil 1966) also proposed six tribes (equivalent to subfamilies of other authors): Phasiini, Exoristini, Goniini, Dexiini, Voriini, and Tachininae (O Hara 2013). Crosskey (1976) classification is very similar to Mesnil (1966), but he also used Verbeke (1962) concept of Dufouriinae. Crosskey (1976) proposed five subfamilies based on chaetotaxy and pruinosity characteristics: Phasiinae, Dufouriinae, Proseninae, Tachininae, Goniinae.

On the other hand, Herting (1984) recognized again four subfamilies the same way he did before, only the name Echino myiinae had to be changed to Tachininae. Herting

(1984) used egg morphology, oviposition, and parasite-host relationship as classification criteria for the four subfamilies: Exoristinae, Tachininae, Phasiinae, and Dexiinae (O Hara 2013). Finally, Wood (1987) assembled the information given by Mesnil (1966) and Herting (1984) and acknowledged the same four subfamilies as Herting (1984). Today the classification of Tachinidae in four subfamilies is the most widely accepted (Herting, 1984; Wood, 1987; Stireman, 2002; O'Hara & Wood, 2004; Tachi & Shima, 2010; Cerretti *et al.,* 2014; Winkler *et al.,* 2015; Blaschke *et al.,* 2018; Stireman *et al.,* 2019).

Although the monophyly and composition of Tachinidae are relatively stable, their origin and relationship within Oestroidea are not. In particular, the identity of the Tachinidae sister-group remains uncertain, as several studies showed different hypothesis, such as Sarcophagidae (Pape, 1992; Rognes, 1997; Tachi & Shima, 2010), Calliphoridae (Wiegmann *et al.,* 2011), Oestridae (Zhang *et al.,* 2016), Mesembrinellidae (Marinho *et al.,* 2012; Junqueira *et al.,* 2016), Rhiniidae (Marinho *et al.,* 2017), Rhiniidae + Rhinophoridae (Cerretti *et al.,* 2014), Rhinophoridae + Polleniidae (Kutty *et al.,* 2010), and Polleniidae (Nelson *et al.,* 2012; Singh & Wells, 2013; Winkler *et al.,* 2015; Cerretti *et al.,* 2017; Blaschke *et al.,* 2018; Cerretti *et al.,* 2019; Kutty *et al.,* 2019; Stireman *et al.,* 2019; Buenaventura *et al.,* 2020).

As a highly diverse family, Tachinidae has a complex and a somewhat confusing internal classification. Tachinidae still presents many monotypic genera, and the classification within the family has been changed substantially by specialists(O Hara 2013). The four subfamilies have been put together in various forms in recent phylogenetic studies (Fig. 1) such as follows: (Exoristinae + Dexiinae) + (Phasiinae + Tachininae) (Stireman, 2002) (Fig. 1A); Dexiinae + (Phasiinae + (Tachininae + Exoristinae)) (Tachi & Shima, 2010) (Fig. 1B); Exoristinae + (Dexiinae + (Tachininae + Phasiinae)) (Singh & Wells, 2013) (Fig. 1C); Phasiinae + (Dexiinae + Exoristinae) with no Tachininae sampled species (Li *et al.,* 2017) (Fig. 1F); and Tachininae + (Dexiinae + (Phasiinae + Exoristinae)) (Cerretti *et al*., 2017) (Fig. 1E). In our study, we recovered the relation of the subfamilies as (Phasiinae + Dexiinae) + (Tachininae + Exoristinae), which is recovered both morphological (Cerretti *et al.,* 2014) and molecular studies (Cerretti *et al.,* 2017; Blaschke *et al.,* 2018; Kutty *et al.,* 2019; Stireman *et al.,* 2019; Buenaventura *et al.,* 2020) (Fig. 1D, 1G-J).

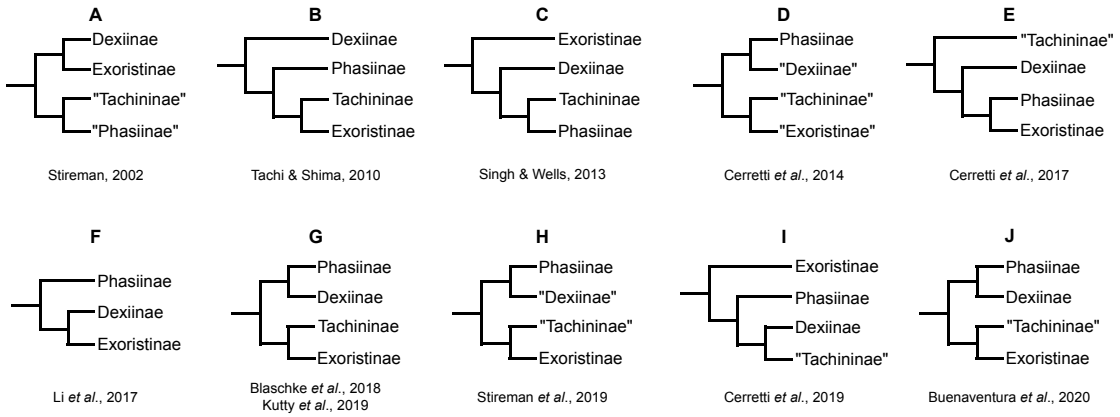

Figure 1. Previous phylogenetic hypothesis of Tachinidae. Nodes from the original studies have been collapsed to show the subfamily-level relatioship. Non-monophyletic subfamilies are in quotes.

Knowing these uncertainties regarding the subfamilies monophyletism and their relationship with each other, our goal is to better understand the relationship between the subfamilies of Tachinidae and the position of tachinids within the Oestroidea clade using a different approach. Here we use transcriptomic data and ortholog genes to reconstruct a reliable and robust molecular phylogeny of the family Tachinidae. Our focus is to attest the monophyly of Tachinidae and the four subfamilies (Phasiinae, Dexiinae, Tachininae, and Exoristinae). Furthermore, we intend to explore the relationship between the subfamilies and the families of Oestroidea to confirm the sister-group for Tachinidae.

2. Materials and Methods

2.1. *Taxon sampling, RNA extraction, and transcriptome sequencing*

We sequenced 30 species of Tachinidae (3 Phasiinae; 7 Dexiinae; 9 Tachininae; 11 Exoristinae) and seven species of other oestroid families for the outgroup (2 "Calliphoridae"; 1 Mesembrinellidae; 1 Rhinophoridae; 3 Sarcophagidae) (Supplementary Table 1). Other six species of Tachinidae (2 Phasiinae; 1 Dexiinae; 2 Tachininae; 1 Exoristinae) and 16 species from the outgroup (7 "Calliphoridae"; 1 Polleniidae; 1 Mystacinobiidae; 1 Rhiniidae; 3 Rhinophoridae; 4 Sarcophagidae) were obtained from the GenBank (Supplementary Table 2). Totalizing 59 species (Table 1). The analyses were rooted in *Mesembrinella bicolor* since in every previous study Mesembrinellidae and Ulurumyiidae form a basal clade in the Oestroidea clade (Cerretti *et al.,* 2017, 2019; Kutty *et al.,* 2019; Buenaventura *et al.,* 2020).

Specimens were collected alive and identified at the species level whenever possible. The individuals were sacrificed in a cryogenic tube then stored in RNALater. According to the manufacturer's information, RNA extraction was performed with a part of only one specimen using TRIzol® (Life Technologies). The libraries were built using NEBNext® Poly (A) mRNA Magnetic Isolation Module kits to isolate only the poly (A) + RNA set. The cDNA library was prepared using NEBNext® Ultra RNA Library Prep Kit for Illumina. The necessary adapters and primers for sequencing were added using NEBNext® Multiplex oligos for Illumina Index Primers Set 1.

The extractions were quantified using the Qubit RNA HS kit and the libraries with the Qubit dsDNA HS kit. Extractions were done at the Molecular Evolution Laboratory of the Department of Zoology (Institute of Biosciences - University of São Paulo - USP). The sequencing was done with the NextSeq 500 platform (Illumina) at the Center for Supporting Research Facilities (CEFAP-USP).

Table 1. List of species, number of assembled contigs, number of genes from orthology prediction in the complete dataset, with 75% coverage and 50% coverage.

Family	Subfamily	Tribe	Species name	Author name	Data generated	Assembled contigs	Orthologs in full dataset (2622)	Orthologs in 75% cover (1304 genes)	Orthologs in 50% cover (1890 genes)
Tachinidae	Phasiinae	Cylindromyiini	*Cylindromyia carinata*	Townsend, 1927	DZ	38754	1608	1178	1495
Tachinidae	Phasiinae	Cylindromyiini	*Cylindromyia sp.*	Meigen, 1803	SRX3697544	71817	2053	1220	1718
Tachinidae	Phasiinae	Gymnosomatini	*Gymnoclytia paulista*	Townsend, 1929	DZ	48928	1639	1220	1521
Tachinidae	Phasiinae	Gymnosomatini	*Gymnosoma nitens*	Meigen, 1824	SRX3697542	62433	2099	1266	1793
Tachinidae	Phasiinae	Phasiini	*Phasia* sp.	Robineau-Desvoidy, 1830	DZ	50645	1885	1243	1710
Tachinidae	Dexiinae	Eutherini	*Euthera bicolor*	Coquillett, 1902	SRX798067	41128	808	713	782
Tachinidae	Dexiinae	Epigrimyiini	*Beskia aelops*	Brauer & Bergenstamm, 1889	DZ	63625	2162	1251	1788
Tachinidae	Dexiinae	Telothyriini	*Eutelothyria itaquaquecetubae*	Townsend, 1931	DZ	39702	2060	1254	1757
Tachinidae	Dexiinae	Dexiini	*Trichodura* sp.	Macquart, 1843	DZ	51707	1969	1257	1744
Tachinidae	Dexiinae	Dexiini	*Trichodura* sp.	Macquart, 1843	DZ	59365	1141	933	1073
Tachinidae	Dexiinae	Dexiini	*Zelia* sp.	Townsend, 1919	DZ	40669	1627	1230	1540
Tachinidae	Dexiinae	Dexiini	*Zelia* sp.	Townsend, 1919	DZ	30706	1188	1003	1131
Tachinidae	Dexiinae	Voriini	*Voria ruralis*	Fallén, 1810	DZ	69256	1954	1260	1748
Tachinidae	Tachininae	Iceliini	*Iceliini* sp.	Townsend, 1931	DZ	27299	1111	979	1062
Tachinidae	Tachininae	Leskiini	*Stomatodexia campestris*	Nunez, 2005	DZ	43960	1261	1077	1197
Tachinidae	Tachininae	Leskiini	*Stomatodexia campestris*	Nunez, 2006	DZ	38132	1471	1166	1386
Tachinidae	Tachininae	Minthoini	*Mintho rufiventris*	Fallén, 1817	SRX3697541	124012	2079	1173	1673
Tachinidae	Tachininae	Myiophasiini	*Cholomyia inaequipes*	Bigot, 1884	DZ	40053	1648	1215	1538
Tachinidae	Tachininae	Myiophasiini	*Gnadochaeta* sp.	Macquart, 1850	DZ	40226	1740	1236	1579
Tachinidae	Tachininae	Ormiini	*Ormia* sp.	Robineau-Desvoidy, 1830	DZ	137108	2200	1236	1748
Tachinidae	Tachininae	Tachinini	*Archytas* sp.	Jaennicke, 1867	DZ	51343	2062	1256	1766
Tachinidae	Tachininae	Tachinini	*Copecrypta* sp.	Townsend, 1908	DZ	44910	1989	1252	1746
Tachinidae	Tachininae	Tachinini	*Tachinini* sp.		DZ	55415	2118	1259	1785
Tachinidae	Tachininae	Bigonichetini	*Triarthria setipennis*	Fallén, 1810	SRX314906	57389	1985	1248	1730
Tachinidae	Exoristinae	Blondeliini	*Calodexia sp.*	Wulp, 1891	DZ	54008	2017	1247	1759
Tachinidae	Exoristinae	Exoristini	*Chetogena sp.*	Rondani, 1856	DZ	40559	1973	1282	1759
Tachinidae	Exoristinae	Goniini	*Belvosia* sp.*1*	Robineau-Desvoidy, 1830	DZ	32601	1254	1031	1182
Tachinidae	Exoristinae	Goniini	*Belvosia* sp.*2*	Robineau-Desvoidy, 1830	DZ	30776	1238	1040	1177
Tachinidae	Exoristinae	Goniini	*Belvosia* sp.*3*	Robineau-Desvoidy, 1830	DZ	28809	1347	1141	1300
Tachinidae	Exoristinae	Goniini	*Houghia* sp.	Coquillett, 1897	DZ	59948	2032	1255	1760
Tachinidae	Exoristinae	Goniini	*Leschenaultia* sp.	Robineau-Desvoidy, 1830	DZ	42833	1674	1246	1591
Tachinidae	Exoristinae	Goniini	*Pseudogonia rufifrons*	Wiedemann, 1830	SRX3697555	72505	1946	1240	1707
Tachinidae	Exoristinae	Goniini	*Spallanzania brasiliensis*	Townsend, 1927	DZ	27897	1217	1045	1163
Tachinidae	Exoristinae	Masiphyini	*Masiphya* sp.	Brauer & Bergenstamm, 1891	DZ	59789	2125	1246	1762
Tachinidae	Exoristinae	Euthelairini	*Neomintho macilenta*	Wiedemann, 1830	DZ	38297	2030	1268	1762
Tachinidae	Exoristinae	Winthemiini	*Winthemia pinguis*		DZ	48471	2038	1270	1775
Calliphoridae	Ameniinae		*Amenia* sp.	Robineau-Desvoidy, 1830	SRX3697546	54629	1928	1266	1738
Calliphoridae	Bengaliinae		*Verticia nigra*	Malloch, 1927	SRX3697551	77138	1961	1204	1673
Calliphoridae	Helicoboscinae		*Eurichaeta muscaria*	Meigen, 1826	SRX3697545	68155	1993	1251	1742
Calliphoridae	Chrysomyinae		*Hemilucilia semidiaphana*	Rondani, 1850	DZ	43290	1942	1274	1752

Calliphoridae	Chrysomyinae	*Protophormia terraenovae*	Robineau-Desvoidy, 1830	DRX081803	124649	2105	1230	1733
Calliphoridae	Luciliinae	*Lucilia cuprina*	Wiedemann, 1830	SRX907164	72583	1579	1188	1470
Calliphoridae	Luciliinae	*Lucilia sericata*	Meigen, 1826	SRX3371917	72383	2193	1275	1803
Calliphoridae	Calliphorinae	*Calliphora vomitoria*	Linnaeus, 1758	SRX798052	58033	1255	1036	1185
Calliphoridae		*Calliphoridae* sp.	Townsend, 1915	DZ	35259	1718	1226	1586
Polleniidae	Polleniinae	*Pollenia* sp.	Robineau-Desvoidy, 1830	SRX5055105	74991	1819	1224	1633
Mesembrinellidae		*Mesembrinella bicolor*	Giglio-Tos, 1893	DZ	26574	1048	896	993
Mystacinobiidae		*Mystacinobia zelandica*	Holloway, 1976	SRX3697552	88914	2087	1239	1758
Rhiniidae		*Stomorhina subapicalis*	Macquart, 1847	SRX798118	65703	1470	1138	1368
Rhinophoridae		*Shannoniella cuspidata*	Townsend, 1939	DZ	36835	1606	1179	1485
Rhinophoridae		*Bixinia winkleri*	Cerretti, Lo Giudice & Pape, 2014	SRX3697547	49117	1524	1174	1438
Rhinophoridae		*Stevenia* sp.	Robineau-Desvoidy, 1830	SRX3697549	57227	2033	1266	1775
Sarcophagidae	Miltogramminae	*Gymnoprosopa* sp.	Townsend, 1892	SRX5055106	47248	1508	1092	1350
Sarcophagidae	Sarcophaginae	*Sarcophaga peregrina*	Robineau-Desvoidy, 1830	SRX3371908	89869	2159	1273	1792
Sarcophagidae	Sarcophaginae	*Sarcophaga* sp.	Meigen, 1826	SRX3697548	67097	1828	1201	1610
Sarcophagidae	Sarcophaginae	*Neobellieria bullata*	Parker, 1916	SRX2822440	37484	929	756	870
Sarcophagidae	Sarcophaginae	*Peckia intermutans*	Walker, 1861	DZ	48393	985	830	929
Sarcophagidae	Sarcophaginae	*Sarcophaginae* sp. *2*		DZ	36444	1691	1212	1567
Sarcophagidae	Sarcophaginae	*Sarcophaginae* sp. *1*		DZ	32918	1897	1269	1719

2.2. *Assemblies and orthology prediction*

The sequence quality was assessed using FASTQC (Andrews, 2010). Bases with quality less than 15 were cut with Trimmomatic (Bolger *et al.,* 2014) using a 4bp sliding window size. The minimum length of trimmed reads was set at 36bp. The *de novo* transcriptome assembly was done with Trinity (Haas *et al.,* 2013). Only reads paired-end were assembled, and contigs smaller than 200bp were removed.

The translation of nucleotide sequences into amino acid was carried out using TransDecoder (http://transdecoder.github.io). This software was also used to find the minimum length of open reading frames (ORFs). The longest ORFs were chosen for each transcript sequence found. CD-HIT (Li & Godzik, 2006) software removed the redundancies in the longest ORFs, leaving only one copy of each longest ORF. Orthology prediction was carried out using BUSCO (Simão *et al.,* 2015). High quality, single-copy orthologs were selected using a series of Bash commands. We created FASTA files for each ortholog containing sequence data from taxa that presented that particular gene.

The quality selection was made based on BUSCO tables and gene classification. This software generates a table with quality indexes for each query sequence compared to sequences from the OrthoDB database from BUSCO, DipteraOdb10. The database is composed of 26 species of flies, such as the following: *A. aegypti, A. albimanus, A. atroparvus, A. culicifacies, A. epiroticus, A. gambiae, B. cucurbitae, B. dorsalis, B. antarctica, C. capitata, C quinquefasciatus, D. grimshawi, D. melanogaster, D. mojavensis, D. virilis, D. willistoni, G. austeni, G. brevipalpis, G. fuscipes, L. cuprina, M. destructor, M. domestica, P. papatasi, P. nubifer, P. vanderplanki*. All genes were selected based on e-value, the main quality parameter (Karlin & Altschul, 1990). The e-value parameter uses values from 0 (when the query and database sequences are identical) to e^{-n} (e.g., e^{-10}, e^{-50}, e^{-100}, e^{-200}). We made a conservative selection in cases where the e-value is not 0, the ideal value. Only genes with an e-value lower than e^{-100} were selected.

The FASTA files containing amino acid sequences were aligned using MAFFT (Katoh, 2002) with the global pairwise alignment (G-INS-i) algorithm. The gaps sequences were cut by TrimAl (Capella-Gutierrez *et al.,* 2009) with the automated algorithm. After aligned and trimmed, the ortholog sequences were treated with Python scripts developed in the Department of Zoology of the University of São Paulo. Gaps at the ends of the sequences were replaced by the letter "X" in amino acid sequences and “N” in the nucleotide sequences. Sites with more than 25% of missing data were trimmed.

Four matrices were designed with different quantities of coverage, three for amino acids and one for nucleotides: a matrix of 100% coverage (0 missing sequences), a 75% coverage matrix (25% of missing sequences), and a 50% coverage matrix (50% of missing sequences) for amino acid, and a 75% coverage matrix for nucleotides. These three different matrices were designed to evaluate the balance between the number of genes and missing data and phylogenetic signal (Supplementary Figure 1).

2.3. *Phylogenomic analyses*

The amino acid dataset was analyzed using Maximum Likelihood (ML) and Maximum Parsimony (MP), and the nucleotide dataset was analyzed using only ML. The ML analyses with the three amino acid matrices and the nucleotide matrix were carried out with IQ-TREE (Nguyen *et al.,* 2015) on CEFAP (Research Facilities Center) computer cluster. The model was chosen using IQ-TREE ModelFinder (Kalyaanamoorthy *et al.,* 2017) with the AICc criteria. The best ML tree search was carried out using the JTT+F+R10 model for every amino acid matrix and GTR+F+R9 for nucleotide matrix. Ultrafast bootstrap (UBS) analyses assessed the node support (Hoang *et al.,* 2018).

Parsimony analyses with the matrix of amino acid were carried out in TNT v.1.5 (Goloboff, 1999) using new technologies search. We used the following commands: 100 replications as starting point, each replication initially autoconstrained (previous and Wagner), each replication with constraint, random and exclusive sectorial searches, with ratchet (20 iterations), tree-drifting (20 iterations), no hybridization, and fusing (20 rounds). Node support was assessed by jackknife (JK) resampling (1000 replicates), bootstrap (BB) resampling (1000 replicates), and Bremer.

3. Results

3.1. *Data analyses*

The total RNA of 30 tachinids and 7 outgroups species was extracted with high quality (Table 1) and well sequenced. The *de novo* transcriptome assembly with Trinity produced an average of 45,824 (26,574 – 137,108) contigs with an average of 48,584,154 (20,799,956 – 17,2547,924) base pairs per sample. We identified an average of 1,694 (1,608 – 2,200) ortholog genes (Table 1) per sample. We successfully built four matrices with three different gene coverage (Supplementary Fig. 1), with 100% coverage and no missing data (Supplementary Figure 1A), with 75% coverage (Supplementary Fig. 1B, Fig. 1 and Fig. 2) and 15% of missing data, and one with 50% coverage (Supplementary Fig. 1C) and 50% of missing data, and one with 75% coverage for nucleotides (Supplementary Fig. 2).

The 100% coverage dataset was made with 92 single-copy protein-coding genes (alignment length: 37,447 sites of amino acids) and resulted in an ML tree (Supplementary Fig. 1A). The matrix of 75% coverage is composed of 1,304 single-copy protein-coding genes (alignment length: 645,489 sites of amino acids and 4,185,231 sites for nucleotides). This dataset resulted in one ML tree (Supplementary Fig.1B and Fig.1) and a MP tree (Fig. 2) for amino acids and on ML tree for nucleotides (Supplementary Fig. 2). The 50% coverage dataset was made with 1,890 single-copy protein-coding genes (alignment length: 1,250,855 sites of amino acids), resulting in an ML tree (Supplementary Fig. 1C).

3.2. *Phylogenetic analyses*

Datasets having different coverage presented very similar topologies in ML analyses (Supplementary Fig. 1). Significant differences between topologies A, B, and C were the phylogenetic position of internal branches. *Copecrypta* sp. and *Archytas* sp. (Tachininae), *Spallanzania brasiliensis* and *Pseudogonia rufifrons* (Exoristinae) exchanged places in the topologies. Moreover, the results of the nodes support were very different (Table 2).

Table 2. Comparison of ultrafast bootstrap node support across different amino acids dataset coverage with maximum likelihood analyses.

Node support	Dataset coverage		
	100%	75%	50%
100	42	54	53
90-99	6	2	-
65-99	4	-	3
< 65	4	-	-

After the analyses with the three different matrices, we chose the 75% matrix to develop other analyses (Supplementary Fig. 1 and 2). This matrix showed a better balance between the number of genes and missing data. The 75% coverage tree had better results with high support values in every node than the other matrices (Supplementary Fig. 1B and Table 2).

We recovered a highly resolved amino acid ML tree (75% matrix) with almost every node with 100% ultrafast bootstrap support (UBS). The only two nodes different than 100 presents UBS=99 and UBS=94. In both analyses, Mesembrinellidae is the tree root, making the family the basal clade and sister to the remaining Oestroidea. In ML analyses, Mystacinobiidae has been recovered sister to Sarcophagidae and this clade sister to the remaining families. Calliphoridae is the only oestroid family recovered as paraphyletic, with Rhiniidae nested within it, sister group to Bengaliinae, and Rhinophoridae sister to Helicoboscinae and Ameniinae.

Tachinidae family is monophyletic with the well-supported Polleniidae as sister-group. The relationship recovered among the subfamilies is (Phasiinae + "Dexiinae") + ("Tachininae" + "Exoristinae"). Phasiinae appears as monophyletic and sister to Dexiinae. Dexiinae is recovered as paraphyletic with the genus *Masiphya* (Masyphyini, Exoristinae) nested within the tribe and sister to *Trichodura* sp. The clade Dexiinae and *Masiphya* is strongly supported as monophyletic. Exoristinae appears as polyphyletic with Masyphyini within Dexiinae, and the tribe Iceliini (Tachininae) nested within Exoristinae and sister to *Winthemia* sp. Tachininae is also found as polyphyletic, with the Myiophasiini tribe as sister to the remaining Tachinidae and Iceliini nested within Exoristinae.

We recovered a strongly resolved Maximum Likelihood tree (MLT) with nucleotide data (Sup. Fig. 2) with 54 out of 58 nodes with 100% support for ultrafast bootstrap. Three nodes have support value of UBS=82 and one node UBS=96. The topologies of the different matrices are almost identical, with only two changes in internal

branches. *Sarcophaga peregrina* and *Neobellieria bullata* (Sarcophagidae), and *Spallanzania brasiliensis* and *Pseudogonia rufifrons* (Exoristinae, Goniini) exchanged their positions in the nucleotide tree in comparison with the amino acids tree.

We recovered a well resolved MP tree (75% matrix of amino acid), with 46 of 58 nodes with 100% support for both jackknife (jn) and bootstrap (bb) and Bremer support ranging from 125 to 18442. Other nodes presented small variation, eight have jackknife or bootstrap values between 90 and 99 and Bremer support ranging from 92 to 382. Two nodes have jn and bb varying between 49 and 70, and 52 and 65 for the Bremer support. Only one node is recovered with very low support in every support analyses jn=16, bb=11, Bremer=8 (Fig. 2). The ML and MP analyses of the 75% coverage matrix are very similar (Fig. 1 and Fig. 2, respectively). The major differences between the two topologies were:

1. Changed position of *Neobellieria bullata* (Sargophaginae, Sarcophagidae):

In the MLT *Neobellieria bullata* is recovered sister to *Sarcophaga* sp. and this clade sister to *Sarcophaga peregrina*. In the Maximum Parsimony tree (MPT) the relationship among the members of the Sarcophaginae is different. *Neobellieria bullata* is sister to the clade *Sarcophaga* sp. and *Sarcophaga peregrina*.

2. Changed position of *Peckia intermutans* (Sargophaginae, Sarcophagidae):

In the MLT *Peckia intermutans* is recovered sister to *Sarcophaginae* sp. 2 and this clade sister to *Sarcophaginae* sp. In the MPT the relationship among these taxa is contrasting. *Peckia intermutans* is sister to the clade *Sarcophaginae* sp. and *Sarcophaginae* sp.2.

3. Phasiinae paraphyletic and position of *Cylindromyia* (Cylindromyiini):

In the MLT, Phasiinae is monophyletic with Cylindromyiini sister to the clade Phasiini and Gymnosomatini. On the other hand, on the MPT, Phasiinae is paraphyletic regarding Dexiinae, and Cylindromyiini has been recovered sister to the clade Epigrimyiini and Eutherini (Dexiinae).

4. *Eutelothyria itaquaquecetubae* (Telothyriini sister to *Voria ruralis* (Voriini)):

In the MLT Telothyriini is recovered sister to the clade Voriini + (Zeliini + (Masyphyini + Dexiini)). The MPT recovered a different result, in which Telothyriini is sister to Voriini and this clade is sister to (Zeliini + (Masyphyini + Dexiini)).

5. Position of *Chetogena* sp. (Exoristini):

The MLT recovered Exoristini sister to all Goniini and *Calodexia* sp. Differently, the MPT retrieved Exoristini grouped within Goniini, with *Chetogena* sp. sister to the clade *Calodexia* sp. and *Houghia* sp.

6. Changed position of *Pseudogonia rufifrons* (Goniini) and *Spallanzania brasiliensis* (Goniini):

The MLT found *Pseudogonia rufifrons* sister to the clade *Spallanzania brasiliensis* + *Belvosia* sp. However, MPT presents a different relationship among those taxa, in which *Spallanzania brasiliensis* is sister to the clade *Pseudogonia rufifrons* + *Belvosia* sp.

The tree datasets of 0, 15%, and 50% missing data produced almost identical topologies with ML analyses (Supplementary Fig. 1). With variation only in some internal branches and the support values (Supplementary Fig. 1 and Table 2).

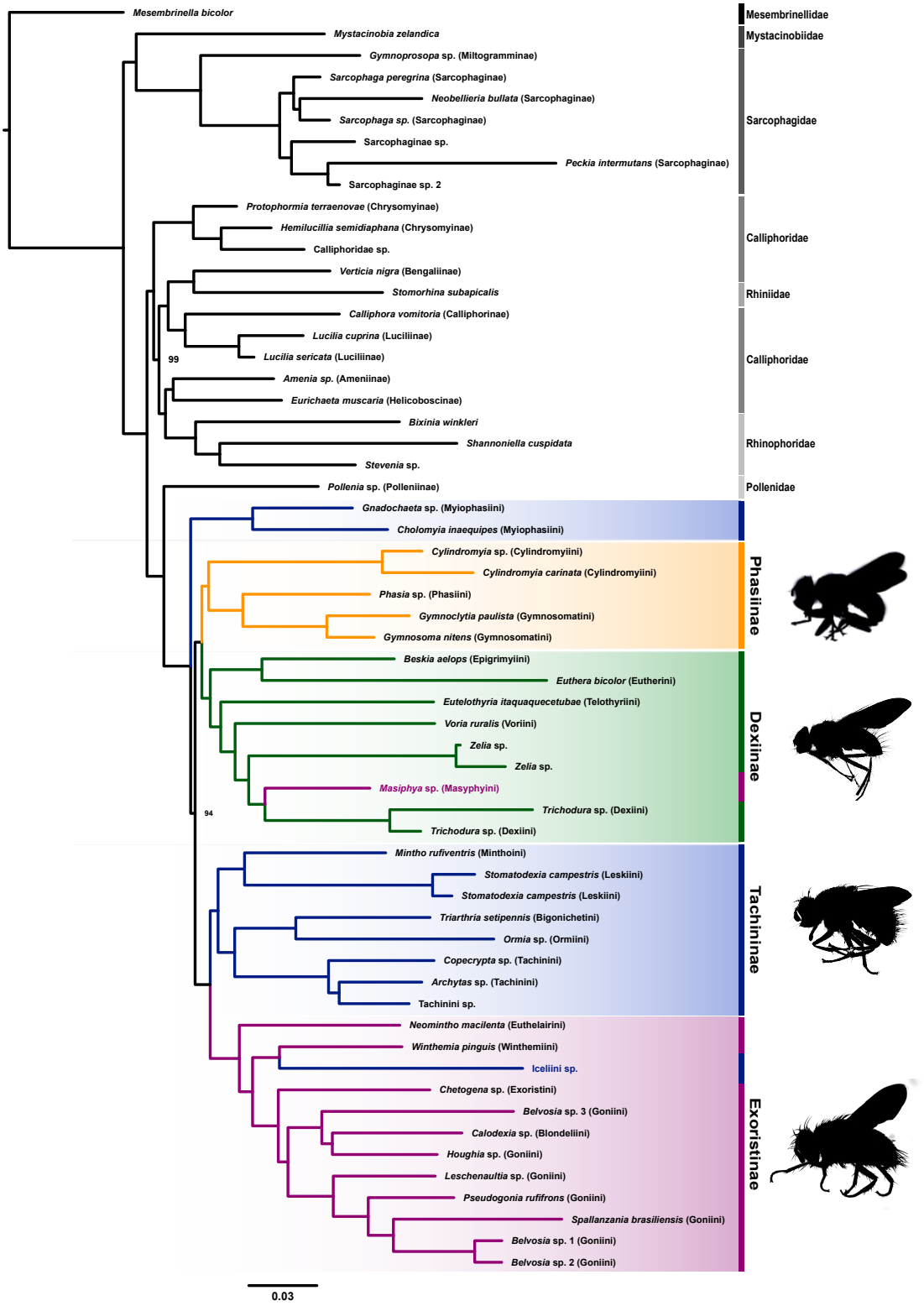

Figure 2. Phylogeny of Tachinidae flies. Maximum likelihood analysis from IQ-TREE with JTT+F+R10 model inferred from the amino acid dataset having a maximum of 15% of missing data. Node support is provided for nodes with ≤99% ML ultrafast bootstrap support.

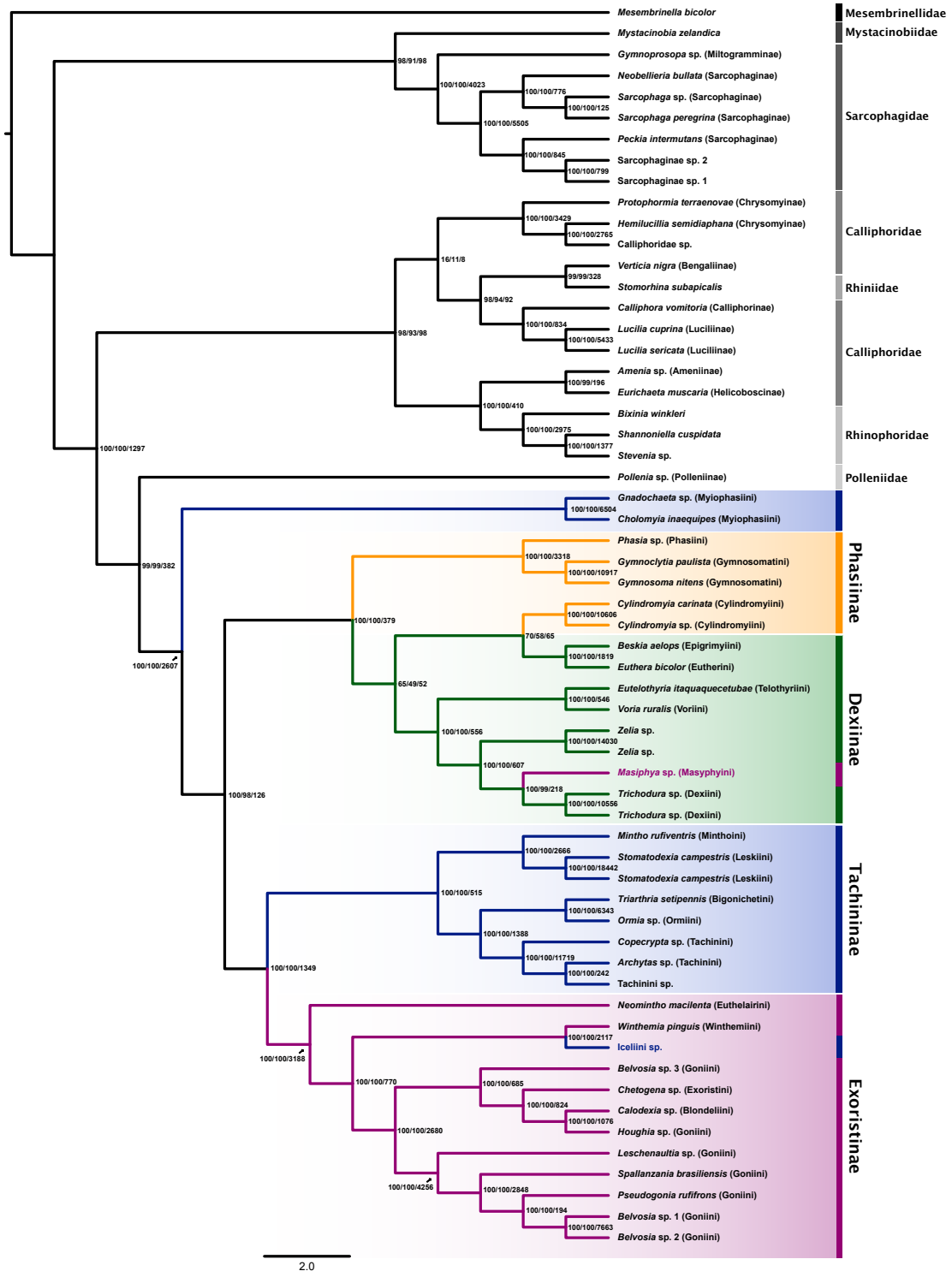

Figure 3. Phylogeny of Tachinidae flies. Maximum parsimony analyses using TNT inferred from the amino acid dataset having a maximum of 15% of missing data. With support values present in the nodes (jackknife/bootstrap/Bremer).

4. Discussion

We present a phylogenomic hypothesis of the Tachinidae family based on transcriptomic data. This type of data became very used for phylogenetic analyses recently because it is possible to recover a strongly supported phylogeny with low coverage sampling of the taxa (Misof *et al.,* 2014; Kutty *et al.,* 2019; Bossert *et al.,* 2019; Buenaventura *et al.,* 2020). Our phylogenomic analyses resulted in well-resolved topologies and high support for all levels of relationships inside Tachinidae. Most nodes obtained in ML and MP analyses are strongly supported and congruent with previous works with morphological (Cerretti *et al.,* 2014) and molecular data (Tachi & Shima, 2010; Blaschke *et al.,* 2018; Kutty *et al.,* 2019; Stireman *et al.,* 2019; Buenaventura *et al.,* 2020). The trees based on amino acids data (likelihood with different coverage and parsimony) and nucleotides data (likelihood) have very similar topology, with few differences on internal nodes and support (Supplementary Fig. 1 and 2).

On the MLT for amino acid data, 56 out of 58 nodes have support values of 100, the other two nodes have support values in the range 90-99 (Fig. 1). On the MLT for nucleotide data, 54 out of 58 nodes have support value of 100, one node presented support value of 96, and three nodes of 82. On the MPT, 47 out of 58 nodes have support values of 100 in jackknife or bootstrap analyses, seven nodes have support values in the range of 60-99, and only one node has values minors than 60 in both analyses of support (Fig. 2). On the other hand, Kutty *et al*. (2019) discussed the need for additional support measures for analyses based on phylogenomic data. They found most nodes (30 out of 39) with high resampling supports (jackknife/bootstrap), although Bremer support has varied significantly for those 100% nodes, ranging from 123 to 3427. Here, in MPT, nodes with 100% support for both jackknife and bootstrap had Bremer support ranging from 125 to 18442.

4.1. *Oestroid relationships and the sister group of Tachinidae*

The Oestroidea relationships obtained in this study show consistency with recent works that focus on Oestroidea families and/or Calyptratae (Tachi & Shima, 2010; Marinho *et al.,* 2012; Nelson *et al.,* 2012; Singh & Wells, 2013; Winkler *et al.,* 2015; Marinho *et al.,* 2017; Cerretti *et al.,* 2017, 2019; Kutty *et al.,* 2019; Stireman *et al.,* 2019; Buenaventura *et al.,* 2020). Eight out of ten families of Oestroidea recognized to date

were sampled and analysed here. Only Ulurumyiidae and Oestridae were not included in our study. Below we present a family-by-family discussion of the results obtained here and compared them to previous works.

Mesembrinellidae Here we used Mesembrinellidae to root the obtained trees. This is congruent with other morphological and molecular phylogenies (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; Buenaventura *et al.,* 2020). Some studies found Mesembrinellidae inside (Kutty *et al.,* 2010) or sister to Tachinidae (Marinho *et al.,* 2012; Junqueira *et al.,* 2016). Other molecular and morphological studies found Mesembrinellidae sister to Ulurumyiidae and the clade (Mesembrinellidae + Ulurumyiidae) sister to the remaining Oestroidea (Cerretti *et al.,* 2017; Michelsen & Pape, 2017; Cerretti *et al.,* 2019; Kutty *et al.,* 2019; Stireman *et al.,* 2019). It is important to note that both studies, ours and Buenaventura *et al*. (2020), have not sampled any Ulurumyiidae species, which could show different results.

Sarcophagidae. This family has been associated with some different sister-group in various studies. Sarcophagidae was considered to be sister-group to Tachinidae (Pape, 1992; Tachi & Shima, 2010), to Calliphoridae (Marinho *et al.,* 2012; Nelson *et al.,* 2012), to Polleniidae (Marinho *et al.,* 2017), and to Oestridae (Winkler *et al.,* 2015; Cerretti *et al.,* 2017, 2019; Stireman *et al.,* 2019; Buenaventura *et al.,* 2020). Sarcophagidae was also considered to be sisters with the clades (Oestridae + (Mesembrinellidae + Tachinidae)) (Junqueira *et al.,* 2016), and Mystacinobiidae + Oestridae (Singh & Wells, 2013; Kutty *et al.* 2019). We recovered Sarcophagidae sister to Mystacinobiidae, which is consistent with the results shown in Kutty *et al*. (2010). It is important to note that we did not sample any Oestridae species, which could also show different results.

Calliphoridae. We present Calliphoridae as polyphyletic which is congruent with the extensive literature confirming the family as non-monophyletic (Rognes, 1997; Kutty *et al.,* 2010; Marinho *et al.,* 2012; Nelson *et al.,* 2012; Singh & Wells, 2013; Winkler *et al.,* 2015; Zhang *et al.,* 2016; Cerretti *et al.,* 2017, 2019; Kutty *et al.,* 2019; Stireman *et al.,* 2019; Buenaventura *et al.,* 2020). Due to the fact that Calliphoridae is polyphyletic this family presents different lineages in the phylogenetic tree. The lineage recovered most times as monophyletic includes the tribes Calliphorinae, Lucillinae, Toxotarsinae and Melanomyinae that can be considered Calliphoridae *sensu stricto* (Kutty *et al.,* 2010; Singh & Wells, 2013; Marinho *et al.,* 2017; Kutty *et al.,* 2019; Buenaventura *et al.,* 2020). We did not sample all the four tribes. However, our results formed a clade containing

Calliphorinae and Lucillinae. Here, the tribe Chrysomyinae forms a distinct lineage, and this arrangement is recovered most times in the literature (Kutty *et al.,* 2010; Singh & Wells, 2013; Marinho *et al.,* 2017; Kutty *et al.,* 2019; Buenaventura *et al.,* 2020). We found Bengaliinae sister to *Stomorhina subapicalis* (Rhiniidae) forming another clade together. This relationship has been recovered before (Singh & Wells, 2013; Kutty *et al.,* 2019; Buenaventura *et al.,* 2020). Bengaliinae has also been recovered as sister to the remaining Calliphoridae (Marinho *et al.,* 2017) and sister to Chrysomyinae in part (Kutty *et al.,* 2010). Helicoboscinae has been found as sister to Calliphoridae *sensu stricto* (Singh & Wells, 2013). On the other hand, Helicoboscinae is recovered as sister to the clade Ameniinae and Rhinophoridae (Kutty *et al.,* 2019) and sister to Rhinophoridae in part (Kutty *et al.,* 2010). In our results, Helicoboscinae is recovered sister to Ameniinae and this clade sister to Rhinophoridae.

Rhiniidae. The Rhiniidae family was associated with different sister-groups in various studies. Rhiniidae was considered to be sister to Tachinidae (Marinho *et al.,* 2017), to the clade Sarcophagidae + Calliphoridae (Marinho *et al.,* 2012) and even closely related to Calliphoridae, Tachinidae and Rhinophoridae (Kutty *et al.,* 2010). Here we present Rhiniidae as sister-group to Bengaliinae (Calliphoridae) which is recovered several times (Cerretti *et al.,* 2017, 2019; Kutty *et al.,* 2019; Stireman *et al.,* 2019; Buenaventura *et al.,* 2020).

Rhinophoridae. This family was recovered sister with Tachinidae (Pape, 1992) and to some clades, such as Tachinidae + Polleniidae (Cerretti *et al.,* 2017), and Rhiniidae + Calliphoridae (Cerretti *et al.,* 2019). We recovered Rhinophoridae as sister-group to Ameniinae + Helicoboscinae, which is consistent with various studies (Kutty *et al.,* 2010; Singh & Wells, 2013; Winkler *et al.,* 2015; Kutty *et al.,* 2019; Stireman *et al.,* 2019; Buenaventura *et al.,* 2020).

Polleniidae. This group is a former subfamily of Calliphoridae, Polleniinae, raised to the family's rank by Cerretti *et al*. (2019). Back when this group was still a subfamily within the blowflies, Polleniidae was recovered multiple times with different sister-groups, such as Rhinophoridae (Kutty *et al.,* 2010), Sarcophagidae (Marinho *et al.,* 2017), and as sister to the clade Rhinophoridae + Rhiniidae (Cerretti *et al.,* 2014). As a subfamily within Calliphoridae or a family, Polleniidae has been recovered as the sister-group to Tachinidae multiple times (Nelson *et al.,* 2012; Singh & Wells, 2013; Winkler *et al.,* 2015; Cerretti *et al.,* 2017; Blaschke *et al.,* 2018; Cerretti *et al.,* 2019; Kutty *et al.,* 2019;

Stireman *et al.,* 2019; Buenaventura *et al.,* 2020), which is consistent to what we recovered in this study.

The relationships within Oestroidea have yielded contrasting results among the families (Pape, 1992; Rognes, 1997; Kutty *et al.,* 2010; Marinho *et al.,* 2012; Singh & Wells, 2013; Kutty *et al.,* 2019; Buenaventura *et al.,* 2020). Therefore, the identity of the sister-group of the tachinids is uncertain until recent times. Throughout history, Tachinidae was paired with different groups as Sarcophagidae (Pape, 1992; Rognes, 1997; Tachi & Shima, 2010), Oestridae (Zhao *et al.,* 2013), Mesembrinellidae (Marinho *et al.,* 2012; Junqueira *et al.,* 2016), Polleniidae + Rhinophoridae (Kutty *et al.,* 2010), and Rhiniidae + Rhinophoridae (Cerretti *et al.,* 2014). Polleniidae had been indicated as sister-group to Tachinidae in some earlier publications (Nelson *et al.,* 2012; Singh & Wells, 2013), but recently it has been consistently recovered (Winkler *et al.,* 2015; Cerretti *et al.,* 2017; Blaschke *et al.,* 2018; Cerretti *et al.,* 2019; Kutty *et al.,* 2019; Stireman *et al.,* 2019; Buenaventura *et al.,* 2020). We recovered Polleniidae as sister to Tachinidae with a bootstrap support value of 100 in MLT, and with a Bremer support value of 382, and jackknife and bootstrap resampling probability of 99 in MPT, which is consistent with most of the work with this family (Nelson *et al.,* 2012; Singh & Wells, 2013; Winkler *et al.,* 2015; Cerretti *et al.,* 2017; Blaschke *et al.,* 2018; Cerretti *et al.,* 2019; Kutty *et al.,* 2019; Stireman *et al.,* 2019; Buenaventura *et al.,* 2020).

4.2. *Tachinidae and its subfamilies*

The monophyly of Tachinidae is here corroborated, which is congruent with the recently published phylogenetic hypothesis based on morphological (Cerretti *et al.,* 2014) or molecular data (Stireman 2002; Tachi & Shima 2010; Winkler *et al.* 2015; Blaschke *et al.* 2018; Stireman *et al.* 2019, 2021) with this family.

4.2.1. *Phasiinae*

Phasiinae has been recovered as monophyletic or paraphyletic. Our MLT has recovered Phasiinae as monophyletic and sister to Dexiinae with strong support (UBS = 100), which is consistent with some recent phylogenetic studies (Tachi & Shima, 2010; Cerretti *et al.,* 2014; Kutty *et al.,* 2019; Stireman *et al.,* 2019) and Blaschke *et al*. (2018)

that details the phylogeny of this group with four-gene data. However, we recovered Phasiinae as paraphyletic in the MPT with Bremer support of 379 and jackknife and bootstrap of 100. Furthermore, Cylindromyiini closely related to Dexiinae with median support (Bremer = 65, jn = 70, bb = 58) which is congruent with Cerretti *et al*. (2017).

The tribes Phasiini and Gymnosomatini are recovered as sister-group in our analyses, corroborating Cerretti *et al*. (2014), Blaschke *et al*. (2018), and Stireman *et al*. (2019). Cylindromyiini is found sister to the clade Phasiini and Gymnosomatini in the ML analyses corroborated by most previous studies (Cerretti *et al.,* 2014; Blaschke *et al.,* 2018; Stireman *et al.,* 2019). On the other hand, Cylindromyiini is grouped with Dexiinae in the MP analysis, sister to the clade Epigrimyiini and Eutherini. This close relationship between Cylindromyiini and Dexiinae, and even closer to the tribes Epigrimyiini and Eutherini can also be found in Cerretti *et al*. (2017).

4.2.2. *Dexiinae*

The paraphyly of Dexiinae has been consistently recovered in different studies, including in the present one. Cerretti *et al*. (2014) classified Dexiinae as paraphyletic concerning Phasiinae with Dufouriini sister to Phasiinae, and the tribe Eutherini clustered with Exoristinae. Stireman *et al*. (2019) also recovered the subfamily as paraphyletic, with the tribes Palpostomatini and Imitomyiini as sister to the clade Dexiinae + Phasiinae. On the other hand, we recovered Dexiinae with Masiphyini as monophyletic with very high MLT support (UBS = 100), congruent with most studies (Tachi & Shima, 2010; Cerretti *et al.,* 2017; Blaschke *et al.,* 2018). In MPT, Dexiinae is recovered as paraphyletic with relatively low support (Bremer = 52, jn = 65, bs = 49). However, Cerretti *et al*. (2017) also recovered Cylindromyiini (Phasiinae) sister to Dexiinae. Unfortunately, we did not sample Dufouriini, Palpostomatini, and Imitomyiini to confirm those other paraphyletic pieces of evidence for Dexiinae provided by Cerretti *et al.* (2014) and Stireman *et al*. (2019).

The tribe Eutherini is grouped sister to Epigrimyini, a result found in two previous studies (Blaschke *et al.,* 2018; Stireman *et al.,* 2019). Dexiini has a controversy systematic placement within Dexiinae since previous studies have found different relationships for this tribe. Here we recovered Dexiini sister to Masyphyini (Exoristinae) and this clade sister to Zeliini. Cerretti *et al*. (2014) found Dexiini within Voriini *sensu* Herting (1984), with Dexiini sister to *Stormina*, which diverges with our results. Blaschke *et al*. (2018)

differ from our findings and Cerretti *et al*. (2014) as they recovered Dexiini sister to Rutiliini. Stireman *et al*. (2019) found a result that corroborated Blaschke *et al*. (2018), Dexiini is polyphyletic with a close relationship with Rutiliini, Doleschellini, Sophiini, and Ernestiini (previously in Phasiinae). We also found Telothyriini sister to the clade Voriini + (Zeliini + (Dexiini + Masyphyini)). Stireman *et al*. (2019) did not corroborate our results since they found Voriini polyphyletic and part of the tribe that contains *Voria ruralis* closely related to Uramyini. On the other hand, in this study, another part of Voriini is closely related to Telothyriini. Blaschke *et al*. (2018) recovered Voriini paraphyletic and one portion sister to Dufouriini and the other part sister to Uramyini. They did not sample Telothyriini or Zeliini.

4.2.3. *Tachininae*

Tachininae is a very complex subfamily, and Tschorsnig (1985) regarded it as the most weakly supported tachinid group. Tachininae has been recovered monophyletic only when taxonomic sampling is very low (Tachi & Shima, 2010; Cerretti *et al.,* 2017; Blaschke *et al.,* 2018). On the other hand, Cerretti *et al*. (2014) recovered Tachininae as polyphyletic with (*Gnadochaeta* (Myiophasiini) + Palpostomatini) and Ormiini as sister to the remaining Tachinidae. Stireman *et al*. (2019) also recovered Tachininae as paraphyletic with Macquartiini and Myiophasiini as sister to the remaining Tachinidae. We did not sample Macquartiini. Stireman *et al*. (2019) also divided Tachininae into two major lineages: one with Graphogastrini, Minthoini, Leskiini, Brachymerini, and the other with the remaining tribes. Although, with fewer sampled tribes, our analyses converge in these two lineages, with Minthoini + Leskiini sister to a clade with Bigonichetini, Ormiini, and Tachinini.

The other tribal relationships in this study are only partially congruent with previous works. Here, we recovered Bigonichetini as sister to Ormiini and this clade sister to Tachinini. Stireman *et al*. (2019) recovered Tachinini sister to a clade with 11 other tribes. In this study, Bigonichetini was not sampled. However, Stireman *et al*. (2019) classified *Triarthria setipennis* (Bigonichetini in O'Hara & Handerson, 2020) as a part of the Loewiini group. Stireman *et al*. (2019) recovered Loewiini sister to a clade composed of Ormiini and Ernestiini. On the other hand, in Cerretti *et al*. (2014), Bigonichetini is grouped with Neaerini and part of Ernestiini, and Ormiini. Ormiini is recovered within the basal tribes of the family, sister to all remaining Tachinidae. Moreover, Tachinini is

presented as sister to the clade Brachymerini and Siphonini. Thus, despite our reduced sampling, our work presents the relative positions of tribes similar to those given by Stireman *et al*. (2019).

This small Neotropical endemic tribe Iceliini had never been sampled in a phylogenetic study before, either with morphological or molecular data. Initially, Iceliini was allocated inside Dexiinae by Townsend (1936). However, the classification of this tribe is very controversial. Thompson (1963) disagreed with the placement of Iceliini within Dexiinae and found larvae features to place the tribe within Tachininae. The features included narrow overlapping plates on the dorsum, hair-like sensoria, and a recurved clavate organ (Thompson, 1963). Guimarães (1976) and O'Hara & Wood (2004) used adult morphology to consider the tribe within Tachininae. Some of the adult features are body elongate and narrowed, no facial carina, antennae with the second segment elongate, about as long as the third segment, eyes bare, two strong lateral scuttellars and two decussate apicals, legs brown, tarsi elongate (Guimarães, 1976). Iceliini was considered close to Tachinini by Tschorsnig (1985) based on similarities in male terminalia, including sternite 6 asymmetrical, cerci fused without suture and pointed, surstyli expanded apical, pregonite lobe-like, and postgonite narrow and pointed. Unlike any previous study, we recovered the Iceliini tribe grouped with Exoristinae and close to Winthemiini. None previous study considered the possibility of Iceliini being part of Exoristinae.

4.2.4. *Exoristinae*

Exoristinae was recovered as polyphyletic. Our study recovered the Masiphyini tribe grouped within Dexiinae and close to tribe Dexiini, which contrasts with Stireman *et al.* (2019) that found Masiphyini inside Exoristinae. This result also differs from Tschorsnig (1985), in which the tribe would be placed within Phasiinae using similarities in male post-abdomen. Neither Cerretti *et al.* (2014) nor Stireman (2002) and Tachi & Shima (2010) sampled any species from the Masiphyini tribe. Without this tribe, Exoristinae and Iceliini is also highly supported as monophyletic, consistent with most studies (Stireman, 2002; Tachi & Shima, 2010; Cerretti *et al.,* 2014, 2017; Blaschke *et al.,* 2018; Stireman *et al.,* 2019).

Here, we reconstructed a basal Euthelairini, sister to the remaining Exoristinae. Stireman *et al.* (2019) recovered Euthelairini sister to Ethillini and this clade sister to the

remaining Exoristinae. Our work presents Winthemiini sister to Iceliini (previously in Tachininae) and this clade sister to Exoristini + (Goniini + *Calodexia* sp.). We found Blondeliini (*Calodexia* sp.) grouped within Goniini. The relationship among the tribes is very similar to those presented by previous authors. Stireman (2002) reconstructed Winthemiini + Exoristini sister to Blondeliini + (Eryciini + Goniini). Tachi and Shima (2010) showed Winthemiini grouped with Ethillini and this clade sister to the remaining Exoristinae in the following relationship: Exoristini + (Blondeliini + (Eryciini + Goniini)). Cerretti *et al.* (2014) presented a similar position with Winthemiini sister to Ethillini, and Exoristini sister to this clade. Stireman *et al.* (2019) recovered Winthemiini sister to the clade Blondeliini + (Exoristini + (Blondeliini + (Eryciini + Goniini))).

4.3. *The fifth lineage of Tachinidae*

The tribe Myiophasiini (Tachininae) was reconstructed as sister to the remaining Tachinidae. This fifth lineage of Tachinidae has been recovered in multiple works (Cerretti *et al.,* 2014; Stireman *et al.,* 2019; Buenaventura *et al.,* 2020). Cerretti *et al.* (2014) recovered Tachininae as polyphyletic and the Coleoptera-parasitizing clade (*Gnadochaeta* (Myiophasiini) + Palpostomatini) and the highly derived Ormiini as sister to the remaining Tachinidae. Cerretti *et al.* (2014) also indicated the greatly variable position of the genus *Macroposopa* (Macquartiini) across the phylogenetic analyses, assuming positions either within Tachininae or, most commonly, at the base of the family, sister to the remaining Tachinidae. Cerretti *et al.* (2014) pointed out similarities in the terminalia between *Macroposopa* and *Gnadochaeta* (Myiophasiini) and Palpostomatini. Thus, they concluded that the position of *Macroposopa* in the base of Tachinidae with *Gnadochaeta* (Myiophasiini) and Palpostomatini is more accurate than within Tachininae.

Recently, Stireman *et al.* (2019) also recovered Tachininae as polyphyletic with the tribes Macquartini and Myiophasiini as sisters to the remaining Tachinidae. Interestingly, Palpostomatini was placed as a Dexiinae tribe. However, part of Palpostomatini and Imitomyiini were reconstructed with strong support as the sister-group to Dexiinae + Phasiinae. The results from Stireman *et al.* (2019) recovered Palpostomatini a basal dexiine, and perhaps further analyses should be done with these tribes to evaluate their certain placement within the family.

This basal lineage composed of Myiophasiini and Macquartiini, and possibly also Palpostomatini, has been consistently recovered based on morphology (Cerretti *et al.,* 2014), PCR-amplified sequences (Stireman *et al.,* 2019), and transcriptomes (present study). It indicates that we have a fifth lineage (subfamily) within the Tachinidae family.

5. Conclusions

We obtained a well-supported phylogeny based on transcriptomic data and ortholog genes. The obtained results present strong support for monophyly of Tachinidae and Polleniidae as its sister-group. Phasiinae is monophyletic, Dexiinae is paraphyletic, and Exoristinae and Tachininae are polyphyletic. The tribes Masyphyini and Iceliini are reconstructed with different relationships than previously found. Perhaps a more thorough study should be done with these tribes to precise their position within the subfamilies. The fifth lineage of Tachinidae is a result that has been recovered a couple of times recently, including in the present study. Increasing the taxon sampling for the tribes in this lineage can give us a definitive answer for this position, and in the future, perhaps a fifth subfamily can be established.

Our results are very similar to the recent studies, sampling and/or focusing on Tachinidae. Nevertheless, increase the taxon sampling and expand the types of data (e.g., morphology, ultra-conserved elements, physiology) are the next step for resolving the open and unclear questions. With that, we will have a phylogenetic understanding of Tachinidae at subfamily and tribe levels that will certainly contribute to comprehend further questions, like diversification history, biogeography, and parasitism evolution.

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References

Andrews, S. (2010) *FastQC: a quality control tool for high throughput sequence data.* Available from: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

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): Phasiine phylogenetics and piercer evolution. *Systematic Entomology* 43, 218–238. https://doi.org/10.1111/syen.12272.

Bolger, A.M., Lohse, M. & Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Bossert, S., Murray, E.A., Almeida, E.A.B., Brady, S.G., Blaimer, B.B. & Danforth, B.N. (2019) Combining transcriptomes and ultraconserved elements to illuminate the phylogeny of Apidae. *Molecular Phylogenetics and Evolution* 130, 121–131. https://doi.org/10.1016/j.ympev.2018.10.012.

Buenaventura, E., Lloyd, M.W., Perilla López, J.M., González, V.L., Thomas-Cabianca, A. & Dikow, T. (2020) Protein-encoding ultraconserved elements provide a new phylogenomic perspective of Oestroidea flies (Diptera: Calyptratae). *Systematic Entomology*. https://doi.org/10.1111/syen.12443.

Capella-Gutierrez, S., Silla-Martinez, J.M. & Gabaldon, T. (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25, 1972–1973. https://doi.org/10.1093/bioinformatics/btp348.

Cerretti, P., O'Hara, J.E., Wood, D.M., Shima, H., Inclan, D.J. & Stireman, J.O. (2014) Signal through the noise? Phylogeny of the Tachinidae (Diptera) as inferred from morphological evidence: Phylogeny of Tachinidae. *Systematic Entomology* 39, 335–353. https://doi.org/10.1111/syen.12062.

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., 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: Calyptratae: Oestroidea) and the dating of oestroid divergences M. Friedman (Ed). *PLOS ONE* 12, e0182101. https://doi.org/10.1371/journal.pone.0182101.

Crawford, J.E., Guelbeogo, W.M., Sanou, A., Traoré, A., Vernick, K.D., Sagnon, N. &

Lazzaro, B.P. (2010) De Novo Transcriptome Sequencing in Anopheles funestus Using Illumina RNA-Seq Technology P. Awadalla (Ed). *PLoS ONE* 5, e14202. https://doi.org/10.1371/journal.pone.0014202.

Crosskey, R.W. (1976) A taxonomic conspectus of the Tachinidae (Diptera) of the Oriental Region. *Bulletin of the British Museum (Natural History), Entomology* 26, 357.

Gillung, J.P., Winterton, S.L., Bayless, K.M., Khouri, Z., Borowiec, M.L., Yeates, D., Kimsey, L.S., Misof, B., Shin, S., Zhou, X., Mayer, C., Petersen, M. & Wiegmann, B.M. (2018) Anchored phylogenomics unravels the evolution of spider flies (Diptera, Acroceridae) and reveals discordance between nucleotides and amino acids. *Molecular Phylogenetics and Evolution* 128, 233–245. https://doi.org/10.1016/j.ympev.2018.08.007.

Goloboff, P.A. (1999) Analyzing Large Data Sets in Reasonable Times: Solutions for Composite Optima. *Cladistics* 15, 415–428. https://doi.org/10.1111/j.1096-0031.1999.tb00278.x.

Guimarães, J.H. (1976) A Review of the Tribe Iceliini (Diptera, Tachinidae) with Descriptions of one new Genus and two new Species from Brazil. *Studia Entomologica* 19, 173-186.

Guimarães, J.H. (1977) A systematic revision of the Mesembrinellidae, stat. nov. (Diptera, Cyclorrhapha). *Arquivos de Zoologia* 29, 1–109.

Haas, B.J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P.D., Bowden, J., Couger, M.B., Eccles, D., Li, B., Lieber, M., MacManes, M.D., Ott, M., Orvis, J., Pochet, N., Strozzi, F., Weeks, N., Westerman, R., William, T., Dewey, C.N., Henschel, R., LeDuc, R.D., Friedman, N. & Regev, A. (2013) De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature Protocols* 8, 1494–1512. https://doi.org/10.1038/nprot.2013.084.

Herting, B. (1984) Catalogue of Paleartic Tachinidae (Diptera). *Stuttgarter Beiträge zur Naturkunde* 369, 228.

Hittinger, C.T., Johnston, M., Tossberg, J.T. & Rokas, A. (2010) Leveraging skewed transcript abundance by RNA-Seq to increase the genomic depth of the tree of life. *Proceedings of the National Academy of Sciences* 107, 1476–1481. https://doi.org/10.1073/pnas.0910449107.

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q. & Vinh, L.S. (2018)

UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Molecular Biology and Evolution* 35, 518–522. https://doi.org/10.1093/molbev/msx281.

Jiang, F., Pan, X., Li, X., Yu, Y., Zhang, J., Jiang, H., Dou, L. & Zhu, S. (2016) The first complete mitochondrial genome of *Dacus longicornis* (Diptera: Tephritidae) using next-generation sequencing and mitochondrial genome phylogeny of Dacini tribe. *Scientific Reports* 6, 36426. https://doi.org/10.1038/srep36426.

Junqueira, A.C.M., Azeredo-Espin, A.M.L., Paulo, D.F., Marinho, M.A.T., Tomsho, L.P., Drautz-Moses, D.I., Purbojati, R.W., Ratan, A. & Schuster, S.C. (2016) Large-scale mitogenomics enables insights into Schizophora (Diptera) radiation and population diversity. *Scientific Reports* 6, 21762. https://doi.org/10.1038/srep21762.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A. & Jermiin, L.S. (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 14, 587–589. https://doi.org/10.1038/nmeth.4285.

Karlin, S. & Altschul, S.F. (1990) Methods for assessing the statistical significance of molecular sequence features by using general scoring schemes. *Proceedings of the National Academy of Sciences* 87, 2264–2268. https://doi.org/10.1073/pnas.87.6.2264.

Katoh, K. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30, 3059–3066. https://doi.org/10.1093/nar/gkf436.

Kutty, S.N., Meusemann, K., Bayless, K.M., Marinho, M.A.T., Pont, A.C., Zhou, X., Misof, B., Wiegmann, B.M., Yeates, D., Cerretti, P., Meier, R. & Pape, T. (2019) Phylogenomic analysis of Calyptratae: resolving the phylogenetic relationships within a major radiation of Diptera. *Cladistics*. https://doi.org/10.1111/cla.12375.

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.

Li, W. & Godzik, A. (2006) Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* 22, 1658–1659. https://doi.org/10.1093/bioinformatics/btl158.

Li, X., Ding, S., Hou, P., Liu, X., Zhang, C. & Yang, D. (2017) Mitochondrial genome analysis of *Ectophasia roundiventris* (Diptera, Tachinidae). *Mitochondrial DNA*

Part B 2, 457–458. https://doi.org/10.1080/23802359.2017.1357447.

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. (2017) The first phylogenetic study of Mesembrinellidae (Diptera: Oestroidea) based on molecular data: clades and congruence with morphological characters. *Cladistics* 33, 134-152.

Marinković, M., de Leeuw, W.C., de Jong, M., Kraak, M.H.S., Admiraal, W., Breit, T.M. & Jonker, M.J. (2012) Combining Next-Generation Sequencing and Microarray Technology into a Transcriptomics Approach for the Non-Model Organism Chironomus riparius M. Watson (Ed). *PLoS ONE* 7, e48096. https://doi.org/10.1371/journal.pone.0048096.

Michelsen, V. & Pape, T. (2017) Ulurumyiidae - a new family of calyptrate flies (Diptera): A new calyptrate family. *Systematic Entomology* 42, 826–836. https://doi.org/10.1111/syen.12252.

Misof, B., Liu, S., Meusemann, K., Peters, R.S., Donath, A., Mayer, C., Frandsen, P.B., Ware, J., Flouri, T., Beutel, R.G., Niehuis, O., Petersen, M., Izquierdo-Carrasco, F., Wappler, T., Rust, J., Aberer, A.J., Aspock, U., Aspock, H., Bartel, D., Blanke, A., Berger, S., Bohm, A., Buckley, T.R., Calcott, B., Chen, J., Friedrich, F., Fukui, M., Fujita, M., Greve, C., Grobe, P., Gu, S., Huang, Y., Jermiin, L.S., Kawahara, A.Y., Krogmann, L., Kubiak, M., Lanfear, R., Letsch, H., Li, Y., Li, Z., Li, J., Lu, H., Machida, R., Mashimo, Y., Kapli, P., McKenna, D.D., Meng, G., Nakagaki, Y., Navarrete-Heredia, J.L., Ott, M., Ou, Y., Pass, G., Podsiadlowski, L., Pohl, H., von Reumont, B.M., Schutte, K., Sekiya, K., Shimizu, S., Slipinski, A., Stamatakis, A., Song, W., Su, X., Szucsich, N.U., Tan, M., Tan, X., Tang, M., Tang, J., Timelthaler, G., Tomizuka, S., Trautwein, M., Tong, X., Uchifune, T., Walzl, M.G., Wiegmann, B.M., Wilbrandt, J., Wipfler, B., Wong, T.K.F., Wu, Q., Wu, G., Xie, Y., Yang, S., Yang, Q., Yeates, D.K., Yoshizawa, K., Zhang, Q., Zhang, R., Zhang, W., Zhang, Y., Zhao, J., Zhou, C., Zhou, L., Ziesmann, T., Zou, S., Li, Y., Xu, X., Zhang, Y., Yang, H., Wang, J., Wang, J., Kjer, K.M. & Zhou, X. (2014) Phylogenomics resolves the timing and pattern of insect evolution. *Science* 346, 763–767.

https://doi.org/10.1126/science.1257570.

Nelson, L.A., Lambkin, C.L., Batterham, P., Wallman, J.F., Dowton, M., Whiting, M.F., Yeates, D.K. & Cameron, S.L. (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. (2013) History of tachinid classification (Diptera, Tachinidae). *ZooKeys* 316, 1–34. https://doi.org/10.3897/zookeys.316.5132.

O'Hara, J.E. & Henderson, S.J. (2020) World genera of the Tachinidae (Diptera) and their regional occurrence. Version 11.0. PDF document, 90 pp. Available from: http://www.nadsdiptera.org/Tach/WorldTachs/Genera/Gentach_ver11.pdf (accessed on 9 March 2021).

O'Hara, J.E. & Wood, D.M. (2004) Catalogue of the Tachinidae (Diptera) of America north of Mexico. Associated Publishers, Gainesville, Fla, 410 pp.

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

Rognes, K. (1997) The Calliphoridae (Blowflies) (Diptera: Oestroidea) are Not a Monophyletic Group1. *Cladistics* 13, 27–66. https://doi.org/10.1111/j.1096-0031.1997.tb00240.x.

Santis, M.D. (2016) Análise cladística da tribo Dufouriini (Diptera, Tachinidae). Programa de Pós Graduação em Zoologia. Universidade de São Paulo.

Simão, F.A., Waterhouse, R.M., Ioannidis, P., Kriventseva, E.V. & Zdobnov, E.M. (2015) BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31, 3210–3212. https://doi.org/10.1093/bioinformatics/btv351.

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.

Stireman, J.O. (2002) Phylogenetic relationships of tachinid flies in subfamily Exoristinae (Tachinidae: Diptera) based on 28S rDNA and elongation factor-1α:

Phylogeny of Exoristinae (Tachinidae). *Systematic Entomology* 27, 409–435. https://doi.org/10.1046/j.1365-3113.2002.00187.x.

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.

Stireman, J.O., O'Hara, J.E. & Wood, D.M. (2006) TACHINIDAE: Evolution, Behavior, and Ecology. *Annual Review of Entomology* 51, 525–555. https://doi.org/10.1146/annurev.ento.51.110104.151133.

Tachi, T. & Shima, H. (2010) Molecular phylogeny of the subfamily Exoristinae (Diptera, Tachinidae), with discussions on the evolutionary history of female oviposition strategy. *Systematic Entomology* 35, 148–163. https://doi.org/10.1111/j.1365-3113.2009.00497.x.

Thompson, W.R. (1963) The Tachinids of Trinidad: II. Echinomyiines, Dexiines, and allies. *Canadian Journal of Zoology* 41, 335–576.

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

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. (1987) Tachinidae. In: J. F. McAlpine (Ed), *Manual of Neartic Diptera*. Biosystematics Research Centre, Ottawa, Ontario, pp. 1193–1269.

Yong, H.-S., Song, S.-L., Lim, P.-E., Chan, K.-G., Chow, W.-L. & Eamsobhana, P. (2015) Complete mitochondrial genome of *Bactrocera arecae* (Insecta: Tephritidae) by next-generation sequencing and molecular phylogeny of Dacini tribe. *Scientific Reports* 5, 15155. https://doi.org/10.1038/srep15155.

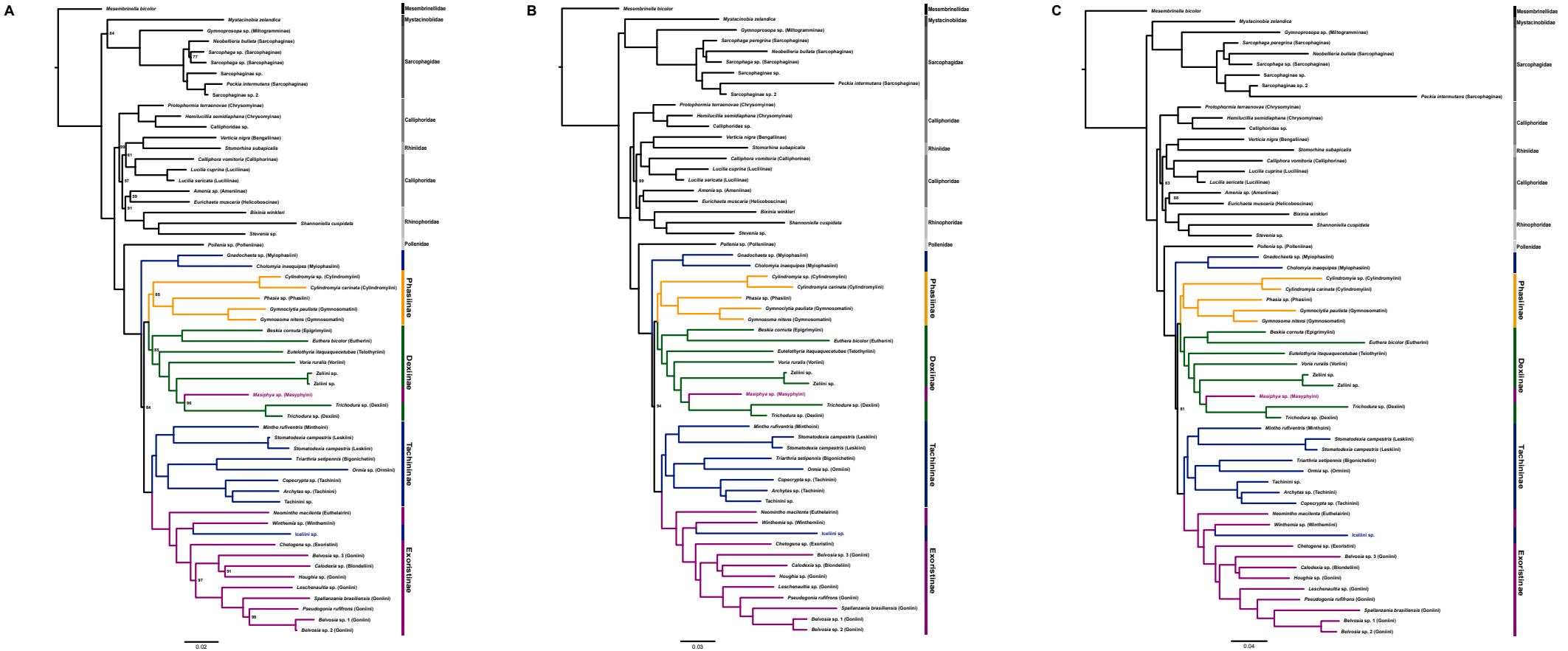
Young, A.D., Lemmon, A.R., Skevington, J.H., Mengual, X., Ståhls, G., Reemer, M., Jordaens, K., Kelso, S., Lemmon, E.M., Hauser, M., De Meyer, M., Misof, B. &

Wiegmann, B.M. (2016) Anchored enrichment dataset for true flies (order Diptera) reveals insights into the phylogeny of flower flies (family Syrphidae). *BMC Evolutionary Biology* 16, 143. https://doi.org/10.1186/s12862-016-0714-0.

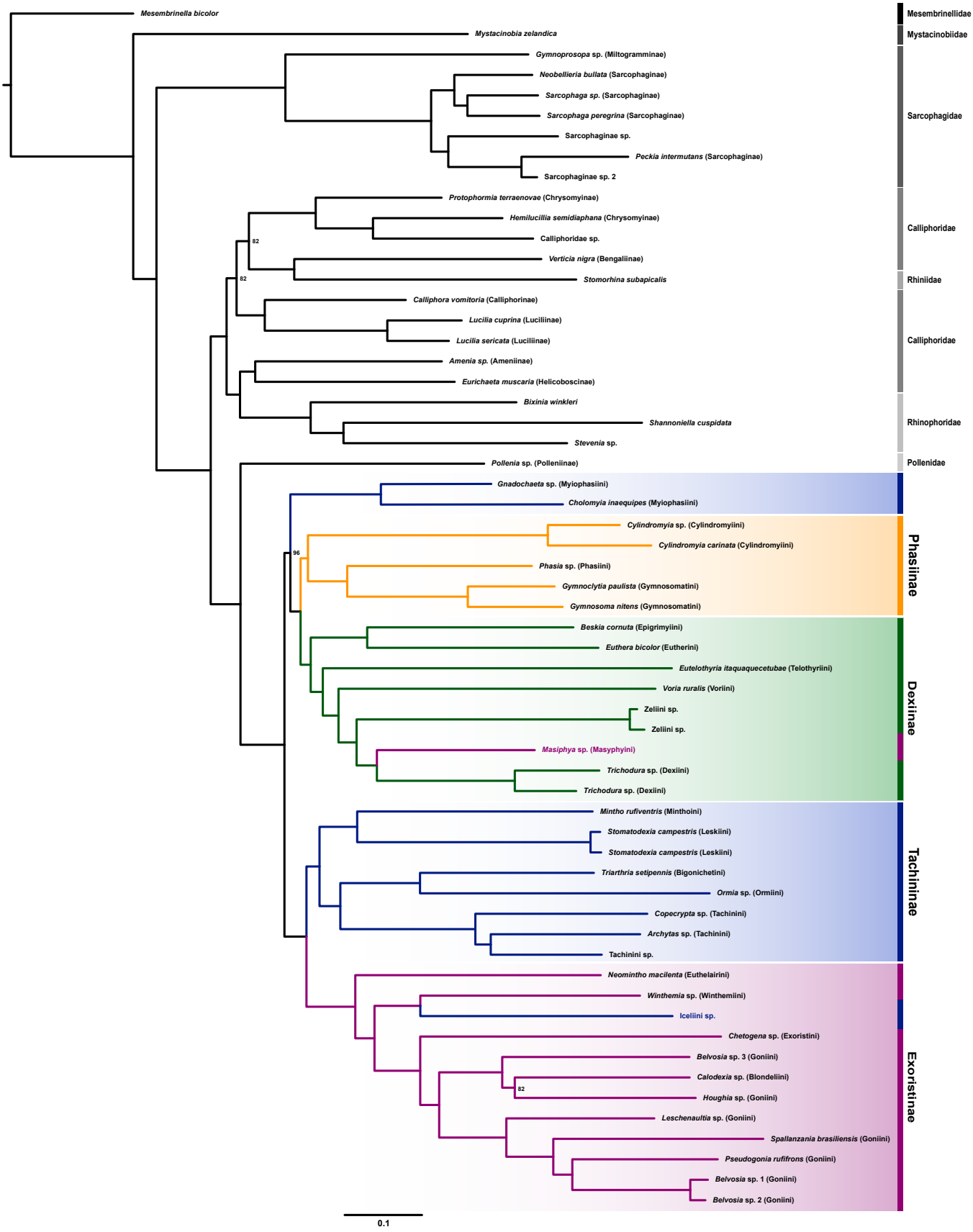
Zhang, D., Yan, L., Zhang, M., Chu, H., Cao, J., Li, K., Hu, D. & Pape, T. (2016) Phylogenetic inference of calyptrates, with the first mitogenomes for Gasterophilinae (Diptera: Oestridae) and Paramacronychiinae (Diptera: Sarcophagidae). *International Journal of Biological Sciences* 12, 489–504. https://doi.org/10.7150/ijbs.12148.

Zhao, Z., Su, T., Chesters, D., Wang, S., Ho, S.Y.W., Zhu, C., Chen, X. & Zhang, C. (2013) The Mitochondrial Genome of *Elodia flavipalpis* Aldrich (Diptera: Tachinidae) and the Evolutionary Timescale of Tachinid Flies D. Doucet (Ed). *PLoS ONE* 8, e61814. https://doi.org/10.1371/journal.pone.0061814.

Supplementary Material



Supplementary Figure 1. Phylogeny of Tachinidae flies. IQ-TREE best tree estimated with JTT+F+R10 model from the concatenated datasets having no missing data (A), a maximum of 15% (B), and 50% (C) of missing data. Node support is provided for nodes with ≤99% ML ultrafast bootstrap support.



Supplementary Figure 2. Phylogeny of Tachinidae flies. IQ-TREE best tree estimated with GTR+F+R9 model from concatenated dataset of 75% coverage of nucleotides. Node support is provided for nodes with ≤99% ML ultrafast bootstrap support.

Supplementary Table 1. Collection information of the species sequenced by the project.

Species	Collector	Collection date	Location
Cylindromyia carinata	LaSBI	24.Nov.2017	Brazil, São Paulo, São Paulo, University of São Paulo, campus
Gymnoclytia paulista	Rodrigo Dios	31.Oct.2018	Brazil, São Paulo, São Paulo, University of São Paulo, campus
Phasia sp.	Letícia Baldassio	12.Dec.2018	Brazil, São Paulo, São Paulo, University of São Paulo, campus
Beskia cornuta	LaSBI	24.Nov.2017	Brazil, São Paulo, São Paulo, University of São Paulo, campus
Eutelothyria itaquaquecetubae	LaSBI	14-17.Jan.2018	Brazil, São Paulo, Salesópolis, Estação Biológica de Boracéia
Trichodura sp.	LaSBI	14-17.Jan.2018	Brazil, São Paulo, Salesópolis, Estação Biológica de Boracéia
Trichodura sp.	Silvio Nihei, Marco Antônio Marinho, Marcelo Santis & Thalles Pereira	11-13.Dec.2015	Brazil, São Paulo, Salesópolis, Estação Biológica de Boracéia
Zeliini	LaSBI	14-17.Jan.2018	Brazil, São Paulo, Salesópolis, Estação Biológica de Boracéia
Zeliini	LaSBI	14-17.Jan.2018	Brazil, São Paulo, Salesópolis, Estação Biológica de Boracéia
Voria ruralis	LaSBI	24.Nov.2017	Brazil, São Paulo, São Paulo, University of São Paulo, campus
Iceliini sp.	Marco Antônio Menezes	30.Nov-11.Dec.2018	Brazil, Pará, Belterra, Fazenda Treviso
Stomatodexia campestris	LaSBI	14-17.Jan.2018	Brazil, São Paulo, Salesópolis, Estação Biológica de Boracéia
Stomatodexia campestris	LaSBI	14-17.Jan.2018	Brazil, São Paulo, Salesópolis, Estação Biológica de Boracéia
Cholomyia inaequipes	LaSBI	14-17.Jan.2018	Brazil, São Paulo, Salesópolis, Estação Biológica de Boracéia
Gnadochaeta sp.	Letícia Baldassio	12.Dec.2018	Brazil, São Paulo, São Paulo, University of São Paulo, campus
Ormia sp.	Rodrigo Dios	14.Oct.2015	Brazil, São Paulo, São Paulo, University of São Paulo, campus
Archytas sp.	Deivys Alvarez & Marcelo Santis	3-9.Feb.2019	Brazil, Minas Gerais, Parque Nacional Grande Sertão Veredas
Copecrypta sp.	Letícia Baldassio	6.Nov.2018	Brazil, São Paulo, São Paulo, University of São Paulo, campus
Tachinini sp.	Deivys Alvarez	19-21.Oct.2018	Brazil, São Paulo, Itatiaia, Parque Nacional de Itatiaia
Calodexia sp.	LaSBI	14-17.Jan.2018	Brazil, São Paulo, Salesópolis, Estação Biológica de Boracéia
Chetogena sp.	Deivys Alvarez & Marcelo Santis	3-9.Feb.2019	Brazil, Minas Gerais, Parque Nacional Grande Sertão Veredas
Belvosia sp.1	Rodrigo Dios	31.Oct.2018	Brazil, São Paulo, São Paulo, University of São Paulo, campus
Belvosia sp.2	Deivys Alvarez & Marcelo Santis	3-9.Feb.2019	Brazil, Minas Gerais, Parque Nacional Grande Sertão Veredas
Belvosia sp.3	Deivys Alvarez & Marcelo Santis	3-9.Feb.2019	Brazil, Minas Gerais, Parque Nacional Grande Sertão Veredas
Houghia sp.	LaSBI	14-17.Jan.2018	Brazil, São Paulo, Salesópolis, Estação Biológica de Boracéia
Leschenaultia sp.	LaSBI	24.Nov.2017	Brazil, São Paulo, São Paulo, University of São Paulo, campus
Spallanzania brasiliensis	LaSBI	24.Nov.2017	Brazil, São Paulo, São Paulo, University of São Paulo, campus

Masiphya sp.	LaSBI	14-17.Jan.2018	Brazil, São Paulo, Salesópolis, Estação Biológica de Boracéia
Neomintho macilenta	LaSBI	14-17.Jan.2018	Brazil, São Paulo, Salesópolis, Estação Biológica de Boracéia
Winthemia "maguila"	LaSBI	24.Nov.2017	Brazil, São Paulo, São Paulo, University of São Paulo, campus
Hemilucilia semidiaphana	LaSBI	24.Nov.2017	Brazil, São Paulo, São Paulo, University of São Paulo, campus
Calliphoridae sp.	Deivys Alvarez & Marcelo Santis	3-9.Feb.2019	Brazil, Minas Gerais, Parque Nacional Grande Sertão Veredas
Mesembrinella bicolor	LaSBI	14-17.Jan.2018	Brazil, São Paulo, Salesópolis, Estação Biológica de Boracéia
Shannoniella cuspidata	LaSBI	14-17.Jan.2018	Brazil, São Paulo, Salesópolis, Estação Biológica de Boracéia
Peckia intermutans	Pedro Souza-Dias & Marcela Monné	30.Nov-11.Dec.2018	Brazil, Pará, Belterra, Fazenda Treviso
Sarcophaginae sp. 2	LaSBI	14-17.Jan.2018	Brazil, São Paulo, Salesópolis, Estação Biológica de Boracéia
Sarcophaginae sp. 1	Deivys Alvarez & Marcelo Santis	3-9.Feb.2019	Brazil, Minas Gerais, Parque Nacional Grande Sertão Veredas

Supplementary Table 2. Information about the species used from GenBank.

Family	Subfamily	Species name	NCBI Accession
Tachinidae	Phasiinae	*Cylindromyia sp.*	SRA:SRR6724166
Tachinidae	Phasiinae	*Gymnosoma nitens*	SRA:SRR6724168
Tachinidae	Dexiinae	*Euthera bicolor*	SRA:SRR1695343
Tachinidae	Tachininae	*Mintho rufiventris*	SRA:SRR6724169
Tachinidae	Tachininae	*Triarthria setipennis*	SRA:SRR921652
Tachinidae	Exoristinae	*Pseudogonia rufifrons*	SRA:SRR6724155
Calliphoridae	Ameniinae	*Amenia sp.*	SRA:SRR6724164
Calliphoridae	Bengaliinae	*Verticia nigra*	SRA:SRR6724159
Calliphoridae	Helicoboscinae	*Eurichaeta muscaria*	SRA:SRR6724165
Calliphoridae	Chrysomyinae	*Protophormia terraenovae*	SRA:DRR087980
Calliphoridae	Luciliinae	*Lucilia cuprina*	SRA:SRR1853101
Calliphoridae	Luciliinae	*Lucilia sericata*	SRA:SRR6265710
Calliphoridae	Calliphorinae	*Calliphora vomitoria*	SRA:SRR1695328
Polleniidae	Polleniinae	*Pollenia sp.*	SRA:SRR8236846
Mystacinobiidae		*Mystacinobia zelandica*	SRA:SRR6724158
Rhiniidae		*Stomorhina subapicalis*	SRA:SRR1695394
Rhinophoridae		*Bixinia winkleri*	SRA:SRR6724163
Rhinophoridae		*Stevenia sp.*	SRA:SRR6724161
Sarcophagidae	Miltogramminae	*Gymnoprosopa sp.*	SRA:SRR8236844
Sarcophagidae	Sarcophaginae	*Sarcophaga peregrina*	SRA:SRR6265701
Sarcophagidae	Sarcophaginae	*Sarcophaga sp.*	SRA:SRR6724162
Sarcophagidae	Sarcophaginae	*Neobellieria bullata*	SRA:SRR5559336

Conclusão Geral

Nós obtivemos uma filogenia muito bem suportada baseada em dados transcriptômicos e genes ortólogos. Encontramos um alto suporte para a monofilia de Tachinidae e para Polleniidae como seu grupo-irmão. Nossas análises recuperaram Phasiinae como monofilética, Dexiinae como parafilética, e Exoristinae e Tachininae como polifiléticas. As tribos Masyphyini e Iceliini são recuperadas com relações diferentes do que encontrado anteriormente na literatura. Um estudo mais detalhado deve ser feito com essas tribos para confirmar suas posições filogenéticas dentro das subfamílias. A quinta linhagem de Tachinidae é um resultado que foi encontrado algumas vezes recentemente, incluindo nesse estudo. O aumento de amostragem para as tribos dessa linhagem pode nos dar uma resposta definitiva para sua posição na família, e no futuro, a possível criação de uma nova subfamília.

Nossos resultados são muito semelhantes aos estudos recentes, que amostraram e/ou tem enfoque em Tachinidae. No entanto, um aumento da amostragem e uma ampliação nos tipos de dados usados nas análises (e.g., morfologia, elementos ultra conservados, fisiologia) seria o próximo passo para resolver as questões que ainda estão em aberto. Com isso, nós teremos um entendimento filogenético de Tachinidae nos níveis de subfamília e tribos. Certamente, esse entendimento irá contribuir para a compreensão de questões futuras, como diversidade histórica, origem do parasitismo e até uma possível mudança na classificação interna da família.