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Molecular Phylogeny of the Oestroidea

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ABSTRACT

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The superfamily Oestroidea includes approximately 9% (≈14,000 spp.) of the order Diptera. Many members of the superfamily are of medical, veterinary, agricultural, and forensic importance. Although Oestroidea has been the subject of much scientific scrutiny, the exact patterns of phylogenetic relationships among the key groups of the superfamily are unresolved and controversial. For a better understanding of oestroid evolutionary hypotheses, phylogenies of Oestroidea were reconstructed at several taxonomic levels using DNA sequence data. The specific aims were to determine the phylogeny of the 1) genus *Chrysomya* (Chrysomyinae); 2) subfamily Chrysomyinae (Calliphoridae); 3) family Oestridae (Oestroidea); and 4) family Calliphoridae (Oestroidea). Sequence data from both mitochondrial and nuclear genes from 99 representative species were analyzed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian (BA) methods. Trees obtained from the different phylogenetic methods were almost identical. The status of family Calliphoridae (the blow flies) has for years been the central systematic problem of the superfamily. These results show that Calliphoridae is polyphyletic, with the phylogenetic position of Mesembrinellinae still uncertain but clearly outside the lineage that includes other Calliphoridae and some non-calliphorids, and Polleniinae is the sister group of the family Tachinidae. Strong support for a sister group relationship between Rhiniinae and traditional calliphorid subfamilies undermines a recent proposal to give Rhiniinae family status. The *Chrysomya* and Chrysomyinae phylogenies were well resolved and suitable for testing a number of existing evolutionary hypotheses. The Chrysomyinae colonization of the neotropics apparently involved a single ancestral species, and the traditional chrysomyinae tribal classification should be abandoned. Tuberculate larvae evolved twice within *Chrysomya*, and published hypotheses about the evolution of sex determination, eye morphology, and genome size within the genus appear to be incorrect. Efforts to resolve the relationships of the Oestroid families were largely inconclusive, although the monophyly of the superfamily was strongly supported.

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ABBREVIATIONS

BA	Bayesian method
bp	Base pair
BP	Bootstrap percentage
BS	Bremer support
CAD	Carbamoylphosphate synthetase, aspartate transcarbamylase, and dihydroorotase
COI	Cytochrome oxidase subunit one
COII	Cytochrome oxidase subunit two
CPS	Carbamoylphosphate Synthetase
Cyt. b	Cytochrome b
DDC	Dopa-decarboxylase
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EF-1α	Elongation factor one alpha
G	Gamma
GTR	General time reversible
I	Invariable site
LBA	Long branch attraction
ML	Maximum likelihood
MP	Maximum parsimony
MPT	Most parsimonious tree
mtDNA	Mitochondrial DNA
NaCl	Sodium chloride
NNI	Nearest neighbor interchange
PAUP	Phylogenetic analysis using parsimony
PCR	Polymerase chain reaction
PEPCK	Phosphoenolpyruvate carboxykinase
PP	Posterior probability
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
SDS	Sodium dodecyl sulphate
TBR	Tree-bisection-reconnection
TE	Tris-EDTA

CHAPTER 1: Introduction

1.1 Superfamily Oestroidea

Oestroidea is a superfamily of calyptrate flies, which are characterized mainly by a vertical row of bristles on the meron (McAlpine, 1989). More than 14,000 species of the Oestroidea are known from all zoogeographical regions (Kutty *et al.*, 2010). Rognes (1997) considered the families, Mystacinobiidae (New Zealand bat fly), Axiniidae (Axe flies), Tachinidae (Tachinid flies), Rhinophoridae (Woodlouse flies), Sarcophagidae (Flesh flies), Oestridae (Bot flies), and Calliphoridae (Blow flies), to be in the Oestroidea.

1.1.1. Family Mystacinobiidae (New Zealand bat fly)

Mystacinobiidae is a highly distinct family, as flies in this family are wingless, haltere-less, physogastric, communal and guano feeders (Holloway, 1976). This family is represented by a single species *Mystacinobia zelandica*, which is endemic to New Zealand and shows a phoretic relationship with the New Zealand short tailed bat*, Mystacina tuberculata*. A female lays eggs in two batches and then dies. *Mystacinobia* eggs are characterized by the presence of respiratory horns and chorionic denticles. Although respiratory horns on eggs are present in other dipteran families (such as Sepsidae and Drosophilidae), *Mystacinobia* eggs differ from them, in not being hydrophobic (Holloway, 1976). Finally, *Mystacinobia* is distinguished from other Diptera in that it pupates inside an empty bat-fly puparium (Holloway, 1976).

1.1.2. Family Axiniidae (Axe flies)

Axiniids are very small size (2.5-6.0 mm) flies of Australia and New Guinea (Colless, 1994). There are only four genera and 15 species. Most of the information we know about this family is from adult males. Although no immature specimen has been reported, limited information obtained from female genitalia, indicates that the larvae may be parasitic (Colless, 1994). More than 8 synapomorphic characters (appendix 1) support monophyly of the group (Colless, 1994). As some characters (such as 3 separate spermathecal ducts) of axiniids are similar to family Tachinidae, Crosskey

(1977) suggested axiniids be placed within Tachinidae, but as they lack characters typical of tachinids (such as a strong subscutellum, and bristled thoracic surfaces) Colless (1994) raised this group to the family level within Oestroidea. Phylogenetic analysis based on morphological data suggests axiniids are either the sister group of all other oestroids except *Mystacinobia* (Colless, 1994; Rognes, 1997) or a member of family Rhinophoridae (Pape & Arnaud, 2001). Intrafamiliar relationships are still not well known (Colless, 1994).

1.1.3. Family Tachinidae (Tachinid flies)

Tachinids are small to large flies (2-20 mm), often extensively bristled, which can be easily identified by the presence of a strongly developed subscutellum. Worldwide, 10,000 species of tachinids are known, which makes it the second largest family of Diptera after Tipulidae (Belshaw, 1993; Irwin *et al.*, 2003). The majority of tachinids are ovolarviparous (Wood, 1987c). All tachinid larvae are endoparasitic on arthropods (mainly insects), which make this family very important from a pest management point of view (Efil & Kara, 2004; O'Hara, 2008). Although the monophyly of the family is well supported based on the characters listed in appendix 2 (Wood, 1987c; Pape, 1992; Rognes, 1997), classification within the family has been revised several times (Townsend, 1934-1942; Crosskey, 1980; Herting, 1984). Most recently, O'Hara (2008) divided this family into four subfamilies: Phasiinae, Dexiinae, Exoristinae and Tachininae. Phasiinae and Dexiinae are less speciose subfamilies whereas Exoristinae and Tachininae are more speciose. The monophyly of Dexiinae is well supported, as they possess a characteristic hinge connection between basiphallus and distiphallus of the aedeagus, but monophyly of other tachinid subfamilies is still unclear (O'Hara, 2008).

1.1.4. Family Rhinophoridae (Woodlouse flies)

Rhinophoridae is a comparatively small family, with only 150 described species, with maximum diversity in the Mediterranean region and in Africa (Herting, 1961; Crosskey, 1977; Kutty *et al.,* 2010). They are oviparous and the larvae are endoparasites of woodlice (Crustacea: Isopoda) (Thompson, 1934). They are the only Diptera that parasitize Crustacea (Crosskey, 1977). They appear morphologically

similar to tachinid flies but differ as they have a weakly developed postscutellum and narrow calypteres (Wood, 1987b). Monophyly of the Rhinophoridae is supported (excluding *Angioneura*, *Melanomya*, and *Morinia*) based on the synapomorphies listed in appendix 3, but its exact relationship with other members of the Oestroidea remains unclear (Crosskey, 1977; Pape, 1986).

1.1.5. Family Sarcophagidae (Flesh flies)

Sarcophagids are robust flies varying in size from 2.5 to 18 mm. Mostly they appear dull gray in color. All sarcophagids are either ovoviviparous (larvae emerges immediately after deposition of eggs that are in advanced stage of embryonic development) or larviparous (larvae hatches inside female body before deposition (Shewell, 1987; Meier *et al.*, 1999). Many sarcophagid larvae mature on carrion and hence play an important role as a forensic indicator species for the estimation of a postmortem interval (PMI) (Wells *et al.*, 2001; Pape *et al.*, 2007). Approximately 2600 species of sarcophagids are known from all over the world that belong to three subfamilies: Sarcophaginae, Miltogramminae and Paramacronychiinae (Pape *et al.*, 2007). Sarcophaginae includes mainly New World flesh flies whereas Miltogramminae includes mainly Old World flesh flies (Pape *et al.*, 2007). Paramacronychiinae is a very small subfamily, which is restricted to the Holarctic region (Pape *et al.*, 2007). Many characters (appendix 4) support monophyly of the Sarcophagidae (Pape, 1992). Even recent molecular analysis supported monophyly of the Sarcophagidae (Kutty *et al.*, 2010).

1.1.6. Family Oestridae (Bot flies)

Oestridae (bot flies) is very well studied family because of their importance in the area of medical and veterinary entomology (Papavero, 1977; Pape, 2001). About 150 species of Oestridae are widely distributed in Africa and central Asia and have a limited distribution in North America (Wood, 1987a; Kutty *et al.*, 2010). They are medium to large (9-25 mm), heavy bodied flies with reduced mouthparts and a pilose bristleless body. Adults do not feed, but rather survive on fat reserves (Wood, 1987a). Although the meron does bear a patch of setae, the characteristic row of bristles is lacking, as present in other families of the Oestroidea. All bot fly larvae are obligate parasites of

mammals, including humans, with a high level of host specificity (Wood, 1987a; Catts, 1982). Most species are oviparous with the exception of members of the subfamily Oestrinae, which are larviparous (Catts, 1964; Anderson, 1975). Larvae develop within the host, and upon reaching maturity they leave the host to pupate in the soil.

Oestridae consists of four subfamilies: Cuterebrinae, Gasterophilinae, Hypodermatinae and Oestrinae (Wood, 1987a). Although extensive morphological data (appendix 5) and the mitochondrial cytochrome oxidase subunit one gene support monophyly of the Oestridae (Pape, 1992; 2001; Otranto *et al.*, 2003), 16S and 18S ribosomal RNA genes suggest non-monophyly of the Oestridae (Nirmala *et al.*, 2001). Also, subfamiliy relationships differ when inferred from morphological (Pape, 2001) compared to molecular analysis (Otranto *et al.*, 2003).

Cuterebrinae is exclusively American (Guimarães, 1967), and its larvae are mainly subcutaneous parasites of rodents and lagomorphs. Third larval instars of Cuterebrinae are unique among all oestrids because they are entirely encrusted with small-flattened plate-like spines. Gasterophilinae are distributed worldwide with no species endemic to the Americas. However, some *Gasterophilus* spp. were introduced to the Americas along with horses. Gasterophilinae larvae are gut parasites of horses, zebras, rhinos and elephants. Sometimes they puncture the stomach wall or obstruct the stomach, which may lead to death. The Hypodermatinae has a worldwide distribution with the highest diversity seen in Central Asia (Zumpt, 1965). Three species belonging to the genus *Hypoderma* are known from North America, to which they were introduced along with cattle. Like Cuterebrinae, Hypodermatinae larvae are subcutaneous parasites, but they lack the small plate like spines on the integument. Hypodermatinae larvae enter the skin through enzymatic dissolution of the tissue, supposedly caused by salivary secretions (Wolfe, 1959). Members of the Oestrinae are distributed worldwide but most species are in Asia and Africa. Their larvae parasitize the host respiratory passages.

1.1.7. Family Calliphoridae (Blow flies)

The Calliphoridae include a diverse group of flies varying in size from 2.5 mm to 19 mm and also varying in color (e.g. metallic blue, green or purple to non-metallic

black, gray, brown or yellow) (Rognes, 1998). Approximately 1450 species of calliphorid flies (including 400 species of Rhiniinae) are known from all continents except Antarctica (Verves, 2005; Kutty *et al.*, 2010). Adult blow flies are known to frequent flowers, feces, carrion and wounds for nutrition. They also serve as vectors of disease, where they transport bacteria, viruses, protozoans and helminths (Greenberg, 1971). Although most blow flies are oviparous, some are either multilarviparous (e.g. *Onesia, Bellardia, Eggisops,* etc.) or unilarviparous (Helicoboscinae, Mesembrinellinae, Ameniinae and Phumosiinae). Since the majority of blow fly larvae are carrion breeders, they play an important role both as an indicator species in forensic entomology and as prominent decomposers (Schumann, 1965). Some larvae are endoparasitic (*Pollenia*, *Bellardia*, etc.) whereas a few show blood-sucking habits (*e.g. Auchmeromyia, Protocalliphora* etc.) (Rognes, 1998). Historically, the family Calliphoridae has been divided into different numbers of subfamilies by different authors (Lehrer, 1970; Hennig, 1973; Pont, 1980; Kurahashi, 1989), but Ameniinae, Auchmeromyiinae, Bengaliinae, Phumosiinae, Mesembrinellinae, Helicoboscinae, Toxotarsinae, Calliphorinae, Luciliinae, Melanomyinae, Polleniinae, Rhiniinae, Chrysomyinae, Aphyssurinae, and Prosthetosomatinae have been recognized by most recent authors (Pape, 1992; Norris, 1999; Rognes, 1991; 1997; Kutty *et al.*, 2010). Monophyly of Calliphoridae is uncertain. Some believe it is monophyletic (Lehrer, 1970; Pape, 1992; Stevens, 2003) while others believe in its non-monophyly (Rognes, 1997; Kutty *et al.*, 2010). Morphological data supports the monophyly of each subfamily (See appendix 6), but the placement of some subfamilies is unclear (Rognes, 1991; 1997). The sister group relationships Chrysomyinae + Toxotarsinae and Auchmeromyiinae + Bengaliinae are well supported, whereas the positions of Rhiniinae, Bengaliinae, Mesembrinellinae, Helicoboscinae, and Polleniinae are uncertain (Pape, 1992; Rognes, 1997).

1.1.7.1 Subfamily Ameniinae

Members of subfamily Ameniinae are medium to large (5 mm to 20 mm) sized metallic green flies that are distributed in Oriental and Australasian regions (Crosskey, 1965). Appendix 6.1 lists the characters that support monophyly of the group. Members of this subfamily have characteristic white spots on the thorax and abdomen. Because of its large size, metallic coloration, comparatively developed subscutellum and strong

facial carina, this group superficially resembles tribe Rutiliini of family Tachinidae, but other characters, such as presence of a tuft of long black hairs on the anterior lappet of metathoracic spiracle, *in-utero* larval development (Macrolarviparity) and larvae parasitic on living mollusks excludes any possibility of its inclusion in the Tachinidae (Crosskey, 1965). Molluscan parasitism among oestroid flies is present in both Calliphoridae and Sarcophagidae, but because of resemblance of male genitalia and morphology (large metallic in color) with calliphorid flies, it was included in the Calliphoridae (Crosskey, 1965).

1.1.7.2 Subfamily Auchmeromyiinae

Auchmeromyiinae are yellow to brown Afrotropical and Oriental flies that are either obligate parasites of mammals or associated with ants and termites (Zumpt, 1965). Pape (1992) put members of subfamily Auchmeromyiinae within the subfamily Bengaliinae because of the shared character of elongated anal veins. However, Rognes (1991) created the new subfamily Auchmeromyiinae, because the aedeagus is very distinct from the members of the subfamily Bengaliinae. Appendix 6.2 lists the characters that support monophyly of the group.

1.1.7.3 Subfamily Bengaliinae

Bengaliinae are also yellow to brown Afrotropical and Oriental flies, with large mouthparts (Senior-White *et al.*, 1940). Most bengaline larvae are primarily predators of ants, termites and wasp larvae and pupae (Pont, 1980; Kurahashi, 1989). Some members (such as *Verticia fasciventris*) are parasitoids of termites (Tsang *et al.*, 2008). Appendix 6.3 lists the characters that support monophyly of the group.

1.1.7.4 Subfamily Phumosiinae

Phumosiinae are metallic green to blue Afrotropical, Oriental and Australian flies that are distinguished by the characters listed in appendix 6.4. Rognes (1997) included a single genus *Phumosia* in this subfamily, but it has been suggested that the genus *Euphumosia,* which is non-metallic, be included within the Phumosiinae (Kurahashi, 1987; Rognes, 1991). The breeding habit of *Euphumosia* is still unknown (Ferrar, 1978).

1.1.7.5 Subfamily Mesembrinellinae

Mesembrinellinae are exclusively Neotropical flies, which can be characterized by the features listed in appendix 6.5. Guimarães (1977) erected the family Mesembrinellidae for this group based on the presence of a very large reniform metathoracic spiracle with single operculum, but most other authors consider this group as a subfamily of the Calliphoridae based on similarity of male genitalia and other morphological characters (Shannon, 1923; Hall, 1948; James, 1970; Hennig, 1973; Rognes, 1991; Pape, 1992).

1.1.7.6 Subfamily Helicoboscinae

Helicoboscinae are Western Palaearctic and Western Himalayan flies with only two genera and four species (Rognes, 1993). Characters listed in appendix 6.6 support it as a monophyletic group (Rognes, 1991; Rognes, 1993). Verves (1986) and Shewell (1991) listed *Eurychaeta* in Sarcophagidae, but Rognes (1986) erected the subfamily Helicoboscinae within Calliphoridae for *Eurychaeta*. Based on morphological data, Pape (1992) supported its position within Calliphoridae.

1.1.7.7 Subfamily Toxotarsinae

Toxotarsinae is a Neotropical group of flies characterized by the autapomorphies listed in appendix 6.7. Some members are sarcophagid-like, but they differ from them in many aspects, such as presence of a telescopic ovipositor and large paralobes in the aedeagus (Dear, 1979). External characters that support its exclusion from Sarcophagidae include setulose stem vein and presence of two notopleural setae (Dear, 1979).

1.1.7.8 Subfamily Calliphorinae

Calliphorinae is a comparatively large subfamily, which is worldwide in distribution (Rognes, 1991). Rognes (1991) suggested synapomorphies (see appendix 6.8) for the monophyly of the Calliphorinae in a restricted sense. The suggested autapomorphy of setae on the lower calypter is also present in a few other blow fly genera (e.g. *Pachychoeromyia*, *Chrysomya* etc.) (Dear, 1985; Rognes, 1991). *Tricycleopsis* spp. also have a few setae on the lower calypter, and may be members of

Calliphorinae, but some authors consider it a member of Melanomyinae (Kurahashi, 1970; Rognes, 1991). Most members of subfamily Calliphorinae are oviparous, but there are some larviparous species belonging to genera *Bellardia, Onesia, Polleniopsis* and *Tainanina* (Onesia group of Kurahashi, 1972) and some subgenera of *Calliphora* (Kurahashi, 1989).

1.1.7.9 Subfamily Luciliinae

Subfamily Luciliinae, also known as the greenbottle flies, have worldwide distribution (Rognes, 1991). Characters that support monophyly of this group are listed in appendix 6.9 (Rognes, 1991; 1997). It includes the following genera: *Dyscritomyia*, *Hypopygiopsis*, *Hemipyrellia* and *Lucilia*. All members are oviparous (Rognes, 1991).

1.1.7.10 Subfamily Melanomyinae

Melanomyinae are Palaearctic, Nearctic and Oriental flies. Diagnostic characters are listed in appendix 6.10 (Kurahashi, 1970; Shewell, 1987; Rognes, 1991). The biology of the members of this subfamily is not well known, but limited information indicates that larvae of these flies are parasitic on snails (Rognes, 1991). Rognes (1991) tentatively included *Angioneura*, *Eggisops*, *Glutoxys*, *Melanomya*, *Melinda*, *Opsodexia*, *Paradichosia*, *Pseudopsodexia* and *Tricycleopsis* under this subfamily; however *Melinda*, *Paradichosia*, *Eggisops*, and *Angioneura* are traditionally considered as a part of Calliphorinae (Kurahashi, 1970; Schumann, 1986; Shewell, 1987).

1.1.7.11 Subfamily Polleniinae

Polleniinae contains Holarctic, Oriental and Australian non-metallic flies that can be characterized by comparatively strong facial carina, unarmed acrophallus of aedeagus and many other characters as listed in appendix 6.11(Rognes, 1997). Polleniinae includes: *Dexopollenia, Melanodexia, Morinia, Nesodexia, Pollenia, Xanthotryxus* and *Wilhelmina*, but the inclusion of many genera remains tentative, as information about them is limited (Rognes, 1991). Placement of *Nesodexia* in Polleniinae is still not well supported as its aedeagus has an armed acrophallus (Rognes, 1991).

1.1.7.12 Subfamily Rhiniinae

Subfamily Rhiniinae is mostly Afrotropical, Oriental and Australian flies that can be characterized by the features listed in appendix 6.12. Most members are oviparous (Rognes, 1991; Peris, 1952) and larvae are predators of ants, termites, and wasps (Dear, 1977; Kurahashi, 1989).

1.1.7.13 Subfamily Chrysomyinae

The subfamily Chrysomyinae is distributed in all zoogeographical regions except Antarctic. Characters that support monophyly of the subfamily are listed in appendix 6.13 (Rognes, 1991). Most members of the subfamily are saprophagous but *Protocalliphora* and *Trypocalliphora* are bloodsucking parasites of nestling birds, and *Chrysomya bezziana* and *Cochliomyia hominivorax* feed on living mammals (Rognes, 1991; Dear, 1985). Most genera under the subfamily Chrysomyinae are probably monophyletic with well-defined autapomorphies (Dear, 1985; Whitworth, 2006). *Trypocalliphora*, *Phormia*, *Phormiata*, *Chloroprocta,* and *Chrysopyrellia* are monotypic genera whereas *Protocalliphora* with 28 species and *Chrysomya* with 36 species are the most diverse genera of the subfamily Chrysomyinae (Dear, 1985; Whitworth *et al.,* 2007; Boyes & Shewell, 1975; Wells & Kurahashi, 1996; Ullerich & Schottke, 2006).

1.1.7.14 Aphyssurinae

Aphyssurinae is newly added subfamily of the Calliphoridae, which is distributed only in Australia. These fly are very small in size (5 mm) and are larviparous. Life history is not well-known, but there is an indication that they may be associated with ants (Norris, 1999). Aphyssurinae is represented by single genus *Aphyssura* Hardy and 28 species (Norris, 1999).

1.1.7.15 Prosthetosomatinae

Prosthetosomatinae are known only from the larval stage and are distributed in Neotropical and Afro-tropical regions (Pont, 1980). These larvae infest nest-mounds of termites (Pont, 1980). Pont (1980) transferred this subfamily from family Muscidae to family Calliphoridae based on similarities in morphological structures and larval habits.

1.2 Morphological versus molecular systematics

Both morphological and molecular systematic approaches have been used for inferring phylogeny at different systematic levels of the Oestroidea (Pape, 1992; Rognes, 1997; Pape, 2001; Otranto *et al.*, 2003, McDonagh, 2009; Kutty *et al.*, 2010). Both methods complement each other and both have some advantages and disadvantages. Molecular data are good because large numbers of molecular characters are available for phylogenetic analysis, whereas in morphological data we have limited number of characters for inferring phylogeny of a particular group. For phylogenetic reconstructions, useful characters must demonstrate heritable variation as seen in most molecular characters, whereas some morphological characters may show non-heritable variation. There are also some disadvantages to using molecular data. The first is the problem of Long Branch Attraction (LBA), which is an erroneous grouping of rapidly evolving non-sister taxa, as a sister taxa, because of interpretation of homoplasies as a synapomorphies (Bergsten, 2005). Morphological characters are less commonly affected by LBA than are molecular characters (Grant & Kluge, 2003), as molecular characters (DNA sequence data) have only four possible character states, whereas morphological characters may have more possible character states (Jenner, 2004).The second is the cost. It is much cheaper to do morphological systematic analysis than to do molecular systematic studies. The third is the specimen availability. Many species of Oestroidea are rare in collection and are available only in museums. For these species only morphological systematic analysis is possible. There are some oestroid taxa (such as *Adipterites, Cretaphormia*) that are known only from fossils (Rognes, 1991). Phylogenetic analysis of these taxa with existing oestroid taxa is only possible by morphological systematic analysis.

1.3 Mitochondrial versus nuclear genes

Mitochondrial and nuclear genes have been extensively used in phylogenetic studies of different groups of flies (Wells & Sperling, 1999; Moulton & Wiegmann, 2004 Kutty *et al.*, 2008; 2010; McDonagh, 2009). Mitochondrial genes have many advantages over nuclear genes such as easy amplification from a variety of taxa (because universal primers are available for many mitochondrial genes), easy direct PCR product sequencing (as it is haploid), its usefulness in the study of closely related taxa

(because, mitochondrial genes frequently evolve at a faster rates than nuclear genes in animals) (Moritz *et al.*, 1987; Lin & Danforth, 2004). This faster rate of evolution helps in resolving species level and genus level phylogeny, but it may not be as good for deeper divergences. However, in one case a whole mitochondrial genome analysis helped resolve a phylogeny of the Diptera (Cameron *et al.*, 2007). Species-level polyphyly due to incomplete lineage sorting is less of concern for mitochondrial genes than for nuclear genes, because effective population size (Ne) of mitochondrial genes is theoretically one-fourth that of nuclear genes (Heckman *et al.*, 2007).

Mitochondrial gene based phylogeny can be affected by horizontal gene transfer, introgression and presence of pseudogenes (Funk & Omland, 2003). In some insect groups among-site rate variations are more heterogeneous in mitochondrial genes than in nuclear genes, which suggests more homoplasious nature of data obtained from mitochondrial genes than from nuclear genes (Lin & Danforth, 2004). Also in general, mitochondrial genes show a more biased base composition than nuclear genes (Lin & Danforth, 2004). Commonly used mitochondrial genes in insect systematics are *cytochrome oxidase subunit one* (*COI*), *cytochrome oxidase subunit two* (*COII*), *cytochrome b* (*Cyt. b*), *12S ribosomal RNA*, and *16S ribosomal RNA* (Wells & Sperling, 1999; Kutty *et al.*, 2008; 2010). Most nuclear protein coding genes are present in single copy and nuclear ribosomal genes are present in multiple copies. Nuclear ribosomal genes (18S, 5.8S and 28S) demonstrate diverse rate of genetic evolution, which make these genes better markers for elucidating phylogenetic relationships between recent as well as old divergences (Larson, 1991; Stevens & Wall, 2001). Universal primers are abundant and hence it is easy to amplify and sequence ribosomal genes but it is difficult to align these sequences (Hickson *et al.*, 2000). Single copy nuclear genes are difficult to amplify but easier to align as these genes are protein coding genes. Single copy nuclear genes commonly used for insect systematics are *Elongation factor one alpha* (*EF-1α*) (Cho *et al.*, 1995), *Phosphoenolpyruvate Carboxykinase* (*PEPCK*) (Leys *et al.*, 2002), *Dopa-decarboxylase* (*DDC*) (Fang *et al.,* 2000), *Wingless* (Brady, 2003), *White* (Baker *et al.*, 2001), *Opsin* (Kawakita *et al.,* 2004), *Hunchback* (Baker & DeSalle, 1997), *Period* (Regier *et al.,* 1998), *Carbamoylphosphate Synthetase* (*CPS*) (Moulton & Wiegmann, 2004), *Enolase* (Farrell *et al.,* 2001) and *Arginine Kinase* (Kawakita *et al.,*

2004). Danforth *et al.* (2005) suggested use of nuclear ribosomal genes along with nuclear protein coding genes for better resolution at deeper nodes. As incongruence of gene and species trees are not uncommon (Cummings, 1994), use of various independent loci gives more accurate evolutionary history of a group of organisms (Rubinoff & Holland, 2005). Combined analysis of mitochondrial and nuclear genes gives better support, stability, and resolution than separate analysis of any of them (Baker & DeSalle, 1997).

1.4 Phylogenetic methods

Several methods are available for phylogenetic analysis of morphological and molecular data but maximum parsimony (MP), maximum likelihood (ML) and Bayesian methods (BA) are more commonly used methods as these methods are character based, use optimality criteria for tree selection and are truly cladistic in nature. MP is a simple non-parametric statistical method that assumes that evolution is rare and the best tree is that which has the least total length. ML is a parametric statistical method that assumes independent evolution of nucleotide sites. ML estimates the probability of obtaining the observed sequence (data) given a tree (hypothesis) under the chosen evolutionary model. The tree that gives highest likelihood is the best. BA is the most recently developed parametric statistical method of phylogenetic inference that is based on Bayes theorem of posterior probability, which uses a Monte Carlo Markov Chain to sample a tree from a posterior probability distribution. BA estimates the probability of obtaining a tree given the data (sequence alignment) and model.

Although all these methods have different optimality criteria, under most sets of realistic conditions they perform similarly and often result in congruent tree topology (Reed *et al.*, 2002). No method is perfect and each one has some advantages and disadvantages (Swofford & Olsen, 1990; Archibald *et al.*, 2003). For morphological data, MP method is the only option because no stochastic model for non-molecular data is available. MP method is more sensitive to LBA, than model based ML and BA methods (Felsenstein, 1978). BA provides clade support in terms of frequency of occurrence of a particular clade among all trees sampled and hence it is less time consuming than ML with bootstrap analysis. Although BA has advantages in terms of speed and reliability it

requires information on prior distribution of data, which is sometimes not easy to provide.

1.5 Objectives of the present study

With more than 14,000 described species (≈ 9% of all known Diptera), Oestroidea is one of the most important superfamilies of Diptera. Some members, such as, flesh flies, blow flies, and tachinid flies, are commonly encountered in day to day life, while others such as New Zealand bat fly are rare but very unusual in terms of morphology and behavior. Many members of the superfamily are agricultural and veterinary pests and some cause myiasis (Zumpt, 1951; Pape, 2001; O'Hara, 2008). Some members are widely used in forensic entomology as an indicator species for estimation of time since death (Goff & Odum, 1987).

Although the superfamily Oestroidea has been the subject of much scientific scrutiny, the exact patterns of phylogenetic relationships among the key groups of the superfamily remain the subject of continuing revision (McAlpine, 1989; Pape, 1992; Rognes, 1997; McDonagh, 2009; Kutty *et al.*, 2008; 2010). Except Calliphoridae, the monophyly of each oestroid family is well-supported (Rognes, 1997). Extensive morphological cladistic analysis of the Oestroidea by Pape (1992) and Rognes (1997), disagrees on monophyly of the Calliphoridae and also on interfamilial relationships within Oestroidea. It is difficult to understand evolution and phylogeny of the Calliphoridae without understanding the evolution and phylogeny of the Oestroidea and vice versa (McAlpine, 1989; Rognes, 1997). Uncertain systematic positions of *Mystacinobia* and the Rhinophoridae along with uncertain monophyly of the Calliphoridae are major unresolved oestroid systematic questions (McAlpine, 1989; Pape, 1992; Rognes, 1997; Kutty *et al.*, 2010). Even within Oestroidea, there are many taxonomic groups (families, subfamilies and genera) that require separate detailed systematic study, but these studies are constrained because of difficulty in collection of these flies (Rognes, 1997; Pape, 2001; Otranto *et al.*, 2003; Nirmala *et al.*, 2001; Kutty *et al.*, 2010).

The main objective of this dissertation was to infer the evolution and phylogeny of the Oestroidea with an emphasis on the family Calliphoridae using DNA sequence data.

This will involve the consideration of systematic questions of Oestridae, Calliphoridae, Chrysomyinae and *Chrysomya*. I chose Oestridae, Calliphoridae, Chrysomyinae and *Chrysomya* for detailed systematic studies, because they have many unresolved systematic questions that need an answer and, also, because I had better taxon sampling from these groups.

Therefore, the objectives of this dissertation are to determine 1.) intrageneric relationships of the genus *Chrysomya,* 2.) intergeneric relationships of the subfamily Chrysomyinae, 3.) intrafamilial relationships of the family Oestridae, and 4.) intrafamilial relationships of the family Calliphoridae, using both mitochondrial and nuclear genes.

Each chapter was written as a publishable manuscript, which addresses the above mentioned objectives separately. Although I tried to avoid unnecessary repetition between different chapters, there are still some repetitive sections because each chapter must be independent of the other. Although this dissertation is my original work, at many places, I have used “We” instead of “I” to give credit to people who have contributed significantly during different stages of this study and are co-authors on the manuscripts.

CHAPTER 2 : Molecular Phylogeny of the Chrysomya (Calliphoridae: Chrysomyinae).

Singh, B., Kurahashi, H. & Wells, J. D. (2010) Molecular phylogeny of the blowfly genus *Chrysomya. Medical and Veterinary Entomology*, DOI: 10.1111/j.1365-2915.2010.00914.x

2.1 Introduction

Chrysomya Robineau-Desvoidy is a genus of blow fly native to the Old World tropics and subtropics. Like other familiar calliphorids, most *Chrysomya* spp. breed in carrion and putrefied wounds (Fuller, 1934**;** Norris, 1965)**.** *Ch. bezziana* Villeneuve larvae feed on healthy warm-blooded vertebrates and are serious medical and veterinary pests (Zumpt, 1951; 1965). With about 36 species (Boyes & Shewell, 1975; Wells & Kurahashi, 1996; Ullerich & Schottke, 2006), the genus is not unusually large for the Calliphoridae. However, compared to other blow fly genera, the *Chrysomya* are notably diverse in morphology and habits, and some of their unique features may have contributed to the ability of several species to spectacularly succeed in invading new geographic regions.

Several authors have proposed hypotheses about evolutionary trends within *Chrysomya*. Ullerich & Schottke (2006) quantified genome size from nine species of *Chrysomya*. Based on karyotype and distribution of species-specific inversions, they concluded that genome size increased over evolutionary time.

The larvae of several species have prominent spiny tubercles not found in other calliphorids (Kitching, 1976; Kitching & Voeten, 1977; Erzinclioglu, 1987; Sukontason *et al.*, 2003; 2005), and are sometimes referred to as “hairy maggots”. All tuberculate species with known natural history are also facultative predators on other maggots (Froggatt, 1918 (as cited in Fuller, 1934); Fuller, 1934; Senior-White *et al.*, 1940; Ullyett, 1950). Hairy maggots are dorso-ventrally flattened, and in one species we have observed, *Ch. rufifacies* (Macquart), will curl and present the spines rather like a hedgehog when disturbed. Therefore, we suspect that the tubercles serve to reduce cannibalism. The ability to both scavenge carrion and eat one’s competitors make these *Chrysomya* powerful ecological competitors for the carrion resource, and the introduction of both hairy and smooth *Chrysomya* to new regions appears to have reduced populations of native calliphorids (Hanski, 1977; Guimarães *et al.*, 1979;

Baumgartner & Greenberg, 1984; Wells & Greenberg, 1992a). Lunt (2002), after considering a limited number of *Chrysomya* species suggested cuticular tubercules evolved twice in *Chrysomya*.

Some *Chrysomya* adult males show a normal compound eye, where ommatidium size decreases gradually from top to bottom, while others show a form of male compound eye, not seen in other blow flies, but not too rare in lower Diptera. This involves a sharply demarcated pattern of enlarged upper and small lower ommatidia (Fig. 2.1) (Kurahashi, 1982; Wells *et al.,* 1994). Kurahashi (1982) theorized that this derived eye evolved in *Ch. megacephala* in response to the development of anthropogenic habitats. *Ch. megacephala* is a hugely successful urban pest that has invaded all of the world's warm regions (Wells, 1991). The possible association between the derived eye and synanthropy has not been so well studied in other *Chrysomya* spp., but what is known does not contradict that pattern (Wells *et al.,* 1994).

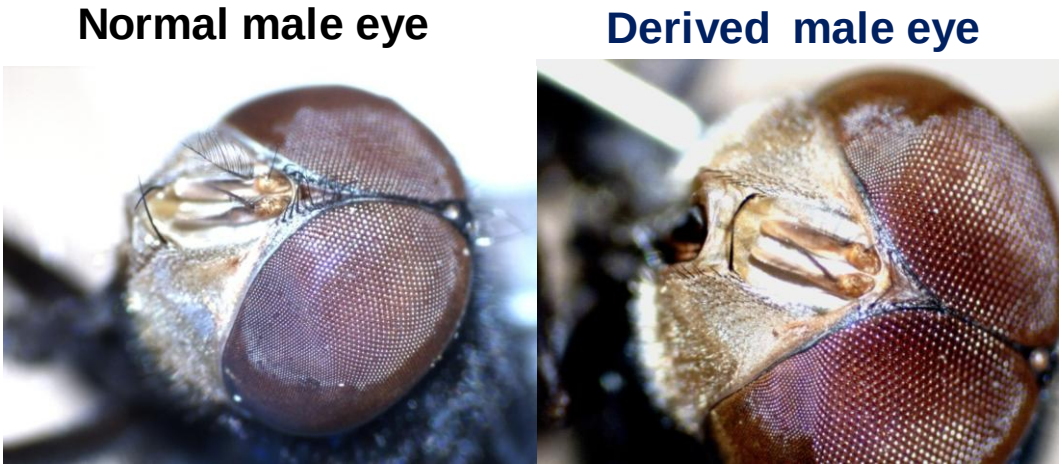

FIGURE 2.1: Male compound eye of *Chrysomya* showing two kinds of compound eye. Left: normal male eye of *Ch. pacifica*; Right: derived male eye of *Ch. megacephala*.

Perhaps most striking is that some *Chrysomya* have a unique mode of sex determination. In the so-called monogenic *Ch. rufifacies* and *Ch. albiceps* Wiedemann, sex is determined by the mother's genotype, i.e. a female produces offspring of a single sex (Ullerich, 1958; 1963; Ullerich & Schottke, 2006). Ullerich (1976) suggested monogeny evolved from amphogeny because monogenic species had what he believed was a derived karyotype. Alternatively, Puchalla (1994) argued that amphogeny evolved

from monogeny in *Chrysomya*, as he observed a homosequencial band pattern in monogenic *Ch. rufifacies* and the outgroup *Lucilia cuprina* (Wiedemann). Such evolutionary scenarios have been difficult to evaluate, because there is no robust phylogeny that can be used to infer such things as the number of times a character has evolved or whether a condition is primitive or derived within the genus (Wiens *et al.*, 2006).

Similarly, taxonomic divisions within *Chrysomya* have not been shown to be true clades. Proposed subgenera are *Microcalliphora* (Townsend) [*Ch. varipes* (Macquart)*, Ch. flavifrons* Aldrich], *Eucompsomyia* (Malloch) [*Ch. semimetallica* (Malloch), *Ch. latifrons* (Malloch)*, Ch. sabroskyi* (Theowald)], *Achoetandrus* (Bezzi) or *villeneuvi* group of Kurahashi & Magpayo (1987) [*Ch. albiceps*, *Ch. rufifacies*, *Ch. yayukae* Kurahashi et Magpayo, *Ch. villeneuvi* Patton, *Ch. sulcifrons* James, and *Ch. schoenigi* Kurahashi et Magpayo] and *Ceylonomyia* (Fan) [*Ch. nigripes* Aubertin]. Kurahashi & Magpayo (1987) recognized, the *megacephala* group, including *Ch. defixa* (Walker), *Ch. megacephala* Fabricius, *Ch. pinguis* (Walker), *Ch. saffranea* Bigot, and *Ch. thanomthini* Kurahashi et Tumrasvin*.*

Kurahashi (1982) produced a morphological phenogram of the *megacephala* group. More recently, DNA sequence data mostly generated for identifying forensic evidence (Wells & Sperling, 2001; Harvey *et al.*, 2003; Wallman *et al.*, 2005; Wells & Williams, 2007; Nelson *et al.*, 2007; Nelson *et al.*, 2008; Harvey *et al.*, 2008) were used to produce phylogenies of *Chrysomya* and other calliphorids. However, these data were insufficient for testing evolutionary hypothesis of *Chrysomya,* as they included few *Chrysomya* species, and the fast-evolving mitochondrial genes used didn't resolve deeper nodes.

The main purpose of this study was to develop a robust phylogeny of the *Chrysomya.*

2.2 Materials and Methods

2.2.1. Specimens and DNA extraction

A total of 27 blowfly specimens were included in this study (See Table 2.1 for collection details). 22 specimens represented the ingroup genus *Chrysomya* and five served as outgroups. All the specimens except *Cochliomyia hominivorax* (Coquerel)

(see below) were preserved live in 95% ethanol. For DNA extraction approximately 20 mg of thoracic muscles of the selected taxa were cut into smaller pieces and then mixed with 500 µl of lysis buffer containing Tris-Cl (pH=9.0) 100 mM, NaCl = 100mM, EDTA (pH=8.0) =50mM and Sucrose =200mM, 50 µl of 10% SDS and 20 µl of 20 mg/ml proteinase K. After overnight incubation at 55^{o}C, DNA purification was done using a phenol:chloroform:isoamyl alcohol (25:24:1) method as described in Moulton and Weigmann (2004). DNA was washed in 70% ethanol and precipitated in equal volume of 100% iso-propanol. Pelleted DNA was dried in a vacuum centrifuge at room temperature for about 20 minutes. The dried pellet was re-suspended in 100 µl of TE buffer (Tris-Cl 10 mM and 1 mM EDTA). DNA quantification was done using a NanoDrop^{TM} 1000 spectrophotometer (Thermo Scientific, Waltham, MA).

Approximately, 4000 to 6000 ng of DNA was obtained from each specimen and was stored at -20^{0}C. T. T. Torres (Universidade Estadual de Campinas, Brazil) supplied DNA for *Co. hominivorax*. Voucher specimens were deposited to West Virginia University Entomological collections, and voucher identification numbers from the same were submitted to GenBank (See Table 2.1 for voucher ID).

2.2.2. PCR amplification

2.2.2.1 Carbamoylphosphate synthetase (CPS)

CPS is an enzymatic element in the fused *carbamoylphosphate synthetase*, *aspartate transcarbamylase*, and *dihydroorotase* (CAD) protein involved in pyrimidine biosynthesis. An 850 bp fragment of CPS was amplified from 26 of the study taxa using primers listed in Table 2.2. Published degenerate CPS primers 787F and 1098R and Schizophora specific degenerate primers Shiz 3' and Shiz 5' (sequences were supplied by Dr. J. K. Moulton (The University of Tennessee, Knoxville,TN) worked for some blow fly taxa. The resulting sequences were edited and aligned using Sequence Navigator (Applied Biosystems, Foster city, CA) and used to design *Chrysomya* specific primers (Table 2.2), using primer 3 software (Steve & Helen, 2000). All primers were purchased from either Integrated DNA Technologies, Inc. (Coralville, IA) or Operon Biotechnologies, Inc. (Huntsville, AL).

TABLE 2.1: Specimen details, collection information and GenBank accession numbers.

Sr. #	Species name	Location	Date	Collected by	Voucher number	Accession number	
						CPS	COI
1	*Chrysomya albiceps* Wiedemann, 1819	Bloemfontein, South Africa	15/V/1997	T.C. Vander Linde	WVU2009-017-1	FJ169339	AF083657[g]
2	*Chrysomya cabrearai* Kurahashi et Salazar,1977	SERAM Manusela National Park, Indonesia	27/IX/1996	H. Kurahashi	WVU2009-017-2	FJ169349	FJ195378
3	*Chrysomya chani* Kurahashi, 1979	Lam Dong Prov.,Vietnam	05/XI/2000	H. Kurahashi	WVU2009-017-30	FJ169348	FJ195377
4	*Chrysomya putoria* (Weidemann, 1830)	Near Chilibre, Panama	03/IX/1995	J. Mendez	WVU2009-017-3	FJ169353	FJ195384
5	*Chrysomya defixa* (Walker,1856)	Chumphon, South Thailand	30/I/2004	H. Banziger	WVU2009-017-4	FJ169358	FJ195375
6	*Chrysomya greenbergi* Wells et Kurahashi,1996	Mt. Nokilalaki, Sulawesi, Indonesia	31/VIII/1996	H. Kurahashi	WVU2009-017-5	FJ169354	FJ195385
7	*Chrysomya incisuralis* (Macquart,1851)	Kuranda, Qld, Australia	24/VI/2002	J. F. Wallman	WVU2009-017-6	FJ169337	FJ195373
8	*Chrysomya latifrons* (Malloch,1927)	Mt. Kiera, New South Wales, Australia	--/II/2005	J.F. Wallman	WVU2009-017-7	FJ169338	FJ195374
9	*Chrysomya marginalis* (Weidemann, 1830)	Bloemfontein, South Africa	15/V/1997	T.C. Vander Linde	WVU2009-017-8	FJ169347	FJ195380
10	*Chrysomya megacephala* Fabricius, 1794	Guam	31/IV/2002	P. Erwin	WVU2009-017-9	FJ169350	AF092761[g]
11	*Chrysomya nigripes* Aubertin,1932	Toili, Sulawesi, Indonesia	3-11/IX/1996	H. Kurahashi	WVU2009-017-10	FJ169346	FJ195379
12	*Chrysomya norrisi* James,1971	Wau, Papua New Guinea	19/IV/1995	J. D. Wells	N/A	FJ169344	AF295552[g]
13	*Chrysomya pacifica* Kurahashi, 1991	Laboratory colony originated from New Britain, Papua New Guinea	27/V/1995	H. Kurahashi	WVU2009-017-11	FJ169355	FJ195383

14	*Chrysomya flavifrons* Aldrich,1925	Kuranda, Qld, Australia	NA	NA	ABTC78506	NA	AY842615 [g]
15	*Chrysomya pinguis* (Walker,1858)	Sarangkot, Nepal	29/V/2007	J.D. Wells	WVU2009-017-12	FJ169357	FJ195381
16	*Chrysomya rufifacies* (Macquart, 1843)	Montgomery, AL, USA	05/X/2005	J.D. Wells	WVU2009-017-13	FJ169341	AF083658 [g]
17	*Chrysomya semimetallica* (Malloch, 1927)	Near Hoskins, New Britain, Papua New Guinea	25/IV/1995	J. D. Wells	N/A	FJ169345	AF295562 [g]
18	*Chrysomya thanomthini* Kurahashi et Tumrasvin, 1977	Lao Cai, Sapa, Vietnam	02/X/1995	H. Kurahashi	WVU2009-017-14	FJ169356	FJ195386
19	*Chrysomya varipes* (Macquart, 1851)	Adelaide, Australia	03/X/1997	J.D. Wells	WVU2009-017-15	FJ169343	AF295556 [g]
20	*Chrysomya villeneuvi* Patton, 1922	Lam Dong Prov., Vietnam	05/XI/2000	H. Kurahashi	WVU2009-017-31	FJ169342	FJ195382
21	*Chrysomya yayukae* Kurahashi et Magpayo, 1987	Toili, Sulawesi, Indonesia	3-11/IX/1996	H. Kurahashi	WVU2009-017-16	FJ169340	FJ195376
22	*Chrysomya bezziana* Villeneuve,1914	Institute Haiwan, Malaysia	09/XI/2001	J. Stevens	WVU2009-017-17	FJ169351	AF295548 [g]
23	*Phormia regina* (Meigen, 1826)	Laboratory colony originated from Pullman, WA, USA	29/V/2008	C.J. Picard	WVU2009-017-18	FJ169331	AF295550 [g]
24	*Cochliomyia macellaria* (Fabricius,1775)	Laboratory colony originated from Morgantown, WV, USA	14/IX/2006	B. Singh	WVU2009-017-19	FJ169333	AF295555 [g]
25	*Cochliomyia hominivorax* (Coquerel, 1858)	Brazil	NA	T.T. Torres	N/A	FJ169334	AF260826 [g]
26	*Lucilia sericata* (Meigen, 1826)	Morgantown, WV, USA	13/V/2005	J.D. Wells	WVU2009-017-20	FJ169332	AJ417717 [g]
27	*Calliphora vomitoria* (Linnaeus, 1758)	Morgantown, WV, USA	14/VIII/2005	J. Hall	WVU2009-017-21	FJ169335	EU418569 [g]

TABLE 2.2: List of *CPS* gene primers used in this study.

Sr. #	Name of primer	Nucleotide Sequence (5' to 3')	Reference
1	787F	GGDGTNACNACNGCNTGYTTYGARCC	Moulton and Wiegmann, 2004
2	1098R	TTNGGNAGYTGNCCNCCCAT	Moulton and Wiegmann, 2004
3	Shiz 3'	TTKGCAATNAKYTGCATGTTRAA	Moulton JK (Personal communication)
4	Shiz 5'	CANGTGGCYGGAGAA TGG CC	Moulton JK (Personal communication)
5	CALCAD192F	AACCCACCGATAAGAGACCA	Designed for this study
6	CALCAD1180R	TTCCTTGGCTTCCGTTAAGA	Designed for this study
7	CH109F	TCCGTCAATGGTTTTGATCC	Designed for this study
8	CH431F	TGGGCATTATTCCGTTTGTT	Designed for this study
9	CH634F	TTGAGAAATCTGGGCAAACC	Designed for this study
10	CH584R	ATGCGGTAAACACCTGAACC	Designed for this study
11	CH653R	GGTTTGCCCAGATTTCTCAA	Designed for this study
12	CH856R	CCAAAACCTTGGCTTGTTGT	Designed for this study
13	CH878R	TCAATGGATTCGGGAGAAGT	Designed for this study
14	CH1044R	CATAGCAGCACCCGACAATA	Designed for this study

Other PCR reagents were purchased from Promega Corp. (Madison, WI). Each 25 µl PCR reaction was prepared with 12.5 µl of 2X Promega Master Mix (Catalog # M7505), 2 µl of each primer (5 pmol/µl), 1-3 µl of template (10-30ng) and enough water to complete the total 25 µl volume. Amplification was done using the touchdown PCR program of Moulton and Wiegmann (2004) using a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA).

2.2.2.2 Cytochrome oxidase subunit one (COI) gene

A 1536 bp fragment of the widely studied COI gene was amplified using the primers and thermal cycler program described by Wells & Sperling (1999). Table 2.1 lists those species that were sequenced for this study and those for which published COI sequence data were available. The PCR reaction mix was prepared as given above for CPS.

2.2.3. Sequencing

PCR product was cleaned either by using a QIAquick PCR purification kit (Qiagen Inc., Valencia, CA) or by using ExoSAP-IT® (USB Corporation, Cleveland, Ohio) as per manufacturer's instructions. Direct sequencing of one or both strands of the cleaned PCR product involved the same primers used for its amplification. Cycle sequencing was performed on a GeneAmp® *PCR* System 9700 (Applied Biosystems, Foster City, CA) with the Big Dye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster city, CA). Each cycle sequencing reaction was prepared with 0.7 to 1.2 µl of cleaned PCR product, 1 µl of 5X sequencing buffer, 1 µl of primer (2.5 pmol/µl), 0.5 µl of BigDye^{TM} and enough water to complete the total 5 µl volume. Cycling conditions included initial denaturation at 96^{o}C for 1 minutes followed by 30 cycles of 96 ^{o}C for 10 seconds, 50^{o}C for 5 seconds, and 60^{o}C for 4 minutes, followed by a 4^{o}C hold. Cycle sequencing product was cleaned either by using spin columns filled with Sephadex G-50 beads (Sigma-Aldrich, Milwaukee, WI) or by ethanol precipitation. For the ethanol precipitation method, 25 µl of a mixture containing 120 µl 3M Sodium acetate (pH=5.2) in 3 ml of absolute alcohol, was added to each well containing 5 µl sequencing product and then centrifuged at 4000 rpm for 20 minutes. The supernatant was removed by inverting the plate. The pellet was washed with 100 µl of 70% ethanol and centrifuged at 4000 rpm for 12 minutes. The supernatant was again removed, initially by inverting the plate and then by wrapping the plate in several layers of kimwipe followed by inverted centrifugation at 700 rpm for 1 minute. All centrifugation steps were performed at 4^{o}C in a Thermo Scientific Sorvall® Legend RT centrifuge. Plates with purified cycle sequencing products were air-dried at room temperature for 15 minutes. 10 µl of Hi-Di formamide was added into each well and sequencing products were then separated on an Applied Biosystems 3130xl genetic analyzer (Foster City, CA). Sequence files were edited and aligned using Sequence Navigator (Applied Biosystems, Foster City, CA). All new CPS and COI DNA sequences were submitted to the GenBank database (See Table 2.1 for accession numbers).

2.2.4. Phylogenetic analyses

Phylogenetic analyses were performed on separate and combined *CPS* and *COI* sequences, a total of 2386 aligned bases. More than one tree-building method was

used to test if phylogenetic results were robust to different analytical assumptions. Maximum parsimony (MP) and maximum likelihood (ML) analyses were performed in PAUP 4.0b10 (Swofford, 2002), and Bayesian analysis (BA) was performed using Mr.Bayes v.3.0b4 (Huelsenbeck & Ronquist, 2001). Outgroup taxa included other chrysomyine blow flies (*Co. macellaria* (Fabricius), *Co. hominivorax*, *Phormia regina* (Meigen)) and representatives of the calliphorid subfamilies Luciliinae (*Lu. sericata* (Meigen)) and Calliphorinae (*Calliphora vomitoria* (Linnaeus)). Branch support for MP and ML analysis was assessed with bootstrapping (BP) and also by Bremer support (BS) for MP analysis. For BA, branch support was assessed as posterior probabilities (PP). MP analysis included 1000 bootstrap replicates of equally weighted heuristic searches using addseq=random nreps=10 swap=nni hold=1 commands in the PAUP block. Other branch swapping options gave the same tree topology. BS (Bremer, 1994) values were calculated for the most parsimonious tree using TreeRot.v3 (Sorenson & Franzosa, 2007) and PAUP4.0b10 (Swofford, 2002). A best-fit model was selected for ML analysis using Modeltest 2 (Posada & Crandall, 1998) and for BA using MrModelTest 2.2 (Nylander, 2004). ML was performed with GTR+I+r model for 100 bootstrap replications using ML optimality criteria. BA was performed using general time reversible model with invariable site and discrete gamma distribution (GTR+I+r) for 1.5 million generations, sampling every 100 generations. After observing the likelihood plot, burn-in value was set as 12000 and posterior probabilities were calculated from the remaining trees by means of a majority rule consensus tree.

2.3 Results

MP bootstrap majority rule consensus trees obtained from separate analyses of the COI and CPS genes lacked resolution at deeper nodes (not shown). A combined analysis of both genes yielded strong support for a monophyletic *Chrysomya*, and three lineages within *Chrysomya* we designated clades I-III (Figs. 2.2 & 2.3). The ML and Bayesian trees based on combined data were identical. This tree differed from the MP tree at a few nodes (as indicated by "≠" in Fig. 2.3), but these nodes in the MP tree were weakly supported by bootstrap and Bremer support values (Fig. 2.2 and Fig. 2.3).

2.4 Discussion

2.4.1. Taxonomy

Microcalliphora (*Ch. varipes* + *Ch. flavifrons*) was monophyletic. *Ch. semimetallica* and *Ch. latifrons*, the two species of *Eucompsomyia* examined here (Malloch, 1927; Bezzi, 1927), formed the basal clade I. *Achoetandrus* i.e. the *villeneuvi* group of Kurahashi & Magpayo (Bezzi, 1927; Lehrer, 1970; Kurahashi & Magpayo, 1987) was also monophyletic (Figs. 2.2 & 2.3), as was Kurahashi's *megacephala* species group. However, we did not recover Kurahashi's *defixa* and *megacephala* subgroups of the *megacephala* group (Kurahashi, 1982). Relationships between the subgenera *Achoetandrus*, *Microcalliphora* and *Ceylonomyia* were not resolved in the MP tree, but the BA and ML trees supported a sister group relationship between *Achoetandrus* and *Microcalliphora* + *Ch. norrisi* James + *Ceylonomyia*. BA and ML trees also supported a sister group relationship between C*eylonomyia* and *Microcalliphora* + *Ch. norrisi*. Both the BA and ML trees (Fig. 2.3) support *Ch. pacifica* Kurahashi and *Ch. megacephala*, as sister species, as proposed earlier (Kurahashi, 1982; Kurahashi, 1991), but the MP tree showed a slightly different topology (Fig. 2.2).

The close relationship between *Ch. putoria* (Weidemann) and *Ch. marginalis* (Weidemann) was in agreement with Ullerich & Schottke (2006), who based their conclusions on karyotype patterns. We observed strong branch support for the species pairs: *Ch. semimetallica* -*Ch. latifrons*; *Ch. varipes* - *Ch. flavifrons* and *Ch. rufifacies* - *Ch. albiceps*, close relationships that had been supported by previous authors. (Wells & Sperling, 2001; Harvey *et al.*, 2003; Wallman *et al*., 2005; Nelson *et al*., 2007; Wells & Williams, 2007; Harvey *et al.,* 2008).

2.4.2. Evolution

The most parsimonious explanation is that a tuberculate *Chrysomya* larva evolved independently in *Ch. varipes* and in the *villeneuvi* group (Fig. 2.2), as proposed by Lunt (2002). The apical positions of the monogenic *Ch. rufifacies* and *Ch. albiceps* (Fig. 2.2), with all other species for which the reproductive system is known being amphogenic, strongly indicate that monogeny is derived rather than the suggested ancestral state in *Chrysomya* (Puchalla, 1994; Ullerich & Schottke, 2006).

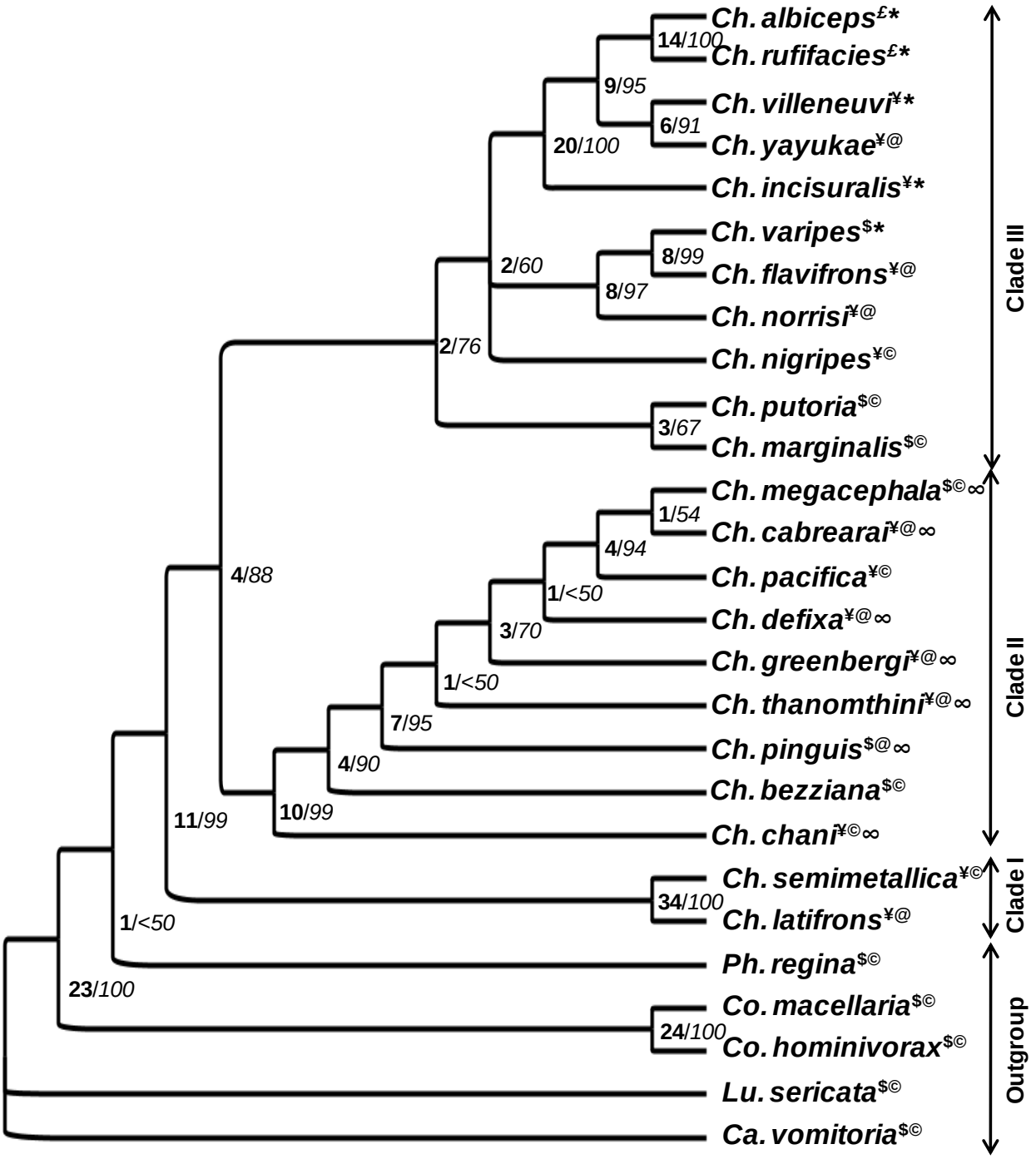

FIGURE 2.2: Strict consensus of the two most parsimonious trees of the Chrysomya based on 2386 bp of combined COI and CPS DNA sequences. A number in bold indicates Bremer support and number in italics indicates bootstrap supports for individual branches. £ = Monogenic spp.; $ = Amphogenic spp.; ¥ = Species with unknown sex determination system; * = Species that produces hairy maggots; © = Species that produces smooth maggots; @ = Species with unknown larval structure; ∞ = Species showing derived male eye condition; Ch. = Chrysomya; Ph. = Phormia; Co. = Cochliomyia; Lu. = Lucilia; Ca. = Calliphora.

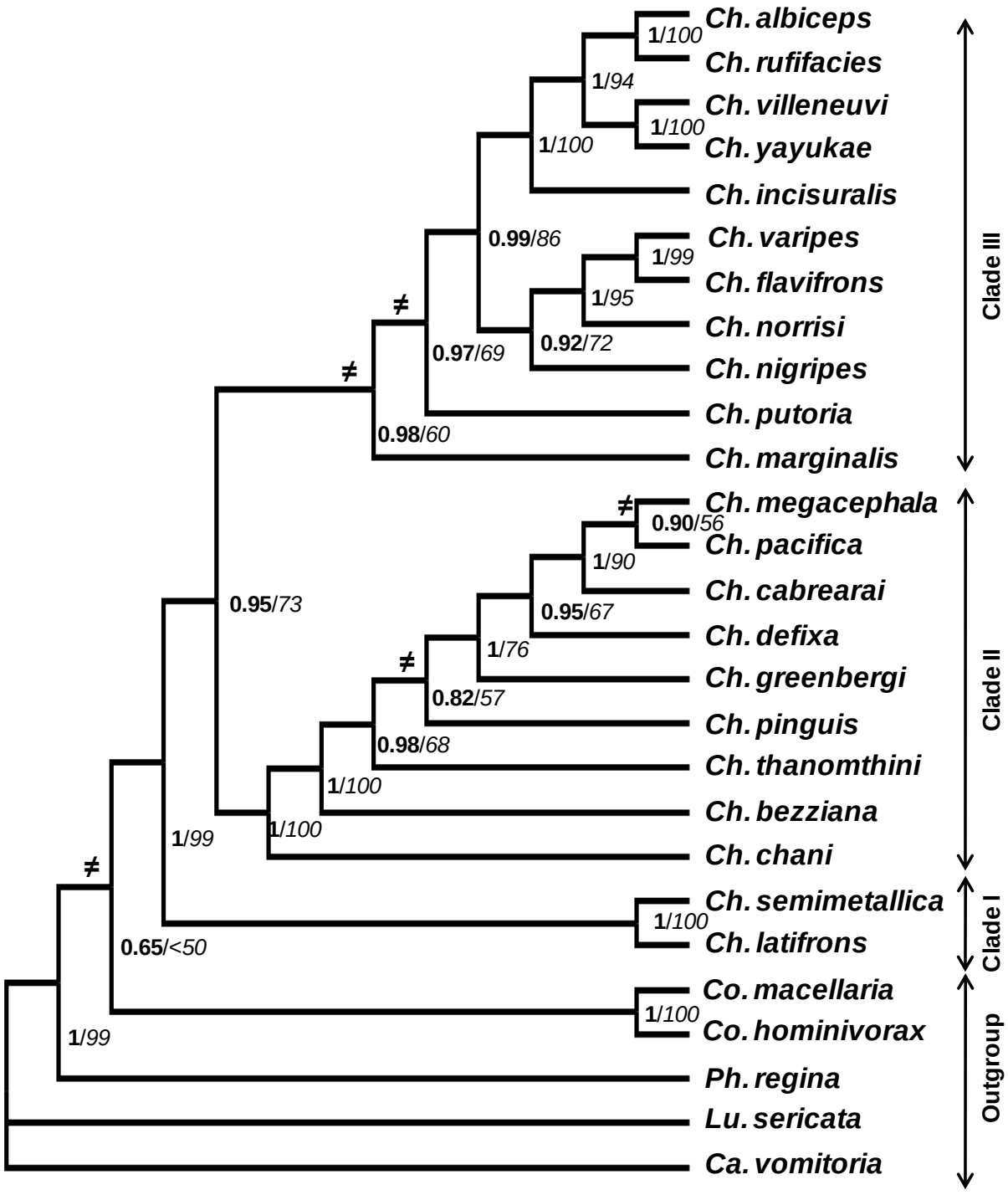

FIGURE 2.3: Maximum-likelihood tree of the *Chrysomya* based on 2386 bp of combined COI and CPS DNA sequences. Number in bold indicates posterior probability value (in BA) and number in italics indicates bootstrap supports (in ML analysis) for individual branches. Nodes not shared with MP tree indicated by "≠". *Ch. = Chrysomya*; *Co. = Cochliomyia*; *Ph. = Phormia*; *Lu*. = *Lucilia*; *Ca. = Calliphora.*

Our result contradicts the Kurahashi (1982) theory about the evolution of derived eye condition in *Ch. megacephala*. He proposed that the derived eye in *Ch. megacephala* evolved in response to the development of anthropogenic landscapes and hence derived eye is apomorphic in *Ch. Megacephala*. In contrast, our result suggests derived eye as a plesiomorphic character state in megacephala group, and the loss of derived eye in *Ch. pacifica* may be because of its movement towards dense forest.

Similarly, the hypothesis of Ullerich & Schottke (2006), that the average 2C DNA content in *Chrysomya* spp. increased over evolutionary time (see table 1 in Ullerich & Schottke (2006) for DNA content values), appears to be incorrect. Species with lower DNA content (*Ch. varipes* 1.04 pg; *Ch. rufifacies* 1.14 pg; *Ch. albiceps* 1.44 pg; *Ch. megacephala* 1.70 pg), occupy apical positions, whereas species with higher DNA content (*Ch. marginalis* 1.63 pg; *Ch. putoria* 1.81pg; *Ch. pinguis* 2.31pg) are basal. Genome size has significant effects on phenotypic traits (Petrov, 2001). For example, Gregory & Johnston (2008) observed a positive correlation between genome size and the developmental time for different species of *Drosophila* at a constant temperature. Because blow fly development rate is of forensic importance (Smith, 1986), the discovery of a similar pattern for *Chrysomya* might make it possible to estimate development rate with a simple measurement of genome size. Developmental data are available for many species of *Chrysomya*: *Ch. marginalis* (Lunt, 2002); *Ch. putoria*, *Ch. chloropyga* (Wiedemann) (Lunt, 2002; Richards *et al.,* 2009); *Ch. albiceps* (Marchenko, 2001; Grassberger *et al.,* 2003; Richards *et al.*, 2008); *Ch. rufifacies* (O'Flynn, 1983; Byrd & Butler, 1997); *Ch. megacephala* (O'Flynn, 1983; Wells & Kurahashi, 1994; Jenson & Miller, 2001; Richards & Villet, 2009), *Ch. bezziana* (Spradbery, 1992), *Ch. varipes* (O'Flynn, 1983) and *Ch. nigripes* Aubertin (O'Flynn, 1983; Jenson & Miller, 2001). Although, variation between published studies in experimental conditions make meaningful comparisons difficult, data available for the single temperature of 25^{o}C: *Ch. putoria* 1.81 pg, 11.28±0.23 days from egg hatching to adult emergence; *Ch. marginalis* 1.63 pg, 12.1 days (Lunt, 2002; Ullerich & Schottke,2006); *Ch. albiceps* 1.44 pg, 12.2±0.84 days (Grassberger *et al.*, 2003; Richards *et al.*, 2008; Ullerich & Schottke, 2006); *Ch. rufifacies*: 1.14 pg, 11.54 days (Byrd & Butler,1997; Ullerich & Schottke,

2006), doesn't suggest to us any obvious correlation between developmental time and genome size in *Chrysomya*.

CHAPTER 3: Molecular phylogeny of the Chrysomyinae (Diptera: Calliphoridae).

Singh, B. & Wells, J. D. (2011) Chrysomyinae (Diptera: Calliphoridae) is monophyletic: A molecular systematic analysis. *Systematic Entomology*, DOI: 10.1111/j. 1365-3113.2011.00568.x

3.1 Introduction

The Calliphoridae subfamily Chrysomyinae is split traditionally into two tribes: the Phormiini (*Phormia* Robineau-Desvoidy, *Protophormia* Townsend, *Protocalliphora* Hough, *Trypocalliphora* Peus, *Phormiata* Grunin) and the Chrysomyini (*Chrysomya* Robineau-Desvoidy; *Chrysopyrellia* Seguy; *Cochliomyia* Townsend; *Compsomyiops* Townsend; *Hemilucilia* Brauer; *Paralucilia* Brauer & Bergenstamm and *Chloroprocta* Wulp) (Hall, 1948; Dear, 1985; Rognes, 1991). Phormiini are distributed in the temperate Holarctic zone, whereas Chrysomyini occur in tropical and subtropical regions. Except *Chrysomya* and *Chrysopyrellia*, all Chrysomyini are Neotropical. Most chrysomyines breed in carrion (Fuller, 1934**;** Norris, 1965), but *Protocalliphora* and *Trypocalliphora* larvae are obligate bloodsucking parasites of nestling birds, and *Chrysomya bezziana* Villeneuve and *Cochliomyia hominivorax* (Coquerel) larvae attack a variety of live mammals (Zumpt, 1951; Dear, 1985; Rognes, 1991). Consequently, many Chrysomyinae are of interest to medical/veterinary entomologists, forensic investigators and ecologists (Kamal, 1958; Norris, 1965; Wells & Greenberg, 1992b; Byrd & Butler, 1997; O' Brien *et al.*, 2001; Baudry *et al.*, 2003; Ng *et el.*, 2003; Gomez *et al.*, 2003). Some *Chrysomya* have both unusual tuberculate larvae (Erzinclioglu, 1987; Sukontason *et al.*, 2003) and a unique mechanism of sex-determination (Ullerich, 1958).

There are unresolved systematic issues concerning these flies. Morphologically, the monophyly of Chrysomyinae is well supported (Rognes, 1991). The authors of a recent large-scale molecular systematic analysis concluded otherwise, in finding Bengaliinae nested within Chrysomyinae (Kutty *et al.*, 2010). However, the Kutty *et al.* (2010) analysis concerned broader questions of calyptrate fly systematics, and few Chrysomyinae were examined. Relationships between various genera of the subfamily remain unclear (Rognes, 1991). The commonly accepted tribal classification reflects the influential legacy of Hall (1948), who accepted Phormiini and Chrysomyini. However Hall was considering only the American fauna, and Dear (1985) noted that previously

proposed diagnostic characters could not assign every chrysomyine species reliably to one of those two tribes, and that this could only be done using additional morphological characters and distribution data. In particular, Dear (1985) singled out *Phormia regina* (Meigen) as a "non-conformist phormiine". Furthermore, Rognes (1985) found that consideration of aedeagus morphology made it difficult to place *Phormia* with the other Phormiini. Rognes (1991) abandoned Hall's (1948) tribal classification, because characters that supposedly indicate monophyly of each tribe either were unreliable or plesiomorphic at the tribal level. A phylogeny based on mitochondrial DNA did not support monophyly of either tribe (Wells & Sperling, 2001).

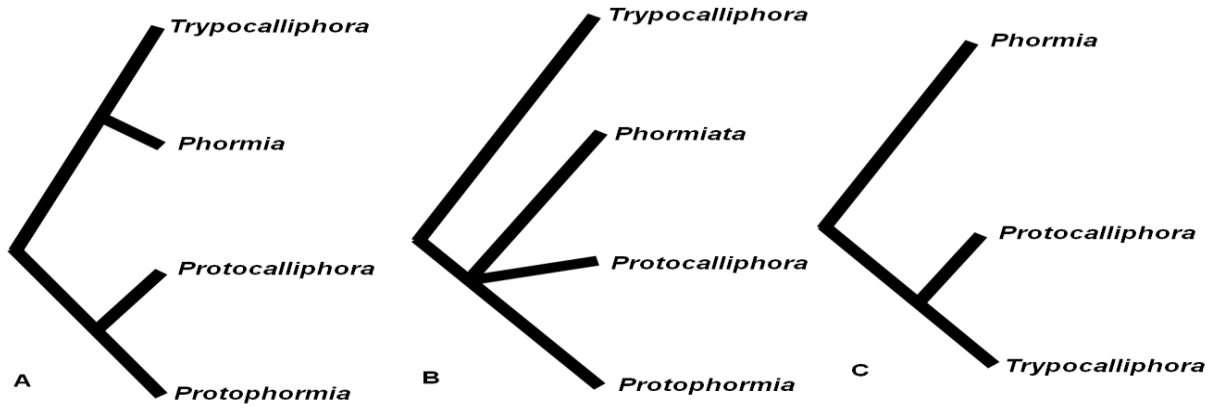

FIGURE 3.1: Published hypotheses of phylogenetic relationships between Phormiini genera based on morphological (A-B) and molecular (C) data. (A) modified from Peus, 1960, (B) modified from Rognes, 1985, and (C) modified from Whitworth *et al.*, 2007.

Various hypotheses have been proposed concerning the taxonomy and relationships of the bird-parasitic chrysomyine genera (Fig. 3.1). Peus (1960) erected the monotypic genus *Trypocalliphora* for all blow fly larvae that cause subcutaneous myiasis in nestlings and divided Phormiini into: 1.) *Phormia* +*Trypocalliphora* and 2.) *Protophormia* (including *Boreellus*) + *Protocalliphora* (Fig. 3.1-A). *Trypocalliphora* was accepted as a valid genus because of its distinct aedeagal structure by Rognes (1985), and he suggested synapomorphies for the monophyly of group *Trypocalliphora* +

(*Protocalliphora* + *Protophormia* (including *Boreellus*) + *Phormiata*) (Fig. 3.1-B). Several authors (Zumpt, 1965; Sabrosky, 1967; Sabrosky *et al*., 1989) included *Trypocalliphora* in *Protocalliphora*. However, in an examination of *Trypocalliphora* specimens used by Sabrosky *et al.* (1989), Whitworth (2003) did not find the reported prothoracic fringe, reportedly characteristic of *Protocalliphora*. Based on adult morphology, Rognes (1991) concluded that *Trypocalliphora* + *Protocalliphora* is monophyletic. However he did not advocate synonymizing the generic names given that the two are rather distinct in form and larval habit. Sabrosky *et al*. (1989) argued that differences in the larval habits of *Protocalliphora* and *Trypocalliphora,* although generally true, are not fixed. Feeding *Trypocalliphora* have been found in the nest material, and some *Protocalliphora* have been found embedded in host tissue. Molecular systematic analysis based on a limited number of genera found that *Trypocalliphora* + *Protocalliphora* was a monophyletic group (Fig. 3.1-C) and *Trypocalliphora braueri* (Hendel) did not fall within *Protocalliphora* (Whitworth *et al.*, 2007).

Resolution of all of the above taxonomic issues would be aided by a robust phylogeny of the Chrysomyinae. In this dissertation we propose one based on the nuclear carbamoylphosphate synthetase (CPS) and mitochondrial cytochrome oxidase subunit one (COI) genes.

3.2 Materials and Methods

3.2.1. Specimens and DNA extraction

A total of 17 exemplar species belonging to 10 genera of Chrysomyinae were included in this study. We were unable to obtain the rare monotypic chrysomyine genera *Chrysopyrellia* and *Phormiata*. As outgroups, we included four species belonging to the calliphorid subfamilies Bengaliinae (*Verticia orientalis* Malloch), Toxotarsinae (*Sarconesia versicolor* Bigot), Luciliinae (*Lucilia sericata* (Meigen)) and Calliphorinae (*Calliphora vomitoria* (Linnaeus)). All specimens, except *Protocalliphora occidentalis* Whitworth and *Protocalliphora sialia* Shannon and Dobroscky, were identified morphologically by the collector (Table 3.1). *Protocalliphora occidentalis* and *Protocalliphora sialia* were identified by Dr. T. L. Whitworth (Whitworth Pest Solutions Inc., USA).

TABLE 3.1: Specimen details, collection information and GenBank accession numbers.

Sr. #	Species name	Location	Date	Voucher #	Collected by	Accession number	
						CPS	COI
1	*Chrysomya putoria* (Weidemann, 1830)	Near Chilibre, Panama	03/IX/1995	WVU2009-017-3	J. Mendez	FJ169353[g]	FJ195384[g]
2	*Chrysomya megacephala* Fabricius, 1794	Guam	31/IV/2002	WVU2009-017-9	P. Erwin	FJ169350[g]	AF092761[g]
3	*Chrysomya rufifacies* (Macquart, 1843)	Montgomery, AL, USA	05/X/2005	WVU2009-017-13	J.D. Wells	FJ169341[g]	AF083658[g]
4	*Chrysomya bezziana* Villeneuve,1914	Institute Haiwan, Malaysia	09/XI/2001	WVU2009-017-17	J. Stevens	FJ169351[g]	AF295548[g]
5	*Cochliomyia macellaria* (Fabricius,1775)	Laboratory colony originated from Morgantown, WV, USA	14/IX/2006	WVU2009-017-19	B. Singh	FJ169333[g]	AF295555[g]
6	*Cochliomyia hominivorax* (Coquerel, 1858)	Brazil	N/A	N/A	T.T. Torres	FJ169334[g]	AF260826[g]
7	*Compsomyiops callipes* Bigot,1877	CA, Contra Costa CO Martinez.	30/VI/1999	WVU2009-017-22	S. D. Wicks	HM639979	AF295549[g]
8	*Compsomyiops fulvicrura* Robineau-Desvoidy, 1830	N/A	N/A	RMBR#102682	N/A	FJ025571[g]	FJ025607[g]
9	*Chloroprocta idioidea* Robineau-Desvoidy, 1830	Brasilia, DF, Brazil	17/VIII/2009	WVU2009-017-23	C. Kosmann	HM639980	HM639976
10	*Hemilucilia segmentaria* (Fabricius,1805)	Campinas, Brazil	18/V/1998	WVU2009-017-24	Lucilia Carvalho	N/A	HM639977
11	*Paralucilia paraensis* (Mello, 1969)	Manaus, AM, Brazil	12/X/2008	WVU2009-017-25	Alex De Souza	N/A	HM639978
12	*Phormia regina* (Meigen, 1826)	Laboratory colony originated from Pullman, WA, USA	29/V/2008	WVU2009-017-18	C. J. Picard	FJ169331[g]	AF295550[g]

13	*Protophormia terraenovae* Robineau-Desvoidy, 1830	Mandore Co. CA, USA	3/VI/2002	WVU2009-017-26	J.D. Wells	HM639981	AF295553[g]
14	*Protophormia atriceps* Zetterstedt, 1845	Sognefjell, Norway	N/A	N/A	N/A	N/A	AF295560[g] AF295561[g]
15	*Trypocalliphora braueri* (Hendel, 1901)	Wheeling, WV, USA	Summer, 2004	WVU2009-017-27	T. L. Whitworth	HM639982	HM639974
16	*Protocalliphora sialia* Shannon and Dobroscky, 1924	Near Saskatoon, SK, Canada	10/VII/2004	WVU2009-017-28	Marie-line Gentes	HM639983	AF295559[g]
17	*Protocalliphora occidentalis* Whitworth, 2003	Near Prince George, BC, Canada.	Summer, 2004	WVU2009-017-29	Russ Dawson	HM639984	HM639975
18	*Verticia orientalis* Malloch, 1927	Selangor, Malaysia	20/II-20/III/1997	WVU2009-017-32	B. Viklund	HQ248106	HQ248105
19	*Lucilia sericata* (Meigen, 1826)	Morgantown, WV, USA	13/V/2005	WVU2009-017-20	J.D. Wells	FJ169332[g]	AJ417717 [g]
20	*Sarconesia versicolor* Bigot, 1857	N/A	N/A	RMBR # 103610	N/A	GQ409291[g]	GQ409319[g]
21	*Calliphora vomitoria* (Linnaeus, 1758)	Morgantown, WV, USA	14/VIII/2005	WVU2009-017-21	J. Hall	FJ169335[g]	GQ223336 [g]

All specimens except *Paralucilia paraensis* (Mello) were preserved live in 95% ethanol. *Paralucilia paraensis* was preserved in 70% ethanol. DNA was extracted from thoracic tissues (with the exception of *P. paraensis*, see below) using an organic extraction method as described in Singh *et al.* (2010). DNA extraction from *Paralucilia paraensis* was done from crushed head tissue using a Qiagen DNeasy blood and tissue Kit (Qiagen, Valencia, CA) as per the manufacturer's protocol. A NanoDrop™ 1000 spectrophotometer (Thermo Scientific, Waltham, MA) was used for DNA quantification. Voucher specimens from each individual were deposited in West Virginia University's Entomological Collection (See Table 3.1 for voucher ID).

3.2.2. PCR amplification

PCR amplification was carried out for 850 bp of the *nuclear carbamoylphosphate synthetase* (*CPS*) gene and 1435 bp of the mitochondrial *cytochrome oxidase subunit one* (*COI*) gene. *CPS* was amplified using primers listed in Singh *et al.* (2010) using the PCR program described in Moulton & Wiegmann (2004). For *COI* gene amplification, we followed the protocols in Wells & Sperling (1999). All primers were purchased from either Integrated DNA Technologies, Inc. (Coralville, IA) or Operon Biotechnologies, Inc. (Huntsville, AL). Other PCR reagents were purchased from Promega Corp. (Madison, WI). PCR reactions for both CPS and COI genes were prepared for 25 µl final volume using PCR reagents following Singh *et al.* (2010). All PCR amplifications were carried-out in a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA).

3.2.3. Sequencing

The amplified products were cleaned by using ExoSAP-IT® (USB Corporation, Cleveland, Ohio) as per manufacturer's instructions. Direct sequencing was performed on the purified product with the same primers used for PCR and using a Big Dye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The sequencing protocol was as described in Singh *et al.* (2010). The sequencing product was separated on an Applied Biosystems 3130xl genetic analyzer (Foster City, CA). Sequence files were edited and aligned using Sequence Navigator (Applied Biosystems, Foster City, CA). *COI* gene sequence of *Lucilia sericata* (AJ417717; Stevens *et al.*, 2002) and *CPS* gene sequence of *Epalpus signifier* (Walker) (AY280680;

Moulton & Wiegmann, 2004) were used as a reference sequences for alignment. All sequences were indel free and simple to align manually. New CPS and COI DNA sequences were submitted to the GenBank database (See Table 3.1 for accession numbers).

3.2.4. Phylogenetic analyses

A total of 2285 bp of concatenated data from both genes was used for phylogenetic analysis by maximum parsimony (MP), maximum likelihood (ML) and Bayesian methods (BA). Different phylogenetic methods were used to test robustness of the tree obtained under each set of assumptions. MP analysis was performed for 1000 bootstrap replications using an equally weighted heuristic search and tree-bisection-reconnection (TBR) branch swapping options in PAUP 4.0b10 (Swofford, 2002). Bremer support (Bremer, 1994) was calculated as branch support for the most parsimonious tree using TreeRot.v3 (Sorenson & Franzosa, 2007) and PAUP4.0b10 (Swofford, 2002). ML analysis was performed for 1000 bootstrap replications using the equally weighted heuristic search option in PAUP4.0b10 (Swofford, 2002). A general time reversible model with variable sites and discrete gamma distribution (GTR+I+r) was selected as a best-fit model for ML and BA analysis. Best-fit model for ML and BA analysis was selected using the Akaike Information Criterion (AIC) in Modeltest 3.7 (Posada & Crandall, 1998) and in MrModelTest 2.3 (Nylander, 2004) respectively. BA was performed for two concurrent sets of six independent chains (5 heated and one cold) for 1.5 million generations sampling every 100 generations using the default parameters in Mr. Bayes v.3.1.2 (Huelsenbeck & Ronquist, 2001). After observing the likelihood plot, out of 15001 trees, 12000 trees were discarded as the burnin, and remaining trees (3001) were used for generation of the 50% majority rule consensus tree. Posterior probabilities were calculated as branch support from all trees that remained after setting burnin. All trees were edited using software MrEnt v.2.2 (Zuccon & Zuccon, 2010).

3.3 Results

We were unable to amplify *CPS* for *Paralucilia paraensis* and *Hemilucilia segmentaria* (Fabricius)**.** ML, BA and MP analyses resulted in almost identical trees, but some nodes in the MP tree were unresolved (Fig. 3.2). All analyses produced a

monophyletic Chrysomyinae and a paraphyletic Chrysomyini. Interestingly, the Neotropical genera *Chloroprocta* + *Hemilucilia* + *Paralucilia* + *Compsomyiops* + *Cochliomyia* were strongly supported as a monophyletic group in all analyses, suggesting a single ancestral colonization of the New World tropics. *Protocalliphora* + *Trypocalliphora* + *Phormia* + *Protophormia* + *Chrysomya* was also monophyletic in all analyses. Monophyly of Phormiini was supported by BA/ML but this was not resolved by MP. Similarly, sister groups *Chloroprocta* + *Hemilucilia* and *Cochliomyia* + *Compsomyiops* were supported in BA and ML, but these were not resolved in MP. In all analyses *Protocalliphora* + *Trypocalliphora* was clearly a monophyletic group, and all genera for which we examined >1 species were monophyletic (Fig. 3.2).

3.4 Discussion

Given these results and Rognes' (1991) morphological analysis, we conclude that the evidence currently favours chrysomyine monophyly. This conflicts with Kutty *et al.* (2010), who found that Bengaliinae was the sister group of Chrysomyinae excluding *Protocalliphora*. Differences in experimental design between Kutty *et al.* (2010) and this study make it difficult to explain the different topology: they used more genes, including short sections of *COI* and *CPS* for many taxa, but only five chrysomyine species and, from the standpoint of Chrysomyinae, many more outgroups. However, concerning what was only a small portion of Kutty *et al*.'s phylogeny, we have confidence in our more complete taxon sample. Because we included only one species each from outgroup subfamilies Bengaliinae, Toxotarsinae, Calliphorinae, and Luciliinae, this was not a strong test of broader aspects of calliphorid systematics (Rognes, 1997), but our results do agree with Kutty *et al.* (2010) in that Toxotarsinae is more closely related to Calliphorinae than to Chrysomyinae, as was sometimes indicated by morphology (Pape, 1992; Rognes, 1997). A close relationship between Toxotarsinae and Calliphorinae was suggested also by Greenberg & Szyska (1984) based on larval morphology and developmental rate.

The subfamily Chrysomyinae includes two major lineages that do not correspond to the traditional tribal classification. Instead chrysomyine evolutionary history reflects geographic distribution: Neotropical and Holarctic/Paleotropical (and perhaps originally Old World).

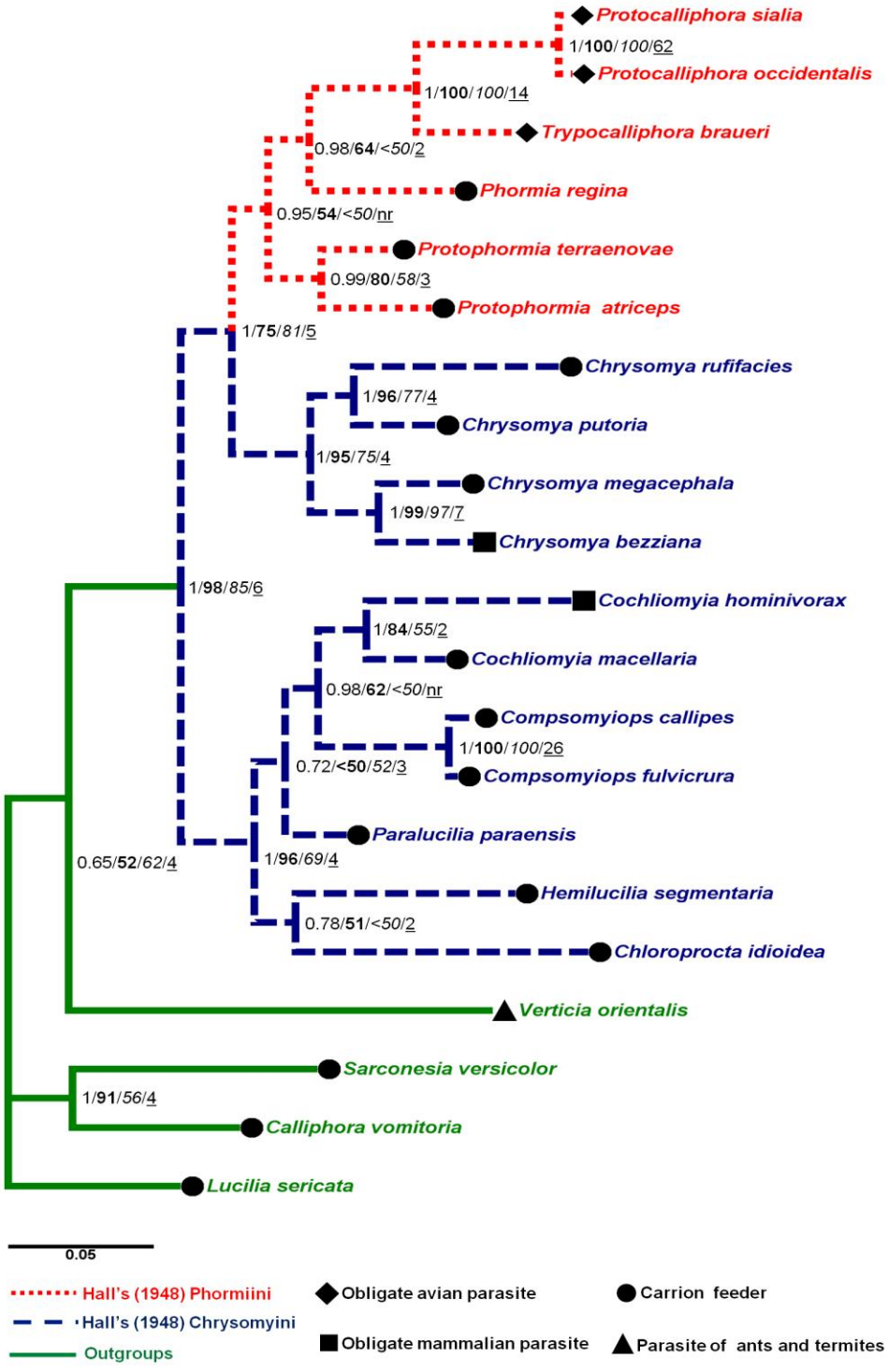

FIGURE 3.2: Bayesian phylogram of the Chrysomyinae based on 2285 bp of combined COI and CPS DNA sequences. Branch support values: Standard font = Bayesian posterior probability; Bold = maximum likelihood % bootstrap; Italics = maximum parsimony % bootstrap; Underlined = Bremer support in maximum parsimony; nr = node not recovered by maximum parsimony.

If our phylogenetic conclusions are correct, then a taxonomic subdivision of the Chrysomyinae would require tribal names for each of these two major lineages. However, we believe that a better policy would be to drop the use of chrysomyine tribes altogether (see also Rognes 1991), because a revised system (Article 23, 24 & 29.4, ICZN, 1999) would involve a new and confusing definition for at least one taxonomic name familiar to many non-systematists who are interested in these flies. Both Phormiini (Phorminae of Shannon, 1923; Sabrosky, 1999) and Chrysomyini (Shannon, 1923; Sabrosky, 1999) have equal priority for *Protocalliphora* + *Trypocalliphora* + *Phormia* + *Protophormia* + *Chrysomya.* Either Cochliomyiini (Lehrer, 1970) or Hemiluciliini (Lehrer, 1970) would designate the Neotropical lineage.

Although *Phormia regina* has been difficult to objectively group with other traditional Phormiini based on anatomy (Dear, 1985; Rognes 1985), the association was unambiguous in our analyses.

The most parsimonious explanation is that bird parasitism (in *Protocalliphora* and *Trypocalliphora*) evolved once within the Chrysomyinae. Whether these two bird blow fly lineages are distinct enough to be separate genera is unclear from the molecular data in that the branches are of similar length. Therefore, this would instead depend on judgement of the importance of differences between *Protocalliphora* and *Trypocalliphora* in morphology and larval habits (Rognes, 1991).

CHAPTER 4: Molecular Phylogeny of the Oestridae (Diptera: Oestroidea).

Singh, B., Otranto, D. & Wells, J. D. (In preparation) Is Oestridae (Diptera: Oestroidea) a natural assemblage?

4.1 Introduction

Oestridae is a small (≈150 species) but well known family of the Oestroidea. All oestrid larvae cause obligate myiasis of mammals including humans, with high level of host specificity (Catts, 1982; Wood, 1987a). Obligate myiasis by these flies results in huge economical loss to the livestock industry both in developed and developing countries (Otranto *et al.*, 2003). These flies are mainly distributed in Africa and central Asia but have a limited distribution in North America (Catts, 1982; Wood, 1987a).

Most recent author, recognize four subfamilies (Cuterebrinae, Gasterophilinae, Hypodermatinae, and Oestrinae) within the Oestridae (Wood, 1987a; Pape, 1992; 2001; Otranto *et al.*, 2003). Cuterebrinae larvae are mainly subcutaneous parasites of rodents and lagomorphs, whereas Gasterophilinae larvae are gut parasites of horses, zebras, rhinos and elephants. Like Cuterebrinae, Hypodermatinae larvae also penetrate the skin, but they lack the small plate-like spines on the integument, characteristic of the Cuterebrinae. Oestrinae larvae parasitize the host respiratory passages. Most species are oviparous with the exception of the members of the subfamily Oestrinae, which are larviparous (Catts, 1964; Anderson, 1975; Papavero, 1977; Anderson & Nilssen, 1990). Larvae develop within the host for several weeks, and, upon reaching maturity, they leave the host to pupate in the soil (Otranto *et al.*, 2003).

Although Oestridae is one of the most investigated families of Diptera, only a few studies were dedicated for the systematics of these flies (Zumpt, 1957; Papavero, 1977; Sabrosky, 1986; Pape, 1992; 2001; Otranto *et al.*, 2003; Kutty, 2008; Kutty *et al.*, 2010; Pollock, 2010). These studies raised many systematic questions, which are still unanswered.

The first and the most important question is whether Oestridae is monophyletic. It seemed that the morphological evidence for monophyly was overwhelming (Pape, 1992; Pape, 2001) but recent comparative anatomical study suggested that Glossinidae

+ Hippoboscidae is nested withn Oestridae, and that this entire lineage belongs to the Acalyptratae (Pollock, 2010). However, molecular systematic analyses strongly support placement of Glossinidae, Hippoboscidae and Oestridae within Calyptratae (Nirmala *et al.,* 2001; Peterson *et al.*, 2007; Kutty, 2008; Kutty *et al.*, 2010; Wiegmann *et al.*, 2011) although they differ on monophyly of the Oestridae. Nirmala *et al.* (2001) observed Oestrinae + (Gasterophilinae + Hypodermatinae) more closely related to either Muscidae or Tachinidae than to Cuterebrinae, in combined *16S rRNA* and *18S rRNA* genes analysis. Kutty (2008) included only one oestrid, *Cuterebra baeri* Shannon& Greene in a molecular systematic analysis of Calyptratae. Although it fell within Oestroidea, she excluded other members of the Oestridae from her analysis, because they showed affinity with members of the superfamily Hippoboscoidea and had leaf-stability value below the threshold set at 75.

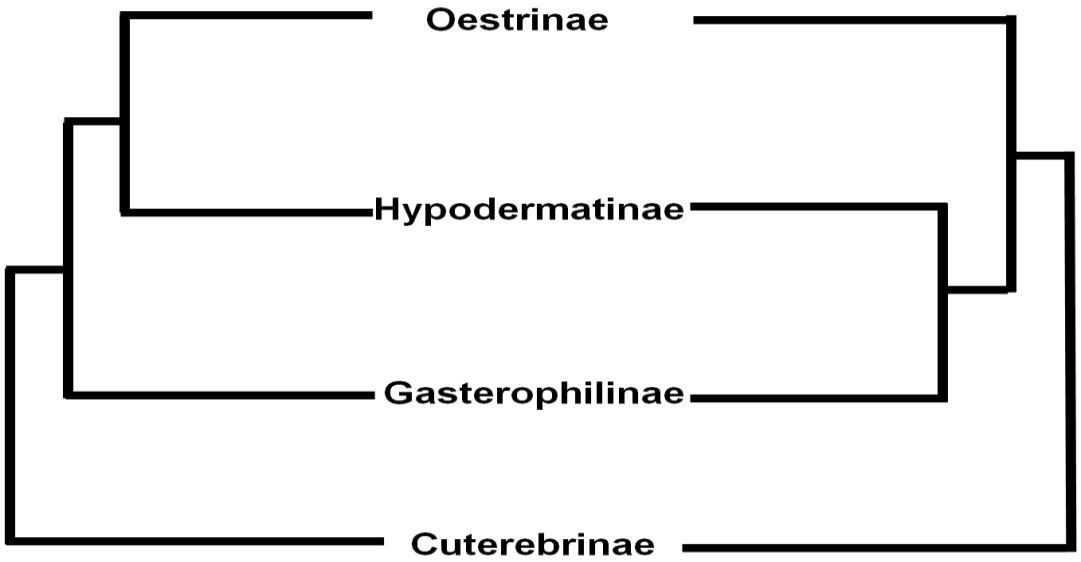

FIGURE 4.1: Published alternate hypotheses of phylogenetic relationship between different subfamilies of the Oestridae. Left: based on morphological data (modified from Pape, 2001) and, right: based on mitochondrial *cytochrome oxidase subunit one* gene (modified from Otranto *et al.*, 2003).

Otranto *et al.* (2003) observed monophyletic Oestridae based on 688 bp of mitochondrial *cytochrome oxidase subunit one* (*COI*) gene but it was not a robust test for the monophyly of the Oestridae because all species that caused non-monophyly of the Oestridae in Nirmala *et al.* (2001) were not included as outgroups in their analysis.

Even if one accepts oestrid monophyly, there are conflicting hypotheses of the subfamily relationships (Pape, 1992; 2001; Nirmala *et al.*, 2001; Otranto *et al.*, 2003). Morphological analysis suggests Hypodermatinae + Oestrinae as a sister group of Gasterophilinae (Pape, 1992; 2001), whereas molecular analysis suggests Hypodermatinae + Gasterophilinae as a sister group of Oestrinae (Nirmala *et al.*, 2001; Otranto *et al.*, 2003) (Fig. 4.1).

Resolution of all of the above taxonomic issues requires extensive molecular systematic analysis based on both mitochondrial and nuclear genes. Here we proposed one based on mitochondrial cytochrome oxidase subunit one (*COI*) and nuclear 28S ribosomal RNA (*28S rRNA*) and Elongation factor one alpha (*EF1α*) genes.

4.2 Materials and Methods

4.2.1. Specimens and DNA extraction

A total of 20 ingroup taxa and 8 outgroup taxa were included in this study. Extracted DNA from all ingroup taxa, except *Cuterebra fontinella* Clark, *Cuterebra* spp. and *Hypoderma lineatum* De Villers, were supplied by the second author. DNA was extracted from a teneral adult of the *Cuterebra* spp. using Qiagen DNeasy blood and tissue Kit (Qiagen, Valencia, CA) as per the manufacturer's protocol. Species of Glossinidae, Muscidae, Tachinidae, Rhinophoridae, Mystacinobiidae, Sarcophagidae and Calliphoridae were included as outgroups (See Table 4.1) (Nirmala *et al.*, 2001; Pape, 2001; Kutty, 2008).

4.2.2. PCR amplification

PCR amplifications were carried out for 688 bp of the mitochondrial *cytochrome oxidase subunit one* (*COI*) gene, 550 bp of *elongation factor one alpha* (*EF1α*) gene and for approximately 750 - 800 bp of D1-D2 region of the *28S rRNA* gene. *COI*, *Ef1α,* and *28S rRNA* genes were amplified by using primers and protocol as described in Wells & Sperling (1999), McDonagh (2009), and Stevens & Wall (2001) respectively. *EF1α* genes of *Hypoderma tarandi* Brauer and *Hypoderma bovis* (Linnaeus) were amplified by using newly designed *Hypoderma* Latreille specific *EF1α* primers (hn19f : 5'- AAC TCG CCC AAC TGA CAA AC -3' and hn503r: 5'- CAG CGT CAC CAG ATT TCA AG-3').

TABLE 4.1: List of species included in this study.

Sr. #	Species name	Family	Subfamily	GenBank accession numbers		
				COI	28S rRNA	EF1α
Hippoboscoidea						
1	*Glossina morsitans* Westwood, 1851	Glossinidae	Glossininae	JF439541[a]	JF439566[a]	JF439518[a]
Muscoidea						
2	*Musca domestica* Linnaeus, 1758	Muscidae	Muscinae	AF104622[a]	AJ551427[a]	DQ567113[a]
Oestroidea						
3	*Mystacinobia zelandica* Holloway, 1976	Mystacinobiidae	NA	JF439542[a]	JF439567[a]	NA
4	*Epalpus signifer* (Walker,1849)	Tachinidae	Tachininae	JF439543[a]	JF439566[a]	JF439519[a]
5	*Rhinophora lepida* (Linnaeus, 1824)	Rhinophoridae	NA	JF439546[a]	JF439571[a]	JF439522[a]
6	*Sarcophaga crassipalpis* Macquart, 1839	Sarcophagidae	Sarcophaginae	JF439547[a]	JF439567[a]	JF439523[a]
7	*Calliphora vomitoria* (Linnaeus, 1758)	Calliphoridae	Calliphorinae	GQ223336[a]	AJ300133[a]	JF439527[a]
8	*Chrysomya rufifacies* (Macquart, 1843)	Calliphoridae	Chrysomyinae	AF083658[a]	AJ551436[a]	JF439532[a]
9	*Hypoderma bovis* (Linnaeus, 1758)	Oestridae	Hypodermatinae	AF497761[a]	New	New
10	*Hypoderma lineatum* (De Villers, 1789)	Oestridae	Hypodermatinae	AF497762[a]	JF718830[a]	JF439526[a]
11	*Hypoderma tarandi* (Linnaeus, 1758)	Oestridae	Hypodermatinae	AF497764[a]	New	New
12	*Hypoderma actaeon* Brauer, 1858	Oestridae	Hypodermatinae	AF497765[a]	NA	NA
13	*Hypoderma diana* Brauer, 1858	Oestridae	Hypodermatinae	AF497763[a]	NA	NA
14	*Przhevalskiana silenus* Brauer, 1858	Oestridae	Hypodermatinae	AF497766[a]	New	New
15	*Oestrus ovis* (Linné 1761)	Oestridae	Oestrinae	AF497767[a]	AJ551428[a]	New
16	*Rhinoestrus phacochoeri* Rodhain & Bequaert, 1915	Oestridae	Oestrinae	AF497772[a]	New	New
17	*Rhinoestrus usbekistanicus* (Gan, 1947)	Oestridae	Oestrinae	AF497771[a]	NA	NA
18	*Cephenemyia trompe* (Modeer, 1786)	Oestridae	Oestrinae	AF497769[a]	New	New
19	*Cephenemyia stimulator* (Clark, 1815)	Oestridae	Oestrinae	AF497768[a]	New	NA
20	*Cephenemyia ulrichii* Brauer, 1862	Oestridae	Oestrinae	AF497770[a]	New	NA
21	*Gasterophilus intestinalis* (de Geer, 1776)	Oestridae	Gasterophilinae	AF497773[a]	AJ551429[a]	NA
22	*Gasterophilus pecorum* (Fabricius, 1794)	Oestridae	Gasterophilinae	AF497776[a]	New	New
23	*Gasterophilus haemorrhoidalis* (Linnaeus, 1758)	Oestridae	Gasterophilinae	AF497774[a]	NA	NA
24	*Gasterophilus nasalis* (Linnaeus, 1758)	Oestridae	Gasterophilinae	AF497775[a]	NA	NA

25	*Cuterebra fontinella* Clark, 1827	Oestridae	Cuterebrinae	JF439549 [a]	JF439574 [a]	JF439525 [a]
26	*Cuterebra baeri* Shannon& Greene, 1926	Oestridae	Cuterebrinae	AF497777 [a]	New	GQ409458 [a]
27	*Cuterebra jellisoni* Curran, 1942	Oestridae	Cuterebrinae	AF497778 [a]	NA	NA
28	*Cuterebra spp.*	Oestridae	Cuterebrinae	New	New	New

[a] = DNA sequences downloaded from GenBank.

NA= Not available.

These primers were designed from *EF1α* sequence of *H. lineatum* using default options in the program Primer3 (Steve & Helen, 2000). All primers were purchased from either Integrated DNA Technologies, Inc. (Coralville, IA) or Operon Biotechnologies, Inc. (Huntsville, AL). PCR amplifications were carried out in 25 ul total volume containing 12.5 ul of 2X Promega Master Mix (Catalogue # M7505, Promega Corp., Madison, WI), 2 ul (10 pmol) of each primer (Integrated DNA Technologies, Inc. Coralville, IA), 1-3 ul (≈ 10 - 30 ng) of template DNA and remaining water. PCR amplifications were also performed in smaller total volume (10 µl-15 µl) for some precious taxa with less concentrated template DNA. All other reaction components were in the same proportions. GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA) was used for all PCR amplifications.

4.2.3. Sequencing and sequence alignments

The amplified products were cleaned by using ExoSAP-IT® (USB Corporation, Cleveland, Ohio) as per manufacturer's instructions. Direct sequencing was performed on the purified product with the same primers used for PCR and using a Big Dye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The sequencing protocol was as described in Singh *et al.* (2010). The sequencing product was separated on an Applied Biosystems 3130xl genetic analyzer (Foster City, CA). Sequence files were edited in Sequence Navigator (Applied Biosystems, Foster City, CA). *COI* and *EF1α* genes were aligned manually in Sequence Navigator (Applied Biosystems, Foster City, CA) using *COI* gene sequence of *Lucilia sericata* (Meigen) (AJ417717; Stevens *et al.*, 2002) and *EF1α* gene sequence of *Chrysomya rufifacies* (Macquart) (JF439532; unpublished) respectively. All *COI* and *EF1α* gene sequences were easy to align and indel free. Alignment of all *28S rRNA* gene sequences was carried out using the program Muscle (Edgar, 2004) as implemented in MEGA 5 (Tamura *et al.*, in press) using default parameters. After multiple sequence alignment, ambiguous hypervariable regions of *28S rRNA* gene were excluded from the alignment and only 716 bp of *28S rRNA* gene was used for further phylogenetic analysis. All newly generated DNA sequences are indicated as a new in Table 4.1.

4.2.4. Phylogenetic analyses

Phylogenetic analyses were carried out using maximum parsimony (MP), maximum likelihood (ML) and Bayesian methods (BA). Different phylogenetic methods were used to test robustness of the tree obtained under each set of assumptions. MP analysis was performed for 1000 bootstrap replications using an equally weighted heuristic search and tree-bisection-reconnection (TBR) branch swapping options in PAUP 4.0b10 (Swofford, 2002). ML analysis was performed for 100 (*COI* gene) and 1000 (*Ef1α* and *28S rRNA* genes) bootstrap replications using the equally weighted heuristic search option in PAUP4.0b10 (Swofford, 2002). Best-fit models (SYM+G for *EF1α*, GTR+I+G for *COI* and TVM+I+G for *28S rRNA* gene) for ML analysis were selected using Akaike Information Criterion (AIC) in Modeltest 3.7 (Posada & Crandall, 1998). Similarly, best-fit models (SYM+G for *EF1α,* GTR+I+G for *COI* and *28S rRNA* gene*s*) for BA were selected using AIC in MrModeltest 2.3 (Nylander, 2004). BA was performed for two concurrent sets of six independent chains (5 heated and one cold) for 1.5 million generations (*EF1α* & *28S rRNA* gene*s*) and for 3 million generation (*COI* gene), sampling every 100 generations using the default parameters in Mr. Bayes v.3.1.2 (Huelsenbeck & Ronquist, 2001). After observing the likelihood plot, 80% of the trees were discarded as the burnin, and the remaining 20% of the trees from each run were used for generation of the 50% majority rule consensus tree. Posterior probabilities were calculated as branch support from all trees that remained after setting burnin. All trees were edited using software MrEnt v.2.2 (Zuccon & Zuccon, 2010). Although nodes with Bayesian posterior probability (PP) ≥ 0.65 are shown on the trees (Figs. 4.2- 4.4), only nodes having PP ≥ 0.95 were deemed significant (Huelsenbeck & Ronquist, 2001). Phylogenetic analyses of concatenated data were not performed, because for many species nuclear genes-overlap was low (Table 4.1).

4.3 Results

These analyses failed to resolve the question of Oestridae monophyly (Figs. 4.2, 4.3, 4.4). In general there was strong support for the monophyly of the subfamilies, although this was less clear for Oestrinae on the *28S rRNA* tree (Figs. 4.3, 4.4), and only one gasterophiline *EF1α* sequence was included.

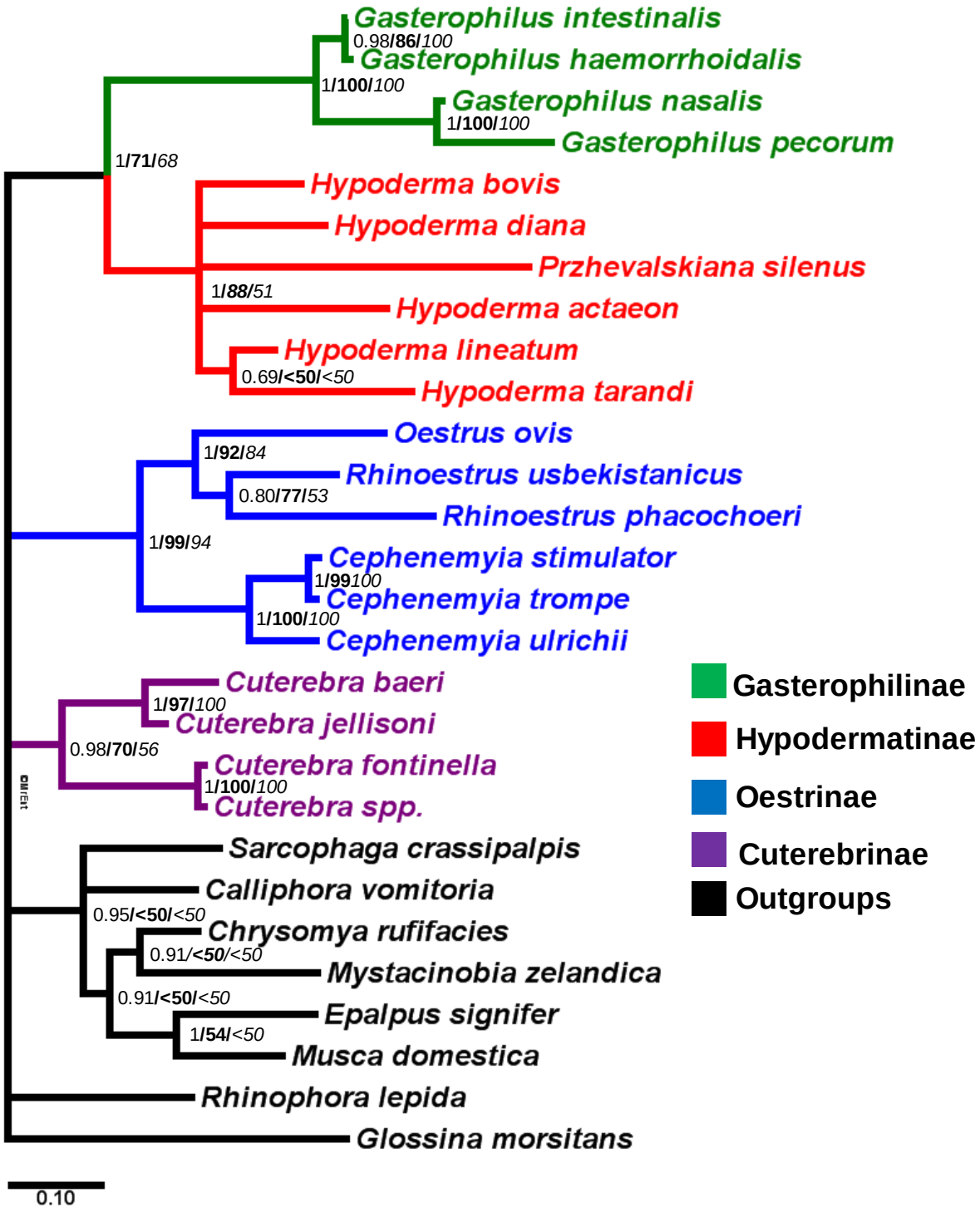

Figure 4.2: Bayesian phylogram of the Oestridae based on 688 bp of *COI* DNA sequences. Branch support values: Standard font = Bayesian posterior probabilities; Bold = maximum likelihood % bootstrap; Italics= maximum parsimony % bootstrap.

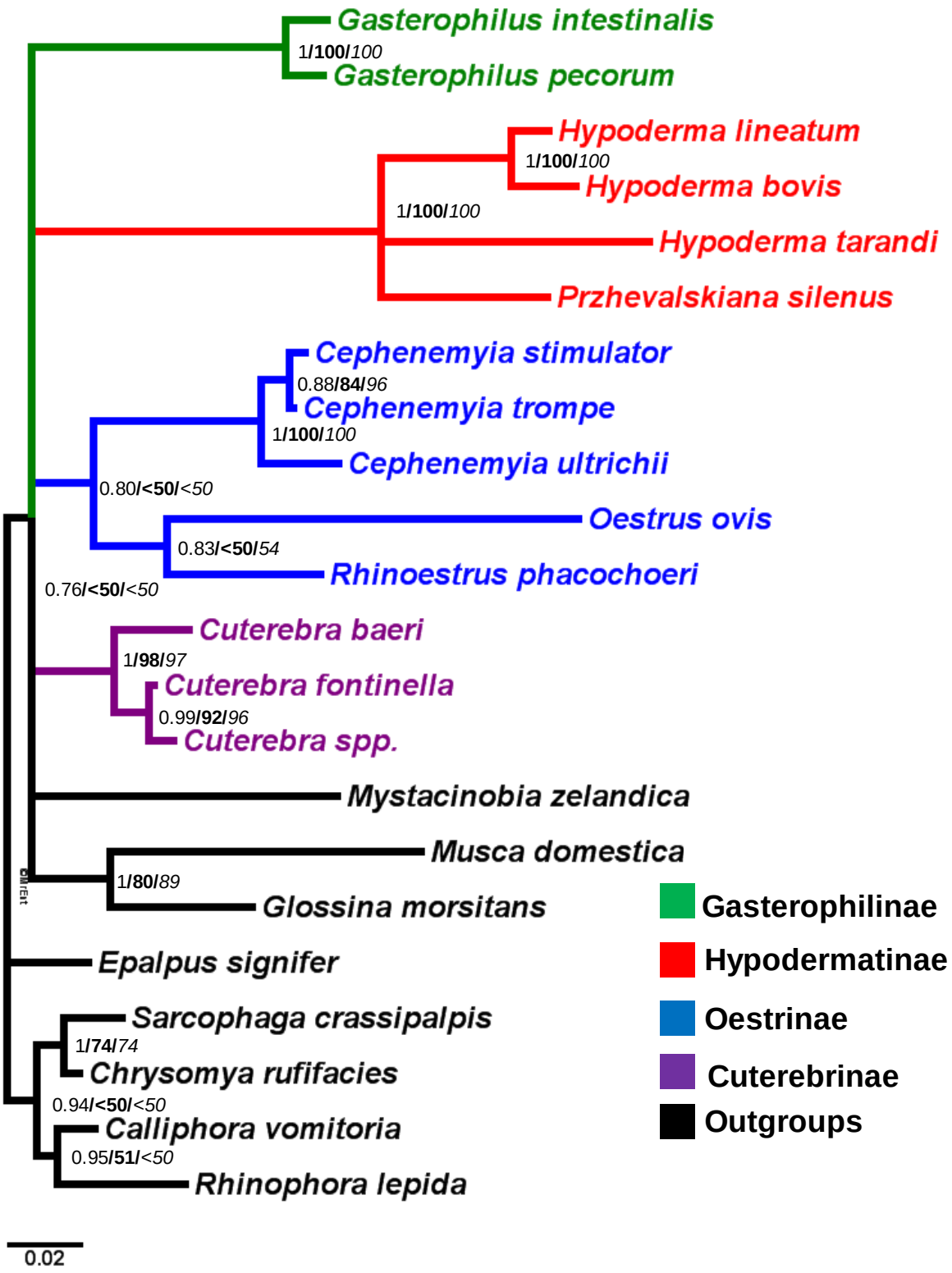

Figure 4.3: Bayesian phylogram of the Oestridae based on 716 bp of *28S rRNA* gene sequences. Branch support values: Standard font = Bayesian posterior probabilities; Bold = maximum likelihood % bootstrap; Italics= maximum parsimony % bootstrap.

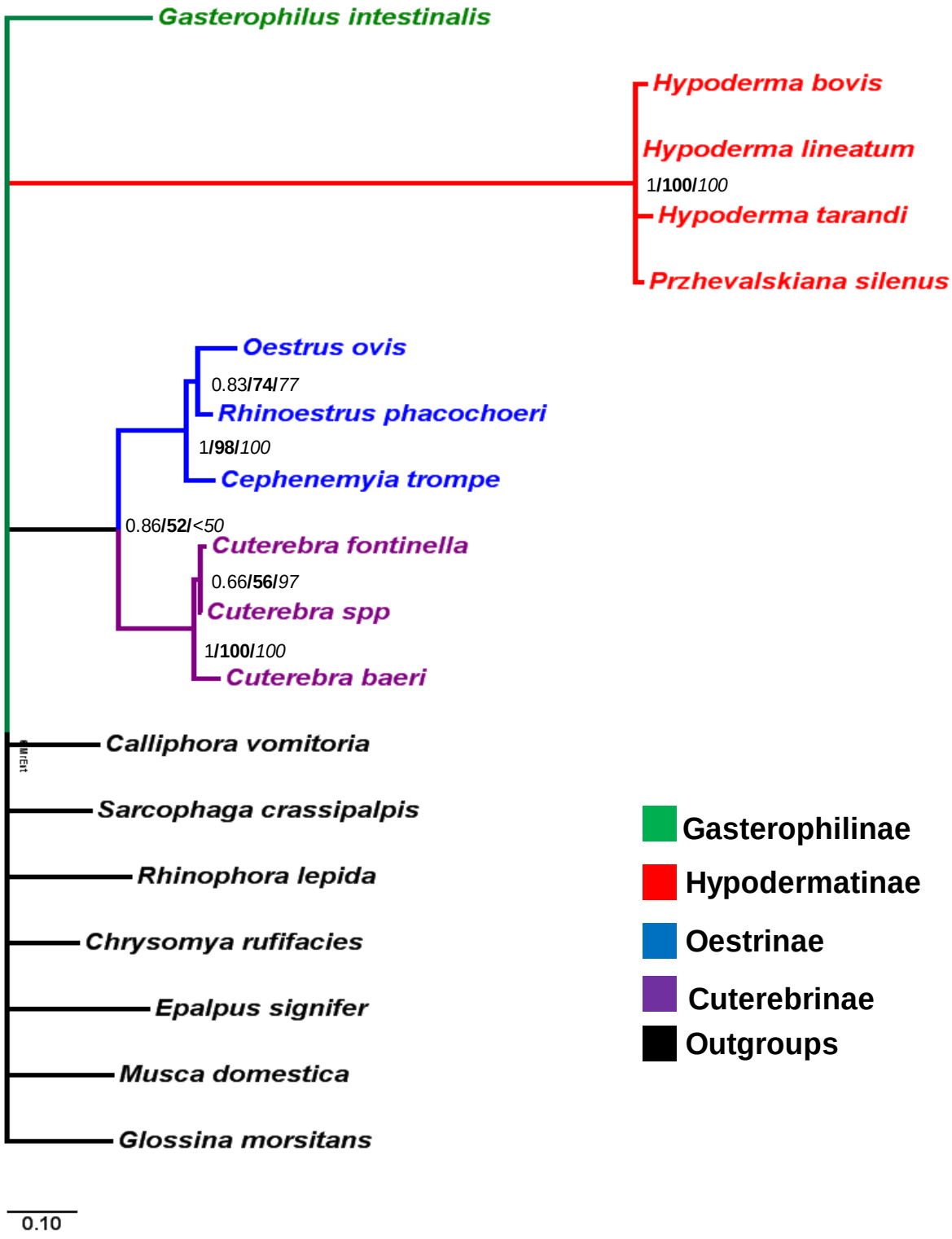

Figure 4.4: Bayesian phylogram of the Oestridae based on 550 bp of *EF1α* gene sequences. Branch support values: Standard font = Bayesian posterior probabilities; Bold = maximum likelihood % bootstrap; Italics= maximum parsimony % bootstrap.

As reported by Otranto *et al.* (2003), the *COI* gene supported a Gasterophilinae + Hypodermatinae lineage, but there was no other resolution of subfamilily relationships (Fig. 4.2). All genera for which we had more than one species were monophyletic with the exception of *Hypoderma*. Monophyly of *Oestrus* Linnaeus + *Rhinoestrus* Brauer was well-supported by *COI* (Fig. 4.2) but support was weak in nuclear gene analyses (Figs. 4.3, 4.4).

4.4 Discussion

There are several potential reasons why we didn't find good resolution at deeper nodes. First, it may be because of lack of sufficient phylogenetic signal, as these groups have diverged in a very short span of time (Wiegmann *et al.*, 2011). Second, because these lineages evolved at a much higher rate, they may have accumulated many homoplasious characters, which in turn is affecting resolution at deeper nodes (Hypsa, 2006). Third, because of high sequence heterogeneity among taxa, many PCR reactions failed and hence available data may not be sufficient for resolution at deeper nodes (Otranto *et al.*, 2003). Molecular systematic analysis with much larger data sets from different mitochondrial and nuclear genes may resolve deeper nodes of oestrid phylogeny, but it will be better strategy to explore alternate methods other than primary sequences such as mobile elements, micro-RNA, gene rearrangements etc. The well-supported sister group relationship between Glossinidae and Muscidae in *28S rRNA* gene analysis (Fig. 4.3) undermines hypotheses of a close relationship between Oestridae and Glossinidae (Kutty, 2008; Pollock, 2010). The unusually long-branch observed for Hypodermatinae in *Ef1α* gene analysis, suggests to us that Hypodermatinae is most probably the fastest evolving lineage of the Oestridae.

CHAPTER 5: Molecular Phylogeny of the Calliphoridae (Diptera: Oestroidea).

Singh, B., Pape, T. & Wells, J. D. (In preparation) Rhiniidae is a subfamily of Calliphoridae (Diptera: Oestroidea): Evidence from one mitochondrial and three nuclear genes.

5.1 Introduction

The Calliphoridae (blow flies) are a diverse group of flies of forensic, medical and veterinary importance. Approximately 1450 species of Calliphoridae (including 400 species of Rhiniinae) are known from all continents except Antarctica (Verves, 2005, Kutty *et al.*, 2010). Adult calliphorids are known to frequent flowers, feces, carrion and wounds for nutrition. They also serve as vectors of disease, where they transport bacteria, viruses, protozoans and helminths (Greenberg, 1971). Most blow flies are oviparous but some are unilarviparous (Helicoboscinae, Mesembrinellinae, Ameniinae and Phumosiinae) or multilarviparous (e.g. *Onesia* Robineau-Desvoidy*, Bellardia* Robineau-Desvoidy*, Eggisops* Rondani etc.). The majority of blow fly larvae are carrion breeders and hence play an important role both as an indicator species in forensic entomology and as prominent decomposers (Schumann, 1965; Smith, 1986). Some blow fly larvae cause obligate myiasis to vertebrates (e.g. *Chrysomya bezziana* Villeneuve, *Cochliomyia hominivorax* (Coquerel), *Protocalliphora* Hough*, Trypocalliphora* Peus, *Auchmeromyia* Brauer & Bergenstamm) whereas a few other are endoparasitic on invertebrates (e.g. *Pollenia* Robineau-Desvoidy, *Onesia* etc.) (Rognes, 1998; Stevens, 2003).

A variety of calliphorid subfamilies have been recognized (Lehrer, 1970; Hennig, 1973; Pont, 1980; Kurahashi, 1989), but most recent authors use Ameniinae, Auchmeromyiinae, Bengaliinae, Phumosiinae, Mesembrinellinae, Helicoboscinae, Toxotarsinae, Calliphorinae, Luciliinae, Melanomyinae, Polleniinae, Rhiniinae, Chrysomyinae, Aphyssurinae, and Prosthetosomatinae (Pape, 1992; Rognes, 1991; 1997; Norris, 1999; Kutty *et al.*, 2010). The monophyly of each subfamily is well supported based on morphological characters, but the placement of Mesembrinellinae, Rhiniinae, Toxotarsinae, Bengaliinae, Helicoboscinae, Calliphorinae and Polleniinae are uncertain (Pape, 1992; Rognes, 1991; 1997; Kutty *et al.*, 2010). Recent molecular

systematic analysis suggested non-monophyly of Calliphorinae (Kutty *et al.*, 2010; Singh & Wells, 2011).

Most importantly, the monophyly of Calliphoridae itself has long been in serious doubt (Griffith, 1982; McAlpine, 1989; Pape, 1992; Rognes, 1997; Kutty *et al.*, 2010). Because calliphorids are essentially the oestroid species that could not be assigned to another family, the phylogenetic status of the Calliphoridae is the central systematics problem for the entire superfamily (McAlpine, 1989; Rognes, 1997). Oestroidea includes the Mystacinobiidae (New Zealand bat flies), Axiniidae (axe flies), Rhinophoridae (woodlouse flies), Tachinidae (tachinid flies), Sarcophagidae (flesh flies), Oestridae (bot flies), and Calliphoridae (blow flies) (Rognes, 1997). Recent molecular systematic analysis suggested that the unclassified McAlpine's fly is a member of the Oestroidea (Kutty *et al.*, 2010). Morphological analysis supports the monophyly of the non-calliphorid oestroid families (Pape, 1992; Rognes, 1997). Monophyly of Calliphoridae is uncertain as some taxonomists believe that Calliphoridae is a monophyletic assemblage (Fig. 5.1) (Lehrer, 1970; McAlpine, 1989; Pape, 1992; Stevens, 2003), while others believe otherwise in absence of satisfactory autapomorphic characters for the whole group (Griffiths, 1982; Rognes, 1997; Pape & Arnaud, 2001; Kutty *et al.*, 2010).

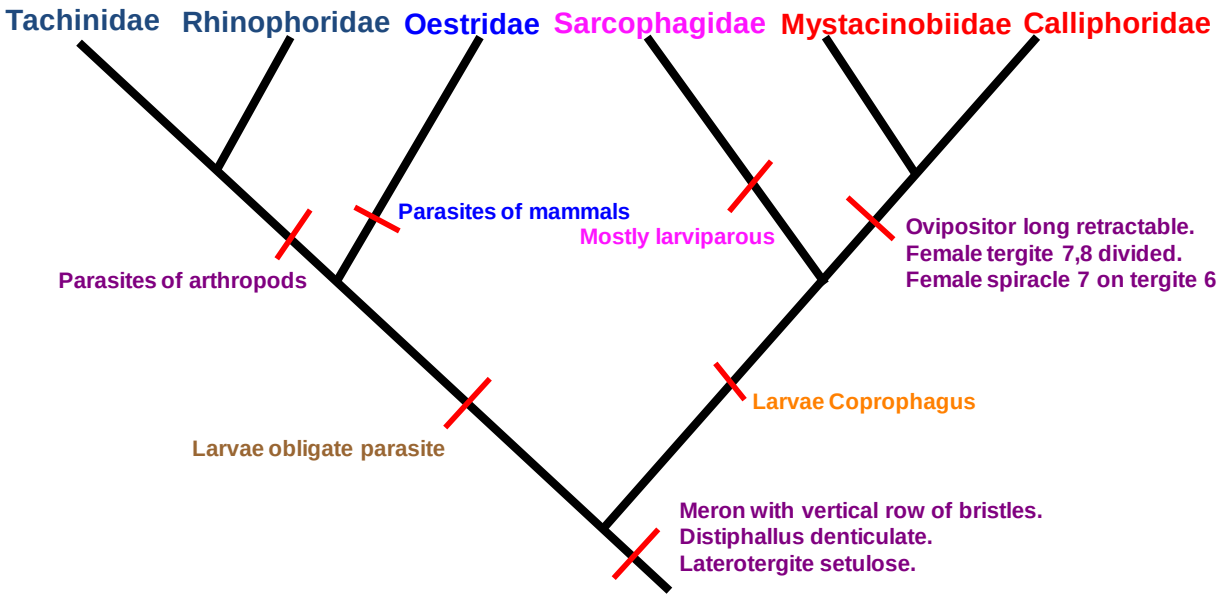

FIGURE 5.1: Phylogenetic tree, showing relationships between different families of the Oestroidea (From McAlpine, 1989).

Oestroid taxa of uncertain phylogenetic position:

Rhiniinae

Rhiniinae have been placed within a monophyletic Calliphoridae (Pape, 1992) or as a sister group to Sarcophagidae + Tachinidae (Rognes, 1997). The latter hypothesis inspired recent authors to raise its rank from subfamily to family (Evenhuis *et al.*, 2008). Recent molecular systematic analysis also supported a family level upgrade of Rhiniinae as a sister group of Calliphoridae + Tachinidae + Oestridae + Rhinophoridae (Kutty *et al.*, 2010).

The more obscure calliphorid subfamilies

Toxotarsinae was grouped with Chrysomyinae based on morphology (Pape, 1992; Rognes, 1997), and with Calliphorinae + Melanomyinae based on DNA sequences (Kutty *et al.*, 2010; Singh & Wells, 2011). Similarly, the phylogenetic positions of Bengaliinae, Helicoboscinae, Calliphorinae, Polleniinae, and Mesembrinellinae differ in the two most extensive morphological analyses of the Oestroidea (Fig. 5.2) (Pape, 1992; Rognes, 1997). In recent molecular systematic analysis Bengaliinae was nested within Chrysomyinae, Helicoboscinae was sister group of Rhinophoridae, Calliphorinae (including Melanomyinae) was sister group of Toxotarsinae, and the systematic position of Polleniinae and Mesembrinellinae were unstable (Kutty *et al.*, 2010).

Rhinophoridae

Some authors consider Rhinophoridae a subfamily of the Calliphoridae (Herting, 1957; Bedding, 1973; Kutty *et al.*, 2008) while others consider it a separate family (Brues *et al.*, 1954; Rohdendorf, 1967; Hennig, 1973; Crosskey, 1977; Tschorsnig, 1985; Pape, 1986, 1992; McAlpine, 1989; Rognes, 1997; Kutty *et al.*, 2010). As a subfamily, it generates nomenclatural difficulty because the name predates Calliphoridae, which might then have to be changed to Rhinophoridae as per International Code of Zoological Nomenclature (ICZN) guidelines. Even as a separate family, its relationship with other oestroid families remains unclear. Rohdendorf (1967)

favored monophyly of Rhinophoridae + Sarcophagidae; Tschorsnig (1985) favored monophyly of Rhinophoridae + Calliphoridae; McAlpine (1989) favored monophyly of Rhinophoridae + Tachinidae; Pape (1992) considered it a sister group of Calliphoridae + Oestridae; Rognes (1997) thought it was either a sister group of Calliphoridae + Oestridae or of all other Oestroidea except *Mystacinobia* and Axiniidae, and Pape & Arnaud (2001) placed Rhinophoridae (including Axiniidae) as a sister group of Rhiniinae.

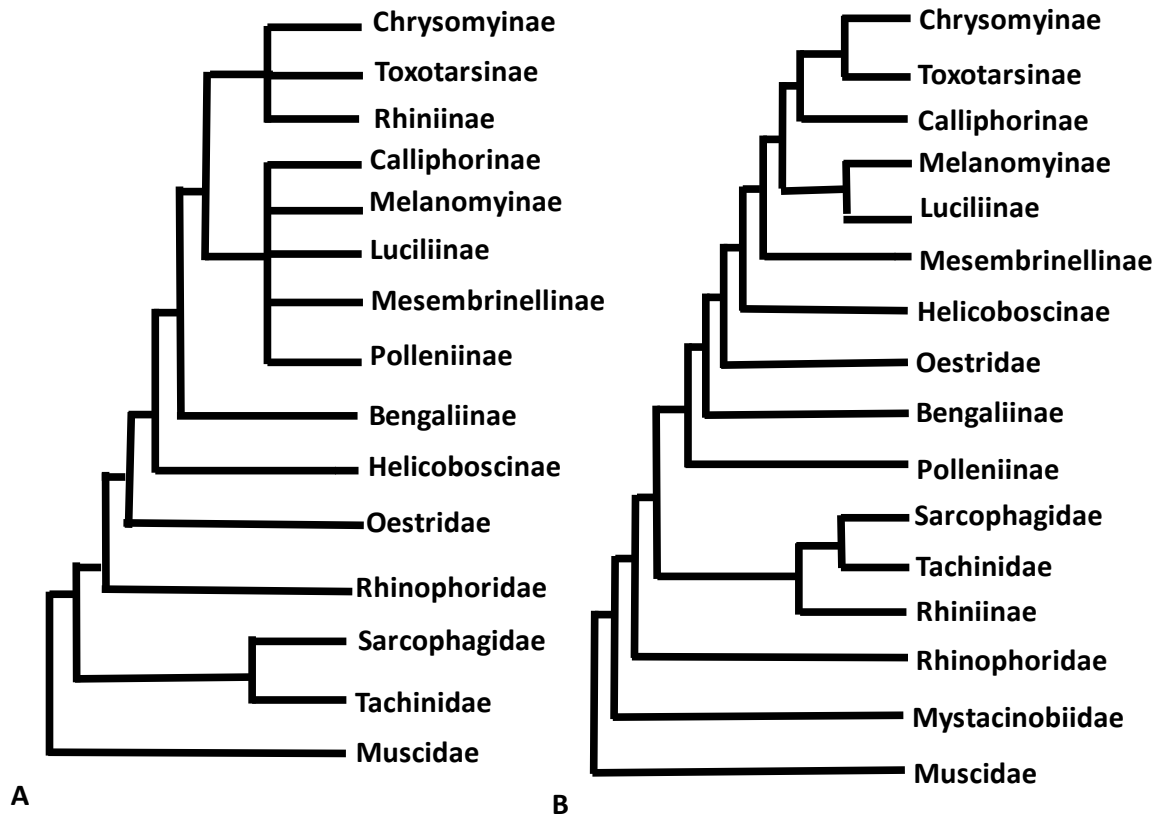

FIGURE 5.2: Published hypotheses of phylogenetic relationship between different groups of oestroid flies based on morphological data. (A) modified from Pape (1992; fig. 8); (B) modified from Rognes (1997; fig. 3).

Mystacinobia Holloway

These flies are so specialized that a particularly wide range of systematic hypotheses have been proposed. Maa (1971) placed them in the superfamily Hippoboscoidea based on superficial resemblance to bat ectoparasites. Holloway

(1976) placed *Mystacinobia* in superfamily Ephydroidea (as Drosophiloidea) based on various head, thorax and leg features. As these body parts are highly modified in *Mystacinobia* for phoresy, Griffiths (1982) didn't consider these characteristics reliable and proposed its inclusion within Oestroidea based on various abdominal characters.

Although, inclusion of *Mystacinobia* within Oestroidea is now well-supported both by morphology and DNA based cladistic analyses (Rognes, 1997; Kutty *et al.*, 2010), its relationship with other oestroid taxa is still uncertain. Griffiths (1982) placed *Mystacinobia* in the Calliphoridae based on the structure and number of spermathecal ducts, as did Gleeson *et al.* (2000) in a limited study using a 511 bp *16S ribosomal RNA* gene. Kurahashi (1989) placed it without explanation in a separate subfamily Mystacinobiinae within Calliphoridae. The large number of autapomorphies in almost all life stages led recent authors to accept a separate family Mystacinobiidae (McAlpine, 1989; Rognes, 1997). Extensive morphological cladistic analysis supports *Mystacinobia* as the most basal oestroid (Rognes, 1997), whereas extensive molecular systematics analysis support it a sister-group of Sarcophagidae (Kutty *et al.*, 2010).

McAlpine's fly

McAlpine's fly is an unusual and undescribed calyptrate fly that was named after its collector Dr. D. K. McAlpine (Ferrar, 1979). Adult females are unilarviparous and feed on dung (Ferrar, 1979). Ferrar (1979) placed it in superfamily Muscoidea based on its affinity with family Anthomyiidae but he also observed that its male genitalia was very similar to calliphorids. Recent molecular systematic analysis favored its inclusion within Oestroidea, as a sister group of *Mystacinobia* (Kutty *et al.*, 2010).

The purpose of this study is to generate a robust phylogeny of the Calliphoridae including all of the problematic taxa mentioned above.

5.2 Materials and Methods

5.2.1. Specimens and DNA extraction

A total of 51 representative species from all six families of the Oestroidea were included in this study. We followed Pape's (1992) suggestion for selection of outgroups, using five representative species from three calyptrate (Glossinidae, Faniidae, and Muscidae) and one acalyptrate (Drosophilidae) families (Table 5.1). DNA was extracted

from thoracic tissues (except *Oestrus ovis* Linnaeus, *Hypoderma lineatum* De Villers, and *Mystacinobia zelandica* Holloway) (see below) of 95% ethanol preserved specimens by organic extraction method as described in Singh *et al.* (2010). For *O. ovis* and *H. lineatum*, DNA was extracted from internal tissues of third and second larval instars respectively. DNA from *M. zelandica* was extracted from leg tissue. For some specimens, DNA extraction was also carried out by using a Qiagen DNeasy blood and tissue Kit (Qiagen, Valencia, CA) as per the manufacturer's protocol. Extracted DNA was quantified using NanoDrop^{TM} 1000 spectrophotometer (Thermo Scientific, Waltham, MA). Voucher specimens from each individual along with collection details were deposited in West Virginia University's Entomological Collection (See Table 5.1 for voucher ID).

5.2.2. DNA amplification

A total of 64 new sequences were generated from one mitochondrial (*Cytochrome oxidase subunit one* (*CO I*)) and three nuclear (*carbamoylphosphate synthetase* (*CPS*), *Elongation factor one alpha* (*EF1α*), and *28S ribosomal RNA* (*28S rRNA*)) genes (See Table 5.1 for accession numbers with *). 1435 bp of the *COI* gene was amplified using primers and protocol as mentioned in Wells & Sperling (1999). 850 bp of CPS gene was amplified using the primers and PCR protocol of Moulton & Wiegmann (2004) and Singh *et al.* (2010). Additional *CPS* gene primers were designed with the help of primer 3 software (Steve & Helen, 2000) for those taxa that didn't amplify using published *CPS* gene primers (Table 5.2). 1240 bp of *EF1α* gene were amplified using primers and PCR protocol as described in McDonagh (2009). Approximately 2200 bp of *28S rRNA* gene were amplified using primers and protocol as mentioned in Stevens & Wall (2001). All primers were purchased from either Integrated DNA Technologies, Inc. (Coralville, IA) or Operon Biotechnologies, Inc. (Huntsville, AL). Other PCR reagents were purchased from Promega Corp. (Madison, WI).

TABLE 5.1: List of taxa and GenBank accession numbers.

Sr. #	Species name	Subfamily	Voucher number	GenBank accession numbers			
				COI	CPS	28S	Ef1a
Ephidroidea: Drosophilidae							
1	*Drosophila melanogaster* Meigen, 1830	Drosophilinae	N/A	NC_001709	NM206765	EF531127	NM165850
Hippoboscoidea: Glossinidae							
2	*Glossina morsitans* Westwood, 1851	Glossininae	WVU2011-018-1	JF439541*	EF531178	JF439566*	JF439518*
Muscoidea: Fanniidae							
3	*Fannia canicularis* (Linnaeus, 1761)	Fanniinae	RMBR#102705	DQ657037	EF531184	DQ656961	N/A
Muscoidea: Muscidae							
4	*Musca domestica* Linnaeus, 1758	Muscinae		AY526196	AY280689	AJ551427	DQ657113
5	*Hydrotaea cyrtoneurina* (Zetterstedt, 1845)	Azeliinae	RMBR# 102715	FJ025622	FJ025578	FJ025526	FJ025678
Oestroidea: Mystacinobiidae							
6	*Mystacinobia zelandica* Holloway, 1976	N/A	WVU2011-018-2	JF439542*	N/A	JF439567*	N/A
Oestroidea: Tachinidae							
7	*Epalpus signifer* (Walker,1849)	Tachininae	WVU2011-018-3	JF439543*	AY280680	JF439568*	JF439519*
8	*Gymnocheta viridis* (Fallén, 1810)	Tachininae	RMBR#103759	GQ409327	GQ409296	GQ409240	GQ409463
9	*Cyrtophleba nitida* Curran, 1930	Dexiinae	WVU2011-018-4	JF439544*	JF439554*	JF439569*	JF439520*
10	*Nemorilla floralis* (Fallén, 1810)	Exoristinae	WVU2011-018-5	JF439545*	JF439555*	JF439570*	JF439521*
11	*Gymnosoma nitens* Meigen, 1824	Phasiinae	RMBR#103754	GQ409326	GQ409295	GQ409239	GQ409462
12	*Phania funesta* (Meigen, 1824)	Phasiinae	RMBR#103756	GQ409349	GQ409306	GQ409260	GQ409477
Oestroidea: Rhinophoridae							
13	*Rhinophora lepida* (Linnaeus, 1824)		WVU2011-018-6	JF439546*	JF439556*	JF439571*	JF439522*
14	*Stevenia hertingi* Kugler, 1978		RMBR#103671	GQ409374	GQ409311	GQ409282	GQ409493
15	*Stevenia atramentaria* (Meigen, 1824)		RMBR#103670	GQ409373	N/A	GQ409281	GQ409492
Oestroidea: Sarcophagidae							
16	*Sarcophaga crassipalpis* Macquart, 1839	Sarcophaginae	WVU2011-018-7	JF439547*	JF439557*	JF439572*	JF439523*

17	*Notochaeta* sp. Aldrich, 1916	Sarcophaginae	RMBR#103715	GQ409339	GQ409301	GQ409252	GQ409473
18	*Metopia campestris* (Fallén, 1810)	Miltogramminae	WVU2011-018-8	JF439548*	JF439558*	JF439573*	JF439524*
19	*Sarcophila meridionalis* Verves, 1982	Paramacronychiinae	RMBR#103689	GQ409368	N/A	GQ409277	GQ409489
Oestroidea: Oestridae							
20	*Cuterebra fontinella* Clark, 1827	Cuterebrinae	WVU2011-018-9	JF439549*	JF439559*	JF439574*	JF439525*
21	*Cuterebra baeri* Shannon & Greene, 1926	Cuterebrinae	RMBR#103661	GQ409320	GQ409294	N/A	GQ409458
22	*Hypoderma lineatum* De Villers, 1789	Hypodermatinae	WVU2011-018-10	AF295558	JF439560*	JF718830*	JF439526*
23	*Oestrus ovis* Linnaeus, 1758	Oestrinae	WVU2011-018-11	AF497767	N/A	AJ551428	JF439540*
Oestroidea: Calliphoridae							
24	*Cynomya cadaverina* Robineau-Desvoidy, 1830	Calliphorinae	WVU2011-018-12	AF259505	JF439561*	AJ300135	FR719230
25	*Calliphora vomitoria* (Linnaeus, 1758)	Calliphorinae	WVU2009-017-21	GQ223336	FJ169335	AJ300133	JF439527*
26	*Onesia tibialis* Macquart, 1846	Calliphorinae	N/A	AY842605	N/A	FR719319	FR719263
27	*Bellardia vulgaris* (Robineau-Desvoidy, 1830)	Calliphorinae	RMBR#103605	GQ409316	GQ409290	GQ409231	GQ409452
28	*Lucilia sericata* (Meigen, 1826)	Luciliinae	WVU2009-017-20	AJ417717	FJ169332	AJ300140	JF439528*
29	*Hypopygiopsis infumata* (Bigot, 1877)	Luciliinae	WVU2011-018-13	JF439550*	JF439562*	JF439575*	JF439529*
30	*Hemipyrellia fernandica* (Macquart, 1855)	Luciliinae	N/A	FR719160	N/A	FR719291	FR719235
31	*Dyscritomyia robusta* (Grimshaw, 1901)	Luciliinae	N/A	AY074899	N/A	FR719289	FR719233
32	*Phormia regina* (Meigen, 1826)	Chrysomyinae	WVU2009-017-18	AF295550	FJ169331	JF713458*	JF439530*
33	*Protophormia terraenovae* Robineau-Desvoidy, 1830	Chrysomyinae	WVU2009-017-26	AF295553	HM639981	AJ300142	JF439531*
34	*Chrysomya rufifacies* (Macquart, 1843)	Chrysomyinae	WVU2009-017-13	AF083658	FJ169341	AJ551436	JF439532*
35	*Chrysomya megacephala* Fabricius, 1794	Chrysomyinae	WVU2009-017-9	AF092761	FJ169350	JF439576*	JF439533*
36	*Cochliomyia macellaria* (Fabricius,1775)	Chrysomyinae	WVU2009-017-19	AF295555	FJ169333	AJ551438	JF439534*

37	*Cochliomyia hominivorax* (Coquerel, 1858)	Chrysomyinae	N/A	AF260826	FJ169334	AJ551437	FM867797
38	*Compsomyiops fulvicrura* Robineau-Desvoidy, 1830	Chrysomyinae	RMBR#102680	FJ025607	FJ025571	FJ025504	FJ025667
39	*Protocalliphora sialia* Shannon & Dobroscky, 1924	Chrysomyinae	WVU2009-017-28	AF295559	HM639983	AJ558190	JF439535*
40	*Pollenia sp.* Robineau-Desvoidy, 1830	Polleniinae	WVU2011-018-14	JF439551*	JF439563*	AJ558192	JF439536*
41	*Pollenia amentaria* (Scopoli, 1763)	Polleniinae	RMBR#103622	GQ409350	N/A	GQ409262	GQ409478
42	*Verticia orientalis* Malloch, 1927	Bengaliinae	WVU2009-017-32	HQ248105	HQ248106	JF439577*	JF439537*
43	*Bengalia peuhi* Villeneuve, 1914	Bengaliinae	RMBR#102672	FJ025601	FJ025566	FJ025501	N/A
44	*Bengalia depressa* Walker, 1857	Bengaliinae	N/A	FR719154	N/A	FR719270	FR719214
45	*Auchmeromyia luteola* (Fabricius, 1805)	Auchmeromyiinae	N/A	FR719153	N/A	FR719269	FR719213
46	*Cordylobia anthropophaga* (Blanchard & Berenger-Feraud, 1872)	Auchmeromyiinae	N/A	FR719158	N/A	FR719285	FR719229
47	*Melinda viridicyanea* Robineau-Desvoidy, 1830	Melanomyinae	RMBR#103618	GQ409335	GQ409299	GQ409248	GQ409469
48	*Mesembrinella* sp. Giglio-Tos	Mesembrinellinae	WVU2011-018-15	JF439552*	JF439564*	JF439578*	JF439538*
49	*Eumesembrinella quadrilineata* Fabricius, 1805	Mesembrinellinae	RMBR#103619	GQ409336	N/A	GQ409249	GQ409470
50	*Sarconesia versicolor* Bigot, 1857	Toxotarsinae	RMBR#103610	GQ409319	GQ409291	N/A	GQ409456
51	*Sarconesia chlorogaster* (Wiedemann, 1830)	Toxotarsinae	RMBR#103609	GQ409359	GQ409310	N/A	GQ409482
52	*Isomyia gomezmenori* (Peris, 1951)	Rhiniinae	WVU2011-018-16	JF439553*	JF439565*	JF439579*	JF439539*
53	*Rhyncomya nigripes* (Séguy, 1933)	Rhiniinae	RMBR#103626	GQ409356	GQ409308	GQ409268	GQ409481
54	*Metallea erinacea* Fang & Fan, 1984	Rhiniinae	RMBR#103625	GQ409337	GQ409300	N/A	GQ409471
55	*Eurychaeta palpalis* (Robineau-Desvoidy, 1830)	Helicoboscinae	RMBR#102703	FJ025612	FJ025575	FJ025512	FJ025672
56	McAlpine's fly		RMBR#104423	GQ409334	N/A	GQ409247	GQ409468

* = Newly sequenced DNA sequence.

TABLE 5.2: List of newly designed *carbamoylphosphate synthetase* (*CPS*) gene primers.

Sr. #	Name of Primer	Nucleotide sequence (5'- 3')
1	Calcad207F	GAC CAT TTG TTT TGG CTG CT
2	Calcad908R	AAT CAA TGG ATT GGG GAG AA
3	Calcad627F	AGT TCG ATT GGT GTG CTG TG
4	Calcad1011R	CAG AAA GCT ATG GCC GAT TC
5	Oescad300F	AAC CCA CCG ATA AGA GAC CA
6	Oescad594F	TCG ATA CTG TGG CTG GTG AA
7	Oescad770F	TTG AGA AAT CTG GGC AAA CC
8	Oescad1287R	TCC TTG GCT TCC GTT AAG AA
9	CadT2207F	AGT GTG GGC GAG GTA ATG TC
10	CadT2370F	CTG CCT TGA AAG CCA ACT TC
11	CadT3156R	CAG AAA GCT ATG GCC GAC TC

Most PCR reactions consisted of 25 ul total volume containing 12.5 ul of 2X Promega Master Mix (Catalogue # M7505), 2 ul (10 pmol) of each primers, 1-3 ul (10 - 30 ng) of template DNA and remaining water. A smaller volume (10-15 µl) was used for some taxa with precious template DNA. All reaction components were in the same proportion. All DNA amplifications were carried-out in a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA).

5.2.3. Sequencing and sequence alignments

The amplified products were cleaned by using ExoSAP-IT® (USB Corporation, Cleveland, Ohio) as per manufacturer's instructions. Direct sequencing was performed on the purified product with the same primers used for PCR and using a Big Dye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The sequencing protocol was as described in Singh *et al.* (2010). The sequencing product was separated on an Applied Biosystems 3130xl genetic analyzer (Foster City, CA). Sequence files were edited using Sequence Navigator (Applied Biosystems, Foster city, CA). Many overlapping regions of these gene sequences were also obtained from the GenBank database (Table 5.1). Protein coding *COI*, *CPS*, and *EF1α* genes were aligned manually in Sequence Navigator (Applied Biosystems, Foster City, CA) using sequences of *Lucilia sericata* (AJ417717; Stevens *et al.*, 2002), *Epalpus signifier*

(Walker) (AY280680; Moulton & Wiegmann 2004), and *Chrysomya rufifacies* (Macquart) (JF439532; this study), as a reference sequence respectively. All newly sequenced protein coding genes were indel free. For the alignment of *28s rRNA* genes, a conservative approach was applied. Initially, different overlapping fragments of *28S rRNA* gene sequence from each species were edited and aligned manually in Sequence Navigator (Applied Biosystems, Foster City, CA). Because a high degree of length heterogeneity among different taxa often creates problems in multiple sequence alignments (De Rijk *et al.*, 1995; Gillespie, 2005), we performed multiple sequence alignments (MSA) in two steps. Initially, MSA was performed in Muscle v3.8.31 (Edgar, 2004) using default parameters, for most of the species with comparatively complete *28S rRNA* gene sequences. After multiple sequence alignment (MSA), ambiguous hypervariable regions of *28S rRNA* gene were excluded from the alignment using MEGA 5 (Tamura *et al.*, in press) and only 1664 bp of *28S rRNA* gene was used for further phylogenetic analysis. Some species with comparatively smaller *28S rRNA* gene sequence lengths were aligned separately by Muscle (Edgar, 2004) as implemented in MEGA 5 (Tamura *et al.*, in press) using already cropped 1664 bp sequences of closely related taxa as a reference sequence. Final MSA of the *28S rRNA* gene was checked by eye for sequence homology. Aligned and nexus files for all genes are available on request from the first author.

5.2.4. Phylogenetic analyses

Phylogenetic analyses were carried out on 5189 characters from 56 taxa using maximum parsimony (MP), maximum likelihood (ML) and Bayesian methods (BA). For all analyses, indels were coded as missing data and not as a fifth character, because indel coding generally has negligible effects on tree topology (Kutty *et al.*, 2008; 2010). MP analysis was performed in PAUP v.4b10 (Swofford, 2002), for 1000 bootstrap replications using an equally-weighted heuristic search and tree-bisection-reconnection (TBR) branch swapping options. ML analysis was carried out for 100 bootstrap replications in Garli v.1 (Zwickl, 2006) with default options. Best fit model (GTR+I+G) for ML analysis was determined in Modeltest v.3.7 (Posada & Crandall, 1998) based in Akaike Information Criterion (AIC). Similarly, best fit model (GTR+I+G) for BA was determined in MrModeltest (Nylander, 2004) based on AIC. BA was performed for two

concurrent sets of six independent chains (5 heated and one cold) for 3.5 million generations sampling every 100 generations using the default parameters in Mr. Bayes v.3.1.2 (Huelsenbeck & Ronquist, 2001). After 3.5 million generations, standard deviation of the split frequencies was 0.007, much lower than the generally recommended level of 0.01. After observing the likelihood plot, out of 35001 trees from each run, 28000 trees were discarded as the burnin, and the remaining trees (7001) were used for generation of the 50% majority rule consensus tree. Posterior probabilities were calculated as branch support from all trees that remained after setting burnin. Although nodes with Bayesian posterior probability (PP) ≥ 0.65 are shown on the tree (Fig. 5.3), only nodes having PP ≥ 0.95 were deemed significant (Huelsenbeck & Ronquist, 2001). All trees were edited using software MrEnt v.2.2 (Zuccon & Zuccon, 2010).

5.3 Results

Out of 5189 characters, 1423 were parsimony informative. MP, ML, and BA analyses produced almost identical trees, but the Bayesian tree (Fig. 5.3) was more resolved at deeper nodes (PP≥0.95). Oestroidea was monophyletic, as were Rhinophoridae and Tachinidae in BA and ML, but not in MP (Fig. 5.3). The other families were not recovered and consequently there was little resolution of oestroid family relationships. However, there was strong evidence against calliphorid monophyly in that 1) Messembrinellinae fell outside of a well-supported Calliphoridae (excluding Mesembrinellinae) + Rhinophoridae + Tachinidae clade, and 2) Polleniinae was the sister-group of Tachinidae (Fig. 5.3). Chrysomyinae was found to be closely allied to the mostly social insect predator/parasite taxa Auchmeromyiinae, Bengaliinae, and Rhiniinae rather than to the other familiar blow flies. Luciliinae + (Calliphorinae/Toxotarsinae/Melanomyinae) was strongly supported. All calliphorid subfamilies (except Calliphorinae and Bengaliinae) and genera for which we had more than one species were monophyletic (Fig. 5.3).

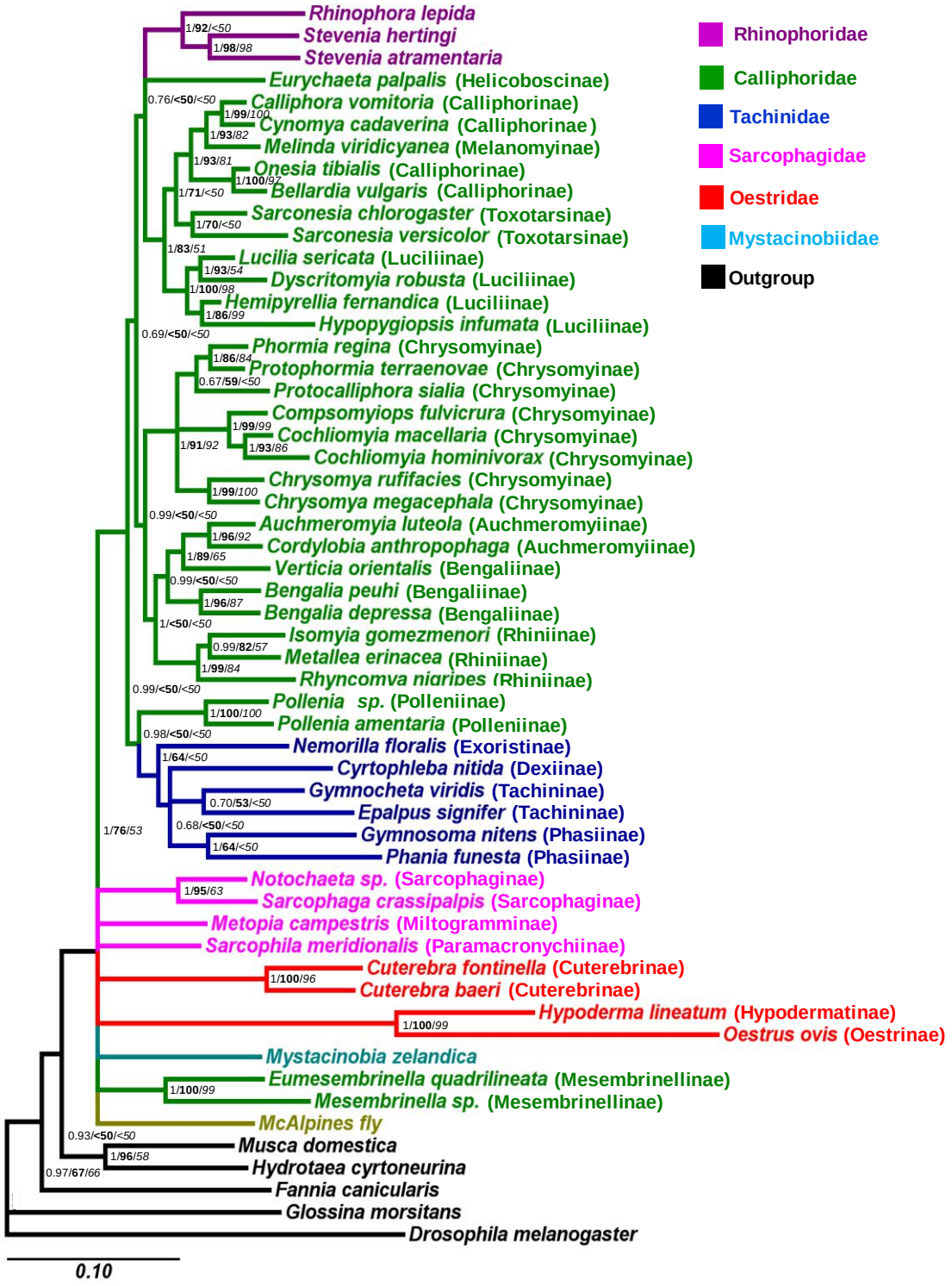

FIGURE 5.3: Bayesian phylogram of the Oestroidea based on 5189 bp of combined *COI*, *CPS*, *28S rRNA*, and *EF1a* DNA sequences. Branch support values: Standard font = Bayesian posterior probability; Bold = maximum likelihood % bootstrap; Italics = maximum parsimony % bootstrap.

5.4 Discussion

5.4.1. Relationships between oestroid families

We confirmed the earlier conclusion of oestroid monophyly (Pape, 1992; Rognes, 1997; Kutty *et al.*, 2008; 2010). Our well-supported Calliphoridae (excluding Mesembrinellinae) + Rhinophoridae + Tachinidae is in agreement with Wiegmann *et al.* (2011) but at odds with the hypothesis that Tachinidae is the sister group of Sarcophagidae (Pape, 1992; Rognes, 1997; Tachi & Shima, 2010), and also suggests to us that Rhinophoridae is either closely related to Calliphoridae (Tschorsnig, 1985; Rognes, 1986; 1991; Kutty *et al.*, 2008) or Tachinidae (Wood, 1987c; McAlpine, 1989) and not to Sarcophagidae (Rohdendorf, 1967). Morphologically, Rhinophoridae looks intermediate between typical calliphorids and typical tachinids (Pape, 1986).

The systematic position of *Mystacinobia* Holloway within Oestroidea is not well-resolved in our analysis but it is certainly not a member of Calliphoridae as sometimes suggested (Griffith, 1982; Gleeson *et al.*, 2000; Kurahashi, 1989). Although, we didn't recover the *Mystacinobia* + McAlpine's fly reported by Kutty *et al.* (2010), our results do agree with them in that McAlpine's fly is a member of the Oestroidea.

Relationships between oestroid families were mostly unresolved as was observed in previous studies (Kutty *et al.*, 2010; Wiegmann *et al.*, 2011). It may be that these groups evolved recently in a very short span of time and hence lack enough character accumulation for any phylogentic resolution (Wiegmann *et al.*, 2011). These results add to a growing body of work supporting rhinophorid monophyly (Pape, 1992; Pape & Arnaud, 2001; Kutty *et al.* 2010). They are in conflict with the Rhiniinae + (Sarcophagidae + Tachinidae) clade proposed by Rognes (1997) and they undermine the recent elevation of Rhiniinae to family rank (Evenhuis *et al.*, 2008; Kutty *et al.*, 2010, see next section).

5.4.2. Relationships within and between calliphorid subfamilies

Our result favors the earlier DNA analysis of Kutty *et al.* (2010) in that Melanomyinae is nested within Calliphorinae, and this combined lineage is sister to Toxotarsinae. This is an unsurprising result for Melanomyinae, the species of which have often been classified as Calliphorinae (Kurahashi, 1970; Schumann, 1986;

Shewell, 1987). But a sister-group relationship between Calliphorinae and Toxotarsinae rejects morphology based well-supported groups such as Chrysomyinae + Toxotarsinae (Rognes, 1997) or Chrysomyinae + Rhiniinae + Toxotarsinae (Pape, 1992). Monophyly of Calliphorinae + Toxotarsinae was also advocated by Greenberg & Szyska (1984) based on larval morphology and developmental rate and by Singh & Wells (2011) based on molecular data.

A monophyletic Luciliinae is in agreement with previously proposed hypotheses (Rognes, 1991; Wallman *et al.*, 2005; McDonagh, 2009; Kutty *et al.*, 2010). Within Luciliinae monophyly of *Lucilia* + *Dyscritomyia* favours the hypothesis proposed by Wells *et al.* (2002).

Strong support for monophyly of Chrysomyinae in our analysis is in agreement with morphological (Rognes, 1991; 1997) and recent molecular systematic analyses (McDonagh, 2009; Singh & Wells, 2011) but differ with Kutty *et al.*'s (2010) extensive molecular systematic analysis of the Calyptratae. Within Chrysomyinae, monophyly of *Phormia* + *Protophormia* and *Cochliomyia* + *Compsomyiops* are well supported, as was observed in previous molecular systematic analysis (McDonagh, 2009; Singh & Wells, 2011). However, we didn't observe support for the sister-group relationship between traditional tribe Phormiini and *Chrysomya* as was suggested in recent molecular systematic analysis of these flies (Singh & Wells, 2011). This may be because we included only a few members of Chrysomyinae in our analysis.

Morphologically, Bengaliinae and Auchmeromyiinae have always been considered as a natural assemblage (Pape, 1992; Rognes, 1997) and here we support it based on molecular data. Similarly, strong support for the monophyly of *Auchmeromyia* + *Cordylobia* is in agreement with Stevens (2003), who proposed its monophyly based on *28S rRNA* gene. Paraphyly of Bengaliinae with respect to Auchmeromyiinae suggest to us that Auchmeromyiinae may be a member of subfamily Bengaliinae as was proposed by Pape (1992) based on shared elongated anal veins. Members of both subfamilies are similar in color (yellow to brown) and have the same geographical distribution (Afrotropical and Oriental).

Verves (1986) and Shewell (1991) placed *Eurychaeta* within Sarcophagidae, while Rognes (1986) and Pape (1992) thought it was within or the sister-group, respectively, of Calliphoridae. Although systematic position of Helicoboscinae is not well-resolved in our analysis, it is certainly not a member of Sarcophagidae (Fig. 5.3).

Mesembrinellinae has similarly been difficult to place because these flies show so many unique characters (Shannon, 1923; Hall, 1948; James, 1970; Hennig, 1973; Rognes, 1991; Pape, 1992). We found that it is not a subfamily of Calliphoridae and is therefore probably worthy of the family status proposed by Guimarães (1977). Pape (1986) argued that family status of Mesembrinellinae implied an adjustment to commonly understood morphological character polarity, that would in turn make Mesembrinellidae the sister group of all other oestroid flies. Although systematic position of the Mesembrinellinae within Oestroidea is not well-resolved in our analysis, it is certainly not a member of Calliphoridae (Pape, 1986; 1992; Rognes, 1997) or Rhinophoridae or Tachinidae (Kutty *et al.*, 2010) (Fig. 5.3).

5.4.3. Evolution

A majority of calliphorid larvae are carrion breeders, but several members of this family also cause myiasis in vertebrate and invertebrate hosts (Stevens, 2003). This result in combination with Singh & Wells (2011) suggests to us that obligate parasitism of warm-blooded animals by calliphorid larvae evolved at least four times independently and hematophagy evolved independently in *Protocalliphora* and in *Auchmeromyia* (Stevens, 2003; McDonagh, 2009). Strong support for the sister group relationship between Polleniinae and Tachinidae suggest that earthworm parasitism among calliphorid flies evolved independently in Polleniinae and in *Onesia*-group of Calliphorinae. Similarly, our results suggest at least two independent evolutions of snail parasitism (Helicoboscinae and Melanomyinae) in Calliphoridae. Interestingly, all calliphorid subfamilies that cause parasitism to ants and termites (Bengaliinae, Auchmeromyiinae and Rhiniinae) (Senior-White *et al.*, 1940; Zumpt, 1965; Dear, 1977; Pont, 1980; Kurahashi, 1989) emerged as a monophyletic group in our analysis (Fig. 5.3), which suggests to us that ant and termite parasitic behavior evolved once in the Calliphoridae.

CHAPTER 6: Conclusions

With total 94 representative ingroup taxa, this work is the most extensive molecular systematic analysis performed so far for the Oestroidea, an important clade in the dipteran tree of life. Earlier studies were conducted on systematics of Oestroidea based on both morphological and molecular data, but morphological systematic analyses differed considerably with each other and molecular systematic analyses lacked support for many deeper nodes. This was mainly due to limited taxon sampling from the calliphorid grade, the most important group for better understanding of the Oestroidea phylogeny, and/or lack of overlapping gene regions for many important taxa. Our results included more than one representative species from all major subfamilies of the Calliphoridae.

In addition to being the most extensive molecular systematic analysis of the Oestroidea, this study also developed several new single copy protein coding nuclear gene primers and demonstrated usefulness of these primers in solving systematic questions of the Oestroidea. Although it is difficult to amplify single copy protein coding nuclear genes (especially if DNA quality is not good), these have advantages over mitochondrial genes in being better in resolving deeper nodes (a major problem in Oestroidea systematics) and over ribosomal genes because of its easy alignments.

With respect to Oestroidea systematics, this study reconfirmed: 1) monophyly of the Oestroidea, Tachinidae, and Rhinophoridae, 2) non-monophyly of the Calliphoridae, 3) placement of *Mystacinobia* and McAlpine's fly within Oestroidea, 4) monophyly of all oestrid subfamilies, 5) Melanomyinae as a part of the Calliphorinae and 6) Toxotarsinae as a sister group of Calliphorinae. These results supplemented existing knowledge of the Oestroidea systematics by suggesting: 1) Rhiniidae is more closely related to traditional calliphorid subfamilies than to either Sarcophagidae or Tachinidae, 2) Auchmeromyiinae is a part of the Bengaliinae, 3) Chrysomyinae and *Chrysomya* is monophyletic, 4) Chrysomyini is paraphyletic, 5) Polleniinae is a sister group of Tachinidae, and 6) Mesembrinellinae most probably merits family rank within Oestroidea.

Well-supported phylogenies obtained from this study also helped in testing several evolutionary hypotheses, including: 1) single origin of bird parasitism, ant and

termite parasitism, and two independent origins of hematophagy, earthworm parasitism and snail parasitism in the calliphorid grade, 2) two independent origins of tuberculate larvae in *Chrysomya*, 3) a decrease in genome size over evolutionary time in *Chrysomya* and 4) recent origin of monogeny compared to amphogeny in *Chrysomya*. These studies also suggest to us that all Neotropical chrysomyine genera share a common ancestral lineage.

Results from this study will help in filling several existing knowledge gaps in the Diptera tree of life, which will provide a framework for further comparative studies on these economically and socially important groups of flies. For example, genome size is positively correlated with developmental time at constant temperature in *Drosophila*. If we find a similar pattern for *Chrysomya*, then it will be much easier to estimate development rate with a simple measurement of genome size, which in turn will help in estimation of post-mortem interval (PMI) in forensic investigations, because these flies are of forensic importance. These results will also help in better understanding of how traits have evolved with time and with respect to environmental conditions. For example, the *Chrysomya* tree suggested that loss of male eye dimorphism in *Chrysomya pacifica* was because of its movement towards dense forest and not otherwise.

Although we were able to resolve many systematic questions of the Oestroidea, there are many that need further attention. Mainly, the systematic position of *Mystacinobia*, McAlpine's fly, Rhinophoridae, Mesembrinellinae, Sarcophagidae, and Oestridae within Oestroidea remained uncertain. Relationships between some calliphorid subfamilies were weakly-supported. Monophyly of the Oestridae is still unsupported by DNA analysis. As these lineages diverged in a short span of time, they may not have accumulated sufficient characters for well-resolved oestroid phylogeny. More extensive taxon (which will not be an easy task, considering many groups are rare in collections) and data sampling may help in better resolution of the oestroid deeper nodes, but we think alternate approaches such as use of mobile elements, micro-RNA's, gene rearrangement etc. may be a better strategy, as these methods are less prone to homoplasy.

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APPENDIX A: List of morphological characters that support monophyly of various oestroid groups.

Appendix 1: Characters that support monophyly of the Axiniidae (Colless, 1994).

1. Characteristic antennal segment 3 (axe-head type or fissicorn type) in male.
2. Antennal segment 2 with long apical projections that articulate with segment 3.
3. Small but distinct bristles on middle of fronto-orbital plate.
4. Fronto-orbital plates broad.
5. Subcosta of wing with no sub-basal kink.
6. Fork of Rs unswollen and without setulae.
7. Male surstylus with pointed inner and broad outer lobe.

Appendix 2: Characters that support monophyly of the Tachinidae (Wood, 1987c; Pape, 1992; Rognes, 1997).

1. Subscutellum well-developed.
2. Mandibles reduced in first instar larvae.
3. Labrum fused and contiguous with rest of cephalopharyngeal skeleton in first instar larvae

Appendix 3: Characters that support monophyly of the Rhinophoridae (Crosskey, 1977; Pape, 1986; Rognes, 1991; 1997).

1. Mandible toothed in the first instar larva.
2. Ventral plate of the distiphallus fused and well-developed.
3. All members parasitic on woodlice.

Appendix 4: Characters that support monophyly of the Sarcophagidae (Pape, 1992).

1. Margin of tergite 1 and 2 covered by abdominal sternite 2.
2. No alpha setae.
3. 10^{th} sternite in male divided, small, and positioned at a right angle to median plane.
4. Posterior spiracles are located in the cavity and peritreme are incomplete in the second and third instar larvae.

Appendix 5: Characters that support monophyly of the Oestridae (Pape, 2001).

1. Sticky eggs.
2. Several segments of first instar larva with circle of thorn-like band.
3. The puparium opens by splitting of a dorsal valve.
4. Adult mouthparts reduced and non-functional.
5. Adult body pilose and bristleless.
6. Setae absent on anatergite.
7. Subcosta strait and runs parallel to costa before merging into it.
8. Second abdominal sternite and tergal margin are well-separated by folded membrane.

Appendix 6: Characters that support monophyly of different subfamilies of the Calliphoridae.

Appendix 6.1: Ameniinae (Rognes, 1986; 1997).

1. Facial carina very strong
2. 2+1 branching pattern of spermathecal ducts.
3. Subscutellum comparatively developed but weaker than Tachinidae.
4. Sternum (ST) 8 of ovipositor larger in width than in length.

Appendix 6.2: Auchmeromyiinae (Rognes, 1997).

1. Presence of small but unique setae in between first and second post-sutural supraalars.
2. Lateral scutellar marginals in large numbers.

Appendix 6.3: Bengaliinae (Senior-White *et al.*, 1940; James, 1966, Kurahashi, 1987).

1. Humeral setae two.
2. Outer post humeral seta absent.
3. Metakatepisternum with setulae.
4. Posterior side of hind coxa with setae.

Appendix 6.4: Phumosiinae (Rognes, 1997).

1. Aedeagus without epiphallus.
2. Katatergite with long bristles.

Appendix 6.5: Mesembrinellinae (Guimaraes, 1977; Rognes, 1997).

1. Single large reniform operculum on the metathoracic spiracle.
2. Comparatively strong subscutellum.
3. Female with crossed inter-frontals.
4. Translucent wings with strongly curved $M_{1.}$

Appendix 6.6: Helicoboscinae (Rognes, 1991; 1993; 1997).

1. Setae absent on post-alar wall.
2. 2+1+1 or 1+1+1 pattern of katepisternal setae.
3. Anterior projections of the tentoropharygeal sclerite are absent in first instar larvae.
4. Posterior spiracular plates completely sclerotised in third instar larva.

Appendix 6.7: Toxotarsinae (Dear, 1979; Rognes, 1997).

1. Setae present on ventral surface of stem vein (exceptions are *Toxotarsus ambrosianus* Lupes and *T. nigrocyaneus* (Walker))
2. Only two scutellar marginals present.

Appendix 6.8: Calliphorinae (Rognes, 1991).

1. Upper part of lower calypter and lower half of anterior part of anepimeron with bristles.
2. Setae absent on ventral surface of costa beyond the point where R_1 joins it.
3. Katepimeron not well-separated from meron.
4. ST8 incomplete and apically divided into two lobes.

Appendix 6.9: Luciliinae (Rognes, 1991; 1997).

1. No setae on parafacialia.
2. Bristles present in lowermost region of metakatepisternum.
3. Presence of metallic-green setose sclerites on the posterior part of suprasquamal ridge

Appendix 6.10: Melanomyinae (Rognes, 1991).

1. T8 and epiproct fused.
2. Cerci small and wide.
3. Microtrichiae absent on sclerites (except hypoproct) and pleural membrane of ovipositor.
4. ST8 either very reduced or absent.

5. No setae and denticles on acrophallus.

Appendix 6.11: Polleniinae (Rognes, 1991; 1997).

1. Comparatively strong facial carina.
2. Acrophallus unarmed and lacks denticles.

Appendix 6.12: Rhiniinae (Rognes, 1991; 1997).

1. Protruded lower part of the face.
2. Shiny submarginal band on upper half of occiput.
3. Upper side of stem vein with row of setae.
4. Apical spines on the lobes of male sternite 5.
5. Ventral plates fused and form a ring around distiphallus.

Appendix 6.13: Chrysomyinae (Rognes, 1991).

1. Separation of katepimeron and meron not clear.
2. Presence of a row of setae on the antero-ventral edge of the posterior spiracle.
3. Upper side of the stem vein with row of setae.
4. Peritreme incomplete in the third instar larvae.

APPENDIX B: Tables showing pairwise p-distance and pairwise transition-transversion ratio of different genes used in this study.

TABLE A1: Pairwise p-distance of *cytochrome oxidase subunit one* (*CO I*) gene used in *Chrysomya* phylogeny (Chapter 2).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
2	0.079															
3	0.085	0.109														
4	0.098	0.101	0.074													
5	0.087	0.094	0.077	0.101												
6	0.096	0.107	0.087	0.098	0.077											
7	0.102	0.108	0.094	0.104	0.089	0.033										
8	0.096	0.101	0.092	0.1	0.083	0.039	0.048									
9	0.098	0.104	0.093	0.1	0.083	0.044	0.049	0.03								
10	0.105	0.111	0.104	0.11	0.093	0.068	0.07	0.064	0.054							
11	0.094	0.109	0.086	0.102	0.081	0.071	0.078	0.066	0.067	0.088						
12	0.104	0.121	0.101	0.117	0.095	0.085	0.091	0.079	0.081	0.108	0.046					
13	0.088	0.089	0.075	0.094	0.064	0.066	0.071	0.068	0.067	0.076	0.058	0.071				
14	0.092	0.096	0.084	0.099	0.078	0.07	0.078	0.07	0.074	0.091	0.068	0.077	0.055			
15	0.091	0.09	0.078	0.098	0.076	0.068	0.077	0.068	0.07	0.085	0.074	0.092	0.063	0.065		
16	0.082	0.093	0.08	0.104	0.073	0.075	0.074	0.073	0.072	0.085	0.066	0.078	0.058	0.06	0.05	
17	0.078	0.085	0.07	0.092	0.068	0.063	0.069	0.067	0.061	0.079	0.066	0.073	0.05	0.053	0.052	0.053
18	0.09	0.109	0.079	0.098	0.076	0.072	0.08	0.072	0.074	0.093	0.069	0.081	0.061	0.057	0.051	0.057
19	0.091	0.094	0.078	0.096	0.071	0.068	0.078	0.074	0.067	0.084	0.068	0.082	0.061	0.057	0.055	0.053
20	0.083	0.102	0.077	0.095	0.075	0.074	0.08	0.073	0.07	0.087	0.069	0.075	0.052	0.058	0.056	0.054
21	0.087	0.108	0.07	0.099	0.07	0.07	0.076	0.072	0.069	0.085	0.067	0.082	0.053	0.053	0.051	0.051
22	0.087	0.1	0.074	0.094	0.075	0.061	0.07	0.063	0.066	0.079	0.064	0.071	0.055	0.047	0.053	0.051
23	0.082	0.098	0.074	0.09	0.07	0.063	0.074	0.066	0.066	0.083	0.063	0.078	0.052	0.05	0.046	0.049
24	0.087	0.106	0.079	0.094	0.076	0.066	0.077	0.07	0.069	0.087	0.066	0.079	0.056	0.053	0.054	0.057

25	0.082	0.097	0.074	0.091	0.072	0.063	0.074	0.066	0.066	0.082	0.064	0.077	0.051	0.05	0.045	0.048
26	0.079	0.09	0.082	0.095	0.076	0.07	0.074	0.071	0.073	0.085	0.07	0.076	0.062	0.066	0.066	0.061
27	0.074	0.082	0.078	0.091	0.072	0.065	0.075	0.066	0.067	0.077	0.067	0.069	0.056	0.062	0.059	0.057

	17	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**
18	0.051									
19	0.05	0.052								
20	0.044	0.03	0.052							
21	0.04	0.024	0.044	0.026						
22	0.04	0.021	0.044	0.034	0.025					
23	0.042	0.015	0.045	0.03	0.019	0.015				
24	0.046	0.022	0.049	0.036	0.026	0.02	0.007			
25	0.042	0.014	0.045	0.029	0.021	0.015	0.001	0.009		
26	0.059	0.064	0.058	0.065	0.061	0.056	0.061	0.063	0.061	
27	0.053	0.067	0.056	0.065	0.06	0.057	0.058	0.06	0.056	0.022

Taxon key for table A1:

1	*Lucilia sericata*	**10**	*Chrysomya incisuralis*	**19**	*Chrysomya bezziana*
2	*Calliphora vomitoria*	**11**	*Chrysomya varipes*	**20**	*Chrysomya thanomthini*
3	*Cochliomyia macellaria*	**12**	*Chrysomya flavifrons*	**21**	*Chrysomya pinguis*
4	*Cochliomyia hominivorax*	**13**	*Chrysomya norrisi*	**22**	*Chrysomya greenbergi*
5	*Phormia regina*	**14**	*Chrysomya nigripes*	**23**	*Chrysomya cabrearai*
6	*Chrysomya albiceps*	**15**	*Chrysomya putoria*	**24**	*Chrysomya megacephala*
7	*Chrysomya rufifacies*	**16**	*Chrysomya marginalis*	**25**	*Chrysomya pacifica*
8	*Chrysomya villeneuvi*	**17**	*Chrysomya chani*	**26**	*Chrysomya semimetallica*
9	*Chrysomya yayukae*	**18**	*Chrysomya defixa*	**27**	*Chrysomya latifrons*

TABLE A2: Pairwise transition-transversion ratio of *cytochrome oxidase subunit one* (*CO I*) gene used in *Chrysomya* phylogeny (Chapter 2).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
2	1.16																	
3	0.83	0.9																
4	0.92	0.78	2.65															
5	1.13	1	0.98	1.25														
6	1	0.71	1.2	1.21	1.25													
7	1.03	0.85	1.27	1.24	1.45	4.56												
8	0.97	0.64	1.2	1.11	1.27	4.45	3.11											
9	1.01	0.73	1.23	1.04	1.28	2.82	2.32	3.5										
10	1.05	0.93	1.31	1.16	1.56	2.06	1.84	2.39	1.63									
11	1.03	0.92	1.06	1.14	1.6	1.53	1.73	1.55	1.54	1.73								
12	1.43	1.07	1.02	1.04	1.69	1.8	1.78	1.6	1.75	2.26	2.17							
13	1.01	0.68	1.13	1.15	1.61	1.49	1.37	1.5	1.33	1.38	1.97	1.9						
14	0.88	0.96	1.02	1.14	1.31	1.18	1.22	1.16	1.04	1.27	1.36	0.85	1.36					
15	0.88	0.92	1.04	1.1	1.64	1.83	1.91	1.93	1.47	1.79	1.79	1.67	1.82	1.31				
16	1.03	0.98	1.19	1.24	1.72	1.57	1.61	1.56	1.46	1.71	1.57	1.44	1.71	1.23	2.44			
17	0.94	0.89	0.96	1.29	1.69	1.43	1.47	1.51	1.17	1.49	1.76	1.31	1.66	1	1.5	1.62		
18	1.25	1.29	1.73	1.53	2.62	2.79	2.59	2.24	2.11	2.31	2.28	2.14	3.18	1.72	2.76	3.37	3.53	
19	1.26	1.08	1.53	1.45	2.3	2.06	2.05	2.05	1.63	1.78	1.81	1.68	2.48	1.84	2.58	2.45	2.85	10.14
20	0.95	1.05	1.38	1.35	2.26	2.55	2.13	2.03	1.73	1.89	1.83	1.46	2.25	1.53	2.5	2.39	2.1	6.5
21	1.08	1.25	1.4	1.53	2.27	2.57	2.16	2.36	2.03	2.07	1.94	1.91	2.9	1.65	2.56	2.7	2.44	11.33
22	1.15	1.05	1.51	1.5	2.48	2.36	2.06	1.94	1.88	1.88	2	1.64	3	1.18	2.67	2.7	2.88	31
23	1.02	1.05	1.43	1.34	2.31	2.28	2.11	1.94	1.74	1.93	1.79	1.74	2.9	1.24	2	2.38	2.71	10
24	1.13	1.14	1.52	1.36	2.41	2.26	2.11	2	1.78	1.93	2	1.6	2.91	1.25	2.24	2.57	3.18	7.25
25	1.07	1.04	1.48	1.37	2.44	2.34	2.17	2	1.8	1.93	1.88	1.74	2.95	1.26	1.95	2.33	2.82	9.5
26	1.03	1.02	1.29	1.28	1.49	1.33	1.4	1.22	1.2	1.4	1.3	1.42	1.44	1.17	1.65	1.75	1.5	2.34
27	0.95	0.88	1.15	1.16	1.35	1.26	1.55	1.15	1.11	1.28	1.27	1.28	1.21	1.02	1.47	1.68	1.26	2.57

	19	**20**	**21**	**22**	**23**	**24**	**25**	**26**
20	6							
21	7.38	6.8						
22	7.5	9	18.5					
23	6.56	6.33	8.67	21				
24	5.82	5.62	7	9.33	4.5			
25	6.67	6.17	9.67	22	---	6		
26	1.97	1.94	2.32	1.87	1.97	1.94	2	
27	1.9	1.97	2.33	1.93	1.83	1.81	1.8	10

Taxa key for table A2: Same as table A1.

TABLE A3: Pairwise p-distance of *carbamoylphosphate synthetase* (*CPS*) gene used in *Chrysomya* phylogeny (Chapter 2).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
2	0.112															
3	0.139	0.145														
4	0.139	0.14	0.062													
5	0.131	0.127	0.1	0.102												
6	0.134	0.159	0.126	0.13	0.124											
7	0.133	0.16	0.126	0.134	0.128	0.008										
8	0.146	0.165	0.135	0.135	0.133	0.035	0.038									
9	0.144	0.166	0.127	0.132	0.128	0.026	0.03	0.032								
10	0.127	0.158	0.122	0.121	0.119	0.039	0.042	0.054	0.052							
11	0.146	0.157	0.113	0.128	0.138	0.074	0.077	0.085	0.079	0.07						
12	0.147	0.16	0.123	0.128	0.131	0.077	0.077	0.086	0.083	0.08	0.047					
13	0.134	0.167	0.136	0.132	0.135	0.09	0.085	0.092	0.088	0.086	0.103	0.09				
14	0.123	0.158	0.13	0.139	0.125	0.062	0.064	0.074	0.075	0.065	0.084	0.082	0.096			
15	0.146	0.149	0.139	0.126	0.124	0.084	0.083	0.084	0.089	0.087	0.109	0.095	0.105	0.077		
16	0.122	0.149	0.114	0.119	0.115	0.087	0.088	0.1	0.098	0.093	0.11	0.109	0.112	0.085	0.101	
17	0.12	0.154	0.129	0.129	0.122	0.095	0.093	0.111	0.109	0.105	0.109	0.109	0.12	0.091	0.102	0.053
18	0.127	0.156	0.124	0.128	0.125	0.102	0.102	0.118	0.11	0.108	0.117	0.111	0.119	0.099	0.108	0.06
19	0.118	0.147	0.119	0.12	0.112	0.08	0.08	0.094	0.095	0.092	0.108	0.103	0.112	0.081	0.088	0.046
20	0.124	0.152	0.122	0.124	0.119	0.085	0.086	0.105	0.099	0.093	0.109	0.108	0.113	0.089	0.092	0.054
21	0.114	0.147	0.11	0.118	0.112	0.083	0.082	0.099	0.096	0.09	0.108	0.102	0.109	0.077	0.086	0.052
22	0.122	0.152	0.117	0.123	0.111	0.091	0.091	0.11	0.105	0.096	0.112	0.106	0.121	0.087	0.096	0.053
23	0.122	0.158	0.12	0.126	0.119	0.091	0.092	0.112	0.105	0.104	0.11	0.102	0.119	0.088	0.096	0.062
24	0.128	0.16	0.129	0.129	0.124	0.092	0.091	0.108	0.102	0.101	0.115	0.105	0.114	0.093	0.099	0.06
25	0.116	0.139	0.119	0.126	0.114	0.087	0.088	0.103	0.102	0.094	0.104	0.105	0.118	0.092	0.106	0.075
26	0.147	0.148	0.136	0.133	0.116	0.107	0.114	0.113	0.112	0.102	0.114	0.117	0.129	0.114	0.119	0.105

	17	18	19	20	21	22	23	24	25
18	0.051								
19	0.032	0.041							
20	0.044	0.041	0.031						
21	0.041	0.044	0.032	0.04					
22	0.047	0.044	0.034	0.039	0.045				
23	0.047	0.042	0.039	0.036	0.046	0.03			
24	0.041	0.034	0.038	0.04	0.042	0.04	0.031		
25	0.069	0.085	0.057	0.073	0.074	0.079	0.084	0.083	
26	0.113	0.115	0.105	0.113	0.118	0.118	0.122	0.12	0.059

Taxa key for table A3:

1	*Lucilia sericata*	**10**	*Chrysomya incisuralis*	**19**	*Chrysomya thanomthini*
2	*Calliphora vomitoria*	**11**	*Chrysomya varipes*	**20**	*Chrysomya pinguis*
3	*Cochliomyia macellaria*	**12**	*Chrysomya norrisi*	**21**	*Chrysomya greenbergi*
4	*Cochliomyia hominivorax*	**13**	*Chrysomya nigripes*	**22**	*Chrysomya cabrearai*
5	*Phormia regina*	**14**	*Chrysomya putoria*	**23**	*Chrysomya megacephala*
6	*Chrysomya albiceps*	**15**	*Chrysomya marginalis*	**24**	*Chrysomya pacifica*
7	*Chrysomya rufifacies*	**16**	*Chrysomya chani*	**25**	*Chrysomya semimetallica*
8	*Chrysomya villeneuvi*	**17**	*Chrysomya defixa*	**26**	*Chrysomya latifrons*
9	*Chrysomya yayukae*	**18**	*Chrysomya bezziana*		

TABLE A4: Pairwise transition- transversion ratio of *carbamoylphosphate synthetase* (*CPS*) gene used in *Chrysomya* phylogeny (Chapter 2).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
2	1.32																	
3	1.88	1.28																
4	1.81	1.16	1.79															
5	2.47	1.77	1.18	0.98														
6	2.42	2.07	1.97	1.97	2.18													
7	2.18	1.89	2.03	1.94	2.06	2												
8	2.26	1.75	1.67	1.61	2.14	1.31	1.73											
9	2.13	1.82	1.7	1.73	1.66	1.2	1.18	0.8										
10	2.09	1.91	1.89	1.78	2.06	2.3	2.67	2.07	1.75									
11	2.27	1.95	2	2.03	2	2.39	2.47	1.8	1.95	2.22								
12	2.22	2.1	1.94	1.97	1.72	2.1	2.21	1.92	1.79	2.25	2.08							
13	1.78	1.84	1.42	1.11	1.67	1.71	1.68	1.69	1.68	2.04	1.83	1.81						
14	2.13	1.84	1.86	1.82	2.41	2.77	2.92	2.69	1.95	2.64	2.37	2.25	2.17					
15	2.26	1.82	1.74	1.55	1.76	2.09	2.3	1.73	1.81	1.96	1.73	1.41	1.87	1.9				
16	1.97	1.54	1.85	1.73	1.65	2.36	2.5	2.15	1.96	2.59	2.5	2.67	1.97	2.94	2.44			
17	2.4	1.67	2.14	2.06	1.6	1.96	1.85	1.76	1.66	2.3	2.1	2.03	1.62	1.88	2.11	1.37		
18	2.06	1.75	2.09	1.92	1.59	2.58	2.38	2.23	2.1	2.5	3	2.75	1.81	2.39	2.14	1.83	1.53	
19	2.12	1.91	2.06	2	1.64	2.24	2.15	1.86	1.79	2.39	2.87	2.61	1.71	2.37	2.12	1.6	1.7	2.18
20	2	1.69	1.89	1.84	1.46	2	1.96	1.87	1.8	2.04	2	1.9	1.82	1.92	2.39	1.3	1.47	1.5
21	2.13	1.72	1.91	1.86	1.57	2.5	2.25	2.11	2.12	2.45	2.56	2.15	2.07	2.21	1.92	1.75	1.92	3.11
22	2.09	1.78	1.8	1.78	1.45	2.45	2.38	2.17	1.93	2.33	2.29	2.27	1.81	2.4	2.48	2.14	1.6	2.08
23	2.25	1.85	2.52	2.15	1.66	2.35	2.17	2.17	1.87	2.52	2.64	2.32	1.73	2.18	1.93	2.12	2.33	4.14
24	2.21	1.67	2.14	2.06	1.62	2.12	2	2.07	1.64	2.19	2.28	2.04	1.62	2.22	1.8	2	1.92	2.22
25	2.06	1.85	1.38	1.52	1.59	1.7	1.65	1.56	1.46	1.93	1.61	2.11	1.54	1.67	1.97	1.33	1.64	1.63
26	1.78	1.62	1.32	1.51	1.54	1.68	1.81	1.74	1.5	1.9	1.61	1.97	1.62	1.9	1.59	1.97	1.91	1.69

	19	20	21	22	23	24	25
20	1						
21	2.38	1.62					
22	2.11	1.36	1.92				
23	2.3	1.82	2.9	1.78			
24	1.67	1.62	3.5	2	3.33		
25	1.4	1.44	1.58	1.64	1.73	1.5	
26	1.7	2	1.94	2.09	1.97	1.76	2.33

Taxa key for table A4: Same as table A3.

TABLE A5: Pairwise p-distance of *cytochrome oxidase subunit one* (*CO I*) gene used in Chrysomyinae phylogeny (Chapter 3).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
2	0.076														
3	0.094	0.094													
4	0.089	0.084	0.096												
5	0.088	0.088	0.106	0.107											
6	0.088	0.1	0.107	0.109	0.065										
7	0.082	0.109	0.099	0.112	0.081	0.045									
8	0.111	0.097	0.108	0.112	0.086	0.087	0.08								
9	0.111	0.105	0.111	0.112	0.098	0.102	0.097	0.086							
10	0.113	0.106	0.112	0.114	0.099	0.104	0.1	0.086	0.006						
11	0.095	0.106	0.125	0.111	0.107	0.095	0.099	0.111	0.117	0.119					
12	0.086	0.102	0.119	0.11	0.08	0.081	0.099	0.111	0.112	0.116	0.092				
13	0.098	0.098	0.118	0.11	0.104	0.098	0.101	0.113	0.116	0.118	0.093	0.076			
14	0.104	0.105	0.105	0.106	0.091	0.079	0.068	0.086	0.096	0.099	0.114	0.099	0.106		
15	0.089	0.086	0.108	0.1	0.071	0.07	0.065	0.084	0.099	0.102	0.097	0.075	0.095	0.074	
16	0.088	0.103	0.111	0.107	0.078	0.072	0.077	0.092	0.105	0.108	0.097	0.082	0.096	0.077	0.049
17	0.091	0.09	0.106	0.098	0.072	0.069	0.056	0.085	0.096	0.099	0.096	0.079	0.097	0.077	0.049
18	0.091	0.096	0.105	0.105	0.082	0.08	0.085	0.092	0.098	0.101	0.092	0.059	0.084	0.09	0.075
19	0.089	0.098	0.106	0.105	0.082	0.08	0.088	0.096	0.098	0.101	0.088	0.057	0.082	0.095	0.074
20	0.084	0.094	0.097	0.1	0.094	0.095	0.09	0.096	0.112	0.114	0.095	0.085	0.092	0.095	0.094
21	0.078	0.089	0.101	0.097	0.079	0.071	0.083	0.089	0.096	0.098	0.086	0.052	0.079	0.093	0.068

	16	17	18	19	20
17	0.051				
18	0.069	0.067			
19	0.073	0.064	0.006		
20	0.098	0.091	0.078	0.076	
21	0.072	0.073	0.049	0.048	0.071

Taxa key for table A5:

1	*Lucilia sericata*	**12**	*Cochliomyia macellaria*
2	*Calliphora vomitoria*	**13**	*Cochliomyia hominivorax*
3	*Verticia orientalis*	**14**	*Chrysomya rufifacies*
4	*Sarconesia versicolor*	**15**	*Chrysomya putoria*
5	*Phormia regina*	**16**	*Chrysomya megacephala*
6	*Protophormia terraenovae*	**17**	*Chrysomya bezziana*
7	*Protophormia atriceps*	**18**	*Compsomyiops callipes*
8	*Trypocalliphora braueri*	**19**	*Compsomyiops fulvicrura*
9	*Protocalliphora sialia*	**20**	*Hemilucilia segmentaria*
10	*Protocalliphora occidentalis*	**21**	*Paralucilia paraensis*
11	*Chloroprocta idioidea*		

TABLE A6: Pairwise transition- transversion ratio of *cytochrome oxidase subunit one* (*CO I*) gene used in Chrysomyinae phylogeny (Chapter 3).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
2	1.28																			
3	1.18	0.8																		
4	1.09	0.96	0.87																	
5	1.14	1.12	1.14	1.07																
6	1	1.25	1.22	1.06	1.58															
7	1.45	1.15	1.5	0.95	1.79	2.33														
8	1.24	1.06	0.91	0.95	2.24	2.47	2.31													
9	1.16	1.24	1.11	1.08	1.98	2.11	1.56	2.97												
10	1.11	1.16	1.06	1.04	1.82	2.06	1.56	2.81	2											
11	0.81	0.88	0.88	0.79	1.08	1.01	0.81	1.16	1.13	1.11										
12	0.8	1.01	1.06	1.07	0.98	1.23	1.71	1.48	1.48	1.41	1.28									
13	0.87	0.93	0.94	0.9	1.26	1.35	1.06	1.31	1.27	1.2	1.22	2.63								
14	1.04	1.03	1.05	0.84	1.5	1.59	1.5	1.56	1.71	1.61	0.9	1.29	1.24							
15	0.92	1.1	1.04	0.91	1.76	1.44	1.69	2.24	1.96	1.84	0.96	1.04	1.11	1.86						
16	1.07	1.32	1.18	1.12	2.5	1.57	1.68	2.14	2.06	1.94	1.11	1.44	1.3	2.08	2.38					
17	1.2	1.25	1.14	1.08	2.43	1.75	1.64	2.27	2.07	1.94	1.09	1.46	1.4	2.06	2.68	6.3				
18	0.83	0.86	0.91	0.96	1.46	1.4	1.43	1.64	1.75	1.65	1.13	2.04	1.88	1.22	1.38	1.48	1.53			
19	0.78	0.8	0.88	0.94	1.31	1.27	1.43	1.51	1.6	1.55	1.04	1.77	1.62	1.2	1.26	1.45	1.34	---		
20	1	0.79	0.72	0.68	1.02	1.11	1.08	1.03	1.01	0.96	1.18	1.54	1.44	0.96	1.2	1.29	1.31	1.51	1.47	
21	0.8	1.02	0.97	0.85	1.47	1.22	1.52	1.47	1.41	1.34	1	1.52	1.58	1.15	1.18	1.46	1.51	1.56	1.35	1.58

Taxa key for table A6: Same as table A5.

TABLE A7: Pairwise p-distance of *carbamoylphosphate synthetase* (*CPS*) gene used in Chrysomyinae phylogeny (Chapter 3).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
2	0.111																
3	0.152	0.155															
4	0.141	0.104	0.163														
5	0.135	0.131	0.165	0.157													
6	0.134	0.147	0.151	0.166	0.059												
7	0.131	0.134	0.148	0.147	0.079	0.078											
8	0.134	0.131	0.145	0.151	0.076	0.071	0.028										
9	0.135	0.134	0.148	0.147	0.08	0.073	0.027	0.011									
10	0.155	0.148	0.159	0.183	0.118	0.126	0.118	0.125	0.122								
11	0.139	0.144	0.14	0.148	0.1	0.095	0.117	0.114	0.113	0.115							
12	0.139	0.139	0.148	0.153	0.105	0.095	0.112	0.106	0.108	0.124	0.062						
13	0.134	0.161	0.156	0.173	0.127	0.108	0.138	0.127	0.132	0.154	0.124	0.13					
14	0.119	0.15	0.149	0.17	0.126	0.107	0.117	0.113	0.117	0.148	0.122	0.131	0.064				
15	0.122	0.156	0.154	0.167	0.118	0.094	0.132	0.124	0.129	0.155	0.12	0.126	0.093	0.088			
16	0.127	0.157	0.157	0.171	0.124	0.104	0.132	0.126	0.132	0.164	0.124	0.128	0.104	0.097	0.042		
17	0.173	0.18	0.174	0.173	0.12	0.117	0.115	0.119	0.113	0.12	0.087	0.101	0.153	0.157	0.144	0.158	
18	0.163	0.158	0.178	0.162	0.107	0.113	0.115	0.116	0.11	0.124	0.086	0.096	0.15	0.157	0.138	0.151	0.019

Taxa key for table A7:

1	*Lucilia sericata*	**7**	*Trypocalliphora braueri*	**13**	*Chrysomya rufifacies*
2	*Calliphora vomitoria*	**8**	*Protocalliphora sialia*	**14**	*Chrysomya putoria*
3	*Verticia orientalis*	**9**	*Protocalliphora occidentalis*	**15**	*Chrysomya megacephala*
4	*Sarconesia versicolor*	**10**	*Chloroprocta idioidea*	**16**	*Chrysomya bezziana*
5	*Phormia regina*	**11**	*Cochliomyia macellaria*	**17**	*Compsomyiops callipes*
6	*Protophormia terraenovae*	**12**	*Cochliomyia hominivorax*	**18**	*Compsomyiops fulvicrura*

TABLE A8: Pairwise transition- transversion ratio of *carbamoylphosphate synthetase* (*CPS*) gene used in Chrysomyinae phylogeny (Chapter 3).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
2	1.29																
3	1.8	1.49															
4	1.35	1.84	1.5														
5	2.59	1.85	1.59	1.65													
6	2.24	1.47	1.79	1.9	1.06												
7	2.31	1.66	1.86	1.66	2.56	2.62											
8	2.17	1.47	1.46	1.72	2.25	2.07	2.83										
9	2.11	1.48	1.47	1.57	1.96	1.93	2.14	2									
10	1.78	1.18	1.17	1.26	1.42	1.62	1.56	1.56	1.65								
11	1.88	1.26	1.09	1.26	1.18	1.11	1.44	1.26	1.18	1.24							
12	1.81	1.15	1.25	1.42	1.02	1.38	1.53	1.25	1.24	1.68	1.79						
13	2.26	1.98	1.71	1.73	2.09	1.95	2.61	2.09	2.11	1.85	2.09	1.97					
14	2.16	1.82	1.93	1.78	2.59	2.56	3.32	2.44	2.46	1.95	1.97	1.92	2.79				
15	2.25	1.83	1.73	1.35	1.63	2	2.57	2.09	2.14	1.8	2.52	2.15	2.16	2.48			
16	2.06	1.77	1.71	1.41	1.56	2	2.24	1.89	1.95	1.75	2.09	1.92	2.38	2.64	4.14		
17	1.67	1.04	1.06	1.37	0.94	1.2	1	1.13	1.1	1.03	2.2	1.8	1.66	1.81	1.67	1.75	
18	1.47	0.84	0.96	1.27	0.83	1.03	0.84	1	0.97	1.06	1.93	1.45	1.75	1.61	1.37	1.44	2.33

Taxa key for table A8: Same as table A7.

TABLE A9: Pairwise p-distance of *28S ribosomal RNA* (*28S rRNA*) gene used in Oestridae phylogeny (Chapter 4).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1															
2	0.076														
3	0.06	0.079													
4	0.03	0.078	0.061												
5	0.068	0.117	0.092	0.071											
6	0.027	0.076	0.053	0.014	0.065										
7	0.03	0.08	0.056	0.026	0.068	0.017									
8	0.044	0.095	0.068	0.04	0.083	0.032	0.033								
9	0.095	0.179	0.125	0.098	0.113	0.091	0.095	0.113							
10	0.109	0.181	0.134	0.109	0.122	0.099	0.104	0.128	0.029						
11	0.059	0.106	0.081	0.059	0.096	0.057	0.06	0.068	0.002	0.027					
12	0.095	0.133	0.115	0.094	0.13	0.088	0.087	0.094	0.186	0.177	0.105				
13	0.069	0.111	0.077	0.065	0.098	0.059	0.063	0.072	0.096	0.095	0.066	0.089			
14	0.096	0.138	0.111	0.095	0.124	0.093	0.094	0.102	0.195	0.201	0.116	0.134	0.118		
15	0.112	0.156	0.129	0.11	0.139	0.103	0.109	0.118	0.192	0.194	0.129	0.133	0.126	0.083	
16	0.099	0.135	0.114	0.098	0.128	0.096	0.098	0.105	0.182	0.188	0.116	0.133	0.119	0.022	0.086
17	0.099	0.132	0.124	0.103	0.131	0.102	0.102	0.105	0.186	0.185	0.116	0.121	0.116	0.069	0.082
18	0.047	0.085	0.065	0.045	0.079	0.04	0.045	0.056	0.106	0.111	0.065	0.101	0.067	0.105	0.119
19	0.036	0.076	0.056	0.037	0.072	0.033	0.036	0.05	0.102	0.116	0.06	0.099	0.069	0.101	0.119
20	0.042	0.083	0.062	0.044	0.083	0.039	0.044	0.057	0.138	0.152	0.068	0.103	0.078	0.099	0.123
21	0.065	0.108	0.081	0.069	0.092	0.065	0.069	0.089	0.12	0.129	0.085	0.123	0.089	0.134	0.139
22	0.067	0.107	0.08	0.067	0.096	0.065	0.07	0.09	0.123	0.129	0.084	0.118	0.079	0.131	0.135

	16	**17**	**18**	**19**	**20**	**21**
17	0.069					
18	0.108	0.112				
19	0.103	0.11	0.02			
20	0.105	0.109	0.026	0.005		
21	0.138	0.138	0.076	0.07	0.081	
22	0.136	0.131	0.072	0.071	0.081	0.016

Taxa key table A9:

1	*Epalpus signifier*	**9**	*Cephenemyia stimulator*	**17**	*Przhevalskiana silenus*
2	*Musca domestica*	**10**	*Cephenemyia ultrichii*	**18**	*Cuterebra baeri*
3	*Glossina morsitans*	**11**	*Cephenemyia trompe*	**19**	*Cuterebra fontinella*
4	*Sarcophaga crassipalpis*	**12**	*Oestrus ovis*	**20**	*Cuterebra* spp.
5	*Mystacinobia zelandica*	**13**	*Rhinoestrus phacochoeri*	**21**	*Gasterophilus intestinalis*
6	*Chrysomya rufifacies*	**14**	*Hypoderma lineatum*	**22**	*Gasterophilus pecorum*
7	*Calliphora vomitoria*	**15**	*Hypoderma tarandi*		
8	*Rhinophora lepida*	**16**	*Hypoderma bovis*		

TABLE A10: Pairwise transition-transversion ratio of *28S ribosomal RNA* (*28S rRNA*) gene used in Oestridae phylogeny (Chapter 4).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
2	1.3																		
3	0.91	1.2																	
4	0.91	1.08	0.62																
5	0.88	0.93	0.88	0.75															
6	0.9	0.96	0.76	0.43	0.8														
7	1.1	1.07	0.86	1	0.88	1													
8	0.63	1.06	0.88	0.56	0.71	0.69	0.77												
9	1.06	1.03	0.69	1.27	1.22	1.13	0.89	0.74											
10	1.47	0.97	0.84	1.31	1.47	1.43	1.12	1	9										
11	1.56	1.18	0.87	1.56	0.97	1.35	1.21	0.96	---	8									
12	1.06	1.19	0.9	1.03	0.91	1.1	1.07	1.17	0.85	0.84	1.03								
13	1.09	1.08	0.74	0.96	0.84	1.05	1	1	0.67	0.7	0.92	0.82							
14	0.78	0.82	0.9	0.7	0.67	0.63	0.66	0.7	0.79	0.88	1.08	1.17	1.03						
15	0.95	0.89	0.88	0.93	0.83	0.95	0.95	0.91	0.76	0.81	1.2	1.21	1.15	1.15					
16	0.64	0.74	0.76	0.58	0.62	0.52	0.55	0.59	0.72	0.8	0.88	0.98	0.84	1.5	1.03				
17	1.03	1.19	1.1	1.06	0.86	0.97	0.97	0.82	0.91	0.94	1.31	1.24	1.19	1.88	1.23	1.72			
18	1.75	1.27	0.96	1.21	0.96	1.15	1.38	0.95	1.18	1.11	1.5	1.33	1.47	1.06	1.07	0.88	1.29		
19	2.12	1.12	0.95	1	0.85	0.92	1.08	1.06	1.06	1.17	1.33	1.16	0.92	0.97	1.07	0.8	1.26	0.75	
20	1.6	0.96	0.81	0.8	0.89	0.71	0.93	0.94	0.84	0.9	1.1	1	0.92	0.85	1	0.71	1.09	1	0.5
21	1.14	1.34	1.43	1.18	1	1.37	1.29	1.07	0.76	0.92	1.03	1	1.14	0.93	1.11	0.85	1.13	1.3	1.33
22	1.19	1.47	1.39	1.3	0.94	1.37	1.29	1.07	0.83	0.96	1.07	1.1	1.5	1.02	1.14	0.92	1.17	1.5	1.33

	20	**21**
21	1.38	
22	1.38	0.57

Taxa key for table A10: Same as table A9.

TABLE A11: Pairwise p-distance of *cytochrome oxidase subunit one* (*CO I*) gene used in Oestridae phylogeny (Chapter 4).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
2	0.179														
3	0.158	0.138													
4	0.173	0.12	0.135												
5	0.153	0.156	0.134	0.137											
6	0.161	0.137	0.144	0.149	0.158										
7	0.19	0.135	0.124	0.123	0.118	0.126									
8	0.184	0.108	0.119	0.129	0.13	0.111	0.109								
9	0.184	0.158	0.155	0.149	0.149	0.178	0.154	0.142							
10	0.182	0.151	0.159	0.14	0.139	0.146	0.141	0.134	0.083						
11	0.193	0.164	0.17	0.163	0.165	0.166	0.151	0.151	0.084	0.093					
12	0.212	0.193	0.178	0.173	0.19	0.174	0.169	0.169	0.141	0.113	0.147				
13	0.211	0.182	0.198	0.179	0.201	0.182	0.179	0.179	0.126	0.118	0.112	0.164			
14	0.211	0.192	0.2	0.186	0.199	0.191	0.17	0.179	0.151	0.14	0.141	0.19	0.18		
15	0.2	0.186	0.208	0.2	0.211	0.201	0.172	0.202	0.214	0.198	0.205	0.222	0.203	0.205	
16	0.201	0.185	0.177	0.162	0.171	0.191	0.16	0.173	0.192	0.172	0.193	0.199	0.195	0.201	0.167
17	0.195	0.183	0.17	0.161	0.162	0.189	0.158	0.172	0.19	0.173	0.192	0.199	0.19	0.199	0.166
18	0.209	0.177	0.178	0.168	0.165	0.187	0.176	0.183	0.18	0.185	0.186	0.215	0.198	0.203	0.169
19	0.198	0.177	0.182	0.186	0.193	0.184	0.161	0.17	0.188	0.183	0.188	0.199	0.212	0.193	0.163
20	0.215	0.222	0.194	0.181	0.182	0.201	0.188	0.198	0.222	0.203	0.214	0.193	0.215	0.228	0.177
21	0.228	0.173	0.196	0.182	0.201	0.185	0.18	0.173	0.166	0.174	0.166	0.199	0.188	0.208	0.215
22	0.232	0.177	0.2	0.186	0.204	0.186	0.18	0.176	0.17	0.176	0.164	0.202	0.188	0.208	0.217
23	0.261	0.211	0.236	0.227	0.224	0.214	0.202	0.217	0.208	0.198	0.198	0.215	0.228	0.209	0.234
24	0.275	0.228	0.259	0.257	0.259	0.245	0.234	0.251	0.23	0.233	0.222	0.251	0.24	0.228	0.241
25	0.183	0.135	0.143	0.164	0.16	0.159	0.135	0.138	0.161	0.16	0.161	0.189	0.185	0.192	0.179
26	0.166	0.141	0.146	0.152	0.136	0.154	0.125	0.14	0.154	0.158	0.157	0.18	0.173	0.19	0.172
27	0.17	0.151	0.161	0.179	0.147	0.142	0.156	0.132	0.185	0.167	0.192	0.206	0.177	0.232	0.214
28	0.173	0.153	0.144	0.17	0.154	0.153	0.163	0.132	0.184	0.171	0.185	0.193	0.18	0.227	0.201

	16	17	18	19	20	21	22	23	24	25	26	27
17	0.019											
18	0.089	0.087										
19	0.142	0.148	0.161									
20	0.17	0.17	0.18	0.151								
21	0.206	0.206	0.202	0.195	0.222							
22	0.203	0.203	0.201	0.198	0.222	0.009						
23	0.237	0.234	0.224	0.225	0.254	0.094	0.1					
24	0.253	0.251	0.235	0.24	0.27	0.14	0.144	0.09				
25	0.192	0.193	0.182	0.183	0.211	0.196	0.205	0.227	0.247			
26	0.17	0.166	0.17	0.169	0.202	0.186	0.19	0.218	0.234	0.057		
27	0.191	0.186	0.19	0.193	0.202	0.206	0.211	0.237	0.268	0.147	0.12	
28	0.186	0.178	0.181	0.164	0.183	0.197	0.204	0.236	0.273	0.144	0.131	0.014

Taxa key for table A11:

1	*Glossina morsitans*	**11**	*Hypoderma diana*	**20**	*Rhinoestrus phacochoeri*
2	*Musca domestica*	**12**	*Hypoderma tarandi*	**21**	*Gasterophilus intestinalis*
3	*Mystacinobia zelandica*	**13**	*Hypoderma actaeon*	**22**	*Gasterophilus haemorrhoidalis*
4	*Epalpus signifier*	**14**	*Przhevalskiana silenus*	**23**	*Gasterophilus nasalis*
5	*Rhinophora lepida*	**15**	*Oestrus ovis*	**24**	*Gasterophilus pecorum*
6	*Sarcophaga crassipalpis*	**16**	*Cephenemyia stimulator*	**25**	*Cuterebra baeri*
7	*Calliphora vomitoria*	**17**	*Cephenemyia trompe*	**26**	*Cuterebra jellisoni*
8	*Chrysomya rufifacies*	**18**	*Cephenemyia ulrichii*	**27**	*Cuterebra fontinella*
9	*Hypoderma bovis*	**19**	*Rhinoestrus usbekistanicus*	**28**	*Cuterebra* spp.
10	*Hypoderma lineatum*				

TABLE A12: Pairwise transition-transversion ratio of *cytochrome oxidase subunit one* (*CO I*) gene used in Oestridae phylogeny (Chapter 4).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
2	0.55																
3	0.52	0.49															
4	0.41	0.64	0.47														
5	0.58	0.86	0.76	0.45													
6	0.9	1.08	0.69	0.65	1.06												
7	0.58	0.86	0.58	0.69	0.73	0.92											
8	0.66	1.18	0.66	0.54	0.87	0.78	0.88										
9	0.45	0.7	0.73	0.7	0.48	1.04	0.71	0.81									
10	0.46	0.7	0.72	0.67	0.49	0.91	0.7	0.67	1.71								
11	0.63	0.77	1.02	0.82	0.71	0.94	0.79	0.93	3.83	2.37							
12	0.68	0.68	0.8	0.94	0.91	0.82	0.84	0.97	1.49	0.86	1.46						
13	0.73	0.95	1.09	0.92	0.87	0.96	0.98	1.28	2.62	2	3.28	1.4					
14	0.73	1.03	1.17	1.14	1.2	1.04	0.86	0.95	1.31	1.29	1.62	1.26	1.88				
15	0.64	0.75	0.72	0.69	0.68	0.93	0.76	0.76	0.65	0.55	0.74	0.78	0.73	0.76			
16	0.55	0.69	0.66	0.66	0.69	0.62	0.69	0.59	0.59	0.51	0.73	0.67	0.65	0.72	0.92		
17	0.48	0.7	0.62	0.61	0.64	0.63	0.7	0.59	0.6	0.55	0.69	0.65	0.64	0.73	0.87	12	
18	0.61	0.77	0.79	0.85	0.82	0.74	0.92	0.77	0.57	0.72	0.8	0.85	0.81	0.89	1.23	2.81	2.53
19	0.6	0.72	0.68	0.75	0.76	0.86	0.71	0.65	0.59	0.54	0.59	0.76	0.64	0.71	1.33	0.88	0.92
20	0.68	0.68	0.63	0.7	0.57	0.84	0.72	0.72	0.76	0.56	0.81	0.62	0.74	1.12	1.26	0.77	0.75
21	0.76	1.12	1.04	1	1.05	0.96	1.14	1.12	1.28	1.11	1.19	1.04	1.15	1.13	1.08	0.89	0.87
22	0.78	1.18	1.07	1.04	1.07	0.98	1.14	1.16	1.34	1.12	1.17	1.07	1.15	1.13	1.1	0.87	0.84
23	0.76	1.07	0.96	0.86	1.22	0.92	0.88	1.01	1.1	1.03	1	0.92	1.01	0.92	0.85	0.83	0.83
24	0.87	0.94	0.91	0.92	1.3	0.93	0.94	0.99	1.11	1.11	1.1	1.06	1.04	0.96	0.93	1.02	0.99
25	0.53	0.94	0.61	0.74	0.73	0.92	0.79	0.83	0.73	0.69	0.85	0.88	1.05	0.86	0.64	0.55	0.55
26	0.5	0.9	0.69	0.55	0.54	0.85	0.76	0.81	0.63	0.51	0.77	0.94	0.83	0.93	0.74	0.62	0.56
27	0.69	1.02	0.72	0.54	0.78	0.91	0.84	0.85	0.93	0.57	1.12	0.79	0.87	1.18	0.84	0.59	0.57
28	0.71	1	0.67	0.51	0.96	0.83	0.89	0.83	1.05	0.69	1.11	0.76	0.85	1.57	0.95	0.71	0.68

	18	19	20	21	22	23	24	25	26	27
19	1.13									
20	1.07	1.26								
21	0.96	1	0.89							
22	0.94	1.03	0.89	---						
23	0.86	0.87	0.8	0.81	0.92					
24	0.93	1.06	1.11	0.88	0.94	1.14				
25	0.58	0.68	0.79	1.05	1.14	0.95	0.95			
26	0.77	0.71	0.93	1.1	1.15	0.95	1.01	1.05		
27	0.73	0.73	0.71	0.87	0.92	0.81	1.03	1.3	1.09	
28	0.79	0.89	0.77	0.8	0.87	0.72	0.98	1.03	0.96	5

Taxa key for table A12: Same as table A11.

TABLE A13: Pairwise p-distance of *elongation factor one alpha* (*EF1α*) gene used in Oestridae phylogeny (Chapter 4).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
2	0.172																
3	0.2	0.106															
4	0.173	0.063	0.097														
5	0.176	0.104	0.137	0.102													
6	0.178	0.074	0.095	0.053	0.118												
7	0.169	0.07	0.093	0.036	0.089	0.06											
8	0.185	0.143	0.149	0.133	0.158	0.142	0.129										
9	0.18	0.148	0.148	0.137	0.156	0.147	0.133	0.044									
10	0.182	0.138	0.139	0.124	0.157	0.139	0.129	0.005	0.027								
11	0.187	0.178	0.184	0.186	0.196	0.193	0.178	0.213	0.22	0.211							
12	0.28	0.27	0.26	0.263	0.26	0.274	0.258	0.284	0.284	0.287	0.281						
13	0.275	0.261	0.267	0.263	0.258	0.27	0.26	0.282	0.285	0.289	0.282	0.014					
14	0.296	0.273	0.282	0.277	0.272	0.289	0.277	0.293	0.293	0.298	0.297	0.029	0.021				
15	0.289	0.278	0.276	0.282	0.278	0.281	0.279	0.3	0.298	0.293	0.291	0.025	0.01	0.022			
16	0.182	0.168	0.179	0.151	0.165	0.178	0.149	0.16	0.164	0.15	0.218	0.253	0.263	0.282	0.285		
17	0.17	0.15	0.164	0.13	0.142	0.154	0.13	0.137	0.135	0.121	0.215	0.263	0.266	0.286	0.285	0.066	
18	0.176	0.163	0.153	0.142	0.142	0.151	0.138	0.149	0.144	0.138	0.218	0.245	0.259	0.279	0.276	0.085	0.062

Taxa key for table A13:

1	*Glossina morsitans*	**7**	*Chrysomya rufifacies*	**13**	*Hypoderma lineatum*
2	*Musca domestica*	**8**	*Cuterebra fontinella*	**14**	*Hypoderma tarandi*
3	*Epalpus signifier*	**9**	*Cuterebra baeri*	**15**	*Przhevalskiana silenus*
4	*Sarcophaga crassipalpis*	**10**	*Cuterebra* spp.	**16**	*Oestrus ovis*
5	*Rhinophora lepida*	**11**	*Gasterophilus pecorum*	**17**	*Rhinoestrus phacochoeri*
6	*Calliphora vomitoria*	**12**	*Hypoderma bovis*	**18**	*Cephenemyia trompe*

TABLE A14: Pairwise transition-transversion ratio of *elongation factor one alpha* (*EF1α*) gene used in Oestridae phylogeny (Chapter 4).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
2	2																
3	1.75	1.71															
4	2.8	3.25	1.52														
5	3.04	2.73	1.88	7													
6	2.16	1.86	1.26	1.42	4												
7	2.72	2.17	1.43	4	6	1.06											
8	1.91	2.67	2.04	3.56	2.78	2.25	2.94										
9	1.61	1.86	1.53	2.26	2.07	1.79	1.92	2.43									
10	1.68	2.73	2.41	3.25	2.82	2.35	2.79	---	1.75								
11	2.32	2	1.81	2.64	2.72	1.94	3.08	2.44	1.95	2.48							
12	1.24	1.28	1.04	1.21	1.14	1.41	1.07	1.24	1.17	1.15	1.32						
13	1.16	1.26	1.23	1.28	1.1	1.39	1.18	1.22	1.17	1.16	1.41	4					
14	1.08	1.13	1.07	1.09	0.95	1.22	1.02	1.14	1.14	1.12	1.21	2.33	0.8				
15	1.03	1.21	1.13	1.17	1.05	1.3	1.1	1.16	1.06	1.11	1.24	2	1.5	1.25			
16	1.86	2.25	1.8	3.15	2.37	2.5	2.73	3	2.33	2.65	3.29	0.9	0.97	0.89	0.93		
17	2	1.89	1.81	2.63	2.12	2.28	2.63	2.43	2.38	2.12	3.22	0.94	0.97	0.9	0.92	3.38	
18	2.23	2.26	1.8	3.11	2.55	2.61	2.62	3.32	2.59	3.07	3.14	0.96	1.06	0.97	0.99	4.22	3.71

Taxa key for table A14: Same as table A13.

TABLE A15: Pairwise p-distance of *28S ribosomal RNA* (*28S rRNA*) gene used in Calliphoridae phylogeny (Chapter 5).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
2	0.07																
3	0.087	0.052															
4	0.067	0.041	0.056														
5	0.093	0.056	0.042	0.05													
6	0.068	0.044	0.04	0.041	0.048												
7	0.093	0.033	0.037	0.061	0.057	0.044											
8	0.072	0.049	0.033	0.043	0.043	0.037	0.033										
9	0.085	0.051	0.041	0.064	0.05	0.04	0.031	0.03									
10	0.067	0.045	0.051	0.038	0.05	0.032	0.05	0.035	0.043								
11	0.064	0.042	0.04	0.038	0.047	0.031	0.04	0.028	0.03	0.024							
12	0.079	0.048	0.032	0.058	0.045	0.035	0.027	0.019	0.018	0.04	0.025						
13	0.134	0.09	0.066	0.109	0.08	0.072	0.066	0.063	0.059	0.076	0.071	0.052					
14	0.07	0.047	0.041	0.043	0.052	0.035	0.044	0.033	0.037	0.031	0.022	0.027	0.072				
15	0.079	0.051	0.029	0.056	0.044	0.034	0.03	0.025	0.031	0.041	0.026	0.017	0.065	0.019			
16	0.091	0.056	0.043	0.069	0.055	0.044	0.037	0.036	0.031	0.05	0.036	0.019	0.065	0.028	0.018		
17	0.061	0.037	0.025	0.031	0.037	0.023	0.023	0.026	0.024	0.025	0.014	0.013	0.055	0.021	0.014	0.025	
18	0.085	0.054	0.036	0.057	0.042	0.038	0.035	0.028	0.034	0.051	0.027	0.02	0.062	0.033	0.025	0.035	0.007
19	0.068	0.046	0.033	0.041	0.049	0.031	0.039	0.037	0.037	0.034	0.023	0.026	0.069	0.028	0.025	0.037	0.016
20	0.079	0.038	0.028	0.054	0.038	0.03	0.028	0.026	0.028	0.043	0.025	0.02	0.062	0.029	0.021	0.028	0.013
21	0.066	0.04	0.034	0.033	0.04	0.025	0.039	0.031	0.034	0.029	0.022	0.03	0.063	0.025	0.026	0.037	0.014
22	0.089	0.064	0.078	0.07	0.086	0.06	0.075	0.067	0.065	0.062	0.056	0.061	0.105	0.06	0.07	0.069	0.053
23	0.092	0.073	0.085	0.071	0.096	0.064	0.094	0.069	0.074	0.069	0.06	0.068	0.106	0.062	0.073	0.076	0.052
24	0.061	0.034	0.034	0.033	0.04	0.026	0.033	0.026	0.031	0.022	0.017	0.019	0.057	0.016	0.018	0.026	0.015
25	0.062	0.037	0.034	0.034	0.043	0.027	0.033	0.027	0.031	0.022	0.016	0.019	0.06	0.015	0.018	0.027	0.014
26	0.079	0.045	0.034	0.058	0.048	0.034	0.028	0.026	0.022	0.04	0.027	0.009	0.053	0.024	0.018	0.017	0.015
27	0.055	0.033	0.036	0.036	0.046	0.028	0.03	0.029	0.021	0.025	0.02	0.012	0.055	0.019	0.02	0.02	0.017
28	0.059	0.038	0.037	0.036	0.047	0.029	0.039	0.029	0.035	0.024	0.019	0.022	0.068	0.018	0.02	0.031	0.016

29	0.172	0.153	0.109	0.157	0.109	0.141	0.135	0.131	0.111	0.144	0.135	0.098	0.076	0.132	0.096	0.103	0.126
30	0.058	0.037	0.043	0.039	0.054	0.033	0.036	0.032	0.029	0.026	0.022	0.018	0.061	0.022	0.026	0.028	0.02
31	0.044	0.026	0.031	0.032	0.046	0.023	0.022	0.027	0.023	0.023	0.014	0.011	0.045	0.013	0.014	0.009	0.012
32	0.06	0.035	0.026	0.032	0.04	0.023	0.023	0.022	0.024	0.02	0.013	0.013	0.053	0.016	0.014	0.024	0.008
33	0.059	0.035	0.027	0.032	0.038	0.023	0.024	0.022	0.025	0.02	0.014	0.014	0.052	0.016	0.015	0.025	0.009
34	0.059	0.035	0.023	0.031	0.038	0.023	0.025	0.023	0.025	0.02	0.013	0.014	0.054	0.016	0.015	0.025	0.008
35	0.058	0.035	0.029	0.034	0.041	0.026	0.023	0.027	0.018	0.023	0.017	0.007	0.046	0.02	0.018	0.019	0.013
36	0.06	0.035	0.027	0.031	0.04	0.025	0.024	0.023	0.025	0.021	0.014	0.019	0.055	0.019	0.017	0.027	0.011
37	0.06	0.037	0.031	0.036	0.043	0.029	0.025	0.028	0.019	0.025	0.019	0.012	0.045	0.023	0.02	0.021	0.015
38	0.089	0.05	0.035	0.067	0.048	0.035	0.027	0.023	0.022	0.044	0.027	0.011	0.045	0.029	0.021	0.021	0.013
39	0.062	0.038	0.024	0.034	0.039	0.026	0.025	0.025	0.024	0.023	0.015	0.014	0.058	0.018	0.015	0.025	0.01
40	0.059	0.037	0.032	0.034	0.038	0.024	0.028	0.023	0.022	0.023	0.014	0.017	0.051	0.018	0.016	0.027	0.011
41	0.084	0.05	0.036	0.06	0.045	0.037	0.028	0.026	0.022	0.047	0.028	0.013	0.05	0.032	0.022	0.023	0.015
42	0.088	0.055	0.032	0.065	0.043	0.041	0.033	0.028	0.032	0.047	0.027	0.02	0.063	0.028	0.022	0.033	0.013
43	0.063	0.033	0.032	0.044	0.044	0.026	0.024	0.02	0.02	0.029	0.019	0.011	0.052	0.023	0.021	0.023	0.01
44	0.067	0.046	0.043	0.041	0.054	0.032	0.046	0.032	0.043	0.026	0.025	0.028	0.07	0.028	0.029	0.04	0.022
45	0.057	0.038	0.035	0.039	0.042	0.029	0.03	0.03	0.022	0.024	0.02	0.011	0.049	0.022	0.022	0.023	0.018
46	0.057	0.037	0.032	0.039	0.04	0.029	0.027	0.029	0.02	0.024	0.02	0.009	0.044	0.021	0.019	0.021	0.017
47	0.09	0.054	0.04	0.068	0.052	0.042	0.034	0.032	0.027	0.049	0.032	0.015	0.051	0.028	0.022	0.019	0.021
48	0.073	0.052	0.063	0.047	0.065	0.039	0.067	0.044	0.06	0.041	0.035	0.048	0.105	0.037	0.047	0.058	0.029
49	0.109	0.076	0.058	0.082	0.062	0.059	0.061	0.049	0.051	0.074	0.058	0.043	0.087	0.059	0.047	0.052	0.045
50	0.06	0.037	0.034	0.031	0.038	0.026	0.034	0.025	0.032	0.022	0.018	0.022	0.056	0.019	0.022	0.031	0.014
51	0.079	0.053	0.034	0.056	0.038	0.036	0.038	0.024	0.028	0.041	0.021	0.021	0.055	0.022	0.022	0.033	0.013
52	0.082	0.049	0.028	0.058	0.041	0.031	0.03	0.021	0.028	0.039	0.022	0.013	0.055	0.019	0.011	0.023	0.009

	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
19	0.023																
20	0.019	0.021															
21	0.03	0.024	0.02														
22	0.08	0.061	0.059	0.057													
23	0.079	0.062	0.074	0.055	0.08												
24	0.021	0.023	0.022	0.02	0.054	0.058											
25	0.022	0.022	0.022	0.02	0.054	0.058	0.002										
26	0.021	0.024	0.018	0.027	0.059	0.071	0.009	0.01									
27	0.024	0.025	0.02	0.021	0.047	0.054	0.009	0.009	0.002								
28	0.026	0.023	0.024	0.022	0.055	0.061	0.008	0.01	0.017	0.012							
29	0.046	0.131	0.101	0.136	0.172	0.165	0.123	0.123	0.092	0.127	0.121						
30	0.031	0.027	0.026	0.026	0.052	0.06	0.012	0.012	0.011	0.009	0.007	0.122					
31	0.018	0.014	0.017	0.016	0.038	0.049	0.008	0.009	0.004	0.003	0.004	0.138	0.005				
32	0.015	0.019	0.015	0.016	0.052	0.056	0.009	0.008	0.013	0.011	0.01	0.124	0.014	0.01			
33	0.017	0.019	0.017	0.016	0.053	0.057	0.008	0.008	0.014	0.011	0.01	0.125	0.014	0.01	0.001		
34	0.011	0.017	0.012	0.015	0.051	0.055	0.008	0.007	0.012	0.01	0.01	0.124	0.013	0.008	0.002	0.002	
35	0.014	0.022	0.012	0.019	0.046	0.052	0.011	0.011	0.005	0.004	0.012	0.126	0.01	0.005	0.007	0.007	0.006
36	0.013	0.021	0.019	0.016	0.055	0.058	0.012	0.011	0.018	0.013	0.013	0.129	0.016	0.01	0.005	0.005	0.005
37	0.02	0.025	0.017	0.02	0.05	0.055	0.015	0.016	0.012	0.007	0.015	0.132	0.013	0.008	0.009	0.009	0.01
38	0.019	0.027	0.018	0.025	0.072	0.084	0.021	0.023	0.011	0.012	0.024	0.088	0.02	0.011	0.012	0.011	0.014
39	0.014	0.02	0.015	0.019	0.054	0.058	0.012	0.011	0.014	0.013	0.013	0.126	0.016	0.01	0.004	0.005	0.004
40	0.017	0.022	0.017	0.016	0.053	0.059	0.013	0.012	0.019	0.014	0.014	0.129	0.018	0.01	0.008	0.008	0.008
41	0.022	0.029	0.019	0.028	0.064	0.08	0.025	0.026	0.016	0.018	0.027	0.101	0.024	0.016	0.017	0.017	0.019
42	0.022	0.026	0.024	0.033	0.071	0.08	0.021	0.021	0.021	0.021	0.023	0.085	0.028	0.019	0.014	0.015	0.013
43	0.02	0.021	0.018	0.023	0.048	0.055	0.015	0.016	0.011	0.01	0.015	0.132	0.015	0.01	0.011	0.011	0.01
44	0.033	0.029	0.033	0.027	0.061	0.064	0.02	0.019	0.028	0.022	0.02	0.131	0.023	0.015	0.016	0.016	0.015
45	0.022	0.024	0.019	0.022	0.051	0.057	0.016	0.015	0.008	0.009	0.016	0.13	0.015	0.007	0.012	0.012	0.012
46	0.019	0.024	0.016	0.021	0.051	0.055	0.015	0.014	0.006	0.008	0.015	0.128	0.014	0.005	0.011	0.011	0.01
47	0.026	0.032	0.022	0.033	0.066	0.079	0.015	0.015	0.004	0.007	0.023	0.091	0.016	0.008	0.017	0.018	0.016

48	0.064	0.04	0.05	0.035	0.068	0.067	0.031	0.031	0.05	0.034	0.031	0.151	0.035	0.029	0.029	0.03	0.029
49	0.056	0.056	0.05	0.054	0.096	0.104	0.05	0.05	0.044	0.047	0.053	0.115	0.053	0.04	0.042	0.044	0.041
50	0.022	0.023	0.024	0.019	0.055	0.059	0.012	0.011	0.021	0.015	0.014	0.132	0.018	0.013	0.011	0.011	0.01
51	0.019	0.025	0.021	0.025	0.067	0.078	0.018	0.018	0.021	0.022	0.023	0.093	0.029	0.017	0.014	0.015	0.012
52	0.015	0.019	0.017	0.022	0.068	0.074	0.008	0.008	0.008	0.01	0.01	0.071	0.017	0.007	0.006	0.007	0.005

	35	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**
36	0.01																
37	0.005	0.007															
38	0.006	0.01	0.002														
39	0.01	0.007	0.011	0.013													
40	0.012	0.008	0.012	0.012	0.01												
41	0.011	0.018	0.012	0.009	0.019	0.01											
42	0.018	0.019	0.022	0.022	0.01	0.021	0.027										
43	0.007	0.013	0.007	0.01	0.01	0.015	0.016	0.006									
44	0.019	0.018	0.021	0.037	0.019	0.02	0.038	0.031	0.023								
45	0.007	0.013	0.008	0.011	0.015	0.015	0.017	0.017	0.009	0.015							
46	0.005	0.012	0.007	0.008	0.013	0.014	0.014	0.014	0.008	0.015	0.002						
47	0.009	0.024	0.016	0.015	0.019	0.023	0.019	0.026	0.016	0.034	0.014	0.01					
48	0.032	0.032	0.036	0.062	0.032	0.031	0.054	0.057	0.04	0.038	0.036	0.035	0.057				
49	0.04	0.048	0.045	0.045	0.044	0.047	0.048	0.054	0.049	0.061	0.047	0.043	0.046	0.046			
50	0.014	0.011	0.015	0.021	0.014	0.01	0.024	0.026	0.02	0.02	0.015	0.016	0.029	0.032	0.05		
51	0.015	0.018	0.021	0.021	0.014	0.014	0.022	0.02	0.022	0.029	0.015	0.015	0.024	0.052	0.05	0.011	
52	0.007	0.012	0.016	0.014	0.008	0.013	0.019	0.013	0.014	0.023	0.012	0.009	0.012	0.047	0.042	0.018	0.014

Taxa key for table A15:

1	*Drosophila melanogaster*	**14**	*Rhinophora lepida*	**27**	*Onesia tibialis*	**40**	*Pollenia rudis*
2	*Glossina morsitans*	**15**	*Stevenia hertingi*	**28**	*Lucilia sericata*	**41**	*Pollenia amentaria*
3	*Fannia canicularis*	**16**	*Stevenia atramentaria*	**29**	*Hypopygiopsis infumata*	**42**	*Bengalia peuhi*
4	*Musca domestica*	**17**	*Sarcophaga crassipalpis*	**30**	*Hemipyrellia fernandica*	**43**	*Bengalia depressa*
5	*Hydrotaea cyrtoneurina*	**18**	*Notochaeta* spp.	**31**	*Dyscritomyia robusta*	**44**	*Verticia orientalis*
6	*Mystacinobia zelandica*	**19**	*Metopia campestris*	**32**	*Phormia regina*	**45**	*Auchmeromyia luteola*
7	*McAlpine's fly*	**20**	*Sarcophila meridionalis*	**33**	*Protophormia terraenovae*	**46**	*Cordylobia anthropophaga*
8	*Epalpus signifier*	**21**	*Cuterebra fontinella*	**34**	*Chrysomya rufifacies*	**47**	*Melinda viridicyanea*
9	*Gymnocheta viridis*	**22**	*Hypoderma lineatum*	**35**	*Chrysomya megacephala*	**48**	*Mesembrinella* spp.
10	*Cyrtophleba nitida*	**23**	*Oestrus ovis*	**36**	*Cochliomyia macellaria*	**49**	*Eumesembrinella quadrilineata*
11	*Nemorilla floralis*	**24**	*Cynomya cadaverina*	**37**	*Cochliomyia hominivorax*	**50**	*Isomyia gomezmenori*
12	*Gymnosoma nitens*	**25**	*Calliphora vomitoria*	**38**	*Compsomyiops fulvicrura*	**51**	*Rhyncomya nigripes*
13	*Phania funesta*	**26**	*Bellardia vulgaris*	**39**	*Protocalliphora sialia*	**52**	*Eurychaeta palpalis*

TABLE A16: Pairwise transition-transversion ratio of *28S ribosomal RNA* (*28S rRNA*) gene used in Calliphoridae phylogeny (Chapter 5).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
2	0.97																	
3	0.94	1.21																
4	1.38	1.65	1.87															
5	1.31	1	1.67	1.29														
6	1.22	1.28	1.67	1.46	1.79													
7	0.72	1.2	1.3	1.17	1	1.42												
8	1.33	1.28	1.36	1.77	1.06	1.77	0.57											
9	1.09	1.2	1.46	1.7	1.67	1.33	0.75	1.17										
10	1.31	1.59	1.35	1.52	1.35	2.18	1.06	1.76	1.31									
11	1.12	1.12	1.13	1.78	1.53	1.74	0.69	1.76	0.86	1.86								
12	1	0.95	1.5	1.53	1.25	2	0.64	1.29	0.78	1.62	1.33							
13	1.03	1.3	1.75	1.65	1.67	2.27	1.38	1.82	2.33	1.38	1.92	3.5						
14	1.19	1.17	0.94	1.57	1.21	1.68	0.58	1.29	0.6	1.68	1.12	0.77	1.47					
15	0.89	0.91	0.92	1.24	1.19	1.9	0.43	0.91	0.8	1.69	1.44	0.88	2.3	2.2				
16	0.85	0.96	1	1.15	1.1	1.47	0.6	0.88	0.86	1.28	1.14	0.78	2.3	1.88	2			
17	1.08	0.94	1	1.36	1.14	1.71	0.45	1.75	0.62	1.8	1.67	1.75	2.11	1.19	1	0.91		
18	1.04	0.76	1.09	1.31	1.55	2.25	0.55	1.38	1.09	1.83	1.25	2.5	2.1	0.92	1.43	1.18	4	
19	1.02	1.05	1	1.23	1.05	1.43	0.73	1.44	0.78	1.59	1.17	1.44	1.62	1.24	1.33	1.14	1.08	0.88
20	0.89	0.94	0.83	1.14	0.88	1.6	0.58	1.09	0.85	1.18	1	1.12	1.82	0.92	1.25	1.09	0.5	0.62
21	1.18	1.16	1.45	1.39	1.13	1.8	1	2.06	1.42	1.67	2.6	1.78	1.82	1.8	2.14	2	1.67	1.5
22	1.19	1.23	0.97	1.67	1.2	1.45	0.71	1.62	1	1.49	1.56	1.08	1.23	1.72	1.07	1.04	1.42	1.04
23	1.47	1.69	1.96	2.19	1.85	2.42	1.74	2.26	2.2	2.17	2.33	1.81	2.6	2.12	2	2.2	2.48	1.7
24	1.2	1.28	1.25	1.62	1.06	1.87	0.57	1.32	0.59	1.64	1.07	1	1.42	1.08	1.5	1	1.27	1.14
25	1.15	1.26	1.45	1.71	1.06	2	0.69	1.65	0.69	1.64	1.25	1.29	1.73	1.27	2	1.2	1.56	1.5
26	0.97	1	1.25	1.53	1.11	1.9	0.58	1	0.46	1.27	1.09	1	2	0.91	1.5	1	1.17	1.33
27	1.17	1.08	1.15	1.61	1.31	1.71	0.43	1.29	0.38	1.93	1.06	0.67	1.45	1.21	1.12	0.78	1	1
28	1.02	1.25	1.23	1.5	1.24	2	0.73	1.4	0.67	1.67	1.07	1	1.62	1.14	1.43	1.17	1.25	1.25
29	0.99	1.05	1.15	1.12	1.02	1.18	0.89	1.06	0.9	1.08	1.06	1.02	1.64	1.01	1.1	1.02	1.07	0.88
30	1.07	1.35	1.54	1.78	1.39	2	0.71	1.48	0.79	1.62	1.31	1.5	1.73	1.4	1.75	1.56	1.54	1.62
31	1.12	0.88	1.11	0.95	1.23	2.25	0.43	1.21	0.67	2	0.78	0.4	1.5	2	2	2	0.75	0.5
32	1.04	1.11	1.33	1.41	1.06	1.79	0.45	1.57	0.4	1.62	1	0.83	2.38	1.17	1	0.82	0.75	1.2
33	1.06	1.15	1.2	1.35	1.13	1.92	0.42	1.4	0.38	1.75	0.92	0.71	2	1.08	0.86	0.75	0.67	1

34	1.04	1.15	1.25	1.43	1.07	1.92	0.7	1.6	0.57	1.75	1.1	1.4	2.38	1.36	1.6	1.1	0.86	1
35	1.04	1.23	1.3	1.55	1.12	1.75	0.6	1.5	0.45	1.6	1.23	2	2.43	1.36	1.5	1.29	1.1	1.5
36	1.08	1.15	1.75	1.43	1.2	1.73	0.42	1.53	0.83	1.92	1.4	1.29	1.8	1.38	1.14	0.92	1	0.67
37	1.04	1.18	1.27	1.5	1	1.4	0.38	1.3	0.55	1.41	1.07	0.83	1.4	1.11	0.8	0.64	0.79	0.88
38	1.06	1	1.27	1.56	1.06	1.78	0.45	1	0.6	1.58	1.38	1.67	1.88	0.75	1.14	0.88	0.8	1.4
39	1.08	1.21	1.38	1.48	1.13	1.87	0.7	1.47	0.5	1.79	1.08	1.4	2.62	1.31	1.6	1.1	0.89	1.5
40	1.13	1.1	1.17	1.67	1.29	1.35	0.54	1.53	0.82	2.17	1.88	1.5	2	1.31	1.33	1.09	1.11	1.6
41	0.92	0.95	1.33	1.53	1.25	1.5	0.64	0.91	0.64	1.53	1.44	1.75	2.25	1	1.25	1.11	1	1.5
42	1	1.05	1.4	1.58	1.12	2.2	1	1	0.67	1.77	0.91	1	2.2	0.69	1.12	1	1.5	2
43	1.03	0.95	1.27	1.36	1.33	1.82	0.6	0.85	0.55	1.62	0.77	0.5	1.89	0.69	0.8	0.73	0.71	1.33
44	0.88	1	1.2	1.16	1.05	1.45	0.76	1.08	0.85	1.1	1	1.09	1.43	1.09	1.18	1.06	1	1.44
45	1.04	1.17	1.7	1.46	1.43	1.82	0.82	1.5	0.9	2	1.54	2	2	1.12	1.57	1.38	1.31	2
46	1.11	1.1	1.5	1.46	1.29	1.76	0.64	1.4	0.7	1.6	1.2	1.33	1.75	1.06	1.29	1.12	1.15	1.6
47	0.91	0.9	1.14	1.33	1.11	1.58	0.62	0.85	0.54	1.12	1.18	0.83	2.25	0.91	1.67	1	1	1
48	1.22	1.15	1.23	1.44	1	1.32	1.1	1.85	1.22	1.56	2.11	1.5	2	1.58	1.5	1.29	1.45	1.53
49	0.93	0.97	1.39	1.03	0.92	1.44	1	1.4	1	1.2	1.26	1.29	1.75	1.32	1.5	1.29	1.12	1.12
50	1.17	1.26	1.45	1.68	1.07	1.93	0.92	2.15	1.25	2.6	2.75	2.6	1.8	1.46	1.71	1.17	2.43	2.75
51	1	0.91	0.93	1.59	1.14	1.5	0.6	1.22	0.77	1.54	1.43	1.12	1.55	0.64	1.25	1.08	0.83	0.86
52	0.94	0.95	1.33	1.44	1.13	2.43	0.64	1	0.57	1.5	1.12	1	2.11	0.88	2	1.25	1.33	1.5

	19	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
20	1																	
21	1.22	1.43																
22	1.35	0.88	1.66															
23	2.03	2.05	3.14	1.93														
24	1.11	0.9	1.43	1.38	2.2													
25	1.25	1.11	1.75	1.38	2.2	1												
26	1.22	0.88	1.56	0.85	1.9	1	1.67											
27	0.95	0.7	1.19	1.33	2.14	1.33	1.14	0										
28	1.05	0.91	1.4	1.39	2.26	1.8	1.29	1	1.38									
29	1	1	1.1	1.01	1.21	1.08	1.11	1.08	1.04	0.96								
30	1.25	1.2	1.62	1.66	2.38	2.8	2.33	3.5	2	1.75	0.97							
31	0.45	0.57	1.38	1.75	2.5	0.5	0.67	0	0	0.25	0.95	0.5						
32	1.07	0.75	1.36	1.39	2.24	1.14	1.6	0.83	0.8	1.12	1.09	1.56	0.57					

33	1	0.67	1.17	1.35	2.17	1.33	1.33	0.71	0.8	1.29	1.08	1.56	0.57	0				
34	1	0.57	1.5	1.4	2.25	1.33	2	1	0.89	1.29	1.06	1.75	0.5	2	1			
35	1.12	0.83	1.38	1.53	2.48	1.57	1.57	1	0.75	1.5	1.06	2.2	0.5	0.83	0.71	1		
36	1.19	0.89	1.6	1.57	2.43	1.5	2.17	1.29	1.33	1.44	1.09	2	0.83	1.67	1	3.5	1.43	
37	1	0.6	1.2	1.56	2.41	1.27	1.36	0.83	1	1.5	1.04	2	0.8	0.88	0.67	1.29	1	1.2
38	1.25	0.86	1.83	0.96	2.05	1.14	1.67	1.67	1	1.12	1.1	1.8	1	1	0.6	2	3	1.33
39	1.12	0.86	1.58	1.41	2.2	1.5	2	1.4	1	1.44	1.11	1.7	0.83	1.33	1	2	1.29	2
40	1	1	1.7	1.49	2.3	1.1	1.5	1.12	0.92	1.09	1.06	1.42	1	0.86	0.62	1.33	1.22	1.17
41	1.09	1	1.44	1.08	2.14	1	1.33	1.17	0.75	1	1.05	1.38	1	0.75	0.56	1.14	1.25	0.67
42	1.22	0.9	1.78	1.04	1.74	1	1.29	1	0.6	1	1	1.1	0.38	0.83	0.71	1	1.33	1.14
43	0.79	0.6	1.25	1.24	1.87	0.8	0.9	0.5	0.5	1	1.03	1	0.25	0.44	0.44	0.5	0.6	0.88
44	1.09	1.15	1.14	1.2	1.74	0.94	1.07	1.09	0.8	1.06	1	1.11	1.43	0.86	0.8	0.92	0.94	1
45	1.22	1.29	1.47	1.44	2.21	1.36	1.5	1.33	0.88	1.6	1.07	1.5	0.6	1.22	1.22	1.38	1.2	1.62
46	1	1	1.33	1.44	2.14	1.18	1.3	0.67	0.62	1.4	1.09	1.3	0.5	1	1	1.12	0.8	1.38
47	1.09	1	1.67	0.81	1.81	0.83	1.2	0.5	0.25	0.89	1.06	2	0.25	0.86	0.75	1	0.75	1.25
48	1.31	1.15	1.52	1.49	1.87	1.48	1.68	1.33	1.43	1.36	1.1	1.59	1.36	1.45	1.38	1.53	1.41	1.57
49	1.16	1.31	1.67	1	2.12	1.31	1.47	1.36	1.27	1.29	1.18	1.6	1.75	1.13	1.06	1.21	1.14	1.25
50	1.44	1.33	2.2	1.6	2.3	1.5	2.17	1.57	1.4	1.67	1.13	2	1.14	2.6	2	3.25	2.29	3.5
51	0.82	0.7	1.86	1	1.78	0.5	0.67	0.7	0.64	0.73	1	1	0.57	0.5	0.44	0.43	0.62	1.14
52	1.33	0.86	1.83	0.89	1.95	1	2	1	0.6	1	1	1.6	0.33	0.67	0.5	1	1	1.5

	37	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**
38	0														
39	1.11	2													
40	0.82	0.8	1.12												
41	0.25	0.4	1.14	0.33											
42	0.8	1.29	0.6	1.12	1.1										
43	0.5	0.75	0.5	0.8	0.62	1.5									
44	0.94	1.5	1.07	1.2	1.31	1.09	0.93								
45	1.17	6	1.4	1.78	1.6	0.86	0.83	1.08							
46	0.83	4	1.2	1.56	1.2	0.57	0.5	1.08	1						
47	0.86	1.2	1.33	1.25	1.33	0.9	0.5	0.92	1	0.6					
48	1.31	1.32	1.57	1.74	1.44	1.2	1.14	1.13	1.46	1.38	1.33				
49	1.06	1.2	1.36	1.25	1.25	1.22	1.12	1.14	1.43	1.29	1.36	1.36			

50	1.78	2.5	2.83	3.25	1.71	1.86	1.67	1.2	1.78	1.89	1.33	1.79	1.18		
51	0.8	1.14	0.71	2	1.25	0.6	0.64	0.77	0.5	0.62	0.8	1.33	1.06	2	
52	0.86	1.5	2	1.2	1.14	1	0.57	1.12	1.25	0.75	0.8	1.4	1.38	2.5	0.57

Taxa key for table A16: Same as table A15.

TABLE A17: Pairwise p-distance of *cytochrome oxidase subunit one* (*CO I*) gene used in Calliphoridae phylogeny (Chapter 5).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
2	0.126																
3	0.112	0.146															
4	0.116	0.141	0.113														
5	0.115	0.126	0.108	0.118													
6	0.133	0.148	0.148	0.132	0.142												
7	0.171	0.175	0.161	0.154	0.161	0.139											
8	0.12	0.147	0.115	0.128	0.129	0.129	0.154										
9	0.124	0.142	0.13	0.129	0.117	0.14	0.144	0.113									
10	0.118	0.137	0.128	0.133	0.129	0.133	0.163	0.12	0.111								
11	0.127	0.127	0.132	0.139	0.121	0.132	0.162	0.121	0.104	0.119							
12	0.131	0.151	0.118	0.14	0.116	0.153	0.136	0.126	0.118	0.12	0.129						
13	0.145	0.14	0.13	0.154	0.131	0.144	0.166	0.127	0.122	0.129	0.126	0.132					
14	0.126	0.138	0.124	0.142	0.122	0.134	0.17	0.13	0.13	0.125	0.115	0.124	0.127				
15	0.132	0.142	0.126	0.141	0.119	0.132	0.174	0.13	0.111	0.125	0.107	0.11	0.127	0.114			
16	0.129	0.147	0.125	0.146	0.123	0.14	0.146	0.13	0.126	0.113	0.13	0.105	0.116	0.132	0.117		
17	0.154	0.155	0.144	0.134	0.142	0.148	0.164	0.134	0.138	0.15	0.151	0.152	0.155	0.148	0.139	0.144	
18	0.114	0.144	0.14	0.139	0.126	0.136	0.16	0.137	0.122	0.139	0.119	0.134	0.14	0.142	0.124	0.119	0.13
19	0.127	0.131	0.143	0.134	0.114	0.131	0.144	0.125	0.114	0.122	0.106	0.139	0.129	0.123	0.122	0.12	0.144
20	0.149	0.145	0.154	0.143	0.139	0.14	0.16	0.147	0.13	0.15	0.129	0.148	0.15	0.142	0.133	0.146	0.141
21	0.147	0.157	0.151	0.151	0.137	0.153	0.177	0.153	0.144	0.144	0.143	0.156	0.161	0.104	0.142	0.149	0.147
22	0.128	0.143	0.124	0.125	0.128	0.143	0.169	0.137	0.126	0.134	0.129	0.137	0.149	0.127	0.132	0.126	0.148
23	0.132	0.148	0.144	0.14	0.143	0.149	0.172	0.126	0.131	0.134	0.135	0.147	0.134	0.136	0.14	0.14	0.142
24	0.2	0.183	0.206	0.192	0.179	0.205	0.197	0.198	0.191	0.164	0.173	0.169	0.189	0.206	0.186	0.196	0.2
25	0.13	0.154	0.146	0.131	0.126	0.139	0.166	0.125	0.133	0.136	0.128	0.142	0.145	0.126	0.122	0.124	0.13
26	0.114	0.138	0.135	0.119	0.114	0.13	0.156	0.114	0.108	0.127	0.107	0.13	0.125	0.114	0.108	0.107	0.117
27	0.117	0.149	0.426	0.136	0.118	0.144	0.426	0.132	0.104	0.135	0.12	0.149	0.141	0.123	0.089	0.426	0.135

28	0.118	0.136	0.124	0.141	0.122	0.124	0.149	0.12	0.118	0.11	0.11	0.105	0.12	0.101	0.111	0.1	0.133
29	0.118	0.135	0.132	0.114	0.116	0.126	0.145	0.112	0.104	0.128	0.118	0.138	0.128	0.108	0.111	0.119	0.116
30	0.123	0.141	0.117	0.121	0.112	0.144	0.156	0.116	0.116	0.133	0.118	0.13	0.137	0.109	0.113	0.108	0.13
31	0.106	0.129	0.098	0.101	0.102	0.129	0.138	0.114	0.097	0.118	0.111	0.132	0.122	0.106	0.097	0.099	0.121
32	0.134	0.146	0.145	0.134	0.125	0.134	0.154	0.122	0.129	0.142	0.133	0.141	0.143	0.137	0.126	0.114	0.124
33	0.116	0.143	0.131	0.108	0.123	0.134	0.154	0.128	0.126	0.133	0.125	0.136	0.146	0.115	0.122	0.133	0.134
34	0.123	0.154	0.136	0.114	0.126	0.134	0.142	0.123	0.128	0.136	0.131	0.138	0.154	0.122	0.118	0.123	0.126
35	0.112	0.141	0.118	0.11	0.114	0.128	0.144	0.12	0.118	0.129	0.126	0.122	0.14	0.124	0.115	0.116	0.12
36	0.124	0.153	0.138	0.109	0.128	0.137	0.168	0.123	0.127	0.134	0.131	0.132	0.151	0.124	0.123	0.144	0.128
37	0.128	0.148	0.141	0.105	0.134	0.14	0.16	0.128	0.122	0.136	0.126	0.149	0.159	0.123	0.132	0.145	0.138
38	0.128	0.14	0.126	0.129	0.128	0.137	0.146	0.124	0.133	0.141	0.121	0.142	0.15	0.114	0.127	0.138	0.13
39	0.12	0.142	0.12	0.105	0.117	0.14	0.155	0.118	0.113	0.126	0.118	0.128	0.139	0.108	0.117	0.129	0.129
40	0.125	0.15	0.109	0.128	0.125	0.144	0.177	0.123	0.121	0.13	0.123	0.127	0.136	0.12	0.113	0.105	0.14
41	0.128	0.136	0.145	0.131	0.13	0.134	0.161	0.125	0.133	0.14	0.128	0.141	0.149	0.124	0.125	0.143	0.137
42	0.134	0.139	0.138	0.127	0.123	0.145	0.171	0.124	0.12	0.133	0.13	0.137	0.142	0.126	0.12	0.123	0.146
43	0.112	0.139	0.124	0.118	0.112	0.136	0.161	0.114	0.109	0.126	0.114	0.133	0.133	0.117	0.108	0.114	0.12
44	0.123	0.137	0.143	0.125	0.12	0.128	0.147	0.123	0.116	0.131	0.116	0.124	0.144	0.12	0.116	0.125	0.13
45	0.109	0.137	0.122	0.128	0.108	0.135	0.166	0.108	0.098	0.117	0.111	0.123	0.135	0.105	0.1	0.116	0.134
46	0.109	0.143	0.426	0.109	0.12	0.134	0.426	0.123	0.106	0.123	0.122	0.152	0.14	0.115	0.09	0.426	0.128
47	0.109	0.13	0.117	0.122	0.104	0.13	0.15	0.107	0.101	0.117	0.114	0.121	0.126	0.112	0.105	0.121	0.128
48	0.122	0.146	0.12	0.138	0.112	0.118	0.157	0.109	0.116	0.113	0.109	0.093	0.118	0.091	0.112	0.092	0.131
49	0.134	0.147	0.156	0.156	0.129	0.148	0.166	0.139	0.133	0.141	0.133	0.142	0.152	0.139	0.134	0.139	0.151
50	0.116	0.131	0.123	0.13	0.108	0.132	0.174	0.124	0.118	0.126	0.12	0.13	0.133	0.114	0.12	0.128	0.142
51	0.117	0.136	0.124	0.12	0.112	0.141	0.16	0.12	0.108	0.124	0.119	0.121	0.134	0.113	0.077	0.108	0.129
52	0.107	0.122	0.113	0.113	0.104	0.128	0.15	0.11	0.114	0.114	0.116	0.122	0.128	0.11	0.094	0.099	0.122
53	0.119	0.134	0.113	0.116	0.11	0.126	0.166	0.102	0.11	0.112	0.111	0.113	0.132	0.109	0.107	0.103	0.133
54	0.122	0.146	0.119	0.115	0.119	0.133	0.131	0.112	0.108	0.122	0.123	0.127	0.13	0.115	0.112	0.111	0.139
55	0.121	0.13	0.114	0.12	0.109	0.136	0.128	0.113	0.103	0.119	0.11	0.118	0.125	0.113	0.1	0.103	0.136
56	0.137	0.152	0.137	0.141	0.12	0.143	0.146	0.137	0.125	0.135	0.127	0.127	0.128	0.113	0.123	0.118	0.143

	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
19	0.113																
20	0.137	0.122															
21	0.14	0.138	0.145														
22	0.14	0.117	0.132	0.112													
23	0.124	0.135	0.149	0.156	0.159												
24	0.16	0.19	0.189	0.213	0.19	0.209											
25	0.134	0.124	0.133	0.152	0.141	0.14	0.2										
26	0.118	0.118	0.133	0.146	0.123	0.128	0.178	0.059									
27	0.426	0.118	0.129	0.139	0.123	0.147	0.426	0.109	0.083								
28	0.103	0.099	0.117	0.134	0.124	0.13	0.198	0.09	0.082	0.426							
29	0.121	0.113	0.122	0.133	0.126	0.123	0.195	0.091	0.076	0.086	0.103						
30	0.111	0.125	0.133	0.134	0.129	0.132	0.189	0.098	0.084	0.103	0.103	0.071					
31	0.112	0.109	0.12	0.135	0.107	0.122	0.155	0.083	0.072	0.094	0.093	0.062	0.046				
32	0.115	0.129	0.121	0.158	0.152	0.15	0.192	0.099	0.089	0.112	0.107	0.083	0.103	0.089			
33	0.122	0.128	0.137	0.139	0.128	0.134	0.202	0.101	0.088	0.098	0.115	0.088	0.103	0.085	0.113		
34	0.113	0.127	0.143	0.141	0.131	0.141	0.197	0.108	0.1	0.109	0.113	0.088	0.098	0.092	0.115	0.065	
35	0.125	0.123	0.127	0.134	0.117	0.134	0.212	0.117	0.105	0.125	0.111	0.104	0.107	0.101	0.124	0.091	0.079
36	0.135	0.134	0.143	0.146	0.132	0.143	0.211	0.11	0.103	0.116	0.118	0.088	0.099	0.093	0.118	0.078	0.072
37	0.133	0.13	0.132	0.145	0.136	0.149	0.188	0.115	0.102	0.109	0.122	0.086	0.093	0.082	0.123	0.08	0.081
38	0.119	0.13	0.131	0.153	0.137	0.144	0.197	0.113	0.098	0.111	0.115	0.098	0.103	0.098	0.12	0.104	0.098
39	0.132	0.124	0.136	0.132	0.126	0.137	0.198	0.104	0.098	0.11	0.118	0.089	0.089	0.085	0.115	0.082	0.08
40	0.137	0.133	0.135	0.148	0.135	0.143	0.198	0.111	0.105	0.109	0.111	0.111	0.107	0.1	0.121	0.098	0.102
41	0.151	0.119	0.139	0.151	0.138	0.143	0.204	0.121	0.11	0.117	0.123	0.116	0.125	0.115	0.133	0.116	0.111
42	0.14	0.122	0.134	0.152	0.135	0.148	0.199	0.126	0.114	0.125	0.117	0.118	0.124	0.118	0.12	0.129	0.121
43	0.115	0.118	0.127	0.139	0.125	0.128	0.2	0.101	0.09	0.109	0.095	0.086	0.099	0.09	0.107	0.097	0.093
44	0.125	0.111	0.115	0.135	0.124	0.129	0.179	0.103	0.095	0.104	0.099	0.088	0.102	0.085	0.115	0.1	0.093
45	0.122	0.11	0.124	0.14	0.119	0.119	0.168	0.107	0.094	0.098	0.096	0.094	0.103	0.094	0.124	0.106	0.107
46	0.426	0.114	0.111	0.125	0.122	0.134	0.426	0.103	0.094	0.109	0	0.069	0.102	0.077	0.109	0.11	0.093
47	0.117	0.106	0.126	0.135	0.12	0.131	0.179	0.104	0.088	0.1	0.099	0.087	0.098	0.092	0.11	0.104	0.102

48	0.124	0.108	0.132	0.15	0.111	0.131	0.178	0.083	0.066	0.426	0.081	0.101	0.096	0.092	0.103	0.104	0.106
49	0.138	0.132	0.157	0.162	0.143	0.149	0.201	0.132	0.122	0.112	0.127	0.123	0.132	0.116	0.134	0.136	0.137
50	0.117	0.117	0.14	0.143	0.128	0.132	0.192	0.126	0.113	0.116	0.119	0.119	0.118	0.11	0.131	0.118	0.116
51	0.113	0.12	0.131	0.142	0.13	0.13	0.181	0.1	0.084	0.09	0.098	0.089	0.092	0.085	0.116	0.107	0.109
52	0.098	0.107	0.134	0.132	0.119	0.126	0.188	0.091	0.079	0.091	0.084	0.078	0.088	0.075	0.111	0.095	0.099
53	0.125	0.114	0.138	0.132	0.124	0.128	0.187	0.123	0.102	0.116	0.108	0.102	0.106	0.097	0.122	0.109	0.114
54	0.132	0.124	0.119	0.139	0.126	0.123	0.192	0.12	0.109	0.123	0.109	0.104	0.107	0.101	0.129	0.115	0.115
55	0.109	0.112	0.131	0.13	0.113	0.125	0.163	0.122	0.101	0.125	0.1	0.107	0.113	0.094	0.123	0.109	0.115
56	0.129	0.118	0.128	0.141	0.126	0.149	0.191	0.124	0.118	0.115	0.1	0.111	0.116	0.105	0.133	0.126	0.129

	35	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**
36	0.077																
37	0.099	0.082															
38	0.106	0.096	0.076														
39	0.095	0.073	0.057	0.082													
40	0.096	0.105	0.112	0.116	0.098												
41	0.122	0.132	0.128	0.123	0.119	0.125											
42	0.129	0.133	0.125	0.131	0.115	0.129	0.088										
43	0.103	0.101	0.106	0.107	0.09	0.109	0.126	0.12									
44	0.095	0.097	0.102	0.103	0.099	0.112	0.122	0.118	0.077								
45	0.105	0.111	0.119	0.118	0.106	0.111	0.112	0.107	0.091	0.091							
46	0.104	0.097	0.108	0.11	0.096	0.114	0.105	0.102	0.076	0.099	0.085						
47	0.104	0.111	0.117	0.115	0.107	0.122	0.112	0.115	0.084	0.097	0.086	0.077					
48	0.111	0.116	0.123	0.105	0.102	0.105	0.114	0.114	0.097	0.093	0.095	0.426	0.085				
49	0.139	0.142	0.14	0.136	0.141	0.144	0.126	0.132	0.141	0.137	0.13	0.126	0.13	0.129			
50	0.113	0.132	0.125	0.126	0.113	0.127	0.12	0.114	0.116	0.117	0.107	0.123	0.11	0.107	0.112		
51	0.106	0.107	0.11	0.11	0.105	0.112	0.112	0.105	0.1	0.111	0.096	0.09	0.093	0.097	0.122	0.116	
52	0.096	0.1	0.101	0.098	0.091	0.106	0.115	0.112	0.09	0.102	0.092	0.077	0.084	0.091	0.109	0.104	0.076
53	0.11	0.113	0.118	0.114	0.103	0.108	0.123	0.121	0.112	0.103	0.094	0.105	0.103	0.088	0.134	0.115	0.11

54	0.111	0.12	0.114	0.115	0.099	0.111	0.127	0.116	0.104	0.114	0.102	0.095	0.114	0.112	0.145	0.12	0.109
55	0.103	0.116	0.122	0.122	0.102	0.106	0.128	0.123	0.101	0.095	0.094	0.107	0.101	0.089	0.142	0.119	0.107
56	0.123	0.131	0.131	0.126	0.123	0.13	0.129	0.121	0.117	0.121	0.109	0.103	0.12	0.113	0.114	0.124	0.112

	52	**53**	**54**	**55**
53	0.092			
54	0.103	0.096		
55	0.099	0.081	0.087	
56	0.112	0.123	0.113	0.117

Taxa key for table A17:

1	*Drosophila melanogaster*	**15**	*Stevenia hertingi*	**29**	*Lucilia sericata*	**43**	*Bengalia peuhi*
2	*Glossina morsitans*	**16**	*Stevenia atramentaria*	**30**	*Hypopygiopsis infumata*	**44**	*Bengalia depressa*
3	*Fannia canicularis*	**17**	*Sarcophaga crassipalpis*	**31**	*Hemipyrellia fernandica*	**45**	*Verticia orientalis*
4	*Musca domestica*	**18**	*Notochaeta* spp.	**32**	*Dyscritomyia robusta*	**46**	*Auchmeromyia luteola*
5	*Hydrotaea cyrtoneurina*	**19**	*Metopia campestris*	**33**	*Phormia regina*	**47**	*Cordylobia anthropophaga*
6	*Mystacinobia zelandica*	**20**	*Sarcophila meridionalis*	**34**	*Protophormia terraenovae*	**48**	*Melinda viridicyanea*
7	*McAlpine's fly*	**21**	*Cuterebra fontinella*	**35**	*Chrysomya rufifacies*	**49**	*Mesembrinella* spp.
8	*Epalpus signifier*	**22**	*Cuterebra baeri*	**36**	*Chrysomya megacephala*	**50**	*Eumesembrinella quadrilineata*
9	*Gymnocheta viridis*	**23**	*Hypoderma lineatum*	**37**	*Cochliomyia macellaria*	**51**	*Sarconesia versicolor*
10	*Cyrtophleba nitida*	**24**	*Oestrus ovis*	**38**	*Cochliomyia hominivorax*	**52**	*Sarconesia chlorogaster*
11	*Nemorilla floralis*	**25**	*Cynomya cadaverina*	**39**	*Compsomyiops fulvicrura*	**53**	*Isomyia gomezmenori*
12	*Gymnosoma nitens*	**26**	*Calliphora vomitoria*	**40**	*Protocalliphora sialia*	**54**	*Rhyncomya nigripes*
13	*Phania funesta*	**27**	*Bellardia vulgaris*	**41**	*Pollenia rudis*	**55**	*Metallea erinacea*
14	*Rhinophora lepida*	**28**	*Onesia tibialis*	**42**	*Pollenia amentaria*	**56**	*Eurychaeta palpalis*

TABLE A18: Pairwise transition-transversion ratio of *cytochrome oxidase subunit one* (*CO I*) gene used in Calliphoridae phylogeny (Chapter 5).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
2	0.58																	
3	0.67	0.55																
4	0.74	0.75	0.9															
5	0.56	0.59	0.69	0.8														
6	0.58	0.69	0.64	0.74	0.67													
7	0.89	0.7	0.98	1	0.87	1.03												
8	0.52	0.55	0.55	0.71	0.54	0.59	0.6											
9	0.41	0.44	0.43	0.61	0.5	0.6	0.92	0.61										
10	0.52	0.6	0.48	0.7	0.51	0.73	0.91	0.57	0.47									
11	0.48	0.54	0.62	0.74	0.58	0.61	0.89	0.66	0.47	0.54								
12	0.54	0.49	0.32	0.65	0.63	0.61	0.83	0.61	0.58	0.64	0.71							
13	0.57	0.57	0.37	0.7	0.58	0.7	0.62	0.66	0.51	0.52	0.51	0.61						
14	0.7	0.84	0.66	1.01	0.67	0.96	1.17	0.78	0.59	0.79	0.71	0.68	0.72					
15	0.46	0.54	0.59	0.6	0.46	0.61	0.78	0.55	0.38	0.5	0.53	0.66	0.59	0.71				
16	0.6	0.44	0.49	0.63	0.45	0.78	0.79	0.53	0.32	0.53	0.51	0.41	0.58	0.8	0.57			
17	0.74	0.99	0.85	0.99	0.78	0.8	0.79	0.88	0.88	0.96	0.86	0.74	0.85	1.02	0.66	0.94		
18	0.49	0.61	0.61	0.94	0.56	0.64	0.78	0.56	0.66	0.64	0.74	0.41	0.64	0.63	0.5	0.71	0.94	
19	0.6	0.6	0.65	0.75	0.46	0.66	1.03	0.58	0.42	0.54	0.48	0.65	0.64	0.86	0.53	0.6	0.9	0.81
20	0.67	0.81	0.83	0.8	0.73	0.82	1.13	0.87	0.78	0.77	0.99	0.83	0.79	0.97	0.73	0.72	1.17	1.3
21	0.8	0.81	0.89	0.98	0.73	0.96	0.92	0.8	0.67	1.04	0.9	0.86	0.75	0.88	0.78	0.82	0.95	0.71
22	0.64	0.58	0.74	0.77	0.67	0.6	0.96	0.63	0.54	0.58	0.56	0.66	0.56	0.81	0.57	0.51	0.83	0.73
23	0.54	0.65	0.72	0.78	0.55	0.75	1	0.77	0.59	0.6	0.67	0.61	0.73	0.8	0.53	0.8	0.92	0.68
24	0.81	0.68	0.7	0.79	0.79	0.72	0.77	0.69	0.82	0.8	0.8	0.9	0.55	0.68	0.65	0.66	0.93	1.07
25	0.88	0.99	0.94	0.98	0.83	0.92	0.69	1	0.83	0.97	0.99	0.94	0.95	1.31	0.9	0.98	1.02	0.86
26	0.69	0.8	0.76	0.82	0.7	0.77	0.69	0.81	0.72	0.83	0.77	0.8	0.79	1.04	0.71	0.7	1.02	0.76
27	0.69	0.93	---	0.75	0.81	0.91	---	0.88	0.9	0.95	0.89	1.07	0.95	1.1	0.88	---	0.97	---
28	0.58	0.52	0.57	0.75	0.51	0.65	0.93	0.49	0.47	0.68	0.62	0.41	0.55	0.66	0.65	0.56	0.83	0.93
29	0.7	0.86	0.94	0.73	0.89	0.83	0.97	0.85	0.81	0.77	0.75	0.79	0.85	1.02	0.79	0.87	1.06	1.03
30	0.81	0.98	0.71	0.87	0.81	0.94	1.19	0.93	0.92	0.92	0.88	0.8	0.94	1.13	0.78	0.69	1.16	1
31	0.67	0.9	0.65	0.81	0.81	0.87	1.37	0.87	0.84	0.79	0.74	0.93	0.84	1.27	0.69	0.79	1.01	1.09
32	0.73	0.91	0.85	1.09	0.77	0.88	1	0.95	0.76	0.84	0.84	0.82	0.78	1.03	0.6	0.82	1.25	1.07

33	0.91	0.84	1	0.91	0.89	0.93	0.9	0.96	0.78	0.94	1.03	0.84	0.91	1.33	0.9	0.82	1.01	0.72
34	1.02	0.89	1.04	0.98	1.04	0.88	1	0.8	0.9	0.9	1	0.88	0.93	1.24	0.84	0.95	1.06	0.78
35	0.85	0.76	0.87	1	0.8	0.76	0.83	0.69	0.68	0.79	0.77	0.8	0.76	1.04	0.74	0.64	0.91	1.03
36	1.14	1.03	1.13	1.03	1.01	0.96	1.07	0.97	0.95	0.98	1.09	1.03	0.92	1.27	1.04	0.91	1.08	1.19
37	0.94	0.94	1.02	0.91	0.93	1.01	1.07	1.04	0.99	0.96	1.21	0.93	0.89	1.19	0.8	0.82	1.25	1.09
38	0.85	0.84	0.93	0.95	0.91	0.78	1.11	0.98	0.8	0.89	1.22	0.93	0.84	0.94	0.76	0.76	1.12	1
39	0.8	0.79	0.89	0.86	0.7	0.82	0.98	0.79	0.76	0.78	1	0.67	0.73	1.15	0.69	0.82	1.19	0.97
40	0.95	0.82	0.75	1.14	0.83	0.8	0.92	0.99	0.75	0.94	0.95	0.9	0.84	1.08	0.87	0.57	1.03	0.92
41	0.7	0.75	0.63	0.88	0.72	0.71	1.05	0.7	0.78	0.83	0.86	0.81	0.85	1.05	0.76	0.94	0.94	0.6
42	0.55	0.62	0.56	0.62	0.59	0.64	0.89	0.58	0.54	0.53	0.55	0.64	0.68	0.76	0.64	0.76	0.91	0.69
43	0.68	0.8	0.81	0.84	0.85	0.76	0.95	0.68	0.89	0.71	0.82	0.8	0.82	1.12	0.8	0.78	1.01	0.93
44	0.8	0.78	0.68	0.87	0.8	0.78	0.97	0.8	0.8	0.66	0.9	0.9	0.71	1.1	0.81	0.73	1.03	0.91
45	0.56	0.62	0.67	0.79	0.64	0.63	1.05	0.6	0.45	0.5	0.5	0.62	0.55	0.91	0.6	0.61	0.94	0.63
46	0.81	0.94	---	0.81	0.97	0.81	---	1	1.06	0.87	0.85	1.24	1.18	1.33	0.88	---	0.91	---
47	0.6	0.58	0.62	0.86	0.62	0.71	0.86	0.67	0.62	0.57	0.64	0.64	0.72	0.89	0.51	0.6	0.81	0.54
48	0.59	0.49	0.5	0.73	0.39	0.73	0.88	0.64	0.32	0.44	0.6	0.41	0.33	0.53	0.52	0.59	0.88	0.66
49	0.56	0.83	0.68	0.75	0.71	0.82	0.85	0.64	0.68	0.71	0.82	0.8	0.81	0.87	0.7	0.61	0.92	0.71
50	0.49	0.61	0.57	0.7	0.56	0.62	0.84	0.42	0.43	0.46	0.5	0.5	0.55	0.65	0.51	0.54	0.85	0.58
51	0.71	0.68	0.81	0.79	0.55	0.75	0.89	0.65	0.56	0.72	0.63	0.71	0.71	0.77	0.58	0.69	0.81	0.68
52	0.57	0.76	0.54	0.59	0.58	0.73	0.7	0.69	0.59	0.7	0.72	0.66	0.75	0.89	0.68	0.79	0.95	0.72
53	0.51	0.59	0.43	0.71	0.48	0.71	0.72	0.71	0.48	0.53	0.51	0.55	0.56	0.65	0.55	0.38	0.84	0.6
54	0.59	0.68	0.71	0.94	0.7	0.68	1	0.78	0.67	0.73	0.69	0.73	0.83	0.71	0.72	0.7	0.97	0.91
55	0.56	0.56	0.65	1.03	0.65	0.75	0.86	0.68	0.5	0.6	0.69	0.71	0.64	0.92	0.5	0.66	0.88	0.81
56	0.66	0.84	0.85	0.8	0.75	0.73	1	0.67	0.65	0.83	0.8	0.85	0.88	0.85	0.77	0.77	0.93	1.17

	19	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
20	0.82																	
21	0.78	1.06																
22	0.58	0.82	1.23															
23	0.72	0.71	0.81	0.64														
24	0.68	0.86	0.84	0.64	0.56													
25	1.05	1.09	0.95	0.88	0.93	0.87												
26	0.86	1.17	0.94	0.72	0.78	0.79	2.61											
27	0.86	1.03	1.14	0.94	0.8	---	1.3	1.14										

28	0.92	1.22	0.76	0.61	0.63	0.68	0.91	0.79	---									
29	0.84	1.09	0.89	0.78	0.9	0.92	1.36	1.28	1.33	1.03								
30	0.89	1.22	0.93	0.79	0.97	0.88	1.43	1.29	1.57	0.88	1.91							
31	0.74	1.05	1.11	0.68	0.87	1	1.29	1.07	1.25	0.79	1.54	5.62						
32	0.83	1.08	0.94	0.75	0.79	0.91	1.23	1.26	1.11	0.86	1.65	1.81	1.61					
33	0.9	1.13	1.17	0.88	0.92	0.95	1.5	1.12	1.07	0.87	1.14	1.39	1	1.18				
34	0.84	1.12	1.16	0.95	0.84	0.84	1.28	1.25	1.58	0.91	1	1.33	1.21	1.11	1.58			
35	0.71	0.98	0.97	0.83	0.75	0.76	1.21	1.03	1.09	0.88	1.04	1.08	0.93	0.97	1.5	1.59		
36	0.9	1.19	1.22	1.15	0.97	0.88	1.47	1.32	1.54	1.21	1.07	1.22	1.1	1.23	2.5	1.57	2.08	
37	0.93	1.33	1.08	0.99	0.88	0.98	1.36	1.01	1.21	1.07	0.8	1.22	1.11	1.38	0.98	1.23	1.29	1.44
38	0.82	1.06	1.08	0.86	0.94	0.9	1.19	0.93	1.25	0.91	0.87	1.47	1.35	1.04	1.26	1.35	1.24	1.3
39	0.66	1.09	0.95	0.81	0.86	0.8	0.99	0.8	1.18	0.69	0.78	0.95	0.87	1.11	1.31	1.27	1.2	1.45
40	0.87	0.98	0.94	0.76	0.99	0.77	1.52	1.24	1.21	0.65	1.16	1.28	1.09	1.09	1.98	2.11	1.71	2.06
41	0.71	1.06	0.92	0.75	0.78	0.75	1.2	1	1.09	0.76	0.9	1.17	0.82	0.97	1.06	1.39	1.01	1.18
42	0.5	0.95	0.71	0.51	0.66	0.67	0.83	0.74	0.82	0.74	0.94	0.89	0.72	1.08	0.79	0.91	0.71	0.9
43	0.89	1.28	0.99	0.87	0.78	0.87	1.48	1.25	1.58	0.76	1.24	1.23	1.17	1.24	1.34	1.25	1.1	1.5
44	0.72	1.21	1.13	0.85	0.72	0.65	1.33	1.16	1.36	0.8	1.03	1.16	0.96	1.21	1.38	1.2	0.88	1.3
45	0.61	0.97	0.91	0.6	0.63	0.66	1.23	0.8	0.9	0.69	1.18	0.97	0.74	1.03	1.14	1.22	1.05	1.18
46	0.86	0.97	0.87	0.7	0.96	---	1.32	1.36	2.1	---	1.29	1.77	1.16	1.13	1.2	1.32	1.28	1.06
47	0.58	0.88	0.85	0.52	0.71	0.71	1.43	1.18	1.19	0.69	1.32	1.04	0.89	0.98	1.16	1.12	0.95	1.32
48	0.54	0.9	0.79	0.44	0.55	0.75	1.76	1.19	---	0.58	1.09	0.97	0.6	1.18	0.82	0.8	0.73	1.05
49	0.75	0.89	0.75	0.71	0.86	0.75	0.96	0.9	1.21	0.48	0.96	1	0.91	0.84	0.86	1.2	0.93	1.1
50	0.46	0.78	0.72	0.59	0.65	0.71	0.89	0.61	1.06	0.32	0.8	0.69	0.73	0.67	0.76	0.86	0.63	0.81
51	0.74	1.01	0.76	0.69	0.64	0.91	1.2	0.96	0.96	0.82	1.09	1.18	0.96	1.08	1.07	1.06	0.84	1.12
52	0.77	0.9	0.84	0.54	0.73	0.88	1.33	0.91	2.06	0.47	1.1	1.35	1.23	1	0.94	1.1	0.91	1.22
53	0.5	0.8	0.93	0.62	0.7	0.8	1	0.79	1.44	0.43	0.8	0.85	0.9	0.99	1.05	0.99	0.8	1.13
54	0.73	0.88	0.89	0.61	0.81	0.71	1.16	1.13	1.26	0.79	0.99	1.04	1.06	1.12	0.96	1.01	0.91	1.15
55	0.6	0.94	0.91	0.74	0.64	0.7	1.36	1.08	0.97	0.73	1.3	1.14	1.11	1.05	1.29	1.34	1.16	1.48
56	0.75	0.92	0.74	0.68	0.81	0.95	1.08	0.96	1.2	0.84	1.01	1	0.91	0.99	1.02	1.13	0.89	1.09

	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
38	2.63																		
39	1.77	1.62																	
40	1.48	1.27	1.6																
41	1.26	1.24	1.24	1.14															
42	0.91	0.85	0.79	0.81	1.13														
43	1.09	1.08	1.15	1.33	1.05	0.77													
44	1.15	1.04	1.1	1.21	1.26	0.87	2.16												
45	1.06	0.94	0.88	1.11	0.96	0.62	1.27	1.22											
46	1.38	0.97	1.2	1.16	1.11	0.69	1.44	1.09	1.36										
47	0.98	0.81	0.85	1.06	0.92	0.66	1.08	1.19	0.88	2									
48	1	0.76	0.67	0.68	0.78	0.54	0.79	0.81	0.52	---	0.87								
49	1.07	0.97	0.88	0.87	0.89	0.78	0.99	0.93	0.82	1.34	0.8	0.57							
50	0.88	0.8	0.73	0.79	0.81	0.65	0.74	0.69	0.55	1	0.57	0.38	0.82						
51	1.07	0.9	0.94	1.08	0.9	0.66	0.73	0.9	0.87	1.04	0.81	0.79	0.87	0.66					
52	1.13	0.93	0.93	1.01	0.89	0.61	1.02	1.03	0.83	1.61	0.76	0.68	0.88	0.56	1.13				
53	0.97	0.95	0.79	0.87	0.74	0.5	0.96	0.93	0.67	1.21	0.68	0.41	0.76	0.57	0.68	0.66			
54	1.05	0.95	0.85	1.04	0.85	0.74	1.08	0.99	0.71	1.23	0.83	0.68	0.9	0.67	0.87	0.92	0.87		
55	1.21	1.08	1.17	1.07	0.86	0.67	1.13	1.3	0.76	1.37	0.78	0.8	0.93	0.58	0.77	1.03	1	1.07	
56	1.06	1.12	0.89	1.02	1.13	0.85	0.89	0.86	0.67	0.68	0.89	0.65	0.97	0.83	0.87	0.91	0.67	0.9	1.04

Taxa key for table A18: Same as table A17.

TABLE A19: Pairwise p-distance of *carbamoylphosphate synthetase* (*CPS*) gene used in Calliphoridae phylogeny (Chapter 5).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
2	0.326															
3	0.373	0.256														
4	0.279	0.273	0.257													
5	0.335	0.26	0.274	0.209												
6	0.312	0.26	0.257	0.234	0.236											
7	0.343	0.25	0.266	0.211	0.227	0.141										
8	0.319	0.269	0.266	0.216	0.251	0.17	0.18									
9	0.312	0.246	0.245	0.224	0.236	0.166	0.169	0.184								
10	0.35	0.243	0.296	0.249	0.243	0.169	0.149	0.185	0.188							
11	0.368	0.254	0.283	0.271	0.257	0.148	0.141	0.174	0.198	0.162						
12	0.243	0.133	0.131	0.177	0.133	0.207	0.147	0.152	0.14	0.137	0.753					
13	0.345	0.244	0.264	0.244	0.257	0.186	0.195	0.2	0.203	0.185	0.179	0.069				
14	0.318	0.233	0.243	0.208	0.254	0.179	0.197	0.18	0.175	0.204	0.216	0.131	0.196			
15	0.343	0.237	0.256	0.219	0.233	0.18	0.185	0.21	0.185	0.2	0.207	0.125	0.18	0.08		
16	0.325	0.243	0.26	0.207	0.26	0.192	0.19	0.188	0.177	0.191	0.226	0.15	0.177	0.133	0.125	
17	0.262	0.172	0.161	0.183	0.146	0.168	0.162	0.166	0.19	0.176	0.753	0.174	0.197	0.181	0.151	0.144
18	0.317	0.239	0.236	0.237	0.263	0.187	0.212	0.197	0.197	0.194	0.196	0.209	0.207	0.185	0.186	0.19
19	0.311	0.249	0.275	0.245	0.243	0.202	0.232	0.224	0.199	0.218	0.232	0.159	0.203	0.216	0.202	0.2
20	0.307	0.241	0.256	0.227	0.234	0.168	0.168	0.169	0.176	0.169	0.146	0.093	0.143	0.158	0.169	0.16
21	0.299	0.245	0.249	0.214	0.241	0.167	0.179	0.176	0.185	0.178	0.157	0.101	0.157	0.158	0.168	0.169
22	0.309	0.245	0.249	0.219	0.229	0.152	0.147	0.185	0.195	0.16	0.14	0.111	0.16	0.163	0.157	0.183
23	0.314	0.238	0.262	0.214	0.234	0.162	0.182	0.185	0.184	0.182	0.187	0.132	0.178	0.151	0.132	0.161
24	0.326	0.24	0.255	0.208	0.228	0.167	0.172	0.18	0.181	0.175	0.164	0.12	0.154	0.146	0.152	0.149
25	0.292	0.227	0.247	0.227	0.21	0.176	0.151	0.173	0.166	0.17	0.154	0.135	0.169	0.167	0.148	0.161
26	0.337	0.213	0.236	0.237	0.236	0.173	0.157	0.185	0.184	0.179	0.148	0.132	0.17	0.167	0.142	0.174
27	0.331	0.231	0.242	0.232	0.218	0.19	0.164	0.207	0.174	0.183	0.182	0.141	0.177	0.165	0.164	0.171

28	0.328	0.244	0.235	0.227	0.242	0.179	0.187	0.198	0.187	0.206	0.191	0.151	0.189	0.154	0.155	0.155
29	0.294	0.227	0.24	0.215	0.229	0.195	0.185	0.192	0.178	0.188	0.187	0.141	0.176	0.158	0.153	0.171
30	0.304	0.22	0.238	0.221	0.244	0.19	0.179	0.187	0.178	0.178	0.179	0.145	0.18	0.153	0.165	0.163
31	0.356	0.258	0.245	0.247	0.227	0.186	0.175	0.197	0.203	0.178	0.182	0.094	0.172	0.192	0.178	0.206
32	0.294	0.24	0.261	0.223	0.232	0.173	0.166	0.175	0.164	0.16	0.15	0.138	0.171	0.162	0.159	0.16
33	0.301	0.231	0.261	0.224	0.24	0.16	0.176	0.17	0.169	0.184	0.177	0.154	0.188	0.154	0.162	0.179
34	0.327	0.25	0.241	0.223	0.267	0.178	0.209	0.217	0.181	0.181	0.189	0.166	0.162	0.174	0.171	0.159
35	0.319	0.237	0.277	0.223	0.244	0.187	0.195	0.196	0.176	0.176	0.196	0.158	0.181	0.179	0.168	0.162
36	0.344	0.232	0.254	0.237	0.233	0.165	0.167	0.185	0.201	0.169	0.148	0.101	0.137	0.166	0.166	0.176
37	0.326	0.232	0.263	0.217	0.222	0.184	0.17	0.195	0.177	0.168	0.202	0.162	0.192	0.171	0.159	0.16
38	0.344	0.263	0.246	0.239	0.241	0.163	0.175	0.204	0.191	0.171	0.165	0.127	0.183	0.19	0.19	0.197
39	0.326	0.251	0.26	0.224	0.228	0.149	0.178	0.201	0.195	0.172	0.164	0.113	0.172	0.176	0.17	0.196
40	0.333	0.254	0.261	0.238	0.257	0.194	0.202	0.197	0.202	0.199	0.217	0.15	0.216	0.179	0.181	0.183
41	0.364	0.251	0.264	0.251	0.239	0.202	0.2	0.216	0.222	0.216	0.21	0.098	0.212	0.18	0.182	0.188
42	0.366	0.249	0.249	0.239	0.249	0.18	0.199	0.198	0.198	0.202	0.198	0.122	0.21	0.166	0.168	0.165
43	0.376	0.249	0.263	0.25	0.241	0.151	0.169	0.171	0.179	0.15	0.153	0	0.188	0.192	0.191	0.202

	17	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**
18	0															
19	0.171	0.194														
20	0.153	0.198	0.196													
21	0.158	0.186	0.201	0.036												
22	0.122	0.171	0.205	0.075	0.077											
23	0.137	0.193	0.187	0.124	0.112	0.127										
24	0.143	0.188	0.188	0.112	0.119	0.098	0.081									
25	0.141	0.178	0.193	0.126	0.127	0.119	0.131	0.127								
26	0.149	0.163	0.188	0.146	0.149	0.146	0.134	0.128	0.06							
27	0.157	0.173	0.188	0.156	0.16	0.132	0.133	0.131	0.128	0.109						
28	0.144	0.179	0.182	0.15	0.158	0.148	0.122	0.128	0.119	0.094	0.092					

29	0.152	0.18	0.193	0.148	0.145	0.141	0.139	0.139	0.1	0.095	0.126	0.12				
30	0.14	0.178	0.185	0.146	0.14	0.145	0.139	0.137	0.102	0.095	0.134	0.126	0.062			
31	0.105	0.188	0.201	0.158	0.16	0.144	0.163	0.156	0.109	0.113	0.151	0.138	0.086	0.096		
32	0.143	0.174	0.178	0.129	0.132	0.12	0.134	0.133	0.073	0.071	0.128	0.124	0.114	0.106	0.116	
33	0.163	0.181	0.183	0.141	0.146	0.142	0.145	0.137	0.145	0.14	0.182	0.158	0.152	0.144	0.163	0.141
34	0.181	0.192	0.204	0.154	0.158	0.172	0.145	0.13	0.158	0.156	0.16	0.157	0.149	0.165	0.161	0.144
35	0.143	0.197	0.201	0.155	0.156	0.159	0.152	0.155	0.162	0.151	0.157	0.154	0.14	0.148	0.178	0.145
36	0.144	0.186	0.205	0.014	0.037	0.079	0.127	0.113	0.134	0.145	0.156	0.163	0.153	0.149	0.155	0.127
37	0.134	0.172	0.183	0.161	0.171	0.159	0.165	0.16	0.156	0.154	0.158	0.156	0.161	0.165	0.184	0.159
38	0.119	0.167	0.207	0.105	0.106	0.109	0.141	0.127	0.155	0.166	0.175	0.167	0.148	0.153	0.162	0.151
39	0.085	0.168	0.203	0.09	0.095	0.083	0.112	0.102	0.15	0.165	0.168	0.165	0.156	0.145	0.149	0.142
40	0.152	0.186	0.224	0.179	0.182	0.18	0.158	0.153	0.156	0.144	0.177	0.159	0.16	0.158	0.179	0.149
41	0.164	0.186	0.242	0.172	0.168	0.153	0.175	0.164	0.162	0.155	0.181	0.171	0.181	0.177	0.197	0.165
42	0.139	0.182	0.226	0.179	0.176	0.146	0.146	0.152	0.154	0.137	0.16	0.149	0.165	0.155	0.166	0.138
43	0.274	0.186	0.203	0.14	0.157	0.138	0.156	0.141	0.147	0.151	0.166	0.164	0.151	0.145	0.154	0.151

	33	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**
34	0.171									
35	0.166	0.134								
36	0.145	0.146	0.151							
37	0.175	0.191	0.17	0.168						
38	0.162	0.158	0.163	0.101	0.186					
39	0.165	0.165	0.159	0.088	0.184	0.08				
40	0.192	0.148	0.167	0.178	0.182	0.182	0.174			
41	0.192	0.172	0.185	0.169	0.212	0.195	0.193	0.13		
42	0.166	0.15	0.165	0.177	0.191	0.183	0.177	0.104	0.106	
43	0.158	0.159	0.163	0.143	0.191	0.156	0.147	0.184	0.189	0.179

Taxa key for table A19:

1	*Drosophila melanogaster*	**12**	*Rhinophora lepida*	**23**	*Lucilia sericata*	**34**	*Bengalia peuhi*
2	*Glossina morsitans*	**13**	*Stevenia hertingi*	**24**	*Hypopygiopsis infumata*	**35**	*Verticia orientalis*
3	*Fannia canicularis*	**14**	*Sarcophaga crassipalpis*	**25**	*Phormia regina*	**36**	*Melinda viridicyanea*
4	*Musca domestica*	**15**	*Notochaeta* spp.	**26**	*Protophormia terraenovae*	**37**	*Mesembrinella* spp.
5	*Hydrotaea cyrtoneurina*	**16**	*Metopia campestris*	**27**	*Chrysomya rufifacies*	**38**	*Sarconesia versicolor*
6	*Epalpus signifier*	**17**	*Cuterebra fontinella*	**28**	*Chrysomya megacephala*	**39**	*Sarconesia chlorogaster*
7	*Gymnocheta viridis*	**18**	*Cuterebra baeri*	**29**	*Cochliomyia macellaria*	**40**	*Isomyia gomezmenori*
8	*Cyrtophleba nitida*	**19**	*Hypoderma lineatum*	**30**	*Cochliomyia hominivorax*	**41**	*Rhyncomya nigripes*
9	*Nemorilla floralis*	**20**	*Cynomya cadaverina*	**31**	*Compsomyiops fulvicrura*	**42**	*Metallea erinacea*
10	*Gymnosoma nitens*	**21**	*Calliphora vomitoria*	**32**	*Protocalliphora sialia*	**43**	*Eurychaeta palpalis*
11	*Phania funesta*	**22**	*Bellardia vulgaris*	**33**	*Pollenia rudis*		

TABLE A20: Pairwise transition-transversion ratio of *carbamoylphosphate synthetase* (*CPS*) gene used in Calliphoridae phylogeny (Chapter 5).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
2	0.99																	
3	0.8	1.17																
4	1.09	1.2	0.93															
5	1.04	1.24	1.06	1.62														
6	1.07	0.88	0.9	1.27	1.35													
7	1	0.88	0.96	1.18	1.13	1.52												
8	1.04	1.05	0.84	1.09	1.7	1.66	2											
9	0.96	1.11	0.92	1.29	1.38	2.14	1.72	2.53										
10	0.99	0.72	0.93	1.28	1.29	1.37	1.7	1.57	1.68									
11	1.04	0.86	0.82	1.43	1.37	1.68	1.55	2	2	1.37								
12	1.82	0.5	3.33	1.29	2.67	2.58	0.75	1.28	2.54	4	---							
13	1.09	0.92	0.8	1.4	1.32	1.59	1.66	1.29	1.89	1.43	1.29	1.33						
14	1.21	1.47	0.88	1.41	1.46	1.53	1.91	1.53	2.64	1.41	2.27	2.14	1.59					
15	1.11	1.24	0.97	1.63	1.23	1.32	1.5	1.68	2.43	1.55	2	3.67	1.74	3.2				
16	1.11	1.33	1.1	1.25	1.43	1.76	1.72	1.65	2.33	1.51	1.7	2.33	1.53	2.42	2.82			
17	1.47	2.2	0.89	1.43	2.25	1.7	1.67	0.92	2.05	1.8	---	1.89	1.22	2.93	2	2		
18	1.03	1.1	0.84	1.07	1.09	0.94	1.11	0.97	1.7	1.09	1.11	1	1.3	1.43	1.52	1.59	---	
19	0.93	1.15	0.92	1.39	1.24	1.65	1.14	1.42	1.52	1.04	1.19	1.94	1.26	1.59	1.44	1.83	1.95	1.73
20	1.06	0.96	0.8	1.11	1.19	1.35	1.15	1.28	2.11	1.32	0.96	4.17	1.09	1.4	1.43	1.82	1.63	1.64
21	1.03	0.86	0.89	1.34	1.24	1.49	1.19	1.02	2.06	1.1	1	3.86	1.22	1.73	1.49	2	1.89	1.38
22	1.19	1.06	0.78	1.35	1.38	1.38	0.96	1.35	2.2	1.48	1.19	2	1.74	1.41	1.32	1.73	1.33	1.55
23	1.26	1.19	1	1.58	1.31	1.88	1.35	1.51	3.14	1.2	1.74	3.4	1.68	2.2	2.24	2.34	1.65	1.37
24	1.22	0.97	0.98	1.22	1.3	2.46	1.87	1.88	3.17	1.43	1.83	2.33	1.55	1.88	2.16	2.02	1.76	1.3
25	1.07	1.04	0.76	1.41	1.12	1.78	1.44	1.59	2.19	1.22	2.21	2.21	1.53	2.09	2.21	1.8	1.56	0.97
26	1.09	1.11	0.83	1.47	1.18	1.76	1.34	1.55	1.97	1.32	2.28	2.12	1.51	2.26	2.57	2.28	1.42	1
27	1.31	1.16	0.81	1.58	1.29	1.9	1.66	1.8	2.07	1.34	1.77	2.13	2.03	2.12	2.31	2.21	1.55	1.17
28	1.21	1.47	0.8	1.35	1.14	1.92	1.67	1.8	2.77	1.44	2	3.55	1.43	2.85	2.68	2.77	1.94	1.17
29	1	0.95	0.75	1.28	1.05	1.63	1.21	1.44	1.72	0.9	1.35	1.76	1.31	1.98	1.96	2.02	1.63	1
30	1.02	0.98	0.88	1.23	1.08	1.6	1.09	1.26	1.77	0.91	1.33	1.82	1.42	2.02	1.87	1.82	1.65	1.41
31	0.94	1.05	0.91	1.12	0.95	1.46	0.9	1.27	1.26	0.81	1.18	2	1.12	1.68	1.56	1.79	0.57	1.09
32	1.05	1.24	1	1.35	1.11	1.72	1.53	1.47	2.17	1.17	2.33	2.83	1.44	2.14	2.46	1.89	1.94	1.13

33	1.23	1.08	1.05	1.22	1.28	1.6	1.39	1.54	2.24	1.97	1.8	2.19	1.82	1.77	2	1.96	1.79	1.91
34	1.13	1.05	0.98	1.16	1.29	1.33	1.55	1.62	1.67	1	1.55	1.25	1.15	1.5	2.33	1.8	1.2	1.09
35	1.26	1.2	1	1.37	1.18	1.56	1.59	1.5	1.96	1.15	1.52	3	1.41	1.71	2.26	1.71	2.29	1.52
36	1.05	0.95	0.79	1.24	1.23	1	1.1	1.23	1.97	1.28	1	2.67	1.09	1.35	1.53	1.63	0.6	1.65
37	1.11	0.98	0.76	1.24	1.22	1.56	1.32	1.81	2.18	1.41	1.76	2.86	1.36	1.89	1.44	1.82	1.93	1.1
38	0.86	0.97	0.85	1.24	1	1.24	1.33	1.3	1.83	1.47	1.13	2.25	2.06	1.48	1.85	1.91	0.5	1.33
39	0.89	1	0.97	1.3	1.25	1.33	1.56	1.42	2.12	1.62	1.32	2.67	1.47	1.6	1.97	1.97	0.8	1.42
40	1.2	1.16	0.93	1.21	1.24	1.14	1.33	1.33	1.62	1.26	1.69	2.62	1.38	1.82	2.06	1.8	1.58	1.23
41	1.1	1.18	1.03	1.22	1.33	1.02	1	1.51	1.56	1.4	1.68	1.5	1.3	1.26	1.46	1.47	1	0.92
42	1.3	1.3	0.89	1.23	1.48	0.98	0.94	1.31	1.54	1.18	1.44	5.5	1.18	1.51	1.84	1.35	1	0.81
43	0.92	0.88	0.81	1.33	1.1	1.21	1.32	1.77	2.36	1.03	1.9	---	1.39	1.67	1.89	1.71	---	1.07

	19	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
20	1.6																	
21	1.51	1.42																
22	1.79	1.27	1.5															
23	1.69	1.39	1.32	1.16														
24	1.76	1.41	1.52	1.46	2.29													
25	1.52	1.78	1.77	2	2.47	2.09												
26	1.53	1.77	1.5	1.53	2.24	2.21	1.12											
27	1.71	1.75	1.89	1.69	2.18	1.74	2.06	2										
28	1.72	1.77	1.85	1.29	2.25	1.95	1.66	2	2.17									
29	1.65	1.13	1.28	1.09	1.88	1.68	1.18	1.11	2.03	2.52								
30	1.66	1.19	1.16	1.14	1.81	1.7	0.98	1.38	1.94	2.15	1.79							
31	1.08	0.76	0.86	1.04	1.47	1.42	0.87	1.03	1.79	1.37	1.93	1.45						
32	1.32	1.49	1.49	1.6	2.17	1.76	2.1	2.07	2.06	2.09	1.26	1.25	1					
33	1.44	1.41	1.24	1.71	2.05	2.29	1.54	2	2.11	2.02	1.51	1.63	1.33	1.59				
34	1	1.57	1.75	1.33	1.63	1.37	2	1.84	1.75	1.57	1.68	1.48	1.33	1.94	1.81			
35	1.48	1.51	1.51	1.6	1.8	1.59	1.56	1.79	1.76	1.73	1.09	1.25	0.96	1.46	1.59	1.61		
36	1.23	6	1.11	1.6	1.06	0.97	1.65	1.62	1.56	1.36	0.93	1.03	0.72	1.36	1.27	1.5	1.26	
37	1.9	1.84	1.78	1.89	2.12	2.43	2.44	2.03	2.21	1.82	1.67	1.79	1.16	1.86	1.94	1.44	1.7	1.38
38	1.4	2.12	1.89	2.08	1.35	1.54	1.61	1.9	1.76	1.35	1.26	1.42	1.27	1.72	1.71	2.11	1.5	1.89
39	1.39	1.65	1.45	2.11	1.42	1.79	2.25	2.44	1.9	1.66	1.42	1.34	1.03	1.85	2.04	1.64	1.65	1.37
40	1.26	1.74	1.67	1.68	1.94	1.61	1.91	1.84	1.6	1.65	1.65	1.62	1.51	1.71	2.16	1.89	1.64	1.82

41	1.17	1.47	1.35	0.92	1.37	1.17	1.14	1.39	1.32	1.25	1.1	1.21	1.09	1.14	1.46	1.86	1.54	1.55
42	1.16	1.27	1.16	0.88	1.33	1.05	1.19	1.43	1.3	1.26	1.29	1.22	1.05	1.03	1.23	1.26	1.44	1.33
43	1.18	1.24	1.17	1.24	1.71	2.11	1.7	1.78	2	1.96	1.03	1.22	0.94	2.37	1.71	1.71	1.3	1.46

	37	**38**	**39**	**40**	**41**	**42**
38	1.49					
39	1.64	2.15				
40	1.73	1.5	1.57			
41	1.46	1.38	1.36	2.1		
42	1.49	1.16	1.33	1.75	2.12	
43	1.5	1.36	1.58	1.48	1.47	1.14

Taxa key for table A20: Same as table A19.

TABLE A21: Pairwise p-distance of *elongation factor one alpha* (*EF1α*) gene used in Calliphoridae phylogeny (Chapter 5).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
2	0.223														
3	0.151	0.172													
4	0.141	0.175	0.082												
5	0.161	0.19	0.094	0.093											
6	0.154	0.195	0.105	0.122	0.103										
7	0.177	0.185	0.102	0.118	0.099	0.089									
8	0.174	0.184	0.106	0.126	0.105	0.097	0.089								
9	0.157	0.176	0.085	0.09	0.074	0.086	0.075	0.083							
10	0.179	0.183	0.109	0.127	0.121	0.124	0.108	0.116	0.107						
11	0.185	0.191	0.149	0.156	0.138	0.123	0.121	0.126	0.113	0.127					
12	0.183	0.176	0.104	0.111	0.111	0.132	0.119	0.115	0.103	0.118	0.135				
13	0.161	0.178	0.1	0.111	0.105	0.128	0.112	0.109	0.101	0.119	0.137	0.093			
14	0.172	0.185	0.098	0.108	0.099	0.116	0.106	0.1	0.081	0.113	0.114	0.077	0.049		
15	0.153	0.17	0.058	0.084	0.055	0.088	0.077	0.08	0.064	0.096	0.119	0.093	0.085	0.084	
16	0.143	0.185	0.07	0.094	0.069	0.097	0.078	0.085	0.066	0.099	0.106	0.091	0.094	0.081	0.041
17	0.153	0.167	0.078	0.096	0.068	0.089	0.087	0.083	0.074	0.104	0.113	0.096	0.099	0.093	0.043
18	0.145	0.173	0.065	0.086	0.056	0.085	0.074	0.077	0.06	0.095	0.108	0.104	0.092	0.082	0.032
19	0.195	0.187	0.145	0.151	0.147	0.135	0.152	0.134	0.122	0.143	0.16	0.151	0.157	0.144	0.123
20	0.206	0.18	0.145	0.154	0.146	0.143	0.149	0.148	0.136	0.136	0.158	0.154	0.172	0.159	0.133
21	0.271	0.288	0.259	0.249	0.25	0.264	0.28	0.274	0.264	0.263	0.272	0.269	0.284	0.269	0.265
22	0.276	0.292	0.267	0.255	0.264	0.267	0.287	0.284	0.278	0.274	0.285	0.282	0.294	0.28	0.277
23	0.144	0.174	0.069	0.079	0.063	0.082	0.079	0.086	0.067	0.093	0.118	0.102	0.09	0.09	0.049
24	0.147	0.177	0.075	0.089	0.068	0.096	0.085	0.098	0.074	0.104	0.125	0.111	0.098	0.1	0.053
25	0.159	0.191	0.093	0.093	0.068	0.106	0.088	0.091	0.068	0.091	0.124	0.11	0.105	0.101	0.059
26	0.148	0.187	0.08	0.085	0.066	0.086	0.083	0.083	0.066	0.093	0.119	0.105	0.103	0.101	0.054
27	0.139	0.179	0.073	0.092	0.064	0.093	0.077	0.085	0.065	0.096	0.117	0.101	0.091	0.094	0.048
28	0.138	0.181	0.07	0.096	0.066	0.086	0.078	0.087	0.066	0.097	0.116	0.105	0.098	0.101	0.045

29	0.137	0.172	0.065	0.092	0.066	0.085	0.076	0.081	0.062	0.093	0.116	0.103	0.094	0.093	0.044
30	0.139	0.173	0.073	0.088	0.057	0.09	0.078	0.084	0.067	0.096	0.119	0.099	0.096	0.097	0.05
31	0.148	0.175	0.061	0.087	0.06	0.081	0.082	0.076	0.057	0.093	0.12	0.09	0.082	0.079	0.04
32	0.145	0.171	0.059	0.09	0.054	0.078	0.075	0.074	0.054	0.09	0.115	0.085	0.08	0.078	0.04
33	0.148	0.164	0.074	0.091	0.069	0.087	0.082	0.077	0.062	0.104	0.111	0.081	0.08	0.079	0.039
34	0.148	0.162	0.071	0.094	0.07	0.082	0.083	0.077	0.064	0.101	0.111	0.084	0.082	0.077	0.042
35	0.15	0.167	0.065	0.094	0.055	0.086	0.079	0.07	0.056	0.099	0.114	0.089	0.082	0.077	0.033
36	0.15	0.175	0.085	0.105	0.064	0.097	0.084	0.084	0.067	0.115	0.124	0.1	0.096	0.09	0.042
37	0.145	0.169	0.072	0.094	0.055	0.089	0.08	0.081	0.061	0.098	0.113	0.091	0.082	0.078	0.027
38	0.15	0.169	0.073	0.094	0.058	0.087	0.075	0.073	0.062	0.092	0.116	0.085	0.079	0.076	0.049
39	0.149	0.195	0.081	0.116	0.092	0.097	0.092	0.092	0.073	0.107	0.121	0.108	0.108	0.099	0.066
40	0.164	0.183	0.084	0.112	0.087	0.105	0.096	0.098	0.079	0.108	0.118	0.111	0.104	0.097	0.061
41	0.145	0.165	0.068	0.079	0.065	0.092	0.078	0.084	0.062	0.102	0.121	0.084	0.086	0.077	0.042
42	0.157	0.176	0.076	0.109	0.073	0.091	0.082	0.083	0.064	0.107	0.126	0.106	0.099	0.09	0.05
43	0.16	0.171	0.082	0.095	0.058	0.108	0.08	0.091	0.066	0.099	0.116	0.096	0.085	0.085	0.053
44	0.153	0.605	0.054	0.605	0.605	0.067	0.605	0.072	0.056	0.605	0.605	0.605	0.605	0.605	0.044
45	0.162	0.179	0.097	0.1	0.08	0.112	0.09	0.086	0.072	0.111	0.118	0.098	0.097	0.091	0.066
46	0.193	0.207	0.139	0.158	0.111	0.119	0.125	0.12	0.107	0.151	0.176	0.15	0.121	0.13	0.081
47	0.156	0.186	0.097	0.115	0.087	0.098	0.106	0.098	0.09	0.123	0.125	0.111	0.114	0.098	0.067
48	0.157	0.175	0.08	0.11	0.091	0.101	0.079	0.089	0.078	0.107	0.128	0.104	0.105	0.095	0.06
49	0.142	0.182	0.085	0.08	0.076	0.11	0.09	0.105	0.08	0.105	0.128	0.111	0.104	0.104	0.068
50	0.163	0.179	0.084	0.102	0.073	0.093	0.086	0.084	0.065	0.11	0.114	0.108	0.088	0.082	0.054
51	0.157	0.193	0.093	0.083	0.076	0.114	0.092	0.103	0.079	0.11	0.136	0.118	0.112	0.102	0.077
52	0.158	0.187	0.086	0.107	0.078	0.093	0.08	0.082	0.063	0.115	0.115	0.102	0.095	0.083	0.055
53	0.153	0.186	0.081	0.106	0.082	0.115	0.096	0.098	0.092	0.104	0.141	0.111	0.098	0.104	0.074

	16	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
17	0.053														
18	0.035	0.045													

19	0.129	0.124	0.137												
20	0.133	0.138	0.143	0.041											
21	0.261	0.276	0.272	0.286	0.288										
22	0.274	0.287	0.283	0.295	0.294	0.007									
23	0.041	0.061	0.05	0.134	0.14	0.27	0.274								
24	0.046	0.07	0.056	0.143	0.149	0.276	0.28	0.009							
25	0.054	0.069	0.061	0.142	0.142	0.277	0.286	0.044	0.049						
26	0.048	0.062	0.059	0.132	0.149	0.27	0.277	0.027	0.04	0.023					
27	0.043	0.059	0.046	0.127	0.139	0.273	0.282	0.039	0.036	0.04	0.038				
28	0.044	0.056	0.046	0.129	0.142	0.269	0.277	0.037	0.04	0.047	0.043	0.018			
29	0.047	0.055	0.049	0.124	0.135	0.267	0.275	0.033	0.035	0.05	0.04	0.017	0.011		
30	0.051	0.06	0.051	0.129	0.137	0.274	0.279	0.041	0.034	0.045	0.043	0.019	0.02	0.016	
31	0.043	0.048	0.037	0.123	0.137	0.266	0.281	0.049	0.061	0.056	0.049	0.035	0.039	0.036	0.043
32	0.037	0.045	0.026	0.122	0.132	0.268	0.284	0.043	0.053	0.056	0.047	0.038	0.033	0.034	0.041
33	0.041	0.048	0.035	0.117	0.13	0.261	0.274	0.055	0.062	0.064	0.058	0.044	0.049	0.045	0.052
34	0.041	0.047	0.029	0.123	0.126	0.261	0.276	0.053	0.059	0.06	0.057	0.048	0.05	0.05	0.057
35	0.037	0.04	0.032	0.121	0.131	0.272	0.288	0.049	0.055	0.06	0.053	0.042	0.041	0.04	0.045
36	0.056	0.06	0.042	0.121	0.13	0.268	0.283	0.068	0.068	0.078	0.075	0.055	0.056	0.057	0.063
37	0.043	0.046	0.03	0.132	0.133	0.261	0.272	0.052	0.06	0.066	0.065	0.047	0.049	0.049	0.052
38	0.041	0.052	0.032	0.125	0.135	0.266	0.282	0.053	0.058	0.062	0.056	0.048	0.048	0.046	0.053
39	0.071	0.069	0.065	0.136	0.144	0.263	0.272	0.073	0.081	0.078	0.075	0.067	0.068	0.065	0.071
40	0.071	0.073	0.06	0.14	0.133	0.273	0.286	0.077	0.081	0.082	0.089	0.074	0.07	0.072	0.075
41	0.047	0.047	0.043	0.143	0.133	0.27	0.283	0.054	0.059	0.06	0.06	0.051	0.046	0.051	0.05
42	0.053	0.062	0.045	0.134	0.138	0.271	0.284	0.065	0.072	0.071	0.064	0.053	0.047	0.048	0.055
43	0.05	0.058	0.038	0.135	0.136	0.27	0.285	0.059	0.052	0.051	0.055	0.046	0.045	0.042	0.044
44	0.605	0.052	0.605	0.106	0.605	0.605	0.605	0.053	0.605	0.605	0.05	0.058	0.043	0.037	0.057
45	0.069	0.081	0.063	0.144	0.14	0.268	0.278	0.053	0.062	0.06	0.059	0.05	0.055	0.055	0.055
46	0.106	0.096	0.107	0.136	0.166	0.288	0.302	0.097	0.111	0.114	0.1	0.095	0.095	0.094	0.099
47	0.071	0.077	0.068	0.132	0.137	0.253	0.263	0.073	0.082	0.094	0.089	0.079	0.076	0.072	0.071
48	0.059	0.068	0.062	0.144	0.145	0.279	0.291	0.073	0.076	0.08	0.083	0.067	0.069	0.065	0.068

49	0.07	0.085	0.071	0.164	0.158	0.276	0.278	0.047	0.045	0.056	0.05	0.035	0.041	0.039	0.033
50	0.045	0.068	0.047	0.117	0.136	0.268	0.282	0.063	0.074	0.071	0.066	0.061	0.061	0.06	0.069
51	0.066	0.091	0.074	0.163	0.165	0.277	0.279	0.045	0.048	0.054	0.046	0.042	0.046	0.044	0.037
52	0.051	0.07	0.059	0.131	0.134	0.276	0.284	0.064	0.072	0.073	0.069	0.058	0.064	0.065	0.068
53	0.071	0.077	0.069	0.155	0.143	0.27	0.279	0.07	0.076	0.078	0.076	0.07	0.071	0.069	0.071

	31	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**
32	0.015														
33	0.036	0.038													
34	0.04	0.037	0.019												
35	0.022	0.022	0.032	0.036											
36	0.036	0.032	0.04	0.042	0.019										
37	0.03	0.025	0.032	0.035	0.011	0.019									
38	0.033	0.027	0.044	0.041	0.035	0.044	0.037								
39	0.055	0.056	0.062	0.066	0.06	0.07	0.061	0.061							
40	0.059	0.057	0.065	0.06	0.059	0.06	0.055	0.064	0.035						
41	0.041	0.038	0.055	0.049	0.031	0.044	0.034	0.052	0.073	0.066					
42	0.049	0.047	0.059	0.058	0.049	0.058	0.047	0.056	0.072	0.073	0.052				
43	0.043	0.036	0.061	0.069	0.039	0.044	0.031	0.048	0.082	0.092	0.057	0.045			
44	0.047	0.04	0.047	0.048	0.043	0.605	0.605	0.051	0.049	0	0.046	0.049	0.032		
45	0.067	0.056	0.067	0.065	0.057	0.073	0.067	0.063	0.085	0.072	0.059	0.073	0.058	0.605	
46	0.091	0.093	0.096	0.098	0.084	0.095	0.089	0.096	0.116	0.13	0.118	0.096	0.103	0.059	0.125
47	0.072	0.063	0.075	0.072	0.068	0.082	0.064	0.072	0.08	0.081	0.079	0.08	0.076	0.605	0.09
48	0.061	0.057	0.07	0.068	0.061	0.069	0.061	0.058	0.068	0.069	0.061	0.067	0.061	0.605	0.077
49	0.069	0.061	0.073	0.075	0.071	0.084	0.068	0.066	0.095	0.089	0.067	0.082	0.056	0.605	0.069
50	0.056	0.054	0.06	0.061	0.052	0.065	0.057	0.061	0.075	0.077	0.063	0.062	0.078	0.039	0.076
51	0.071	0.067	0.081	0.082	0.076	0.09	0.081	0.075	0.095	0.095	0.071	0.086	0.061	0.605	0.069
52	0.057	0.054	0.059	0.063	0.052	0.072	0.052	0.063	0.07	0.075	0.064	0.063	0.07	0.605	0.068
53	0.075	0.068	0.087	0.081	0.073	0.085	0.071	0.072	0.087	0.092	0.074	0.088	0.082	0	0.081

	46	47	48	49	50	51	52
47	0.103						
48	0.124	0.096					
49	0.113	0.089	0.086				
50	0.098	0.081	0.066	0.09			
51	0.124	0.098	0.089	0.023	0.088		
52	0.104	0.084	0.062	0.08	0.048	0.085	
53	0.143	0.096	0.079	0.088	0.086	0.091	0.085

Taxa key for table A21:

1	*Drosophila melanogaster*	**15**	*Sarcophaga crassipalpis*	**29**	*Hemipyrellia fernandica*	**43**	*Auchmeromyia luteola*
2	*Glossina morsitans*	**16**	*Notochaeta* spp.	**30**	*Dyscritomyia robusta*	**44**	*Cordylobia anthropophagi*
3	*Musca domestica*	**17**	*Metopia campestris*	**31**	*Phormia regina*	**45**	*Melinda viridicyanea*
4	*Hydrotaea cyrtoneurina*	**18**	*Sarcophila meridionalis*	**32**	*Protophormia terraenovae*	**46**	*Mesembrinella* spp.
5	*McAlpine's fly*	**19**	*Cuterebra fontinella*	**33**	*Chrysomya rufifacies*	**47**	*Eumesembrinella quadrilineata*
6	*Epalpus signifier*	**20**	*Cuterebra baeri*	**34**	*Chrysomya megacephala*	**48**	*Sarconesia versicolor*
7	*Gymnocheta viridis*	**21**	*Hypoderma lineatum*	**35**	*Cochliomyia macellaria*	**49**	*Sarconesia chlorogaster*
8	*Cyrtophleba nitida*	**22**	*Oestrus ovis*	**36**	*Cochliomyia hominivorax*	**50**	*Isomyia gomezmenori*
9	*Nemorilla floralis*	**23**	*Cynomya cadaverina*	**37**	*Compsomyiops fulvicrura*	**51**	*Rhyncomya nigripes*
10	*Gymnosoma nitens*	**24**	*Calliphora vomitoria*	**38**	*Protocalliphora sialia*	**52**	*Metallea erinacea*
11	*Phania funesta*	**25**	*Bellardia vulgaris*	**39**	*Pollenia rudis*	**53**	*Eurychaeta palpalis*
12	*Rhinophora lepida*	**26**	*Onesia tibialis*	**40**	*Pollenia amentaria*		
13	*Stevenia hertingi*	**27**	*Lucilia sericata*	**41**	*Bengalia depressa*		
14	*Stevenia atramentaria*	**28**	*Hypopygiopsis infumata*	**42**	*Verticia orientalis*		

TABLE A22: Pairwise transition-transversion ratio of *elongation factor one alpha* (*EF1α*) gene used in Calliphoridae phylogeny (Chapter 5).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
2	1.92																		
3	1.78	2.03																	
4	1.79	1.97	1.88																
5	1.8	2.56	2.8	3															
6	2.18	1.67	1.87	2.24	1.44														
7	1.86	1.73	2.1	2.88	2.28	1.73													
8	2.43	2.05	2.95	2.18	3.13	2	1.58												
9	2.08	1.97	2.68	1.68	1.44	1.52	1.94	2.22											
10	2.28	2.14	2.18	2.55	2.27	2.4	2	2.04	2.85										
11	1.95	1.97	1.9	1.73	2.13	1.55	1.81	1.48	1.88	2.5									
12	2.15	2.86	2.26	2.65	4.17	1.62	2.12	2.33	2.79	2.67	1.73								
13	1.71	1.82	1.61	1.82	2.93	1.38	1.55	2.12	1.8	2.39	2.07	3							
14	1.75	1.9	1.52	1.36	2.06	1.35	1.5	1.56	2.11	2.17	2.4	2.53	2.09						
15	2.65	2.58	3	2.13	3.12	1.81	1.75	2.67	2.16	2.29	2.36	4.91	2.47	2.28					
16	1.74	3.17	2.73	2.12	2.9	1.43	1.65	2.27	2.45	2.8	2.35	5.5	3.15	2.83	11				
17	2.39	2.41	2.76	2.18	3.56	1.84	1.82	2.32	2.41	2.57	2.18	5.8	2.45	2.42	5.62	4.17			
18	1.93	3.17	2.5	1.87	2.5	1.47	1.93	2.82	2.3	2.79	1.86	4.27	2.4	2.21	3.5	3.5	2.12		
19	2.32	1.86	2.31	2.23	2.22	1.91	1.84	2.07	1.85	1.94	1.94	2.47	2.72	2.3	2.38	2.7	2.42	2.75	
20	1.78	1.6	1.54	1.77	1.77	1.64	1.41	2.09	1.69	1.5	1.59	1.97	2.08	1.74	2	1.85	2.23	1.93	2.5
21	1.37	1.11	1.26	1.25	1.21	1.25	1.44	1.08	1.21	1.2	1.13	1.11	1.11	1.11	1.27	1.28	1.45	1.41	1.15
22	1.42	1.02	1.23	1.25	1.16	1.23	1.41	1.11	1.27	1.25	1.1	1.19	1.16	1.15	1.29	1.26	1.41	1.38	1.16
23	2.4	1.92	1.85	4.62	2.17	1.42	1.28	1.76	1.93	1.83	1.39	3	1.74	1.42	1.9	1.18	2.43	1	1.93
24	2.12	1.9	1.59	5.12	2.45	1.14	1.33	1.36	1.5	2.09	1.45	3.22	1.91	1.58	1.33	1.36	1.94	1.21	2.23
25	1.84	2	1.38	3	1.79	1.3	1.38	1.81	1.37	2.28	1.39	2.63	2.05	1.46	1.05	1.38	1.42	1.43	2.29
26	2.45	2.1	1.87	3.36	2.33	1.76	1.57	2.22	1.93	2.05	1.47	3.29	2	1.59	2.19	1.33	2.67	1.75	1.81
27	2.19	2.16	2.21	2.47	3.88	1.82	1.5	2.93	1.96	1.8	1.7	3.73	2.37	2	3.92	3.33	3.56	2.71	2.08
28	2.11	2.11	1.9	2.38	3.44	1.52	1.43	2.86	1.89	1.69	1.57	3.62	2.45	2.04	3.31	2.71	3.12	2.25	2.08
29	2.33	2.05	2	2.47	4	1.67	1.5	2.69	2.17	1.72	1.7	3.87	2.47	1.95	4.78	3.67	3.71	3	2.02
30	2.07	1.97	1.77	2.57	2.89	1.65	1.43	2.59	1.83	1.92	1.47	4	2.4	1.96	2.88	2.33	3.11	1.9	1.91
31	2.39	2.45	2.57	1.72	2.6	1.79	1.76	2.76	1.84	2.72	2.08	3.85	2.8	2.06	3.9	4	3.92	3.2	2.26
32	2.31	2.5	2.77	2	2.67	1.61	1.7	2.58	2	2.88	2.13	4	3	2.24	4.22	4.5	3.42	2.75	2.02

33	2.27	2.45	2.59	1.55	2.42	1.86	1.9	2.52	2.04	2.57	2.14	4.7	3	2.67	3.8	2.43	5	2.17	2.22
34	2.27	2.39	2.47	1.65	2.5	1.69	1.95	2.56	2.29	2.48	2.14	4.9	3.07	2.86	4.2	2.43	4.8	1.67	2.45
35	2.6	2.63	3.5	2.53	2.67	1.8	1.8	2.36	2.19	2.79	2.09	5.3	3.07	2.18	7	10	5	3.5	2.4
36	2.15	2.58	2.64	2.22	2.27	1.48	2.11	2.05	1.76	3	2.57	4.31	2.88	2.1	4	5.4	5.14	3.6	2.65
37	2.07	2.69	2.58	2.25	2.44	1.29	1.94	1.94	1.71	2.59	2.14	5.22	2.4	1.82	16	7.33	8.67	2.4	3.32
38	2.32	2.44	2.75	1.79	2.18	1.86	1.52	2.6	1.81	2.53	2.43	3.29	2.93	2.53	3.36	5	3.06	3.5	2.3
39	2	1.86	2.53	1.83	1.39	1.95	1.6	3.52	2.3	2.17	1.74	2.22	1.7	1.68	2.24	1.28	2.4	1.12	1.88
40	1.81	2.08	2.5	1.7	2	1.64	1.68	1.84	2.05	2.12	2.09	2.48	1.56	1.74	1.5	1.93	2.38	1.54	1.89
41	2.38	2.53	2.38	2.14	3.33	1.57	1.89	2.36	1.89	2.6	1.96	4.8	2.33	1.79	5.17	4.4	4.25	3.8	2.85
42	2.33	2.55	2	1.9	1.75	1.77	1.64	2.37	1.85	2.67	2.12	2.74	2.14	1.86	2.39	2.88	2.41	4	2.13
43	1.97	2.61	2.19	2.42	1.45	1.39	1.53	1.95	1.41	1.47	1.56	4.12	2.7	1.64	3.71	3.4	2.27	3.67	2.07
44	3.57	---	---	---	---	2.62	---	5.2	4.75	---	---	---	---	---	---	---	21	---	1.82
45	1.86	2.45	1.68	2.29	2.83	1.5	1.43	1.52	1.37	2.14	1.19	3.54	2	1.5	2.15	2.33	2.4	2.5	2.3
46	1.97	2.09	1.95	2	1.42	1.4	1.89	2	1.78	2.2	1.54	3.75	2.13	1.78	2.5	2.73	2.87	1.62	1.83
47	2.53	2.14	1.86	2.37	1.94	1.56	2.33	2.1	2	2.86	2.45	3.18	2.22	1.95	3.6	4.12	3.42	2.17	2.48
48	2.11	2.21	2.4	1.9	3.5	1.83	1.62	2.65	2.44	3.11	2.35	3.53	2.45	2.26	5	4.67	3.36	5.8	2.33
49	1.7	2.03	1.84	2.67	2.46	1.43	1.7	1.84	1.7	2.32	1.57	2.6	1.83	1.48	2.07	1.86	2.41	1.79	1.95
50	2.61	2.05	2	1.59	2.67	1.89	1.86	2.15	1.58	2.9	2.68	2.75	2.05	2	3.19	2.71	3.2	2.25	2.22
51	1.75	2.03	2.11	2.54	2.75	1.45	1.74	1.8	1.7	2.17	1.56	3.75	2.32	1.87	2.25	1.92	2.44	2.15	2.17
52	2.12	2.26	1.68	1.81	2.58	1.57	1.48	1.84	1.47	2.57	1.8	2.72	2.1	1.84	3.11	2.62	2.92	2.67	2.91
53	2.34	2.75	2	3.21	3.45	1.82	2	2.94	3.27	2.45	1.83	5.18	2.59	2.05	5.38	3.56	3.82	3.22	2.93

	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
21	1.13																		
22	1.08	---																	
23	1.65	1.37	1.43																
24	1.8	1.37	1.44	---															
25	1.88	1.24	1.28	1.5	1.82														
26	1.64	1.33	1.35	1.67	2	0.88													
27	1.77	1.4	1.4	1.71	1.27	1.08	2.62												
28	1.75	1.32	1.33	1.44	1.25	1.29	2.79	21											
29	1.71	1.35	1.35	1.6	1.18	1.62	3.36	9	3.33										
30	1.67	1.4	1.41	2	1.3	1.58	3.08	5	4	2.8									
31	1.88	1.22	1.25	1.64	1.28	0.95	1.9	2.14	2.2	2.58	1.94								
32	1.91	1.28	1.32	1.27	1.12	1.11	1.62	2.14	1.6	2.33	1.82	7.5							
33	1.84	1.18	1.2	1.83	1.1	1.1	2.43	2.93	3.07	3.5	2.56	4.62	4.5						
34	1.87	1.21	1.24	1.74	1	1	2.38	3.29	3.13	3.92	2.94	5.25	4.38	---					
35	2.07	1.31	1.35	2.22	1.47	1.22	2.37	3.17	2.77	3.8	2.6	5.75	5.5	5.5	6.17				
36	2.07	1.27	1.31	1.67	1.61	1.63	1.94	2.25	2.08	2.42	2	3.33	3.6	3	3.29	3.67			
37	2.32	1.34	1.34	1.36	1.64	1.41	1.93	2.75	2.44	2.88	2	3.75	4.33	4	4.5	6	5		
38	1.79	1.19	1.24	1.42	1.05	1.05	1.76	2.33	2.16	2.44	1.95	4.12	3	3.5	3.25	4.25	3.43	3.6	
39	1.54	1.08	1.14	2.04	1.57	1.32	2.68	2.04	2	2.08	2.22	1.96	1.83	2.3	2.52	2.09	1.67	1.24	2.26
40	1.54	1.14	1.16	1.38	1.41	1.21	1.75	1.62	1.36	1.57	1.55	1.44	1.53	1.82	1.65	1.59	1.86	1.62	1.82
41	2.21	1.27	1.28	1.88	1.86	1.6	2.18	2.83	2.23	3.18	2.54	2.36	2.5	3.45	3	3	3.43	4.25	2.62
42	2	1.41	1.34	1.5	1.35	1.24	2	2.2	1.71	2.11	1.75	1.95	1.65	2.55	2.5	2.22	2.25	1.9	2.09
43	1.54	1.37	1.36	1.64	1.75	1.09	1.83	8.67	6	5.75	3.67	2	1.88	2.8	3.3	2.43	2.17	2.25	1.73
44	---	---	---	6	---	---	5.67	10	7	---	10	18	6.5	---	---	---	---	---	4.25
45	1.72	1.25	1.25	1.62	1.92	2.36	2.7	3	2.89	3.38	4	2	1.77	1.62	1.56	1.77	2.21	1.93	1.67
46	1.54	1.19	1.31	1.95	1.62	1.5	2.16	2.56	2.35	2.44	2.47	2.44	2.47	2.87	2.93	2.85	2.36	2.09	2.22
47	1.9	1.17	1.22	1.94	2.12	1.9	2.53	2.86	2.47	2.57	2.27	2.5	2.38	2.92	2.77	3.27	4.3	4	2.27
48	1.85	1.38	1.38	1.74	1.78	1.74	2.47	3.8	3.45	3.7	2.77	3.3	3.56	3.45	3.36	3.78	4.11	4.43	3.56
49	1.6	1.38	1.38	1.67	1.64	1.57	3.25	1.18	1.55	1.45	1.3	1.61	1.47	1.63	1.68	1.82	1.89	1.8	1.37
50	1.76	1.14	1.18	1.39	1.27	1.42	1.83	2.45	2.3	2.55	2.27	2.45	2.15	2.75	2.75	2.44	2.46	2.5	2.12
51	1.77	1.34	1.34	2.44	2.88	2.18	3.57	1.9	1.91	2	1.89	1.67	1.71	1.89	1.95	2.06	2.11	2.12	1.68
52	2.14	1.28	1.25	1.26	1.47	1.3	1.88	2.55	2.58	3	2.29	2.45	2.7	2.25	2.82	2.5	2.62	2.56	2.5
53	2.21	1.37	1.36	2.77	2.92	1.88	3.08	3.9	3.55	3.9	4.56	3.73	3.8	3.62	3.31	5.38	4.3	4.5	3.17

	39	40	41	42	43	44	45	46	47	48	49	50	51	52
40	1.17													
41	1.78	1.82												
42	2.03	1.57	1.65											
43	1.43	1.71	2.89	1.8										
44	9	---	5	4	3									
45	1.3	1.14	2.08	1.88	1.89	---								
46	1.92	1.62	2.33	1.76	1.93	3.5	1.88							
47	1.79	1.52	2.79	2	2.78	---	2.5	3.56						
48	1.53	2.2	3.3	3.27	3.83	---	2.43	3	3.2					
49	2.25	1.77	2.29	1.67	1.89	---	1.87	1.76	2.11	2.28				
50	1.97	1.43	2.11	2.75	2.2	3.25	1.76	2.47	2.38	2.83	1.77			
51	2.25	1.95	2.5	1.68	2.22	---	2.91	2.06	2.25	2.39	2.2	1.86		
52	0.84	1.33	2.5	2.23	2.75	---	1.41	1.6	2.38	2.33	1.83	1.67	1.84	
53	1.73	2.15	6.29	2.81	3.1	---	3.17	3.07	3.07	3	3.46	2.47	3.29	3

Taxa key for table A22: Same as table A21.