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Where do the Neotropical Empidini lineages (Diptera: Empididae: Empidinae) fit in a worldwide context? ☆

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ABSTRACT

The tribe Empidini (Diptera: Empididae: Empidinae) is a diverse group with fourteen genera, seven of which are exclusive to the Neotropical region: *Bolrhamphomyia* Rafael, *Chilerhamphomyia* Rafael, *Hystrichonotus* Collin, *Lamprempis* Wheeler and Melander, *Macrostomus* Wiedemann, *Opeatocerata* Melander and *Porphyrochroa* Melander. Although Empidini itself is likely paraphyletic, many presumably monophyletic genera and species groups are recognized. Here, we apply DNA sequences from multiple genes to infer the phylogeny of Empidini, focusing on placing the Neotropical lineages within the entire tribe and identifying monophyletic groups. We included 98 Empidini taxa along with 18 outgrous terminals, spanning the diversity within the group. The results from the analyses performed are largely similar, with major groupings of genera in common. Specifically, the analyses recovered a monophyletic Hilarini and a paraphyletic Empidini. Most species from Chile and Argentina (Andean region) are found to belong to an early branching lineage within Empidinae, and are not monophyletic with other Empidini. A large portion of the remaining Neotropical Empidini (not Andean) comprises a single clade that includes four endemic genera and a number of Neotropical *Empis* Linneaus species. *Macrostomus* and *Porphyrochroa* each recovered as monophyletic and sister to one another, although generic placement of a few taxa remains uncertain due to conflicting morphological features. *Lamprempis* + *Opeatocerata* are also found to be sister-taxa in most analyses. Several large genera were found to be polyphyletic or paraphyletic including *Empis* and *Rhamphomyia* Meigen. We evaluate our findings and discuss them in light of current Empidinae taxonomy.

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1. Introduction

The tribe Empidini (Diptera: Empididae: Empidinae) is a worldwide group of flies, which are notably diverse in the Neotropical Region. They are known as dance flies and are recognized to be a lineage of Eremoneura – flies with three larval instars (Wiegmann et al., 2011). Dance flies are named for the characteristic mating swarms formed by males or, in some species, by females to attract mates (Cumming, 1994); where transfer of nuptial prey to the female often occurs in these swarms. Recent ongoing phylogenetic analysis of worldwide Empidinae has defined about 70 monophyletic species groups and several major lineages (e.g. Daugeron and Grootaert, 2003; Daugeron and Winkler, 2010), some of which contradict the current generic classification.

Empidini has fourteen recognized genera, twelve of which occur in the Neotropical Region (broadly defined, including temperate regions) and seven of which are exclusively known from that region. Worldwide, most Empidini species are placed in the large genera *Empis* Linneaus and *Rhamphomyia* Meigen, traditionally distinguished by the presence or absence of a forked R_{4+5} vein near the wing tip. The current generic classification of Neotropical Empidinae was refined in an important, but now dated, work by Collin (1933) on Patagonian dance flies. Collin placed three species possessing a forked R_{4+5} vein similar to that of *Empis*, but distinguished by a horizontal proboscis, in *Sphicosa* Philippi. A further three species, also with a horizontal proboscis but otherwise *Rhamphomyia*-like with an unforked R_{4+5}, were accommodated in

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a new genus, *Clinorhampha* Collin. Four additional Neotropical genera with the R_{4+5} vein unforked (*Macrostomus* Wiedemann and *Porphyrochroa* Melander) or forked (*Lamprempis* Wheeler and Melander and *Opeatocerata* Melander), are mainly distributed in warmer regions, outside of Patagonia, and were noted in this work, but not studied by Collin. Currently, the tribe Empidini is thought to be paraphyletic (e.g. see Daugeron et al., 2009; Daugeron and Winkler, 2010; Winkler et al., 2010) and because of this the classification of whole subfamily needs to be revised. Within the tribe Empidini, the 'traditional' large, worldwide genus *Empis* is undoubtedly polyphyletic, with some temperate South American *Empis* likely more closely related to the tribe Hilarini than to the remaining Empidini (Daugeron and Winkler, 2010; Winkler et al., 2010). Furthermore, the loss of the R_{4+5} fork, which separates *Rhamphomyia* from *Empis*, is now known to occur independently within multiple empidine lineages. This consideration adds to the difficulty of determining the placement of the few Neotropical *Rhamphomyia* and their relationship to *Macrostomus* and *Porphyrochroa*. As a step toward clarifying these relationships, Rafael (2010) placed two described species of *Rhamphomyia* from Chile and Bolivia into two new genera, *Chilerhamphomyia* Rafael and *Bolrhamphomyia* Rafael, respectively, and described characters of the male postabdomen separating them from *Rhamphomyia*, *Macrostomus*, and *Porphyrochroa*.

As seen for other Neotropical taxa, (e.g., Muscidae (Löwenberg-Neto and de Carvalho, 2009; Nihei and de Carvalho, 2007), and for Curculionidae (Morrone, 1994)) the genera of Neotropical Empidinae form two, mostly distinct, assemblages: one in temperate Chile and Argentina (Andean region of Morrone (2001)), and another in warmer parts of the Neotropics. The Andean assemblage is dominated by the related tribe Hilarini, but also includes the South American endemic genera *Sphicosa* Philippi (9 species), *Hystrichonotus* Collin (1 species), and *Clinorhampha* (3 species), all in Empidini. This assemblage shows clear affinities with taxa from Australia and New Zealand; for example, species from both South America and New Zealand have been placed in the small genus *Empidadelpha* Collin (3 species), and the newly revised *Empis macrorrhyncha* group (Daugeron et al., 2009) includes Australian, as well as South American, species. The other, more tropically distributed assemblage includes four endemic genera: *Macrostomus* (29 species), *Opeatocerata* (6 species), *Porphyrochroa* (54 species), and *Lamprempis* (22 species), as well as a number of *Empis* species. In contrast to the Gondwanan affinities of Andean Empidini, these genera share some features of the north temperate *Empis* and *Rhamphomyia* (Rafael and Cumming, 2004), although their precise relationships with the temperate fauna are unclear. Our study focuses primarily on the latter assemblage, which for simplicity we hereafter refer to as the Neotropical (as opposed to Andean) Empidini.

Despite the relatively small number of described species of Neotropical (non Andean) Empidini – about 100 in all four endemic genera plus about 75 species of *Empis* – there are many others to be described. For example, in the Canadian National Collection, (CNC, Ottawa) there are more than one hundred putative new species in *Porphyrochroa* that have not been described (M. Watts, pers. obs.). The two richest lineages in the Neotropical Region, *Macrostomus* and *Porphyrochora*, are receiving more attention here because of their greater abundance and diversity, and also because we wish to test their monophyly and relationship, which have never been the subject of explicit phylogenetic study, though they are clearly closely related to each other. *Porphyrochroa* was described by Melander (1928) and Smith (1967) synonymized it with *Macrostomus*. Rafael (2001) affirmed they were distinct lineages and he revalidated *Porphyrochroa* based on characters of external morphology. *Macrostomus* and *Porphyrochroa* have well defined morphological characters to separate them, and according to Rafael and Cumming (2004), the monophyly of *Porphyrochroa* is well established and the two genera are sister groups. Since 2001, many species were described in both genera: Rafael and Ale-Rocha (2002) described new species of *Porphyrochroa* from Dominican Republic; Rafael and Cumming (2006, 2009, 2010, 2012) described new species of *Macrostomus* from the Amazon; Mendonça et al. (2007, 2008) published a revision of *Porphyrochroa* from the Amazon; and Mendonça (2010) described additional species from South Brazil.

Here, we present the first robust molecular phylogenetic analysis of Empidini, focusing on the endemic Neotropical genera *Lamprempis*, *Macrostomus*, *Opeatocerata*, and *Porphyrochroa*. Our objective is to elucidate relationships between these genera and establish their connections with other Neotropical and worldwide Empidini lineages. This well-sampled phylogenetic framework allows interpretation of morphological and ecological diversification among Neotropical Empidini to be further clarified and establishes a basis for much-needed revisionary and descriptive work considered necessary for the large diversity of flies still uncataloged from this region.

2. Materials and methods

2.1. Taxon sampling

The ingroup, Empidini, includes 66 sampled Andean and Neotropical taxa representing the four Neotropical genera (*Lamprempis*, *Macrostomus*, *Opeatocerata*, and *Porphyrochroa*), plus four Andean genera (*Clinorhampha*, *Empidadelpha*, *Hystrichonotus*, and *Sphicosa*), and species of *Empis* and *Rhamphomyia* from both of those regions. Thirty-two additional *Empis* and *Rhamphomyia* species were included from other regions to more fully represent known lineages of Empidini. We attempted to include congeners from different subgenera or different species groups wherever possible, particularly for the larger genera, *Empis*, *Rhamphomyia*, *Macrostomus*, and *Porphyrochroa*. Most of the Neotropical *Empis* species we included are undescribed or extremely difficult to identify to species. These were included in analyses as exemplars of the rich diversity of undiscovered or undersampled empidines from major geographic regions of Central and South America (Costa Rica, French Guiana, Brazil, Peru, Bolivia, Chile, and Argentina).

Exemplars included from *Macrostomus* and *Porphyrochroa* are also undescribed and unidentifiable to species. Both genera are considered monophyletic based on morphology, and they are likely to be close relatives (Rafael and Cumming, 2004). We included multiple morphospecies from both groups to test their monophyly and placement in the tree; the first author has been working on the description of the *Porphyrochroa* species, which will be published in future taxonomic treatments.

Eleven taxa, representing five genera of the tribe Hilarini (*Aplomera*, *Atrichopleura*, *Hilara*, *Hilarempis*, and *Hilarigona*) were also included, and seven other, representing the subfamilies Ocydromiinae (*Oropezella*), Trichopezinae (*Hyperperacera*) and the genera *Hesperempis*, *Hormopeza*, *Iteaphila*, *Oreogeton*, and *Philetus*, all classified as *incertae sedis* within the Empidoidea (Sinclair and Cumming, 2006), served as outgroups, since they are often interpreted as having groundplan features of the Empididae *s.str.* (Sinclair and Cumming, 2006), i.e., representative of the plesiomorphic condition for Empidini adult morphology, especially complex features of the male genitalia.

2.1.1. Identity of terminals

Because our focus is on fauna of the Neotropical region, we sought to include as much of the known, but largely undescribed, diversity as possible. Only about 25% of the species included here