Molecules and morphology in Empidoidea (Diptera)
Phylogenetic analysis and taxonomical implications

Emma Wahlberg

Names such as dance flies, dagger flies, balloon flies, long legged flies and smoke flies all reflect on the many different appearances and behaviours of species within the superfamily Empidoidea. They belong to the insect order Diptera, commonly known as flies and mosquitoes. There are over 10,000 known species within this very large species group and the diversity of some of the included families and genera is highest in temperate areas, but members of the superfamily occur worldwide with the exception of Antarctica. The classification and evolutionary history have been far from straightforward subjects. Morphology based studies have been unable to answer many questions raised by both systematists and biogeographers, as well as ecologists and ethologists. Partly due to morphological modifications and adaptations difficult to interpret. In this thesis I use genetic data, DNA, to investigate and explore the phylogeny of the superfamily in whole and also of the genus Wiedemannia. I also build upon these results for further morphological studies in the herein erected family Ragadidae. Finally, I contribute with new and extended data of both the taxonomy and the Swedish biodiversity.
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Phylogenetic analysis and taxonomical implications

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Abstract

The classification and evolutionary history of the superfamily Empidoidea (Diptera) have been far from straightforward subjects. Morphology based phylogenies of the superfamily have been unable to answer many questions raised by both systematists and biogeographers, as well as ecologists and ethologists, partly due to morphological modifications and adaptations difficult to interpret. In paper I a phylogeny is presented which was the first published that was based on molecular data with multiple loci and with high support. We sampled several groups never included in molecular analyses before and were able to resolve several relationships and recover several species groups that were previously unplaced. Atelestidae is confirmed as the sister group to remaining families. Hybotidae is sister group to Dolichopodidae, Ragadidae stat. n. and Empididae. Species included in the family Brachystomatidae are found to be nested within Empididae and Brachystomatidae is therefore lowered to subfamily. The erection of Ragadidae to family, sister to Empididae, is based on genetic distances between the present families and subfamilies. Paper II deals with the genus Wiedemannia (Empididae: Clinocerinae), also using molecular data but also put into a spatial and temporal context with molecular dating. The subgenera are found to be non-monophyletic and are therefore suggested to be rejected as accepted names. The dated tree, based on molecular data and known fossil records, suggests an initial diversification 50 million years ago followed by further diversification events that may be linked to changes in sea levels on a global scale. We also provide a Species Distribution Model map suggesting areas of potential hot spots as based on climatic variables. Following the results in paper I, the internal relationships of Ragadidae are revised in paper III. The lack of DNA data, partly caused by few available specimens and the rarity of some species, motivated a morphological approach together with a maximum parsimony analysis to investigate the monophyly of species groups and genera. The flower feeding genera Iteaphila and Anthepiscopus are found to form a distinct monophyletic clade together with Hormopeza. The internal relationships are revised, updated diagnostic characters are provided as well as a determination key to the genera. In paper IV we deal with the taxonomical issue of Chvalaea sopianae and C. rugosiventris, two species in the family Hybotidae. Both species were originally described from single female specimens. However, the sex of C. rugosiventris was mistaken in the original description, and the differential characters of the two species are based on sexual dimorphism. The two species are synonymized and the male of C. rugosiventris redescribed. We also provide additional geographic data on the hybotid species Allanthalia pallida and Leptodromiella crassiseta. The final paper, paper V, presents records of new species to Sweden and new records within Sweden of a large number of species of Hybotidae, Empididae and Ragadidae. We also provide a substantial addition of genetic barcodes of the Swedish fauna, shared publicly in The Barcode of Life Database. This increases the coverage of Swedish taxa in the database with 71% for Empididae and 13% for Hybotidae.

Keywords: Empidoidea, Empididae, Hybotidae, phylogeny, taxonomy, systematics, molecular data, morphology, revision.

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MOLECULES AND MORPHOLOGY IN EMPIDOIDEA (DIPTERA)

Emma Wahlberg
Molecules and morphology in Empidoidea (Diptera)
Phylogenetic analysis and taxonomical implications

Emma Wahlberg
It's been a long road, getting from there to here.
It's been a long time, but my time is finally near.
And I will see my dream come alive at last.
I will touch the sky.
And they're not gonna hold me down no more,
no they're not gonna change my mind.

Cause I've got faith of the heart.
I'm going where my heart will take me.
I've got faith to believe. I can do anything.
I've got strength of the soul.
And no one's gonna bend or break me.
I can reach any star.
I've got faith.
I've got faith, faith of the heart.

Where My Heart Will Take Me (Diane Warren)
– title song of Star Trek: Enterprise
Abstract

The superfamily Empidoidea (Diptera) comprises over 10,000 known taxa and is known from all parts of the world. This high diversity and wide distribution have tempted many researchers in taxonomy and systematics to try to entangle the evolutionary history. The classification and evolutionary history have been far from straightforward subjects. Morphology based phylogenies of the superfamily have been unable to answer many questions raised by both systematists and biogeographers, as well as ecologists and ethologists, partly due to morphological modifications and adaptations difficult to interpret. In paper I a phylogeny is presented which was the first published that was based on molecular data with multiple loci and with high support. We sampled several groups never included in molecular analyses before and were able to resolve several relationships and recover several species groups that were previously unplaced. Atelestidae is confirmed as the sister group to remaining families. Hybotidae is sister group to Dolichopodidae, Ragadidae stat. n. and Empididae. Species included in the family Brachystomatidae are found to be nested within Empididae and Brachystomatidae is therefore lowered to subfamily. The erection of Ragadidae to family, sister to Empididae, is based on genetic distances between the present families and subfamilies. Paper II deals with the genus Wiedemannia (Empididae: Clinocerinae), also using molecular data but also put into a spatial and temporal context with molecular dating. The subgenera are found to be non-monophyletic and are therefore suggested to be rejected as accepted names. The dated tree, based on molecular data and known fossil records, suggests an initial diversification 50 million years ago followed by further diversification events that may be linked to changes in sea levels on a global scale. We also provide a Species Distribution Model map suggesting areas of potential hot spots as based on climatic variables. Following the results in paper I, the internal relationships of Ragadidae are revised in paper III. The lack of DNA data, partly caused by few available specimens and the rarity of some species, motivated a morphological approach together with a
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**Keywords:** Empidoidea, Empididae, Hybotidae, phylogeny, taxonomy, systematics, molecular data, morphology, revision.
The thesis is based on the following articles, which are referred to in the text by their Roman numerals:


V. **Wahlberg, E.**, Rhodén, C., Johanson, K. A. 2019. New records of dance flies (Hybotidae) and dagger flies (Empididae) in Sweden and a significant addition of genetic barcodes of the Swedish empidoid fauna. Entomologisk Tidsskrift 140(2), 133–144.

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The nomenclatural acts in this thesis are not issued for permanent scientific records or for purposes of zoological nomenclature and are not regarded as published within the meaning of the International Code of Zoological Nomenclature (ICZN, ed. 4, article 8.2).
**Candidate contributions to thesis articles***

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**Contribution explanation**

Minor: contributed in some way, but contribution was limited.

Significant: provided a significant contribution to the work.

Substantial: took the lead role and performed the majority of the work.
Related articles that are not included in the thesis, but have been prepared during the course of the PhD studies:


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Introduction

Diptera and the empidoids – an irresistible temptation

In a world with around 1,000,000 known species of insects (Roskov et al. 2019) the order Diptera, or flies, represents almost 160,000 species (Pape et al. 2011). The estimated number of insect species varies greatly, but a mean estimation is around 7,000,000 (Stork 2017). This kind of enumeration of described and estimated number of species is standard in almost all taxonomic and systematic papers published. It might at first seem like self-justification of in-depth studies and research into the authors’ favourite organism groups. However, the number of known and estimated species, the biodiversity, is the quintessential element in the exploration of evolution. Mayhew (2007) gives a thorough review of the evolutionary mechanisms behind the observed biodiversity. In short, the richness of species is a direct effect of e.g. variety of ecological niches, morphological adaptations and sexual competition and conflict. There is no method to travel in time and observe past evolutionary events, but we can observe present and fossil species in regard to the examples above. With an increase in number of species this data source expands, and with more data the picture becomes both more complex and detailed. This is why the number of species as well as where they are and how they are recognized are not only formal statements, but also the main source of our understanding of evolution.

The Diptera superfamily Empidoidea Latreille, 1809 includes over 10,000 described species (Moulton & Wiegmann 2007; Pape et al. 2011). The species diversity of some of the included families and genera is highest in temperate areas, but members of the superfamily occur worldwide with the exception of Antarctica (Sinclair & Cumming 2006). The sheer number of species and wide distribution makes this group a tempting subject to both taxonomic and systematic research.
Brauer (1883) was the first to establish the grouping within Empidoidea, comprising the families Dolichopodidae Latreille, 1809 and Empididae Latreille, 1809. Since this classification the internal relationships as well as the relationship between Empididae and Dolichopodidae has been debated and revised multiple times. Most hypotheses and all revisions have been based on morphological characters, characters that have been notoriously difficult to interpret because of extreme modifications and adaptations. Chvála (1983) presented a classification that gained wide acceptance, constituting the families Atelestidae Chvála, 1983, Dolichopodidae, Microphoridae Collin, 1960, Empididae and Hybotidae Fallén, 1816, as well as definitions of a number of subfamilies and tribes. Even though this classification as well as some modifications by e.g. Sinclair & Cumming (2006) are mainly followed, Yang et al. (2006; 2007) used the older two-family classification. The disagreements in classifications may be daunting at first, but also presents an opportunity to examine difficult as well as stimulating systematic and taxonomic questions.

Figure 1. *Empis pennipes* Linnaeus, 1758, ♀, with very long mouthparts and pennate (flattened and leaflike) bristles on mid and hind legs. Photo: E. Wahlberg.
The first empidoids appear in the fossil record during the mid-Jurassic period (ca 160 million years ago) and the diversification of families are found in fossils from early Cretaceous (ca 125 million years ago) (Grimaldi & Cumming 1999; Grimaldi & Engel 2005). The rich abundance of flies as well as a widespread radiation of species, also within other insect orders, during this period has been regarded as a possible link to the evolution of flowering plants and new ecological niches (Zhang & Wang 2017). Time calibration of phylogenetic trees is however difficult and large gaps in the fossil record raises concerns of uncertainty.

Getting to know Empidoidea and why it’s meaningful
Empidoidea together with the group Cyclorrhapha constitutes the group Eremoneura. The Eremoneura is monophyletic on the basis of characters in the mouthparts in the larvae, adult wing venation and male and female genitalia (Sinclair & Cumming 2006). Both the taxonomical and morphological diversity within the superfamily makes a short and precise diagnosis of Empidoidea difficult, a comparison of a combination of characters with other closely related taxa is almost always necessary for the non-specialist to recognize a species as belonging to the superfamily. The classification in following paragraphs follow that of Wahlberg & Johanson (2018, paper I) and the numbers of species and genera are based on the 2019 Annual Checklist from Species 2000 & ITIS Catalogue of Life (Roskov et al. 2019).

The Atelestidae is the smallest family with only 11 species, assigned to 4 genera in 2 subfamilies. They are generally small, dark flies with a humped appearance. Almost nothing is known about their biology, including larval stages. The males are holoptic, meaning having eyes that meet on top front of the head, while the females are dichoptic, meaning separated eyes. This character is typical to dipteran species with swarming behaviour, which also have been recorded for species in this family. They are seldom collected, but when they are, they are found in vegetation often close to the ground.
In contrast to Atelestidae the Dolichopodidae is the largest empidoid family with 7,200+ described species organized in 230 genera. For most species, the name long-legged flies describe their appearance well, however some deviations from this description are obvious. The males are sometimes equipped with ostentatious ornamented mouthparts, antennae or forelegs, used in intraspecific interactions such as courting (Sivinski 1997). Adults are predatorial and occupy a vast range of habitats depending on species. Some are terrestrial in vegetation or tree trunks, while others are epi-aquatic skating and hunting on water surfaces. A few species are adapted to live in marine environments. As in adults, larvae are found in a wide spectre of habitats, and individual species are adapted to different diets.

The Empididae, also called dance or dagger flies, are often equipped with long mouthparts giving them their latter vernacular name (Fig. 1). However, this is not the case for all species and there are some species in the family having little pronounced mouthparts. The family comprises 3,200 known species in 3 subfamilies and more than 80 genera. Apart from the prominent mouthparts they are also known for their swarming behaviour. Males are sometimes holoptic, as in the Atelestidae. Within the family, specifically in the genera Empis Linnaeus, 1758, Hilara Meigen, 1822 and Rhamphomyia Meigen, 1822, the females can be ornamented with pennate legs (Fig. 1), broad maculated wings and inflatable abdomen, also linked to swarming behaviour.

Courtship in these genera may also involve a prey delivered from the male to the female as a nuptial gift. Courtship rituals with the male carrying inedible seeds have been observed in some species (Chvála 1976). Some species (i.e. in the genus Hilara) the nuptial gift is packaged in a silken cocoon; hence the name balloon flies. The cocoons however can sometimes be empty or be recycled cocoons used in previous mating attempts (Chvála 1976; Marshall 2012). A study by Downes (1970) showed that mating swarms and swarms where males gather to catch prey often are separate (Fig. 2). Visual markers in the environment are often of high importance in some species and mating swarms can be stationary at those positions. In other species within Empididae swarming behaviour is absent, however conglomeration of individuals on
various surfaces where both male to male and female to female interactions occur have been observed (Rogers 1981; Sinclair 1994). The habitat preference in adults vary, most notable between subfamilies. Empidinae Latreille, 1809 are often found in terrestrial environments, from pastureland to dense forest. Clinocerinae Collin, 1928 are often riparian, found close to water bodies. The larvae are also found in various environments, however the aquatic larvae of Clinocerinae are some of the most studied thanks to their varying requirements of water quality and turbulence (Sinclair 1995; Werner & Pont 2003; Ivković et al. 2013).

Figure 2. Males of *Rhamphomyia nigrita* Zetterstedt, 1838 hunt at a swarm of mosquitoes (right of center), fly towards a swarm of females (upper left) and the two land during mating (far right). Illustration from Downes (1970).
With 1,965 species in 6 families and 69 genera the Hybotidae is the second largest family, after Dolichopodidae. It is perhaps also the morphologically most diverse group. Most adults are predators, some apparently highly adapted to capture prey, with robust mouthparts and spines on powerful legs (Fig. 3). Some species feed on pollen and are delicate, with hardly distinguishable mouthparts. Mating behaviour varies among species, some form aerial swarms while other swarm on ground and in some cases swarming behaviour is absent (Chvála 1983).

Figure 3. *Platypalpus cursitans* (Fabricius, 1775), ♀, with mid legs equipped with strong bristles and spines that are used for catching and holding prey. Photo: E. Wahlberg.
With a few exceptions, females of species in the subfamilies Hybotine Fallén, 1816 and Bicellariinae Sinclair & Cumming, 2006, are holoptic. Rather than an adaptation to swarming for mating purposes, this have been suggested to be an adaptation to hunting prey in the air from below (Chvála 1976; Sinclair & Cumming 2006). Very little is known about larval stages and their habitats, but some are recorded from soil, dung, wood and water (Chvála 1983).

The family Ragadidae, with 57 species in 7 genera, contain the group where the biology maybe is least known. The morphology of mouthparts and spines on legs and lower part of head indicate a strong predatory behaviour in adults. Adults in the genera *Iteaphila* Zetterstedt, 1838 and *Anthepiscopus* Becker, 1891, however, are evidently flower visitors based on both observations and mouthpart morphology. Adult males and females of species in the genus *Hormopeza* Zetterstedt, 1838 are attracted to smoke. Whether this is a que to aggregate into mating swarms, for oviposition or for feeding is however unknown (Sinclair & Cumming 2006). Immature biology is unknown.

Different life histories, distinct prospects and similar challenges

Empidoidea is not a homogenous group. As far as we know morphology, life histories and habitat vary greatly between families, subfamilies, tribes and genera. Some species may as adults depend on several different patches for feeding, hunting, breeding and depositing eggs. While as larvae yet other habitats are required. Following the past classification within Empidoidea, the research on the biology is scattered and gapped. A lack of compiled and thorough synthesis of life histories may be the cause in many cases, as identification and correct taxonomic treatment is far from trivial.

The potential of using species in the Empidoidea as bioindicators, i.e. organisms for analysing and assessing ecological parameters such as habitat type and complexity, was briefly discussed by Pollet (2009). The family Empididae was suggested due to pronounced requirements of mosaic habitats, in comparison to e.g. Dolichopodidae.
However, arguments against Diptera as biological indicators include the difficulty in identification, specifically to species level.

The large number of species in some families with the general lack of available and updated identification keys is problematic (Orford et al. 2015). There are tools that may ease this challenge, a study on the use of DNA sequences for identification increased the identification of taxa in invertebrates compared to manual identification by non-specialists (Sweeney et al. 2011). This does not however solve the problem as only just above 1000 species are available with DNA sequences in the Barcode of Life Data Systems (BOLD) database (Ratnasingham & Hebert 2007), the most utilized database for species determination using DNA data. With 90% percent of the species missing the task is upon taxonomists to provide if not DNA sequences but at least available classifications and literature to aid in correct taxonomic assignments.

Aims and structure of this thesis
The objectives of this thesis are to first infer the phylogenetic tree of the superfamily Empidoidea based on molecular methods, and thereafter further examine subgroups and finally present new records and data regarding taxa in Sweden. In paper I the molecular phylogenetic backbone is analysed, and the classification revised in part, and in paper II additional molecular as well as biogeographical analysis is carried out of the genus Wiedemania Zetterstedt, 1838 (Empididae: Clinocerinae). In paper III the results from paper I are followed up with a morphological revision of the family Ragadidae. Finally, based on the material examined during the preparations of papers I – III, taxonomical data and distributional records are presented in papers IV – V.
Material and methods

The SMTP and additional sampling
The majority of the material used in this thesis was collected in the Swedish Malaise Trap Project (SMTP, Karlsson et al. 2005) (Fig. 4). The collection during SMTP ranged from 2003 to 2006, with 75 traps placed throughout Sweden. Additional material was provided by Catharina & Mathias Jaschhof (Station Linné, Öland), as well as Julia Stigenberg and Kjell Arne Johanson (Swedish Museum of Natural History), and a few aquatic species were provided by Dr Marija Ivković (University of Zagreb, Croatia).

Additional material was borrowed from MTD (Museum für Tierkunde, Dresden, Germany), NHMW (Naturhistorisches Museum Wien, Vienna, Austria), MZLU (Lund University, Lund, Sweden), NZAC (New Zealand Arthropod Collection, Landcare Research, Auckland, New Zealand) and USNM (National Museum of Natural History, Smithsonian Institution, Washington DC., USA). Field sampling was carried out at several occasions in Tottsjömo (Västergötland, Sweden) and Torpesta kvarn (Södermanland, Sweden) (Fig. 4).

Photography of specimens required different equipment depending on their size and preparation type. For complete specimens, dried or in alcohol, a Nikon DS-Ri2 mounted on a motorized Nikon SMZ microscope was used, and automatic focus stacking carried out directly in Nikon NIS-Elements 5.10 connected to the camera. For dissected objects a Nikon DS-Fi1 camera on a Nikon Eclipse 80i microscope or a Nikon D7100 mounted on a Leitz Orthoplan large field microscope was used with manual focus stacking, and thereafter automatically aligned and stacked in Helicon Focus 6 (Helicon Soft Ltd., Dominica). All photos were edited and finalized in Adobe Photoshop CC.
All material, including vouchers and DNA extracts, is stored at the Swedish Museum of Natural History. A total of 3,000 specimens were examined, representing almost 200 species.

Figure 4. Map of Sweden with administrative borders. Blue circles represent sampled locations within the Swedish Malaise Trap Project and red squares represent additional collecting sites, Tottsjömo (A) and Torpesta kvarn (B).

Molecular data
Several molecular markers were evaluated during the work on paper I. Two mitochondrial gene fragments, mitochondrial cytochrome β (Cytβ) and cytochrome oxidase c subunit I (COI), were found to separate taxa on species level well. However, to resolve nodes separating families, subfamilies, tribes
and genera the nuclear markers nuclear carbomoylphosphate synthase domain of rudimentary (CAD), elongation factor-1α (EF-1α) and isocitrate dehydrogenase (IDH) were added in the matrices. Published primer sequences were in most cases sufficient, however for Cytβ exchanging a few nucleotides to inosine were needed to amplify the fragment in some taxa. A new primer pair for the fragment of EF-1α was also designed. Established protocols for Sanger sequencing were followed in both paper I and paper II. The COI gene fragment was also used for producing species barcodes, and the same protocols were used in paper V for species that were not already sequences earlier in the project.

Morphological data

Due to the diverse morphology both in size and morphology among families and genera different approaches were taken for studying characters. For non-type specimens stored in alcohol maceration of genitalia was carried out during DNA extraction, as the abdomen of large species or whole body of small species was used and incubated overnight in proteinase K. Dry specimens were softened in a humid chamber, and thereafter dissected. Dissected genitalia and other parts were placed in either Euparal or glycerol and photographed, and thereafter either transferred back to alcohol or for dry specimens stored in micro vial in glycerol attached to the pinned specimen. Type specimens that were unsuitable for dissection were studied undissected or by examining previously dissected parts attached to the pin of the specimen.

A collection of morphological characters was aggregated from previous publications (Chvála 1983; Sinclair 1999, 2016; Sinclair & Cumming 2006) and combined with a number of new characters for diagnosis and analysis. Terminology of morphological characters follow Cumming et al. (1995) for male terminalia, McAlpine (1981) for general adult and wing morphology, Stuckenberg (1999) for antennae. Cumming & Wood (2017) published a newer interpretation of the second anterior branch of the cubitus in the wing, and this interpretation has been followed herein.
Phylogenetic analysis

Both paper I and paper II deals with molecular data as basis for the phylogenetic analysis, using Bayesian inference. The methods differ regarding the partitioning and tree inference steps. In paper I the partition scheme is based on an entropy-based index of substitution saturation of codon 1+2 and 3 (Xia et al. 2003; Xia & Lemey 2009). In paper II PartitionFinder 2.1.1 (Lanfear et al. 2016) is used with Akaike information criterion (AICc). The results differ slightly between the methods, but in both cases the third codon in all genes is suggested to be treated separately.

Rather than choosing a substitution model a priori, in both papers the model selection is handled more dynamically. In paper I the inference is carried out in MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) and set to integrate over all available models using the mixed setting (Huelsenbeck et al. 2004). In paper II on the other hand the inference is carried out in BEAST 2.4.6 (Bouckaert et al. 2014) and by using bModelTest (Bouckaert & Drummond 2017) to infer the model during the MCMC analysis. The approaches are similar in both cases, and the advantages over selecting model in a separate step before inference are that a separate simple tree that might not be correct during model selection is avoided and that the uncertainties of the model are co-estimated during the Bayesian phylogenetic analysis. In paper II the inferred tree is also time calibrated using data from previously published fossil records and a log-normal clock model.

A different approach is taken in paper III, where morphology is applied to produce a cladogram. A matrix of 28 of morphological characters were used in a maximum parsimony analysis carried out in PAUP* 4.0a164 (Swofford 2003). A heuristic search option with random stepwise addition and 1000 replicates and TBR branch swapping with MulTrees enabled was selected together with 1000 bootstrap replications. Thereafter MacClade 4 (Maddison & Maddison 2001) was used to map the characters onto the resulting strict concensus tree.
Summaries of the papers

Paper I
Up until now all previous classifications of the superfamily Empidoidea have been based on morphological data. Many relationships among several groups have been unknown or tentative, and several taxa have been unplaced leaving many evolutionary questions unanswered. The many characters providing the backbone to current classification are often difficult to examine (e.g. internal and genital structures) making placement of newly discovered taxa difficult. Only a very few molecular studies have been performed earlier. Tentative results have been interesting with higher resolution than morphology-based studies. Collins & Wiegmann (2002) used two genetic markers (28S ribosomal DNA and elongation factor-1α) and corroborated, but with low support, Chvála’s (1983) hypothesis. Moulton & Wiegmann (2004) used one genetic marker (carbamoylphosphate synthase domain of rudimentary) and found high support for many higher groups. Later Moulton & Wiegmann (2007) used one additional marker (28S ribosomal DNA) and found support for major groups as well, and as in Collins & Wiegmann (2002) it was similar to the hypothesis by Chvála (1983). However, all these studied lacked taxon representation from many subfamilies and tribes and also lacked sufficient resolution to provide an updated classification.

In this paper we use two mitochondrial, cytochrome oxidase c subunit I (COI) and cytochrome β (Cytβ), and three nuclear genetic markers, carbamoylphosphate synthase domain of rudimentary (CAD), elongation factor-1α (EF-1α) and isocitrate dehydrogenase (IDH), to reconstruct the phylogenetic tree of the Empidoidea utilizing Bayesian inference. In this study we choose to focus on all families except Dolichopodidae. Our results agree with the classifications of several groups proposed by Sinclair & Cumming (2006). But there are also several interesting discrepancies. Firstly, the family
Atelestidae is found to be sister group to all remaining Empidoidea, a result most like other molecular analyses but different from those based on morphology. The second pattern previously seen in molecular studies is the position of the genus *Bicellaria* Macquart, 1823 at the root in the family Hybotidae. The support for Brachystomatidae as a separate family outside Empididae is not supported. Another more surprising find is that the subfamilies Empidinae and Hemerodromiinae Schiner, 1862 (Empididae) are recovered as non-monophyletic. These have since long been regarded as sister groups. Another notable result is the sister relationship between the subfamily Ragadinae Sinclair, 2016 together with a species group called the *Iteaphila*-group by Sinclair & Cumming (2006) and the family Empididae. Based on the genetic distances, i.e. the branch lengths, we raised Ragadinae to family status and include the *Iteaphila*-group as a new subfamily, *Iteaphilinae* **subfam. n.** A revised classification with diagnoses for all higher groups within Empidoidea, except for Dolichopodidae, are provided following the results from the molecular inference (Fig. 5).

Figure 5. New classification of taxa in Empidoidea; numbers at nodes represent support as posterior probabilities in percentage based on the Bayesian inference.
Paper II

In paper II we focus on a group within Empididae, the genus Wiedemannia in the subfamily Clinocerinae. Species in Wiedemannia are often found tightly associated to lotic environments. Species in Clinocerinae mostly prefer water with temperature constant and below 20°C, and higher species richness is found in alpine environments (Wagner & Gathmann 1996; Ivković et al. 2013). Within Clinocerinae this is the most species rich genus, with 121 described species from the Palaeartic, Nearctic, Afrotropical and Oriental regions. As in other groups in the superfamily males are readily determinable to species using genital characters, females however are difficult to distinguish further (Sinclair 1995).

The subgenera Chamaedipsia Mik, 1881, Eucelidia Mik, 1881, Philolutra Mik, 1881, Pseudowiedemannia Engel, 1918, Roederella Engel, 1918 and Wiedemannia (sensu stricto) has generally been recognized previously (Chvála & Wagner 1989; Sinclair 1995). However, recently the subgeneric concepts have been questioned as many described species does not fit either subgenus (Sinclair 1995).

In this paper we use two mitochondrial (cytochrome oxidase c subunit I and cytochrome β) and two nuclear (carbomoylphosphate synthase domain of rudimentary and elongation factor-1α) gene fragments to examine the internal relationships within Wiedemannia as well as test the monophyly of the genus.

As in paper I we perform a Bayesian inference, however this time we also conduct a molecular dating of the tree using fossil taxa. Furthermore, we analyse the current global distribution using a Species Distribution Model (SDM), which also provides mapping of regions with suitable habitat based on current climatic data.

What we find is that the subgeneric concepts indeed does not hold for testing, with most of the subgenera being non-monophyletic. We can also infer several speciation events that might coincide with geological events, such as changes in sea level and climate events. The first diversification is seen in Eocene (ca 50 million years ago), with further diversification seen at ca 30 million years ago.
Figure 6. Potential world distribution of the genus *Wiedemannia*, based on bioclimatic variables (Hijmans et al. 2005). Triangles (▴) indicate published records.
These events coincide with drops in global sea level. Periods of flooding and draught might have distinct effects on the dispersal and speciation of aquatic species groups, such as species in *Wiedemannia*. Lastly, we identify several potential hotspots based on the SDM that highlight regions interesting for further investigation (Fig. 6).

**Paper III**

The genera *Anthepiscopus*, *Hormopeza*, *Gloma* Meigen, 1822, *Iteaphila*, *Oreogeton* Schiner, 1860 and *Ragas* Walker, 1837 formed the subfamily Oreogetoninae as erected by Chvála (1976) and has been regarded as sister group to remaining subfamilies in Empididae. Later another arrangement including *Dipsomyia* Bezzi, 1909, *Hormopeza*, *Hydropeza* Sinclair, 1999, *Ragas* and *Zanclotus* Wilder, 1982 was proposed forming the ‘Ragas-group’ by Sinclair (1999). The monophyly of this later grouping was supported in Sinclair & Cumming (2006), but its placement within the superfamily still unresolved. The ‘Ragas-group’ was later erected to subfamily within Empididae by Sinclair (2016). The genera *Iteaphila* and *Anthepiscopus* has been treated as unplaced within Empidoidea.

In **paper I** we found that *Ragas* together with *Iteaphila* and *Anthepiscopus* form a separate clade. We raised the Ragadinae to family level and included *Iteaphila* and *Anthepiscopus* as a new subfamily, Iteaphilinae, within it. However, the internal relationships within this group were still unresolved, because of the lack of molecular data for representatives of the genera. Following up on this I continued the investigation, applying morphological methods. The aim of **paper III** was to analyse the internal relationships, update the diagnostic characters and produce a determination key applicable to both male and female specimens. A parsimony analysis was performed in PAUP* 4.0a164 (Swofford 2003), including 28 morphological characters. The characters were also mapped to the cladogram using MacClade 4 (Maddison & Maddison 2001).

The strict consensus tree showed a monophyletic Ragadidae, with *Anthepiscopus*, *Iteaphila* and *Hormopeza* together form a distinct clade.
separate from remaining genera. The characters supporting this clade are characters in the male genitalia. *Iteaphila* and *Anthepiscopus* are flower feeding, lacking the specialized mouthparts, epipharyngal blades, used for cutting prey present in other Ragadidae. They also have a straight labrum, in contrast to recurved in other Ragadidae. *Hormopeza* (Fig. 7), however, possess the specialized mouth parts and the recurved labrum in the female only. All three genera lack stout spine like setae, present in the other genera. Females of *Hormopeza* are predacious but feeding habits in males are unknown. Relationships between remaining genera were however not possible to resolve.

The paper concludes with a transfer of *Hormopeza* to Iteaphilinae, an updated determination key and current diagnoses as well as detailed photographs of characters.

Figure 7. *Hormopeza obliterata* Zetterstedt, 1838, ♂, holotype, habitus, lateral view (MZLU 5863:1). Photo: E. Wahlberg.
Paper IV

During the preparations of paper I, we found an interesting taxonomical issue regarding a species in the family Hybotidae. *Chvalaea sopiana*e Papp & Földvári, 2002 was described from a single female specimen. The previously single known species of this genus is *C. rugosiventris* (Strobl, 1910), also described from a single female. However, this original description was erroneous regarding the sex. It is actually male. The male genitalia including the phallus is normally hidden, with the phallus folded inwards (Fig. 8). The basal part of the phallus extending posteriorly was previously interpreted as an ovipositor. We discovered that the female *C. sopiana* is differentiated from *C. rugosiventris* only by sexually dimorphic characters. The description of *C. sopiana* by Papp & Földvári (2002) was the first description of the female *C. rugosiventris*. Therefore we choose to synonymize the *C. sopiana* with *C. rugosiventris* and carried out a formal redescription of the species.

In addition to this we report new locality data of the species *Allanthalia pallida* (Zetterstedt, 1838) and *Leptodromiella crassisetum* (Tuomikoski, 1932), from Russia and Sweden.

Figure 8. *Chvalaea rugosiventris* (Strobl, 1910), ♂, terminalia, dorsal view. Photo: E. Wahlberg.
Paper V

The large amount of material processed during the work with this thesis has resulted in new data on many species of the Swedish empidoid fauna. In paper V we presented new species for Sweden but also for many Swedish regions, focusing on Empididae, Hybotidae and Ragadidae. Three species, Rhamphomyia erythrophthalma Meigen, 1830, R. lamellata Collin, 1926 and Platypalpus pygialis Chvala, 1973 are new to Sweden. Two of the species, R. erythrophthalma and P. pygialis are previously known in Europe but not north of Denmark. The third species, R. lamellata, was recently recorded from Norway. These three represent species that in a relatively modern time has migrated northwards. The fauna of southern Sweden was studied thoroughly by Milan Chvála in the 1970’s and 1980’s, and the range shift is very possible to be recent. The 61 new regional records within Sweden are in some cases expected, such as closing gaps between northern and southern records. However, some represent southern records of northern species and vice versa. Finally, we also present sequences of the COI, cytochrome oxidase c subunit I, barcoding gene. A total of 106 COI sequences from both male and female specimens representing 71 unique species (Fig. 9). This is a much needed addition of the Swedish fauna to The Barcode of Life database (BOLD, Ratnasingham & Hebert 2007). This resulted in an increase of the number of species records in BOLD for Empididae with 71% and for Hybotidae 13%.
Figure 9. Number of barcoding sequences uploaded from this project to BOLD per family and genus. The inclusion of *Ragas* and *Iteaphila* within Empididae in the graph is due to databases that are not updated to current classification.
Discussion and conclusions

During the first part of this thesis I demonstrated that molecular data is a great source of information in exploring and testing phylogenetic hypotheses, even where studies in morphological characters has seemingly come to a dead end. The results from the phylogenetic analysis of the superfamily are in large parts in agreement with previous classifications, but with additional information about groups that previously have been regarded as unplaced. Even though many groups are supported by the analysis herein as well other studies of not only molecular data but also morphology, some groups will need to be further investigated. One such group is the subfamily Empidinae within Empididae. The non-monophyletic Empidinae in regard to Hemerodromiinae is surprising. This is not yet supported robustly by morphological characters. The complex situation of the genera *Empis* and *Rhamphomyia* has been raised before, e.g. by Watts *et al.* (2015) where several genera including the two mentioned above were found to be either polyphyletic or paraphyletic. This should indicate that we need to revise and reclassify these genera. However, they hold a large number of subgenera and species, the latter almost 2,000, which leaves a reclassification to become a rather comprehensive task.

The revision of the *Wiedemannia* presented here is an equal approach to similar problems. While the complications with the subgeneric concepts had been raised previously, no phylogenetic analysis had previously been performed for this group. The molecular data herein did not support the previously accepted subgeneric division. Adding to that the diversification can be put into a geological and temporal context by using fossil dating of the tree. This DNA data provides information on major splits as well as a background to how regions of diversity hotspots may arise through for example varying sea levels and orogenic processes. The usage of current climatic data for present distribution can be of use for identifying potential hotspots of diversity. In our study the Species Distribution Model recovered potential
areas with habitats suitable for *Wiedemannia*. Some of them are geographically plausible, adjacent or close to known records. Other are far from the known distribution and colonisation of those might be less plausible. However, such regions might still be of interest for studying species with similar requirements but with another biogeographical history.

Molecular data aside, morphological data has much to tell. Especially when regarded within smaller and more distinct groups. Because of the rarity of some taxa within Ragadidae, and the age and condition of the previously collected material, DNA-based analysis was not suitable within this project. I was interested in the general morphology of species in this group and a morphological approach to this family was therefore appropriate. The genera in the subfamily Ragadinae have several features linking them together, most notably the synapomorphy stout and spine-like setae on fore coxa. The genera *Ragas* and *Zanclotus* also have spine-like setae on the postgena, a synapomorphy for these two taxa also pointed out by Sinclair (1999). The spine-like setae in combination with the epipharyngal blades are plausible to be adaptation to a predatory lifestyle. The Iteaphilinae, including *Hormopeza*, lack these spine-like setae. However, species in *Hormopeza* possess the specialized mouth parts at least in females. Hence a loss of predatory lifestyle is not synapomorphy for this group. A still open question within this group is the relationship between *Anthepiscopus* and *Iteaphila*. The monophyly of *Iteaphila* has been questioned both by Sinclair & Shamshev (2018) and in the phylogenetic results in paper I in this thesis. Even though it’s a relatively small group further studies incorporating DNA data and broader taxon sampling will undoubtedly yield more interesting information. Steps taken here with a suggested classification and updated determination key, will hopefully lead to this group being more accessible for further studies.

During the work with the three first papers of this thesis I handled a large amount of material, from the collections at the Swedish Museum of Natural History, external museum collections, as well as private collections. It is inevitable that interesting taxonomical and biogeographical subjects are revealed.
As in the case of the misidentified specimens of *Chvalaea* in paper IV. Furthermore, the number of new records in paper V indicate that there is still much to discover and record of the Swedish fauna.

Without an international network of expertise and the collaboration with colleagues much of this work would have taken a much longer time, if at all possible, to carry out. A common source for discussion and sometimes heated arguments is the discussion of morphology versus molecules. At the extreme ends one might claim that the morphological characters are the only valid for phylogenetic inference, while at the other end the claim might be that these characters are insufficient. Less extreme arguments surround the cost effectiveness of DNA extraction and sequencing compared to many years of taxonomic training. In practice the different branches of modern systematics and taxonomy are however more intertwined, with less noticeable boundaries. In this thesis I often combine the two sources of methodology to strengthen the hypotheses and conclusions, based on as much unlinked data as possible.

On this subject it is also relevant to discuss the alleged death of taxonomy. Almost every year someone has expressed this concern, e.g. McClain (2011), Drew (2011), Higgs (2016) and most recently Saunders (2019). The points in these discussion pieces are that taxonomy is in catastrophic decline, millions of dollars globally pushed in projects with measurable results, and research in species and classifications is not “sexy” enough. Some point out that taxonomy has a communication problem. The unawareness of taxonomy in decision making is problematic. Suggestions on what is replacing taxonomy includes molecular methods rendering manual identification superfluous, identification without even collecting (e.g. through DNA fragments in soil and water; environmental DNA) and automated identification and description based on machine learning algorithms.

The “new” taxonomist is a person with access to the latest laboratory equipment, has cutting edge knowledge in programming and bioinformatics and is at least part of an integrated team where everyone is a specialist. And they produce results that have a value that is directly measurable in physical resources. This is often far from what the traditional taxonomist is
accustomed to. During my work on this thesis this discussion has been evident in the community, but I have also concluded that I disagree with the given dire future of a profession. In a pilot study on using DNA fragment deposited by organisms in the environment for species identification, environmental DNA (eDNA), I set out to test the matureness of this method and how I as a taxonomist may still be relevant. During the study I sampled soil, sediment and water at a freshwater stream, with the aim to identify the dipteran fauna (Wahlberg 2019). I was able to identify 58 flies to species level. However, a straight list of taxa did not give me all the answers one could seek during an inventory of a specific habitat. Only by researching the taxonomic literature with species descriptions including habitat and geographic data the taxon composition makes sense. For example, within Diptera species in one group might occupy a mosaic of habitats, as exemplified in the introduction of this thesis. Hence a species name itself does not tell the complete story of the species. Furthermore, I was unable to identify 128 taxa.

The most commonly used database for molecular reference sequences, used in identification of taxa using DNA barcodes, is the Barcode of Life Data Systems (BOLD Systems). Within BOLD only 700 of the 8,000 known species of Diptera have barcode sequences. And despite the effort reported in paper V, with a substantial addition of barcode sequences of empidoid taxa, there are still over 200 species of Empididae and Hybotidae known from Sweden that are not barcoded. That is over half of the known fauna. Modern molecular methods are hampered by the lack of taxonomical representation in reference databases. There is an apparent urgency in giving priority to closing this gap, an endeavour that requires both taxonomical and morphological expertise, as well as knowledge of molecular methods. This also includes providing resolved phylogenetic data, more stable classifications and accessible determination keys. With these tools the array of biological methods can be combined and extend our knowledge of evolution and biodiversity.
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When I decided to pursue and begin my PhD education I was prepared for the immense workload, not only of the scientific research but also teaching, administration, mental and physical maintenance and effects on social life. Or, so I thought. In reality, it has been a challenging and sometimes frustrating experience. But at the same time amazingly awarding, fun and exciting. And not at least educating. There have been many people involved during the years, in various way. First and foremost, I would like to thank all the wonderful people at the zoology department at the Swedish Museum of Natural History. While I might have been quite alone as a PhD student there for most of the time, I have not been lonely.

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Svensk sammanfattning

Överfamiljen Empidoidea i ordningen Diptera, tvåvingar, består av över 10,000 arter spridda över hela världen. Denna stora artrikedom och geografiska utbredning har lockat flertalet forskare i taxonomi och systematik. Klassificering av artgrupper och den evolutionära historien inom överfamiljen har dock varit allt annat än okomplicerat. Fylogenetiska undersökningar baserade på morfologi har lämnat många frågor obesvarade, delvis på grund av modifieringar och anpassningar som har varit svåra att tolka.


I artikel II undersöks släktet Wiedemannia (Empididae: Clinocerinae), även här med hjälp av genetiska data men även i ett biogeografiskt och tidsmässigt sammanhang. Undersläktena visar sig inte vara monofyletiska, och vi rekommenderar därför att dessa förkastas. Den daterade fylogenin, baserad på genetiska data och kända fossil, tyder på en differentiering av artgrupper för ca 50 miljoner år sedan följt av ytterligare differentieringar i intervaller som möjligtvis kan kopplas till varierande global havsnivå. Vi presenterar också en karta baserad på modellering av artutbredning med hänsyn till klimatologiska varibler, med exempel på potentiella ”hot spots”.

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Baserat på resultaten i första artikeln undersöks relationerna inom familjen 
Ragadidae i artikel III. Avsaknaden av genetiska data i kombination med 
visa arters sällsynthet gör en morfologisk undersökning mest lämplig i 
kombination med släktskapsanalys. Artena i släktena *Iteaphila* och 
*Antheepiscopus* lever främst av pollen och nektar, och bildar tillsammans med 
släktet *Hormopeza* en egen naturlig grupp. I artikeln revideras relationerna 
mellan släktena, diagnoserna med morfologiska egenskaper uppdateras och en 
bestämningsnyckel till släktena i Ragadidae presenteras.

De två arterna *Chvalaea sopiana* and *C. rugosiventris* i familjen 
Hybotidae undersöks i artikel IV. Båda arterna beskrevs från början från 
enstaka exemplar av honor. Könet på *C. rugosiventris* misstogs dock i 
originalbeskrivningen, och de morfologiska egenskaper som särskiljer de två 
arterna är egentligen skillnader mellan könen. De två arterna synonymiseras 
on vi ombeskriver hanen av *C. rugosiventris*. Vi bidrar även med nya 
geografiska observationer av arterna *Allanthalia pallida* och *Leptodromiella 
crassiseta* tillhörande samma familj.

I den sista artikeln, artikel V, presenterar vi observationer av nya arter för 
Sverige och nya observationer inom Sverige för redan bofasta arter i 
familjerna Hybotidae, Empididae och Ragadidae. Vi kan också bidra med ett 
betydande tillskott av genetiska streckkoder till databasen The Barcode of Life 
Database. Detta ökar antalet representerade arter från Sverige med 71% för 
Empididae och 13% för Hybotidae.