



# A new perspective on the phylogeny of *Polietina* Schnabl & Dziedzicki (Diptera: Muscidae): integrating morphological and molecular evidence

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## Abstract

*Polietina* is a genus of Muscidae found in the New World, particularly in the Neotropical region. The genus is currently classified within the subfamily Muscinae and consists of 15 species whose phylogenetic relationships have been previously studied using morphological data. In our study, we conducted a phylogenetic analysis based on 37 morphological characters sourced from the literature and eight molecular markers (12S, 16S, 18S, 28S, *cytochrome C* oxidase subunit I, *cytochrome b*, region 4 of carbamoyl-phosphate synthetase-aspartate transcarbamoylase dihydroorotase, and elongation factor 1- $\alpha$ ), totaling 5366 characters. We obtained molecular data for eight species of the genus and four outgroup taxa from original sequencing and public repositories. We used Bayesian posterior probabilities to estimate the topology, as follows: (*P. flavidicincta* ((*P. rubella* (*P. concina* (*P. wulpi*, *P. orbitalis*))) (*P. steini* ((*P. flavithorax* (*P. ponti*, *Polietina* sp.)) (*P. major* (*P. prima* (*P. bicolor* (*P. minor* (*P. univittata*, *Polietina* sp.)))))))). The reciprocal monophyly of all species for which more than one identified sequence was available is supported by our results. Our analysis largely supports the previously published hypotheses regarding the phylogeny of *Polietina*. However, major differences were observed between the locations of *P. flavidicincta* and *P. flavithorax*. Additionally, we discuss the identity of unidentified *Polietina* specimens with sequences published in GenBank or new sequences produced as part of our study.

## Key words

Bayesian posterior probabilities, biodiversity, COI, Genbank, house flies, Muscinae, Neotropical region

## 1. Introduction

*Polietina* Schnabl and Dziedzicki, 1911 is a genus of flies primarily found in the tropical forests of Central and South America, with three species also occurring in Mexico and the Nearctic region (Löwenberg-Neto and de Carvalho 2013). This genus belongs to Muscinae subfamily (Diptera, Muscidae) (Couri and de Carvalho 1997; Nihei and de Carvalho 2007b; Haseyama et al. 2015) and is considered monophyletic based on both morphological and molecular evidence (Nihei and de Carvalho 2007a, b; Haseyama et al. 2015; de Carvalho and Haseyama 2018). *Polietina* has been revised twice over the last 30 years (Couri and de Carvalho 1997; Nihei and de Carvalho 2007a) with a single new species described since the last revision (*Polietina ponti* de Carvalho and Haseyama, 2018). Additionally, de Carvalho and Haseyama (2018) proposed a synonymy between *Polietina prima* (Couri and Machado, 1990) and *Polietina nigra* (Couri and de Carvalho, 1996), resulting in a genus currently comprising 15 species.

The phylogenetic relationships of *Polietina* were first examined using morphological characteristics by Nihei and de Carvalho (2007a), who analyzed 13 of the 15 recognized species at the time due to the unavailability of specimens of *Polietina basicincta* (Stein, 1904) and *Polietina mellina* (Stein, 1904). Their dataset included 39 characters and was analyzed using parsimony with equal, implied, and successive character weightings. De Carvalho and Haseyama (2018) reanalyzed this dataset with the inclusion of *P. ponti* using equal weighting parsimony, reaffirming previously published relationships (Nihei and de Carvalho 2007a), except for the addition of the new species. They also examined the cytochrome *c* oxidase subunit I (COI) sequences from GenBank for five *Polietina* species, confirming the reciprocal monophyly of two species with multiple sequences available (*P. prima* and *Polietina orbitalis* Stein, 1904).

Recent phylogenetic analyses of the Muscidae at the subfamily or family level have predominantly utilized morphological (de Carvalho 1989; Couri and Pont 2000; Couri and de Carvalho 2003; Savage and Wheeler 2004; Nihei and de Carvalho 2007b; de Carvalho et al. 2019; Sorokina and Ovtshinikova 2020; Gomes et al. 2020) or molecular evidence (Schuehli et al. 2007; Kutty et al. 2008, 2010, 2014; Haseyama et al. 2015; Ren et al. 2019; Grzywacz et al. 2021; Li et al. 2023; Walczak et al. 2023). Conversely, studies focusing on species-level phylogenetic inference have primarily relied on morphological data (de Carvalho 1999; de Carvalho and Couri 2002; Soares and de Carvalho 2005; Schuehli and de Carvalho 2005; de Carvalho and Pont 2006; Couri et al. 2007; Nihei and de Carvalho 2007a, 2011; Couri and de Carvalho 2008; Haseyama and de Carvalho 2012; de Carvalho and Haseyama 2018; Gomes et al. 2018a, 2018b; Patitucci et al. 2023; Pérez et al. 2023). Examples of species-level studies based on molecular evidence include those on *Hydrotaea* Robineau-Desvoidy, 1830 (Grzywacz et al.

2017), the *Hydrotaea dentipes* species group (Vikhrev 2024), *Lispe* Latreille, 1796 (Gao et al. 2022; Walczak et al. 2025), and *Stomoxys* Geoffroy, 1762 (Dsouli et al. 2011). To our knowledge, Savage et al. (2004) were the only to propose a phylogenetic analysis using combined morphological and molecular evidence for species relationships within a muscid genus, specifically for *Thricops* Rondani, 1856.

In this study, we conducted the first combined analysis of morphological and molecular evidence to explore species relationships within *Polietina*.

## 2. Materials and Methods

### 2.1. Dataset design and data acquisition

The species studied here included four outgroups and all *Polietina* species, except *P. basicincta* and *P. mellina*, due to the loss of their type material and the absence of recognized specimens (Nihei and de Carvalho 2007a) (Table S1). We utilized the morphological dataset published by Nihei and de Carvalho (2007a) with modifications proposed by de Carvalho and Haseyama (2018). The character list follows Nihei and de Carvalho (2007a, pages 500–501), excluding characters 19 and 24. Remaining characters were renumbered subsequently. Outgroup terminals were combinations of different species based on the availability of molecular data: *Fannia bahiensis* Albuquerque, 1957 was combined with *F. canicularis* (Linnaeus, 1761) sequences, *Delia platura* (Meigen, 1826) with *D. radicum* (Linnaeus, 1758); *Cyrtoneuropsis multomaculta* (Stein, 1904) with *C. maculipennis* (Macquart, 1843); *Morellia xanthoptera* Pamplona, 1986 with *M. nigricosta* Hough, 1900. For in-group analysis, individual specimens were used as terminals when multiple specimens were available for the same species. In these cases, the individuals were specified using voucher numbers; in cases where this was not available, we used the initials of the authors, as specified in GenBank. Specimens used for the acquisition of molecular data were identified using the key provided by Nihei and de Carvalho (2007a) and the original species descriptions.

We amplified eight molecular markers for *Polietina* and outgroup species, including protein-coding and ribosomal genes. For nuclear markers, we amplified: region 4 of carbamoyl-phosphate synthetase-aspartate transcarbamoylase dihydroorotase (CAD), elongation factor 1- $\alpha$  (EF1- $\alpha$ ), 18S rRNA, and 28S rRNA. As mitochondrial markers, we targeted cytochrome *C* oxidase subunit I (COI), cytochrome *b* (*Cytb*), 12S rRNA, and 16S rRNA. We complemented our data with sequences from GenBank, primarily from the studies by Schuehli et al. (2007) and Haseyama et al. (2015). We used all available sequences for the target markers, even if the voucher was not identified at the species level. The only

exceptions were the sequences of *P. orbitalis* associated with voucher DZUP459957 (AJ879599, AJ871209) and *P. steini* associated with voucher DZUP459958 (AJ879598, AJ871208); these sequences were excluded due to the uncertainty of their identities (de Carvalho and Haseyama 2018).

For specimens collected for DNA extraction, three legs from each specimen were separated, placed in a vial, preserved in absolute ethanol, and frozen. Depending on the specimen size, one to three legs were dried, macerated, and incubated overnight in 300  $\mu$ L of sodium dodecyl sulfate lysis solution (Sigma-Aldrich, Merck) with 5  $\mu$ L of proteinase K 20 mg/mL (Thermo Fisher Scientific Inc.) at 55 °C. Protein and DNA were precipitated using ammonium acetate (Sigma-Aldrich, Merck) and isopropyl alcohol (Labsynth), respectively. The final DNA was eluted in 50  $\mu$ L of Tris-HCl-EDTA buffer.

PCR reactions were performed with GoTaq™ G2 Flexi DNA Polymerase (Promega Corporation) according to the manufacturer's protocol. The primers and annealing temperatures used are listed in Table S2. All PCR reactions consisted of an initial step of 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, annealing temperature for 30 sec and 72°C for 1 min, then a final step of 72°C for 7 min and hold at 4°C. Amplified products were purified using Ampure XP (Beckman Coulter). Sequencing reactions utilized BigDye® Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific Inc.) and were precipitated with 3 M sodium acetate (Labsynth). The samples were sequenced on a 3730 sequencing machine (Thermo Fisher Scientific Inc.) at GateLab (<https://gatelab.ib.usp.br>), and chromatograms were assembled using CodonCode Aligner software (<https://www.codoncode.com>). All contigs were analyzed using the BLASTn suite on the NCBI website to identify potential contamination. Additionally, all coding genes (CAD, COI, *Cytb*, and EF1- $\alpha$ ) were translated into proteins to check for stop codons and possible errors. No issues were detected in either analysis. Finally, the sequences were deposited in GenBank (Table S1).

## 2.2. Data analysis

The sequences were aligned using the MAFFT 7 online server (Kato et al. 2019) with default settings. Each marker's alignment was subsequently trimmed to minimize missing data using TRIMAL 1.3 (Capella-Gutiérrez et al. 2009) via PHYLEMON 2.0 (Sánchez et al. 2011) with the “automated1” option. Individual alignments were then concatenated using SEQUENCEMATRIX v.1.7.8 (Vaidya et al. 2010). For individuals that were not identified to the species level, their morphological datasets were completed with questionnaires.

Initially, we analyzed the molecular data using the maximum likelihood optimality criterion implemented in IQTREE v1.6.12 (Nguyen et al. 2015). We considered the entire sequence of ribosomal genes and individual codon positions of protein-coding genes as start-

ing partitions. Then we used MODELFINDER within IQTREE (Kalyaanamoorthy et al. 2017) with the “-m MFP+MERGE” option to determine the best partitioning scheme. The best-fit model of nucleotide evolution for each partition was determined using MODELFINDER with default settings. We also analyzed the data without partitioning, and since both yielded identical topologies, a combined analysis (morphological and molecular data) was accomplished without partitioning the molecular data.

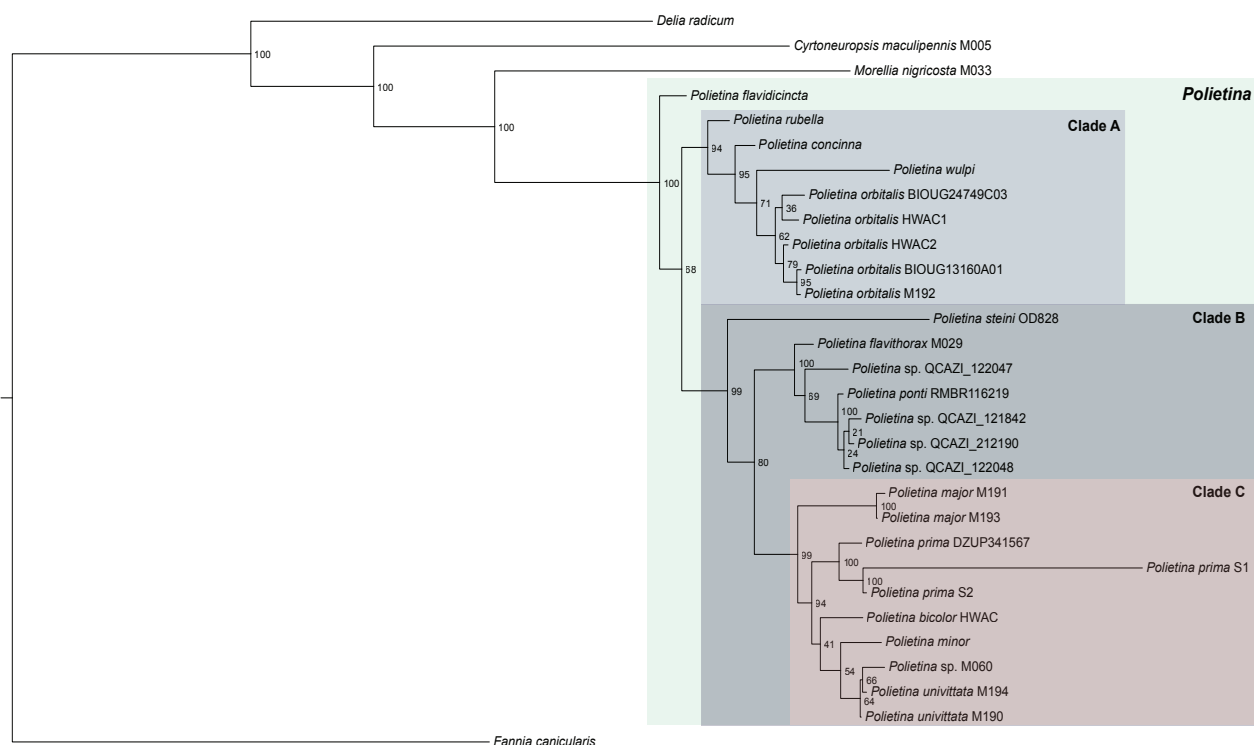
For analysis of the combined data, Bayesian posterior probabilities were chosen as the optimality criterion, and the analysis was conducted using MRBAYES 3.2.5 (Ronquist et al. 2012) with the Metropolis-coupled Markov Chain Monte Carlo algorithm. We performed two simultaneous runs, each with four chains, for 10 million generations, saving samples every 1000 generations. Convergence was assessed using the average standard deviation of the split frequencies (below 0.01), and the effective sample size of the analysis parameters was verified using TRACER 1.7.2 (Rambaut et al. 2018). For morphological partitioning, we used the MRBAYES model for standard discrete data based on Lewis (2001), specifying the following options: coding = variable coding = informative rates = gamma ngammacat = 8. For molecular partitioning, we utilized the GTR+G+I model suggested by MODELFINDER. The consensus type was set to default (File S1). The resulting tree was visualized using FIG-TREE 1.4 (Rambaut and Drummond 2012), with *Fannia* used to root the tree. WINCLADA (Nixon 2002) was used to optimize the synapomorphies in the combined tree.

We estimated pairwise genetic distances using MEGA XI (Tamura et al. 2021) and Kimura-2P with default settings based on COI sequences.

## 3. Results

We obtained new data from 10 muscid specimens, eight of which belonged to six *Polietina* species (Table S1). When combined with the data from GenBank, we obtained molecular information for eight out of the 15 species within the genus. Five of these species were sequenced for the first time. Five specimens could not be identified at the species level. The combined final matrix included 37 morphological characters and 5329 nucleotide characters for 17 species and five unidentified specimens.

Bayesian posterior probability analysis of the combined molecular and morphological data produced a tree that recovered *Polietina* monophyletic and divided it into two main clades, A and B, with clade C nested within clade B (Fig. 1). However, *P. flavidicincta* is a sister group of all other *Polietina* species. Our findings support the reciprocal monophyly of all species for which information is available. The divergence values varied from 0.0 to 0.1446 within the in-group (Table S3).



**Figure 1.** Bayesian molecular phylogenetic hypothesis of 13 *Polietina* species (25 specimens) and four outgroup taxa, based on Bayesian posterior probabilities derived from eight molecular markers (12S rRNA, 16S rRNA, 18S rRNA, 28S rRNA, cytochrome C oxidase subunit I, cytochrome *b*, region 4 of carbamoyl-phosphate synthetase-aspartate transcarbamoylase dihydroorotase, and elongation factor 1- $\alpha$ ; 5329 bp, model = GTR+I+G) and 37 discrete morphological characters (model = GTR+G). Specimens with molecular data have voucher numbers associated with its name, except for *Fannia canicularis* and *Delia radicum* (please refer to Table S1 for details). Numbers represent Bayesian posterior probability values.

## 4. Discussion

The Bayesian posterior probability analysis of the combined molecular and morphological data generated a tree (Fig. 1), which was largely consistent with two previous studies on *Polietina* phylogeny (Nihei and de Carvalho 2007a; de Carvalho and Haseyama 2018), with notable exceptions regarding the position *P. flavidicincta* and *P. flavithorax*. In our current analysis, *P. flavidicincta* emerged as the sister group to all other *Polietina* species, whereas in previous analyses, it was positioned as the sister group of Clade A, which included (*P. rubella* (*P. concinna* (*P. wulpi*, *P. orbitalis*))) (Fig. 1). Since there were no molecular data for *P. flavidicincta*, we believe that this difference was due to the chosen analysis method. Females of this species are the only females in the genus without median anterodorsal setae on the foretibia, whereas *P. orbitalis* and *P. bicolor* females are polymorphic in this character (Fig. S1 [character 29]). The structure of Clade A was consistent with that in the literature. Notably, molecular data were available only for *P. orbitalis* within this clade. Consequently, the topology of Clade A was mainly based on morphological characters, resulting in relationships that were nearly identical to those previously reported.

In Clade B (Fig. 1), all species had molecular data for at least one specimen, except for *P. minor*. *Polietina steini* was recovered as a sister group to other species, which is consistent with the previous hypotheses. *Polietina prima* was estimated to be a sister group of (*P. bicolor* (*P. univittata* and *P. minor*)). This topology aligns with previously published hypotheses, which had a lower resolution for most of Clade B (Nihei and de Carvalho 2007a; de Carvalho and Haseyama 2018). However, our tree strongly supports *P. major* as the sister group of the remaining Clade C, whereas previous hypotheses had placed this species as the sister group of *P. flavithorax*. According to de Carvalho and Haseyama (2018), *P. major* and *P. flavithorax* share three unique traits within the genus, all related to hind tibia chaetotaxy: five or more anteroventral setae on male hind tibia; four anteroventral setae on female hind tibia; and a series of posteroventral setae on male hind tibia. In contrast, the new grouping of Clade C had no morphological support in the combined analysis (Figs S1–S3). *Polietina flavithorax* was recovered as a sister group to the clade containing *P. ponti* and unidentified specimens of *Polietina* (*Polietina* sp. QCAZI samples), in contrast to the results of de Carvalho and Haseyama (2018), in which *P. ponti* was in an uncertain position within Clade B. According to our combined analysis, *P. flavithorax* shares with *P. ponti*

one homoplastic synapomorphy, three postsutural dorso-central setae (Fig. S2 [character 7]). WINCLADA also pointed to the presence of four anteroventral setae on the female hind tibia as a synapomorphy for the clade; however, *P. ponti* is known only in males. Therefore, the character was coded as an unknown state for *P. ponti*, leading to errors in the program. *Polietina ponti* was found in a clade with unidentified *Polietina* specimens (sequences from GenBank). The COI sequences for specimens QCAZI\_212190 and QCAZI\_122048 were identical to *P. ponti*, whereas QCAZI\_121842 had a single-variable site. Considering the evidence provided by this single marker, as no other sequences were available, these three specimens were very likely *P. ponti*. Another unidentified specimen, QCAZI\_122047, could also be *P. ponti*, although it had a longer branch and its position had low support. Its genetic distance from *P. ponti* was 0.014, which was very similar to the distance found within the most divergent pair of *P. orbitalis* (0.010, BIOUG24749-C03, and M192). These unidentified specimens were collected in Ecuador, whereas the only known *P. ponti* specimens were from the type series from Peru.

Previous studies have shown that *P. bicolor*, *P. minor* and *P. univittata* form an unresolved clade (Nihei and de Carvalho 2007a; de Carvalho and Haseyama 2018). Here, we found the same clade, but *P. minor* was the sister-group to the clade *P. univittata* + *Polietina* sp. M060, although there are no molecular data for *P. minor*. The molecular data indicated that *P. univittata* M194 and *Polietina* sp. M060 is probably the same species since the COI sequences of M194 and M060 are identical, whereas *P. univittata* M190 has a single-variable site. The female specimen, M060 (*Polietina* sp.), was previously identified as *P. minor*. Males of *P. minor* and *P. univittata* closely resemble each other and are distinguished only when the lower spinning processes in the cercal plate are examined. Every published key characterizes *P. minor* as having upwardly oriented spines and *P. univittata* as having downwardly oriented spines (Couri and de Carvalho 1997; Nihei and de Carvalho 2007a; de Carvalho and Haseyama 2018). *Polietina minor* is supposedly the only species of the genus with upwardly oriented spines; however, a recent examination of a paratype brings new light to the original description provided by Albuquerque (1956). Dr. Márcia Couri examined the genitalia of a paratype deposited at the Museu Nacional (Rio de Janeiro) and confirmed that *P. minor* spines were actually oriented downward, similar to other *Polietina* species. Additionally, Dr. Couri recognized the lower spinned process of *P. univittata* with three spines subequal in length (despite one being stronger), while *P. minor* has one very long and strong spine, and two shorter and weaker ones. Therefore, despite the inaccuracies of previous studies, males of the two species could be distinguished through genital examination. Females of *P. minor* and *P. univittata* can be distinguished by a brown infuscation on the apex of R<sub>1</sub> and Sc (absent in *P. minor* and present in *P. univittata*) and the number of anteroventral setae on the hind

tibia (three in *P. minor* and two or three in *P. univittata*). As the latter trait varies in *P. univittata*, there is a single characteristic that can be used in all cases to distinguish females. Besides, we must also consider that the conspecific association of males and females under a valid name is not a simple and objective task in Muscidae species, as well as in *Polietina* species. Therefore, it is likely that females of *P. minor* and *P. univittata* are not well defined or described. Further studies are required to distinguish the females of *P. univittata* and *P. minor* properly. Therefore, we opted not to assert the species identity of *Polietina* sp. M060.

## 5. Declarations

**Competing interests.** The authors have declared that no competing interests exist.

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## 7. References

- Albuquerque DO (1956) Fauna do Distrito Federal. XIII. Sobre o gênero *Polietina* Schnabl & Dziedzicki, 1911, com descrições de espécies novas (Diptera, Muscidae). Boletim do Museu Nacional (Nova Série, Zoologia) 139: 1–31.
- Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25(15): 1972–1973.
- Couri MS, de Carvalho CJB (1997) Revision of *Polietina* Schnabl & Dziedzicki (Diptera, Muscidae) and considerations on its new systematic position. *Revista Brasileira de Zoologia* 14(2): 255–281. <https://doi.org/10.1590/S0101-81751997000200002>
- Couri MS, de Carvalho CJB (2003) Systematic relations among *Philornis* Meinert, *Passeromyia* Rodhain & Villeneuve and allied genera (Diptera, Muscidae). *Brazilian Journal of Biology* 63(2): 223–232. <http://dx.doi.org/10.1590/S1519-69842003000200007>
- Couri MS, de Carvalho CJB (2008) A review of the Neotropical genus *Drepanocnemis* Stein (Diptera, Muscidae), with phylogenetic analysis and biogeographic considerations of its species. *Journal of Natural History* 42(41–42): 2659–2678.
- Couri MS, de Carvalho CJB, Löwenberg-Neto P (2007) Phylogeny of *Philornis* Meinert species (Diptera: Muscidae). *Zootaxa* 1530: 19–26. <https://doi.org/10.11646/zootaxa.1530.1.2>

- Couri MS, Pont AC (2000) Cladistic analysis of Coenosiini (Diptera: Muscidae: Coenosiinae). *Systematic Entomology* 25(3): 373–392. <https://doi.org/10.1046/j.1365-3113.2000.00125.x>
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772–772. <https://doi.org/10.1038/nmeth.2109>
- Dsouli N, Delsuc F, Michaux J, De Stordeur E, Couloux A, Veuille M, Duvallet G (2011) Phylogenetic analyses of mitochondrial and nuclear data in haematophagous flies support the paraphyly of the genus *Stomoxys* (Diptera: Muscidae). *Infection, Genetics and Evolution* 11(3): 663–670. <https://doi.org/10.1016/j.meegid.2011.02.004>
- de Carvalho CJB (1989) Classificação de Muscidae (Diptera): uma proposta através da análise cladística. *Revista Brasileira de Zootaxia* 6 (4): 627–648. <http://dx.doi.org/10.1590/S0101-81751989-00400009>
- de Carvalho CJB (1999) Revision, cladistics and biogeography of the Neotropical genus *Souzalopesmyia* Albuquerque (Diptera: Muscidae). *Proceedings of the Entomological Society of Washington* 101(1): 123–137.
- de Carvalho CJB, Couri MS (2002) Part I. Basal groups. In: de Carvalho CJB (Ed.) *Muscidae (Diptera) of the Neotropical Region: Taxonomy*. Editora Universidade Federal do Paraná, Curitiba, 17–132.
- de Carvalho CJB, Haseyama KLF (2018) A new species of *Polietina* (Diptera: Muscidae) from South America, with an updated phylogeny of the genus and a review of species' identity in GenBank. *Zootaxa* 4407(3): 415–426. <https://doi.org/10.11646/zootaxa.4407.3.8>
- de Carvalho CJB, Haseyama KLF, Gomes LRP, Zafalon-Silva S (2019) New genus and new species of Muscidae (Diptera) from the Andes highlands and discussion of its phylogenetic position based on morphological evidence. *Austral Entomology* 58(3): 484–497. <https://doi.org/10.1111/aen.12400>
- de Carvalho CJB, Pont AC (2006) Taxonomy, cladistics and biogeography of the South American genus *Brachygasterina* Macquart (Diptera: Muscidae). *Zootaxa* 1151: 1–26.
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5): 1792–1797. <https://doi.org/10.1093/nar/gkh340>
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3(5): 294–299.
- Gao Y, Ge Y, Yan L, Vikhrev NE, Wang Q, Butterworth NJ, Zhang D (2022) Phylogenetic analyses support the monophyly of the genus *Lispe* Latreille (Diptera: Muscidae) with insights into intrageneric relationships. *Insects* 13(11): 1015. <https://doi.org/10.3390/insects-13111015>
- Giribet G, Carranza S, Baguñà J, Riutort M, Ribera C (1996) First molecular evidence for the existence of a Tardigrada + Arthropoda clade. *Molecular Biology and Evolution* 13(1): 76–84. <https://doi.org/10.1093/oxfordjournals.molbev.a025573>
- Goloboff PA, Farris JS, Nixon KC (2008) TNT, a free program for phylogenetic analysis. *Cladistics* 24(5): 774–786. <https://doi.org/10.1111/j.1096-0031.2008.00217.x>
- Gomes LRP, Couri MS, de Carvalho CJB (2018) A new species of *Drepanocnemis* (Diptera, Muscidae) from Andes in Peru, with an updated phylogenetic analysis of species. *Revista Brasileira de Entomologia* 62(1): 51–56. <https://doi.org/10.1016/j.rbe.2017.10.001>
- Gomes LRP, de Carvalho CJB (2018) *Souzalopesmyia* Albuquerque, 1951 (Diptera: Muscidae): new species from South America with an updated phylogeny based on morphological evidence. *Zoosystema* 40(sp1): 539–546. <https://doi.org/10.5252/zoosystema2018v40a25>
- Gomes LRP, Fogaça JM, de Carvalho CJB (2020) New genus of Muscidae: Coenosiinae (Diptera) from the Mexican Transition Zone and its phylogenetic position based on morphological evidence. *Insect Systematics & Evolution* 52(1): 110–124. <https://doi.org/10.1163/1876312X-bja10003>
- Grzywacz A, Wallman JF, Piwczynski M (2017) To be or not to be a valid genus: the systematic position of *Ophyra* R.-D. revised (Diptera: Muscidae). *Systematic Entomology* 42(4): 714–723. <https://doi.org/10.1111/syen.12240>
- Grzywacz A, Trzeciak P, Wiegmann BM, Cassel BK, Pape T, Walczak K, Piwczynski M (2021) Towards a new classification of Muscidae (Diptera): a comparison of hypotheses based on multiple molecular phylogenetic approaches. *Systematic Entomology* 46(3): 508–525. <https://doi.org/10.1111/syen.12473>
- Haseyama KLF, de Carvalho CJB (2012) A new species of *Cyrtoneuropsis* (Diptera: Muscidae) with considerations on the phylogeny of the genus. *Zoologia* 29(6): 549–556. <https://doi.org/10.1590/S1984-46702012000600006>
- Haseyama KLF, Wiegmann BM, Almeida EAB, de Carvalho CJB (2015) Say goodbye to tribes in the new house fly classification: a new molecular phylogenetic analysis and an updated biogeographical narrative for the Muscidae (Diptera). *Molecular Phylogenetics and Evolution* 89: 1–12. <https://doi.org/10.1016/j.ympev.2015.04.006>
- Hedin M, Derkarabetian S, McCormack M, Richart C, Shultz JW (2010) The phylogenetic utility of the nuclear protein-coding gene EF-1a for resolving recent divergences in Opiliones, emphasizing in-tron evolution. *The Journal of Arachnology* 38(1): 9–20. <https://doi.org/10.1636/HA09-49.1>
- Kalyaanamoorthy S, Minh BQ, Wong TK, Von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature methods* 14(6): 587–589.
- Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in bioinformatics* 20(4): 1160–1166.
- Kutty SN, Pape T, Pont AC, Wiegmann BM, Meier R (2008) The Muscoidea (Diptera: Calyptratae) are paraphyletic: Evidence from four mitochondrial and four nuclear genes. *Molecular Phylogenetics and Evolution* 49(2): 639–652. <https://doi.org/10.1016/j.ympev.2008.08.012>
- Kutty SN, Pape T, Wiegmann BM, Meier R (2010) Molecular phylogeny of the Calyptratae (Diptera: Cyclorhapha) with an emphasis on the superfamily Oestroidea and the position of Mystacinobiidae and McAlpine's fly. *Systematic Entomology* 35(4): 614–635.
- Kutty SN, Pont AC, Meier R, Pape T (2014) Complete tribal sampling reveals basal split in Muscidae (Diptera), confirms saprophagy as ancestral feeding mode, and reveals an evolutionary correlation between instar numbers and carnivory. *Molecular Phylogenetics and Evolution* 78: 349–364. <https://doi.org/10.1016/j.ympev.2014.05.027>
- Lewis PO (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology* 50(6): 913–925. <https://doi.org/10.1080/106351501753462876>
- Li X, Cai X, Ding S, Wang L, Li W, Liu X, Yang D (2023) Phylogeny and evolutionary timescale of Muscidae (Diptera: Calyptratae) inferred from mitochondrial genomes. *Insects* 14(3): 286. <https://doi.org/10.3390/insects14030286>
- Löwenberg-Neto P, de Carvalho CJB (2013) Muscidae (Insecta: Diptera) of Latin America and the Caribbean: geographic distribution

- and checklist by country. *Zootaxa* 3650: 1–147. <https://doi.org/10.11646/zootaxa.3650.1.1>
- Marinho MAT, Junqueira ACM, Paulo DF, Esposito MC, Villet MH, Azeredo-Espin AML (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(3): 840–854. <https://doi.org/10.1016/j.ympev.2012.08.007>
- Medlin L, Elwood HJ, Stickel S, Sogin ML (1988) The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene* 71(2): 491–499. [https://doi.org/10.1016/0378-1119\(88\)90066-2](https://doi.org/10.1016/0378-1119(88)90066-2)
- Moulton JK, Wiegmann BM (2004) Evolution and phylogenetic utility of CAD (rudimentary) among Mesozoic-aged Eremoneuran Diptera (Insecta). *Molecular Phylogenetics and Evolution* 31(1): 363–378. [https://doi.org/10.1016/S1055-7903\(03\)00284-7](https://doi.org/10.1016/S1055-7903(03)00284-7)
- Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32(1): 268–274.
- Nihei SS, de Carvalho CJB (2007a) Systematics and biogeography of *Polietina* Schnabl & Dzierdzicki (Diptera, Muscidae): Neotropical area relationships and Amazonia as a composite area. *Systematic Entomology* 32(3): 477–501. <https://doi.org/10.1111/j.1365-3113.2006.00376.x>
- Nihei SS, de Carvalho CJB (2007b) Phylogeny and classification of Muscini (Diptera, Muscidae). *Zoological Journal of the Linnean Society* 149(4): 493–532. <https://doi.org/10.1111/j.1096-3642.2007.00252.x>
- Nihei SS, de Carvalho CJB (2011) Taxonomy and cladistic analysis of the subgenus *Xenomorellia* Malloch (Diptera: Muscidae: Morellia Robineau-Desvoidy) with description of two new species. *Journal of Natural History* 45(41–42): 2627–2644.
- Nixon KC (2002) WinClada version 1.00.08. Published by the author, Ithaca.
- Nixon KC, Carpenter JM (1993) On outgroups. *Cladistics* 9(4): 413–426. <https://doi.org/10.1111/j.1096-0031.1993.tb00234.x>
- Palumbi S (1996) Nucleic acids II: the polymerase chain reaction. In: Hillis D, Moritz C, Mable B (eds), *Molecular Systematics*, 2<sup>nd</sup> edn. Sinauer Assoc., Sunderland, MA, 205–247.
- Patitucci LD, Mulieri, PR, Couri, MS, Domínguez, MC (2023) Phylogeny of the old and fragmented genus *Austrocoenosia* Malloch reveals new evidences on the morphology and evolution of the genera *Coenosia* Meigen and *Neodexiopsis* Malloch (Diptera: Muscidae). *Arthropod Systematics & Phylogeny* 81: 485–495. <https://doi.org/10.3897/asp.81.e104969>
- Pérez S, Fogaça JM, Wolff M, de Carvalho CJB (2020) Morphological phylogeny of *Reinwardtia* Brauer & Bergenstamm (Diptera, Muscidae), with the description of a new species from the Neotropical region. *Systematics and Biodiversity* 18(5): 485–495. <https://doi.org/10.1080/14772000.2020.1776782>
- Pinto-da-Rocha R, Bragagnolo C, Marques FPL, Antunes JM (2014) Phylogeny of harvestmen family Gonyleptidae inferred from a multitocus approach (Arachnida: Opiliones). *Cladistics* 30(5): 519–539. <https://doi.org/10.1111/cla.12065>
- Rambaut A, Drummond A (2012) FigTree: Tree figure drawing tool, v1.4.2. Institute of Evolutionary Biology, University of Edinburgh.
- Ren L, Shang Y, Yang L, Shen X, Chen W, Wang Y, Guo Y (2019) Comparative analysis of mitochondrial genomes among four species of muscid flies (Diptera: Muscidae) and its phylogenetic implications. *International Journal of Biological Macromolecules* 127: 357–364. <https://doi.org/10.1016/j.ijbiomac.2019.01.063>
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Sánchez R, Serra F, Tárraga J, Medina I, Carbonell J, Pulido L, de María A, Capella-Gutiérrez S, Huerta-Cepas J, Gabaldón T, Dopazo J, Dopazo H (2011) Phylemon 2.0: a suite of web-tools for molecular evolution, phylogenetics, phylogenomics and hypotheses testing. *Nucleic acids research* 39(suppl. 2): W470–W474. <https://doi.org/10.1093/nar/gkr408>
- Savage J, Wheeler TA (2004) Phylogeny of the Azeliini (Diptera: Muscidae). *Studia dipterologica* 11(1): 259–299.
- Schuehli GS, de Carvalho, CJB (2005) Revision and cladistics of the Neotropical genus *Pseudoptilolepis* Snyder (Diptera, Muscidae). *Revista Brasileira de Zoologia* 22(1): 23–34.
- Schuehli GS, de Carvalho CJB, Wiegmann BM (2007) Molecular phylogenetics of the Muscidae (Diptera: Calyptratae): new ideas in a congruence context. *Invertebrate Systematics* 21(3): 263–278. <https://doi.org/10.1071/IS06026>
- Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87: 651–701.
- Sorokina VS, Ovtshinnikova OG (2020) The position of the Azeliinae in the Muscidae (Diptera) based on musculature of the male terminalia. *ZooKeys* 975: 87–108. <https://doi.org/10.3897/zookeys.975.55502>
- Sorokina VS, Tridrikh NN, Shaikovich EV (2023) Clarifying the taxonomic status and exploring hidden diversity in *Graphomya minor* Robineau-Desvoidy, 1830 (Diptera: Muscidae: Mydaeinae) using molecular and morphological evidence. *Annales de la Société entomologique de France (NS)* 59(4): 233–248. <https://doi.org/10.1080/00379271.2023.2222026>
- Tamura K, Stecher G, Kumar S (2021) MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution* 38(7): 3022–3027. <https://doi.org/10.1093/molbev/msab120>
- Vaidya G, Lohman DJ, Meier R (2010) SequenceMatrix: concatenation software for the fast assembly of multigenic datasets with character set and codon information. *Cladistics* 27(2): 171–180. <https://doi.org/10.1111/j.1096-0031.2010.00329.x>
- Vikhrev NE (2024) Review of *Hydrotaea dentipes* species group (Diptera, Muscidae). *Amurian Zoological Journal* XVI(4): 1008–1020. <https://www.doi.org/10.33910/2686-9519-2024-16-4-1008-1020>
- Walczak K, Pape T, Ekanem M, Szpila K, Grzywacz A (2023) Insights into the systematics of *Alluaudinella* and allied *Aethiopomyia* and *Ochromusca* (Muscidae, Diptera). *Zoologica Scripta* 52(3): 279–297. <https://doi.org/10.1111/zsc.12584>
- Walczak K, Piwczynski M, Pape T, Johnston NP, Wallman JF, Szpila K, Grzywacz A (2025) Unravelling phylogenetic relationships within the genus *Lispe* (Diptera: Muscidae) through genome-assisted and de novo analyses of RAD-seq data. *Molecular Phylogenetics and Evolution* 204: 108291. <https://doi.org/10.1016/j.ympev.2025.108291>

## Supplementary Material 1

### Figures S1–S3

**Authors:** Haseyama KLF, de Carvalho CJB, Soares EDG, Nihei SS (2025)

**Data type:** .pdf

**Explanation notes:** **Figure S1.** Bayesian molecular phylogenetic hypothesis of 13 *Polietina* species (25 specimens) and four outgroup taxa with synapomorphies plotted using fast optimization. — **Figure S2.** Bayesian molecular phylogenetic hypothesis of 13 *Polietina* species (25 specimens) and four outgroup taxa with synapomorphies plotted using slow optimization. — **Figure S3.** Bayesian molecular phylogenetic hypothesis of 13 *Polietina* species (25 specimens) and four outgroup taxa with synapomorphies plotted using unambiguous optimization.

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**Link:** <https://doi.org/10.3897/asp.83.e144844.suppl1>

## Supplementary Material 2

### Tables S1–S3

**Authors:** Haseyama KLF, de Carvalho CJB, Soares EDG, Nihei SS (2025)

**Data type:** .zip

**Explanation notes:** **Table S1.** List of species used in this study, including voucher ID, GenBank number, voucher repository, sex of the specimen, and data collection (only for original sequences). — **Table S2.** List of primers used in this study, with annealing temperatures. — **Table S3.** Pairwise sequence distances between *Polietina* specimens and outgroup taxa estimated by MEGA XI (model = Kimura 2P).

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## Supplementary Material 2

### File S1

**Authors:** Haseyama KLF, de Carvalho CJB, Soares EDG, Nihei SS (2025)

**Data type:** .nex

**Explanation notes:** List Data matrix and options used to run MrBayes.

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