Molecular phylogenetics reveals novel relationships within Empidoidea (Diptera)

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Abstract. Empidoidea represent a large and diverse superfamily of true flies, and to date no stable hypothesis on the phylogeny exists. Previous classifications have been based on morphological data and the relationships among several groups are still unknown. Using the mitochondrial genes cytochrome oxidase c subunit I (COI) and cytochrome β (Cytβ) and the nuclear genes carbomoylphosphate synthase domain of rudimentary (CAD), elongation factor-1α (EF-1α) and isocitrate dehydrogenase (IDH) in a Bayesian analysis, we tested the support of higher taxonomic groups within this large superfamily of flies. We re- evaluated previous hypotheses of evolution within the group and present a highly supported phylogenetic hypothesis. Atelestidae, Dolichopodidae, Empididae and Hybotidae were supported as monophyletic families, with Atelestidae as sister group to the remaining Empidoidea. Within the family Hybotidae, Bicellarinae stat.n. formed the sister group to the other subfamilies. The family Ragadidae stat.n. is established to include the subfamily Ragadinae and the new subfamily Iteaphilinae sub-fam.n.; Ragadidae was sister group to the Empididae. Dolichopodidae was found to form a sister group to Ragadidae plus Empididae. Within Empididae, Hemerodromiinae was found to be a nonmonophyletic group. The tribes Hilarini and Hemerodromiini stat.rev. were recovered as sister groups, as were Empidini and Chelipodini stat.rev. The former family Brachystomatidae was found to be nested within Empididae. A revised classification and diagnoses of nondolichopodid families, subfamilies and tribes are provided.

Introduction

The superfamily Empidoidea Latreille (Insecta: Diptera) includes over 10 000 described species (Pape et al., 2011) and is represented in all biogeographical regions except Antarctica. According to fossil records (Grimaldi & Engel, 2005) the group existed in the Jurassic, with families and subfamilies already diversified in the Early Cretaceous (Grimaldi & Cumming, 1999). Several species have gained interest from biologists owing to elaborate mating rituals (e.g. representatives of the genus Empis Linnaeus), and from ecologists due to freshwater life histories (e.g. some Clinocerinae). A vast diversity in morphology in combination with being closely related to the extremely diverse Cyclorrhapha has sparked a number of hypotheses about the evolution of the group. The internal classification of Empidoidea has been subject to a number of revisions and discussion since Brauer (1883) first considered that Empididae Latreille and Dolichopodidae Latreille were two closely related families. Chvála (1983), Wiegmann et al. (1993), Collins & Wiegmann (2002) and Sinclair & Cumming (2006) all provided detailed historical reviews of the superfamily. The two relatively recent classifications that have gained most acceptance are those by Chvála (1983) (Fig. 1A) and Sinclair & Cumming (2006) (Fig. 1B). Even though both have been used simultaneously, the latter has taken over as the dominant classification scheme. In the analysis by Sinclair & Cumming (2006), only parts of the phylogeny of Empidoidea were resolved. Two more recent publications by Yang et al. (2006) and Yang et al. (2007) use an older classification, dividing Empidoidea in two families (Dolichopodidae and Empididae), with several rearrangements. All previous classifications and most phylogenetic hypotheses comprising Empidoidea have been based on morphological data. Tentative studies producing a phylogenetic hypothesis based on molecular data were first carried out by Collins & Wiegmann (2002) using 28S ribosomal DNA and elongation factor-1α (EF-1α). Their phylogeny corroborated Chvála’s (1983) hypothesis, but with low support. They were
unable to resolve within-family relationships. Later Moulton & Wiegmann (2004) explored a large part of a region of the CAD gene in phylogenetic reconstruction of the superfamilies of the Eremoneura. They produced high support for many higher groups, but taxon sampling was insufficient to draw conclusions regarding relationships below family level. The same authors continued their exploration by adding 28S ribosomal DNA to the analysis (Moulton & Wiegmann, 2007) (Fig. 1C). Support for major groups, in favour of the family classification by Chvála (1983), was increased compared with their previous study. However, questions regarding relationships within families and among tribes remained and increased sampling of both taxa and molecular data was suggested. In this paper we test support for the monophyly of families, subfamilies and tribes, with a focus on the families Empididae and Hybotidae Meigen.

Material and methods

All currently recognized empidid and hybotid families and tribes, as well as several incertae sedis taxa sensu Sinclair & Cumming (2006) were included in the analysis, represented by 83 ingroup taxa (Table S1). An additional six taxa from both lower Brachycera and Cyclorrhapha were selected to serve as outgroups. The samples were made available mainly from the Swedish Malaise Trap Project (SMTP) (Karlsson et al., 2005), and staff and associates of the Swedish Museum of Natural History (SMNH). All material has been stored at approximately −20°C. As ground-living and aquatic species were rare in the Malaise trap samples, additional collection methods such as using sweep nets, pit fall traps and light traps were carried out to increase the taxonomic coverage. Some specimens in the subfamily Clinocerinae were donated to the project by Dr Marija Ivković (University of Zagreb, Croatia). Gene fragments from each of mitochondrial cytochrome $β$ (Cyt$β$) and cytochrome oxidase c subunit I (COI), and the nuclear carbomoylphosphate synthase domain of rudimentary (CAD), elongation factor-1$α$ (EF-1$α$) and isocitrate dehydrogenase (IDH) were included in the analysis. The primers used are listed in Table S2. Difficulties in amplifying Cyt$β$ in several taxa were solved by exchanging a few nucleotides to inosine in the primer sequence (Table S2). A new set of primers for amplifying EF-1$α$ were designed (Table S2) for the same reason, using previous published sequences in GenBank (Clark et al., 2015) of Empidoidea and close relatives. Photography of morphological characters were carried out on specimens after lysis, and produced with manual focus stacking, using a Nikon DS-Fi1 camera on a Nikon Eclipse 80i microscope. Photos were automatically aligned and stacked in HELICON FOCUS 6 (Helicon Soft Ltd., Dominica) and were thereafter edited and finalized in Adobe PHOTOSHOP CC 2017.1.1.

Extraction and sequencing

Following the manufacturer’s protocols, the KingFisher™ Duo (Thermo Scientific, Waltham, MA, U.S.A.) extraction robot was used together with KingFisher™ Cell and Tissue DNA Kit (Thermo Scientific) to extract DNA. For large specimens the abdomen was removed from the body and used for extraction, and thereafter kept together with the rest of the body. For small specimens, the whole body was used for extraction. Lysis was performed in 56°C overnight. All extracted material is kept in 80% ethanol as vouchers at the Swedish Museum of Natural History (SMNH). The PCR reactions were performed using Ready-To-Go PCR Beads (Amersham Biosciences, U.K.). The mixture for PCR reactions was set up with $21\mu L$ H$_2$O, $1\mu L$ each of reverse and forward primer and $2\mu L$ DNA extract, except for when amplifying IDH and EF-1$α$, where $3\mu L$ of DNA extract was required to obtain best results. PCR reaction protocols were optimized regarding temperature and number of cycles for each primer pair and are listed in Table S3. PCR products were cleaned using Exo-Fast (QiaQuick PCR Purification Kit; Qiagen, Inc., Valencia, CA, U.S.A.) and sequencing reactions were carried out by Macrogen Inc. (South Korea) using the same primer pairs as for PCR reaction. The resulting gene fragments were assembled into contigs in GENEIOUS 8.1.8 (Kearse et al., 2012) where they were inspected and primer sequences removed. The raw sequences were thereafter stored in voseq 1.7.4 (Peña & Malm, 2012), which was also used for managing sequences and creating datasets for analyses. To detect contamination, sequences were compared with known sequences of relatives as well as the GenBank database using BLAST (Madden, 2013).

Alignment and partitioning

Sequences were aligned using MAFFT 7.017 (Katoh et al., 2002) implemented in GENEIOUS 8.1.8. The mitochondrial genes COI and Cyt$β$ were aligned with the G-INS-1 method, while the nuclear genes CAD, EF1-α and IDH contained regions of gaps and were aligned using the E-INS-i method. In both cases, sequence direction adjustment was selected while other options were left at default. Long gaps or highly divergent regions generate potential alignment problems in the nuclear genes and were removed using the software Gblocks 0.91b (Castresana, 2000; Talavera & Castresana, 2007) under the default (stringent) options setting. Substitution saturation for codon 1+2 and 3 was assessed using Xī’s method (Xī et al., 2003; Xī & Lemey, 2009) in DAMBE 6.4.29 (Xī, 2013), an entropy-based index of substitution saturation.

Phylogenetic analysis

Bayesian analysis was carried out using MRBAYES 3.2.6 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) on the CIPRES Science Gateway (Miller et al., 2010). Following the results from the saturation assessment, datasets for analysis were set up using a partitioned scheme with codon positions 1+2 and 3 for each gene assigned to separate subsets. Rate variation across sites was set to gamma distribution with a proportion of invariable sites. To integrate over substitution model space, a mixed setting was used (nst = ‘mixed’).

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(Huelsenbeck et al., 2004). Temperature was set to 0.11 and number of chains to 4, and two parallel runs were performed. The analysis ran for 100 million generations and a sampling frequency of 10 000. The average standard deviation of split frequencies was used as a first measure of convergence (< 0.005), and secondly using the log files in TRACER 1.6 (Rambaut et al., 2014) for convergence and effective sample size (ESS). The ESS was, for all parameters, at least 500 for separate runs and at least 1300 combined. The majority rule tree was generated after removing the first 25% burn-in trees. The majority rule consensus tree was viewed in and exported from FIGTREE 1.4.2 (Rambaut, 2014), and annotated and finalized in Adobe ILLUSTRATOR cc 19.2.1.

Results

Of the 89 included taxa, sequences were obtained from 85 individuals for the Cytb gene, 88 for COI, 82 for CAD, 45 for EF-1α, and 59 for the IDH gene. GenBank accession codes for successfully sequenced genes for each individual taxon are available in Table S1. Matrix and the resulting tree file were deposited to TreeBase (accession number S22207). Amplification of nuclear data from Atelestus pulicarius Fallén proved to be unsuccessful, and CAD and EF-1α data were instead supplemented from previously published sequences of the same regions available from GenBank (accession codes in Table S1). Aligned nucleotide lengths of gene fragments were 433 for Cytb (60% variable, 51% parsimony-informative), 658 for COI (47% variable, 43% parsimony-informative), 1111 for CAD (60% variable, 45% parsimony-informative), 785 for EF-1α (52% variable, 42% parsimony-informative) and 1021 for IDH (48% variable, 38% parsimony-informative). Alignment lengths for nuclear genes after processing in Gblocks 0.91b were 776 for CAD (63% variable, 53% parsimony-informative), 519 for EF-1α (44% variable, 40% parsimony-informative) and 699 for IDH (67% variable, 54% parsimony-informative). The total dataset included 3085 nucleotides.

The saturation test using the ratio of observed entropy to the entropy of full substitution saturation is summarized in detail in Table S4. First and second codon positions show little saturation in all genes (index of substitutional saturation (Iss) < critical index of substitutional saturation (Iss.c), P < 0.05). The third codon position of the nuclear genes CAD, EF-1α and IDH show little saturation assuming a symmetrical topology (Iss < Iss.c, P < 0.05), but are very poor for phylogenetics assuming an asymmetrical topology (Iss > Iss.c, P > 0.05). The third codon position of COI shows saturation for both, assuming a symmetrical (Iss < Iss.c, P > 0.05) and asymmetrical (Iss > Iss.c, P < 0.05) topology, while the same position for Cytb shows saturation assuming an asymmetrical topology (Iss > Iss.c, P < 0.05). Based on these results, showing saturation of the third codon position in all genes, the third codon position was partitioned and modelled independently in the Bayesian analysis.

The majority rule consensus tree from the Bayesian inference showed strong support for all higher taxa (Fig. 2). Empidoidea formed a monophyletic superfamily with Atelestidae as the sister group to all other empidoid groups. The family Hybotidae was sister group to the remaining Empidoidea, excluding Atelestidae. Within Hybotidae, the subfamily Hybotinae, herein represented by the genera Bidens, Cyclophthalminus and Tachydrimini, Empidinae was recovered as a monophyletic subfamily, with Drapetini as sister group to Symballophtalmini and Tachydrimini. Empidinae was recovered as a monophyletic subfamily with Drapetini as sister group to Symballophtalmini and Tachydrimini. Empidinae was recovered as a monophyletic subfamily, with Drapetini as sister group to Symballophtalmini and Tachydrimini. Empidinae was recovered as a monophyletic subfamily, with Drapetini as sister group to Symballophtalmini and Tachydrimini. 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Fig. 2. Majority rule tree based on 15,000 post-burn-in trees from MrBayes (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) on a partitioned dataset of the genes cytochrome b (Cytb), cytochrome oxidase c subunit I (COI), carboxylglycophosphate synthase domain of rudimentary (CAD), elongation factor-1α (EF-1α) and isocitrate dehydrogenase (IDH). Numbers above branches represent posterior probability values. Classification on the right side follows Sinclair & Cumming (2006), and groups found as nonmonophyletic in the analysis herein are indicated by an asterisk. [Colour figure can be viewed at wileyonlinelibrary.com].
Rhamphomyia Meigen was nested within Empis. The sister group to the Empididae and the Ragas and Iteaphila groups was Dolichopodidae, with Microphorinae as the sister group to the remaining dolichopodids.

**Discussion**

**Phylogenetic relationships and taxonomic implications**

The results from the analyses confirm that many groups in the classification by Sinclair & Cumming (2006) (Fig. 1B) are monophyletic. Hennig (1970) first proposed that Atelestinae is closely related to Hybotidae and Microphorinae Collin. Chvála (1983), however, raised the group to family level and placed it as sister group to the Cyclorrhapha. Wiegmann *et al.* (1993) detected a close relationship between Atelestidae and Hybotidae in their analysis using morphological characters, and Sinclair & Cumming (2006) found Atelestidae as the sister group to Hybotidae. However, the finding that Atelestidae form the sister group to all remaining Empidoidea is coherent in all studies using molecular data (Collins & Wiegmann, 2002; Moulton & Wiegmann, 2004, 2007) (e.g. Fig. 1C), including the present analysis.

The position of *Bicellaria* near the root of Hybotidae supports Moulton & Wiegmann (2007) (Fig. 1C) when using combined CAD and 28S gene data. Chvála (1983) (Fig. 1A) placed *Bicellaria* within Trichini in Ocydromiinae, while Sinclair & Cumming (2006) created the tribe Biculariini and included it in Hybotinae. Based on the results herein we suggest that Biculariini form a group outside Hybotinae and raise it to subfamily Biculariinae stat.n.

Brachystomatidae comprise the three subfamilies Ceratomerinae, Trichopoezinae and Brachystomatinae as combined by Sinclair & Cumming (2006), who also raised it to its present status as family. Cumming *et al.* (1995) hypothesized a close relationship of this group to Dolichopodidae + Microphorinae. This was corroborated in the results by Sinclair (1995). Molecular analysis by Collins & Wiegmann (2002), however, did not support that Brachystomatidae represent the sister group to Dolichopodidae + Microphorinae, results further corroborated by Moulton & Wiegmann (2007). Even though the sample size is too small and restricted to Trichopoezinae to investigate the monophyly of Brachystomatidae, maintaining family-level status for Brachystomatidae would necessitate extensive changes in the classification of the remaining empidids with several clades raised to family rank as well. We feel that this would create an overly split classification, so we choose a conservative approach and move this group into Empididae.

Empidinae and Hemerodromiinae have previously been recognized as sister taxa, due to the presence of heavy sclerotized male cerci with clasping abilities in species of both groups (Ulrich, 1975; Chvála, 1983; Cumming *et al.*, 1995). Hemerodromiinae is distinct from Empidinae by the presence of a long, tubular fore coxae and raptorial forelegs widely separated from the middle and rear pairs. Hemerodromiini and Chelipodini were formalized by Melander (1947). Wiegmann *et al.* (1993) excluded Chelipodini and found that Hemerodromiini were most closely related to Brachystomatinae + Clinocerinae. Cumming *et al.* (1995), on the other hand, found that Hemerodromiinae is most closely related to Empidinae because they share the same male cercal morphology, but the authors did not include characters to the test the monophyly of the subfamilies.

Collins & Wiegmann (2002) included one taxon from each tribe and recovered Hemerodromiinae as paraphyletic yet closely related to Empidinae. The close relationship to Empidinae was also confirmed by Sinclair & Cumming (2006), on the basis of the presence of an upcurved phallus and widely separated epandrial lamellae in the two groups. They also recovered a monophyletic Hemerodromiinae; however, their representative of Chelipodini, *Chelipodozus* Collin, is problematic in the sense that it differs from the other genera in the tribe by having a bare laterotergite (Plant, 2008). This state is considered a synapomorphy for Hemerodromiini.

Based on morphological characters, Plant (2011b) found *Chelipodozus* nested within Hemerodromiini and Chelipodini genera *Afrodromia* Smith and *Drymodromia* Becker as near-basal taxa, preceding the remaining Chelipodini + Hemerodromiini. Moulton & Wiegmann (2007) recovered Hemerodromiinae as a monophyletic clade based on an analysis using fragments of CAD and 28S. While Hemerodromiinae formed the sister group to the Empidinae, it rendered Hemerodromiini paraphyletic. Based on our study including multiple taxa from both groups, we include the Hemerodromiini and Chelipodini as tribes in the Empidinae.

The *Ragas* group, as proposed by Sinclair (1999a) and supported by Sinclair & Cumming (2006) (including the genera *Dipsomyia* Bezzi, *Hormopeza* Zetterstedt, *Hydropeza* Sinclair, *Ragas* and *Zanclotus* Wilder), and the *Iteaphila* group (including *Anthepticus* and *Iteaphila*) have previously been grouped together with *Oreogeton* Schiner, *Gloma* Meigen and *Hesperempis* Melander in the subfamily Oreogetoninae (Chvála, 1976). Chvála (1983) hypothesized this subfamily to be a primitive group within Empididae. The results here do not support Oreogetoninae sensu Chvála (1976), but instead corroborate the results by Wiegmann *et al.* (1993), Collins & Wiegmann (2002), Sinclair & Cumming (2006) and Moulton & Wiegmann (2007). Sinclair (2016) erected the subfamily Ragadinae for the *Ragas* group but did not discuss its phylogenetic placement other than keeping it as incertae sedis within Empididae sensu Sinclair & Cumming (2006). The results here show that the *Ragas* and *Iteaphila* clade is sister group to remaining empidids, similar to the results by Moulton & Wiegmann (2007), where *Hormopeza* was also recovered within the clade. Based on diagnostic characters as well as the genetic differences separating this group we recognize the Ragadinae and *Iteaphila* group as distinct subfamilies within the family Ragadidae stat.n.

**New classification and diagnoses in Empidoidea**

Based on our new phylogenetic hypothesis, a revised classification of the Empidoidea is presented below, with subfamilies and tribes shown for all families except Dolichopodidae. The
Fig. 3. New internal classification of Empidoidea; numbers at nodes represent support as posterior probabilities in percentage based on the Bayesian analysis. [Colour figure can be viewed at wileyonlinelibrary.com].

genera *Gondwanamyia* Sinclair, *Homalocnemis* Philippi, and *Oreogeton* have been placed *incertae sedis* within the superfamily and remain unplaced until further studies resolving the placement of these are clarified. A simplified cladogram based on our analysis showing the proposed classification and hypothesized relationships is presented in Fig. 3. Morphological characters applied in the diagnoses for the various taxa are mainly derived from Chvála (1983) and Sinclair & Cumming (2006). The terminology follows Cumming et al. (1995) for male terminalia, McAlpine (1981) for adult morphology, and Stuckenberg (1999) for antennae.

**Atelestidae Hennig**
Atelestinae Hennig, 1970: 1. Type genus *Atelestus* Walker.

Atelestidae Chvála (1983: 85).

*Diagnosis.* Similar to Empididae and Ragadidae by the presence of symmetrical male terminalia without rotation (Fig. 5A) and origin of vein R₅ at a distance from humeral crossvein (h) as long or longer than length of h (Fig. 6A). Distinguished from Ragadidae by having a costa ending at or near M₁₂, and from Empididae by having prosternum separated from proepisternum (Fig. 7A).

*Comments.* Although this group has consistently been closely associated with Hybotidae in morphological studies, the molecular evidence showed that it formed the sister group to all remaining groups in the superfamily. Atelestidae was formally expanded to include the genus *Nemedina* Chandler as the single genus in the subfamily *Nemedininae* Sinclair & Cumming.
Subfamilies included. Atelestinae and Nemedininae (Sinclair & Cumming, 2006).

Atelestinae Hennig
Atelestinae Hennig, 1970: 1. Type genus Atelestus Walker.

Diagnosis. Separated from Nemedininae by the presence of a straight r-m and R₁ not distinctly short (Fig. 6A).

Comments. This group have been considered a monophyletic group based on morphology by both Chvála (1983) and Sinclair & Cumming (2006).

Genera included. Acarteroptera Collin, Alavesia Waters & Arillo, Atelestus Walker, and Meghyperus Loew (Hennig, 1970; Chvála, 1983; Sinclair & Kirk-Spriggs, 2010).

Nemedininae Sinclair & Cumming
Nemedininae Sinclair & Cumming, 2006: 77. Type genus Nemedina Chandler.

Diagnosis. Distinguished from Atelestinae by having arched vein r-m and a short R₁.

Comments. Including only one extant genus, but several fossil taxa have been described as closely related (Grimaldi & Cumming, 1999; Sinclair & Shamshev, 2003).

Genus included. Nemedina (Sinclair & Cumming, 2006).

Dolichopodidae Latreille
Dolichopodes [sic] Latreille, 1809: 239. Type genus Dolichopus Latreille.

Dolichopodidae Agassiz (1846: 128).

Diagnosis. Male genitalia rotated, distinguished from Hybotidae by being dextrally rotated between 90° and 180°, including segment 8 and sometimes also segment 7 (Fig. 5B). Wings with simple R₄ including segment 8 and sometimes also segment 7 (Fig. 5B). A costa that ends at, or just beyond, R₄₊₅, or continues along the wing margin. Except for Niphogenia Melander spp. and Philetus Melander spp., distinguished from both Ragadidae and Atelestidae by the presence of a prosternum that is fused with proepisternum and forming a precoxal bridge (Fig. 7C).

Comments. Brachystomatidae is here brought back as a subfamily within Empididae and the subfamilies of the former family are consequently lowered to tribes. The incertae sedis genera Brochella Melander, Dryodromia Rondani, Hesperempis Melander and Philetus were not included in our analysis and remain unplaced until further studies confirm their placement.

Subfamilies included. Brachystomatinae, Clinocerinae, Empidinae.

Brachystomatinae Melander stat. rev.
Brachystomatinae Melander, 1908: 202. Type genus Brachystoma Meigen.

Brachystomatidae Sinclair & Cumming (2006: 80).

Diagnosis. Variable group with wing with or without anal lobe; CuA₂ joining the A₁ before crossvein bm-cu (Fig. 6H) in Ceratomerini and Trichipezini, beyond in Brachystomatini. Scape at most slightly longer than the pedicel, twice as long in Ceratomerini; conus of the pedicel absent (Fig. 7K) in Brachystomatini and Trichipezini, present in Ceratomerini. Male cercus developed but simple in structure (cf. Fig. 5F). Females with acanthophorites on tergite 10 (Fig. 5J).

Comments. The group was originally treated as a subfamily of Empididae, but was elevated to family level by Sinclair & Cumming (2006). The status as a subfamily is re-established here. The tribes within this subfamily have previously been included as subfamilies within Empididae, but were excluded from the Empididae by Sinclair & Cumming (2006).

Tribes included. Brachystomatini, Ceratomerini, Trichipezini (Sinclair & Cumming, 2006).

Brachystomatini Melander stat.n.
Brachystomatinae Melander, 1908: 202. Type genus Brachystoma Meigen.

Diagnosis. Narrow wings as Ceratomerini, but distinct in in the vein CuA₂ that is joining A₁ beyond the crossvein bm-cu.
Scape at most slightly longer than the pedicel, never twice as long as in Ceratomerini, and pedicel without conus (cf. Figs. 7I, J). Male cercus is articulated but simple in structure. Female tergite 10 is divided into a pair of lobes with stout spine-like setae (acanthophorites) (cf. Fig. 5J).

Comments. According to Sinclair (1995) the tribe comprises the two genera Anomalempis Melander and Brachystoma Meigen. When Sinclair & Cumming (2006) raised Brachystomatinae to family level, the nominate subfamily was defined to include Xanthodromia Saigusa as a third genus.

Genera included. Anomalempis, Brachystoma, and Xanthodromia (Sinclair & Cumming, 2006).

Ceratomerini Collin stat.n.
Ceratomerinae Collin, 1928: 1. Type genus Ceratomerus Philippi.

Diagnosis. Narrow wings and absence of an anal lobe (cf. Fig. 6D); and CuA₂ joins A₁ before the crossvein bm-cu. Scape twice as long as, or longer than, the pedicel; pedicel with a fingerlike conus. Male cercus well developed but simple in structure (cf. Fig. 5F). Female tergite 10 with acanthophorites (cf. Fig. 5J).

Comments. The tribe Ceratomerini includes Ceratomerus Philippi, Glyphidopa Sinclair, and Zealandicessa Koçak & Kemal. It was included in Empidinae and transferred to Brachystomatidae by Sinclair & Cumming (2006).

Genera included. Ceratomerus, Glyphidopa, and Zealandicessa (Sinclair, 1997; Koçak & Kemal, 2010).

Trichopezini Vaillant stat. rev.
Trichopezini Vaillant, 1981: 353. Type genus Trichopeza Rondani.

Trichopezinae Sinclair (1995: 720).

Diagnosis. Wings with well-developed anal lobe; and CuA₂ joining the A₁ before crossvein bm-cu (Fig. 6H). Scape at most slightly longer than the pedicel, twice as long in a few species, the conus of the pedicel absent (Fig. 7K). Male cercus simple (cf. Fig. 5F). Females with acanthophorites on tergite 10 (Fig. 5J).

Comments. This tribe was originally proposed by Vaillant (1981) within Hemerodromiinae, including the single genus Trichopeza Rondani. Sinclair (1995) raised the status to subfamily and included several genera previously included in Clinocerinae and Oreogotoninae. Sinclair & Cumming (2006) moved the subfamily to the Brachystomatidae.

Genera included. Afropeza Sinclair & Shamshev, Adipsomyia Yang et al., Apalocnemis Philippi, Boreodromia Coquillett, Ceratempis Melander, Ephidrempis Saigusa, Gloma, Heleodromia Haliday, Heteroplebus Philippi, Hyperperaceria Collin, Niphogenia, Pseudheleodromia Wagner, Rubistella Garrett Jones, Sabroskyella Wilder, Sematopoda Collin, Sinotrichoepza Yang et al., and Trichopeza (Vaillant, 1981; Sinclair, 1995; Yang et al., 2007; Sinclair, 2011b).

Clinocerinae Collin
Clinocerinae Collin, 1928: 2. Type genus Clinicera Meigen. Clinoceratinae Melander (1928: 10).

Diagnosis. Wing narrow without anal lobe; and the CuA₂ joins A₁ before the crossvein bm-cu (Fig. 6D). Scape at most slightly longer than the pedicel, pedicel lacks conus (Fig. 7I). Male cercus well developed and simple in structure (Fig. 5F). Female tergite 10 is complete or divided, without stout spine-like setae (Fig. 5H).

Comments. According to previous analyses based on morphology, as well as our molecular analysis, this subfamily is monophyletic and forms the sister group to all other subfamilies in Empididae.

Genera included. Afroclinocera Sinclair, Asymphylotera Collin, Bergensstammi Mik, Clinicera Meigen, Clinocerella Engel, Dolichocephala Macquart, Hypenella Collin, Kowarzia Mik, Oreothalia Melander, Pheoebalia Mik, Prougomyia Collin, Proclinopyga Melander, Rhyacodromia Saigusa, Roedriodes Coquillett, Trichoclinocera Collin, and Wiedemanni Zetterstedt (Sinclair, 1995, 1999b, 2008).

Empidinae Latreille
Empides [sic] Latreille, 1809: 189. Type genus Empis Linnaeus. Empidinae Schiner (1862: lii).

Hemerodromiinae Schiner, 1862 syn.n.

Diagnosis. Characteristic in having a well-developed anal lobe; the CuA₂ is joining A₁ before crossvein bm-cu (Fig. 6C); scape usually only slightly longer than, rarely twice as long as, pedicel; conus of the pedicel absent (Fig. 7J). Distinguished from other Empidinae in the males by the presence of highly sclerotized cercus involved in clasping during mating; or reduced and membranous cercus (Fig. 5G). Female tergite 10 complete and without spine-like setae (Fig. 5I).

Comments. This subfamily includes the tribes Hemerodromini and Chelipodini previously assigned to Hemerodromiinae. The genera Afrodromia and Drymodromia were removed from Chelipodini by Plant (2011a) and placed incertae sedis within Hemerodromiinae. Following our analysis these two taxa are now placed within the subfamily Empidinae.

Tribes included. Empidini, Chelipodini, Hemerodromini, Hilarini.
Chelipodini Hendel stat. rev.
Chelipodini Hendel, 1936: 1929. Type genus Chelipoda Macquart.

Diagnosis. Wings similar to those of the Hemerodromiini in that the anal lobe is absent; the wings are narrow at the base; and the costa circumambient (Fig. 6E). Forelegs are raptorial with elongated fore coxa; fore femur with at least one row of strong setae, similar to those in Hemerodromiini (Fig. 7N). Distinguished from Hemerodromiini by the strong setae on scutellum and the setose laterotergite (Fig. 7Q) (Fig. 8A).

Comments. Plant (2011a) indicated that the genus Chelipodozus belongs to Hemerodromiini, but with low support. It is herein still considered belonging to Chelipodini until evidence about its relationship is revealed.

Genera included. Achelipoda Yang et al., Anaclastoctedon Plant, Chelipoda Macquart, Chelipodozus, Monodromia Collin, Phyllodromia Zetterstedt, Pitophyllodromia Bezzi, and Sororserexa Plant (Melander, 1947; Smith, 1967, 1975, 1980, 1989; Sinclair & Cumming, 2006; Yang et al., 2007; Plant, 2011a,c).

Empidini Collin (Fig. 4C)
Empidini [sic] Latreille, 1809: 189. Type genus Empis Linnaeus.
Empidini Collin (1961: 326).

Diagnosis. Characteristic in having broad wings with well-developed anal lobe; wing costa ending near R₅ (Fig. 6C). Forelegs with slender first tarsomere and legs without raptorial adaptations (Fig. 7O). Scutellum with at least one pair of strong setae (cf. Fig. 7S), and laterotergite has setae (Fig. 8B).

Comments. This tribe has been hypothesized to be most closely related to Hilarini, but we found it to be the sister group to the Chelipodini.

Genera included. Bolhrhamphomyia Rafael, Chilerhamphomyia Rafael, Clinarcompha Collin, Edenophorus Smith, Empidadelpha Collin, Empis, Hystrichonotus Collin, Lamprempis Wheeler & Melander, Macrostomus Wiedemann, Opeatocerata Melander, Porphyrochroa Melander, Rhamphella Malloch, Rhamphomyia, and Spicosa Philippi (Smith, 1980; Chvála & Wagner, 1989; Rafael & Cumming, 2004; Sinclair & Cumming, 2006).

Hemerodromiini Schiner stat. rev.
Hemerodromiinae [sic] Schiner, 1862: li. Type genus Hemerodromia Meigen.
Hemerodromiinae Wheeler & Melander (1901: 376).

Diagnosis. Similar to Chelipodini in the wings, with anal lobe absent; wings are generally narrower; and the costa circumambient (Fig. 6F). Forelegs are raptorial as in the Chelipodini, with elongated fore coxa, and fore femur with one or more rows of strong setae (cf. Fig. 7N). Separated from Chelipodini by scutellum with at most weakly developed setae (Fig. 7R), and the laterotergite bare (Fig. 8C).

Comments. This tribe was formerly included in the subfamily Hemerodromiinae which is a synonym of Empidinae.

Genera included. Antipodromia Plant, Chelifera Macquart, Cladorromia Bezzi, Colabris Melander, Doliodromia Collin, Hemerodromia Meigen, Metachela Coquillett, and Neoplasta Coquillett (Melander, 1947; Smith, 1967, 1975, 1980, 1989; Sinclair & Cumming, 2006; Plant, 2011b).

Hilarini Collin
Hilarini Collin, 1961: 326. Type genus Hilara Meigen.

Diagnosis. Similar to Empidini in having broad wings and with well-developed anal lobe. Distinguished from Empidini by the presence of circumambient costa in the wings (Fig. 6G). Forelegs in the males often with enlarged first tarsomere.
Fig. 5. Pictures of posterior part of abdomen. (A–E) male genitalia in dorsal view, *Atelestus pulicarius* (A), *Neurigona saturalis* (B), *Empis vitripennis* (C), *Hybos grossipes* (D), *Ragas unica* (E); (F, G) male genitalia in dorsal view, *Clinocera nivalis* (F), *E. vitripennis* (G); (H–J), female genitalia in dorsal view, *C. nivalis* (H), *E. vitripennis* (I), *Trichopezia longicornis* (J).
Fig. 6. Photographs of right wing. (A) Atelestus pulicarius; (B) Neurigona suturalis; (C) Empis vitripennis; (D) Clinocera rivalis; (E) Chelipoda vocatoria; (F) Hemerodromia sp.; (G) Hilara litoriae; (H) Trichopeza longicornis; (I) Hybos grossipes; (J) Bicellaria salcata; (K) Leptopeza flavipes; (L) Oedalea stigmatella; (M) Tachydromia umbrarum; (N) Drapetis parilis; (O) Symballophthalmus fascitarsis; (P) Trichina bilobata; (Q) Ragas unica. Abbreviations: h, humeral crossvein; R5, radial sector; r-m, radial-medial crossvein; M1, first medial vein; M1+2, first and second medial vein (unbranched); R4, fourth radial vein; R5, fifth radial vein; R4+5, fourth and fifth radial vein (unbranched); A1, anal vein; CuA2, second anterior branch of cubital vein; bm-cu, basal medial-cubital crossvein; dm, discal medial cell; bm, basal medial cell; cup, posterior cubital cell (anal cell).

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Fig. 7. Detailed photos of prosternum, antenna, foreleg and scutellum. (A–H) prosternum in anterior view, Atelestus pulicarius (A), Neurigona suturalis (B), Enpis vitripennis (C), Hybos grossipes (D), Drapetis parilis (E), Symballophthalmus fasciatus (F), Tachydromia umbrarum (G), Ragas unica (H); (I–M), antenna in lateral view, Clinocera nivalis (I), E. vitripennis (J), Trichoeca longicornis (K), Itachila macquarti (L), R. unica (M); (N–P) foreleg in lateral view, Chelipoda vocatoria (N), E. vitripennis (O), Hilara litorea (P); (Q–S) scutellum in dorsal view, C. vocatoria (Q), Hemerodromia sp. (R), H. litorea (S).
Fig. 8. Details of laterotergite, head and proboscis. (A–D) laterotergite in lateral view, Chelipoda vocatoria (A), Empis vitripennis (B), Hemerodromia sp. (C), Hilara litorea (D); (E–K) head in lateral view, Bicellaria sulcata (E), Hybos grossipes (F), Leptopeza flavipes (G), Oedalea stigmatella (H), Tachydromia umbrarum (I), Drapetis parilis (J), Trichina bilobata (K); (L–M), proboscis in lateral view, Itaephila macquarti (L), Raga unica (M).
(Fig. 7P); scutellum with strong or weak setae (Fig. 7S). Distinguished from those in the Empidini by the absence of setae on the laterotergite (Fig. 8D).

Comments. This tribe is morphologically similar to Empidini, but it was recovered as sister group to Hemerodromiini in our analysis.

Genera included. Afroempis Smith, Allochorus Collin, Amictoides Bezzi, Aplomera Macquart, Attrichopleura Bezzi, Bandella Bickel, Canomyia Bickel, Deuteragenosta Philippi, Eugowra Bickel, Gynatoma Collin, Hilara Meigen, Hilaremphis Bezzi, Hilarigona Collin, Hybomyia Plant, Munburra Bickel, Pastrichotus Collin, Thinempis Bickel, and Trichohilara Collin (Smith, 1980; Bickel, 1996; Sinclair & Cumming, 2006).

Hybotidae Meigen
Hybotinae Meigen, 1820: X. Type genus Hybos Meigen. 
Hybotidae Macquart (1827: 136).

Diagnosis. Share similarities with those in the Dolichopodidae, particularly in rotation of genitalkia and wing characters. Male terminalia rotated dextrally between 45° and 90°, excluding segment 7 (Fig. 5D), in the Dolichopodidae rotated between 90° and 180°. Wings of the Hybotidae always with a simple vein R45; costa ends near or at M1/M12 but may also end at or near R1+5/R5 (Fig. 6L). Distinguished from Dolichopodidae also by the point of origin of vein R9, being at a distance from the humeral crossvein (h) equal to or longer than length of h.

Comments. The diagnosis of the tribus Bicellariini, here elevated to subfamily, and of the subfamily Hybotinae, are revised below, following the results from our analysis.

Subfamilies included. Bicellariinae Sinclair & Cumming, Hybotinae Meigen, Oedaleinae Chvála, Tachydromiinae Meigen, and Trichininae Chvála.

Bicellariinae Sinclair & Cumming stat.n.
Bicellariini Sinclair & Cumming, 2006: 79. Type genus Bicellaria Macquart.

Diagnosis. Similar to those in the Tachydromiini in that the wings lack cell dm. Separated by having the M1+2 vein branched into M1 and M2 (Fig. 6J); M1 and M2 may be faint or abbreviated at base; and the cup cell present and shorter than the bm cell, or as long as the bm cell. Proboscis oriented ventrally (Fig. 8E) while in the Tachydromiinae it is oriented posteriorly or dorsally.

Comments. The tribe Bicellariini is removed from Hybotinae and raised to a distinct hybotid subfamily based on our analysis, i.e. it is not closely related to Hybotini. Instead it forms the sister group to remaining Hybotidae and constitutes a few morphologically homogenous genera.

光阴 included. Bicellaria, Hoplocyrtoma Melander, and Leptocyrtoma Saigusa (Sinclair & Cumming, 2006).

Hybotinae Meigen
Hybotinae Meigen, 1820: 346. Type genus Hybos Meigen.

Diagnosis. Characteristic wing characters, like the presence of a dm cell; the unbranched M1+2; presence of a cup cell being as long as, or longer than the bm cell (Fig. 6I). Proboscis oriented strictly anterad (Fig. 8F).

Comments. The taxa previously included in Hybotinae as Bicellariini are here placed into a separate subfamily.

Genera included. Acarterus Loew, Afrohybos Smith, Chilcottomyia Saigusa, Ceratohybos Bezzi, Euhybus Coquillett, Hybos, Lactistomyia Melander, Lamachella Melander, Neohybos Ale-Rocha & Carvalho, Smithybos Ale-Rocha, Stenoproctus Loew, Syndyas Loew, and Syneches Walker (Smith, 1967, 1975, 1980; Sinclair, 2011a).

Ocydromiinae Schiner
Ocydromiinae Schiner, 1862: lii. Type genus Ocydromia Meigen. 
Ocydromiinae Melander (1908: 222).

Diagnosis. Characteristic by having wings with intact dm cell; a branched M1+2 together with a short M1 or M2 is absent; and the cup cell shorter than the bm cell (Fig. 6K). Proboscis oriented ventrad or slightly ventro-caudad (Fig. 8G).

Comments. This subfamily historically has included the tribes Oedaleini and Trichinini until Sinclair & Cumming (2006) elevated the two into separate subfamilies.

Genera included. Aboccippita Plant, Apterodorima Oldroyd, Austropeza Plant, Chvalaeca Papp & Földvári, Hoplopeza Bezzi, Leptodromia Sinclair & Cumming, Leptodromiella Tuomikoski, Leptopeza Macquart, Leptopezella Sinclair & Cumming, Neohybos Meigen, Orypezza Collin, Pseudoscelolabes Collin, Scelolabes Philippi, and Stylocyrtoma Saigusa (Chvála, 1983; Plant, 1989; Sinclair & Cumming, 2000, 2006).

Oedaleinae Chvála
Oedaleini Chvála, 1983: 112. Type genus Oedalea Meigen. 
Oedaleinae Sinclair & Cumming (2006: 78).

Diagnosis. Similar to those of the Trichininae, particularly in the wings including the presence of dm cell; the branched M1+2; the complete M1 which reaches the wing margin; and the cup cell being shorter than bm cell (Fig. 6L). Distinguished from the Trichininae by the presence of anterad projecting and often long proboscis (except for species in Allanthalia Melander which have a scarcely visible proboscis) (Fig. 8H).

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**Comments.** This group was previously classified as a tribe within the Ocydromiinae by Chvála (1983), but was elevated to subfamily by Sinclair & Cumming (2006). The latter classification was supported in our analysis. Oedaleinae formed a sister group to the Hybotinae.

**Genera included.** Allanthalia, Anthalia Zetterstedt, Euthyneura Macquart, and Oedalea Meigen (Chvála, 1983; Sinclair & Cumming, 2006).

**Tachydromiinae Meigen**

Tachydrominae [sic] Meigen, 1822: vii. Type genus Tachydromia Meigen.

**Diagnosis.** Similar to the Bicellariinae in the wings by the absence of the dm cell, but vein M₁₂ is unbranched (Fig. 6M). Unique in the family by the absence of a cup cell or the cell very small, at most reaching to half the length of bm cell; and the anal vein often faint. Proboscis oriented ventrad or ventrocaudad (Fig. 8I).

**Comments.** This subfamily is distinguished by its wing characters. The taxon forms a sister group to the other Hybotidae subfamilies excluding Bicellariinae. Collin (1961) first divided the subfamily into Drapetini and Tachydromiini and Sinclair & Cumming (2006) introduced the third tribe Symballophthalmini.

**Genera included.** Drapetini, Symballophthalmini, and Tachydromiini (Collin, 1961; Sinclair & Cumming, 2006).

**Drapetini Collin**

Drapetini Collin, 1961: 26. Type genus Drapetis Meigen.

**Diagnosis.** Similar to Tachydromiini by nonbroadened wing (Fig. 6N), different in having pubescent eyes (Fig. 8J) and prosternum fused with proepisternum (Fig. 7E).

**Comments.** Drapetini was recovered as a sister group to Tachydromiini by Sinclair & Cumming (2006), contrasting Chvála (1975) who hypothesized it as being the sister group to Tachydromiini + Symballophthalmini. The hypothesis by Chvála (1975) was supported in our analysis.

**Genera included.** Allodromia Smith, Atodrapetis Plant, Austrodrapetis Smith, Austrodromia Collin, Baeodromia Cumming, Chaetodromia Chillcott & Teskey, Chersodromia Walker, Crossopalpus Bigot, Dasmetina Gil, Drapetis Meigen, Elaphropeza Macquart, Isodrapetis Collin, Megaplecta Melander, Micrempis Melander, Nanodromia Grootaert, Ngaheremyiia Plant & Didham, Pontodromia Grootaert, Sinodrapetis Yang et al., and Stilpon Loew (Chvála, 1975; Cumming, 2006; Plant & Didham, 2006).

**Symballophthalmini Sinclair & Cumming**

Symballophthalmini Sinclair & Cumming, 2006: 79. Type genus Symballophthalus Becker.

**Diagnosis.** Characteristic by having wings that broaden apically (Fig. 6O). Eyes are bare as in Tachydromiini, distinguished by having a prosternum separate from proepisternum (Fig. 7F).

**Comments.** This tribe was erected by Sinclair & Cumming (2006) to include the genus Symballophthalus Becker. 1889. The tribe was found to represent the sister group to the remaining Tachydromiinae by Sinclair & Cumming (2006). It was in our analysis recovered as sister group to Tachydromiini, similar to the hypothesis by Chvála (1975).

**Genera included.** Symballophthalus (Sinclair & Cumming, 2006).

**Tachydromiini Meigen**

Tachydrominae [sic] Meigen, 1822: vii. Type genus Tachydromia Meigen.

**Tachydromiini Collin**

Collin, 1961: 27. Type genus Tachydromia Meigen.

**Diagnosis.** Similar to those of the Drapetini by nonbroadened wing (Fig. 6M). Distinguished from Drapetini by the presence of bare eyes (Fig. 8I) and prosternum fused with proepisternum into a precoxal bridge (Fig. 7G).

**Comments.** The Tachydromiini was in our analysis recovered as a sister group to the Symballophthalmini, supporting the hypothesis by Chvála (1975), and refuting the sister relationship to Drapetini proposed by Sinclair & Cumming (2006).

**Genera included.** Ariasella Gil, Dysaletria Loew, Pieltainia Arias, Platypalpus Macquart, Tachydromia Meigen, Tachyempis Melander, and Tachyspeza Meigen (Chvála, 1975; Sinclair & Cumming, 2006; Shamshev & Grootaert, 2012).

**Trichininae Chvála**

Trichinini Chvála, 1983: 112. Type genus Trichina Meigen.

**Trichininae Sinclair & Cumming**

Sinclair & Cumming (2006: 77).

**Diagnosis.** Similar to those in Oedaleinae in that the dm cell is present, the M₁₂ vein branched, and the cell cup shorter than the bm cell (Fig. 6P). Distinguished from the Oedaleinae by the presence of a short proboscis pointing ventrally (Fig. 8K).

**Comments.** This group was removed from Ocydromiinae by Sinclair & Cumming (2006). The species in our analysis formed a monophyletic group sister to (Ocydromiinae, (Hybotinae, Oedaleinae)).

**Genera included.** Trichina Meigen and Trichinomyia Tuomikoski (Chvála, 1983).
Ragadidae Sinclair stat.n.
Ragadinae Sinclair, 2016: 2. Type genus Ragas Walker.

Diagnosis. Similar to those in the Empididae and Atelestidae by having symmetrical and straight terminalia (Fig. 5E), as well as the point of origin of vein Rs situated at a distance from the humeral crossvein (h) as long as or longer than h (Fig. 6Q). Separated from those in the Atelestidae by the presence of circumambient costa in the wing (Fig. 6Q). Distinguished from those in the Empididae by having a prosternum separated from proepisternum (Fig. 7H), except for Hydropeza spp. that can be separated by the recurved labrum, which is otherwise straight in Empididae.

Comments. Excluding Iteaphila and Anthepiscopus from Empididae, Sinclair & Cumming (2006) suggested this group to be a distinct higher taxon. It was formally published as a new subfamily within Empididae by Sinclair (2016). The group (Iteaphila, Anthepiscopus), Ragadinae) is here recognized as forming the sister group to Empididae. Ragadinae is raised to family level, based on genetic differences separating the group from Empididae. It is expanded to include a new subfamily Iteaphilinae, comprising the two genera Iteaphila and Anthepiscopus.

Subfamilies included. Iteaphilinae subfam.n. and Ragadinae Sinclair.

Iteaphilinae subfam.n.
http://zoobank.org/urn:lsid:zoobank.org:act:CD19C12F-C00C-419B-9B79-937119F175AC
Type genus Iteaphila Zetterstedt.

Diagnosis. Distinguished from those in the Ragadinae by the absence of ventroapical sclerites (epipharyngal blades) on the labrum (Fig. 8L); absence of a ventroapical comb on the labrum; and the third segment of antenna (postpedicel) at least three times longer than wide (Fig. 7L).

Comments. This new subfamily includes two very similar genera previously placed as incertae sedis within the superfamily Empidoidea by Sinclair & Cumming (2006). It was in our analysis found to be sister group to the subfamily Ragadinae.

Genera included. Anthepiscopus and Iteaphila.

Ragadinae Sinclair
Ragadinae Sinclair, 2016: 2. Type genus Ragas Walker.

Diagnosis. Very similar to those of the Iteaphilinae, separated by having a labrum with ventroapical sclerites (epipharyngal blades) (Fig. 8M) and ventroapical comb; third antennal segment (postpedicel) at most two and a half times as long as broad (Fig. 7M).

Comments. This group comprises a number of genera as proposed by Sinclair & Cumming (2006) and later classified in a separate subfamily as described by Sinclair (2016).

Genera included. Dipsomyia, Hormopeza, Hydropeza, Ragas, and Zanclotus (Sinclair, 2016).

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

Table S1. Included taxa with voucher and GenBank accession codes.

Table S2. Primer names and sequences used in amplification of genes in this study.

Table S3. PCR reaction protocols for each gene fragment.

Table S4. Complete statistics from substitution saturation tests for codon 1+2 and 3 using Xia’s method (Xia et al., 2003; Xia & Lemey, 2009) in DAMBE 6.4.29 (Xia, 2013).

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References
Agassiz, J.L.R. (1846) Nomina systematica generum Dipterorum (Pt. 4). Nomenclator zoologicus continens nomina systematica generum animalium tam viventium quam fossilium, secundum ordinem alphabeticum disposita, adiectis auctoribus, libris, in quibus reperiuntur, anno editionis, etymologia et familiis, ad quas pertinent, in singulis classibus., p. 393. Jent & Gassmann, Solothurn.

Bickel, D.J. (1996) Thinempis, a new genus from Australia and New Zealand (Diptera: Empididae), with notes on the tribal classification of the Empidinae. Systematic Entomology, 21, 115–128.

Brauer, F. (1883) Die Zweiflügler des kaiserlichen Museums zu Wien. III. Systematische Studien auf Grundlage der Dipteren-Larven nebst einer Zusammenstellung von Beispielen aus der Literatur über dieselben und Beschreibung neuer Formen. Denkschriften der Mathematisch-Naturwissenschaftlichen Klasse der kaiserlichen Akademie der Wissenschaften, Vol. 47, pp. 1–100. Wien.

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