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# Taxonomy and systematics of the Australian Sarcophaga s.l. (Diptera: Sarcophagidae)

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# Taxonomy and systematics of the Australian *Sarcophaga s.l.* (Diptera: Sarcophagidae)

A thesis submitted in fulfillment of the requirements for the award of the degree

**Doctor of Philosophy**

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*by*


**Kelly Ann Meiklejohn**

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# Thesis Certification

I, Kelly Ann Meiklejohn declare that this thesis, submitted in fulfillment of the requirements for the award of Doctor of Philosophy, in the School of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Kelly Ann Meiklejohn

**31st of August 2012**

# Table of Contents

# List of Figures

# List of Tables

# List of Appendices

# List of Abbreviations

| | |
|---|---|
| A | adenine |
| AATS | alanyl-tRNA synthetase |
| ABRS | Australian Biological Resources Study |
| ACT | Australian Capital Territory |
| AFP | Australian Fedral Police |
| ALA | Atlas of Living Australia |
| AMS | Australian Museum, Sydney, NSW, Australia |
| ANIC | Australian National Insect Collection, Canberra, ACT, Australia |
| Apr | April |
| ARC | Australian Research Council |
| ATP | adenosine triphosphate |
| Aug | August |
| BLAST | Basic Local Alignment Search Tool |
| BOLD | Barcode of Life Database |
| bp | base pair |
| C | cytosine |
| CAD | carbamoylphosphate synthetase/aspartate transcarbamylase/dihydroorotase |
| cm | centimetres |
| COI | cytochrome oxidase subunit I |
| COII | cytochrome oxidase subunit II |
| CPS | carbamoylphosphate synthetase |
| CSIRO | Commonwealth Scientific and Industrial Research Organisation |
| cyt b | cytochrome oxidase subunit b |
| Dec | December |
| dNTP | deoxyribonucleotide |
| E | east |
| EDTA | ethylendiaminetetraacetic acid |
| EF-1$\alpha$ | elongation factor - 1 $\alpha$ |
| EMBOSS | European Molecular Biology Open Software Suite |
| ESE | east-south-east |
| Feb | February |
| *g* | relative centrifugal force |
| G | guanine |
| h | hour |
| $H_2O$ | water |

| | |
|---|---|
| HCl | hydrochloric acid |
| HPC | high performance computing |
| ITS | internal transcribed spacer |
| Jan | January |
| Jul | July |
| Jun | June |
| K2P | Kimura 2-Parameter |
| km | kilometres |
| M | molar |
| Mar | March |
| *MEGA* | Molecular Evolutionary Genetics Analysis |
| mg | milli-gram |
| min | minute |
| ml | milli-litre |
| mM | milli-molar |
| mtDNA | mitochondrial DNA |
| N | north |
| NaCl | sodium chloride |
| NADH | nicotinamide adenine dinucleotide |
| NCBI | National Center for Biotechnology Information |
| ND4 | NADH dehydrogenase subunit 4 |
| ND5 | NADH dehydrogenase subunit 5 |
| NE | north-east |
| NJ | neighbour-joining |
| nM | nano-molar |
| Nov | November |
| NP | national park |
| NRM | Natural History Museum of Sweden (Naturhistoriska riksmuseet), Stockholm, Sweden |
| NSW | New South Wales |
| NT | Northern Territory |
| NUMT | nuclear mitochondrial pseudogene |
| $^{o}$C | degrees celsius |
| Oct | October |
| *PAUP** | Phylogenetic Analysis Using Parsimony (* and other methods) |
| PCR | polymerase chain reaction |
| PMI | post-mortem interval |
| PP | posterior probability |

| | |
|---|---|
| QDPC | Queensland Department of Primary Industries, Indooroopilly, Qld, Australia |
| Qld | Queensland |
| QM | Queensland Museum, Brisbane, Qld, Australia |
| RDH | rabbit haemorrhagic disease |
| RNA | ribonucleic acid |
| rRNA | ribosomal RNA |
| s | second |
| SEM | scanning electron microscopy |
| *s.l.* | *sensu lato* |
| SA | South Australia |
| SDS | sodium dodecyl sulphate |
| Sep | September |
| T | thymine |
| Tas | Tasmania |
| TE | Tris-EDTA |
| TNT | Tree analysis using New Technology |
| TPI | triosephosphate isomerase |
| Tris | tris(hydroxymethyl)aminomethane |
| tRNA | transfer RNA |
| U | unit |
| UOW | University of Wollongong, Wollongong, NSW, Australia |
| UQIC | University of Queensland, St Lucia, Qld, Australia |
| USA | United States of America |
| UV | ultra-violet |
| Vic | Victoria |
| WA | Western Australia, Perth, Western Australia, Australia |
| WAM | Western Australian Museum |
| ZMUC | Natural History Museum of Denmark, Zoological Museum, University of Copenhagen, Copenhagen, Denmark |
| µg | micro-gram |
| µl | micro-litre |
| µm | micro-metre |

# Abstract

The flesh flies (Diptera: Sarcophagidae) are a globally distributed family of over 3,000 species classified into three subfamilies and 173 genera. Almost 25% of sarcophagids belong to the genus *Sarcophaga* (*sensu lato*), which are further classified into 132 subgenera. The validity of, and relationships between these *Sarcophaga s.l.* subgenera remain questionable. Interestingly, many *Sarcophaga s.l.* species are of potential forensic importance, as they are attracted to and possibly breed in carrion. Despite this, the use of *Sarcophaga s.l.* specimens in forensic casework has been limited, as morphological species-level identification at any life stage is very challenging. Considering this, this PhD research was focussed on developing methods for the identification of the Australian *Sarcophaga s.l.*, but also on evaluating the utility of various markers for resolving relationships within *Sarcophaga s.l.*

Prior to developing a range of tools for the identification of the Australian *Sarcophaga s.l.*, it was necessary to define and clarify the fauna. Based on the current world catalogue of sarcophagids and various online catalogues for sarcophagids of the Australasian/Oceanian regions, 80 species have been documented from Australia, from the subfamilies Miltogramminae and Sarcophaginae. In 2009-2010, broad taxon sampling of sarcophagids across Australia was undertaken and locality information was documented from curated sarcophagids in a range of collections. From the information obtained, distribution records for 31 Australian species were updated and six new species records for Australia documented. Three new species as new to science from the large genus *Sarcophaga s.l.* have also been identified, with two of these described in this thesis. Additionally four new synonymies for the Australian flesh flies have been reported. Overall, the current Australian sarcophagid fauna comprises 84 species: 17 miltogrammines and 67 sarcophagines (with 55 being *Sarcophaga s.l.* species).

Little taxonomic work has been undertaken on the Australian Sarcophagidae since the 1950-70s, and the literature for the Australian species is now outdated. An updated key is provided for the Australian sarcophagids, allowing their separation into subfamilies and genera, along with the identification of all species of *Sarcophaga s.l.* A computer-based interactive LUCID key was also produced for the identification of the Australian *Sarcophaga s.l.*, for use by non taxonomists. In a LUCID key, the user is able to choose morphological features that they are familiar with for identifications, instead of stipulated features which are found in traditional taxonomic keys. Included in both the updated taxonomic key and the LUCID key is a comprehensive database of illustrations and photographs of male terminalia, as well as updated biological and distributional information for each species of *Sarcophaga s.l.*

The molecular-based approach of DNA barcoding, which utilises a 648-bp fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene, was comprehensively evaluated for species-level identification of Australian sarcophagids. In a pilot study, barcoding effectively discriminated between 16 adult Australian sarcophagids. The current study evaluated barcoding on a larger taxon set of 588 adult Australian sarcophagids. A total of 39 of the 84 known Australian species were represented by 580 specimens, which includes 92% of species with potential forensic importance. A further eight specimens could not be reliably identified, but were included nonetheless as six unidentifiable taxa. A neighbour-joining (NJ) phylogenetic tree was generated and nucleotide sequence divergences were calculated using the Kimura-two-Parameter (K2P) distance model. All species except *Sarcophaga* (*Fergusonimyia*) *bancroftorum*, known for its high morphological variability, were resolved as reciprocally monophyletic (99.2% of cases), with most having bootstrap support of 100. Excluding *bancroftorum*, the mean intraspecific and interspecific variation ranged from 0-1.12% and 2.81-11.23%, respectively, allowing for species discrimination. This study also investigated whether DNA could be extracted and COI barcode sequences obtained for molecular identification of each immature life stage of the forensically important Australian flesh fly, *Sarcophaga* (*Sarcorohdendorfia*) *impatiens* (Walker). Genomic extracts were prepared from all larval instars and puparia. Amplifications of the barcoding region were successful from all extracts, but puparial amplicons were weak. All sequences were identified as *S. impatiens* with 99.95% confidence using the Barcode of Life Database (BOLD). Importantly, crop removal was necessary to eliminate PCR inhibition for specimens from the late second and early third instars. DNA barcoding was therefore validated as a suitable method for the molecular identification of all life stages of Australian sarcophagids.

This PhD research was also focussed on evaluating the usefulness of three sources of data for resolving relationships between 39 species from 14 *Sarcophaga s.l.* subgenera: the mitochondrial COI barcode region, ~800bp of the nuclear gene CAD and 110 morphological characters. Bayesian analyses were performed on all data combinations, but the most well resolved phylogeny was obtained when all three data sets were combined (78% of nodes with posterior probability (PP) of >0.90). Strong support at the species-level was provided by COI and the morphological data (PP of 1.00), and CAD facilitated high resolution at basal levels (PP of 0.93-1.00); support between most *Sarcophaga s.l.* subgenera was poor. Notably, the only *Sarcophaga s.l.* subgenera that were resolved as monophyletic were *Liopygia* and *Parasarcophaga*. The monophyly and relationships between the remaining *Sarcophaga s.l.* subgenera sampled remain questionable. The markers that were evaluated in this study provide greater phylogenetic signal when combined, rather than when used singly or as pairs. It is suggested that future phylogenetic studies on the genus *Sarcophaga s.l.* continue to combine data sets, and at least use COI and CAD, along with morphological data, for their analyses.

# Acknowledgements

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often than not! I also thank Christopher Kavazos, who shared all of his knowledge on using the Leica Automontage System, but also the LUCID software with me. Without his help, I do not think that LUCID key for the Australian *Sarcophaga s.l.* would have been achievable.

To my family, Mum, Dad and Leslie, thank you for providing me with unwavering support from the very beginning and helping me to achieve this goal. I don't think they ever envisioned that after our family road trip in January 2008, where we stopped at road kill to collect sarcophagids along the way, I would develop such a love and passion for flies. Finally, I thank my fiancé David. When I met David in 2009, our first conversation included that I worked on flies and cultured maggots! That didn't deter David and he has been a tower of strength throughout my PhD. I am positive without the endless computing assistance that he has given me, I would have taken substantially longer to finish than I have.

# CHAPTER 1: **General Introduction**

## 1.1 Preamble

The Sarcophagidae (Diptera), or the flesh flies, are a globally distributed family comprising approximately 173 genera and 3,000 species (Pape *et al.* 2011). Previously, the sarcophagids were treated as a subfamily of Calliphoridae (Diptera), however it is now accepted as a distinct family divided into three subfamilies: Miltogramminae, Paramacronychiinae and Sarcophaginae (Pape 1992; Pape 1996). Generally, the non-miltogrammine sarcophagids possess longitudinal stripes on the thorax, a checkerboard abdomen and a heavily bristled body, allowing for straight-forward family-level identification (Pape 1996; Shewell 1987).

As most of this thesis has been published or prepared/submitted for publication, each chapter has been written as a journal article or compilation of journal articles. Consequently there is some repetition between the General Introduction with the introduction of each chapter, along with the General Conclusions and the conclusions of each chapter. Nevertheless, to keep this to a minimum, the General Introduction (Chapter 1) and General Conclusions (Chapter 5) have been kept brief, focussing on broader issues. There is also some repetition among the chapters of the methodology used. The title page of each chapter details publication information, along with authorship contributions. All references have been compiled within a single list at the end of the thesis.

## 1.2 Distribution, characteristics and life histories of the subfamilies

### 1.2.1 Miltogramminae

The Miltogramminae are small to medium-sized flies, with over 650 described species classified into approximately 35 genera (Pape 1996). Miltogrammines are commonly found in subtropical regions of the northern hemisphere, along with arid regions of Africa and Asia (Pape 1996). Australia has a small miltogrammine fauna with only 17 species classified into six genera (*Aenigmetopia*, *Amobia*, *Metopia*, *Miltogramma*, *Protomiltogramma*, and *Senotainia*), despite the continent having vast arid areas. Miltogrammines have characteristic features which allow for straight-forward subfamily classification, including: arista bare or with fine trichiae; gena narrow, at most 0.2 of eye height; coxopleural streak present; longitudinal vittae on thorax not distinctive; hind coxa bare on posterior surface; male abdominal sternites 2-4 partly concealed by overlapping margins of corresponding tergites; abdomen with transverse bands/median stripe with lateral spots/three distinct spots-no clear checkerboard patterning (Pape 1996) (Figure 1a). The vast majority of miltogrammines are characterised as kleptoparasites of Hymenoptera, specifically solitary wasps and bees. Female miltogrammines generally deposit first-instar larvae onto the food source within the nest, allowing the larva to feed on either pollen balls or hymenopteran immatures. However, not all miltogrammines share this biology, with some having associations with locusts and termites, as either predators or internal parasites (O'Hara *et al.* 1999; Pape 1996).

### 1.2.2 **Paramacronychiinae**

The Paramacronychiinae is the smallest of the three subfamilies, with only 20 genera and approximately 90 described species. There is no clear distributional trend of paramacronychiine species; however, the majority are found in the non-tropical localities of the northern hemisphere. Species of this subfamily are generally medium-sized and absent from the neotropical region, except for a single species endemic to the Galapagos Islands, *Galopagomyia inoa* (Pape 1996). No species have been recorded from Australia, New Zealand, or Papua New Guinea. Most species have abdominal patterning consisting of median stripes and dark lateral spots, and a fusing of both the surstylus and epandrium along with tergite 6 and syntergosternite 7-8 (Pape 1996) (Figure 1b). paramacronychiine species encompass a broad range of biologies, with some species documented as parasitoids of butterflies, grasshoppers, locusts, bumble bees and snails, with others known as scavengers and insect predators (Pape 1996).

### 1.2.3 **Sarcophaginae**

The largest of the three subfamilies, the Sarcophaginae comprises over 2,200 species segregated into 51 genera (Pape *et al.* 2011). Most species are medium to large in size and are well distributed in both the Palaearctic and Neotropical regions, as opposed to the Afrotropical and Australasian/Oceanian regions (based on species/area ratio). It has been proposed that the Sarcophaginae arose from the New World, as *Blaesoxipha*, *Ravinia*, and *Sarcophaga* (*sensu lato*) are the only non-introduced genera found in the Old World (Pape 1996). The largest sarcophagine genus, *Sarcophaga s.l.*, comprises approximately 132 subgenera globally, with the validity of these questionable. The Australian Sarcophaginae comprises four genera, *Blaesoxipha*, *Oxysarcodexia*, *Sarcophaga s.l.* and *Tricharaea*, encompassing close to 70 species. The species of *Sarcophaga s.l.* represent approximately two-thirds of the entire Australian fauna, and are classified into 14 of the 27 subgenera known from the Australasian and Oceanian zoogeographic regions. However, as comprehensive sampling and taxonomy of the sarcophagids has not been undertaken in the last 60 years, it is plausible that the number and distribution of Australian Sarcophaginae species is substantially greater.

Diagnostic features of the majority of sarcophagines include: a plumose arista; gena broad, more than 0.2 of eye height; coxopleural streak absent; thorax generally with three distinct longitudinal vittae; hind coxa setose on posterior surface; male abdominal sternites 2-4 exposed and overlapping lateral margins of corresponding tergites; abdomen with distinct checkerboard patterning (exception of *Blaesoxipha* with median and lateral stripes) (Pape 1996) (Figure 1c). There is a vast diversity in the biology of the Sarcophaginae, given the large number of genera and species. Some species can cause myiasis in vertebrates such as frogs, toads, lizards, turtles and sheep, making such species potential economic pests. Members of the genus *Blaesoxipha* are known parasitoids, especially of grasshoppers, but also of mantids, cockroaches, millipedes and beetles. Species of the *Oxysarcodexia* and *Ravinia* genera have been associated with dung, and select *Sarcophaga s.l.* subgenera are known parasitoids of spider egg sacs (*Baranovisca*), snails (*Heteronychia*) and earthworms (*Sarcophaga* s.str.) (Pape 1996).


**Figure 1.** Representatives from each of the Sarcophagidae subfamilies: a) Miltogramminae (© P. Cerretti); b) Paramacronychiinae (© S. Hart); c) Sarcophaginae (© K.A. Meiklejohn).

## ***Forensic applications of the Sarcophaginae***

Many species of the genus *Sarcophaga s.l.* are documented to have feeding and breeding preferences for decomposing vertebrate carcasses, including human corpses (Byrd and Castner 2010; O'Hara *et al.* 1999; Pape 1996). Immatures are generally deposited by females onto a meat source during the initial stages of decomposition, in some cases within minutes of death. Forensic entomologists can use insect evidence present on a corpse to estimate the minimum time since death, or post-mortem interval (PMI), in criminal investigations. Additionally, PMI estimations can aid in the possible identification of the deceased, with correlation to the missing persons database (Catts 1992; Wells and LaMotte 2010).

Early decomposition progression is the preferred method for PMI estimation, and can be implemented when corpse decomposition is relatively limited (Catts 1992; Wells and LaMotte 2010). This method relies on careful examination of the insect immatures collected from the corpse upon discovery (Catts and Goff 1992). Early corpse colonisers (i.e. Diptera) are used preferentially over late colonisers (i.e. Coleoptera [beetles]) for estimation by this method, due to their presence during initial corpse decomposition (Catts 1992; Catts and Goff 1992; Wells and LaMotte 2010).

Despite all fly immatures from the one corpse theoretically being subjected to the same environmental conditions prior to collection, it cannot be assumed that all immatures present will be at the same stage of development (i.e. eggs; first, second and third larval instars; and pupae) (Amendt *et al.* 2004; Catts 1992). Progression through these stages can be different among Diptera, due to varying temperature thresholds or optima for growth, feeding preferences based on the condition of food source decomposition, and importantly the time spent in a particular development stage (Amendt *et al.* 2004; Catts and Goff 1992; Wells and LaMotte 2010). Therefore the specific development stage of each immature must be determined. This generally can be done through size (width or length), weight, and by the examination of the posterior spiracular slits (Catts 1992; Catts and Goff 1992; Wells and LaMotte 2010). Once the developmental stage of an immature is determined, species-level identification is essential to confirm age (attained through examination of relevant developmental profiles) (Grassberger and Reiter 2001; Wells and LaMotte 2010; Wells *et al.* 2001b). Correlation between the immature ages of several different dipteran species can establish a more accurate and conclusive PMI estimate for use in forensic investigations (Catts 1992; Wells and LaMotte 2010).

Sarcophagids are one dipteran family involved in initial corpse colonisation, and hence have the potential to be vital PMI estimators. It can be argued that sarcophagids have the ability to provide a more accurate PMI estimate than the Calliphoridae (blow flies), based on differences in their lifecycles (Wells *et al.* 2001b). Sarcophagids are viviparous, whereby they deposit live larvae directly onto a meat source, providing instantly developed immatures for corpse decomposition (Shewell 1987; Zehner *et al.* 2004). By contrast, most calliphorids are oviparous, laying eggs onto a meat source which will only hatch into larvae once the correct environmental conditions are met (Amendt *et al.* 2004; Catts 1992; Catts and Goff 1992; Greenberg 1991).

## 1.3 Species identification of the Sarcophagidae

### 1.3.1 Morphological species identification

Despite family and subfamily level identifications being straightforward for all sarcophagids, species-level identifications require examination of subtle morphological variation. Species-level identifications of larvae are almost impossible, as the examination of spine band arrangement is generally not diagnostic for sarcophagids (Cantrell 1981; Wells *et al.* 2001b; Zehner *et al.* 2004). Only taxonomic experts can identify adult sarcophagids, which requires meticulous examination of diagnostic features including: regional setulae presence and colour, body pigmentation, setae length, placement and abundance, and male terminalia.

The inability to accurately identify sarcophagids at any life stage has meant that their use in forensic investigations has been overshadowed by the calliphorids. To use sarcophagids in forensic entomology, a method for easy yet accurate species-level identification at any life stage is required. Several molecular approaches that assist with species-level identification have been proposed.

### 1.3.2 **Molecular species identification**

A molecular approach to species-level identification of organisms requires the examination and comparison of rapidly evolving genes or nucleotide regions (Hebert *et al.* 2003a; Hebert and Gregory 2005). Historically, protein markers were used for screening genetic variation rather than for molecular taxonomic identifications. Comparatively, deoxyribonucleic acid (DNA) is especially informative for species identifications and diagnosis, with DNA markers currently employed for most molecular species identifications.

#### ***Protein markers***

Allozymes are variant forms of an enzyme that are coded for by different alleles (alternative forms of a gene which are located at a specific position on specific chromosomes) at the same locus, and are a common protein marker for species identification (Loxdale and Lushai 1998). Allozymes are separated on starch, polyacrylamide or cellulose acetate media, and are subsequently stained using enzyme specific reaction mixtures (Loxdale and Lushai 1998; Sperling *et al.* 1994). Differences in banding patterns that appear upon staining can allow for species identification, with commonly targeted enzymes including carboxylesterases peptidases, phosoglucomutase and phosphoglucoisomerase (Loxdale and Lushai 1998; Sperling *et al.* 1994). Allozymes are inexpensive, with a quick running time and long shelf life for reaction materials (Loxdale and Lushai 1998; Sperling *et al.* 1994). Despite this, weak banding patterns, expression only in specific developmental stages and the requirement of fresh or deep frozen biological materials ($<-25^{o}$C), has meant that the use of allozymes have been superseded by DNA approaches (Sperling *et al.* 1994; Wallman and Donnellan 2001).

#### ***DNA markers***

The use of DNA as a molecular identification tool is ideal as it provides a vast amount of information for diagnostic purposes, is present in all biological tissues, and is highly resistant to processes of degradation, in comparison to most other biological molecules (Benecke and Wells 2001; Sperling *et al.* 1994).

*Nuclear DNA*

The nuclear genome is a source of DNA which can be used to discriminate between species and specimens, although there are some concerns surrounding the ease of its use. For example, nuclear genes contain an increased number of introns and non-coding regions, which can easily interrupt a potentially informative 600-1000 base pair (bp) reading frame (Dasmahapatra and Mallet 2006; Hebert *et al.* 2003a; Hurst and Jiggins 2005). There is a lack of universal nuclear gene primers, making amplification and sequencing of non-characterised, but potentially phylogenetically informative gene regions difficult (Hebert *et al.*, 2003; Hurst *et al.*, 2005; Dasmahapatra *et al.*, 2006). Finally, the diploid (two copies of each locus) inheritance mode of the nuclear genome leaves it susceptible to recombination, possibly complicating clear species identification by molecular techniques (Hebert *et al.* 2003a; Hebert and Gregory 2005).

*Mitochondrial DNA*

A second source of cellular DNA is mitochondrial DNA (mtDNA). In animals, mtDNA is generally approximately 17,000 bp in size, and consists of: two ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes, along with 13 protein coding genes that code for subunits of the enzymes and structural proteins required in ATP synthesis or the electron transport chain (Moritz *et al.* 1987) (Figure 2). Simple in structure and organisation, mtDNA has few introns and non-coding regions, along with a unique mode of inheritance: it is haploid (only one copy of each locus) and is strictly maternally inherited (Hebert *et al.* 2003a; Hebert and Gregory 2005; Moritz *et al.* 1987). Additionally, mtDNA has a high copy number within cells, making it both easy to isolate and amplify, and generally has a high A-T content (Benecke and Wells 2001; de Oliveira *et al.* 2005; Lunt *et al.* 1996; Sperling *et al.* 1994).

The utilisation of mtDNA for molecular species identification can be problematic however, since portions of the mitochondrial genome can become integrated into the nuclear genome, known as nuclear mitochondrial pseudogenes (NUMTs), and cases of heteroplasmy and introgression have also been documented (Blanchard and Lynch 2000; Hurst and Jiggins 2005; Mortiz *et al.* 1987; Moulton *et al.* 2010; Song *et al.* 2008; Zhang and Hewitt 1996). The phenomenon of NUMTs can lead to the existence of distinct copies of some mitochondrial genes within an individual, which can be amplified equally (Hurst and Jiggins 2005; Zhang and Hewitt 1996). In such cases, species discriminations based on these genes may provide misleading results ( Song *et al.* 2008; Zhang and Hewitt 1996). Most nuclear copies can however be recognised by several identifiable characteristics and eliminated prior to data analysis (Blanchard and Lynch 2000; Zhang and Hewitt 1996).

Species discrimination from mtDNA generally involves the comparison of specific gene regions that show sufficient phylogenetically informative nucleotide substitutions. D-loop sequences (comprising the main non-coding region) are commonly used to distinguish between humans, as this represents the fastest evolving region of the genome (Benecke and Wells 2001; Moritz *et al.* 1987). However, as easy amplification of this non-coding region is difficult, protein coding genes are often used to recognise and discriminate between species (Benecke and Wells 2001; Dawnay *et al.* 2007). A range of vertebrate species have been effectively identified using the cytochrome b gene (cyt b) (Benecke and Wells 2001; Branicki *et al.* 2003; Castresana 2001; Hajibabaei *et al.* 2006; Loxdale and Lushai 1998). Portions or complete sequences of the cytochrome oxidase subunits one and two genes (COI and COII) have proven effective for insect species determination, where COI has been highly informative for dipteran species (DeBry *et al.* 2012; Malgorn and Coquoz 1999; Meiklejohn *et al.* 2011; Nelson *et al.* 2007; Otranto *et al.* 2003; Saigusa *et al.* 2005; Wallman and Donnellan 2001; Wells *et al.* 2001b; Wells and Sperling 2001; Wells and Williams 2005). COI is composed of a mixture of both conserved and variable gene regions: conserved regions allowing for the design of robust universal primers, with variable regions permitting distinction between species.

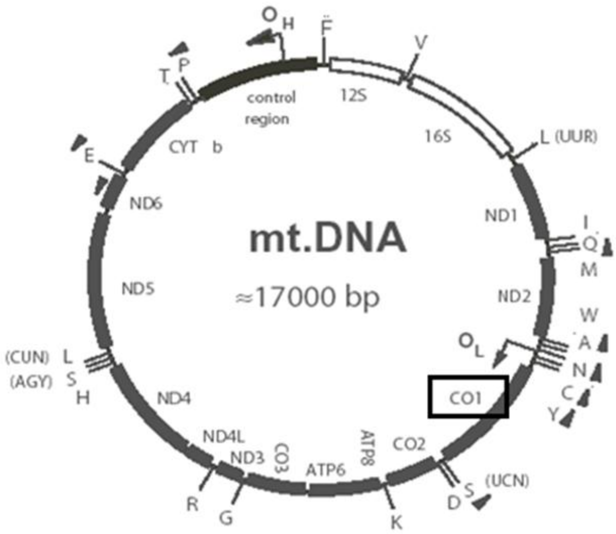


**Figure 2.** Map of the typical mitochondrial genome of animals. The relative positions of rRNA (12S, 16S), tRNA (1–letter abbreviations outside the circle) and 13 protein coding genes (letter and number combinations within the circle) are shown. The position of the cytochrome c oxidase subunit I (COI) gene is highlighted; adapted from Arnason and Janke (2002).

### *DNA barcoding*

DNA barcoding is now a commonly accepted method for molecular species identification, which utilises a 648 bp region from the 5' end of COI (Hebert *et al.* 2003a; Hebert and Gregory 2005). Numerous studies have evaluated the effectiveness of barcoding, with the approach shown to be unreliable for some Diptera (Meier *et al.* 2006; Whitworth *et al.* 2007) but also proven successful for many groups of invertebrates, including springtails (Collembola) (Hogg and Hebert 2004), butterflies (Lepidoptera) (Hebert *et al.* 2004), mayflies (Ephemeroptera) (Ball *et al.* 2005), scuttle flies (Diptera: Phoridae) (Boehme *et al.* 2010), black flies (Diptera: Simuliidae) (Rivera and

Currie 2009), the forensically important blow flies of eastern Australia (Nelson *et al.* 2007), and 16 species of Australian Sarcophagidae (Meiklejohn *et al.* 2011).

Aside from permitting species identifications, barcoding can also be used to reveal previously non-described species, clarify synonymy problems and refine existing taxonomic hypotheses (Hebert *et al.* 2003a; Hebert and Gregory 2005; Schindel and Miller 2005). Caution must be used when using barcoding to identify cryptic and allopatric species. Studies suggest that integrative approaches, using morphology, geography, ecology or a multilocus investigation using coalescent-based species delimination tools, be employed to confirm such findings (Fujita *et al.* 2012; Yeates *et al.* 2011). Species identifications obtained through barcoding reduces the pressure for taxonomic expertise, by removing the required interpretation of outdated taxonomic literature by non-specialists (Hebert *et al.* 2003a; Hebert and Gregory 2005). Additionally, complete adult specimens are not required, as is generally necessary for accurate morphological identifications (Hebert *et al.* 2003a; Stevens and Wall 2001). This is advantageous when incomplete or fragments of specimens, such as legs, are obtained for identification, particularly common in forensic cases (Schindel and Miller 2005; Stevens and Wall 2001).

## 1.4 Phylogenetic relationships within the genus *Sarcophaga* (*sensu lato*)

The Sarcophagidae is one of six dipteran families belonging to the superfamily Oestroidea, with its monophyly, and that of its three subfamilies, well supported. The sister family to the Sarcophagidae remains questionable, and is likely to be either the Calliphoridae or the Tachinidae, or the unnamed McAlpine's fly (Kutty *et al.* 2010; McAlpine 1989; Nelson *et al.* 2012b; Pape 1992; Rognes 1997). Minimal work has been undertaken to examine the relationships between sarcophagid genera, subgenera and species. Phylogenetic studies of sarcophagids have previously focused solely on morphological data, with a common emphasis on the male terminalia (Roback 1954; Sugiyama and Kano 1984; Verves 1989).

With advances in technology and lower costs for producing molecular data, both mitochondrial and nuclear genes have been used effectively for the phylogenetic inference of a range of dipteran families. Genes such as COI and COII, cyt b, 12S/16S rRNA and NADH dehydrogenase 1/2/4/4L/5 from the mitochondrial genome have been phylogenetically informative for some dipteran families (Bernasconi *et al.* 2000; Han and Ro 2005; Wallman *et al.* 2005). In addition, a number of nuclear genes such as 28S, internal transcribed spacer II (ITS II), triose phosphate isomerase (TPI), alanyl-tRNA synthetase (AATS), elongation factor-1α (EF-1α) and the carbamoylphosphate synthetase (CPS) region of the fusion protein CAD (rudimentary), have been especially useful in resolving higher level relationships (Bertone *et al.* 2008; Moulton and Wiegmann 2004; Moulton and Wiegmann 2007). Also, an increasing number of studies are utilising a combination of nuclear and mitochondrial genes for phylogenetic inference, with great success (Dsouli *et al.* 2011; Ekrem *et al.* 2010; Gibson *et al.* 2010; Huang and Cheng 2011; Kehlmaier and Assmann 2010; Singh *et al.* 2011).

Close to 25% of the global sarcophagid fauna has been classified into a single sarcophagine genus, *Sarcophaga s.l.* The monophyly of *Sarcophaga s.l.* has been supported using both morphological and molecular data (Giroux *et al.* 2010; Kutty *et al.* 2010; Wells *et al.* 2001b; Zehner *et al.* 2004), however the classification of its 132 subgenera remain questionable. Recently, Giroux (2010) utilised 73 morphological characters, including 41 male terminalia features, for the phylogenetic reconstruction of 72 Sarcophaginae species, representing 19 genera and 31 *Sarcophaga s.l.* subgenera. Kutty (2010) used a range of mitochondrial (12S, 16S, COI and cyt b) and nuclear (18S, 28S, CAD and EF-1α) gene sequences to infer the phylogeny of the Calyptratae, which included 46 sarcophagid species. Kutty (2010) did not however focus on the genus *Sarcophaga s.l.*, only including representatives from seven subgenera.

## 1.5 Conclusions

There is a pressing need to develop a range of methods for the species-level identification of Australian sarcophagids, especially for members of the forensically important genus, *Sarcophaga s.l.* Before undertaking this, the Australian sarcophagid fauna needs to clarified, in terms of current species distributions along with documenting new sarcophagid records from Australia. Species and distributional information collected will also contribute to the documentation and assessment of biodiversity. It is also plausible that there are numerous species that remain new to science requiring description. As comprehensive studies of the Australian flesh fly fauna have not been undertaken since H. de Souza Lopes in the 1950-70s, it is not surprising that the current taxonomic literature for the Australian Sarcophagidae is somewhat obsolete, and requires updating. To allow for sarcophagid identification by non-taxonomists, the generation of a computer-based LUCID key, along with a comprehensive evaluation of DNA barcoding for the molecular identification of sarcophagids, is required. With reliable and accurate methods for the identification of *Sarcophaga s.l.* species, it is plausible that the use of flesh fly specimens for PMI estimation will increase. Also a pilot study is necessary to evaluate the utility of both molecular and morphological markers for resolving relationships within the genus *Sarcophaga s.l.*, prior to commencing a comprehensive study

## 1.6 Aims

### 1. To update current distribution ranges for the Australian Sarcophagidae along with documenting new Australian records of sarcophagids (Chapter 2)

Comprehensive taxon sampling of the Australian Sarcophagidae has not been previously undertaken, and as a result, current distribution and species records have not been maintained. It is especially imperative that records are maintained for species considered economic pests, along with those of known potential forensic importance.

### 2. To update the nomenclature of the Australian Sarcophagidae and describe new species (Chapter 2)

As the Australian Sarcophagidae are a neglected fauna, there are numerous collected specimens that represent species new to science. It is vital that such species be described, so that an accurate catalogue for flesh flies is maintained, on both a national and global scale.

### 3. To revise the taxonomic key for the Australian *Sarcophaga s.l.* (Chapter 2)

The current taxonomic literature for the Australian flesh flies dates to the 1950-70s, and only facilitates the identification of approximately 60% of the Australian species. Morphological descriptions of females are not complete for a number of species, making identifications of female specimens even more challenging. A revised key will be presented, which allows for separation into subfamilies and genera, along with the identification of all species of *Sarcophaga s.l.*

### 4. To produce an interactive computer-based LUCID key for the Australian *Sarcophaga s.l.* (Chapter 2)

For non-specialists, identifications made using a taxonomic key can be demanding, requiring a sound knowledge of fly anatomy and taxon specific diagnostic features. A LUCID key enables species identifications based on informative character states, rather than examination of basic morphological features. To allow for straight-forward identification of flesh flies, a LUCID key will be generated focussed on the Australian *Sarcophaga s.l.*, and made publically accessible via the internet.

### 5. To comprehensively evaluate DNA barcoding as a molecular method for species identification of forensically important Australian Sarcophagidae (Chapter 3)

A previous pilot study tested the principle that DNA barcoding could distinguish between adults of forensically important species, using 16 sarcophagids from eastern Australia. This study will comprehensively evaluate DNA barcoding on a larger taxon set, including specimens from a range of geographical locations across Australia.

### 6. To assess the effectiveness of DNA barcoding to identify all immature life stages of a forensically important Australian flesh fly (Chapter 3)

To assist with taxonomic identifications, immature sarcophagid specimens are generally reared to adults, however this is not always possible. A molecular based approach that allows for accurate flesh fly identification from any life stage is needed. The effectiveness of DNA barcoding for the identification of all immature life stages will be assessed.

### 7. To evaluate the utility of molecular and morphological markers for resolving relationships within the genus *Sarcophaga s.l.* (Chapter 4)

Almost 25% of sarcophagids belong to the genus *Sarcophaga s.l.* However little is known about the validity of, and relationships between the 132 *Sarcophaga s.l.* subgenera. A pilot study will evaluate the usefulness of three sources of data for resolving relationships between species from *Sarcophaga s.l.* subgenera: the mitochondrial COI barcode region, ~800 bp of the nuclear gene CAD and morphological characters.

# CHAPTER 2: **Taxonomy of the Australian Sarcophagidae (Diptera)**

## 2.1 Updates on the distribution and records of Australian Sarcophagidae

*This section is slightly modified, in terms of the layout and numbering of figures and tables, from the paper:*

Meiklejohn, K.A., Dowton, M., and Wallman, J.F. (2012a) Notes on the distribution of 31 species of Sarcophagidae (Diptera) in Australia, including new records in Australia for eight species. *Transactions of the Royal Society of South Australia* **136**, 56-64.

The contributions of each author to the research described are as follows: MD and JFW designed the research and provided advice and feedback on the manuscript; KAM composed the research as a manuscript for publication, undertook field collections across Australia and documented the locality of sarcophagid curated material from a range of Australian collections.

**Please note:** After this manuscript was published, additional information was obtained regarding two of the species documented as new records in Meiklejohn (2012a). *Sarcophaga* (*Sarcosolomonia*) *crinita* Parker, 1919 was recorded as a new species for Australia, but it has since been established that *Sarcophaga* (*Sarcosolomonia*) *synia* Johnston and Tiegs, 1923 is a junior synonym of *crinita* (Chapter 2.2). Additionally, *Sarcophaga* (*Harpagophalla*) *kempi* Senior-White, 1924 was incorrectly reported in Meiklejohn (2012a). After closely examining the terminalia of the two male specimens labeled *kempi* in the ANIC, they were identified as *Sarcophaga* (*Sarcosolomonia*) *crinita*. The new records for *crinita* and *kempi* published in Meiklejohn (2012a) have subsequently been removed from this thesis.

## 2.1.1 Introduction

Sarcophagidae, or flesh flies, are common and globally distributed, with over 3,000 species known from over 173 genera (Pape *et al.* 2011). The vast majority of sarcophagid species inhabit areas with a tropical or warm temperate climate, with only a few known to inhabit arctic regions (Byrd and Castner 2010; Moribayashi *et al.* 2001; Pape 1996). Sarcophagids are known for their striking appearance, with a large proportion of the species having longitudinal stripes on the thorax and a checkerboard abdomen, allowing for straight-forward family-level identification. However, accurate species-level identifications for taxa globally are difficult, due to a lack of comprehensive and well illustrated taxonomic keys.

Currently, Australia has 80 documented species, which includes many endemics from two of three subfamilies: Miltogramminae and Sarcophaginae. Some species found in Australia are known to feed on the nectar of flowers, while others are parasitoids of snails and insects, including wasps, bees, butterflies and grasshoppers (Byrd and Castner 2010; McKillup *et al.* 2000; Pape 1996). Other Australian sarcophagids are considered medically and economically important, as they are known to cause myiasis in vertebrates. Interestingly, over 50% of the Australian fauna, mainly species from the large genus *Sarcophaga s.l.*, are potentially forensically important, as they prefer to both feed and breed in decomposing carcasses (Pape 1996). Only three sarcophagids have been documented from forensic casework in Australia, *Sarcophaga* (*Liopygia*) *crassipalpis*, *Sarcophaga* (*Sarcorohdendorfia*) *impatiens* and *Sarcophaga* (*Sarcorohdendorfia*) *praedatrix* (JFW, *pers. comm.*). Considering most sarcophagids collected are not identified, there is the potential that other known carrion-breeding species have and will feature in criminal investigations. Levot (2003), who documented insects infesting the bodies of murder victims in New South Wales (NSW) over a 17-year period, found that sarcophagids (species unknown) featured in 16% of all cases (n=132), but were the only family present in just a single case.

## 2.1.2 Methods

Current distributions for 31 Australian species are given, updating the records presented in the current catalogue of Australasian/Oceanian sarcophagids published in 2007 (available online at http://hbs.bishopmuseum.org/aocat/sarcophagidae.html based on Pape 1996) and the Australian Faunal Directory (AFD) (available online at http://www.environment.gov.au/biodiversity/abrs /onlineresources/fauna/afd/taxa/SARCOPHAGIDAE) (Table 1.) The first Australian records for six species have been documented, taking the total number of Australian sarcophagids to 86. This was achieved by documenting locality information for pinned sarcophagids in a range of collections, which appear to have been missed in previous online resources. Collections examined included the Australian National Insect Collection (ANIC; Canberra, ACT, Australia), Australian

Museum (AMS; Sydney, NSW, Australia), Queensland Museum (QM; Brisbane, Qld, Australia), Queensland Department of Primary Industries (QDPC; Indooroopilly, Qld, Australia) and the University of Queensland (UQIC; Brisbane, Qld, Australia). Specimens were also collected by KAM as a result of broad sampling of sarcophagids across Australia during 2009-2010 (currently stored in the Diptera Collection in the School of Biological Sciences, University of Wollongong (UOW; Wollongong, NSW, Australia), yielding new distribution records for a range of species.

### 2.1.3 **Results**

***New Australian records***

*Miltogramminae*

***Amobia pelopei* (Rondani)**

*Sphixapata pelopei* Rondani, 1859

*Amobia pelopei* is a parasite on wasp nests, specifically *Eumenes pyriformis petiolaris*, *Sceliphron destillatorium, S. omissum, S. spirifex, Rhynchium atrium* and *Chloridea obsolete* (Kurahashi 1974). *Amobia pelopei* was previously only known from the Palaearctic (Afganistan, Armenia, Azerbaijan, Austria, Czech Republic, France, Germany, Gruzia, Hungary, Italy, Poland, Russia, Switzerland, Turkmenistan, Uzbekistan) and Afrotropical (Malawi, South Africa, Zimbabwe) regions (Pape 1996). Specimens have now been collected in the Australasian and Oceanian regions, from the Australian Capital Territory, New South Wales, the Northern Territory and Queensland (Mt Coree, ACT, 13 Jan 1969 (ANIC); Pymble, NSW (ANIC); Cahills Crossing, NT, 07 May 1973 (ANIC); Darwin, NT, 25 Apr 1905 (ANIC); Somerset, Qld (ANIC); Woombye, Qld, 17 Dec 1970 (ANIC); Townsville, Qld, 13 Apr 1968 (ANIC); and Gordonvale, Qld (ANIC)).

***Metopia sauteri* (Townsend)**

*Chaetanicia sauteri* Townsend, 1933

*Metopia sauteri* is a parasitoid of the spider wasp *Episyron arrogans* (Endo 1980). *Metopia sauteri* has previously been documented within the Palaearctic (China, Japan) and Oriental (China, Japan, India, Laos, Nepal, Taiwan) regions, but not the Australasian and Oceanian regions (Pape 1996). There are three specimens of *M. sauteri* which were collected from the Northern Territory (Baroalba Creek Springs (19km NE by E of Mt Cahill), 28 Oct 1972, collector: unknown (ANIC)), Queensland (Woombye, 17 Dec 1970, D. H. Colless (ANIC)) and Western Australia (Mining Camp Mitchell Plateau, 09 Jan 1983, collector: unknown (ANIC)).

*Sarcophaginae*

***Oxysarcodexia varia* (Walker)**

*Sarcophaga varia* Walker 1836

*Oxysarcodexia varia*, commonly known as the striped dung fly, has been documented from a range of countries from the Neotropical region, including Argentina, Bolivia, Brazil, Chile and Uruguay. *Oxysarcodexia varia* has also been recorded from the Australasian and Oceanian regions, but limited to Fiji, French Polynesia, New Zealand and Norfolk Island (Pape 1996). *Oxysarcodexia varia* was collected at carrion-baits, comprising rotten minced kangaroo meat and sheep's liver, in the temperate Australian states of New South Wales, Tasmania and Victoria (Holbrook, NSW, 01 Mar 2007, A. Johnson (UOW); Bagdad, Tas, 29 Jan 2010, KAM (UOW); Fentonbury, Tas, 29 Jan 2010, KAM (UOW); Little Swanport, Tas, 30 Jan 2010, KAM (UOW); Waratah, Tas, 31 Jan 2010, KAM (UOW); Stanley, Tas, 31 Jan 2010, KAM (UOW); Richmond, Tas, 31 Jan 2010, KAM (UOW); Poatina, Tas, 01 Feb 2010, KAM (UOW); Seven Mile Beach, Tas, 01 Feb 2010, KAM (UOW); Orbost, Vic, 03 Mar 2007, A. Johnson (UOW); Brodribb River, Vic, 05 Mar 2007, A. Johnson (UOW); Metung, Vic, 06 Mar 2007, A. Johnson (UOW); and Harrietville, Vic, 07 Mar 2007, A. Johnson (UOW)). This species is considered medically and forensically important as specimens have been trapped at dung and carcasses, and is supposedly linked to rabbit haemorrhagic disease (RDH) (Henning *et al.* 2005).

***Sarcophaga* (*Lioproctia*) *multicolor* Johnston and Tiegs**

*Sarcophaga multicolor* Johnston and Tiegs, 1922

*Sarcophaga multicolor* was previously recorded from the Australasian and Oceanian regions, but limited to Indonesia and Papua New Guinea (Pape 1996). Records for *multicolor* can now be extended into Queensland, Australia. All specimens were collected by KAM from northern tropical rainforest environments using carrion-baits: this species therefore has potential forensic importance (Byfield NP, Qld, 15 Jan 2010 (UOW); Finch Hatton Gorge, 20 Jan 2010 (UOW); Conway NP, Qld, 22 Jan 2010 (UOW); and Misty Mountains, Qld, 23 Jan 2010 (UOW)).

***Sarcophaga* (*Sarcosolomonia*) *sumunensis* (Lopes)**

*Bezziola sumunensis* Lopes, 1967

*Sarcophaga sumunensis*, similar to *crinita*, was previously documented within the Australasian and Oceanian regions, however specimens had only been collected from Papua New Guinea and Indonesia (Pape 1996). The distribution of *sumunensis* is now extended to Queensland, Australia (Yeppoon, Qld, 10 May 1955, K. R. Norris (ANIC)).

**Table 1.** Updated distributional information for 31 Australian species of Sarcophagidae. Abbreviations for Australian states and territories include: ACT, Australian Capital Territory; NSW, New South Wales; NT, Northern Territory; Qld, Queensland; SA, South Australia; Tas, Tasmania; Vic, Victoria; and WA, Western Australia. Symbols denote museums/collections where specimens are stored: [1]Australian National Insect Collection (ANIC), [2]Australian Museum (AMS), [3]Queensland Department of Primary Industries (QDPC), [4]Queensland Museum (QM) and [5]University of Wollongong (UOW). Species considered potentially forensically important are denoted by *, and species endemic to the continent of Australia denoted by #. International regions of distribution are documented from Pape (1996), with previously documented Australian distribution range sourced from the current catalogue of Australasian/Oceanian sarcophagids published in 2007 (http://hbs.bishopmuseum.org/aocat/sarcophagidae.html) and the Australian Faunal Directory (AFD) (http://www.environment.gov.au/biodiversity/abrs/online-resources/fauna/afd/taxa/SARCOPHAGIDAE).

| Classification | International regions | Previously documented Australian range | New Australian range extensions | Biology |
|---|---|---|---|---|
| **Miltogramminae**<br>*Aenigmetopia*<br>*fergusoni* Malloch, 1930 # | Australasian and Oceanian | WA | NT[1], Qld[1] | No known information. |
| *Amobia*<br>*auriceps* (Baranov, 1935) | Australasian and Oceanian, Oriental | Not specified. | NSW[2], Qld[3] | No known information. |
| *burnsi* (Malloch, 1930) # | Australasian and Oceanian | NT and Qld | SA[1], WA[1] | Kleptoparasite of potter wasps (Malloch 1930). |
| *Metopia*<br>*nudibasis* (Malloch, 1930) | Afrotropical, Australasian and Oceanian, Palaearctic | ACT, NT, Qld | NSW[1] | Bred from nests of the wasp *Gastrosericus asilivorus* (Pape and Blasco-Zumeta 1996). |
| *Miltogramma*<br>*rectangularis* Malloch, 1930 # | Australasian and Oceanian | NSW | Qld[3] | Brood parasite of the Australian native bee, *Amegilla dawsoni* (Alcock 2000). |
| *Protomiltogramma*<br>*laticeps* Malloch, 1930 # | Australasian and Oceanian | ACT, NSW | NT[1], Qld[1,2,3], Tas[1], Vic[1], WA[1] | No known information. |
| *plebeia* Malloch, 1930 # | Australasian and Oceanian | NSW | Qld[3] | No known information. |
| *Senotainia*<br>*navagatrix* de Meijere, 1910 | Australasian and Oceanian, Oriental | NT, Qld | WA[1] | Likely kleptoparasite in nests of solitary aculeate Hymenoptera (Pape and Kurahashi 1995). |
| **Sarcophaginae**<br>*Blaesoxipha*<br>*Blaesoxipha*<br>*rufipes* (Macquart, 1839) | Afrotropical, Australasian and Oceanian, Oriental, Palaearctic | NSW, NT, Qld | ACT[1], SA[1], WA[1] | Internal parasite of Orthoptera, primarily of *Pyrgomorphidae* and *Acrididae* (Cantrell 1978). |

| | | | | |
|---|---|---|---|---|
| *Sarcophaga* *Australopierretia* *australis* (Johnston and Tiegs, 1921) * | Australasian and Oceanian | ACT, NSW, NT, Qld, WA | SA[1,5] | Reared from decaying meat (Johnston and Tiegs 1921). |
| *Bercaea* *africa* (Wiedemann, 1824) * | Afrotropical, Australasian and Oceanian, Nearctic, Neotropical, Oriental, Palaearctic | NSW | Qld[5], SA[5], WA[5] | Known to cause myiasis and collected from faeces and carrion (Park 1977; Bänziger and Pape 2004). |
| *Boettcherisca* *peregrina* (Robineau-Desvoidy, 1830)* | Australasian and Oceanian, Oriental, Palaearctic | NSW, NT, Qld, SA, WA | ACT[1] | Reared from decaying meat (Johnston and Tiegs 1921). |
| *Fergusonimyia* *bancroftorum* Johnston and Tiegs, 1921 # | Australasian and Oceanian | ACT, NSW, Qld | NT[1] | Collected in open forests (Johnston and Tiegs 1921). |
| *Johnstonimyia* *lincta* (Lopes, 1959) # | Australasian and Oceanian | Qld | NSW[1,3] | No known information. |
| *Lioproctia* *spinifera* Hardy, 1932 # | Australasian and Oceanian | Qld | NSW[3] | No known information. |
| *torvida* (Lopes, 1959) # | Australasian and Oceanian | WA | NT[1,5], Qld[1,4], SA[1] | No known information. |
| *Liopygia* *crassipalpis* Macquart, 1839 * | Afrotropical, Australasian and Oceanian, Nearctic, Neotropical, Oriental, Palaearctic | NSW, Qld, SA, WA | ACT[1,5], Vic[1] | Known to cause myiasis (Martínez-Sánchez *et al.* 2006; Castro *et al.* 2010) and has been collected from human corpses (JFW *pers. comm.*). |
| *ruficornis* (Fabricius, 1794) * | Afrotropical, Australasian and Oceanian, Nearctic, Neotropical, Oriental, Palaearctic | NT, Qld | NSW[3] | Carrion-breeder and causes myiasis in dogs, horses and mules (Amoudi *et al.* 1994). |
| *Liosarcophaga* *eta* Johnston and Tiegs, 1921* | Australasian and Oceanian | Qld | NT[1] | Reared from fish carcasses, and also attracted to decaying meat (Johnston and Tiegs 1921). |
| *sigma* Johnston and Tiegs, 1921* | Australasian and Oceanian, Oriental | ACT, NSW, Qld | NT[1,5], Tas[5], Vic[1,5], WA[1,4,5] | Reared from decaying meat (Johnston and Tiegs 1921). |

| | | | | |
|---|---|---|---|---|
| *Parasarcophaga*<br>*misera* Walker, 1849 * | Australasian and Oceanian, Oriental, Palaearctic | Qld | NSW[1,3], NT[1,5] | Reared from decaying meat and horse manure (Johnston and Tiegs 1921). |
| *taenionota* (Wiedemann, 1819) * | Australasian and Oceanian, Oriental, Palaearctic | Qld | ACT[1], NSW[1,3,5], NT[1,5], WA[1,5] | Caught on decaying meat, human faeces and carrion (Johnston and Tiegs 1921; Park 1977). |
| *Sarcorohdendorfia*<br>*bidentata* (Lopes, 1953) # | Australasian and Oceanian | NT, Qld, WA | NSW[3] | No known information. |
| *bifrons* Walker, 1853 * | Australasian and Oceanian, Oriental | ACT, NSW, Qld, WA | SA[1] | Captured around decaying meat (Johnston and Tiegs 1922). |
| *furcata* Hardy, 1932 # | Australasian and Oceanian | Qld | ACT[1], SA[1], WA[5] | No known information. |
| *impatiens* Walker, 1849 * | Australasian and Oceanian | ACT, NSW, NT, Qld, Tas | SA[5], Vic[1,5] | Reared from rotten meat and sheep's liver (Johnston and Tiegs 1921) and collected from human corpses (JFW *pers. comm.*). |
| *longifilia* Salem, 1946 | Australasian and Oceanian, Oriental | WA | NSW[1] | No known information. |
| *Sarcosolomonia*<br>*fabea* (Lopes, 1959) * # | Australasian and Oceanian | Vic | NSW[1] | Found on a dead sheep (Lopes 1959). |
| *versatilis* (Lopes, 1959) # | Australasian and Oceanian | NT | Qld[1] | No known information. |
| *Taylorimyia*<br>*aurifrons* Macquart, 1846 * # | Australasian and Oceanian | Qld | NSW[1,5], SA[1,5], WA[1,5] | Reared from decaying meat (Johnston and Tiegs 1921). |
| *Tricharaea*<br>*Tricharaea*<br>*brevicornis* (Wiedemann, 1830) * | Australasian and Oceanian, Neotropical | ACT, NSW, Vic | Tas[1,5] | Carrion-breeding (Bänziger and Pape 2004). |

***Sarcophaga* (unplaced) *simplex* (Lopes)** (not assigned to a subgenus, Pape 1996)

*Heteronychia simplex* Lopes, 1967

*Sarcophaga simplex* was previously documented from the Oriental (Indonesia), Australasian and Oceanian regions (Pape 1996). The distribution of *simplex* within the Australasian and Oceanian regions was however restricted to Papua New Guinea, but now extends into Queensland, Australia (Mulgrave River Valley, Gordonvale, Qld, 15 Nov 1981 (ANIC)).

### 2.1.4 **Discussion**

Australia is a large continent, with diverse environments consisting of tropical rainforests, sandy deserts, temperate forests and coastal habitats. As a result, the biodiversity of the Australian flora and fauna is rich, containing numerous endemic species. Despite the range of sarcophagid species found within Australia, specific preferences for certain climates and vegetation types can influence their distribution. The presence of biogeographical boundaries, such as the Great Dividing Range and the Nullabor Desert, can also restrict the emergence of new populations of species. With further field work, it is likely that additional new species or distribution records of sarcophagids from Australia will be obtained. As extensive work has not been done on the Australian taxa since the 1950-70s, it is vitally important that any information obtained is documented. It is especially imperative that current records are maintained for species with potential forensic importance, as distributional information can be used to determine possible corpse relocation. The Australian sarcophagid fauna is currently being updated in various online and published formats, and a revised taxonomic key, including all known Australian *Sarcophaga s.l.* species, will follow in a subsequent publication.

## 2.2 Nomenclature and species descriptions

*This section is slightly modified, in terms of the layout and numbering of figures and tables, from a paper prepared for submission to* Zootaxa*:*

Meiklejohn, K.A., Wallman, J.F., and Pape, T. Updates on the nomenclature of the Australian Sarcophagidae (Diptera) along with descriptions of two new species of *Sarcophaga* (*sensu lato*).

The contributions of each author to the research described are as follows: JFW and TP designed the research and provided advice and feedback on the manuscript; TP composed the differential diagnosis, etymology, subgeneric affiliation and nomenclature sections of the manuscript; KAM composed the research as a manuscript for publication and prepared the descriptions of both species.

**Please note:** Scientific names and nomenclatural acts used in this thesis should not be considered as validly published in the sense of the ICZN, and they are to be excluded for purposes of zoological nomenclature. However for consistency and ease of understanding, the names proposed in this chapter have been carried throughout the remainder of the thesis.

## 2.2.1 Introduction

The taxonomy and nomenclature of Australian Sarcophagidae (Diptera) is still far from mature. Early authors like F. Walker and P. J. M. Macquart described only a very few species with Australian type localities, and these mostly remained ignored or neglected until recently (Lopes and Kano 1979b; Pape 2008). Early Australian flesh fly taxonomy had an applied profile and may be considered to date from Skuse's (1891) description of a grasshopper parasite (*Blaesoxipha pachytyli*) or from the "sheep-maggot fly" (*Sarcophaga froggatti*) described by Taylor (1917). Parker (1922) explicitly planned a series of papers describing several Australian species, but he probably saw himself overtaken (and his two new species narrowly antedated) by the very productive Australian flesh fly workers T. H. Johnston, C. W. Tiegs and G. H. Hardy, who in a bout of papers in the early 1920s quickly created a solid taxonomic base. From the 1950s up until 1990, H. de Souza Lopes undertook the last comprehensive studies on the Australian fauna, producing several taxonomic keys in addition to species descriptions. A few supplementary species were added by Cantrell (1986), Verves (1987) and Pape (1994). For many Australian species, detailed morphological descriptions of females are still lacking and published illustrations of male terminalia vary drastically in quality. Given this partly insufficient documentation, it is not surprising that some species have been described more than once, creating numerous synonymies. Pape (2008) resolved six synonymies for Australasian/Oceanian sarcophagids, and in the current paper I present another four synonymies for the Australian flesh flies. Additionally I describe two species as new to science, both in the large genus *Sarcophaga* (*sensu lato*).

## 2.2.2 Materials and methods

Holotype and paratype material was collected on separate collection trips undertaken by D. E. Hansen, B. D. Lessard, K. A. Meiklejohn, T. Pape and the QM. Specimens were collected on hilltops or using decayed meat baits, and killed in ethyl acetate or 100% ethanol, respectively. The terminalia of pinned male specimens were extended prior to drying. For ethanol-stored specimens, the terminalia were detached and studied in glycerin and subsequently stored in a microvial pinned with the source specimen. Photographs of terminalia were taken by D. K. B. Cheung (ZMUC), using a BK Lab System by Visionary Digital and using Zerene Stacker (Zerene Systems LLC, Richland, Washington, USA) for focus stacking.

*Abbreviations for specimen depositories.*

Specimens studied have been deposited in the following institutions:

ANIC Australian National Insect Collection, Canberra, Australia.

NRM Natural History Museum of Sweden (Naturhistoriska riksmuseet), Stockholm, Sweden.

| | |
|---|---|
| QM | Queensland Museum, Brisbane, Queensland, Australia. |
| ZMUC | Natural History Museum of Denmark, Zoological Museum, University of Copenhagen, Copenhagen, Denmark. |

## 2.2.3 Results

***Species descriptions***

***Sarcophaga* (*Sarcosolomonia*) *collessi*** sp. nov. **(Figure 3)**

**Type material.**

Holotype (male): Australia: Queensland/Carnarvon NP. 1097m/ Mt. Moffatt, summit/ 25°03.6'S 148°02.6'E/12.x.2002 D.E. Hansen (ZMUC:00022172). Paratypes: 1 (male): Australia: Queensland/Great Basalt Wall NP./19°58' S 145°34'E/15.xii.2006 Queensland Museum (QM) (ZMUC:00022176); 1 (female): Australia: Queensland/ Munduberra, 25°30' S 151°17' E/ 10.i.2010 B. D. Lessard & K. A. Meiklejohn (ZMUC00022175). The holotype is deposited at ZMUC, the paratypes at QM.

**Differential diagnosis.**

*Sarcophaga collessi* is exclusively diagnosable within *Sarcosolomonia* Baranov, 1938 by its very robust and, in lateral view, almost right-angled antero-basal part of distiphallus, which in combination with the abruptly curved juxta gives the distiphallus a rectangular appearance. Also, the ribbon-like branches of the sclerotised vesica are curled or convoluted, which is unique within the subgenus.

**Description.**

**Male.**

*Length.* 12.0 mm (n=2).

*Colour.* Ground colour brownish black, with dense light-grey (holotype) or dull yellow (paratype) microtrichosity on the palps, parafacials, fronto-orbital plate, occiput, postgena, thorax and abdomen. Thorax with three longitudinal brownish-black vittae; microtrichosity of the abdomen forming the typical checkerboard patterning changing with the incidence of light. Cercus brown, but black at apex; surstylus, phallus and gonites brown.

*Head.* Arista dark brown, setose and thickened on approximately basal 0.3. Proclinate orbital setae absent and 10-11 frontal setae present on each side. Lateral vertical setae only slightly longer and stronger than longest postocular seta. Parafacial, at its narrowest point, about 0.20-0.25 the width of the eye and with black setulae. Sub- and supra-vibrissa with only black setulae, approximately 0.15-0.2 the length of the vibrissa. Gena with a well defined facial ridge and black setulae. Occipital setulae yellow below the first few rows of black.

*Thorax*. Scutum with 2-3 presutural acrostichal and 2-3 dorsocentral setae; 1 postsutural acrostichal and 4 dorsocentral setae; 3 intraalar and 3 supraalar setae. Scutellum with a pair of apical setae and 2 pairs of subapical setae. Propleuron bare. *Legs*. Hind tibia with long setulae. *Wing*. Costal spine well developed with small setae along the entire length. Vein $R_1$ without setulae and vein $R_{4+5}$ with numerous setulae on dorsal margin.

*Abdomen.* Tergite 3 with (paratype) or without (holotype) median marginal setae, and with one pair of lateral marginal setae. Tergite 4 with median marginal setae and two pairs of lateral marginal setae. Abdominal sternites 1-2 with black setulae. *Terminalia*. Sternite 5 with numerous short black setae clustered closely along inner margin. Approximately 4-5 long setae on each apex, with some long setulae on both the surface and apex. Protandrial segment with black setulae mostly confined to the posterior margin. Epandrium with black setulae on entire surface. Cercus with a gentle inward curve ventrally on apical 0.25 and with a distinct ventral subapical swelling or knob that almost makes the tip look bifid. Dorsal half with setulae 0.3-0.5 times the length of the cercus, ventral half with setulae only 0.1-0.2 times the length of the cercus. Surstylus triangular not particularly modified, with black setulae confined to the ventral 0.6. Pre- and postgonites both curved inwards, with a few black setulae present on ventral margin of the pregonite. Distiphallus: the ventral (or anterior) part of the base (ventral plate) is strongly sclerotized and bulging, forming an angle of $90^{o}$, and extending downwards (or distally) to become membranous along distal margin; juxta, curved anteriorly with a deep median cleft and the distal margin membranous; vesica taking the shape of sclerotized, ribbon-like, curled branches; styli long and narrow, following the juxta to its tip.

**Female.** *NB: only differences to male holotype given.*

*Length.* 10.0 mm (n=1).

*Colour.* $6^{th}$ abdominal tergite brown.

*Head.* Arista black, setose and thickened on approximately basal 0.2. Proclinate orbital setae present and 8 frontal setae present on each side. Parafacial at its narrowest point, about 0.2 the width of the eye. Genal ridge not well defined and gena with thinly dispersed black setulae.

*Thorax.* Scutum with 2-3 presutural acrostichal setae and 3-4 intraalar setae. Scutellum without a pair of apical setae.

*Abdomen. Terminalia.* $6^{th}$ abdominal tergite entire with 12-14 setae along inner margin. $7^{th}$ abdominal sternite with 7-8 setae along a slightly concaved hind margin. Abdominal sternites 2-5 with setae surpassing the hind margin of the next sternite.

**Distribution.**

AUSTRALASIAN/OCEANIAN – Australia (Queensland).

**Figure 3.** *Sarcophaga* (*Sarcosolomonia*) *collessi* **sp. nov.** Male terminalia in left lateral view, cercus with tip hidden behind phallus (Photo: D. K. B. Cheung).

**Etymology.**

For the esteemed Australian dipterist, the late Donald H. Colless.

**Subgeneric affiliation.**

Lopes (1958a) erected *Bezziola* to contain a single Micronesian species, *Sarcophaga stricklandi* (Hall and Bohart 1948), but when revising larger Australasian material he realised (Lopes 1967) that this nominal genus would fall as a synonym of *Sarcosolomonia* with the type species *S. tulagiensis* Baranov, 1938. Lopes and Kano (1969) divided *Sarcosolomonia* into *Sarcosolomonia* (s.str.) and their newly erected *Parkerimyia* and provided features to support the monophyly of each. Since then, several species have been described under the genus-group name *Sarcosolomonia*, and usually with little or no further discussion of the circumscription of the taxon (e.g., Shinonaga and Kurahashi 1969; Pape and Bänziger 2003).

The taxon *Sarcosolomonia* does not have any remarkable autapomorphies but still appears coherent from similarities in the shape of the phallus. There is no hinge or similar separation of the juxta, which curves forward as an elongate, tapering and usually mostly membranous structure. Lateral styli are inserted rather close to the hinge between basi- and distiphallus, which means that the juxta occupies more than half the length of the distiphallus. Lateral styli are thread-like and extend along the curvature of the juxta. The antero-proximal part of the distiphallus (ventral plate) is projecting and curving distally, being membranous at least at the tip.

*Sarcophaga collessi* would seem to fall within the *Parkerimyia* species-group as defined by the male cercus having a ventral swelling or process subapically, giving the impression of an almost bifid tip. This appears to be further supported by the shape of what is here interpreted as the vesica, which in the *Parkerimyia* species-group is divided into two biramous parts (compare Pape and Bänziger 2003, Figure 3).

## ***Sarcophaga* (*Sarcorohdendorfia*) *clavus*** sp. nov. **(Figure 4)**

**Type material.**

Holotype (male): Australia: Qld/Carnarvon NP/ Mt. Moffatt, 1097m/10.x.2002. T. Pape (ZMUC:00022173). Paratype: 1 (male): same data as holotype (ZMUC00022174) [abdomen dissected from specimen and glued to a piece of card beneath the source specimen]. The holotype is deposited in ANIC, the paratype in ZMUC.

**Differential diagnosis.**

*Sarcophaga clavus* is exclusively diagnosable within *Sarcorohdendorfia* Baranov, 1938 by the unique shape of its vesica with a pair of lateral 'nail head' shaped projections from the distal margin. Also, the vesica is unique within the subgenus by its rather simple shape with a distal tapering process.

**Description.**

**Male.**

*Length.* 10.0 mm (n=2).

*Colour.* Ground colour black, with dense dull yellow microtrichosity on palps, parafacials, fronto-orbital plate, occiput, postgena, thorax and abdomen. Thorax with three longitudinal black vittae; microtrichosity of the abdomen forming the typical checkerboard patterning changing with the incidence of light. Cercus, surstylus, phallus and gonites reddish brown.

*Head.* Arista reddish brown, setose and thickened on approximately basal 0.25-0.3. Proclinate orbital setae absent and 8 frontal setae present on each side. Lateral vertical setae the same length and strength as the longest postocular seta. Parafacial, at its narrowest point, about 0.25 the width of the eye and with only black setulae. Sub- and supra-vibrissa with only black setulae, approximately 0.3 the length of the vibrissa. Gena and occiput with only yellow setulae.

*Thorax.* Scutum with 1 presutural acrostichal and 4 dorsocentral setae; 4 postsutural dorsocentral setae with acrostitchal setae absent; 2 intraalar and 3 supraalar setae. Scutellum with a pair of apical setae and 2 pairs of subapical setae. Propleuron with yellow setulae. *Legs.* Hind tibia with short setulae. *Wing.* Costal spine well developed with small setae along the entire length. Vein $R_1$ without setulae and vein $R_{4+5}$ with numerous setulae on dorsal margin.

*Abdomen.* Tergite 3 without median marginal setae, but with one pair of lateral marginal setae present. Tergite 4 with strong median marginal setae and two pairs of lateral marginal setae. Abdominal sternites 1-2 with black setulae. *Terminalia.* Inner margins of sternite 5 with evenly dispersed short and medium setae, with a slight medial projection half way along the margin. At apex, 2-3 long setae and numerous long setulae. Surface of sternite 5 also with long setulae. Protandrial segment black with grey microtrichosity and black setulae on entire surface. Epandrium dark reddish brown or blackish, with black setulae on entire surface. Cercus hollowed out on posterior surface and curved inward on apical 0.3, apex with a small hooked tip. Dorsal half with setulae ~0.8 times the length of the cercus, ventral half with setulae ~0.6 times the length. Surstylus subtriangular with black setulae confined to the ventral 0.6. Pre- and postgonite both curved inwards: pregonite wide along length with black setulae present on ventral margin; postgonite with a pointed apex and some black setulae along dorsal margin. Distiphallus: vesica 'bowl' shaped and inwardly curved with a short pointed extension distally; juxta, distally with 'nail head' shaped lateral projections.

**Female.**

Unknown.

**Figure 4.** *Sarcophaga* (*Sarcorohdendorfia*) *clavus* **sp. nov.** Male terminalia in left lateral view (Photo: D. K. B. Cheung).

**Distribution.**

AUSTRALASIAN/OCEANIAN – Australia (Queensland).

**Etymology.**

From the Latin *clavus* = nail or spike. The species epithet is a noun in apposition and refers to the shape of the lateral projections of the phallic juxta being reminiscent of the head of a nail.

**Subgeneric affiliation.**

Pape (2000) gave a preliminary diagnosis of the subgenus *Sarcorohdendorfia*, which in a slightly reworded version will include members of the genus *Sarcophaga s.l.* (see diagnosis in Pape 1996) with 4-6 dorsocentral setae of which at least the anterior two are rather short; propleuron setose; terminalia black or dark brown to reddish; harpes short, rarely forming arm-like processes; juxta well developed and arching anteriorly; vesica distally with a median finger-like projection or recurving hook as well as a more proximal smaller process, never shaped like medially apposed plates; and lateral styli well developed, slender, often elongated, never short and stout. The shape of the vesica may be considered particularly diagnostic, and the condition in *clavus* may in this respect not be particularly well fitting, as the distal projection is straight and there is at most an indication of the proximal process. However, a similar condition is seen in *emuensis* (Lopes and Kano 1979a) and the subgeneric affiliation within *Sarcorohdendorfia* is currently the best supported. As noted by Pape and Kurahashi (2004), the subgenus *Sarcorohdendorfia* may still be insufficiently circumscribed in relation to certain other Australasian subgenera, in particular *Johnsonimyia* Lopes, 1959 and *Lioproctia* Enderlein, 1928.

## ***Nomenclature***

*Notes on synonymy:*

*Sarcophaga fergusonina* Hardy, 1940 — junior synonym of *Sarcophaga* (*Sarcorohdendorfia*) *assimilis* Macquart, 1851, **syn.n**.

REMARKS: The type material of *fergusonina* was sought for in both ANIC and QM without success, and this synonymy is therefore based almost entirely on the rather sketchy illustration of the male distiphallus provided by Hardy (1943, Figure 16). The synonymy is in agreement with Lopes (1954), who mentioned that the nominal species "seems to be close" to *assimilis* (as *Tricholioproctia hardyi*, Johnston and Tiegs, 1922).

*Sarcophaga horti* Blackith and Blackith, 1988 — junior synonym of *Sarcophaga* (*Fergusonimyia*) *bancroftorum* Johnston and Tiegs, 1921, **syn.n**.

REMARKS: The species *Sarcophaga* (*Fergusonimyia*) *bancroftorum* appears to be morphologically more variable than any other Australian flesh fly, as already pointed out by Lopes

(1958b). The new synonymy is proposed because *horti*, which was described from Indonesia, Sulawesi, appears to fall within the range of variation of *bancroftorum*, except for one feature: a setulose propleuron. Material examined from Papua New Guinea (in NRM) has: a low number of setulae anteriorly on the propleuron; but the structure of the male terminalia and a colour of the antennal flagellomere (varying from black to orange) match what has been observed in material of *bancroftorum* from Australia. Considering the general morphological variability of *bancroftorum*, the low number of propleural setulae alone is not considered justification for a specific separation. Blackith and Blackith (1988) compare *horti* with "closely related" species, which in Pape (1996) are placed in the subgenera *Mehria* Enderlein, 1928 and *Myorhina* Robineau-Desvoidy, 1830. This led Pape (1996) to place the nominal species in the subgenus *Myorhina*, but as the evidence for this is very inconclusive, the monotypic subgenus *Fergusonimyia* Lopes, 1958b is maintained.

*Sarcophaga synia* Johnston and Tiegs, 1923 — junior synonym of *Sarcophaga* (*Sarcosolomonia*) *crinita* Parker, 1919.

REMARKS: The nominal species *Sarcophaga synia* Johnston and Tiegs, 1923 was listed as a valid species by Lopes (1959, in *Bezziola* Lopes; 1989, in *Sarcosolomonia*), but was listed as a synonym of *Sarcosolomonia crinita* by Nandi (2002). The latter author does not state if this is a new synonymy, but within the literature there is no documented earlier occurrence.

*Sarcophaga triplex* Hardy, 1943 — junior synonym of *Sarcophaga* (*Sarcorohdendorfia*) *furcata* Hardy, 1932, **syn.n**.

REMARKS: The nominal species *Sarcophaga triplex* Hardy, 1943 has never been revised and was included in Lopes (1954) with a mention that it would be close to *furcata*. It was dismissed by Lopes (1959) as "unknown", but subsequently listed as a valid species in the catalogues of Lopes (1989) and Pape (1996). The present synonymy is based on a photograph of the holotype (courtesy of Dr Christine Lambkin and Mr Geoff Thompson, QM).

*Sarcophaga brevicornis* Ho, 1934 — junior synonym of *Sarcophaga* (*Liosarcophaga*) *kohla* Johnston and Hardy, 1923a, **syn.n**.

REMARKS: Somewhat surprisingly, this synonymy has not previously been proposed, and the two nominal species have not even been compared in the literature in spite of the obvious similarities, see e.g., relevant figures in Lopes (1954) and Ho (1934). For example, Sugiyama (1990) describe *Sarcophaga mimobrevicornis* from Malaysia with the remark that it is "very closely related to *Sarcophaga brevicornis* Ho", but with no mention of *kohla*. Interestingly, Baranov (1934a,b) recorded *S. kohla* (in *Thyrsocnema* Enderlein, 1928) from the Solomon Islands, which probably was missed (or dismissed) by Lopes (1989) and Pape (1996). Also, Nandi (2002) reported "Australia" under the distribution of *brevicornis*. I was not able to find morphological evidence for a separate Australian species and therefore propose this synonymy.

*Notes on rank:*

*Sarcophaga* (*Sarcorohdendorfia*) *piva* Roback, 1952 and *Sarcophaga* (*Sarcorohdendorfia*) *howensis* Johnston and Hardy, 1923b are treated as valid species following Pape (1996) rather than as subspecies of *Sarcophaga* (*Sarcorohdendorfia*) *antilope* Böttcher, 1913 as in Lopes (1989).

REMARKS: Lopes (1989) listed four subspecies under *Sarcophaga antilope*: *antilope*, *howensis*, *piva* and *variabilis* (Lopes 1958a) (all in *Sarcorohdendorfia*), two of which, *howensis* and *piva*, have been reported from Australia. Justification for applying a concept of subspecies considering the sparse material available and the lack of extensive studies of morphological variation, has not been noted. Both taxa appear to be diagnosable and adult male specimens examined are unambiguously identifiable, and the taxa therefore warrant recognition as valid species.

It is important to highlight that the current Australian sarcophagid fauna comprises 84 species: 17 miltogrammines and 67 sarcophagines (with 55 being *Sarcophaga s.l.* species).

## 2.3 A revised key of the Australian Sarcophagidae (Diptera) with special emphasis on *Sarcophaga* (*sensu lato*)

*This section is slightly modified, in terms of the layout and numbering of figures and tables, from a paper prepared for submission to* Zootaxa*:*

Meiklejohn, K.A., Dowton, M., Pape, T., and Wallman, J.F. A revised key of the Australian Sarcophagidae (Diptera) with special emphasis on *Sarcophaga* (*sensu lato*).

The contributions of each author to the research described are as follows: JFW and MD designed the research and provided advice and feedback on the manuscript; TP provided guidance and knowledge on morphological characters to include in the matrix, but also provided some of the terminalia photographs; KAM composed the research as a manuscript for publication, collected all data for the character matrix, developed the revised key, generated the morphological, distributional and biological information for each species, took some of the terminalia photographs and collated the terminalia illustrations from a range of manuscripts.

## 2.3.1 Introduction

The Sarcophagidae (Diptera), or flesh flies, are a globally distributed family comprising approximately 173 genera and 3,000 species (Pape *et al.* 2011). Previously, the sarcophagids were treated as a subfamily of Calliphoridae (Diptera), however they are now accepted as a distinct family divided into three subfamilies: Miltogramminae, Paramacronychiinae and Sarcophaginae (Pape 1996). Species of the largest Sarcophaginae genus, *Sarcophaga s.l.*, possess a checkerboard abdomen, heavily bristled body and longitudinal stripes on the thorax.

The Australian Sarcophagidae comprises 84 known species, from both the Miltogramminae and Sarcophaginae. The miltogrammines account for ~20% of Australian sarcophagids, classified into six genera (*Aenigmetopia*, *Amobia*, *Metopia*, *Miltogramma*, *Protomiltogramma*, and *Senotainia*), with most species known to be kleptoparasites of Hymenoptera, specifically solitary wasps and bees (O'Hara *et al.* 1999; Pape 1996). The remaining ~80% of the Australian flesh flies are sarcophagines, classified into four genera: *Blaesoxipha*, *Oxysarcodexia*, *Sarcophaga s.l.*, and *Tricharaea*. Both *Oxysarcodexia* and *Tricharaea* are present within the Australian fauna, but are only represented by a single species. However the genus *Blaesoxipha*, the species of which are predominantly parasitoids of Orthoptera, comprises ten known species. The 55 species of *Sarcophaga s.l.* represent close to two-thirds of the entire Australian flesh fly fauna, and these are classified into 14 subgenera. Many *Sarcophaga s.l.* species have documented feeding and breeding preferences for decomposing vertebrate carcasses, including human corpses (Pape 1996).

Little work has been undertaken on the taxonomy of the Australian miltogrammines. The most recent key, which only facilitates separation of genera, was published by Malloch (1930). A range of taxonomists have worked more extensively on the Australian Sarcophaginae, such as T. H. Johnston, C. W. Tiegs, G. H. Hardy and H. de Souza Lopes, producing several keys for their identification (Hardy 1934; Johnston and Hardy 1923; Lopes 1954; Lopes 1959; Lopes and Kano 1979a). The current taxonomic keys for the Australian sarcophagines were produced by H. de Souza Lopes during the 1950-1970s. These keys do not account for intraspecific variation in all species, nor do they allow for discrimination between *Blaesoxipha* species. Several new species have also been described since the 1970s, but these have not been incorporated into a single comprehensive key. In addition to this, morphological descriptions are not complete for those ~40% of the species where the female is still unknown or insufficiently documented. Males can be reliably identified by examining the terminalia for diagnostic features.

## 2.3.2 **Methods**

The revised key presented in this paper was constructed after generation of a character matrix. To account for intraspecific variation, the 110 characters (including 55 characters from the male terminalia) were scored for five males and five females from each species, based on curated specimens from a range of collections and museums. From this matrix, characters variable between species were identified and used for species discrimination.

As there are fewer male terminalia characters to draw upon for miltogrammines and *Blaesoxipha,* morphological species identifications are especially problematic. Consequently, the revised key only allows for separation of miltogrammine genera and to the recognition of the genus *Blaesoxipha* for both sexes. The key does however facilitate identification of males of all species of *Sarcophaga s.l.*, along with *Oxysarcodexia varia* and *Tricharaea* (*Tricharaea*) *brevicornis*. Female terminalia are generally insufficiently studied and many features of the head, thorax and abdomen provide overlapping variation. Therefore, this key only enables reliable identification of females for the *Sarcophaga s.l.* subgenera *Australopierretia*, *Bercaea*, *Boettcherisca*, *Fergusonimyia*, *Hardyella*, *Liopygia* and *Sarcorohdendorfia*, along with the genera *Oxysarcodexia* and *Tricharaea*. An attempt has been made to introduce *Sarcophaga* (*Heteronychia*) *penicillata* Villeneuve into Australia as a biological control agent for the snail *Cochlicella acuta* (Hopkins and McDonald 2007). However, there is no firm evidence that a natural population of this species has been established, and so it is not included in the revised key. Similarly, *Sarcophaga* (*Sarcorohdendorfia*) *irrequieta* Walker has not been included in the revised key, as the type specimen could not be sourced for verification of this species.

The remainder of the paper is formatted as follows: 1) the revised key, incorporating illustrations of the head, thorax, abdomen and male/female terminalia, with key features highlighted; illustrations and photos of the male terminalia for all *Sarcophaga s.l.* species, along with *Oxysarcodexia varia* and *Tricharaea* (*Tricharaea*) *brevicornis*; and 2) information for each species in alphabetical order by genus and subgenus, including morphological characters, distribution and known biology. A complete list of synonyms for each species is not given; only synonyms for specimens collected in Australia are listed. It is important to note that the terms ‘setae’ and ‘setulae’ are used within the key to identify well developed hair-like structures possessing a socket and shaft. For use in this key, the boundary between the gena and postgena is defined roughly as a vertical line flush with the posterior margin of the eye. For some couplets which may require clarification, necessary features have been highlighted with arrows in the pertinent figures and referenced in the key. Within the morphological description of each species, unless otherwise stated, each species has: apical scutellar setae present in males but absent in females; short setulae on the hind tibia in both sexes; and the 1$^{st}$ and 2$^{nd}$ abdominal sternites with long setulae only black.

## 2.3.3 Revised key

**1)** Arista bare or with small fine trichiae; gena narrow, at most 0.2 of eye height; coxopleural streak present; longitudinal vittae on thorax not distinctive; hind coxa bare on posterior surface; male abdominal sternites 2-4 partly concealed by overlapping margins of corresponding tergites; abdomen with transverse bands/median stripe with lateral spots/three distinct spots – no clear checkerboard patterning ........................................................................ **MILTOGRAMMINAE (2)**

Arista plumose; gena broad, more than 0.2 of eye height; coxopleural streak absent; thorax generally with three distinct longitudinal vittae; hind coxa setose on posterior surface; male abdominal sternites 2-4 exposed and overlapping lateral margins of corresponding tergites; abdomen with distinct checkerboard patterning (exception of *Blaesoxipha* with median and lateral stripes)............................................................................................................. **SARCOPHAGINAE (7)**

**2)** Lunule bare ..........................................................................................................................................**(3)**

Lunule setose ......................................................................................................................................**(6)**

**3)** Propleuron with black setulae confined to the anterior margin.............................. ***SENOTAINIA***

Propleuron bare .....................................................................................................................................**(4)**

**4)** Vibrissa reduced........................................................................................................ ***MILTOGRAMMA***

Vibrissa well developed...........................................................................................................................**(5)**

**5)** Proclinate orbital setae fine/hair like, more than five ......................................................... ***AMOBIA***

Proclinate orbital setae strongly developed, less than five ............... ***PROTOMILTOGRAMMA***

**6)** Two reclinate and two proclinate orbital setae; lunule often setose; tegula black........ ***METOPIA***

Only proclinate orbital setae present; lunule bare; tegula yellow .................... ***AENIGMETOPIA***

**7)** Frontal setae in rows parallel or gradually diverging near lunule .....................................................**(8)**

Frontal setae in rows strongly diverging near lunule....................................... ***SARCOPHAGA* (10)**

**8)** Postalar wall bare; males with at least one strong proclinate orbital seta ............................................
........................................................................................... ***TRICHARAEA* (*Tricharaea*) *brevicornis***

Postalar wall setose; males without proclinate orbital setae..............................................................**(9)**

**9)** Gena and postocular with silver microtrichosity; male mid femur with ctenidium of normal (non-flattened) spines ........................................................................................................ ***BLAESOXIPHA***

Head with bright yellow microtrichosity at gena and silver microtrichosity at postocular; male mid femur with ctenidium of flattened spines ....................................... ***OXYSARCODEXIA varia***

**10)** 2nd and 3rd antennomere at least partly yellow ..................................................................... **(11)**

2nd and 3rd antennomere at most reddish black ..................................................................... **(14)**

**11)** Ground colour of terminalia at most reddish black (males: protandrial segment-epandrium-cercus; females: 6th abdominal tergite)................................................................................... **(12)**

Ground colour of terminalia red or orange.............................. ***Sarcophaga* (*Liopygia*) *ruficornis***

**12)** Katepisternum and 1st and 2nd abdominal sternites with setulae only black ................................ **(13)**

Katepisternum and 1st and 2nd abdominal sternites with some setulae yellow/white ........................ ............................................................................................... ***Sarcophaga* (*Lioproctia*) *torvida***

**13)** Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally........................ ***Sarcophaga* (*Fergusonimyia*) *bancroftorum***

Postocular with setulae only yellow/white ............................ ***Sarcophaga* (*Johnstonimyia*) *lincta***

**14)** Basal 0.5 of wing vein $R_1$ setulose ........................................................................................ **(15)**

Basal 0.5 of wing vein $R_1$ bare.............................................................................................. **(16)**

**15)** Propleuron with setulae only black; 1st and 2nd abdominal sternites setulae only black.................... ............................................................................. ***Sarcophaga* (*Australopierretia*) *australis***

Propleuron with setulae mostly yellow/white; 1st and 2nd abdominal sternites with setulae only yellow/white ............................................................ ***Sarcophaga* (*Sarcorohdendorfia*) *meiofilosia***

**16)** Ratio of width of eye to width of head (at widest parts) no more than 0.5 (Figure 15a).................. ............................................................................................. ***Sarcophaga* (*Hardyella*) *littoralis***

Ratio of width of eye to width of head (at widest parts) generally 0.75 (Figure 15b).................. **(17)**

**17)** Ground colour of terminalia red or orange (males: protandrial segment-epandrium-cercus; females: 6th abdominal tergite) ...................................................................................................... **(18)**

Ground colour of terminalia at most reddish black.......................................................................... **(20)**

**18)** Propleuron with only one or at most a few scattered setulae ................................................................
................................................................................................ ***Sarcophaga* (*Boettcherisca*) *peregrina***
Propleuron uniformly bare ........................................................................................................ **(19)**

**19)** Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally; surstylus reduced in size and pointed; cercus in lateral view with lateral projections near apex (Figures 22b, 22c) ........................ ***Sarcophaga* (*Bercaea*) *africa***
Postocular with setulae only yellow/white; surstylus rounded; cercus in lateral view with posterior projections near apex (Figure 33b) ....................... ***Sarcophaga* (*Liopygia*) *crassipalpis***

**20)** Propleuron with only one or at most a few scattered setulae <u>(males and females)</u> .................. **(21)**
Propleuron uniformly bare <u>(males only)</u> ............................................................................ **(22)**
Propleuron uniformly setulose <u>(males and females)</u> ....................................................... **(44)**

**21)** Males: vesica membranous with a series of spines (Figure 23a); cercus without enlargement at apex; females: 7th abdominal sternite very concave on hind margin ....................................................
................................................................................................ ***Sarcophaga* (*Boettcherisca*) *peregrina***
Males: vesica sclerotised without a series of spines; cercus with enlargement at apex (Figure 24b); females: 7th abdominal sternite tear drop shaped ........................................................................
..................................................................................... ***Sarcophaga* (*Fergusonimyia*) *bancroftorum***

**22)** Katepisternum with setulae only black .................................................................................. **(23)**
Katepisternum with setulae mostly black, but at least some yellow/white ........................................
........................................................................................................ ***Sarcophaga* (*Johnstonimyia*) *kappa***

**23)** 1st to 4th abdominal sternites and ventral surface of tergites with dense very long setulae .............
........................................................................................................ ***Sarcophaga* (*Taylorimyia*) *aurifrons***
At least the ventral surfaces of the 1st to 4th abdominal tergites with short setulae .................... **(24)**

**24)** Juxta, anteroventral part with a pair of elongate arms with a bifid tip (Figures 35a, 37a, 38a) ........
....................................................................................................................................................... **(25)**
Juxta, anteroventral part not modified ................................................................................ **(27)**

**25)** Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally ........................................................................................ **(26)**
Postocular with setulae only yellow/white ............................... ***Sarcophaga* (*Liosarcophaga*) *dux***

**26)** Gena with setulae only black ................................................ ***Sarcophaga* (*Liosarcophaga*) *sigma***

Gena with setulae a mix of black and yellow/white ........... ***Sarcophaga* (*Liosarcophaga*) *kohla***

**27)** Vesica connected to the distiphallus by means of a narrow, stalk-like connection (Figures 39a, 40a, 41a)........................................................................................................................... **(28)**

Vesica broadly connected to the distiphallus ........................................................................ **(30)**

**28)** Juxta with long arms bent inwards at 90° (Figures 39a, 41a); cercus without medial projections near apex (Figure 39b) ......................................................................................................... **(29)**

Juxta with short arms curved inwards (Figure 40a); cercus with medial projections at apex (Figure 40b)............................................................................ ***Sarcophaga* (*Parasarcophaga*) *misera***

**29)** Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally................................. ***Sarcophaga* (*Parasarcophaga*) *albiceps***

Postocular with setulae only yellow/white ............... ***Sarcophaga* (*Parasarcophaga*) *taenionota***

**30)** Lateral styli narrow and thread-like along entire length and greatly extending past the juxta (Figures 65b, 67a, 68a) ...........................................................................................................**(31)**

Lateral styli not narrow and thread-like along entire length, nor greatly extending past the juxta... .......................................................................................................................................... **(33)**

**31)** 3rd abdominal tergite lacking median marginal setae........ ***Sarcophaga* (*Sarcosolomonia*) *crinita***

3rd abdominal tergite with median marginal setae ............................................................ **(32)**

**32)** Cercus bent inward 90° along apical 0.3-0.4, becoming very slender (Figure 68b)........................... ....................................................................................... ***Sarcophaga* (*Sarcosolomonia*) *sumunensis***

Cercus curved inward along apical 0.25, retaining width at apex (Figure 67b)................................... .......................................................................................... ***Sarcophaga* (*Sarcosolomonia*) *papuensis***

**33)** 3rd abdominal tergite lacking median marginal setae....................................................................... **(34)**

3rd abdominal tergite with median marginal setae ...................... ***Sarcophaga* (unplaced) *simplex***

**34)** Hind tibia with short setulae.................................................................................................. **(35)**

Hind tibia with long setulae .................................................................................................. **(40)**

**35)** Gena with setulae only yellow/white ................................................................................ **(36)**

Gena with setulae a mix of black and yellow/white ........................................................... **(38)**

**36)** Parafacial with setulae only black; cercus not bent backwards........................................................ **(37)**

Parafacial with setulae a mix of black and yellow/white; cercus bent backwards (Figure 69c) ........
........................................................................................ ***Sarcophaga* (*Sarcosolomonia*) *versatilis***

**37)** Vesica with a cleft forming lateral projections distally (Figure 30a) ....................................................
............................................................................................... ***Sarcophaga* (*Lioproctia*) *multicolor***

Vesica entire (Figure 70) ..................................... ***Sarcophaga* (*Sarcosolomonia*) *collessi*** sp. nov.

**38)** 5th sternite with setulae on surface; head with bright yellow microtrichosity ....................................
......................................................................................... ***Sarcophaga* (*Baranovisca*) *cyrtophorae***

5th sternite without setulae on surface; head without bright yellow microtrichosity.................. **(39)**

**39)** 5th sternite with setae long at apex .................................. ***Sarcophaga* (*Baranovisca*) *arachnivora***

5th sternite with setae short at apex ......................................... ***Sarcophaga* (*Baranovisca*) *reposita***

**40)** Juxta with setulae like processes dense distally (Figure 29a)........ ***Sarcophaga* (*Lioproctia*) *imita***

Juxta without setulae like processes distally.......................................................................................**(41)**

**41)** Juxta with numerous thin finger-like projections at base (Figure 31a)...............................................
................................................................................................... ***Sarcophaga* (*Lioproctia*) *spinifera***

Juxta without numerous thin finger-like projections at base ......................................................... **(42)**

**42)** Vesica long and extending past the juxta (Figure 36a) ............ ***Sarcophaga* (*Liosarcophaga*) *eta***

Vesica short.................................................................................................................................... **(43)**

**43)** Vesica with a cleft forming lateral projections distally (Figure 30a) ....................................................
............................................................................................... ***Sarcophaga* (*Lioproctia*) *multicolor***

Vesica entire (Figure 66a) ...................................................... ***Sarcophaga* (*Sarcosolomonia*) *fabea***

**44)** Males ............................................................................................................................................ **(45)**

Females ........................................................................................................................................... **(80)**

**45)** Propleuron with setulae only yellow/white ..................................................................................... **(46)**

Propleuron with setulae only black .................................................................................................... **(67)**

Propleuron with setulae a mix of black and yellow/white............................................................... **(75)**

**46)** Hind tibia with short setulae........................................................................................................... **(47)**

Hind tibia with long setulae ............................................................................................................... **(48)**

**47)** Parafacial with setulae only black.................... ***Sarcophaga* (*Sarcorohdendorfia*) *clavus*** sp. nov.
Parafacial with setulae a mix of black and yellow/white........................................................................
........................................................................................ ***Sarcophaga* (*Sarcorohdendorfia*) *emuensis***

**48)** Median patch of dense setae on 4th abdominal tergite absent...................................................... **(49)**
Median patch of dense setae on 4th abdominal tergite present (Figure 16)................................ **(66)**

**49)** 1st and 2nd abdominal sternites with setulae only black ................................................................. **(50)**
1st and 2nd abdominal sternites with setulae only yellow/white ..................................................... **(55)**

**50)** Parafacial with setulae only black............................................................................................**(51)**
Parafacial with setulae a mix of black and yellow/white........................................................................
........................................................................................ ***Sarcophaga* (*Sarcorohdendorfia*) *spinigera***

**51)** Cercus with spines absent................................................................................................... **(52)**
Cercus with spines present (Figure 48b) ............................................................................ **(53)**

**52**) Lateral styli narrow and thread-like, greatly extending past the juxta (Figure 53a) ...........................
........................................................................................ ***Sarcophaga* (*Sarcorohdendorfia*) *longifilia***
Lateral styli not narrow and thread-like, not greatly extending past the juxta ....................................
........................................................................................ ***Sarcophaga* (*Sarcorohdendorfia*) *assimilis***

**53)** Juxta with 4-6 finger-like lateral projections distally; apex of cercus bifid (Figure 64) ....................
........................................................................................ ***Sarcophaga* (*Sarcorohdendorfia*)** sp. nov.
Juxta without finger-like lateral projections distally; apex of cercus not bifid ............................. **(54)**

**54)** Lateral styli jagged along anterior margin and not extending past the juxta (Figure 50a)................
........................................................................................***Sarcophaga* (*Sarcorohdendorfia*) *furcata***
Lateral styli not jagged along anterior margin but extending past the juxta (Figure 46a) .................
........................................................................................***Sarcophaga* (*Sarcorohdendorfia*) *bifrons***

**55)** Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally.......................................................................................... **(56)**
Postocular with setulae only yellow/white ........................................................................ **(57)**

**56)** Apex of cercus bifid (Figure 42b) ............................ ***Sarcophaga* (*Sarcorohdendorfia*) *assimilis***
Apex of cercus not bifid (Figure 46b) ..........................***Sarcophaga* (*Sarcorohdendorfia*) *bifrons***

**57)** Parafacial with setulae only black ............................ ***Sarcophaga* (*Sarcorohdendorfia*) *assimilis***

Parafacial with setulae only yellow/white ........................................................................... **(58)**

Parafacial with setulae a mix of black and yellow/white ................................................................ **(60)**

**58)** Apex of cercus bifid (Figure 45b) .......................... ***Sarcophaga* (*Sarcorohdendorfia*) *bidentata***

Apex of cercus not bifid ......................................................................................................... **(59)**

**59)** Cercus curved inward along apical 0.25; surstylus elongated (Figure 58b) ....................................... ..................................................................................... ***Sarcophaga* (*Sarcorohdendorfia*) *praedatrix***

Cercus nearly straight; surstylus triangular (Figure 49b)......................................................................... ..................................................................................... ***Sarcophaga* (*Sarcorohdendorfia*) *froggatti***

**60)** Apex of surstylus bifid (Figure 61b) ...................... ***Sarcophaga* (*Sarcorohdendorfia*) *villisterna***

Apex of surstylus not bifid ..................................................................................................... **(61)**

**61)** Cercus with spines absent ......................................................................................................... **(62)**

Cercus with spines present (Figure 48b) ........................................................................................ **(63)**

**62)** Distiphallus with a large membranous region along posterior margin, encased by a thin sclerotisation (Figure 56a) ........................................... ***Sarcophaga* (*Sarcorohdendorfia*) *omikron***

Distiphallus with a small membranous region at posterior margin, encased by a thick sclerotisation (Fig 59a) ............................................... ***Sarcophaga* (*Sarcorohdendorfia*) *spinigera***

**63)** Surstylus elongated (Figure 58b) ........................... ***Sarcophaga* (*Sarcorohdendorfia*) *praedatrix***

Surstylus not elongated ........................................................................................................ **(64)**

**64)** Apex of cercus bifid (Figure 45b) .......................... ***Sarcophaga* (*Sarcorohdendorfia*) *bidentata***

Apex of cercus not bifid ......................................................................................................... **(65)**

**65)** Juxta with a slender membranous projection pointed ventrally; vesica not elongated distally (Figure 49a) .................................................................. ***Sarcophaga* (*Sarcorohdendorfia*) *froggatti***

Juxta lacking a slender pointed membranous projection; vesica elongated distally (Figure 48a)...... ..................................................................................... ***Sarcophaga* (*Sarcorohdendorfia*) *emuensis***

**66)** Parafacial with setulae only black .......................... ***Sarcophaga* (*Sarcorohdendorfia*) *impatiens***

Parafacial with setulae a mix of black and yellow/white ........................................................................ ..................................................................................... ***Sarcophaga* (*Sarcorohdendorfia*) *villisterna***

**67)** 3rd abdominal tergite with median marginal setae .......... **(68)**
3rd abdominal tergite lacking median marginal setae .......... **(69)**

**68)** Vesica curved inward, without hook at apex (Figure 57a) .......... ***Sarcophaga* (*Sarcorohdendorfia*) *piva***
Vesica not curved inward, with hook at apex (Figure 51a) .......... ***Sarcophaga* (*Sarcorohdendorfia*) *howensis***

**69)** Basicosta deep yellow (Figure 14) .......... ***Sarcophaga* (*Sarcorohdendorfia*) *darwiniana***
Basicosta not deep yellow, generally brown .......... **(70)**

**70)** Gena with setulae only black .......... **(71)**
Gena with setulae only yellow/white .......... **(72)**
Gena with setulae a mix of black and yellow/white .......... **(73)**

**71)** Lateral styli narrow and thread-like, greatly extending past the juxta (Figure 53a) .......... ***Sarcophaga* (*Sarcorohdendorfia*) *longifilia***
Lateral styli not narrow and thread-like, not greatly extending past the juxta .......... ***Sarcophaga* (*Sarcorohdendorfia*) *piva***

**72)** 4th abdominal tergite lacking median patch of dense setae .......... ***Sarcophaga* (*Lioproctia*) *alcicornis***
4th abdominal tergite with median patch of dense setae .......... ***Sarcophaga* (*Sarcorohdendorfia*) *zeta***

**73)** Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally .......... ***Sarcophaga* (*Sarcorohdendorfia*) *alpha***
Postocular with setulae only yellow/white .......... **(74)**

**74)** Surstylus elongated; vesica not curved inward (Figures 60a, 60b) .......... ***Sarcophaga* (*Sarcorohdendorfia*) *seniorwhitei***
Surstylus not elongated; vesica curved inward (Figure 57a) .......... ***Sarcophaga* (*Sarcorohdendorfia*) *piva***

**75)** 4th abdominal tergite lacking median patch of dense setae .......... **(76)**
4th abdominal tergite with median patch of dense setae .......... **(79)**

**76)** Lateral styli narrow and thread-like, greatly extending past the juxta (Figure 61a) .................... **(77)**
Lateral styli not narrow and thread-like, not greatly extending past the juxta ....................................
........................................................................................ ***Sarcophaga* (*Sarcorohdendorfia*) *assimilis***

**77)** Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally ......................... ***Sarcophaga* (*Sarcorohdendorfia*) *villisterna***
Postocular with setulae only yellow/white ........................................................................................ **(78)**

**78)** Hind tibia with short setulae ................................ ***Sarcophaga* (*Sarcorohdendorfia*) *megafilosia***
Hind tibia with long setulae ........................................... ***Sarcophaga* (*Sarcorohdendorfia*) *bifrons***

**79)** 1st and 2nd abdominal sternites with setulae only black .. ***Sarcophaga* (*Sarcorohdendorfia*) *beta***
1st and 2nd abdominal sternites with setulae only yellow/white ..........................................................
........................................................................................ ***Sarcophaga* (*Sarcorohdendorfia*) *villisterna***

**80)** 3rd abdominal tergite lacking median marginal setae ........................................................................ **(81)**
3rd abdominal tergite with median marginal setae ............................................................................ **(93)**

**81)** Basicosta deep yellow (Figure 14) ........................ ***Sarcophaga* (*Sarcorohdendorfia*) *darwiniana***
Basicosta not deep yellow, generally brown ........................................................................................ **(82)**

**82)** 4th abdominal tergite lacking median marginal setae ........................................................................
........................................................................................ ***Sarcophaga* (*Sarcorohdendorfia*) *emuensis***
4th abdominal tergite with median marginal setae ............................................................................ **(83)**

**83)** Hind tibia with short setulae ........................................................................................................ **(84)**
Hind tibia with long setulae ........................................................................................................... **(92)**

**84)** Prescutellar acrostichal setae absent ................................................................................................ **(85)**
Prescutellar acrostichal setae present ................................................................................................. **(87)**

**85)** Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally ............................... ***Sarcophaga* (*Sarcorohdendorfia*) *bifrons***
Postocular with setulae only yellow/white ........................................................................................ **(86)**

**86)** Parafacial with setulae a mix of black and yellow/white ........................................................................ ***Sarcophaga* (*Sarcorohdendorfia*) *froggatti*** **or** ***spinigera***
Parafacial with setulae only yellow/white ........................................................................ ***Sarcophaga* (*Sarcorohdendorfia*) *bidentata, froggatti*** **or** ***omikron***

**87)** Propleuron with setulae only yellow/white ........................................ **(88)**
Propleuron with setulae only black ........................................ **(89)**
Propleuron with setulae a mix of black and yellow/white ........................................ **(91)**

**88)** Parafacial with setulae only yellow/white ............. ***Sarcophaga* (*Sarcorohdendorfia*) *villisterna***
Parafacial with setulae only black ...... ***Sarcophaga* (*Sarcorohdendorfia*) *assimilis*** **or** ***impatiens***
Parafacial with setulae a mix of black and yellow/white ........................................................................ ***Sarcophaga* (*Sarcorohdendorfia*) *omikron*** **or** ***villisterna***

**89)** Gena with setulae a mix of black and yellow/white ........................................ **(90)**
Gena with setulae only yellow/white ........................................................................ ***Sarcophaga* (*Sarcorohdendorfia*) *alpha*** **or** ***zeta*** **or** ***Sarcophaga* (*Lioproctia*) *alcicornis***

**90)** Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally .................................... ***Sarcophaga* (*Sarcorohdendorfia*) *piva***
Postocular with setulae only yellow/white .................... ***Sarcophaga* (*Sarcorohdendorfia*) *alpha***

**91)** Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally .................... ***Sarcophaga* (*Sarcorohdendorfia*) *seniorwhitei***
Postocular with setulae only yellow/white . ***Sarcophaga* (*Sarcorohdendorfia*) *assimilis*** **or** ***beta***

**92)** Prescutellar acrostichal setae absent ........................ ***Sarcophaga* (*Sarcorohdendorfia*) *longifilia***
Prescutellar acrostichal setae present ........................... ***Sarcophaga* (*Sarcorohdendorfia*) *furcata***

**93)** Katepisternum with setulae only black .................. ***Sarcophaga* (*Sarcorohdendorfia*) *howensis***
Katepisternum with some yellow/white setulae ........................................................................ ***Sarcophaga* (*Sarcorohdendorfia*) *megafilosia***

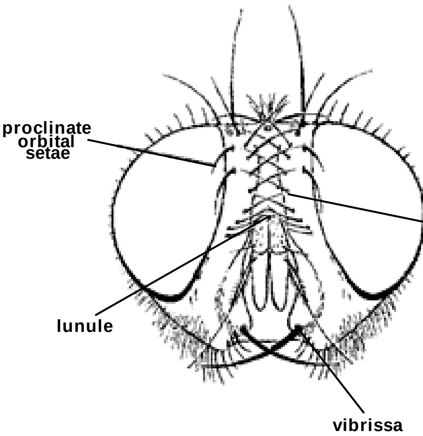


**Figure 5.**

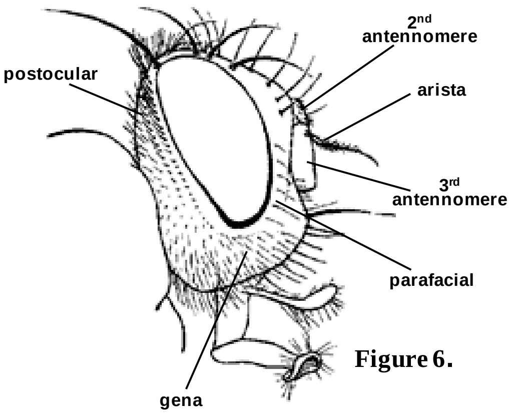


**Figure 6.**

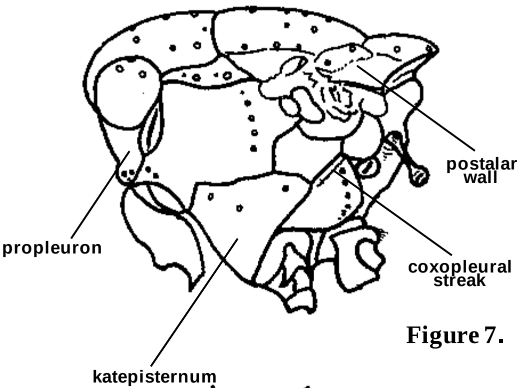


**Figure 7.**

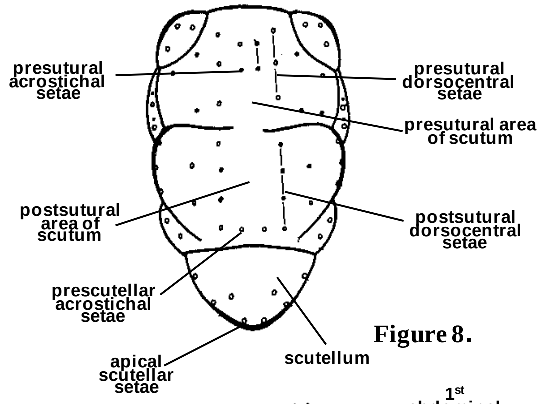


**Figure 8.**

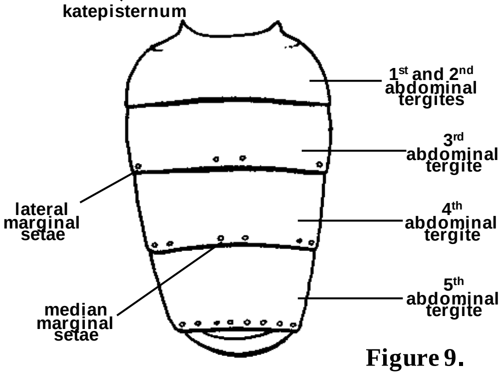


**Figure 9.**

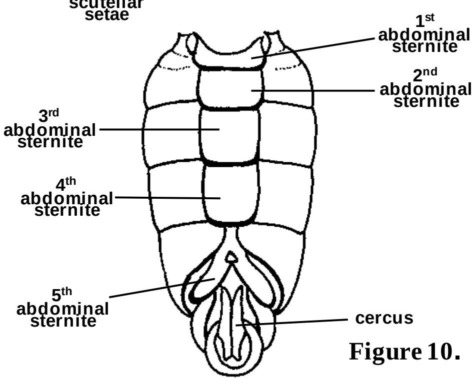


**Figure 10.**

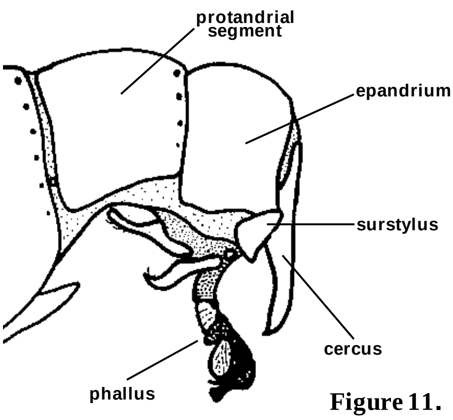


**Figure 11.**

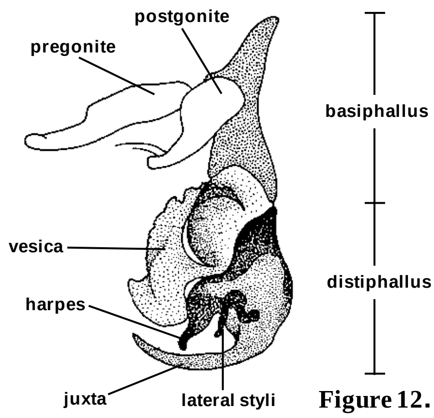


**Figure 12.**

Unless otherwise stated for figures 5-73: a-illustration of phallus, lateral view, b-illustration of entire male terminalia, lateral view, c-photograph of entire male terminalia, lateral view. In figures 5-73, the following superscript letters denote where illustrations were sourced: [a] Johnston and Tiegs, 1921; [b] Johnston and Hardy, 1923a; [c] Hardy, 1932; [d] Lopes, 1954; [e] Lopes and Albuquerque, 1955; [f] Lopes, 1958a; [g] Lopes, 1958b; [h] Lopes, 1959; [i] Lopes, 1967; [j] Shinonaga and Kurahashi, 1969; [k] Lopes, 1973; [l] Park, 1977; [m] Kano and Lopes, 1979; [n] Lopes and Kano, 1979; [o] Lopes, 1985; [p] Cantrell, 1986; [q] Pape, 1987; [r] Verves, 1987; and [s] Pape *et al.* 2000. **Figure 5.** Head, lateral view [q]; **Figure 6.** Head, frontal view [q]; **Figure 7.** Thorax, lateral view [q]; **Figure 8.** Thorax, dorsal view [q]; **Figure 9.** Abdomen, dorsal view [q]; **Figure 10.** Abdomen, ventral view [q]; **Figure 11.** Male terminalia, lateral view [q]; **Figure 12.** Phallus, lateral view [q].

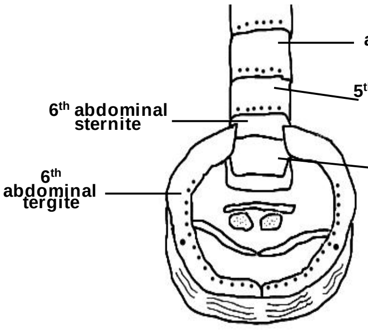


**Figure 13.**

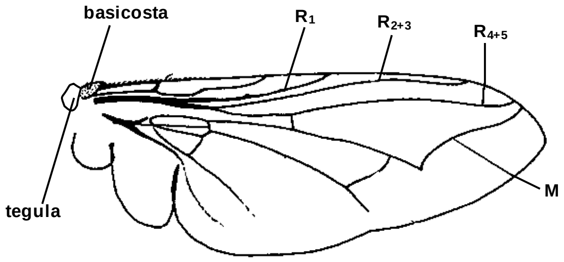


**Figure 14.**

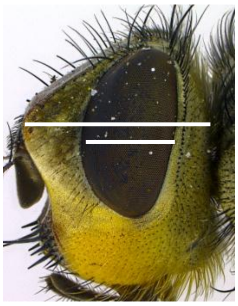

**Figure 15a.**

**Figure 15b.**

**Figure 16.**

**Figure 17a.**

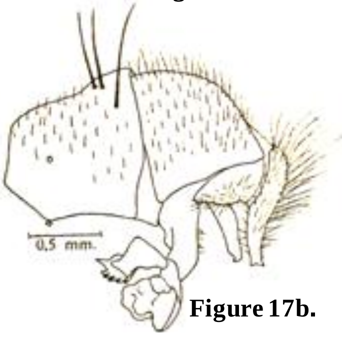


**Figure 17b.**

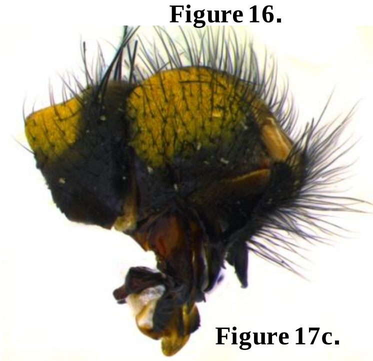

**Figure 17c.**

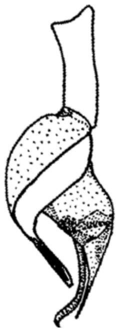

**Figure 18a.**

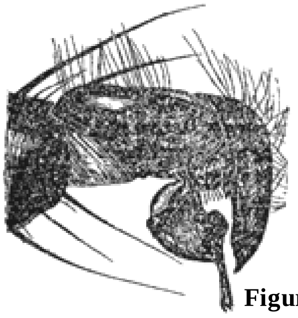

**Figure 18b.**

**Figure 18c.**

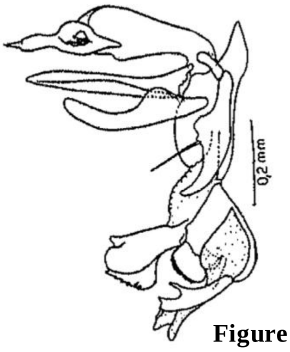


**Figure 19a.**

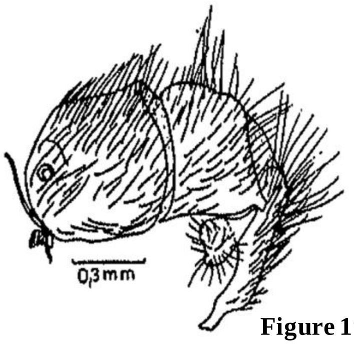


**Figure 19b.**

**Figure 13.** Female terminalia, ventral view; **Figure 14.** Wing [q]; **Figure 15a.** *Sarcophaga* (*Hardyella*) *littoralis* with protuberant head; **Figure 15b.** *Sarcophaga* sp. without protuberant head; **Figure 16.** Median patch of dense setae on 4th abdominal tergite (male); **Figure 17a.b.c.** *Oxysarcodexia varia* [e]; **Figure 18a.b.c.** *Sarcophaga* (*Australopierretia*) *australis* [ar]; **Figure 19a.b.** *Sarcophaga* (*Baranovisca*) *arachnivora* [o].

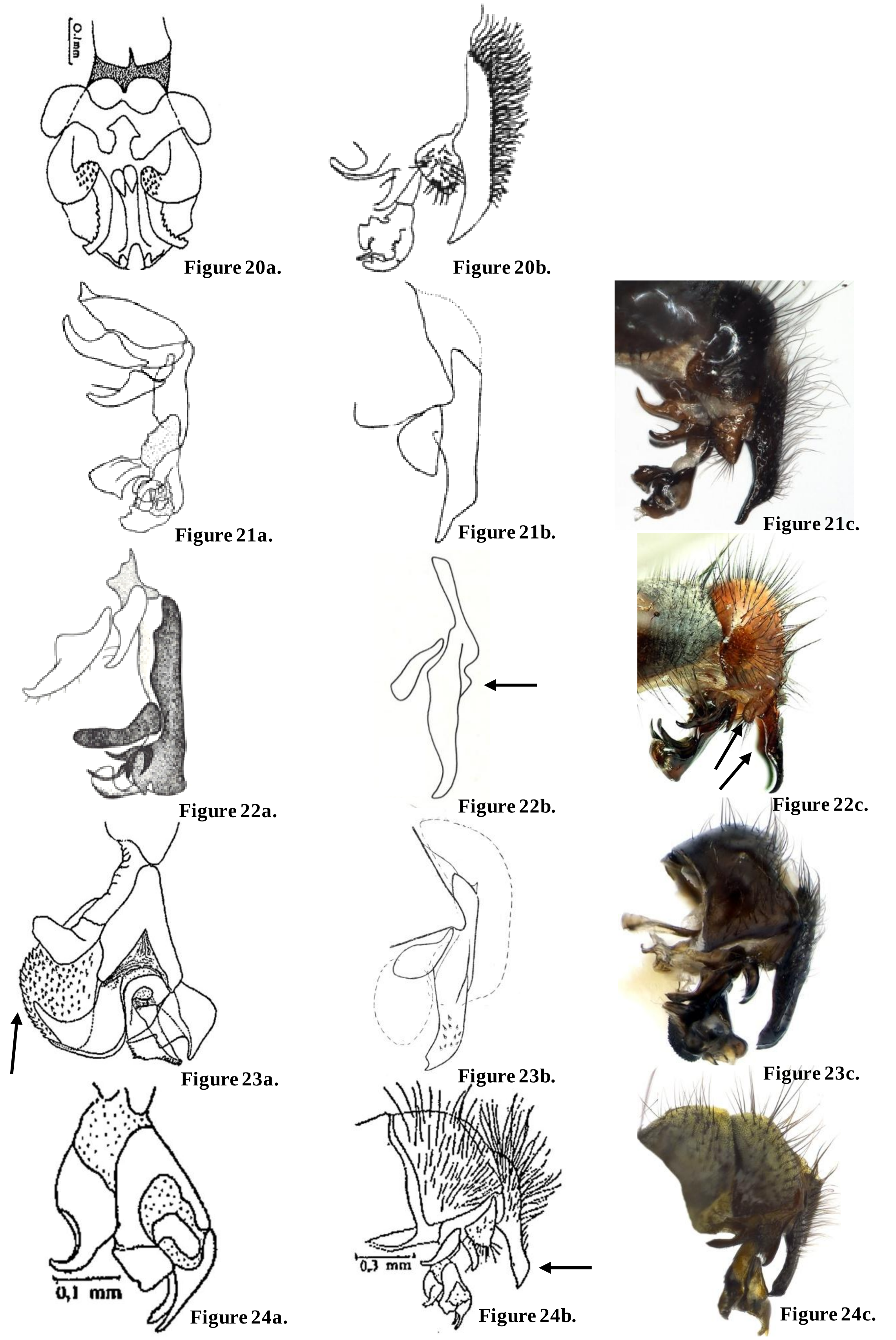


**Figure 20.** *Sarcophaga* (*Baranovisca*) *cyrtophorae* [p] **a.** illustration of phallus, ventral view. **b.** illustration of entire male terminalia, lateral view; **Figure 21a.b.c.** *Sarcophaga* (*Baranovisca*) *reposita* [hp]; **Figure 22a.b.c.** *Sarcophaga* (*Bercaea*) *africa* [q]; **Figure 23a.b.c.** *Sarcophaga* (*Boettcherisca*) *peregrina* [f]; **Figure 24a.b.c.** *Sarcophaga* (*Fergusonimyia*) *bancroftorum* [g].

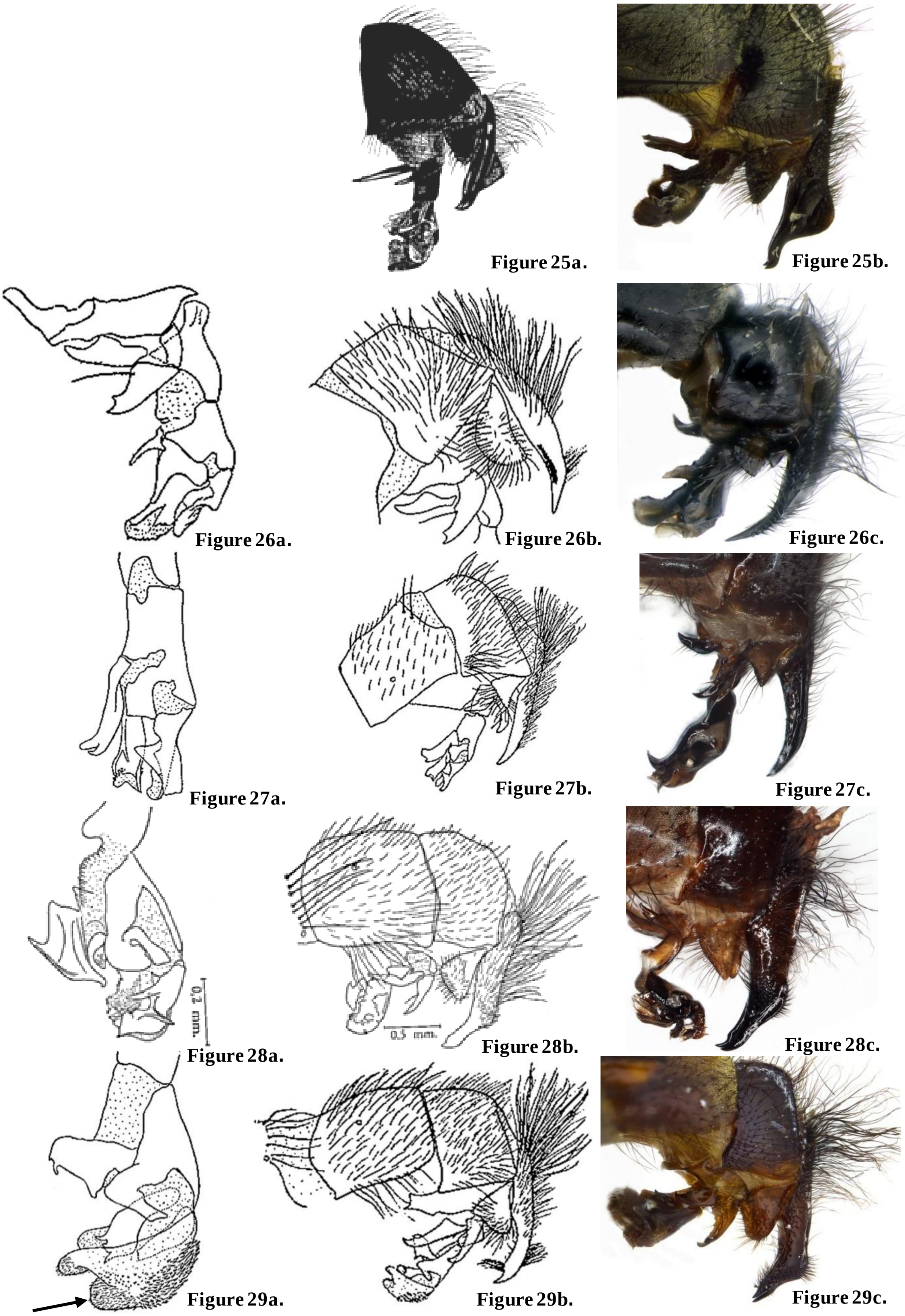


Figure 25a. Figure 25b.
Figure 26a. Figure 26b. Figure 26c.
Figure 27a. Figure 27b. Figure 27c.
Figure 28a. Figure 28b. Figure 28c.
Figure 29a. Figure 29b. Figure 29c.

**Figure 25.** *Sarcophaga* (*Hardyella*) *littoralis* [a]. **a.** illustration of entire male terminalia, lateral view. **b.** photograph of entire male terminalia, lateral view; **Figure 26a.b.c.** *Sarcophaga* (*Johnstonimyia*) *kappa* [h]; **Figure 27a.b.c.** *Sarcophaga* (*Johnstonimyia*) *lincta* [h]; **Figure 28a.b.c.** *Sarcophaga* (*Lioproctia*) *alcicornis* [d]; **Figure 29a.b.c.** *Sarcophaga* (*Lioproctia*) *imita* [h].

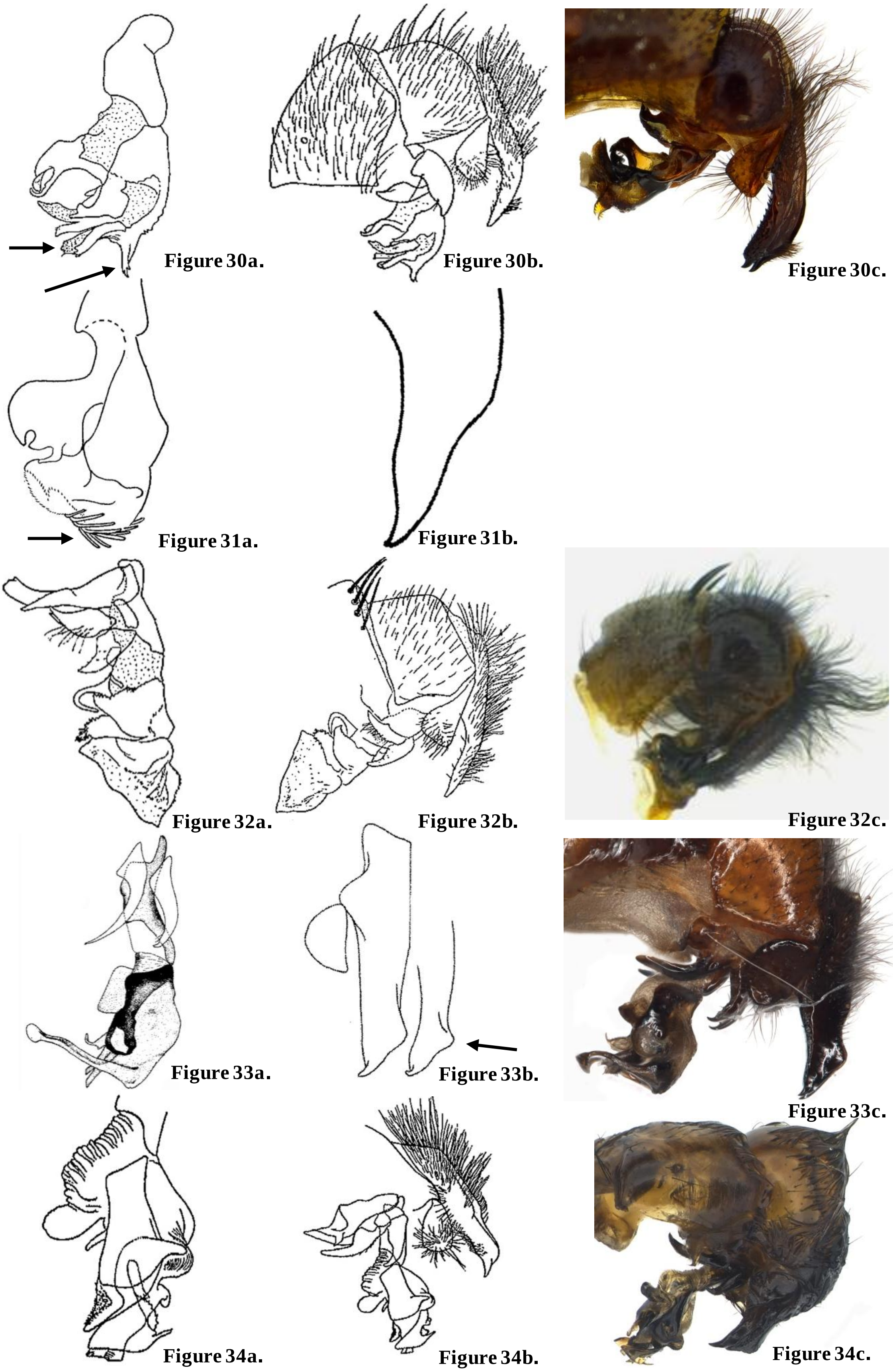


**Figure 30a.b.c.** *Sarcophaga* (*Lioproctia*) *multicolor* [jh]; **Figure 31.** *Sarcophaga* (*Lioproctia*) *spinifera* [c] **a.** illustration of phallus, lateral view. **b.** cercus, lateral view; **Figure 32a.b.c.** *Sarcophaga* (*Lioproctia*) *torvida* [h]; **Figure 33a.b.c.** *Sarcophaga* (*Liopygia*) *crassipalpis* [l]; **Figure 34a.b.c.** *Sarcophaga* (*Liopygia*) *ruficornis* [f].

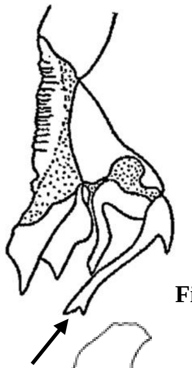
Figure 35a.

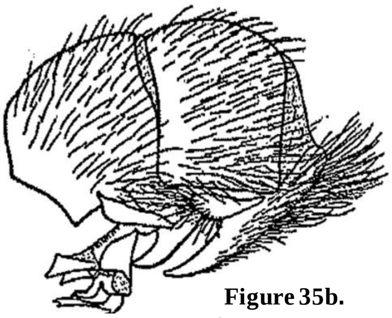
Figure 35b.


Figure 35c.

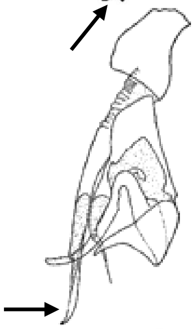
Figure 36a.

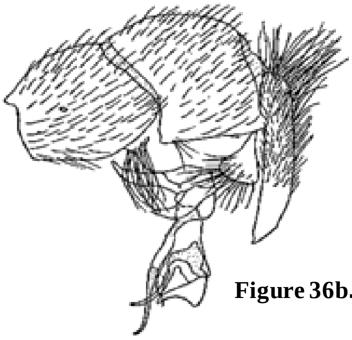
Figure 36b.


Figure 36c.

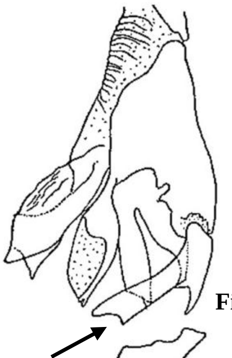
Figure 37a.


Figure 37b.

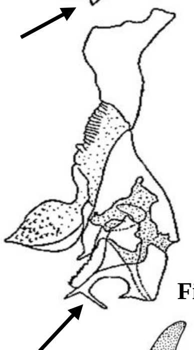
Figure 38a.

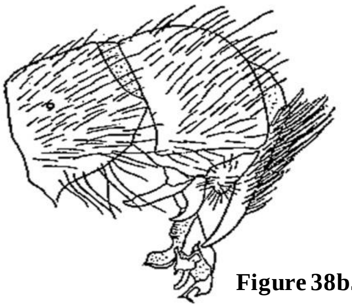
Figure 38b.


Figure 38c.

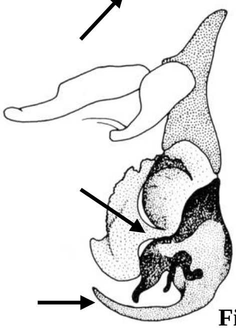
Figure 39a.

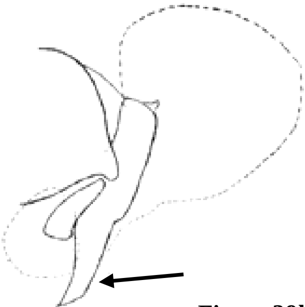
Figure 39b.

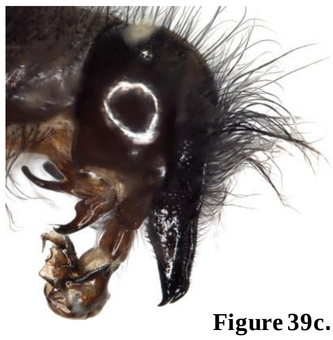
Figure 39c.

**Figure 35a.b.c.** *Sarcophaga* (*Liosarcophaga*) *dux* [f]; **Figure 36a.b.c.** *Sarcophaga* (*Liosarcophaga*) *eta* [h]; **Figure 37.** *Sarcophaga* (*Liosarcophaga*) *kohla* [h]. **a.** illustration of phallus, lateral view. **b.** photograph of entire male terminalia, lateral view; **Figure 38a.b.c.** *Sarcophaga* (*Liosarcophaga*) *sigma* [h]; **Figure 39a.b.c.** *Sarcophaga* (*Parasarcophaga*) *albiceps* [q].

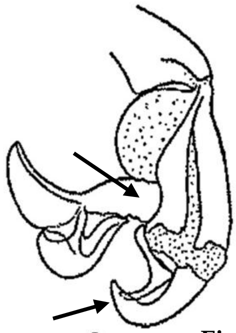

**Figure 40a.**

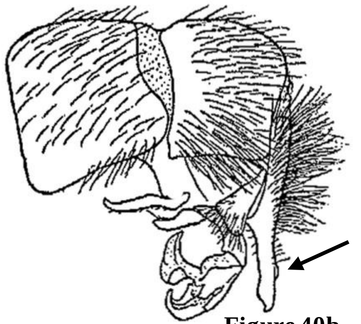

**Figure 40b.**

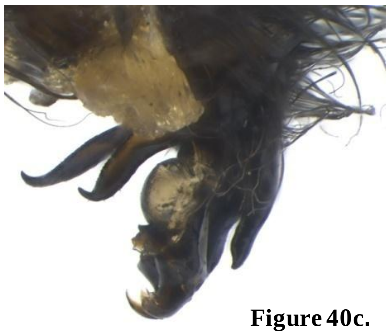

**Figure 40c.**

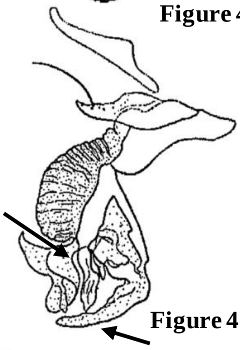

**Figure 41a.**

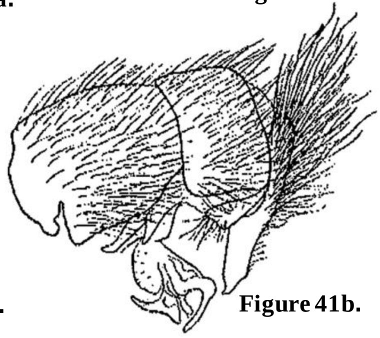

**Figure 41b.**

**Figure 41c.**

**Figure 42a.**

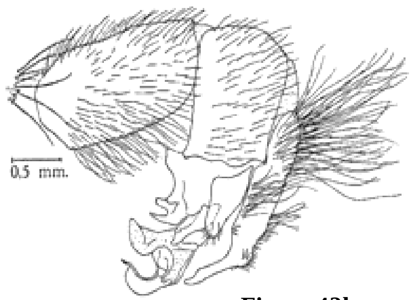


**Figure 42b.**

**Figure 42c.**

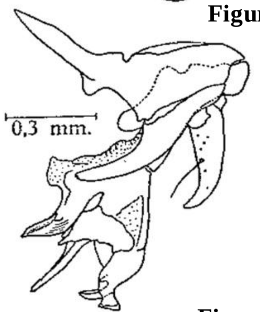


**Figure 43a.**

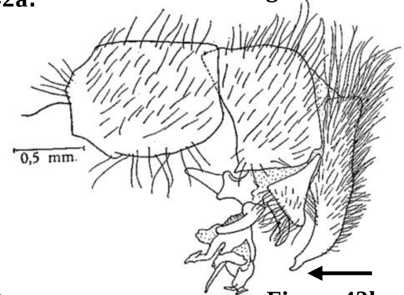


**Figure 43b.**

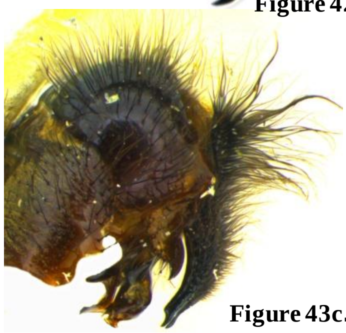

**Figure 43c.**


**Figure 44a.**

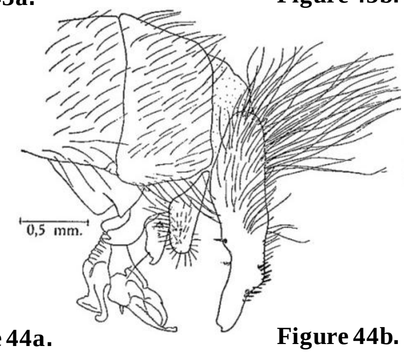


**Figure 44b.**

**Figure 44c.**

**Figure 40a.b.c.** *Sarcophaga* (*Parasarcophaga*) *misera* [f]; **Figure 41a.b.c.** *Sarcophaga* (*Parasarcophaga*) *taenionota* [f]; **Figure 42a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *alpha* [d]; **Figure 43a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *assimilis* [d]; **Figure 44a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *beta* [d].

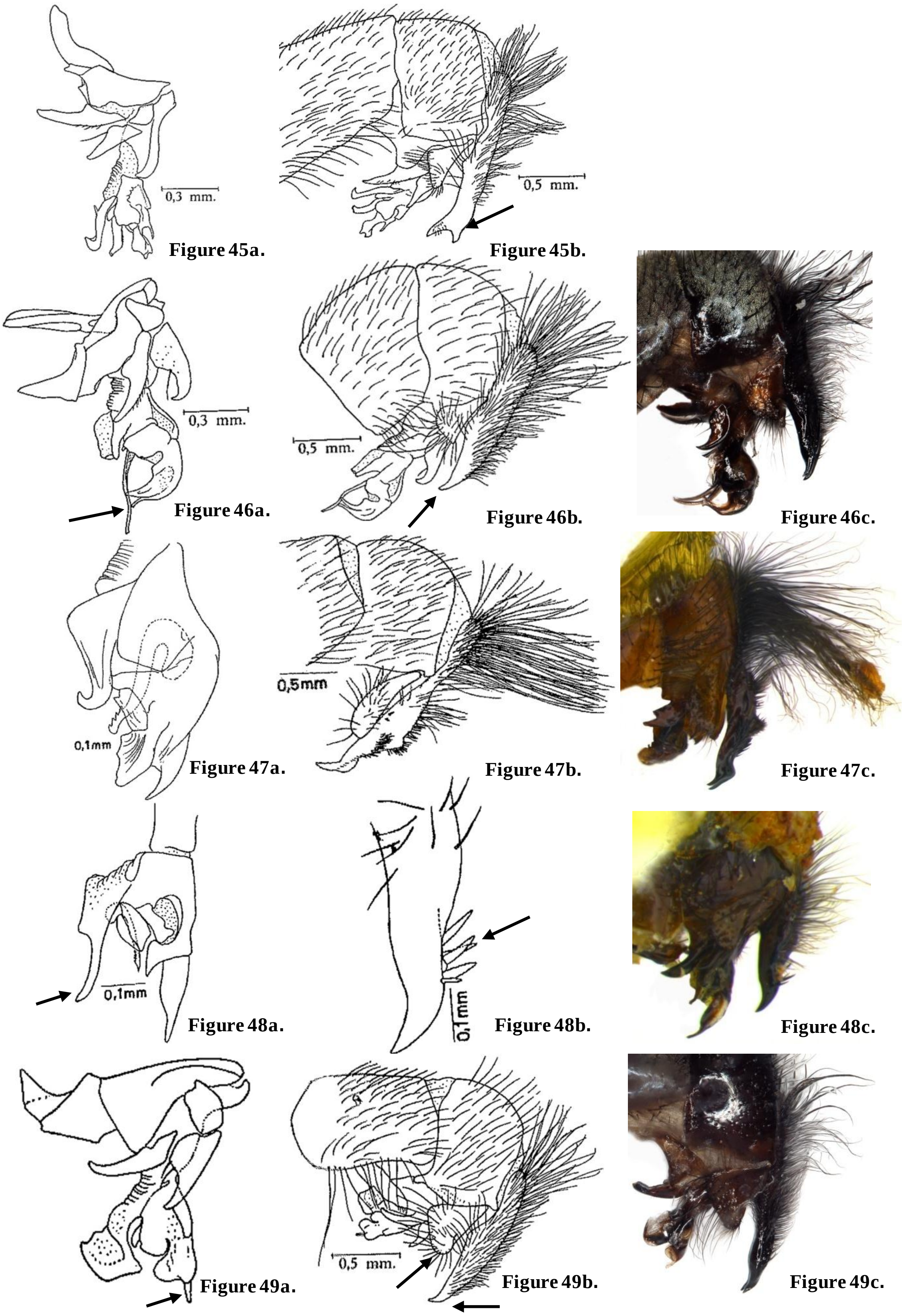


**Figure 45a.b.** *Sarcophaga* (*Sarcorohdendorfia*) *bidentata* [d]; **Figure 46a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *bifrons* [d]; **Figure 47a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *darwiniana* [m]; **Figure 48a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *emuensis* [n]; **Figure 49a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *froggatti* [d].

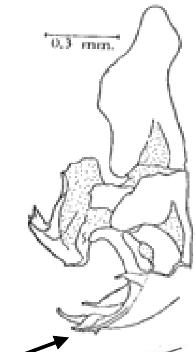

**Figure 50a.**

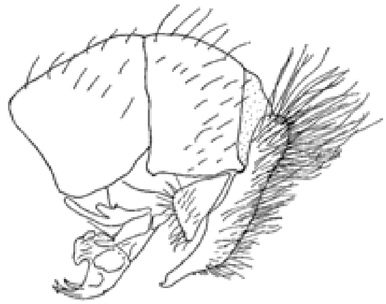

**Figure 50b.**

**Figure 50c.**

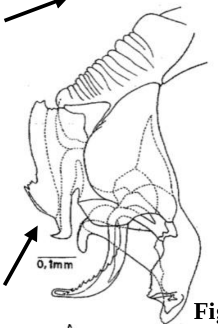

**Figure 51a.**

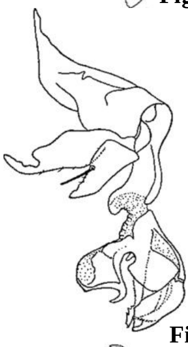

**Figure 52a.**

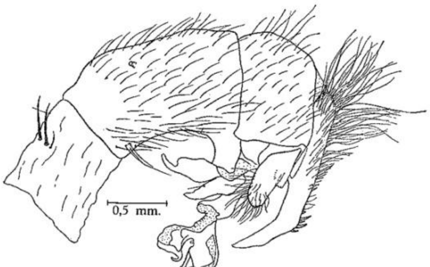

**Figure 52b.**

**Figure 52c.**

**Figure 53a.**

**Figure 53b.**

**Figure 53c.**

**Figure 54a.**

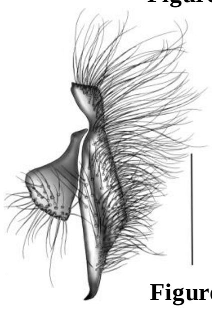

**Figure 54b.**

**Figure 54c.**

**Figure 50a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *furcata* [d]; **Figure 51a.** *Sarcophaga* (*Sarcorohdendorfia*) *howensis* [m]; **Figure 52a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *impatiens* [d]; **Figure 53a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *longifilia* [d]; **Figure 54a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *megafilosia* [s].


Figure 55a.

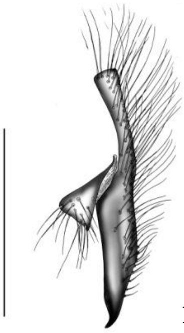
Figure 55b.


Figure 55c.

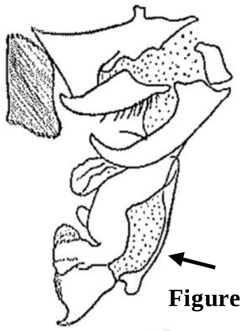
Figure 56a.

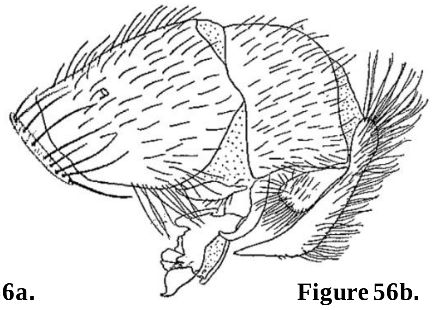
Figure 56b.

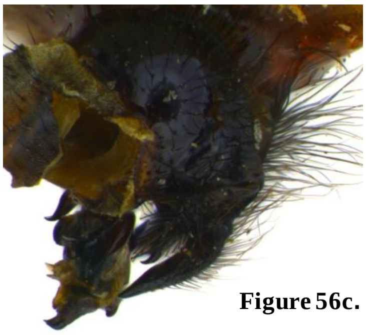
Figure 56c.

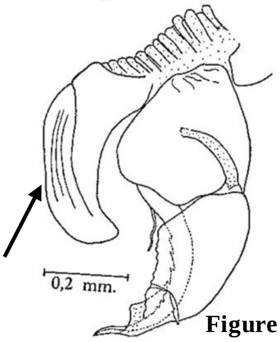

Figure 57a.

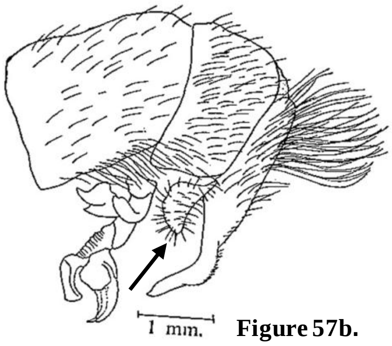

Figure 57b.


Figure 57c.


Figure 58a.

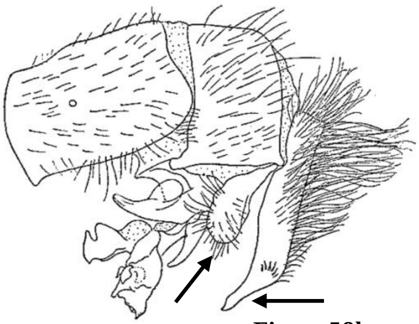
Figure 58b.


Figure 58c.

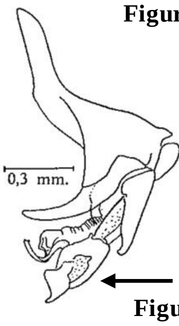

Figure 59a.

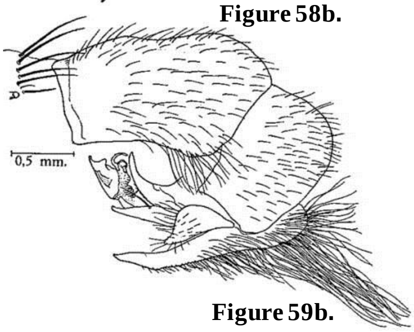

Figure 59b.

**Figure 55a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *meiofilosia* [s]; **Figure 56a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *omikron* [d]; **Figure 57a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *piva* [d]; **Figure 58a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *praedatrix* [d]; **Figure 59a.b.** *Sarcophaga* (*Sarcorohdendorfia*) *spinigera* [d].

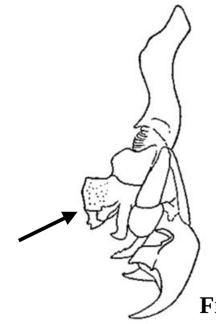

**Figure 60a.**

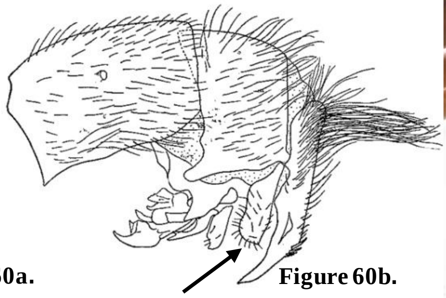

**Figure 60b.**

**Figure 60c.**

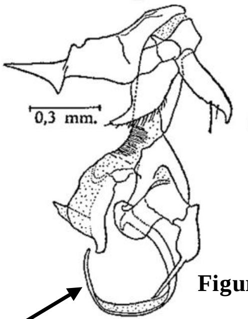


**Figure 61a.**

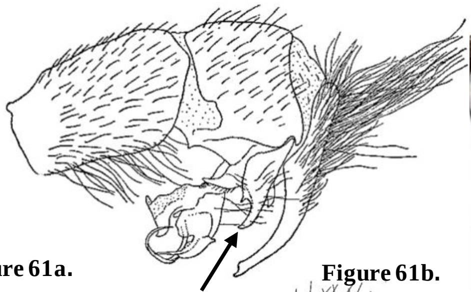

**Figure 61b.**

**Figure 61c.**

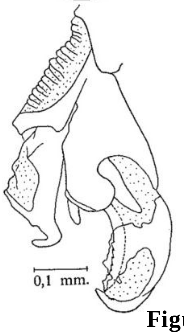


**Figure 62a.**

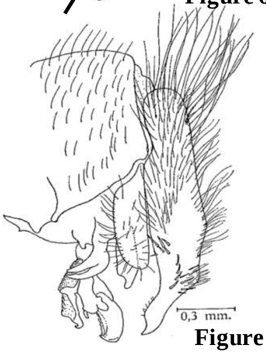


**Figure 62b.**

**Figure 62c.**

**Figure 63.**

**Figure 64.**

**Figure 60a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *seniorwhitei* [d]; **Figure 61a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *villisterna* [d]; **Figure 62a.b.c.** *Sarcophaga* (*Sarcorohdendorfia*) *zeta* [d]; **Figure 63.** *Sarcophaga* (*Sarcorohdendorfia*) *clavus* **sp. nov.**, photograph of entire male terminalia, lateral view; **Figure 64.** *Sarcophaga* (*Sarcorohdendorfia*) **sp. nov.** (unnamed species near *praedatrix*), photograph of entire male terminalia, lateral view.


**Figure 65a.**

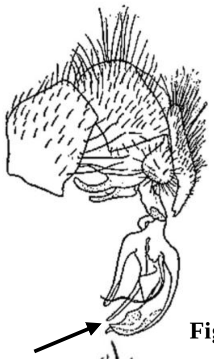
**Figure 65b.**


**Figure 65c.**


**Figure 66a.**


**Figure 66b.**

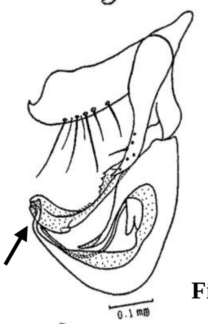

**Figure 67a.**

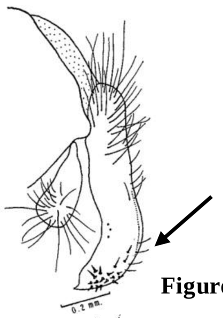

**Figure 67b.**

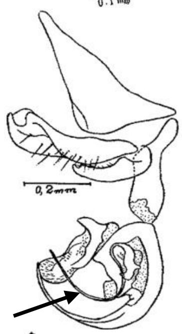

**Figure 68a.**

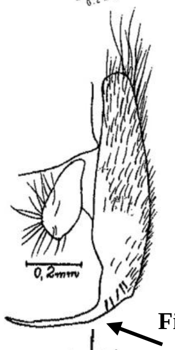

**Figure 68b.**


**Figure 68c.**

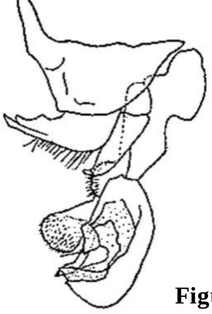
**Figure 69a.**

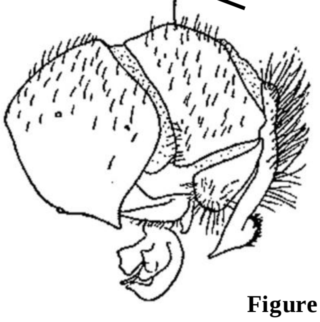
**Figure 69b.**

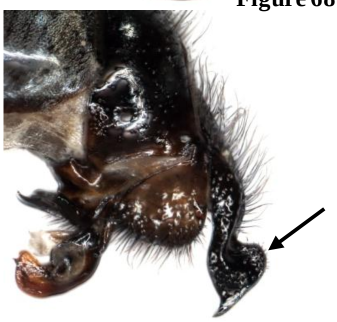
**Figure 69c.**

**Figure 65a.b.c.** *Sarcophaga* (*Sarcosolomonia*) *crinita* [bh]; **Figure 66a.b.** *Sarcophaga* (*Sarcosolomonia*) *fabea* [h]; **Figure 67.** *Sarcophaga* (*Sarcosolomonia*) *papuensis* [j]. **a.** illustration of phallus, lateral view. **b.** illustration of cercus, lateral view; **Figure 68.** *Sarcophaga* (*Sarcosolomonia*) *sumunensis* [i]. **a.** illustration of phallus, lateral view. **b.** illustration of cercus, lateral view. **c.** photograph of entire male terminalia, lateral view; **Figure 69a.b.c.** *Sarcophaga* (*Sarcosolomonia*) *versatilis* [h].

Figure 70.

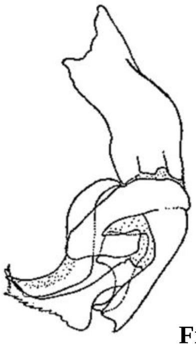

Figure 71a.

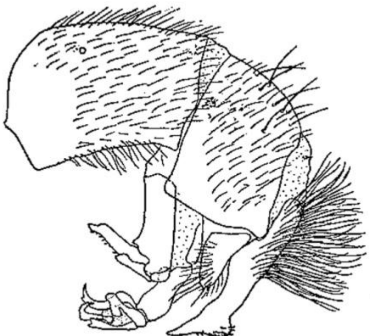

Figure 71b.

Figure 71c.

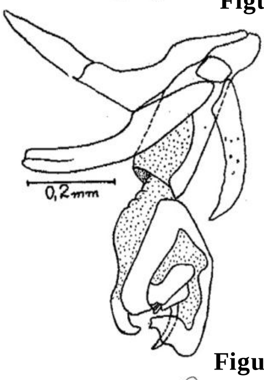


Figure 72a.

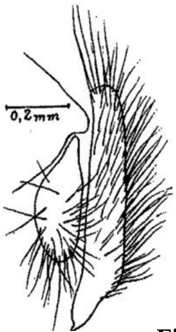


Figure 72b.

Figure 72c.

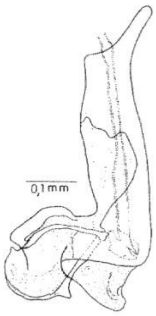


Figure 73a.

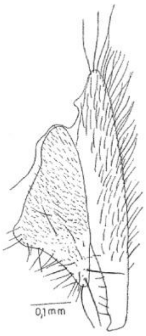


Figure 73b.

**Figure 70.** *Sarcophaga* (*Sarcosolomonia*) *collessi* **sp. nov.**, photograph of entire male terminalia, lateral view; **Figure 71a.b.c.** *Sarcophaga* (*Taylorimyia*) *aurifrons* [h]; **Figure 72a.b.c.** *Sarcophaga* (unplaced) *simplex* [i]; **Figure 73.** *Tricharaea* (*Tricharaea*) *brevicornis* [k]. **a.** illustration of phallus, lateral view. **b.** illustration of cercus, lateral view.

### 2.3.4 Species information

***Oxysarcodexia varia*** **(Walker)**

*Sarcophaga varia* Walker, 1836

*Sarcophaga milleri* Johnston and Tiegs, 1922

#### *Morphological characters*

Ground colour black, with bright gold microtrichosity on the parafacials and fronto-orbital plate, but silver microtrichosity on the occiput. Gena with setulae only black. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal and apical scutellar setae absent or present, depending on the specimen. Propleuron bare. Body 5-10 mm in length.

#### *Geographical distribution*

Australia (New South Wales, Tasmania, Victoria) – AUSTRALASIAN/OCEANIAN, NEOTROPICAL.

#### *Biology*

*Oxysarcodexia varia* has been collected from dung, decayed carrion-baits, open scrub and carcasses (Mulieri *et al.* 2008) and is also proposed to be linked to rabbit haemorrhagic disease (RDH) in New Zealand (Henning *et al.* 2005). This is the only species recorded from this genus in Australia.

***Sarcophaga*** **(*Australiopierretia*)** ***australis*** **(Johnston and Tiegs)**

*Helicobia australis* Johnston and Tiegs, 1921

#### *Morphological characters*

Vein $R_1$ setulose on basal 0.5. Gena with setulae only black. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present. Propleuron not uniformly setulose, with only a few black setulae. Hind tibia with short setulae. Females with short setulae on the 1$^{st}$ and 2$^{nd}$ abdominal sternites. Body 5-10 mm in length.

#### *Geographical distribution*

Australia (Australian Capital Territory, New South Wales, Northern Territory, Queensland, South Australia, Western Australia) – AUSTRALASIAN/OCEANIAN.

#### ***Biology***

*Sarcophaga australis* was reared from decaying meat by Johnston and Tiegs (1921), and has been documented in association with grasshoppers and sea turtle eggs (Fuller 1938; Hall and Parmenter 2008). This species has also been collected at decayed carrion-baits by KAM, and belongs to the monotypic subgenus, *Australopierretia.*

### ***Sarcophaga* (*Baranovisca*) *arachnivora* Lopes**

*Sarcophaga arachnivora* Lopes, 1985

#### ***Morphological characters***

Gena with setulae only black, with apical scutellar setae present in males only. Body 5-10 mm in length.

#### ***Geographical distribution***

Australia (New South Wales) – AUSTRALASIAN/OCEANIAN.

#### ***Biology***

*Sarcophaga arachnivora* has been reared from egg sacs of the spider *Dicrostichus magnificus* (Cantrell 1986; Lopes 1985).

### ***Sarcophaga* (*Baranovisca*) *cyrtophorae* (Cantrell)**

*Parasarcophaga cyrtophorae* Cantrell, 1986

#### ***Morphological characters***

Gena with setulae a mix of black and yellow/white. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present. Propleuron bare. Body 5-10 mm in length.

#### ***Geographical distribution***

Australia (Queensland) – AUSTRALASIAN/OCEANIAN.

#### ***Biology***

*Sarcophaga cyrtophorae* has been reared from egg sacs of the spider *Cyrtophora moluccensis* (Cantrell 1986).

***Sarcophaga* (*Baranovisca*) *reposita* (Lopes)**

*Parasarcophaga reposita* Lopes, 1959

***Morphological characters***

Gena with setulae a mix of black and yellow/white. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present. Propleuron bare. 1$^{st}$ and 2$^{nd}$ abdominal sternites with short setulae. Body 5-10 mm in length.

***Geographical distribution***

Australia (New South Wales, Queensland) – AUSTRALASIAN/OCEANIAN.

***Biology***

*Sarcophaga reposita* has been reared from egg sacs of the spider *Cyrtophora moluccensis* (Cantrell 1986; Lopes 1959).

***Sarcophaga* (*Bercaea*) *africa* (Wiedemann)**

*Musca africa* Wiedemann, 1824

***Morphological characters***

Ground colour of male and female terminalia bright red or orange. Gena with setulae a mix of black and yellow/white. Generally, the postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. However, some specimens with setulae only yellow/white. Prescutellar acrostichal setae absent or present, depending on the specimen. Propleuron bare. 1$^{st}$ and 2$^{nd}$ abdominal sternites with short setulae in females, and only males with long setulae on the hind tibia. Body 5-10 mm in length.

***Geographical distribution***

Australia (New South Wales, Queensland, South Australia, Western Australia) – AUSTRALASIAN/OCEANIAN, AFROTROPICAL, NEARCTIC, NEOTROPICAL, ORIENTAL, PALAEARCTIC.

***Biology***

*Sarcophaga africa* is almost cosmopolitan. It is known to larviposit on faeces (in the presence of carrion) (Bänziger and Pape 2004). It has also been collected from baits consisting of either pieces of raw fish, cut segments of ripe fruit (*Carica papaya* and *Citrus sinensis*), decomposing matter and faeces (Aspoas 1991; Pérez-Moreno *et al.* 2006). It is a noxious insect known for causing myiasis (Park 1977). This species is the only Australian representative of the subgenus *Bercaea* and has also been collected at decayed carrion-baits by KAM.

**_Sarcophaga_ (_Boettcherisca_) _peregrina_ (Robineau-Desvoidy)**

*Myophora peregrina* Robineau-Desvoidy, 1830

***Morphological characters***

Gena with setulae only or mostly black. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present. Propleuron not uniformly setulose, with only a few black setulae. 1st and 2nd abdominal sternites with short setulae in females. Body 5-10 mm in length.

***Geographical distribution***

Australia (Australian Capital Territory, New South Wales, Northern Territory, Queensland, South Australia, Western Australia) – AUSTRALASIAN/OCEANIAN, ORIENTAL, PALAEARCTIC.

***Biology***

*Sarcophaga peregrina* has been observed to breed in carrion, and has been commonly found attracted to faeces, garbage and fish baits (Bänziger and Pape 2004; Das and Dasgupta 1982; Pérez-Moreno *et al.* 2006). This species has also been documented in cases of human oral and nasal myiasis (Kanimura and Arakawa 1986; Lee 1968). Studies have documented *peregrina* as an occasional parasite of earthworms and the locust *Chortoicetes terminifera,* along with being a facultative predator of lepidopteran pupae (Xue *et al.* 2011). The first-, second- and third-instar larvae and puparium were described by Cantrell (1981; referred to as *Boettcherisca peregrina*). This species is the only Australian representative of the subgenus *Boettcherisca*.

**_Sarcophaga_ (_Fergusonimyia_) _bancroftorum_ Johnston and Tiegs**

*Sarcophaga bancrofti* Johnston and Tiegs, 1921

***Morphological characters***

This species has been documented as one of the most morphologically variable Australian flesh flies. High intraspecific variation is common in male terminalia, along with the presence/absence and number of setae on the thorax. 2nd and 3rd antennomere at least partly yellow. Generally with a bare propleuron, but some specimens have a few black setulae. Gena with setulae only black. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present. Apical scutellar setae present in males, but either absent or present in females, depending on the specimen. Some male specimens with long setulae on the hind tibia. Body 5-10 mm in length in females, but 10-15 mm in males.

***Geographical distribution***

Australia (Australian Capital Territory, New South Wales, Northern Territory, Queensland) – AUSTRALASIAN/OCEANIAN.

***Biology***

*Sarcophaga bancroftorum* has been collected at decayed carrion-baits by KAM.

***Sarcophaga* (*Hardyella*) *littoralis* Johnston and Tiegs**

*Sarcophaga littoralis* Johnston and Tiegs, 1922

*Sarcophaga ogilvyi* Salem, 1946

***Morphological characters***

Ratio of width of eye to width of head (at widest parts) no more than 0.5. Gena with setulae only yellow/white. Generally, the postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. However, some specimens with setulae only yellow/white. Prescutellar acrostichal setae absent. Propleuron uniformly setulose with setulae only yellow/white. Some male specimens with long setulae on the hind tibia. 1st and 2nd abdominal sternites with setulae only yellow/white. Body 5-10 mm in length.

***Geographical distribution***

Australia (New South Wales, Queensland) – AUSTRALASIAN/OCEANIAN.

***Biology***

*Sarcophaga littoralis* has been collected at ocean beaches (Johnston and Tiegs 1922) and documented to have emerged from the common purple snail, *Janthina janthina* (ANIC collections). This species has also been collected at decayed carrion-baits by KAM.

***Sarcophaga* (*Johnstonimyia*) *kappa* Johnston and Tiegs**

*Sarcophaga kappa* Johnston and Tiegs, 1921

*Sarcophaga illingworth* Parker, 1922

***Morphological characters***

Gena with setulae only or mostly yellow/white. Generally, the postocular has setulae only yellow/white. However, some specimens have at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present in males, but either absent or present in females, depending on the specimen. Propleuron bare and males with long setulae on the hind tibia. 1st and 2nd abdominal sternites with setulae only yellow/white. Body 10-15 mm in length.

***Geographical distribution***

Australia (New South Wales, Northern Territory, Queensland, Western Australia) – AUSTRALASIAN/OCEANIAN.

***Biology***

*Sarcophaga kappa* has been recorded breeding in rotten meat and carrion (Johnston and Tiegs 1921; Xue *et al.* 2011) and was collected at decayed carrion-baits by KAM.

***Sarcophaga* (*Johnstonimyia*) *lincta* Lopes**

*Sarcophaga lincta* Lopes, 1959

***Morphological characters***

Gena with setulae a mix of black and yellow/white. 2nd and 3rd antennomere at least partly yellow. Postocular with setulae only yellow/white. Prescutellar acrostichal setae present and apical scutellar setae absent. Propleuron bare and males with long setulae on the hind tibia. Body 5-10 mm in length.

***Geographical distribution***

Australia (New South Wales, Queensland) – AUSTRALASIAN/OCEANIAN.

***Biology***

No known information.

***Sarcophaga* (*Lioproctia*) *alcicornis* Hardy**

*Sarcophaga alcicornis* Hardy, 1932

***Morphological characters***

Gena with setulae only yellow/white. Generally, the postocular has setulae only yellow/white. However, some females have at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present. Propleuron uniformly setulose with setulae only black. Males with long setulae on the hind tibia. Body 10-15 mm in length.

#### *Geographical distribution*

Australia (New South Wales, Queensland) – AUSTRALASIAN/OCEANIAN.

#### *Biology*

Labels from pinned specimens from the (ANIC) include: 'ex dead grubs', 'ex corn' and 'ex scrub' (Lopes, 1954). *Sarcophaga alcicornis* has also been collected at decayed carrion-baits by KAM.

### ***Sarcophaga* (*Lioproctia*) *imita* Pape**

*Sarcophaga imita* Pape, 1996

*Johnstonimyia imitatrix* Lopes, 1959

#### *Morphological characters*

Gena with setulae only or mostly black. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present. Propleuron bare and the hind tibia with long setulae in males and females. Body 10-15 mm in length.

#### *Geographical distribution*

Australia (Queensland) – AUSTRALASIAN/OCEANIAN.

#### *Biology*

No known information.

### ***Sarcophaga* (*Lioproctia*) *multicolor* Johnston and Tiegs**

*Sarcophaga multicolor* Johnston and Tiegs, 1922

#### *Morphological characters*

Gena and postocular with setulae only yellow/white. Prescutellar acrostichal and apical scutellar setae both present in females, but either absent or present in males, depending on the specimen. Males and females with long setulae on the hind tibia. Body 10-15 mm in length.

#### *Geographical distribution*

Australia (Queensland) – AUSTRALASIAN/OCEANIAN.

#### *Biology*

*Sarcophaga multicolor* has been collected at decayed carrion-baits by KAM.

***Sarcophaga* (*Lioproctia*) *spinifera* Hardy**

*Sarcophaga spinifera* Hardy, 1932

***Morphological characters***

Gena with setulae a mix of black and yellow/white in males, but with setulae only black in females. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal and apical scutellar setae present. Propleuron bare and the hind tibia with long setulae in both sexes. Body 5-10 mm in length.

***Geographical distribution***

Australia (New South Wales, Queensland) – AUSTRALASIAN/OCEANIAN.

***Biology***

*Sarcophaga spinifera* has been collected at decayed carrion-baits by KAM.

***Sarcophaga* (*Lioproctia*) *torvida* (Lopes)**

*Johnstonimyia torvida* Lopes, 1959

***Morphological characters***

Gena and postocular with setulae only yellow/white. 2nd and 3rd antennomere at least partly yellow. Prescutellar acrostichal setae present. Propleuron bare and the hind tibia with long setulae in males. 1st and 2nd abdominal sternites with setulae only yellow/white. Body 10-15 mm in length.

***Geographical distribution***

Australia (Northern Territory, Queensland, South Australia, Western Australia) – AUSTRALASIAN/OCEANIAN.

***Biology***

*Sarcophaga torvida* has been collected at decayed carrion-baits by KAM.

***Sarcophaga* (*Liopygia*) *crassipalpis* Macquart**

*Sarcophaga crassipalpis* Macquart, 1839

***Morphological characters***

Ground colour of male and female terminalia bright red or orange. Gena with setulae only or mostly yellow/white. Postocular with setulae only yellow/white. Prescutellar acrostichal setae present but apical scutellar setae absent. Propleuron bare and the hind tibia with long setulae in males. 1st and 2nd abdominal sternites with short setulae in females. Body 10-15 mm in length.

***Geographical distribution***

Australia (Australian Capital Territory, New South Wales, Queensland, South Australia, Victoria, Western Australia) – AUSTRALASIAN/OCEANIAN, AFROTROPICAL, NEARCTIC, NEOTROPICAL, ORIENTAL, PALAEARCTIC.

***Biology***

*Sarcophaga crassipalpis* has successfully been reared from pork liver (Sanjean 1956), and is known to complete larval development in vertebrate and invertebrate carcasses (Pérez-Moreno *et al.* 2006). This species is known to cause myiasis in sheep and the spiny-tailed lizard *Uromastyx hardwicki*, and oral, intestinal, cutaneous and ophthalmo-myiasis in humans (Lukin 1989; Martínez-Sánchez *et al.* 2006; Shiota *et al.* 1990). Adults feed on faeces, carrion, leaf sucking hemipteran excreta and flowers (Castro *et al.* 2010). The first- and second-instar larvae were described by Cantrell (1981) and the third-instar by Cantrell (1981) and Ishijima (1967). This species also has featured in forensic cases in Australia (JFW *pers. comm.*).

***Sarcophaga* (*Liopygia*) *ruficornis* (Fabricus)**

*Musca ruficornis* Fabricus, 1794

***Morphological characters***

Ground colour of male and female terminalia bright red or orange, with the 2nd and 3rd antennomeres at least partly yellow. Gena and postocular with setulae only yellow/white. Prescutellar acrostichal setae absent. Propleuron bare. 1st and 2nd abdominal sternites with setulae only black in males but with setulae only yellow/white in females. Body 10-15 mm in length.

***Geographical distribution***

Australia (New South Wales, Northern Territory, Queensland, South Australia, Western Australia) – AUSTRALASIAN/OCEANIAN, AFROTROPICAL, NEARCTIC, NEOTROPICAL, ORIENTAL, PALAEARCTIC.

***Biology***

*Sarcophaga ruficornis* breeds in both faeces and carrion, and has been caught at decayed carrion-baits, rabbit, fish, liver and chicken baits (Amoudi *et al.* 1994; Bänziger and Pape 2004; Das and Dasgupta 1982; Hanan 2010; Shazia *et al.* 2006). It has been reported to cause myiasis in dogs, horses, mules and leprosy patients (Amoudi *et al.* 1994; Sreevatsa *et al.* 1990). It is also a documented parasite of the toad, *Bufo melanostictus* (Roy and Dasgupta 1977).

***Sarcophaga* (*Liosarcophaga*) *dux* Thomson**

*Sarcophaga dux* Thomson, 1869

***Morphological characters***

Gena with setulae a mix of black and yellow/white, but postocular with setulae only yellow/white. Prescutellar acrostichal setae present. Propleuron bare and the hind tibia with long setulae in males. Body 10-15 mm in length.

***Geographical distribution***

Australia (Australian Capital Territory, New South Wales, Northern Territory, Queensland, South Australia, Western Australia) – AUSTRALASIAN/OCEANIAN, AFROTROPICAL, PALAEARTIC, ORIENTAL.

***Biology***

In Thailand, *Sarcophaga dux* is synanthropic and breeds in both faeces and carrion (Bänziger and Pape 2004). This species is regarded as forensically important in Japan and Thailand (Sukontason *et al.* 2003). This species has been observed to readily enter human dwellings and larviposit on carrion and garbage (Hanan 2010; Pérez-Moreno *et al.* 2006; Sukontason *et al.* 2003).

***Sarcophaga* (*Liosarcophaga*) *eta* Johnston and Tiegs**

*Sarcophaga eta* Johnston and Tiegs 1921

***Morphological characters***

Gena with setulae only or mostly black. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present in females, but either absent or present in males, depending on the specimen. Propleuron bare and hind tibia with long setulae in males. 1st and 2nd abdominal sternites with short setulae in females. Body 10-15 mm in length.

***Geographical distribution***

Australia (Northern Territory, Queensland) – AUSTRALASIAN/OCEANIAN.

#### ***Biology***

*Sarcophaga eta* has been bred from fish carcasses, and has also been observed to be attracted to rotten meat in Brisbane (Johnston and Tiegs 1921).

### ***Sarcophaga* (*Liosarcophaga*) *kohla* Johnston and Tiegs**

*Sarcophaga kohla* Johnston and Tiegs, 1921

#### ***Morphological characters***

Gena with setulae a mix of black and yellow/white. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present in females, but either absent or present in males, depending on the specimen. Propleuron bare and the hind tibia with long setulae in males. 1st and 2nd abdominal sternites with short setulae in females. Body 10-15 mm in length.

#### ***Geographical distribution***

Australia (Australian Capital Territory, New South Wales, Northern Territory, Queensland, South Australia, Victoria, Western Australia) – AUSTRALASIAN/OCEANIAN.

#### ***Biology***

*Sarcophaga kohla* has been reared from decaying molluscs by Hardy (1927) and collected at decayed carrion-baits by KAM.

### ***Sarcophaga* (*Liosarcophaga*) *sigma* Johnston and Tiegs**

*Sarcophaga sigma* Johnston and Tiegs, 1921

*Sarcophaga aurifrons* Macquart, 1846

#### ***Morphological characters***

Gena with setulae only black. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae either absent or present in both sexes, depending on the specimen. Apical scutellar setae absent in females, but either absent or present in males, depending on the specimen. Propleuron bare. 1st and 2nd abdominal sternites with short setulae in females. Body 5-10 mm in length.

#### ***Geographical distribution***

Australia (Australian Capital Territory, New South Wales, Northern Territory, Queensland, South Australia, Tasmania, Victoria, Western Australia) – AUSTRALASIAN/OCEANIAN.

AUSTRALASIAN/OCEANIAN, ORIENTAL.

#### *Biology*

Johnston and Tiegs (1921) bred *Sarcophaga sigma* from rotten meat in Brisbane and KAM caught it at decayed carrion-baits.

### ***Sarcophaga* (*Parasarcophaga*) *albiceps* Meigen**

*Sarcophaga albiceps* Meigen, 1826

#### *Morphological characters*

Gena with setulae a mix of black and yellow/white. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present. Propleuron bare and the hind tibia with long setulae only in males. 1st and 2nd abdominal sternites with short setulae in females. Body 10-15 mm in length.

#### *Geographical distribution*

Australia (Queensland) – AUSTRALASIAN/OCEANIAN, ORIENTAL, PALAEARCTIC.

#### *Biology*

*Sarcophaga albiceps* breeds in decaying organic matter and has been observed to larviposit on mutton in India and fish in Pakistan (Shazia *et al.* 2006; Singh and Bharti 2008). Similar observations have been made of this species breeding in faeces in the presence of carrion in Thailand (Bänziger and Pape 2004). *Sarcophaga albiceps* has also been documented causing cutaneous myiasis of buffalo, cows and humans (Castro *et al.* 2010). Larvae of *albiceps* are facultative predators of a variety of lepidopteran pupae and hymenopteran larvae. The third-instar larva was described by Ishijima (1967).

### ***Sarcophaga* (*Parasarcophaga*) *misera* Walker**

*Sarcophaga misera* Walker, 1849

*Sarcophaga gamma* Johnston and Tiegs, 1921

*Sarcophaga brunneopalpis* Johnston and Tiegs, 1922

#### *Morphological characters*

Gena with setulae only or mostly yellow/white. Generally, postocular with setulae only yellow/white. However, some males have at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present. Propleuron bare and the hind tibia with long setulae in males only. 1st and 2nd abdominal sternites with short setulae in females. Body 10-15 mm in length.

***Geographical distribution***

Australia (New South Wales, Northern Territory, Queensland, Victoria, Western Australia) – AUSTRALASIAN/OCEANIAN, ORIENTAL, PALAEARCTIC.

***Biology***

*Sarcophaga misera* has been caught at decayed carrion-baits, human faeces, carcasses and dead fish (Das and Dasgupta 1982; Pérez-Moreno *et al.* 2006). This species has been documented as one of Queensland's sheep myiasis flies (referred to as *Sarcophaga frontalis*; Tyron 1917) and as a predator of the snail *Indoplanorbis exustus* (Parashar *et al.* 1997). The third-instar larva was described by Ishijima (1967).

***Sarcophaga* (*Parasarcophaga*) *taenionota* (Wiedemann)**

*Musca taenionota* Wiedemann, 1819

*Sarcophaga omega* Johnston and Tiegs, 1921

***Morphological characters***

Gena with setulae only or mostly yellow/white and postocular with setulae only yellow/white. Prescutellar acrostichal setae present. Propleuron bare and the hind tibia with long setulae in males. 1st and 2nd abdominal sternites with short setulae in females. Body 10-15 mm in length.

***Geographical distribution***

Australia (Australian Capital Territory, New South Wales, Northern Territory, Queensland, Western Australia) – AUSTRALASIAN/OCEANIAN, ORIENTAL, PALAEARCTIC.

***Biology***

*Sarcophaga taenionota* is commonly found on, and is known to breed in human and cow faeces, along with dead animals (Ferrar 1979; Park 1977; Pérez-Moreno *et al.* 2006; Woolcock 1975). It was also collected at decayed carrion-baits by KAM.

***Sarcophaga* (*Sarcorohdendorfia*) *alpha* Johnston and Tiegs**

*Sarcophaga alpha* Johnston and Tiegs, 1922

***Morphological characters***

Gena with setulae only or mostly yellow/white and postocular with setulae only yellow/white. Prescutellar acrostichal setae present. Apical scutellar setae either absent or present in females, depending on the specimen. Propleuron uniformly setulose with setulae only black and the hind tibia with long setulae in males. In males, median patch of dense setae at the base of the 4th abdominal sternite present. Body 10-15 mm in length.

***Geographical distribution***

Australia (Queensland) – AUSTRALASIAN/OCEANIAN.

***Biology***

*Sarcophaga alpha* has been caught around rotten meat (Johnston and Tiegs 1921) and at decayed carrion-baits by KAM.

***Sarcophaga* (*Sarcorohdendorfia*) *assimilis* Macquart**

*Sarcophaga assimilis* Macquart, 1851

*Sarcophaga hardyi* Johnston and Tiegs, 1922

***Morphological characters***

Gena with setulae only or mostly yellow/white. Generally, postocular with setulae only yellow/white. However, some males with at least one row of black setulae in addition to black postocular setae, with setulae yellow/white ventrally. Prescutellar acrostichal setae present in females, but either absent or present in males, depending on the specimen. Apical scutellar setae present in males, but either absent or present in females, depending on the specimen. Propleuron uniformly setulose with setulae either only yellow/white or with a mix of black and yellow/white, depending on the specimen. Hind tibia with long setulae in males. 1$^{st}$ and 2$^{nd}$ abdominal sternites with setulae either only yellow/white or only black, depending on the specimen. Females with short setulae on the 1$^{st}$ and 2$^{nd}$ abdominal sternites. Body 10-15 mm in length.

***Geographical distribution***

Australia (Tasmania) – AUSTRALASIAN/OCEANIAN.

***Biology***

*Sarcophaga assimilis* has been caught at a dead snake (T. Pape. *pers. obs.*).

***Sarcophaga* (*Sarcorohdendorfia*) *beta* Johnston and Tiegs**

*Sarcophaga beta* Johnston and Tiegs, 1921

*Sarcophaga delta* Johnston and Tiegs, 1921

***Morphological characters***

Gena with setulae only or mostly yellow/white and postocular with setulae only yellow/white. Prescutellar acrostichal setae present. Apical scutellar setae present in males, but either absent or present in females, depending on the specimen. Propleuron uniformly setulose, with setulae a mix of black and yellow/white. Hind tibia in males with long setulae. In males, median patch of dense setae at the base of the 4th abdominal sternite present. Body 10-15 mm in length.

***Geographical distribution***

Australia (Queensland) – AUSTRALASIAN/OCEANIAN.

***Biology***

*Sarcophaga beta* has been bred from rotten meat (Johnston and Tiegs 1921) and also collected at decayed carrion-baits by KAM.

***Sarcophaga* (*Sarcorohdendorfia*) *bidentata* (Lopes)**

*Tricholioproctia bidentata* Lopes, 1953

***Morphological characters***

Gena and postocular with setulae only yellow/white. Prescutellar acrostichal setae present in males but absent in females. Propleuron uniformly setulose, with setulae only yellow/white and the hind tibia with long setulae in males. 1st and 2nd abdominal sternites with setulae only yellow/white. In males, median patch of dense setae at the base of the 4th abdominal sternite present. Body 10-15 mm in length.

***Geographical distribution***

Australia (New South Wales, Northern Territory, Queensland, Western Australia) – AUSTRALASIAN/OCEANIAN.

***Biology***

*Sarcophaga bidentata* has been collected at decayed carrion-baits by KAM.

***Sarcophaga* (*Sarcorohdendorfia*) *bifrons* Walker**

*Sarcophaga bifrons* Walker, 1853

*Sarcophaga epsilon* Johnston and Tiegs, 1922

***Morphological characters***

Gena with setulae only yellow/white or with a mix of black and yellow/white. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae absent in females, and either absent or present in males, depending on the specimen. Propleuron uniformly setulose, with setulae only yellow/white and the hind tibia in males with long setulae. 1st and 2nd abdominal sternites in males with setulae either black or yellow/white depending on the specimen, but females with setulae only yellow/white. Body 10-15 mm in length.

***Geographical distribution***

Australia (Australian Capital Territory, New South Wales, Queensland, South Australia, Western Australia) – AUSTRALASIAN/OCEANIAN, ORIENTAL.

***Biology***

*Sarcophaga bifrons* has been captured around decaying meat (Johnston and Tiegs 1922). The label on pinned specimens in the ANIC reads: 'bred crab' (Lopes 1954).

***Sarcophaga* (*Sarcorohdendorfia*) *clavus*** sp. nov.

[Name and description in Meiklejohn *et al.* (to be submitted to *Zootaxa*)]

***Morphological characters***

Gena and postocular with setulae only yellow/white. Prescutellar acrostichal setae absent. Propleuron uniformly setulose, with setulae only yellow/white. Body 10-15 mm in length. (These characters are only valid for males, as females were not examined).

***Geographical distribution***

Australia (Queensland) – AUSTRALASIAN/OCEANIAN.

***Biology***

No known information.

***Sarcophaga* (*Sarcorohdendorfia*) *darwiniana* Kano and Lopes**

*Sarcophaga darwiniana* Kano and Lopes, 1979

***Morphological characters***

Basicosta deep yellow. Gena with setulae only or mostly yellow/white and postocular with setulae only yellow/white. Prescutellar acrostichal setae present in males, but either absent or present in females, depending on the specimen. Apical scutellar setae present in both sexes. Propleuron uniformly setulose, with setulae only black and the hind tibia of males with long setulae. In males, median patch of dense setae at the base of the 4$^{th}$ abdominal sternite present. Body greater than 15 mm in length.

***Geographical distribution***

Australia (Northern Territory) – AUSTRALASIAN/OCEANIAN.

***Biology***

No known information.

**_Sarcophaga_ (_Sarcorohdendorfia_) _emuensis_ Lopes and Kano**

*Sarcophaga emuensis* Lopes and Kano, 1979

***Morphological characters***

Gena with setulae only or mostly yellow/white and postocular with setulae only yellow/white. Prescutellar acrostichal setae either absent or present in both sexes, depending on the specimen. Propleuron uniformly setulose, with setulae only yellow/white. Hind tibia in males with either long or short setulae depending on the specimen. 1st and 2nd abdominal sternites with setulae only yellow/white. Body 5-10 mm in length.

***Geographical distribution***

Australia (Northern Territory, South Australia, Western Australia) – AUSTRALASIAN/OCEANIAN.

***Biology***

No known information.

**_Sarcophaga_ (_Sarcorohdendorfia_) _froggatti_ Taylor**

*Sarcophaga froggatti* Taylor, 1917

*Sarcophaga theta* Johnston and Tiegs, 1921

***Morphological characters***

Gena and postocular with setulae only yellow/white. Prescutellar acrostichal setae absent in females, but either absent or present in males, depending on the specimen. Propleuron uniformly setulose, with setulae only yellow/white and the hind tibia with long setulae in males. 1st and 2nd abdominal sternites with setulae only yellow/white. Body 10-15 mm in length.

***Geographical distribution***

Australia (New South Wales, Northern Territory, Queensland, Western Australia) – AUSTRALASIAN/OCEANIAN.

***Biology***

*Sarcophaga froggatti* has been bred from decayed meat in Brisbane (referred to as *Sarcophaga theta* n. sp.) (Johnston and Tiegs 1921) and collected at decayed carrion-baits by KAM.

***Sarcophaga* (*Sarcorohdendorfia*) *furcata* Hardy**

*Sarcophaga furcata* Hardy, 1932

***Morphological characters***

Gena and postocular with setulae only yellow/white. Prescutellar acrostichal and apical scutellar setae present. Propleuron uniformly setulose, with setulae only yellow/white and the hind tibia with long setulae in both sexes. 1st and 2nd abdominal sternites with yellow/white setulae in females. Body 10-15 mm in length.

***Geographical distribution***

Australia (Australian Capital Territory, Queensland, South Australia) – AUSTRALASIAN/OCEANIAN.

***Biology***

*Sarcophaga furcata* has been collected at decayed carrion-baits by KAM.

***Sarcophaga* (*Sarcorohdendorfia*) *howensis* Johnston and Hardy**

*Sarcophaga howensis* Johnston and Hardy, 1923b

***Morphological characters***

Gena with setulae only or mostly black. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present. Propleuron uniformly setulose, with setulae only black and the hind tibia with long setulae in males. Median marginal setae present on the 3rd abdominal tergite. In males, median patch of dense setae at the base of the 4th abdominal sternite present. Body 10-15 mm in length.

***Geographical distribution***

Australia (Lord Howe Island) – AUSTRALASIAN/OCEANIAN.

***Biology***

No known information.

***Sarcophaga* (*Sarcorohdendorfia*) *impatiens* Walker**

*Sarcophaga impatiens* Walker, 1849

***Morphological characters***

Gena and postocular with setulae only yellow/white. Prescutellar acrostichal setae present. Apical scutellar setae present in males, but either absent or present in females, depending on the specimen. Propleuron uniformly setulose, with setulae only yellow/white and the hind tibia with long setulae in males. 1st and 2nd abdominal sternites with setulae only yellow/white. In males, median patch of dense setae at the base of the 4th abdominal sternite present. Body 10-15 mm in length.

***Geographical distribution***

Australia (Australian Capital Territory, New South Wales, Northern Territory, Queensland, South Australia, Tasmania, Victoria, Western Australia) – AUSTRALASIAN/OCEANIAN.

***Biology***

*Sarcophaga impatiens* has been bred from rotten meat and sheep's liver (Johnston and Tiegs 1921) and collected at decayed carrion-baits by KAM. It is a common carrion breeder, however is rarely involved in animal or human myiasis. Larval development of *impatiens* was investigated by Roberts (1976). The first-, second- and third-instar larvae were described by Cantrell (1981; referred to as *Tricholioproctia impatiens*). This species has also featured in forensic cases in Australia (JFW *pers. comm.*).

***Sarcophaga* (*Sarcorohdendorfia*) *longifilia* Salem**

*Sarcophaga longifilia* Salem, 1946

***Morphological characters***

Gena with setulae only black in males, but only yellow/white in females. Generally, postocular with setulae only yellow/white. However, some male specimens have at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae absent in females, but either absent or present in males, depending on the specimen. Propleuron uniformly setulose, with setulae only yellow/white in females, but males with setulae either only black or only yellow/white. Hind tibia with long setulae in both sexes, but some male specimens with short setulae. Body 10-15 mm in length.

***Geographical distribution***

Australia (New South Wales, Western Australia) – AUSTRALASIAN/OCEANIAN, ORIENTAL.

#### ***Biology***

*Sarcophaga longifilia* is known to associate with mangroves (T. Pape and J.F. Wallman, *pers. obs.*).

### ***Sarcophaga* (*Sarcorohdendorfia*) *megafilosia* Pape, McKillup and McKillup**

*Sarcophaga megafilosia* Pape, McKillup and McKillup, 2000

#### ***Morphological characters***

Gena with setulae only or mostly yellow/white. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present. Propleuron uniformly setulose, with setulae a mix of black and yellow/white in both sexes, but some female specimens with setulae only yellow/white. 1st and 2nd abdominal sternites with setulae only yellow/white. Body 10-15 mm in length.

#### ***Geographical distribution***

Australia (Queensland) – AUSTRALASIAN/OCEANIAN.

#### ***Biology***

*Sarcophaga megafilosia* is a parasitoid of specimens of the littoral snail *Littoraria filosa*, having shell lengths $\geq$ 10mm (McKillup *et al.* 2000; Pape *et al.* 2000).

### ***Sarcophaga* (*Sarcorohdendorfia*) *meiofilosia* Pape, McKillup and McKillup**

*Sarcophaga meiofilosia* Pape, McKillup and McKillup, 2000

#### ***Morphological characters***

Vein $R_1$ setulose on basal 0.5. Gena with setulae a mix of black and yellow/white. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present. Propleuron uniformly setulose, with setulae mostly yellow/white, however some male specimens have a mix of black and yellow/white. 1st and 2nd abdominal sternites with setulae only yellow/white. Body 5-10 mm in length.

#### ***Geographical distribution***

Australia (Queensland) – AUSTRALASIAN/OCEANIAN.

#### ***Biology***

*Sarcophaga meiofilosia* is a parasitoid of specimens of the littoral snail *Littoraria filosa*, having shell lengths 4 - <10mm (McKillup *et al.* 2000; Pape *et al.* 2000).

***Sarcophaga* (*Sarcorohdendorfia*) *omikron* Johnston and Tiegs**

*Sarcophaga omikron* Johnston and Tiegs, 1921

*Sarcophaga stellata* Salem, 1946

### *Morphological characters*

Gena and postocular with setulae only yellow/white. Presutural acrostichal present in males but absent in females. Prescutellar acrostichal setae present. Propleuron uniformly setulose, with setulae only yellow/white and the hind tibia with long setulae in males. 1$^{st}$ and 2$^{nd}$ abdominal sternites with setulae only yellow/white. Body 10-15 mm in length.

### *Geographical distribution*

Australia (Australian Capital Territory, New South Wales, Northern Territory, Queensland, South Australia) – AUSTRALASIAN/OCEANIAN.

### *Biology*

*Sarcophaga omikron* was bred from wool and rotten potatoes (Johnston and Tiegs 1921) but also collected at decayed carrion-baits by KAM.

***Sarcophaga* (*Sarcorohdendorfia*) *piva* Roback**

*Sarcophaga piva* Roback, 1952

### *Morphological characters*

Gena with setulae mostly yellow/white. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present. Propleuron uniformly setulose, with setulae only black, and the hind tibia with long setulae in males. Some males with median marginal setae on the 3$^{rd}$ abdominal tergite. In males, median patch of dense setae at the base of the 4$^{th}$ abdominal sternite present. Body greater than 15 mm in length.

### *Geographical distribution*

Australia (Queensland) – AUSTRALASIAN/OCEANIAN, ORIENTAL.

### *Biology*

*Sarcophaga piva* has been collected at decayed carrion-baits by KAM.

***Sarcophaga* (*Sarcorohdendorfia*) *praedatrix* Walker**

*Sarcophaga praedatrix* Walker, 1849

*Sarcophaga tyroni* Johnston and Tiegs, 1921

*Sarcophaga queenslandae* Parker, 1922

***Morphological characters***

Gena and postocular with setulae only yellow/white. Prescutellar acrostichal setae absent and apical scutellar setae present in both sexes. Propleuron uniformly setulose, with setulae only yellow/white and hind tibia with long setulae in males. 1st and 2nd abdominal sternites with setulae only yellow/white. Body 10-15 mm in length.

***Geographical distribution***

Australia (New South Wales, Northern Territory, Queensland, South Australia, Western Australia) – AUSTRALASIAN/OCEANIAN.

***Biology***

*Sarcophaga praedatrix*, also referred to as *Sarcophaga tryoni* (Johnston and Tiegs 1921), is a common species throughout Australia. In Brisbane, larvae are not believed to survive the cooler months between June and August (Johnston and Tiegs 1921). The first-, second- and third-instar larvae were described by Cantrell (1981; referred to as *Tricholioproctia tryoni*). *Sarcophaga praedatrix* has been caught at decayed carrion baits by KAM and JFW and has featured in forensic cases in Australia (JFW *pers. comm.*). Note however, that data on *praedatrix* in the literature may include the undescribed species here listed as '*Sarcophaga* (*Sarcorohdendorfia*) **sp. nov.** (unnamed species near to *praedatrix*)'.

***Sarcophaga* (*Sarcorohdendorfia*) *seniorwhitei* Ho**

*Sarcophaga seniorwhitei* Ho, 1938

*Sarcophaga flavinervis* Senior-White, 1924

***Morphological characters***

Gena with setulae mostly yellow/white. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present. Propleuron uniformly setulose, with setulae only black and hind tibia with long setulae in males. In males, median patch of dense setae at the base of the 4th abdominal sternite present. Body 10-15 mm in length.

### *Geographical distribution*

Australia (Northern Territory, Queensland, Western Australia) – AUSTRALASIAN/OCEANIAN, ORIENTAL, PALAEARTIC.

### *Biology*

*Sarcophaga seniorwhitei* has been collected in mixed deciduous forests (Bänziger and Pape 2004) and from mountain ranges in Korea (Park 1977). This species has also been reared from fish, chicken and horse flesh under laboratory conditions (Pérez-Moreno *et al.* 2006).

## ***Sarcophaga* (*Sarcorohdendorfia*) *spinigera* (Lopes)**

*Tricholioproctia spinigera* Lopes, 1953

### *Morphological characters*

Gena and postocular with setulae only yellow/white. Prescutellar acrostichal setae absent in females, but either absent or present in males, depending on the specimen. Propleuron uniformly setulose, with setulae only yellow/white and the hind tibia with long setulae in males. 1$^{st}$ and 2$^{nd}$ abdominal sternites with setulae only yellow/white in females, but either black or yellow/white in males, depending on the specimen. Body 10-15 mm in length.

### *Geographical distribution*

Australia (Queensland, South Australia, Western Australia) – AUSTRALASIAN/OCEANIAN.

### *Biology*

*Sarcophaga spinigera* has been collected at decayed carrion-baits by KAM.

## ***Sarcophaga* (*Sarcorohdendorfia*) *villisterna* Salem**

*Sarcophaga villisterna* Salem, 1946

### *Morphological characters*

Gena with setulae only or mostly yellow/white and postocular with setulae only yellow/white. Prescutellar acrostichal setae present in females, but either absent or present in males, depending on the specimen. Apical scutellar setae present in males, but either absent or present in females, depending on the specimen. Propleuron uniformly setulose, with setulae only yellow/white and the hind tibia with long setulae in males. 1$^{st}$ and 2$^{nd}$ abdominal sternites with setulae only yellow/white. Body 10-15 mm in length.

### *Geographical distribution*

Australia (New South Wales, Northern Territory, Queensland, Western Australia) – AUSTRALASIAN/OCEANIAN.

### *Biology*

*Sarcophaga villisterna* has been collected at decayed carrion-baits by KAM, close to beaches or inland lakes.

## ***Sarcophaga* (*Sarcorohdendorfia*) *zeta* Johnston and Tiegs**

*Sarcophaga zeta* Johnston and Tiegs, 1921

### *Morphological characters*

Gena with setulae only yellow/white. Postocular with setulae only yellow/white in males, but with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally in females. Prescutellar acrostichal setae present. Propleuron uniformly setulose, with setulae only black and the hind tibia with long setulae in males. $1^{st}$ and $2^{nd}$ abdominal sternites in females with short setulae. In males, median patch of dense setae at the base of the $4^{th}$ abdominal sternite present. Body 10-15 mm in length.

### *Geographical distribution*

Australia (New South Wales, Queensland) – AUSTRALASIAN/OCEANIAN.

### *Biology*

*Sarcophaga zeta* has been collected at decayed meat and carrion-baits (Johnston and Tiegs 1921).

## ***Sarcophaga* (*Sarcorohdendorfia*) sp. nov.**

[Unnamed species near *praedatrix* Meiklejohn *et al.* (unpublished)]

### *Morphological characters*

Gena and postocular with setulae only yellow/white. Prescutellar acrostichal setae present. Propleuron uniformly setulose, with setulae only yellow/white and hind tibia with long setulae in males. Body 10-15 mm in length. (These characters are only valid for males as females were not examined).

### *Geographical distribution*

Australia (Queensland) – AUSTRALASIAN/OCEANIAN.

***Biology***

This species was collected at decayed carrion-baits by KAM.

***Remarks***

During the preparation of this revised key, it was realised that what is referred to as *Sarcophaga* (*Sarcorohdendorfia*) *praedatrix* by Lopes (1954) actually covers two different species. The description and naming of this second species will appear in a forthcoming paper.

***Sarcophaga* (*Sarcosolomonia*) *crinita* Parker**

*Sarcophaga crinita* Parker, 1919

***Morphological characters***

Gena with setulae mostly yellow/white in males, but only black in females. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present. Apical scutellar setae present in males, but either absent or present in females, depending on the specimen. Propleuron bare. Body 5-10 mm in length.

***Geographical distribution***

Australia (Northern Territory, Queensland, Western Australia) – AUSTRALASIAN/OCEANIAN, ORIENTAL.

***Biology***

*Sarcophaga crinita* has been caught at rabbit and chicken baits in Pakistan (Shazia *et al.* 2006) and bred from "pieces of beef" in Sulawesi (Blackith 1990).

***Sarcophaga* (*Sarcosolomonia*) *fabea* (Lopes)**

*Bezziola fabea* Lopes, 1959

***Morphological characters***

Gena with setulae only or mostly yellow/white. Generally, postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. However, some males have setulae only yellow/white. Prescutellar acrostichal and apical scutellar setae present in males, females unknown. Propleuron bare and the hind tibia with long setulae in both sexes. Body 5-10 mm in length.

***Geographical distribution***

Australia (New South Wales, Victoria) – AUSTRALASIAN/OCEANIAN.

***Biology***

*Sarcophaga fabea* has been found on dead sheep (Lopes 1959).

***Sarcophaga* (*Sarcosolomonia*) *collessi*** sp. nov.

[Name and description in Meiklejohn *et al.* (to be submitted to *Zootaxa*)]

***Morphological characters***

Gena with setulae only black. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae present. Propleuron bare and median marginal setae on the 3rd abdominal tergite present in some specimens. Body 10-15 mm in length.

***Geographical distribution***

Australia (Queensland) – AUSTRALASIAN/OCEANIAN.

***Biology***

*Sarcophaga collessi* was collected at decayed carrion-baits by KAM.

***Sarcophaga* (*Sarcosolomonia*) *papuensis* Shinonaga and Kurahashi**

*Sarcophaga papuensis* Shinonaga and Kurahashi, 1969

***Morphological characters***

Gena with setulae mostly yellow/white in males, but only black in females. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal and apical scutellar setae present in both sexes. Propleuron bare. Body 5-10 mm in length.

***Geographical distribution***

Australia (Queensland) – AUSTRALASIAN/OCEANIAN.

***Biology***

*Sarcophaga papuensis* was documented emerging from the common swamp pitcher-plant, *Nepenthes mirabilis* (Yeates *et al.* 1989).

***Sarcophaga* (*Sarcosolomonia*) *sumunensis* (Lopes)**

*Bezziola sumunensis* Lopes, 1967

***Morphological characters***

Gena with setulae a mix of black and yellow/white. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal and apical scutellar setae present in both sexes. Propleuron bare. Body 5-10 mm in length.

***Geographical distribution***

Australia (Queensland) – AUSTRALASIAN/OCEANIAN.

***Biology***

No known information.

***Sarcophaga* (*Sarcosolomonia*) *versatilis* (Lopes)**

*Bezziola versatilis* Lopes, 1959

***Morphological characters***

Gena with setulae only yellow/white. Generally, postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. However, some specimens of both sexes have setulae only yellow/white. Prescutellar acrostichal setae present. Propleuron bare and the hind tibia of some male specimens with long setulae. 1$^{st}$ and 2$^{nd}$ abdominal sternites with short setulae in females. Body 5-10 mm in length.

***Geographical distribution***

Australia (Northern Territory, Queensland) – AUSTRALASIAN/OCEANIAN.

***Biology***

No known information.

***Sarcophaga* (*Taylorimyia*) *aurifrons* Macquart**

*Sarcophaga aurifrons* Macquart, 1846

*Sarcophaga iota* Johnston and Tiegs, 1921

### *Morphological characters*

Gena with setulae only or mostly yellow/white and postocular with setulae only yellow/white. Prescutellar acrostichal setae present. Propleuron bare. 1st to 4th abdominal sternites and ventral sides of corresponding tergites with very long setulae in males, but females with short setulae. Body 5-10 mm in length.

### *Geographical distribution*

Australia (Australian Capital Territory, New South Wales, Northern Territory, Queensland, South Australia, Victoria, Western Australia) – AUSTRALASIAN/OCEANIAN.

### *Biology*

*Sarcophaga aurifrons* has been bred from rotten meat in Brisbane (Johnston and Tiegs 1921). The first-, second- and third-instar larvae were described by Cantrell (1981; referred to as *Taylorimyia iota*). This species has also been collected at decayed carrion-baits by KAM.

***Sarcophaga* (unplaced) *simplex* (Lopes)**

*Heteronychia simplex* Lopes 1967

### *Morphological characters*

Gena with setulae only black. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal and apical scutellar setae present. Propleuron bare and median marginal setae present on the 3rd abdominal tergite. (These characters are only valid for males as females were not examined).

### *Geographical distribution*

Australia (Queensland) – AUSTRALASIAN/OCEANIAN, ORIENTAL.

### *Biology*

*Sarcophaga simplex* has been collected at decayed carrion-baits by KAM.

***Tricharaea* (*Tricharaea*) *brevicornis* (Wiedemann)**

*Tachina brevicornis* Wiedemann, 1830

*Tricharaea scatophagina* Thomson, 1869

#### *Morphological characters*

Longitudinal vittae on thorax faint and with at least one strong proclinate orbital seta present in males. Thorax and abdomen covered in long microtrichosity. Gena with setulae only black. Postocular with at least one row of black setulae in addition to black postocular setae, with setulae only yellow/white ventrally. Prescutellar acrostichal setae either absent or present in both sexes, depending on the specimen. Apical scutellar setae absent. Propleuron bare and some male specimens with long setulae on the hind tibia. 1st and 2nd abdominal sternites with setulae only yellow/white. Body 5-10 mm in length.

#### *Geographical distribution*

Australia (Australian Capital Territory, New South Wales, Tasmania, Victoria) – AUSTRALASIAN/OCEANIAN, NEOTROPICAL.

#### *Biology*

*Tricharaea brevicornis* is primarily found in open pastures, open woodlands and along beaches (Ferrar 1979). This species is the only Australian representative of the genus *Tricharaea*, and has been collected at decayed carrion-baits by KAM but is also known to be attracted to and breed in cow dung (Ferrar 1979).

## 2.3.5 Conclusions

This overview of the Australian Sarcophagidae not only focussed on preparing an updated key, but also on providing a comprehensive database of illustrations and photographs of male terminalia, along with updating biological information for the fauna. This revision does not facilitate accurate morphological identification for females of all *Sarcophaga s.l.* species. Future work could focus on examining the microtrichiae found on the intersegmental membranes of the ovipositor, which has been shown to be discriminatory some blow flies (Rognes 1991). Additionally, extensive work needs to be undertaken on the Miltogramminae and the genus *Blaesoxipha*, to develop a reliable key for their identification. It is to be expected that even with a revised key, morphological identifications will still be challenging for non-specialists, especially when complex structures such as the male terminalia need to be examined. Considering this, a LUCID key, which is more user-friendly for non-taxonomists, has been generated for the Australian Sarcophagidae and appears online (http://keys.lucidcentral.org/keys/v3/Sarcophaga/). Also DNA-barcoding has been tested as a molecular-based approach for species resolution of the

Australian Sarcophagidae, and deemed reliable (Meiklejohn *et al.* 2011; Meiklejohn *et al.* 2012c). The new species referred to in this manuscript will published in a subsequent paper.

## 2.4 LUCID key for the Australian *Sarcophaga* (*sensu lato*)

This section is the LUCID key for the Australian *Sarcophaga* (*sensu lato*):

Meiklejohn, K.A., Kavazos, C.R.J., Dowton, M., Pape, T. and Wallman, J.F. (2012) Australian *Sarcophaga* (*sensu lato*) (Diptera: Sarcophagidae). Institute for Conservation Biology and Environmental Management, University of Wollongong, Australia [available online at http://keys.lucidcentral.org/keys/v3/Sarcophaga/].

The contributions of each author to the research described are as follows: CRJK wrote the biological information and took lateral photographs for some of the species, but also provided tutoring for using the LUCID program; JFW and MD designed the research and provided advice and feedback on the key; TP provided guidance and knowledge on morphological characters to include in the matrix; KAM created the LUCID key, collected all data for the character matrix, generated the fact sheets, took lateral species photographs and character photographs, and created map distribution illustrations.

A LUCID key enables identification of specimens based on the users choice of morphological characters, rather than stipulated characters which are found in traditional taxonomic keys. The LUCID key for the Australia *Sarcophaga s.l.* is targeted at users with a general knowledge of fly anatomy, and was created based on the same character matrix used to generate the revised taxonomic key (Chapter 2.3). Similar to the revised taxonomic key, this version of the LUCID key only allows for separation between subfamilies and the recognition of the genus *Blaesoxipha*. As high morphological variation between sexes was noted for most species, separate male and female entities are included for all *Sarcophaga s.l.* species in the key. To facilitate the straightforward identification to the subfamily and subsequently the sarcophagine genera, upon opening the LUCID key only certain characters are available for selection. After a specimen has been identified as belonging to the genus *Sarcophaga s.l.* and the sex is correctly determined, all pertinent morphological characters become available for selection. To assist with identifications, photographs or illustrations are provided for morphological characters and/or states included in the key. After identifying a specimen, it is suggested that the user examine the relevant species fact sheet, which includes information on the known distribution and biology. If it is deemed that the identification is incorrect, it is easy to review the characters/states chosen leading to that identification (bottom left - 'Features Chosen') and correct where necessary. Please access the LUCID key for the Australian *Sarcophaga s.l.* by going to http://keys.lucidcentral.org/keys/v3/Sarcophaga/.

# CHAPTER 3: **Molecular identification of the Australian Sarcophagidae (Diptera)**

## 3.1 Comprehensive evaluation of DNA barcoding

*This section is slightly modified, in terms of the layout and numbering of figures and tables, from the paper:*

Meiklejohn, K.A., Wallman, J.F., Cameron, S.L., and Dowton, M. (2012c) Comprehensive evaluation of DNA barcoding for the molecular species identification of forensically important Australian Sarcophagidae (Diptera). *Invertebrate Systematics*. [*in press*]

The contributions of each author to the research described are as follows: MD and JFW designed the research; SLC, MD and JFW provided advice and feedback on the manuscript; SLC and MD provided training in experimental techniques; KAM performed all the molecular experiments described, analysed the data and composed the research as a manuscript for publication.

### 3.1.1 Introduction

A range of insects present on a corpse can be used as evidence in forensic investigations to estimate the post-mortem interval (PMI). Commonly, estimation of the PMI using insect evidence requires accurate species identification, with subsequent examination of thermobiological profiles to determine age (Amendt *et al.* 2004; Catts 1992; Catts and Goff 1992). For accuracy, forensic entomologists preferentially use evidence from initial corpse colonisers, such as flesh flies (Diptera: Sarcophagidae) and blow flies (Diptera: Calliphoridae) (Amendt *et al.* 2004). Despite the prospective use of sarcophagids in forensic investigations, their use to date has been overshadowed by calliphorids. This is due to the difficulties of morphological species-level identification at any life stage of flesh flies, and a lack of documented thermobiological profiles of these insects.

Adult sarcophagids can be easily identified at the family level, as a large proportion of species share the characteristic features of longitudinal stripes on the thorax and a tessellated/chequered abdominal pattern. However, species-level identification is difficult and requires examination of subtle morphological variation of bristle placement and length, hair colouration, body pigmentation and the male terminalia (Pape 1996; Shewell 1987). Considering this, molecular-based approaches for species identifications have been proposed to eliminate issues with identifications based exclusively on taxonomy (Wells *et al.* 2001b; Zehner *et al.* 2004).

DNA barcoding is now a commonly accepted method for molecular species identification, utilising a 648-bp fragment from the 5' end of the mitochondrial COI gene. Numerous studies have evaluated the effectiveness of barcoding, with the approach shown to be unreliable for some Diptera (Meier *et al.* 2006; Whitworth *et al.* 2007), but also proven successful for many groups of invertebrates, such as springtails (Collembola) (Hogg and Hebert 2004), butterflies (Lepidoptera) (Hebert *et al.* 2004), mayflies (Ephemeroptera) (Ball *et al.* 2005), black flies (Diptera: Simuliidae) (Rivera and Currie 2009), scuttle flies (Diptera: Phoridae) (Boehme *et al.* 2010) and blow flies (Nelson *et al.* 2007), as well as some vertebrates (e.g. Tavares and Baker 2008; Ward *et al.* 2005).

In a previous pilot study, 16 species of Australian Sarcophagidae were successfully resolved using DNA barcoding (Meiklejohn *et al.* 2011). The purpose of this initial study was to test the principle that the barcode region could distinguish between members of this important fauna. The aim of the present study was to substantially increase the earlier level of sampling. A total of 588 sarcophagid specimens were sampled, including representatives from all Australian states and territories. This sample comprised 39 of the 84 known Australian species, represented by 580 specimens, and includes approximately 92% of the potentially forensically important species, mainly of the genus *Sarcophaga* (*sensu lato*) Meigen. The remaining eight specimens that were collected could not be reliably identified, but were included nonetheless as six unidentifiable taxa. It is hoped that the results of this study will assist with the implementation of Australian flesh flies in forensic investigations.

### 3.1.2 Materials and methods

***Specimens***

Trapping at decayed meat baits (comprising sheep's liver and kangaroo mince), hand netting and the 'hill-topping' technique of collecting from leks (Blackith and Blackith 1992), were all employed to collect adult sarcophagid specimens across Australia (Appendix 1; Figure 74). All specimens were collected directly into absolute ethanol and stored at 4°C in the Diptera Collection in the School of Biological Sciences, University of Wollongong, Australia. Morphological species identifications were carried out by KAM for each specimen using the taxonomic keys for the Australian flesh flies (Lopes 1954; Lopes 1959; Lopes and Kano 1979a). To confirm species identifications, the terminalia of each specimen were examined, which required dissections of some male specimens. It is important to highlight, that as specimens used in this study were sourced from fieldwork and not curated collections, it was not possible to ensure that each species was uniformly sampled.

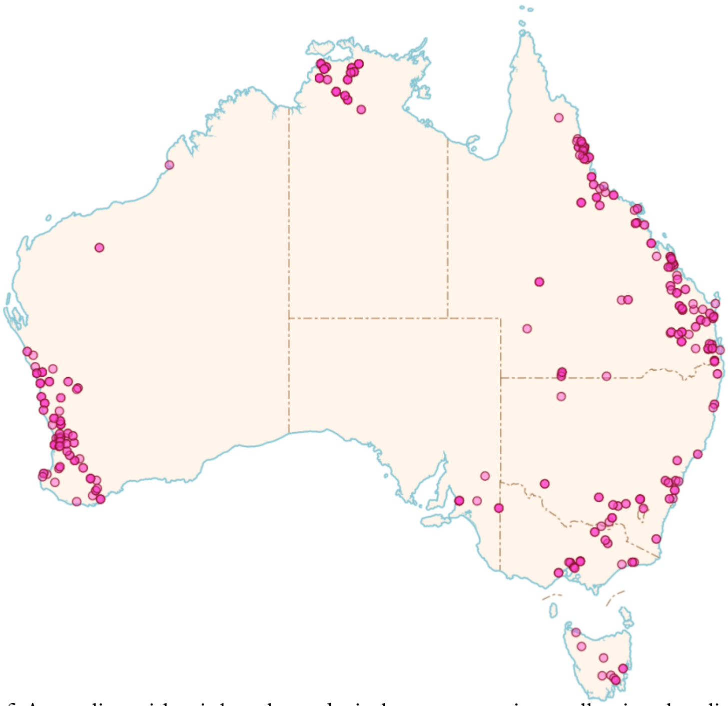

**Figure 74.** Map of Australia, with pink coloured circles representing collection localities for sarcophagid specimens used in this study. Dark and light pink coloured circles indicate localities where more than one specimen or only one specimen was collected, respectively.

***DNA extraction, amplification and sequencing***

Two legs from each adult sarcophagid specimen were used as tissue for total genomic DNA extractions using a previously published protocol (Aljanabi and Martinez 1997). The DNA was resuspended in 50 µl of fresh TE solution (1 mM Tris–HCl (pH 8), 0.1 mM EDTA) and subsequently stored at 4°C. The 648-bp COI barcoding region was amplified and sequenced using the protocol outlined by Meiklejohn (2011).

### ***DNA sequence analysis***

Sequence electropherograms were edited using ChromasPro Version 1.33 (Technelysium, Tewantin, QLD, Australia: available online at www.technelysium.com.au/ChromasPro.html). To confirm that the COI gene had been amplified, each sequence was submitted to both the Barcoding of Life Database (BOLD; available online at www.boldsystems.org) and the Basic Local Alignment Search Tool (BLAST; National Center for Biotechnology Information, Bethesda, MD, USA; available online at http://blast.ncbi.nlm.nih.gov/Blast.cgi). All nucleotide sequences were translated into amino acid sequences using the programme EMBOSS Transeq (available online at http://www.ebi.ac.uk/Tools/st/emboss_transeq/) to determine the correct reading frame. ClustalW within *MEGA* version 4 was used to align all mitochondrial gene sequences (Tamura *et al.* 2007). All sequences were entered into BOLD, where storage and preliminary barcoding analyses were performed.

### ***DNA barcoding analysis***

To obtain a visual representation of the divergence between specimens, a bootstrap (2,000 replicates) neighbour-joining (NJ) analysis was performed using the programme *Phylogenetic Analysis Using Parsimony** (*PAUP** and other methods) Version 4.0b10 (Swofford 2001). A semistrict consensus of the 2,000 NJ bootstrap trees was generated, which only retained relationships that had occurred on >50% of the trees. The two 'unknown' miltogrammine specimens included in the taxon set (KM059 and KM837), along with three blowfly species (Diptera: Calliphoridae, *Calliphora augur*, *Chrysomya rufifacies* and *Lucilia cuprina*), were used as the outgroup sequences. To quantitatively evaluate DNA barcoding for the Australian Sarcophagidae, nucleotide sequence divergences were calculated using the Kimura-two-Parameter (K2P) distance model, available within *PAUP** (Kimura 1980).

## 3.1.3 **Results and discussion**

### ***Morphological species identifications***

In the present study, 588 specimens were collected from across Australia and 39 species of Sarcophagidae were identified. Importantly, some of the taxa used in the present study were from Meiklejohn (2011); some of these were also misidentified. These identifications have been corrected in the current manuscript and appear with the correct species identification and same unique voucher code as in Meiklejohn (2011) (denoted by + at the specimen voucher code; Appendix 1).

Difficulties were encountered in accurately identifying some of the 588 specimens using the available taxonomic keys, as these keys only facilitate the identification of 54 of the possible 84 Australian species. Given that reference barcode sequences for all Australian sarcophagids are not available, correct species identifications of all specimens are vital for subsequent evaluation of the barcoding approach. To assist with the identification of specimens whose identity was uncertain, sarcophagid specialist Associate Professor Thomas Pape (ZMUC) was consulted. Photographs were taken of the lateral, dorsal and head profiles, along with detailed images of terminalia of each specimen. Most of these specimens were confidently identified; however, eight female specimens could not be accurately classified to species. These specimens were nonetheless still included in the taxon set, and are represented as 'unknown', with some of the 'unknown' species comprising multiple individuals that were morphologically identical to one another. These specimens collectively represent six unidentifable taxa: *Miltogramma* Unknown A, *Protomiltogramma* Unknown A; and *Sarcophaga* Unknown A - D. To further assist with identifications, each 'unknown' sequence was submitted to BOLD and NCBI, although no conclusive matches were obtained. The unknown *Sarcophaga* species cannot confidently be associated with a particular subgenus, and it is possible that these could represent new species, given that no extensive work on the Australian fauna has been carried out since the 1950-70s.

*Inclusion of female specimens*

As complete morphological species descriptions are not available for ~40% of female Australian sarcophagids, it could be argued that a male-only taxon set should be used to evaluate DNA barcoding for this fauna, as these are the only specimens that can be reliably identified. However, most species in the literature that lack complete female descriptions are not likely to be carrion breeders. As this study was aimed at probable carrion-breeding Australian sarcophagids, morphological identifications of females in this study can be regarded as reliable. The specimen composition used in this study was 53% female and 47% male.

Of the 39 known and six 'unknown' species in the current taxon set, 33 species are represented by both sexes, three species by males only and eight species by females alone. If this study was based solely on male specimens, nearly 18% of the species diversity would be missed. Similar results have been documented by Ekrem (2010) in chironomids (Diptera: Chironomidae), where females are also considerably more difficult to identify than males. In their study, 304 of 402 specimens collected were males, while 27% of species were represented only by females. Complete female morphological descriptions for difficult species might be obtained in the future, by means of associating them with male specimens of the same species, through a combination of barcoding and further morphological work (Yeates *et al.* 2011).

## ***Evaluation of DNA Barcoding***

To minimise the possibility of amplifying nuclear mitochondrial pseudogenes (NUMTs), taxon-specific primers were used for amplifications and only strongly amplified products were sequenced (Moulton *et al.* 2010). Further evidence that the obtained barcoding sequences were of mitochondrial origin came from the observation that they did not contain base ambiguities, premature stop codons or frameshift mutations upon translation.

To additionally validate the barcoding approach, attempts were made to obtain reference barcode sequences for all 84 Australian sarcophagids, from pinned museum specimens dating as far back as the 1920s. Both Chelex® (BioRad, Gladesville, NSW, Australia) and the Qiagen DNeasy Blood and Tissue kit (Qiagen, Doncaster, Victoria, Australia) extraction methods were trialled, using only one leg in each extraction to retain the integrity of the specimens. Only small ~55bp products of COI were able to be amplified using the primer combination of LCO1490-L (5'-GGTCWACWAATCATAAAGATATTGG-3') and mtD5 (5'-TGTTCCTACTATTCCGGCTCA-3') from the DNA extracted from the pinned specimens, consistent with high levels of DNA degradation. Direct sequencing of these products failed. As this study did not exhaustively examine all methods for DNA extraction and sequencing from pinned museum specimens, future studies should examine a broad range of extraction techniques in order to obtain complete reference sequences for the Australian Sarcophagidae.

This study was aimed at comprehensively evaluating the barcoding approach for species identification of forensically important Australian Sarcophagidae. Two early methods employed for evaluating DNA barcoding for species level resolution include: generation of a NJ tree based on K2P distances, and calculation of the intraspecific (within-species) and interspecific (between-species) sequence variation. More recent approaches have focused on the principles of population genetics to assess barcoding (Matz and Nielsen 2005; Nielsen and Matz 2006; Pons *et al.* 2006). However, to allow for direct comparison with the pilot study which evaluated barcoding for the Australian Sarcophagidae (Meiklejohn *et al.* 2011), the two earlier approaches to evaluate barcoding have been employed in the current comprehensive study.

### *NJ Tree*

In successful barcoding studies, specimens morphologically identified as the same species should be resolved as a single monophyletic group within the NJ tree (Hebert *et al.* 2003a). Australian sarcophagid specimens that were identified as the same species according to their morphology were nearly always resolved as a single monophyletic group on the NJ tree (99.2% of cases) (Figure 75). *Sarcophaga* (*Fergusonimyia*) *bancroftorum*, which appears to be the most morphologically variable Australian sarcophagid, was not monophyletic, rather resolved as five

**Figure 75.** Neighbour-joining (NJ) tree of Kimura-two-parameter (K2P) distances for 623 cytochrome oxidase subunit I (COI) gene sequences from Sarcophagidae: 588 from Australian specimens and 35 from specimens collected outside Australia obtained from GenBank (denoted by asterisk). ***GENERA*** and *subgenera* are given on the right-hand side: white bar at top indicates Miltogramminae, while black bars represent Sarcophaginae. Numbers given on main branches refer to bootstrap proportions among 2,000 bootstrap replicates >50% (internal monophyletic bootstrap values not shown). Morphological species identifications are given for all specimens, with voucher ID and GenBank accession number given for Australian and international specimens, respectively. Outgroups consist of two unidentified species of Miltogramminae (KM059 and KM837) and three species of Calliphoridae (*Calliphora augur, Chrysomya rufifacies* and *Lucilia cuprina*). Evolutionary distance divergence scale bar, 0.1.

distinct clusters (Figure 75). This polyphyly was not surprising given that some morphological variation was noted between the two male clusters: difference between the presence and absence of setulae on the propleuron and shape of the juxta (male terminalia). Specimens identified as the same 'unknown' species were also resolved separately as monophyletic on the NJ tree. Examination of the tree revealed large sequence divergences between most of the monophyletic groups, as they are separated by long branches. The monophyly of most species groups was well supported, having bootstrap values of 100 (Figure 75). However, the species clusters of *Sarcophaga* (*Sarcorohdendorfia*) *omikron* and *Sarcophaga* (*Sarcorohdendorfia*) *spinigera* both had bootstrap support of 88, as they had one specimen more divergent than the others (Figure 75). It was plausible that the divergent sequences, KM311 (*omikron*) and KM260 (*spingera*) may have been NUMTs. However neither sequence, when rechecked, contained premature stop codons or indels. Despite this, an additional NJ tree was generated with the removal of the two divergent specimens (KM311 and KM260). In this tree, the monophyly of *omikron* and *spinigera* was supported with bootstrap values of 100 for each taxon (tree not shown).

To assess the effect of geographic variation on the robustness of the barcoding approach, specimens were collected from various locations across Australia. For some species, such as *Sarcophaga* (*Sarcorohdendorfia*) *praedatrix, Sarcophaga* (*Parasarcophaga*) *taenionota* and *Sarcophaga* (*Taylorimyia*) *aurifrons*, monophyletic groups were resolved even with the inclusion of over 50 specimens obtained from multiple geographically isolated populations, collected over a 3,500 km range. To further test population effects on species resolution, sequences from species represented in the Australian sarcophagid taxon set (but collected outside Australia), were downloaded from BOLD (accessed on 26/8/2011). A total of 35 sequences from seven species were obtained: *Sarcophaga africa, crassipalpis, dux, misera, peregrina, ruficornis* and *taenionota.* These sequences were added to the 588 Australian sequences upon initial generation of the NJ bootstrapped tree, and are denoted by an asterisk (Figure 75). In nearly every case (33 of the 35 sequences), the international sequences were recovered in a monophyletic grouping with their Australian conspecifics (Figure 75). In the remaining two cases, a *dux* (AY879255) specimen was recovered with *Sarcophaga* (*Liosarcophaga*) *kohla,* whereas one *peregrina* (EU815030) specimen grouped among the outgroup sequences. Given how difficult these species are to identify, it is likely that these specimens were misidentified, or mislabelled or the sequence contaminated by the depositor.

<u>*Percentage divergences*</u>

Calculation of the percentage divergence between sequences is used to quantitatively evaluate the success of DNA barcoding. For successful species-level resolution using the barcoding approach, interspecific genetic variation exceeds that of intraspecific variation (Hebert *et al.* 2003a; Hebert *et al.* 2003b). This was the case for most of the Australian Sarcophagidae examined in this study, which is described more fully in the following section. A similar result to this was obtained when the barcoding approach was previously evaluated for Australian sarcophagids (Meiklejohn *et al.* 2011).

*Intraspecific variation*

The mean intraspecific variation for the Australian Sarcophagidae used in this study, excluding *bancroftorum*, ranged from 0-1.12% (Table 2). For 33 of the 36 species, the mean intraspecific variation was lower than 1%. The mean intraspecific variation for the nine specimens morphologically identified as *bancroftorum* was 7.67% (Table 2), which would indicate that these specimens are not a single species. Interestingly, the mean intraspecific variation of *bancroftorum* clusters KM589+KM590+KM813, KM886+KM887 and KM691+KM822, was 0.487%, 0% and 0%, respectively. These results corroborate the separation of these specimens in the NJ tree, and as such it is plausible that specimens identified as *bancroftorum* may represent multiple distinct species.

Some studies have documented that intraspecific variations can be grossly underestimated by the inclusion of numerous specimens from a single species population (Meier *et al.* 2006). Given that the taxon set in this study included specimens from a range of geographical populations across Australia, it is likely that the calculated intraspecific variation has not been biased in this way. For species for which international sequences are available in BOLD, the intraspecific variation was recalculated with the inclusion of these sequences (Table 3). The intraspecific variation for all species except *peregrina*, were lower than 2.6%. Given that one international specimen of *peregrina* (EU815030) did not cluster with its conspecifics, the higher variation of 3.82% was due to the inclusion of this specimen in the calculations (1.33% when EU815030 was removed). Low intraspecific barcode variation was previously documented in *Sarcophaga* (*Liopygia*) *argyrostoma* (0.6-2.6%) and for a range of sarcophagids from both Europe and the USA (<1%) (Draber-Monko *et al.* 2009; Wells *et al.* 2001b; Zehner *et al.* 2004). Overall, the findings indicate that, even with a comprehensive sample of the Australian Sarcophagidae, this fauna mainly has low intraspecific variation, making barcoding an appropriate molecular identification method.

**Table 2.** Summary of the intraspecific COI percentage genetic divergences (using K2P model) among 36 of the Australian Sarcophagidae species studied. Species that were only represented by one specimen are not shown. Symbols given at species' names indicate biology: * denotes that the species is of potential forensic importance, and # denotes that the species is a parasitoid. For species lacking a symbol, the biology is not documented. Additionally, ^ denotes that the species has been documented in Australian forensic cases (JFW *pers. comm.*).

| Sarcophagidae species | Distance (%) | | | | |
|---|---|---|---|---|---|
| | No. of specimens | Minimum | Mean | Maximum | SD |
| *O. varia* * | 15 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *S. africa* * | 13 | 0.0000 | 0.2607 | 1.6945 | 0.6114 |
| *S. alcicornis* * | 7 | 0.0000 | 0.3939 | 1.0770 | 0.3128 |
| *S. alpha* * | 2 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *S. aurifrons* * | 56 | 0.0000 | 0.7907 | 1.7772 | 0.2947 |
| *S. australis* * | 34 | 0.0000 | 0.7653 | 4.5836 | 1.1485 |
| *S. bancroftorum* * | 9 | 0.0000 | 7.6721 | 12.800 | 6.2636 |
| *S. bidentata* * | 26 | 0.0000 | 0.4264 | 1.1682 | 0.3380 |
| *S. crassipalpis* *^ | 23 | 0.0000 | 0.1999 | 0.6144 | 0.1805 |
| *S. cyrtophorae* # | 2 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *S. dux* * | 20 | 0.0000 | 0.4616 | 0.3053 | 0.0756 |
| *S. froggatti* * | 28 | 0.0000 | 1.0423 | 2.7225 | 0.4993 |
| *S. impatiens* *^ | 48 | 0.0000 | 0.8273 | 4.6734 | 0.7633 |
| *S. kappa* * | 16 | 0.0000 | 0.5516 | 1.5873 | 0.3208 |
| *S. kohla* * | 2 | 0.1524 | 0.1524 | 0.1524 | 0.0000 |
| *S. littoralis* *# | 2 | 0.1524 | 0.1524 | 0.1524 | 0.0000 |
| *S. megafilosia* # | 2 | 0.3053 | 0.3053 | 0.3053 | 0.0000 |
| *S. meiofilosia* # | 2 | 0.4587 | 0.4587 | 0.4587 | 0.0000 |
| *S. misera* * | 31 | 0.0000 | 0.3453 | 0.9204 | 0.2055 |
| *S. multicolor* * | 4 | 0.0000 | 0.0762 | 0.1524 | 0.0762 |
| *S. omikron* * | 35 | 0.0000 | 0.7493 | 6.8271 | 1.4322 |
| *S. peregrina* * | 2 | 0.4600 | 0.4600 | 0.4600 | 0.0000 |
| *S. praedatrix* *^ | 70 | 0.0000 | 0.3234 | 0.8959 | 0.1807 |
| *S. ruficornis* * | 6 | 0.0000 | 0.5934 | 1.0770 | 0.3719 |
| *S. simplex* * | 3 | 0.1558 | 0.3074 | 0.4582 | 0.1235 |
| *S. spinigera* * | 11 | 0.0000 | 0.4663 | 2.5647 | 0.9892 |
| *S. sigma* * | 18 | 0.0000 | 0.3390 | 1.0755 | 0.2198 |
| *S. taenionota* * | 61 | 0.0000 | 0.2956 | 1.1662 | 0.1960 |
| *S. torvida* * | 6 | 0.4587 | 0.8667 | 1.5278 | 0.3182 |
| *S. villisterna* * | 3 | 0.6126 | 0.9213 | 1.2310 | 0.2525 |
| *S. zeta* * | 8 | 0.0000 | 0.1159 | 0.3053 | 0.0974 |
| *T. brevicornis* * | 2 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *S.* (*Sarcorohdendorfia*) **sp.nov.** * | 3 | 0.0000 | 0.2178 | 0.3365 | 0.1542 |
| *S. collessi* **sp.nov.** * | 4 | 0.5005 | 1.1210 | 1.3476 | 0.2819 |
| *Sarcophaga* Unknown A * | 2 | 0.3296 | 0.3296 | 0.3296 | 0.0000 |
| *Sarcophaga* Unknown C * | 2 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

**Table 3.** Intraspecific COI percentage genetic divergences (using K2P model) of seven Australian Sarcophagidae with the inclusion of international sequences.

| Sarcophagidae species | Distance (%) | | | | |
|---|---|---|---|---|---|
| | No. of specimens | Minimum | Mean | Maximum | SD |
| Australian *S. africa* vs. international *S. africa* | 15 | 0.0000 | 0.2047 | 1.8507 | 0.4500 |
| Australian *S. crassipalpis* vs. international *S. crassipalpis* | 32 | 0.0000 | 0.2986 | 0.9492 | 0.2484 |
| Australian *S. dux* vs. international *S. dux* | 24 | 0.4587 | 2.5486 | 6.7457 | 1.6960 |
| Australian *S. misera* vs. international *S. misera* | 34 | 0.0000 | 1.2075 | 1.7331 | 0.2749 |
| Australian *S. peregrina* vs. international *S. peregrina* | 12 | 0.0000 | 3.8178 | 17.3083 | 5.6583 |
| Australian *S. ruficornis* vs. international *S. ruficornis* | 10 | 0.0000 | 0.4534 | 1.8451 | 0.5153 |
| Australian *S. taenionota* vs. international *S. taenionota* | 64 | 0.2662 | 1.6609 | 2.5502 | 0.3595 |

*Interspecific variation*

The interspecific variation was calculated for Australian Sarcophagidae species that were clustered closely together on the NJ tree (Table 4). Specimens of *megafilosia* and *meiofilosia* had an interspecific variation of 2.81%. It is known that both of these species are parasitoids of the marine snail *Littoraria filosa*, where *megafilosia* only parasitises snails with shell lengths ≥10mm and *meiofilosia* parasitises snails with shells 4-<10mm long (Pape *et al.* 2000). Due to the small sample size in this comparison (four specimens), it is difficult to draw firm conclusions to explain the low interspecific variation. However, given the similar biology, restricted Queensland distribution and the short branch lengths linking these flesh fly species, it is possible that there may be gene flow between them, which is one hypothesis for the low variation. For the remaining species that were compared, the mean interspecific variation ranged from 3.75-11.23%. This range is in accordance with other studies that have used the COI gene for sarcophagid identification (Wells *et al.* 2001b; Zehner *et al.* 2004).

Meier (2008) have cautioned against using mean interspecific divergences as a method for species discrimination in Diptera. They propose that, with increases in specimen numbers, mean interspecific variations become inaccurately inflated. Accordingly, they suggest that the only correct reflection of species variation is from the minimum interspecific variation (Meier *et al.* 2008). With the exception of the *megafilosia* and *meiofilosia* comparison discussed above, the smallest mean interspecific divergence found was for *crassipalpis* vs. *ruficornis* (3.75%), which is not greatly different from the minimum interspecific divergence (3.61%) for specimens of this pair (Table 4). Similar results are seen for all species pairs, suggesting that the use of mean interspecific variation does not inflate the gap between intraspecific and interspecific variation for Australian sarcophagids.

**Table 4.** Interspecific COI percentage genetic divergences (using K2P model) between those Australian sarcophagids clustered closely together on the NJ tree.

| Sarcophagidae species | Distance (%) | | | | |
|---|---|---|---|---|---|
| | **No. of specimens** | **Minimum** | **Mean** | **Maximum** | **SD** |
| *S. bifrons* vs. *S. spinigera* | 12 | 7.4150 | 7.6151 | 9.6170 | 0.6330 |
| *S. crassipalpis* vs. *S. ruficornis* | 29 | 3.6138 | 3.7548 | 4.0996 | 0.1615 |
| *S. furcata* vs. *S. assimilis* | 4 | 5.6573 | 6.1039 | 6.4306 | 0.3269 |
| *S. froggatti* vs. *S. villisterna* | 31 | 6.8717 | 7.9090 | 8.9440 | 0.4979 |
| *S. kappa* vs. *Sarcophaga* Unknown D | 16 | 11.0493 | 11.2305 | 11.4156 | 0.1159 |
| *S. megafilosia* vs. *S. meiofilosia* | 4 | 2.6529 | 2.8137 | 2.9744 | 0.1608 |
| *S. kohla* vs. *Sarcophaga* Unknown C | 4 | 3.9163 | 3.9976 | 4.0789 | 0.0813 |
| *S. zeta* vs. *Sarcophaga* Unknown A | 10 | 5.0000 | 5.0190 | 5.0697 | 0.0294 |

***Phylogenetic inference***

It is important to emphasise that the NJ tree presented in this manuscript does not attempt to resolve any relationships between the subfamilies, genera, subgenera and species of the Australian Sarcophagidae. Bootstrap support at higher level nodes is not given on the NJ tree as it was <50, indicating a lack of confidence in the relationships depicted. Given that the main application of the COI barcoding region is for molecular species-level identifications, it was also not possible to reliably associate the six unknown *Sarcophaga s.l.* species with a particular subgenus. Substantial work is needed to identify these unknown species, which might be achieved by obtaining sequence data from genes in addition to COI, and employing a range of phylogenetic methods to investigate their relationships.

## 3.1.4 Conclusions

To date, forensic entomologists have not capitalised on using flesh fly evidence for estimating the PMI, due to the difficulties inherent in morphological identifications, along with a lack of thermobiological data. This study focused on comprehensively evaluating DNA barcoding as a molecular approach for the identification of 588 specimens of the Australian Sarcophagidae. Examination of percentage genetic divergences and a NJ tree were used to test barcoding in this study, with the results indicating that it is an effective approach for the accurate species resolution of this fauna, in line with the previous study focused on the east coast (Meiklejohn *et al.* 2011). It is suggested that future barcoding studies on sarcophagids should compare multiple methods for evaluating barcoding, including population genetic approaches. Following the accurate identification of a flesh fly specimen, the relevant thermobiological profile should be examined to determine the specimen's age. Currently, such profiles are not available for the majority of sarcophagids. Future studies should therefore also focus on documenting flesh fly thermobiology, in order to fully facilitate the use of these flies as effective tools in forensic entomology.

## 3.2 Immature identification using DNA barcoding

*This section is slightly modified, in terms of the layout and numbering of figures and tables, from the paper:*

Meiklejohn, K.A., Wallman, J.F., and Dowton, M. (2012b) DNA barcoding identifies all immature life stages of a forensically important flesh fly (Diptera: Sarcophagidae). *Journal of Forensic Sciences*. [doi:10.1111/j.1556-4029.2012.02220.x]

The contributions of each author to the research described are as follows: MD and JFW designed the research and provided advice and feedback on the manuscript; MD provided training in experimental techniques; KAM performed all the molecular experiments described, analysed the data and composed the research as a manuscript for publication.

### 3.2.1 **Introduction**

Insect specimens collected at crime scenes can be used to estimate the minimum post-mortem interval (PMI), season of death, presence of toxins, or corpse transportation (Catts 1992). For accuracy, forensic entomologists, where possible, utilize evidence from initial corpse colonizers, which include carrion-breeding species of blow flies (Diptera: Calliphoridae) and flesh flies (Diptera: Sarcophagidae) (Byrd and Castner 2010). Flesh flies can provide precise PMI estimations as they are viviparous (lay live larvae), producing immatures that are ready to start feeding immediately on the corpse and contribute to its decomposition. Despite the considerable forensic potential of sarcophagids, their use to date in forensic investigations has been limited in comparison with calliphorids, as accurate species-level morphological identification at any life stage is very difficult (Amendt *et al.* 2004; Byrd and Castner 2010; Catts 1992; Catts and Goff 1992).

By contrast, family-level identification of flesh flies is relatively straightforward. The majority of adults generally share the common features of grey black longitudinal stripes on the thorax, a heavily bristled body, and a tessellated abdomen (Pape 1996; Shewell 1987). Larvae have their posterior spiracles in a deep cavity on the last abdominal segment. Adult species-level identification is difficult even for taxonomic experts, because it requires close examination of subtle morphological variation (Pape 1996; Shewell 1987). Identification of immature specimens to the species-level is even more challenging, as there are fewer characters to draw upon, and larval and pupal descriptions for most carrion-breeding species are incomplete. Sarcophagids collected from crime scenes are generally therefore reared to adults to assist with taxonomic identifications; however, this is not always possible (Byrd and Castner 2010; Shewell 1987).

Recent studies have shown that the identification of adult flesh flies can be achieved using molecular methods. Mitochondrial genes have been a focus, including COI and ND4 (Wells *et al.* 2001b; Zehner *et al.* 2004). Additionally, the 648-bp barcoding region of COI has been shown to effectively distinguish between Australian Sarcophagidae (Meiklejohn *et al.* 2011), along with the flesh fly *Sarcophaga* (*Liopygia*) *argyrostoma* (Draber-Monko *et al.* 2009), blow flies of eastern Australia (Nelson *et al.* 2007), butterflies (Lepidoptera) (Hajibabaei *et al.* 2006), blackflies (Diptera: Simuliidae) (Rivera and Currie 2009), mayflies (Ephemeroptera) (Ball *et al.* 2005), and tachinids (Diptera: Tachinidae) (Smith *et al.* 2006).

Despite the success of barcoding, it is important to consider the coamplification of NUMTs, nonfunctional copies of mitochondrial genes found in the nucleus (Hazkani-Covo *et al.* 2010; Richly and Leister 2004; Song *et al.* 2008). The inclusion of NUMT sequences in barcoding studies can overestimate the number of species resolved. To minimize the chance of obtaining NUMTs and allow for accurate evaluation of the barcoding approach, it has been suggested that researchers should extract only mitochondrial DNA and use taxon-specific primers for amplifications (Hazkani-Covo *et al.* 2010; Song *et al.* 2008).

Taking into account the success of DNA barcoding for the recognition of adult Australian sarcophagids, this study investigated whether barcoding sequences could be obtained from immature flesh flies of all life stages (first-, second-, and third-instar larvae, along with puparia). If possible, this technique could be used by forensic entomologists to make accurate species identifications of flesh flies in cases where the immature evidence is incomplete, has been killed prior to rearing, or only the puparia remain.

### 3.2.2 Materials and methods

***Species and culture rearing***

A laboratory culture of the forensically important flesh fly species, *Sarcophaga* (*Sarcorohdendorfia*) *impatiens* (Walker) (Mt. Keira, NSW, Australia), was used to obtain experimental immatures. This endemic species was chosen as it is a carrion-breeder, is frequently collected from crime scenes, and has larvae that cannot be identified morphologically. Gravid females laid larvae on sheep's liver; the larvae were subsequently separated into plastic weigh boats in replicates of 50. Each replicate was provided with sufficient liver for growth and placed in a rearing container on a bed of wheaten chaff. To obtain first-instar larvae, immatures were allowed to develop at 25°C for 12 h, after which they were killed in boiling water. This is the standard approach for killing maggots (Day and Wallman 2008) and also assists with the removal of residual feeding substrate from the skin. The larvae were then stored in absolute ethanol to preserve their DNA for molecular analysis (Day and Wallman 2008). The posterior spiracular slits of each immature were examined using a stereomicroscope (Leica MZ7$_5$; Leica Microsystems, Wetzlar, Germany) to determine the specific developmental stage (first, second, or third instar). This procedure was repeated for larvae aged 24, 48, 72, 96, and 120 h, until pupation occurred at 144 h. Upon pupation, pupae were placed into transparent containers and monitored closely until emergence. Immediately following emergence, intact puparia were collected and placed into open Petri dishes.

***DNA extraction, amplification, and sequencing***

Total genomic DNA was extracted from the immature specimens using a previously published protocol (Sunnucks and Hales 1996). Triplicate extractions were performed for each time point for whole *impatiens* larvae and for whole puparia. These extractions were carried out immediately following emergence, and after both 1 and 2 weeks. As some puparia were missing the puparial cap and lining following adult emergence, any remnants of these were removed for standardization of the samples prior to extraction. Excess ethanol was evaporated from the tissues, and they were subsequently ground with a pestle in a sterile 1.5-mL tube. Ground immature tissue was incubated with an extraction buffer, which consisted of 50 mM Tris-HCl (pH 8), 20 mM ethylenediaminetetraacetic acid (EDTA) (pH 8), 400 mM NaCl, 1.0% sodium dodecyl sulphate (SDS) (pH 8), and 0.2 mg proteinase K (added just prior to extraction). The final volume of the extraction buffer was proportional to tissue size and ranged from 200 to 1000 µL. Importantly, to assist with complete tissue digestion, the final SDS and proteinase K concentrations reported here were higher than those given by Sunnucks and Hales (1996). Each extraction was incubated overnight at room temperature, after which the liquid was decanted into a sterile 1.5-mL tube and sufficient 5 M NaCl added to give a final NaCl concentration of 1.1 M. Upon mixing, the reactions were incubated at room temperature for 10 min to precipitate any undigested proteins. The extractions were then centrifuged for 5 min at 16,100 × *g* (Eppendorf 5415D microcentrifuge; Crown Scientific, Acacia Ridge, Australia), and the resulting supernatants removed and placed into fresh 0.6-mL screw-cap tubes, with an equal volume of absolute ethanol (95%). The mixtures were then incubated at room temperature for 15 min and spun at 13,400 × *g* for 15 min. The supernatants were removed, and the DNA pellets washed with 200 µL of ice-cold 70% ethanol and spun at 13,400 × *g* for 15 min. The ethanol wash was drained, and the DNA pellets were air dried. Each DNA pellet was resuspended in 50 µL of fresh TE [1 mM Tris-HCl (pH 8), 0.1 mM EDTA] solution and stored at 4°C. The 648-bp barcode region of COI was amplified and sequenced with barcoding primers specific to Diptera, following the protocols described in Meiklejohn (2011).

***DNA sequence analysis***

Sequence electropherograms were verified and edited using ChromasPro Version 1.33 (Technelysium Ltd., Helensvale, Queensland, Australia; www.technelysium.com.au/ChromasPro.html). Consensus sequences were submitted to BOLD (www.boldsystems.org) for identification. Each sequence was uploaded into both BOLD and GenBank (accession numbers JN231257-JN231282). Sequences from all immatures were aligned to an adult *impatiens* reference sequence using BioEdit Sequence Alignment Editor Version 7.0.5.3 (Hall 1999), to establish any nucleotide substitution between individuals and immature life stages.

### 3.2.3 Results and discussion

***COI amplification***

This study examined whether DNA extracted from various immature stages of *impatiens* could be used to amplify the COI barcoding region using the primers specific to Diptera. Larval immatures from 12, 24, 96, and 120 h developmental time points strongly amplified the 658-bp region (Figure 76a). This result correlates with studies by Cainé (2006) who extracted DNA from

141 maggots and successfully amplified both 305- and 519-bp portions of COI for the identification of numerous blow fly species.

Interestingly, the barcode region was unable to be consistently amplified from 48- and 72-h larval extracts, which represent late second and early third instars, respectively (Figure 76a, Lanes 8-13). Unpublished studies have shown that *impatiens* larvae show exponential growth at 25°C at these time points, with extended crops having contents that are clearly visible. Campobasso (2003) indicated that at different stages of larval development, the crop may become more prominent and its contents could interfere with successful extraction of larval DNA. Some published studies recommend removal of the crop prior to larval DNA extraction at any larval stage (Wells *et al.* 2001a). This was not performed initially, to establish whether dissection of this structure was mandatory for amplification of the barcoding region.

As a consequence of the above, the crop was subsequently dissected from three larvae from both the 48- and 72-h time points. Eye surgery scissors were used to make a lateral incision along the mid-dorsal region of the anterior of each larva. With fine forceps, the incision was then carefully widened and the entire crop pulled out and cut off at its neck. Larvae from which the crop had been successfully dissected were used as tissue in additional DNA extractions. The barcode region was successfully amplified from five of six of these extracts in which the crop was removed (Figure 76b, Lanes 11-16). This indicated that the presence of the crop in the extraction tissue had prevented the barcode fragment from amplifying. This was also confirmed through an inhibition PCR experiment, in which three separate reactions were set up, each containing different sources of DNA. The sources included either "failed" 48- or 72-h larval DNA or "positive" larval DNA, which had previously amplified COI. In tubes one and two, either the "failed" 48- or 72-h extract was combined with the "positive" larval DNA in equal volumes. The third tube was a positive control and contained only the "positive" larval DNA. In the presence of this "failed" DNA, the "positive" larval DNA source failed to amplify the 658-bp fragment (Figure 76b, Lanes 17-19). Investigations to identify the specific crop inhibitor were not undertaken, as the objective was to establish an effective approach for DNA extraction, allowing for amplification at these problematic time points. Interestingly, no issues with larval DNA amplification were documented by Cainé (2006). As problems in larval extraction were only encountered in this study with late second- and early third-instar larvae, the success by Cainé (2006) may be linked to the age of the immatures that they used, which they did not specify. The crop may not have been entirely dissected from the extract that failed to amplify.

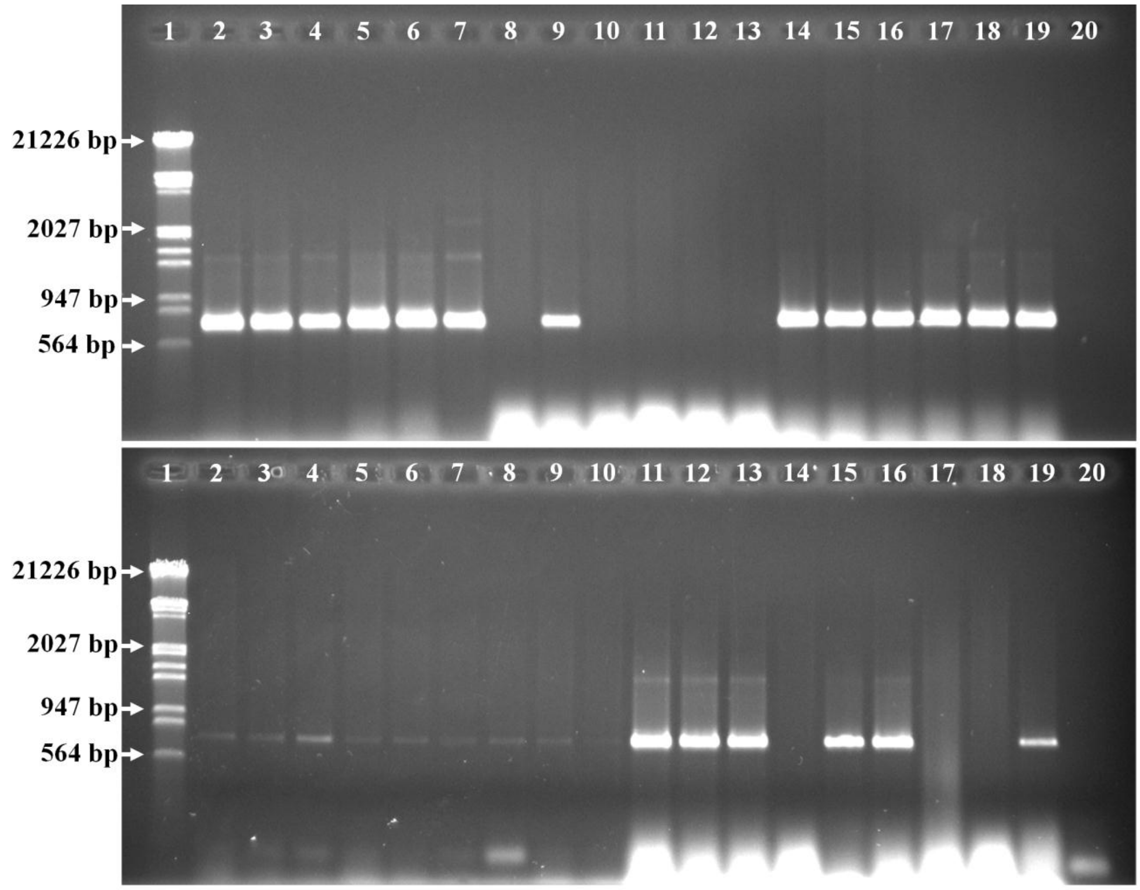


**Figure 76.** Images of COI barcoding amplicons on agarose gels (1%) following electrophoresis, ethidium bromide staining and examination under UV light. Molecular weight marker (lambda DNA digested with *Eco*RI and *Hind*III) is seen in lane 1 and negative control in lane 20 in both gels.

**a.** The amplicons from larval extracts: 12 h (lanes 2-4), 24 h (lanes 5-7), 48 h (lanes 8-10), 72 h (lanes 11-13), 96 h (14-16) and 120 h (17-19).

**b.** The amplicons from puparia extracts: 0 weeks (lanes 2-4), 1 week (lanes 5-7) and 2 weeks (lanes 8-10); larval extracts with crop removed: 48 h (11-13) and 72 h (14-16); inhibition PCR of 48 h 'failed' DNA with 'positive' larval DNA (lane 17), 72 h 'failed' DNA with 'positive' larval DNA (lane 18) and inhibition control of only 'positive larval DNA' (lane 19).

Whole larvae were used as the tissue for DNA extractions for all stages. In casework, it would always be prudent to instead use only a portion of the larva for DNA extraction, leaving most of the cuticular spines, the cephaloskeleton, and the anterior and posterior spiracles intact to assist with identification (Sperling *et al.* 1994).

Puparial extracts from all time points also successfully amplified the barcode region; however, the resultant amplicons were weak (Figure 76b, Lanes 2-10). This result indicated that any possible pupal DNA degradation was not significant enough to prevent amplification in these extracts. Similarly, Mazzanti (2010) showed that a 931-bp region of COI-COII was able to be amplified for 77.4% of dipteran specimens < 5 years old. However, none of the specimens > 5 years old amplified the complete region, which was most likely a result of DNA degradation. However, studies by Dhananjeyan (2010) on *Aedes* mosquitos showed that internal transcribed spacer markers were unable to be amplified from pupal exuviae collected 1–9 days post emergence. These inconsistent results for gene amplification between different pupal exuviae of different families of flies could be linked to the targeted gene region (nuclear or mitochondrial genes), but also the chemical composition of exuviae. It has been documented that insect exuviae contain mostly ash, lipids, proteins, metal ions, and chitin, with the absence of substantial nucleic acids necessary for DNA-based species identification (Dhananjeyan *et al.* 2010; Gilby and McKellar 1976; Golebiowski *et al.* 2010; Gongyin *et al.* 2007; Roseland *et al.* 1985; Teotia and Miller 1974). Considering that the puparial cap and lining were removed in this study prior to extraction, and these are thought to be DNA-rich (Dhananjeyan *et al.* 2010), the production of weak or no amplicons for all extracts was expected. It is important to note that the cap and lining were removed for standardization, as not all puparia remained intact after adult emergence, which is also common in specimens collected in the field (Gongyin *et al.* 2007).

***COI sequencing***

COI barcoding amplicons from larval and puparial extractions were sequenced successfully, with edited sequences producing full-length consensus sequences 648 bp long. Interestingly, sequence data obtained from the 2-week puparial amplicons showed some base ambiguities and decreased signal. This is likely to be due to the sequencing template being limited in quantity. To verify species identity, all immature sequences were searched against the COI animal database available online at BOLD (www.boldsystems.org). The BOLD-ID output indicated 100% confidence that all immature sequences belonged to the genus *Sarcophaga*, and at least 99.95% confidence that the sequences were *impatiens*. This high sequence similarity was also seen after all immature sequences were aligned to an adult sequence, obtained from sequencing the entire mitochondrial genome of *impatiens* (Nelson *et al.* 2012a). Some nucleotide substitutions were seen between sequences and immature life stages, but none changed the amino acid sequence of the COI protein upon translation - all substitutions were transitions and were restricted mostly to the third codon position. Importantly, all sequences, even those obtained from weak products, were verified as *bona fide* mitochondrial sequences: signal background in electropherograms was minimal; unexpected insertions or deletions that cause frameshifts or stop codons did not occur. From this, it can be stated that *impatiens* lacks easily identifiable NUMTs.

### 3.2.4 Conclusions

The results of this study indicate that immatures of *impatiens*, either larvae or puparia, are an adequate tissue source for molecular species-level identification using DNA barcodes. This straightforward approach eliminates the need for difficult morphological identifications by taxonomic experts and is likely to show similar results if applied to other flesh flies or blow flies. It is important to note that this study was completed using intact larvae and puparia. Although immature specimens collected from crime scenes are sometimes incomplete, it is nonetheless suggested that similar successful identifications would be obtained even if smaller tissue amounts were used in initial extractions. For success in future studies, it is suggested that the crop be dissected out prior to larval extractions, especially for specimens in the late second and third instars of development. The ability to obtain an accurate species-level identification of an immature specimen may be vital in a forensic investigation for estimating the minimum PMI, identifying corpse transportation and determining season of death. Given the results of this study, it is hoped that morphologically unidentifiable immature specimens may have increased potential in criminal cases.

# CHAPTER 4: **Phylogenetic relationships within the genus *Sarcophaga* (*sensu lato*)**

*This section is slightly modified, in terms of the layout and numbering of figures and tables, from a paper prepared for submission to* Molecular Phylogenetics and Evolution*:*

Meiklejohn, K.A., Wallman, J.F., Pape, T., Cameron, S.L., and Dowton, M. Utility of CAD, COI and morphological data for resolving relationships within the genus *Sarcophaga* (*sensu lato*) (Diptera: Sarcophagidae): a preliminary study.

The contributions of each author to the research described are as follows: MD and JFW designed the research; SLC, MD, TP and JFW provided advice and feedback on the manuscript; SLC and MD provided training in experimental techniques and programming; TP provided guidance and knowledge on morphological characters to include in the matrix; KAM performed all the molecular experiments described, analysed the data and composed the research as a manuscript for publication.

## 4.1.1 Introduction

The Sarcophagidae (flesh flies) are a globally distributed family of 173 genera and 3,094 species, which are known mainly as carrion-breeders and insect parasitoids (Pape 1996; Pape *et al.* 2011; Shewell 1987). The monophyly of the family and its three subfamilies, Miltogramminae, Paramacronychiinae, and Sarcophaginae, has been well supported (Giroux *et al.* 2010; Kutty *et al.* 2010; Pape 1992; Pape 1996). Little is known about the placement of the Sarcophagidae within the Oestroidea; it has been proposed that the sister family may be either the Tachinidae or the Calliphoridae, or the unnamed McAlpine's fly (Kutty *et al.* 2010; McAlpine 1989; Pape 1992; Rognes 1997).

The largest of the three subfamilies, the Sarcophaginae, comprises approximately 2,200 species segregated into 51 genera (Pape 1996), but generic concepts vary between authors. Close to one-third of these species have been classified into a single genus, *Sarcophaga s.l.* Monophyly of *Sarcophaga s.l.* has been supported based on both molecular and morphological data (Giroux *et al.* 2010; Kutty *et al.* 2010; Wells *et al.* 2001b; Zehner *et al.* 2004); however the circumscription and classification of its 132 subgenera remain questionable. Interestingly, over 50% of *Sarcophaga s.l.* subgenera are monotypic and restricted to the Afrotropical zoogeographic region, and only 25% of the subgenera are represented by more than five species (Pape 1996).

Recently, the phylogeny of the flesh flies was inferred based on both morphological (Giroux *et al.* 2010) and molecular data (Kutty *et al.* 2010). Giroux (2010) used 73 morphological characters (including 41 male genitalic features) for phylogenetic reconstruction of 72 species of Sarcophaginae, representing 19 genera and 31 *Sarcophaga s.l.* subgenera. Kutty (2010) used a range of mitochondrial (12S, 16S, COI and cyt b) and nuclear (18S, 28S, CAD and EF-1α) gene sequences to infer the phylogeny of the Calyptratae, which included 46 sarcophagid species from 28 genera. Kutty (2010), however, did not focus on the genus *Sarcophaga s.l.*, with only seven subgenera represented.

To date, no evolutionary studies of the Sarcophagidae have focussed on *Sarcophaga s.l.*, and those that have included *Sarcophaga s.l.* species did not represent large subgenera with multiple species, making it difficult to draw reliable inferences about subgeneric monophyly. Considering the large size of this genus, the current preliminary study was aimed at evaluating the utility of three sources of data for resolving a small number of subgenera and species, as a precursor to a larger-scale study of the genus: the mitochondrial COI barcode region, ~800bp of the nuclear gene CAD and 110 morphological characters.

## 4.1.2 **Methods**

### ***Molecular data***

*Taxon Sampling*

Sarcophagid specimens used in this study were collected using meat baits, consisting of rotten kangaroo mince and sheep's liver, and the 'hill topping' technique (Blackith and Blackith 1992). They were placed directly into absolute ethanol and stored at 4°C in the Diptera Collection in the School of Biological Sciences, University of Wollongong, Australia. Each specimen was identified morphologically using the taxonomic literature for the Australian Sarcophagidae (Lopes 1954; Lopes 1959; Lopes and Kano 1979a), but also assisted by examination of curated collection material from the ANIC, QDPC, QM and UQIC.

The specimens used to obtain COI and CAD sequences represent 39 species from 14 of the 132 *Sarcophaga s.l.* subgenera (Appendix 1). The subgenera sampled include: three of the four largest subgenera, *Liosarcophaga*, *Sarcorohdendorfia* and *Sarcosolomonia*; seven subgenera which consist of five or more species in total; and four subgenera that are monotypic and restricted to the Australasian/Oceanian zoogeographic regions. *Sarcophaga simplex*, which is found in Australia but has not been reliably classified to a subgenus, is also included. The taxon set also includes two unknown *Sarcophaga s.l.* taxa (*Sarcophaga* Unknown A and B), along with two new species of the *Sarcophaga s.l.* subgenera *Sarcorohdendorfia* and *Sarcosolomonia*, which will be described in a subsequent publication.

*DNA extraction*

Total genomic DNA was extracted from the two front legs of each specimen using a previously published protocol (Aljanabi and Martinez 1997). Pelleted DNA was resuspended in 50 µl of TE (1 mM Tris-HCl (pH 8), 0.1 mM EDTA) and stored at 4°C for subsequent use.

*Gene amplification*

The 648 bp COI barcode fragment was amplified for all specimens as outlined by Meiklejohn (2011). All barcode sequences used in this study have been published previously, either in Meiklejohn (2011) or Meiklejohn (2012c *in press*). Prior to commencing this study, the entire ~4,000 bp CPS domain of CAD was amplified and sequenced for three species from different genera and subgenera: *Oxysarcodexia varia*, *Sarcophaga* (*Parasarcophaga*) *misera*, and *Sarcophaga* (*Sarcorohdendorfia*) *impatiens*. After sequence alignment, the most variable portion of the CPS domain in these species was identified as the initial ~800bp (or fragment one).

Based on this, fragment one of CAD was amplified using the primer combination of 54F (5'-GTNGTNTTYCARACNGGNATGGT-3') and 405R (5'-GCNGTRTGYTCNGGRTGRAAYT G-3') (Moulton and Wiegmann 2004). Each 25 µl reaction contained 2 µl of 10X PCR buffer (with 20mM $MgCl_2$; composition withheld by manufacturer, Scientifix, Cheltenham, Victoria, Australia), 200 µM of each deoxynucleotide triphosphate (dNTP), 800 nM of each primer (Sigma, Castle Hill, NSW, Australia), 1.0 U of *Scientifix*Hot Start DNA Polymerase (Scientifix, Cheltenham, Victoria, Australia), 2 µl of extracted total genomic DNA or distilled $H_2O$ (negative control). The PCR temperature cycling consisted of an initial denaturation at 94$^o$C for 2 min, followed by: 5 cycles of 94$^o$C for 30 s, 52$^o$C for 30 s, 72$^o$C for 2 min; 7 cycles of 94$^o$C for 30 s, 51$^o$C for 1 min, 72$^o$C for 2 min; 37 cycles of 94$^o$C for 30 s, 45$^o$C for 20 s, 72$^o$C for 2 min 30 s; along with a final elongation for 3 min at 72$^o$C.

To establish whether amplifications were successful, all COI and CAD amplicons were resolved by agarose gel electrophoresis (1%). Samples that contained an amplicon of either ~650 bp or ~800 bp for COI and CAD, respectively, were incubated with ExoSAP-IT® at 37$^o$C for 15 min followed by 80$^o$C for 15 min, to digest unincorporated primers and dNTPs (GE Healthcare, Buck, HP8 4SP, UK). The amount of ExoSAP-IT® used to treat samples was altered from the manufacturer's instructions to 0.5 µl of ExoSAP-IT® enzyme with every 5 µl of PCR product.

Sequencing was performed using the ABIPRISM® BigDye™ Terminator Version 3.1 Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on the ExoSAP-IT® treated products. Each sequencing reaction contained 5 µM of a single PCR primer to initiate the sequencing reaction, 0.5 µl of BigDye™ (Applied Biosystems, Foster City, CA, USA), 1 µl of ExoSAP-IT® treated product and 0.5 µl of distilled $H_2O$ for a final volume of 2.5 µl. Cycling conditions for sequencing reactions consisted of 28 cycles of 96$^o$C for 10 s, 50$^o$C for 5 s and 60$^o$C for 4 min.

Purification of individual sequencing reactions was obtained by mixing the entire sequencing reaction volume with 26 µl of a sodium acetate-ethanol solution (final concentration of sodium acetate 120 mM, pH 8). The mixture was incubated at room temperature for 15 min and then centrifuged at 2,500 rpm for 15 min. The supernatant was removed and the resulting pellet washed with 60 µl of 75% ethanol and centrifuged for a further 5 min at 2,500 rpm. The supernatant was again discarded and the pellet allowed to air-dry, before storage at -20$^o$C. Sequencing products were sent to the ACRF Biomolecular Resource Facility (Canberra, ACT, Australia) for separation and generation of electropherograms.

#### Nucleotide alignment

Within 50-100bp of the 3' end of CAD fragment one, there is an intron/exon boundary. As unambiguous full-length sequences were not obtained for all specimens, this intron was not consistently sequenced. In addition to this, only approximately 30-40 bp of the intron sequenced remained in full length sequences, after trimming the 405R primer sequence. Although intron sequences can provide phylogenetic signal, for consistency, the intron/exon boundary was identified in full length sequences based on both the GT-AG rule (Mount 1982) and GenScan outputs (available online at http://genes.mit.edu/GENSCAN.html), and the intron subsequently excised. Individual exon sequences for both COI and CAD were translated into amino acids and separately aligned using Clustal W within *MEGA* version 4 (Tamura *et al.* 2007).

### Morphological data

The total number of characters scored was 110, comprising 50 non-terminalia and 60 terminalia characters (5 and 55 terminalia characters for females and males, respectively). The complete data matrix is shown in Appendix 2. To allow for direct comparison between markers, these characters were scored for the *Sarcophaga s.l.* species from which CAD and COI were sequenced. This was achieved by examining pinned adult male and female curated specimens, borrowed from the ANIC, UQIC, QDPC and QM. Most characters were coded by direct observations, however when structures were missing, the relevant taxonomic literature was consulted. Characters that were either inapplicable or that could not be scored were denoted by a '?'. Characters whose state varied between males and females for a given species, were coded as possessing both (e.g. 01).

### Phylogenetic analysis

Bayesian analyses were conducted for all possible combinations of the molecular and morphological data, along with each data set separately in MRBAYES (Version 3.1.2) (Huelsenbeck and Ronquist 2001). In addition to this, an unweighted parsimony analysis of only the morphological data was performed in TNT (Version 1.1) (Goloboff *et al.* 2003). For all analyses, Miltogramminae specimens (*Miltogramma* Unknown A (KM837) and *Protomiltogramma* Unknown A (KM059)), along with specimens from the sarcophagine genera *Blaesoxipha*, *Oxysarcodexia* and *Tricharaea*, were included to test the monophyly of the Sarcophaginae and *Sarcophaga s.l.*

#### Bayesian analysis

For the molecular data, the Akaike Information Criterion implemented within MRMODELTEST (Version 2.2), was used to determine the most suitable evolutionary model(s) separately for the COI and CAD data (Nylander *et al.* 2004): the data were partitioned by gene and then further partitioned by codon (first-, second- and third-codon position). Based on the study by Lewis (2001), the morphological data were analysed using the discrete model of evolution. It is important to note that morphological characters were not scored for each individual specimen listed in Appendix 2. Instead, for analyses with both molecular and morphological data, the corresponding morphological data scored from the curated museum specimens were added to the molecular data set based on species identity (e.g. the identity of JW221v1 is *Sarcophaga* (*Australopierretia*) *australis*, so the *australis* morphological data were concatenated to the molecular data for this specimen). All Bayesian analyses were run on the High Performance Computing (HPC) cluster at the University of Wollongong (Wollongong, NSW, Australia). The number of generations, sampling frequency, split frequencies and burn-in for each analysis have been summarised in Table 5.

*Parsimony analysis*

Prior to analysis, uninformative characters were removed, leaving 101 characters for reconstruction (uninformative characters denoted by * in Appendix 2). Systematic changes to the default settings for both traditional and new technology searches were implemented to establish the optimal settings for finding the shortest tree. A driven new technology search, using all four methods, found the shortest tree (595) with all the default settings applied except: 1) the number of additional sequences was set at 100; 2) minimum length to be found 25 times; 3) the ratchet weighting probability was 5% with 400 iterations; 4) tree-drifting used 50 cycles. A strict consensus of the 16 shortest trees was generated (Appendix 4). Absolute Bremer support values for all resolved nodes were determined by defining non-monophyletic constraints for each node. Constraints were enforced in separate new technology searches using the above mentioned settings, establishing the number of steps between suboptimal and optimal trees for each node. Following this, character synapomorphies were mapped onto the relevant node of the strict consensus tree.

*Neighbour joining (NJ) analysis*

A neighbour joining (NJ) phylogenetic tree (2,000 replicates) was also constructed, inferred using all three markers, to compare the resolution of the two different methods. The NJ phylogeny, when compared to the Bayesian analyses, resolved relationships at basal nodes with substantially lower support (bootstrap support of <60 with 2,000 replicates), and is not shown.

### 4.1.3 **Results and discussion**

***Marker choice and evaluation***

The gene regions of COI and CAD, along with 110 morphological characters, were evaluated for phylogenetic signal with regards to the genus *Sarcophaga s.l.* in this preliminary study.

The COI barcoding region was chosen as published studies have deemed it as reliable and accurate for species-level identifications (Meiklejohn *et al.* 2011; Meiklejohn *et al.* 2012c) and has been a common component of insect molecular systematics in general (Caterino *et al.* 2000). The CPS domain of CAD has been useful for resolving higher level relationships in Diptera (Moulton and Wiegmann 2004), and so was used to supplement COI in this investigation. The characters used to construct the morphological dataset included those documented as important for species discrimination (Lopes 1954; Lopes 1959; Pape 1996), along with a range of supplementary characters from the head, abdomen, thorax and terminalia.

The results from the Bayesian analyses performed on all data combinations, regarding the monophyly of *Sarcophaga s.l.* subgenera and the percentage of nodes with significant posterior probabilities (PP >0.90), are also given in Table 5. All analyses resolved the sarcophagines as monophyletic (PP of 0.75-1.00), except for the COI phylogeny (Appendix 3b). The morphological data alone produced the most poorly resolved phylogeny, with only 30% of nodes with PP of >0.90 (Appendix 3c). The phylogeny produced with COI and morphological data was well resolved (75% of nodes with PP of >0.90), however support for the monophyly of *Sarcophaga s.l.* was only moderate (PP of 0.70; Appendix 3e). The most well resolved phylogeny was generated by combining both COI, CAD and the morphological data, with basal relationships well supported and 78% of nodes with PP of >0.90 (Table 5; Figure 77). For this reason, the preliminary monophyly and relationships of the *Sarcophaga s.l.* subgenera were examined using the phylogeny depicted in Figure 77. It is important to note that the NJ phylogeny, based on all three markers, resolved relationships at basal nodes with substantially lower support (bootstrap support of <60), when compared to the Bayesian analyses; it is not shown. The strict consensus of the parsimony analysis was largely unresolved (Appendix 4), so this tree was used only to identify characters important for the classification and taxonomy of particular *Sarcophaga s.l.* subgenera.

Three *Sarcophaga s.l.* subgenera (represented by four species), additional to those represented in this study, were included in the molecular phylogeny of the Calyptratae by Kutty (2010). These species were not incorporated here, as only COI sequences were available for those specimens; CAD was sequenced for only four of the 46 sarcophagid species included in Kutty (2010). As higher level relationships were not robustly resolved within the 'COI-only' phylogeny (Appendix 3b), it was decided that including these species would not assist with elucidating the relationships within *Sarcophaga s.l.* Further, the CAD sequences obtained by Kutty (2010) do not overlap with those used in the current study: they focused on CAD fragment 4, whereas CAD fragment 1 was sequenced in this study. Finally, it would not have been possible to score all morphological characters for these species as specimens were not available to us, resulting in an incomplete data matrix.

## ***Sarcophaginae***

The largest subfamily of the Sarcophagidae, containing the genus *Sarcophaga s.l.* among others, is the Sarcophaginae. In this study, the Sarcophaginae are resolved as monophyletic (PP of 1.00; Figure 77), with monophyly of this subfamily also documented in a range of studies (Giroux *et al.* 2010; Kutty *et al.* 2010; Pape 1996).

### *Genus* Sarcophaga s.l.

The genus *Sarcophaga s.l.* was resolved as monophyletic (PP of 0.93; Figure 77). This conforms with several studies that have also supported *Sarcophaga s.l* as monophyletic, based on both molecular and morphological data (Giroux *et al.* 2010; Kutty *et al.* 2010; Wells *et al.* 2001b; Zehner *et al.* 2004). The monophyly of most of the non-monotypic subgenera of *Sarcophaga s.l* sampled still remain questionable.

*Liopygia* was the only subgenus of *Sarcophaga s.l.* consistently resolved as monophyletic in all Bayesian analyses (Table 5). The parsimony analysis identified two characters that could be used for the classification of this subgenus: ground colour of terminalia bright red or orange and the surstylus with a thickened proximal margin (Appendix 4). Zehner (2004) and Wells (2001), who used mitochondrial COI/ND5 and COI genes, respectively, for phylogenetic reconstruction, also resolved *Liopygia* as monophyletic in their studies.

The subgenus *Parasarcophaga* was resolved as monophyletic (PP of 0.99; Figure 77), but not consistently in all analyses (Table 5). Male terminalia characters, such as connection of the vesica to the distiphallus by means of a narrow, stalk-like projection and the proximal part of the vesica drawn out into a pair of divergent processes, were identified as features that can be used to classify this subgenus (Appendix 4). Species of the subgenus *Lioproctia* were polyphyletic (Figure 77). The second largest *Sarcophaga s.l.* subgenus, *Liosarcophaga*, was resolved as paraphyletic, disrupted by a single representative from the subgenus *Boettcherisca* (Figure 77).

*Sarcorohdendorfia*, the most well sampled subgenus included in this study, was not resolved as monophyletic, with one species from the subgenus *Lioproctia*, *alcicornis*, disrupting the monophyly (Figure 77). The placement of *alcicornis* among species of *Sarcorohdendorfia* in the morphological phylogeny (Appendix 3c) was not surprising, as this species possesses the diagnostic features of *Sarcorohdendorfia*: setulae on the propleuron and a '√' shaped vesica (Lopes 1959). As *alcicornis* was similarly clustered when all data sets were combined (PP of 1.00; Figure 77), it is plausible to suggest an assignment of *alcicornis* to the subgenus *Sarcorohdendorfia*.

**Table 5.** Summary of the number of generations, sampling frequency, split frequencies and burn-in for each of the Bayesian analyses performed. An asterisk denotes an analysis in which one of the four runs had substantially worse tree likelihoods, which was the cause for the high split frequencies between the runs. The consensus tree for these analyses therefore excluded this run. Results from each of the analyses, regarding the monophyly status of *Sarcophaga s.l.* subgenera and the percentage of nodes with posterior probability (PP) of >0.90, is also given.

| | **CAD Bayesian analysis (Appendix 3a)** | **COI Bayesian analysis (Appendix 3b)** | **Morphological Bayesian analysis (Appendix 3c)** | **CAD + Morphological Bayesian analysis (Appendix 3d)** | **COI + Morphological Bayesian analysis (Appendix 3e)** | **CAD + COI Bayesian analysis (Appendix 3f)** | **CAD + COI + Morphological Bayesian analysis (Figure 77)** |
|---|---|---|---|---|---|---|---|
| **Number of generations** | 10,000,000 | 5,000,000 | 5,000,000 | 20,000,000 | 20,000,000 | 40,000,000 | 30,000,000 |
| **Sample frequency** | 5,000 | 500 | 500 | 5,000 | 5,000 | 5,000 | 5,000 |
| **Split frequencies** | 0.108207* | 0.013571 | 0.018497 | 0.103381* | 0.055689* | 0.010719 | 0.082900 |
| **Discarded as burn-in** | 20% | 20% | 20% | 15% | 10% | 25% | 27% |
| **Nodes with posterior probability of >0.90** | 48% | 55% | 30% | 56% | 75% | 49% | 78% |
| **SARCOPHAGINAE** | ✓ 1.00 | × | ✓ 1.00 | ✓ 1.00 | ✓ 1.00 | ✓ 0.75 | ✓ 1.00 |
| ***SARCOPHAGA*** | ✓ 0.77 | × | ✓ 1.00 | ✓ 0.88 | ✓ 0.77 | ✓ 0.74 | ✓ 0.93 |
| ***Lioproctia*** | × | × | × | × | × | × | × |
| ***Liopygia*** | ✓ 1.00 | ✓ 1.00 | ✓ 0.57 | × | ✓ 1.00 | ✓ 0.75 | ✓ 1.00 |
| ***Liosarcophaga*** | × | × | × | × | × | × | × |
| ***Parasarcophaga*** | × | × | ✓ 1.00 | × | ✓ 1.00 | × | ✓ 0.99 |
| ***Sarcorohdendorfia*** | × | × | × | × | × | × | × |


**Figure 77.** Bayesian inference of phylogeny for 39 species from 14 of the 132 *Sarcophaga s.l.* subgenera, based CAD, COI and morphological data. *GENERA* and *subgenera* are given on the right-hand side: white bars indicate Miltogramminae, black bars indicate Sarcophaginae (^ denotes subgenera of *Sarcophaga s.l.*). Numbers given at main branches refer to posterior probability values as a proportion. Morphological species identifications are given for all specimens along with voucher IDs. Outgroups are Miltogramminae specimens: *Miltogramma* Unknown A (KM837) and *Protomiltogramma* Unknown A (KM059). Evolutionary distance divergence scale bar, 0.1.

Sarcophaga s.l. *species commonly resolved together*

Two *Sarcorohdendorfia* species, *megafilosia* and *meiofilosia* are supposedly closely related given their similar biologies: both species are parasitoids of the marine snail *Littoraria filosa* (McKillup *et al.* 2000; Pape *et al.* 2000). Interestingly, these species were indeed strongly resolved together (Figure 77), with the lateral styli extending past the juxta identified as a diagnostic character for these species (Appendix 4).

*Classification of unknown species*

Given that this is a preliminary study assessing the utility of a range of markers to resolve members of the genus *Sarcophaga s.l.*, care must be taken with the classification of unknown species. Despite 78% of nodes having PP of >0.90, only two out of the five non-monotypic *Sarcophaga s.l.* subgenera included in this study were resolved as monophyletic (Figure 77). Considering this, subgeneric classifications of the unknowns can only be deemed reliable if the unknown specimens are resolved strongly with multiple species of a single subgenus.

Two morphologically identical female *Sarcophaga s.l.* specimens could not be reliably identified to the species level, but are included in the taxon set as *Sarcophaga* Unknown A (Appendix 1). Subgeneric classification of *Sarcophaga* Unknown A to *Sarcorohdendorfia* was possible, as both specimens possess setulae on the propleuron. This classification was confirmed by placement of this taxon with multiple other *Sarcorohdendorfia* species in the phylogeny (Figure 77). Another female *Sarcophaga s.l.* specimen which was not reliably identified was included as *Sarcophaga* Unknown B, and was resolved with *S.* (*Hardyella*) *littoralis* (PP of 1.00; Figure 77). Given that *Hardyella* is a monotypic subgenus, it is difficult to infer the identity of this Unknown from the analyses. Two new species have been included in the taxon set: *Sarcophaga* (*Sarcorohdendorfia*) **sp. nov.** (unnamed species near to *praedatrix* [based on male terminalia]; specimens KM670, KM672 and KM680) and *Sarcophaga* (*Sarcosolomonia*) *collessi* **sp. nov**. (specimens KM575, KM831 and KM865); with the description of these to follow in a possible subsequent publication. As the new *Sarcosolomonia* species is the only representative of this subgenus in the study, inferences about the monophyly of this particular subgenus cannot be made.

*Placement of monotypic subgenera*

Four monotypic subgenera, known exclusively from the Australasian/Oceanian region, were included in the taxon set: *Australopierretia*, *Fergusonimyia*, *Hardyella* and *Taylorimyia*. None of these monotypic subgenera emerged from within another subgenus (Figure 77), providing some evidence that they are correctly classified as monotypic, however this needs to be confirmed in a more comprehensive study.

Sarcophaga (Fergusonimyia) bancroftorum

*Fergusonimyia* is a monotypic subgenus of *Sarcophaga s.l.*, with its species *bancroftorum*, documented as highly morphologically variable between individuals. There are a few distinctive features that can facilitate identification, such as the male cercus possessing an enlargement at the apex and the 7th abdominal female sternite being tear drop shaped (Lopes 1958b). Additionally, the 2nd and 3rd antennomere of this species is at least partly yellow, sharing this characteristic with only two other sarcophagids included in this study (*S.* (*Lioproctia*) *torvida* and *S.* (*Liopygia*) *ruficornis*). Differences between *bancroftorum* specimens have been documented in the number of presutural dorsocentral and presutural acrostichal setae, absence or presence of setulae on the propleuron, colour and number of setae of the head, length of setulae on hind tibiae, along with the male terminalia (Lopes, 1958). Interestingly, morphologically variable specimens of *bancroftorum* were not resolved as a single species based on the COI barcode approach (Meiklejohn *et al.* 2012c *in press*). Despite this, to date all morphological and molecular variation between *bancroftorum* specimens has been classified as intraspecific variation, with separation into distinct species or subspecies not validated.

Four male specimens were confidently identified as *bancroftorum* and were included in the taxon set (Appendix 1). Variation between these specimens was evident upon examination of the terminalia, with the juxta differing in shape between (KM589+KM590) and (KM886+KM887). The specimens of these two *bancroftorum* forms were distinctly clustered together within a monophyletic clade, but separated by branches longer than expected (PP of 1.00; Figure 77). In addition to this, the taxon set included two female specimens that were identified as possibly *bancroftorum*. This identification is tentative, as the 2nd and 3rd antennomere of these specimens were at least partly yellow, but the presence of a tear drop shaped 7th abdominal sternite could not be confirmed (Appendix 1). Both female specimens, KM842 and KM691, were resolved together within the monophyletic *bancroftorum* clade (PP of 1.00, Figure 77).

These results highlight that there is extensive morphological and molecular variation among *bancroftorum* individuals. Future studies should focus on detailed examination of the morphological variation within the species, and determine whether such variation is in fact sufficient for the classification of additional species or subspecies. Amplification and sequencing of additional genes could assist with confirming different morphological forms prior to the description of new species.

Sarcophaga (*unplaced*) simplex

*Sarcophaga simplex* is one of approximately 25 sarcophagids that remain unplaced within a subgenus of *Sarcophaga s.l.*, and was also included in the taxon set of this study. Lopes (1967) tentatively classified *simplex* within the subgenus *Heteronychia*, however this classification was not accepted by Blackith and Blackith (1988) and Pape (1996), who did not assign this species to a subgenus. As no *Heteronychia* species with complete COI, CAD and morphological data were able to be included in this study, Lopes's tentative classification of *simplex* to this subgenus cannot be refuted. *Sarcophaga simplex* was not resolved within a monophyletic subgenus in the analysis: instead, it was resolved basally within a cluster which includes *Sarcophaga* Unknown B and the monotypic subgenus *Hardyella* (PP of 1.00; Figure 77). Based on this, *simplex* currently cannot be classified to a particular subgenus of *Sarcophaga s.l.*

## 4.1.4 Conclusions

The molecular gene regions of COI and CAD, along with morphological characters, are appropriate markers for use in a future more comprehensive study, examining the relationships within the genus *Sarcophaga s.l.* All possible combinations of these markers were trialled and the most well resolved phylogeny was inferred when the three data sets were combined. The barcode region of COI and the morphological data facilitated the strong support of each species cluster (PP of 1.00), and provided good resolution between some species. Fragment one of CAD facilitated high resolution of the Sarcophaginae and *Sarcophaga s.l.* (PP of 0.93-1.00); however support for nodes at the subgeneric level was poor. Resolution could be improved in future work by including another mitochondrial gene, such as ND4L or ITS2, which have provided good resolution between calliphorid genera and subgenera (Marinho *et al.* 2011; Wallman *et al.* 2005). Future extensive studies of *Sarcophaga s.l.* could also focus on obtaining scanning electron microscopy (SEM) images of male terminalia, to enable more subtle characters to be included within the character matrix. As it appears that the markers evaluated here provide greater phylogenetic signal when combined, it is suggested that future phylogenetic studies on the genus *Sarcophaga s.l.* should continue to combine data sets and at least include COI, CAD and morphological data.

# CHAPTER 5: **General Conclusions**

This study provides fresh insight into the taxonomy and systematics of the Australian Sarcophagidae. The revised taxonomic key produced and presented in Chapter 2 builds on the seminal work undertaken by Lopes in the 1950-1970s. To assist with bringing taxonomy into the 21st century, a computer-based LUCID key was generated (Chapter 2), providing an alternative platform for morphological identifications for non-taxonomists. Work was also undertaken to develop a molecular approach for the identification of sarcophagids. DNA barcoding was deemed effective for discriminating between forensically important adult sarcophagids, but also between all immature life stages of *Sarcophaga* (*Sarcorohdendorfia*) *impatiens* (Chapter 3). Molecular and morphological markers were evaluated for phylogenetic utility in a preliminary study sampling the large genus *Sarcophaga s.l.* (Chapter 4). The most well supported phylogeny was produced when all markers were used for inference, so future comprehensive studies of *Sarcophaga s.l.* should continue to combine data sets, and at least use COI, CAD and morphological data in their analyses.

## 5.1 Defining and updating the records and distribution of the Australian Sarcophagidae

Considerable effort was invested during the early stages of this study to better describe the basic taxonomy of the Australian Sarcophagidae, in terms of genera, subgenera and species, along with updating distributional records. Although records for the Australian sarcophagids will be subsequently updated (found online on the ABRS and ALA websites), it is vital that these records are maintained, especially for those species of potential forensic importance. Within the ANIC there are numerous drawers of unsorted pinned material. Identifying these specimens should be given high priority, as it is very plausible that this material may include undescribed species, along with additional species records for Australia.

## 5.2 Taxonomic revision of the Australian Sarcophagidae

The revised taxonomic key facilitates the species-level identification of all male Australian *Sarcophaga s.l.* species, along with providing illustrations and photographs of their terminalia within a single manuscript. As this study focussed on the *Sarcophaga s.l.*, miltogrammines and species of *Blaesoxipha* were only reliably identified to the genus-level. For inclusion of these groups in the taxonomic key, morphological features of the head and male terminalia could be examined for miltogrammines and species of *Blaesoxipha*, respectively. However, as the miltogrammines and *Blaesoxipha* have not been a major focus of taxonomic studies of Australian sarcophagids, future work should initially target their collection. Given that Australia has a rich fauna of Hymenoptera and Orthoptera, it is likely that the number of taxa currently documented for the miltogrammines and *Blaesoxipha* is a misrepresentation. Following comprehensive collection, a range of alternative morphological characters could be examined more closely, possibly through the use of scanning electron microscopy (SEM) images, to identify those that might allow species-level discrimination.

Species-level resolution of female flesh flies is difficult due to the high amounts of intraspecific morphological variation, but also due to the lack of informative terminalia characters. Consequently, only ~60% of female *Sarcophaga s.l.* species can be reliably identified using the revised key for the Australian Sarcophagidae. Rognes (1991) examined structural differences in microtrichiae found on the intersegmental membranes of the ovipositor, for discrimination between female blow flies. Given the usefulness of this character in blow flies, it is conceivable that similar success might be obtained for species-level separation of female sarcophagids, if examined for all species. Similarly, the immature stages of only relatively few Australian flesh flies have been documented (e.g. Cantrell 1981) because of their perceived taxonomic difficulty. It is plausible that examination of the cephaloskeleton and cuticular spinulation might assist with the recognition and phylogenetic analysis of the immatures of a greater range of sarcophagid taxa.

The LUCID key generated for the Australian *Sarcophaga s.l.* enables identification based on informative character states, rather than examination of stipulated morphological features. This key is available from the LUCID central website (http://keys.lucidcentral.org/keys/v3/Sarcophaga/) for public use. As it is likely that the known Australian flesh fly fauna will continue to increase in numbers, it is vital that the LUCID key is maintained as an identification tool that includes as much of the known fauna as possible. Thankfully, it is straight-forward to release new versions of the key, with the inclusion of extra species after their documentation. It is also possible to include additional morphological characters within the key to assist with the diagnosis of particularly challenging taxa. While these changes are possible, at this stage they will only be able to be done by KAM, as the original builder matrix is not publically available. If it appears that updating the key becomes too laborious, access to the original builder matrix could be given to select taxonomists to assist with managing updates in order to release future, more complete versions.

## 5.3 Molecular identification of the Australian Sarcophagidae

DNA barcoding was evaluated as a molecular approach for the identification of both adult and immature forensically important Australian sarcophagids. After a comprehensive evaluation using ~600 adult Australian sarcophagid specimens collected from across Australia, barcoding was deemed a reliable identification method. Similarly, all sarcophagid immature life stages were consistently identified by barcoding. As sarcophagids are commonly collected as evidence in forensic cases, these results will facilitate the identification of specimens by non-taxonomists such as lab technicans, and hopefully increase their use as PMI indicators.

Reference barcodes are currently not available for all Australian sarcophagids, with some species of potential forensic importance among those for which sequences are lacking. Additional work should be focused on obtaining a complete library of barcodes. The generation of such a library would also assist with alleviating concerns of false-positive misidentifications, especially for forensic specimens, which is possible when not all species have been sequenced. To establish this library, further taxon collecting, or using DNA from curated museum specimens may be required. The current study did include some preliminary work evaluating three different methods for extracting DNA from legs of curated museum specimens. Although DNA was successfully extracted from specimens up to 90 years old, the entire 648 bp barcode fragment could not be amplified in one reaction, with only small ~55bp COI fragments consistently amplified. The work undertaken in this study did not exhaustively test methods for extraction from curated specimens, and it is suggested that a range of non-destructive extraction methods, using whole specimens, be trialed.

Given the success of DNA barcoding for Australian sarcophagids, future studies could also look at testing this approach on a global scale. Several studies, conducted by others, have investigated the use of a broad range of mitochondrial genes for the identification of flesh flies (Bajpai and Tewari 2010; Draber-Monko *et al.* 2009; Guo *et al.* 2010; Wells *et al.* 2001b; Yadong *et al.* 2010; Zehner *et al.* 2004). It would however be beneficial to have a standardised approach, implemented for the global identification of sarcophagids. Once such an approach is accepted by the scientific community, researchers could develop a comprehensive database of COI sequences for all sarcophagids, with an initial focus on those of forensic importance.

Following reliable identification of sarcophagids, species-specific thermodevelopmental data need to be examined to estimate a PMI. Growth data have not been sufficiently documented for most sarcophagids, including species of potential forensic importance. Preliminary growth data have been collected for the forensically important species *Sarcophaga* (*Sarcorohdendorfia*) *impatiens* at 25$^{o}$C (unpublished), however these need to be expanded to include additional temperature conditions. Priority should be given to documenting the thermodevelopmental profiles of another two species known from forensic casework in Australia, *Sarcophaga* (*Sarcorohdendorfia*) *praedatrix* and *Sarcophaga* (*Liopygia*) *crassipalpis* (JFW, *pers. comm.*). In addition, growth rates should be studied under varying conditions (i.e. fluctuating temperature, humidity and light). Until such work has been completed, the use of sarcophagids to estimate PMI will remain restricted.

## 5.4 Preliminary phylogenetic analysis of the genus *Sarcophaga s.l.*

The utility of morphological data, along with the gene regions of COI and CAD, were evaluated for resolving the relationships within the genus *Sarcophaga s.l.* All three markers were deemed to provide useful phylogenetic signals, so future comprehensive studies, sampling more *Sarcophaga s.l.* subgenera and species, should consider combining at least each of these markers. Strong support at the species-level was provided by both COI and the morphological data, and CAD facilitated high resolution at basal levels. To assist with resolving relationships at the subgeneric level, additional markers would need to be added in a more comprehensive study. As the mitochondrial gene ND4L was effective at resolving relationships between *Calliphora* (Diptera: Calliphoridae) subgenera (Wallman *et al.* 2005), this gene region could be a supplementary marker in subsequent studies. Additionally, ITS2 has proven to be informative at the species and genus level within calliphorids (Marinho *et al.* 2011), and could also be evaluated for *Sarcophaga s.l.* SEM images of the male terminalia could also be fundamental for identifying additional characters for subgeneric classifications. Creating a library of SEM images for exemplar species of each subgenus may be challenging, as obtaining high quality images is very time consuming, expensive and sometimes requires the terminalia to be sacrificed. It is likely that with technological advancements, this approach may become more achievable in the future and prove very beneficial.

Once a robust and well sampled phylogeny of the genus *Sarcophaga s.l.* is achieved, it can be used to determine whether the current subgeneric classifications are valid. Considering that nearly 50% of the subgenera are monotypic, it is highly likely that some of these will be reclassified and combined into fewer subgenera. The phylogeny could also be used to explore current hypotheses for some subgenera; for example, *Lioproctia* and *Johnstonimyia* may be a single subgenus (Pape and Kurahashi 2004); and *Australopierretia*, *Boettcherisca, Fergusonimyia, Hardyella* and *Sarcorohdendorfia* may be sister taxa, as all species possess at least some setulae on the propleuron. Studies on *Sarcophaga s.l.* should also endeavour to include as many of the unplaced taxa as possible, to assist with their subgeneric assignment.

This PhD research has made a novel and diverse contribution to our knowledge of the Australian Sarcophagidae, with a special emphasis on *Sarcophaga s.l.* species. Previously, identification of Australian sarcophagids, and sarcophagids in general, was challenging for non-taxonomists. The work documented here provides three alternative methods for identification for a broad range of users: a revised taxonomic key for Diptera experts; a LUCID key for those with general biology/entomology knowledge; and a molecular identification method for those lacking experience in taxonomy. This work has also provided a strong platform for future phylogenetic studies on the genus *Sarcophaga s.l.* by testing both molecular and morphological markers. It is hoped that aspects of this research can be applied to sarcophagids from other zoogeographic regions, and that work on the Australian members of these fascinating flies will continue in the future.

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# Appendices

**Appendix 1.** Specimen information, voucher identification (ID) code, sex (male♂, female♀), COI and CAD GenBank accession numbers and collection localities for the sarcophagids sampled. Collection locations are given with suburb and state, with abbreviations as follows: ACT, Australian Capital Territory; NSW, New South Wales; NT, Northern Territory; Qld Queensland; SA, South Australia; Tas, Tasmania; Vic, Victoria; and WA, Western Australia. * denotes that the species is of potential forensic importance, and # denotes that the species is a parasitoid. For species lacking a symbol, the biology is not documented. Additionally, ^ denotes that the species has been documented in Australian forensic cases (JFW *pers. comm.*) and + specimens incorrectly identified by Meiklejohn (2011).

| Classification SUBFAMILY *GENUS* *Subgenus* *species* | Voucher ID | Sex | GenBank accession No. COI | GenBank accession No. CAD | Collection locality |
|---|---|---|---|---|---|
| **MILTOGRAMMINAE** | | | | | |
| ***MILTOGRAMMA* #** | | | | | |
| **Unknown A** | KM837 | ♀ | JN964689 | JQ290802 | Lamington NP, Qld (28° 8' S, 153° 7' E) |
| ***PROTOMILTOGRAMMA* #** | | | | | |
| **Unknown A** | KM059 | ♀ | JN964698 | JQ290568 | Kakadu NP, NT (12° 54' S, 132° 30' E) |
| | | | | | |
| **SARCOPHAGINAE** | | | | | |
| ***BLAESOXIPHA*** Loew | | | | | |
| ***Blaesoxipha* #** | | | | | |
| ***apoxa*** | KM908 | ♂ | JN964688 | JQ290814 | Kanjini, WA (22° 21' S, 118° 16' E) |
| Pape, 1994 | | | | | |
| | | | | | |
| ***OXYSARCODEXIA*** Townsend | | | | | |
| ***varia* *** | JW229v1 | ♀ | GQ254420 | JQ290538 | Orbost, Vic (37° 43'S, 148° 28'E) |
| (Walker, 1836) | JW229v2 | ♀ | GQ254419 | n/a | Orbost, Vic (37° 43'S, 148° 28'E) |
| | JW230 | ♀ | GQ254423 | n/a | Brodribb River, Vic (37° 43'S, 148° 34'E) |
| | JW231 | ♂ | GQ254422 | n/a | Metung, Vic (37° 49'S, 147° 52'E) |
| | JW232v1 | ♂ | GQ254421 | n/a | Harrietville, Vic (36° 52'S, 147° 4'E) |
| | JW232v2 | ♀ | GQ254425 | n/a | Harrietville, Vic (36° 52'S, 147° 4'E) |
| | JW238 | ♀ | GQ254424 | n/a | Holbrook, NSW (35° 42'S, 147° 19'E) |
| | KM718 | ♂ | JN964697 | JQ290898 | Bagdad, Tas (42° 37'S, 147° 15'E) |
| | KM726 | ♂ | JN964696 | JQ290881 | Fentonbury, Tas (42° 38'S, 146° 45'E) |
| | KM731 | ♀ | JN964695 | JQ290899 | Little Swanport, Tas (42° 20'S, 147° 56'E) |
| | KM734 | ♂ | JN964694 | JQ290777 | Waratah, Tas (41° 24'S, 145° 35'E) |
| | KM735 | ♂ | JN964693 | JQ290901 | Stanley, Tas (40° 48'S, 145° 16'E) |
| | KM738 | ♀ | JN964692 | JQ290932 | Richmond, Tas (42° 44'S, 147° 25'E) |
| | KM745 | ♂ | JN964691 | JQ290778 | Poatina, Tas (41° 53'S, 146° 50'E) |
| | KM746 | ♂ | JN964690 | JQ290902 | Seven Mile Beach, Tas (42° 50'S, 147° 32'E) |
| | | | | | |
| ***SARCOPHAGA*** Meigen | | | | | |
| ***Australopierretia*** | | | | | |
| ***australis* *** | JW210 | ♀ | GQ254431 | n/a | Berry, NSW (34°48'S, 150°46'E) |
| (Johnston and Tiegs, 1921) | JW211v1 | ♀ | GQ254430 | JQ290512 | Yass, NSW (34°50'S, 148°55'E) |
| | JW211v2 | ♀ | GQ254433 | n/a | Yass, NSW (34°50'S, 148°55'E) |
| | JW212 | ♀ | GQ254432 | JQ290526 | Pinaroo, NSW (35°15'S, 140°54'E) |
| | JW221 | ♀ | GQ254429 | JQ290531 | Kensington Gardens, SA (34°55'S, 139°40'E) |
| | KM046 | ♀ | JN964762 | JQ290558 | Kakadu NP, NT (12° 38' S, 132° 34' E) |
| | KM099 | ♀ | JN964761 | JQ290583 | Kakadu NP, NT (12° 25' S, 132° 58' E) |
| | KM102 | ♀ | JN964760 | JQ290584 | Edith Falls, NT (14° 10' S, 132° 11' E) |
| | KM104 | ♂ | JN964759 | n/a | Edith Falls, NT (14° 10' S, 132° 11' E) |
| | KM107 | ♀ | JN964758 | JQ290586 | Umbrawarra Gorge, NT (13° 57' S, 131° 41' E) |
| | KM117 | ♀ | JN964757 | n/a | Umbrawarra Gorge, NT (13° 57' S, 131° 41' E) |
| | KM140 | ♀ | JN964756 | JQ290680 | Darwin, NT (12° 27' S, 130° 50' E) |
| | KM153 | ♀ | JN964755 | JQ290685 | East Point, NT (12° 24' S, 130° 49' E) |
| | KM160 | ♀ | JN964754 | n/a | East Point, NT (12° 24' S, 130° 49' E) |
| | KM168 | ♀ | JN964753 | JQ290691 | Berry Springs, NT (12° 42' S, 131° 0' E) |
| | KM169 | ♀ | JN964752 | n/a | Berry Springs, NT (12° 42' S, 131° 0' E) |
| | KM219 | ♂ | JN964751 | JQ290848 | Kalamundra NP, WA (31° 58' S, 116° 3' E) |
| | KM280 | ♂ | JN964750 | n/a | Chapman River, WA (28° 46' S, 114° 43' E) |
| | KM292 | ♂ | JN964749 | JQ290850 | Kalbarri NP, WA (27° 41' S, 114° 11' E) |

| | | | | | |
|---|---|---|---|---|---|
| | KM300 | ♀ | JN964748 | JQ290597 | Dongara, WA (29° 16' S, 114° 55' E) |
| | KM318 | ♀ | JN964747 | JQ290602 | WA (30° 15' S, 115° 10' E) |
| | KM326 | ♀ | JN964746 | JQ290606 | Orange Springs, WA (30° 59' S, 115° 42' E) |
| | KM328 | ♀ | JN964745 | JQ290851 | Woodridge, WA (31° 18' S, 115° 36' E) |
| | KM426 | ♀ | JN964744 | JQ290624 | Broomehill East, WA (33° 52' S, 117° 46' E) |
| | KM433 | ♂ | JN964743 | JQ290628 | Williams, WA (33° 1' S, 116° 52' E) |
| | KM457 | ♀ | JN964742 | n/a | Wungong Dam, WA (32° 11' S, 116° 3' E) |
| | KM469 | ♀ | JN964741 | JQ290634 | Armadale, WA (32° 7' S, 116° 1' E) |
| | KM501 | ♂ | JN964740 | JQ290925 | Ipswich, Qld (27° 34' S, 152° 46' E) |
| | KM598 | ♀ | JN964739 | JQ290927 | Mt Larcom, Qld (23° 48' S, 150° 59' E) |
| | KM673 | ♂ | JN964738 | JQ290753 | Henrietta Creek, Qld (17° 35' S, 145° 45' E) |
| | KM720 | ♀ | JN964737 | JQ290775 | Bagdad, Tas (42° 36' S, 147° 14' E) |
| | KM762 | ♀ | JN964736 | n/a | Currawinya NP, Qld (28° 43' S, 144° 29' E) |
| | KM847 | ♀ | JN964735 | n/a | Noonbah Station, Qld (24° 8' S, 143° 11' E) |
| | KM862 | ♀ | JN964763 | n/a | Qld (19° 58' S, 145° 34' E) |
| | | | | | |
| ***Baranovisca*** | | | | | |
| ***cyrtophorae*** **#** | KM1032 | ♂ | JN964814 | n/a | Split Rock, Qld (15° 23' S, 144° 18' E) |
| (Cantrell ,1986) | KM1098 | ♀ | JN964813 | n/a | Yeerongpilly, Qld (27° 31' S, 153° 0' E) |
| | | | | | |
| ***Bercaea*** | | | | | |
| ***africa*** ***** | JW200 | ♀ | GQ254444 | JQ290519 | Yass, NSW (34° 50' S, 148° 54' E) |
| (Wiedemann, 1824) | KM336 | ♀ | JN964710 | JQ290609 | Rockingham, WA (32° 16' S, 115° 43' E) |
| | KM461 | ♂ | JN964709 | JQ290642 | Armadale, WA (32° 7' S, 116° 1' E) |
| | KM465 | ♀ | JN964708 | JQ290827 | Armadale, WA (32° 7' S, 116° 1' E) |
| | KM468 | ♀ | JN964707 | JQ290633 | Armadale, WA (32° 7' S, 116° 1' E) |
| | KM471 | ♀ | JN964706 | JQ290828 | Armadale, WA (32° 7' S, 116° 1' E) |
| | KM488 | ♀ | JN964705 | JQ290645 | WA (31° 55' S, 116° 19' E) |
| | KM510 | ♂ | JN964704 | JQ290656 | Dalby, Qld (27° 11' S, 151° 15' E) |
| | KM618 | ♂ | JN964703 | JQ290795 | Yepoon, Qld (23° 9' S, 150° 45' E) |
| | KM974 | ♂ | JN964702 | JQ290819 | Kensington Gardens, SA (34° 55' S, 138° 39' E) |
| | KM977 | ♀ | JN964701 | JQ290883 | Kensington Gardens, SA (34° 55' S, 138° 39' E) |
| | KM981 | ♀ | JN964700 | JQ290820 | Kensington Gardens, SA (34° 55' S, 138° 39' E) |
| | KM982 | ♀ | JN964699 | JQ290821 | Kensington Gardens, SA (34° 55' S, 138° 39' E) |
| | | | | | |
| ***Boettcherisca*** | | | | | |
| ***peregrina*** ***** | JW245 + | ♀ | GQ254478 | n/a | Cairns, Qld (16° 59' S, 145° 46' E) |
| (Robineau-Desvoidy, 1830) | KM714 | ♂ | JN965026 | JQ290773 | Ingham, Qld (18° 35' S, 146° 9' E) |
| | | | | | |
| ***Fergusonimyia*** | | | | | |
| ***bancroftorum*** ***** | KM589 | ♂ | JN965187 | JQ290895 | Cania Gorge NP, Qld (24° 42' S, 150° 59' E) |
| Johnston and Tiegs, 1921 | KM590 | ♂ | JN965186 | JQ290714 | Cania Gorge NP, Qld (24° 42' S, 150° 59' E) |
| | KM813 | ♂ | JN965183 | n/a | Enoggera Reservoir, Qld (27° 26' S, 152° 54' E) |
| | KM886 | ♂ | JN965180 | JQ290938 | Zenith Beach, NSW (32° 43' E, 152° 10' S) |
| | KM887 | ♂ | JN965174 | JQ290906 | Zenith Beach, NSW (32° 43' E, 152° 10' S) |
| | | | | | |
| **possibly** ***bancroftorum*** ***** | KM691 | ♀ | JN965184 | JQ290916 | Ellis Beach, Qld (16° 40' S, 145° 34' E) |
| | KM822 | ♀ | JN965182 | n/a | Qld (19° 41' S, 146° 26' E) |
| | KM838 | ♀ | JN965177 | n/a | Lamington NP, Qld (28° 8' S, 153° 7' E) |
| | KM842 | ♀ | JN965172 | JQ290803 | Lamington NP, Qld (28° 8' S, 153° 7' E) |
| | | | | | |
| ***Hardyella*** | | | | | |
| ***littoralis*** ***** # | KM197 | ♀ | JN964955 | n/a | Berry Springs, NT (12° 42' S, 131° 0' E) |
| Johnston and Tiegs, 1922 | KM710 | ♂ | JN964954 | JQ290847 | Byfield State Forest, Qld (22° 55' S, 150° 40' E) |
| | | | | | |
| ***Johnstonimyia*** | | | | | |
| ***kappa*** ***** | KM031 | ♂ | JN964953 | n/a | Healesville, Vic (37° 41' S, 145° 31' E) |
| Johnston and Tiegs, 1921 | KM038 | ♂ | JN964952 | JQ290918 | Berry Springs, NT (12° 40' S, 131° 3' E) |
| | KM050 | ♀ | JN964951 | JQ290560 | Katherine, NT (14° 24' S, 132° 20' E) |
| | KM069 | ♀ | JN964950 | JQ290921 | Litchfield NP, NT (13° 12' S, 130° 44' E) |
| | KM089 | ♀ | JN964949 | JQ290577 | Kakadu NP, NT (12° 25' S, 132° 58' E) |
| | KM134 | ♀ | JN964948 | JQ290930 | Darwin, NT (12° 27' S, 130° 50' E) |
| | KM155 | ♀ | JN964947 | JQ290686 | East Point, NT (12° 24' S, 130° 49' E) |
| | KM178 | ♀ | JN964946 | JQ290693 | Berry Springs, NT (12° 42' S, 131° 0' E) |
| | KM184 | ♀ | JN964945 | n/a | Berry Springs, NT (12° 42' S, 131° 0' E) |
| | KM204 | ♂ | JN964944 | n/a | Berry Springs, NT (12° 42' S, 131° 0' E) |
| | KM570 | ♂ | JN964943 | n/a | Munduberra, Qld (25° 30' S, 151° 17' E) |
| | KM583 | ♂ | JN964942 | JQ290838 | Eidsvold, Qld (25° 22' S, 151° 8' E) |
| | KM594 | ♂ | JN964941 | JQ290879 | Catfish Creek, Qld (24° 0' S, 151° 1' E) |
| | KM603 | ♂ | JN964940 | JQ290896 | Rockhampton, Qld (23° 22' S, 150° 37' E) |

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| | KM625 | ♂ | JN964939 | JQ290926 | Marlborough, Qld (22° 48' S, 149° 50' E) |
| | KM808 | ♀ | JN964938 | n/a | Qld (19° 41' S, 146° 26' E) |
| | | | | | |
| ***Lioproctia*** | | | | | |
| ***alcicornis**** | KM657 | ♂ | JN964717 | JQ290743 | Paluma NP, Qld (19° 0' S, 146° 16' E) |
| Hardy, 1932 | KM678 | ♀ | JN964716 | JQ290755 | Millaa Millaa, Qld (17° 29' S, 145° 39' E) |
| | KM763 | ♂ | JN964715 | JQ290882 | Qld (19° 58' S, 145° 34' E) |
| | KM766 | ♀ | JN964714 | n/a | Qld (19° 58' S, 145° 34' E) |
| | KM864 | ♂ | JN964713 | n/a | Qld (19° 58' S, 145° 34' E) |
| | KM913 | ♀ | JN964712 | JQ290816 | Qld (16° 32' S, 145° 22' E) |
| | KM993 | ♀ | JN964711 | JQ290934 | Qld (24° 31' S, 151° 28' E) |
| | | | | | |
| ***multicolor**** | KM624 | ♂ | JN964990 | JQ290727 | Byfield NP, Qld (22° 49' S, 150° 37' E) |
| Johnston and Tiegs, 1922 | KM633 | ♂ | JN964989 | JQ290798 | Finch Hatton Gorge, Qld (21° 4' S, 148° 38' E) |
| | KM643 | ♂ | JN964988 | JQ290739 | Conway NP, Qld (20° 17' S, 148° 45' E) |
| | KM911 | ♀ | JN964987 | n/a | Misty Mountains, Qld (17° 39' S, 145° 52' E) |
| | | | | | |
| ***spinifera**** | KM587 | ♂ | JN965099 | JQ290840 | Cania Gorge NP, Qld (24° 42' S, 150° 59' E) |
| Hardy, 1932 | | | | | |
| | | | | | |
| ***torvida**** | KM047 | ♂ | JN965160 | JQ290863 | Katherine, NT (14° 24' S, 132° 20' E) |
| (Lopes, 1959) | KM064 | ♂ | JN965159 | JQ290913 | Kakadu NP, NT (12° 51' S, 132° 42' E) |
| | KM065 | ♀ | JN965158 | n/a | Kakadu NP, NT (12° 51' S, 132° 42' E) |
| | KM100 | ♀ | JN965157 | n/a | Edith Falls, NT (14° 10' S, 132° 11' E) |
| | KM103 | ♀ | JN965156 | n/a | Edith Falls, NT (14° 10' S, 132° 11' E) |
| | KM781 | ♀ | JN965155 | n/a | Currawinya NP, Qld (28° 43' S, 144° 29' E) |
| | | | | | |
| ***Liopygia*** | | | | | |
| ***crassipalpis**^*** | JW198v1 | ♂ | GQ254443 | JQ290516 | Kensington Gardens, SA (34° 55' S, 138° 39' E) |
| Macquart, 1839 | JW198v2 | ♂ | GQ254440 | n/a | Kensington Gardens, SA (34° 55' S, 138° 39' E) |
| | JW199v1 | ♀ | GQ254442 | JQ290518 | Coburg, Vic (37° 44' S, 144° 57' E) |
| | JW199v2 | ♀ | GQ254441 | n/a | Coburg, Vic (37° 44' S, 144° 57' E) |
| | KM004 | ♀ | JN964812 | n/a | Healesville, Vic (37° 40' S, 145° 31' E) |
| | KM207 | ♂ | JN964811 | JQ290866 | Kalamundra NP, WA (31° 58' S, 116° 3' E) |
| | KM334 | ♀ | JN964810 | JQ290608 | Rockingham, WA (32° 16' S, 115° 43' E) |
| | KM339 | ♀ | JN964809 | JQ290611 | Rockingham, WA (32° 16' S, 115° 43' E) |
| | KM340 | ♀ | JN964808 | JQ290612 | Rockingham, WA (32° 16' S, 115° 43' E) |
| | KM341 | ♂ | JN964807 | n/a | Rockingham, WA (32° 16' S, 115° 43' E) |
| | KM342 | ♂ | JN964806 | JQ290789 | Rockingham, WA (32° 16' S, 115° 43' E) |
| | KM343 | ♂ | JN964805 | JQ290872 | Rockingham, WA (32° 16' S, 115° 43' E) |
| | KM351 | ♀ | JN964804 | JQ290791 | Busselton, WA (33° 40' S, 115° 18' E) |
| | KM352 | ♀ | JN964803 | JQ290707 | Dunsborough, WA (33° 38' S, 115° 6' E) |
| | KM353 | ♂ | JN964802 | JQ290873 | Dunsborough, WA (33° 37' S, 115° 7' E) |
| | KM459 | ♂ | JN964801 | JQ290632 | Armadale, WA (32° 7' S, 116° 1' E) |
| | KM464 | ♀ | JN964800 | JQ290826 | Armadale, WA (32° 7' S, 116° 1' E) |
| | KM496 | ♂ | JN964799 | JQ290649 | Nixon Park, Qld (27° 33' S, 152° 58' E) |
| | KM885 | ♀ | JN964798 | JQ290748 | Oakleigh East, Vic (37° 53' S, 145° 6' E) |
| | KM890 | ♂ | JN964797 | JQ290808 | Black Mountain, ACT (35° 16' S, 149° 5' E) |
| | KM894 | ♀ | JN964796 | JQ290809 | Black Mountain, ACT (35° 16' S, 149° 5' E) |
| | KM899 | ♀ | JN964795 | JQ290818 | Mangerton, NSW (34° 25' S, 150° 52' E) |
| | KM967 | ♀ | JN964794 | n/a | Oakleigh East, Vic (37° 53' S, 145° 6' E) |
| | | | | | |
| ***ruficornis**** | KM137 | ♀ | JN965097 | JQ290678 | Darwin, NT (12° 27' S, 130° 50' E) |
| (Fabricius, 1794) | KM138 | ♀ | JN965096 | JQ290679 | Darwin, NT (12° 27' S, 130° 50' E) |
| | KM630 | ♂ | JN965095 | n/a | Mackay, Qld (21° 9' S, 149° 9' E) |
| | KM707 | ♀ | JN965094 | JQ290942 | Cairns, Qld (16° 59' S, 145° 44' E) |
| | KM708 | ♂ | JN965093 | n/a | Cairns, Qld (16° 59' S, 145° 44' E) |
| | KM881 | ♀ | JN965092 | n/a | Yeerongpilly, Qld (27° 31' S, 153° 0' E) |
| | | | | | |
| ***Liosarcophaga*** | | | | | |
| ***dux**** | JW217v1 | ♀ | GQ254447 | JQ290529 | Balranald, NSW (34° 7' S, 143° 30' E) |
| Thomson, 1869 | JW233 | ♂ | GQ254445 | JQ290539 | Perth, WA (31° 56' S, 115° 56' E) |
| | JW246v1 | ♀ | GQ254449 | n/a | Pinaroo, NSW (35° 15' S, 140° 54' E) |
| | KM012 | ♂ | JN964831 | JQ290552 | Fraser Is., Qld (25° 15' S, 153° 10' E) |
| | KM052 | ♀ | JN964830 | JQ290562 | Katherine, NT (14° 24' S, 132° 20' E) |
| | KM158 | ♀ | JN964829 | JQ290689 | East Point, NT (12° 24' S, 130° 49' E) |
| | KM284 | ♀ | JN964828 | JQ290870 | Chapman River, WA (28° 46' S, 114° 43' E) |
| | KM296 | ♂ | JN964827 | JQ290705 | Dongara, WA (29° 16' S, 114° 55' E) |
| | KM297 | ♂ | JN964826 | JQ290908 | Dongara, WA (29° 16' S, 114° 55' E) |
| | KM333 | ♂ | JN964825 | JQ290706 | Rockingham, WA (32° 16' S, 115° 43' E) |

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| | KM338 | ♀ | JN964824 | JQ290610 | Rockingham, WA (32° 16' S, 115° 43' E) |
| | KM507 | ♂ | JN964823 | JQ290655 | Dalby, Qld (27° 11' S, 151° 15' E) |
| | KM610 | ♀ | JN964822 | JQ290722 | Yepoon, Qld (23° 9' S, 150° 45' E) |
| | KM627 | ♀ | JN964821 | JQ290729 | Clareview, Qld (22° 7' S, 149° 32' E) |
| | KM628 | ♂ | JN964820 | JQ290730 | Clareview, Qld (22° 7' S, 149° 32' E) |
| | KM640 | ♀ | JN964819 | JQ290737 | Prosperine, Qld (20° 22' S, 148° 35' E) |
| | KM659 | ♂ | JN964818 | JQ290749 | Ingham, Qld (18° 35' S, 146° 9' E) |
| | KM661 | ♂ | JN964817 | JQ290750 | Innisfail, Qld (17° 32' S, 146° 1' E) |
| | KM705 | ♂ | JN964816 | JQ290769 | Cairns, Qld (16° 59' S, 145° 44' E) |
| | KM903 | ♀ | JN964815 | JQ290813 | Perth, WA (31° 55' S, 115° 58' E) |
| | | | | | |
| ***kohla**** | KM621 | ♂ | JN965185 | JQ290725 | Byfield NP, Qld (22° 49' S, 150° 37' E) |
| Johnston and Hardy, 1923a | KM717 | ♀ | JN965175 | JQ290774 | Ingham, Qld (18° 35' S, 146° 9' E) |
| | | | | | |
| ***sigma**** | JW213 | ♀ | GQ254427 | n/a | Wadonga, Vic (36° 5' S, 146° 42' E) |
| Johnston and Tiegs, 1921 | JW227v1 | ♂ | GQ254426 | JQ290536 | Yass, NSW (34° 50' S, 148° 54' E) |
| | JW228v1 | ♂ | GQ254428 | JQ290537 | Mt Samson, Qld (27° 18' S, 152° 50' E) |
| | KM060 | ♂ | JN964734 | JQ290569 | Kakadu NP, NT (12° 54' S, 132° 31' E) |
| | KM090 | ♀ | JN964733 | JQ290578 | Kakadu NP, NT (12° 25' S, 132° 58' E) |
| | KM224 | ♀ | JN964732 | JQ290641 | Twin Swamp Reserve, WA (31° 44' S, 116° 1' E) |
| | KM283 | ♂ | JN964731 | JQ290884 | Chapman River, WA (28° 46' S, 114° 43' E) |
| | KM480 | ♀ | JN964730 | JQ290643 | Clackline, WA (31° 43' S, 116° 29' E) |
| | KM499 | ♂ | JN964729 | JQ290651 | Ipswich, Qld (27° 34' S, 152° 46' E) |
| | KM519 | ♂ | JN964728 | JQ290894 | Jandowae, Qld (26° 42' S, 151° 16' E) |
| | KM532 | ♂ | JN964727 | n/a | Nanango, Qld (26° 26' S, 152° 3' E) |
| | KM642 | ♂ | JN964726 | JQ290738 | Conway NP, Qld (20° 17' S, 148° 45' E) |
| | KM692 | ♂ | JN964725 | JQ290761 | Ellis Beach, Qld (16° 40' S, 145° 34' E) |
| | KM730 | ♂ | JN964724 | JQ290776 | Little Swanport, Tas (42° 20' S, 147° 56' E) |
| | KM732 | ♀ | JN964723 | JQ290900 | Little Swanport, Tas (42° 20' S, 147° 56' E) |
| | KM761 | ♀ | JN964722 | JQ290782 | Currawinya NP, Qld (28° 43' S, 144° 29' E) |
| | KM777 | ♀ | JN964721 | n/a | Currawinya NP, NSW (29° 55' S, 144° 26' E) |
| | KM866 | ♂ | JN964720 | JQ290805 | Charles Darwin Reserve, WA (29° 30' S, 117° 3' E) |
| | | | | | |
| ***Parasarcophaga*** | | | | | |
| ***misera**** | KM136 | ♂ | JN964986 | JQ290677 | Darwin, NT (12° 27' S, 130° 50' E) |
| Walker, 1849 | KM141 | ♂ | JN964985 | JQ290910 | Darwin, NT (12° 27' S, 130° 50' E) |
| | KM142 | ♂ | JN964984 | n/a | Darwin, NT (12° 27' S, 130° 50' E) |
| | KM145 | ♀ | JN964983 | n/a | Humpty Doo, NT (12° 35' S, 131° 7' E) |
| | KM146 | ♀ | JN964982 | JQ290682 | Humpty Doo, NT (12° 35' S, 131° 7' E) |
| | KM150 | ♂ | JN964981 | JQ290683 | Humpty Doo, NT (12° 35' S, 131° 7' E) |
| | KM159 | ♀ | JN964980 | n/a | East Point, NT (12° 24' S, 130° 49' E) |
| | KM534 | ♀ | JN964979 | JQ290666 | Gympie, Qld (26° 5' S, 152° 20' E) |
| | KM613 | ♀ | JN964978 | JQ290723 | Yepoon, Qld (23° 9' S, 150° 45' E) |
| | KM615 | ♂ | JN964977 | JQ290794 | Yepoon, Qld (23° 9' S, 150° 45' E) |
| | KM631 | ♂ | JN964976 | JQ290797 | Mackay, Qld (21° 9' S, 149° 9' E) |
| | KM636 | ♂ | JN964975 | JQ290735 | Qld (21° 2' S, 148° 43' E) |
| | KM644 | ♂ | JN964974 | JQ290799 | Ayr, Qld (19° 34' S, 147° 24' E) |
| | KM645 | ♀ | JN964973 | JQ290740 | Ayr, Qld (19° 34' S, 147° 24' E) |
| | KM651 | ♂ | JN964972 | JQ290843 | Ayr, Qld (19° 34' S, 147° 24' E) |
| | KM655 | ♂ | JN964971 | JQ290742 | Townsville, Qld (19° 13' S, 146° 37' E) |
| | KM663 | ♂ | JN964970 | JQ290859 | Innisfail, Qld (17° 32' S, 146° 1' E) |
| | KM664 | ♀ | JN964969 | JQ290744 | Innisfail, Qld (17° 32' S, 146° 1' E) |
| | KM667 | ♂ | JN964968 | JQ290745 | Innisfail, Qld (17° 32' S, 146° 1' E) |
| | KM681 | ♀ | JN964967 | JQ290756 | Mareeba, Qld (16° 59' S, 145° 25' E) |
| | KM683 | ♂ | JN964966 | JQ290757 | Mareeba, Qld (16° 59' S, 145° 25' E) |
| | KM687 | ♂ | JN964965 | JQ290758 | Mareeba, Qld (16° 59' S, 145° 25' E) |
| | KM688 | ♀ | JN964964 | JQ290759 | Mt Molloy, Qld (16° 40' S, 145° 19' E) |
| | KM690 | ♀ | JN964963 | JQ290760 | Ellis Beach, Qld (16° 40' S, 145° 34' E) |
| | KM693 | ♂ | JN964962 | JQ290762 | Ellis Beach, Qld (16° 40' S, 145° 34' E) |
| | KM694 | ♂ | JN964961 | JQ290941 | Kuranda, Qld (16° 48' S, 145° 37' E) |
| | KM696 | ♀ | JN964960 | JQ290764 | Kuranda, Qld (16° 48' S, 145° 37' E) |
| | KM698 | ♂ | JN964959 | JQ290765 | Little Mulgrave River, Qld (17° 8' S, 145° 43' E) |
| | KM699 | ♀ | JN964958 | JQ290766 | Little Mulgrave River, Qld (17° 8' S, 145° 43' E) |
| | KM700 | ♂ | JN964957 | JQ290767 | Little Mulgrave River, Qld (17° 8' S, 145° 43' E) |
| | KM706 | ♀ | JN964956 | JQ290770 | Cairns, Qld (16° 59' S, 145° 44' E) |
| | | | | | |
| ***taenionota**** | JW214v1 | ♂ | GQ254491 | JQ290527 | Wangarratta, Vic (36° 21' S, 146° 20' E) |
| (Wiedemann, 1819) | JW214v2 + | ♂ | GQ254498 | n/a | Wangarratta, Vic (36° 21' S, 146° 20' E) |
| | JW215 | ♀ | GQ254497 | JQ290528 | Mullengandra Village, NSW (35° 53' S, 147° 9' E) |
| | JW216v3 + | ♂ | GQ254492 | n/a | Yass, NSW (34° 50' S, 148° 54' E) |

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| | JW217v2 [+] | ♀ | GQ254499 | n/a | Balranald , NSW (34° 7' S, 143° 30' E) |
| | JW222v1 | ♀ | GQ254501 | JQ290532 | Tarcutta, NSW (35° 9' S, 147° 39' E) |
| | JW222v2 [+] | ♂ | GQ254500 | n/a | Tarcutta, NSW (35° 9' S, 147° 39' E) |
| | JW225v1 [+] | ♂ | GQ254494 | n/a | Wagga, NSW (35° 6' S, 147° 22' E) |
| | JW225v2 [+] | ♂ | GQ254493 | n/a | Wagga, NSW (35° 6' S, 147° 22' E) |
| | JW226v1 | ♀ | GQ254488 | JQ290535 | Gundagai, NSW (35° 3' S, 148° 6' E) |
| | JW226v2 [+] | ♀ | GQ254486 | | Gundagai, NSW (35° 3' S, 148° 6' E) |
| | JW236v1 | ♀ | GQ254496 | JQ290542 | Holbrook, NSW (35° 42' S, 147° 19' E) |
| | JW236v2 [+] | ♂ | GQ254495 | n/a | Holbrook, NSW (35° 42' S, 147° 19' E) |
| | JW239v1 [+] | ♂ | GQ254484 | n/a | Narrandera, NSW (34° 45' S, 146° 34' E) |
| | JW239v2 [+] | ♂ | GQ254485 | n/a | Narrandera, NSW (34° 45' S, 146° 34' E) |
| | JW242v1 | ♀ | GQ254490 | JQ290545 | Wallabadah, NSW (33° 1' S, 151° 0' E) |
| | JW242v2 [+] | ♀ | GQ254489 | n/a | Wallabadah, NSW (33° 1' S, 151° 0' E) |
| | JW243 [+] | ♀ | GQ254487 | n/a | Narrandera, NSW (34° 45' S, 146° 34' E) |
| | KM045 | ♂ | JN965154 | JQ290551 | Kakadu NP, NT (12° 38' S, 132° 34' E) |
| | KM068 | ♀ | JN965153 | JQ290571 | Litchfield NP, NT (13° 12' S, 130° 44' E) |
| | KM076 | ♂ | JN965152 | n/a | Litchfield NP, NT (13° 12' S, 130° 44' E) |
| | KM077 | ♂ | JN965151 | n/a | Litchfield NP, NT (13° 12' S, 130° 44' E) |
| | KM101 | ♀ | JN965150 | JQ290920 | Edith Falls, NT (14° 10' S, 132° 11' E) |
| | KM105 | ♂ | JN965149 | n/a | Edith Falls, NT (14° 10' S, 132° 11' E) |
| | KM109 | ♂ | JN965148 | JQ290588 | Umbrawarra Gorge, NT (13° 57' S, 131° 41' E) |
| | KM114 | ♂ | JN965147 | n/a | Umbrawarra Gorge, NT (13° 57' S, 131° 41' E) |
| | KM115 | ♂ | JN965146 | n/a | Umbrawarra Gorge, NT (13° 57' S, 131° 41' E) |
| | KM119 | ♀ | JN965145 | n/a | Umbrawarra Gorge, NT (13° 57' S, 131° 41' E) |
| | KM122 | ♀ | JN965144 | n/a | Umbrawarra Gorge, NT (13° 57' S, 131° 41' E) |
| | KM128 | ♀ | JN965143 | JQ290594 | Kakadu NP, NT (13° 17' S, 132° 20' E) |
| | KM233 | ♂ | JN965142 | JQ290822 | Bindoon, WA (31° 17' S, 116° 5' E) |
| | KM235 | ♂ | JN965141 | JQ290696 | Bindoon, WA (31° 17' S, 116° 5' E) |
| | KM242 | ♂ | JN965140 | JQ290697 | Bindoon, WA (31° 17' S, 116° 5' E) |
| | KM249 | ♂ | JN965139 | JQ290698 | WA (31° 7' S, 116° 3' E) |
| | KM252 | ♂ | JN965138 | JQ290824 | WA (31° 7' S, 116° 3' E) |
| | KM344 | ♂ | JN965137 | JQ290613 | Serpentine, WA (32° 21' S, 115° 59' E) |
| | KM460 | ♂ | JN965136 | JQ290792 | Armadale, WA (32° 7' S, 116° 1' E) |
| | KM466 | ♀ | JN965135 | JQ290854 | Armadale, WA (32° 7' S, 116° 1' E) |
| | KM479 | ♂ | JN965134 | JQ290644 | Clackline, WA (31° 43' S, 116° 29' E) |
| | KM498 | ♂ | JN965133 | JQ290830 | Ipswich, Qld (27° 34' S, 152° 46' E) |
| | KM512 | ♂ | JN965132 | JQ290657 | Dalby, Qld (27° 11' S, 151° 15' E) |
| | KM513 | ♀ | JN965131 | JQ290658 | Chinchilla, Qld (26° 45' S, 150° 37' E) |
| | KM515 | ♂ | JN965130 | JQ290833 | Chinchilla, Qld (26° 42' S, 150° 40' E) |
| | KM517 | ♂ | JN965129 | JQ290660 | Jandowae, Qld (26° 42' S, 151° 16' E) |
| | KM526 | ♂ | JN965128 | JQ290663 | Qld (26° 49' S, 151° 40' E) |
| | KM527 | ♂ | JN965127 | JQ290855 | Maidenwell, Qld (26° 49' S, 151° 4' E) |
| | KM533 | ♂ | JN965126 | JQ290665 | Gympie, Qld (26° 5' S, 152° 20' E) |
| | KM536 | ♂ | JN965125 | JQ290668 | Gympie, Qld (26° 5' S, 152° 20' E) |
| | KM539 | ♂ | JN965124 | JQ290670 | Gympie, Qld (26° 11' S, 152° 39' E) |
| | KM561 | ♂ | JN965123 | n/a | Munduberra, Qld (25° 35' S, 151° 18' E) |
| | KM563 | ♂ | JN965122 | JQ290878 | Munduberra, Qld (25° 30' S, 151° 17' E) |
| | KM581 | ♂ | JN965121 | JQ290713 | Eidsvold, Qld (25° 22' S, 151° 8' E) |
| | KM592 | ♀ | JN965120 | n/a | Callide Dam, Qld (24° 20' S, 150° 37' E) |
| | KM593 | ♂ | JN965119 | JQ290716 | Callide Dam, Qld (24° 20' S, 150° 37' E) |
| | KM616 | ♂ | JN965118 | n/a | Yepoon, Qld (23° 9' S, 150° 45' E) |
| | KM634 | ♀ | JN965117 | JQ290733 | Finch Hatton Gorge, Qld (21° 4' S, 148° 38' E) |
| | KM635 | ♀ | JN965116 | JQ290734 | Qld (21° 2' S, 148° 43' E) |
| | KM637 | ♂ | JN965115 | JQ290736 | Qld (21° 2' S, 148° 43' E) |
| | KM652 | ♂ | JN965114 | JQ290844 | Bowling Green Bay NP, Qld (19° 25' S, 146° 56' E) |
| | KM713 | ♀ | JN965113 | JQ290772 | Ingham, Qld (18° 35' S, 146° 9' E) |
| | KM815 | ♂ | JN965112 | JQ290801 | The Tombs, Qld (25° 4' S, 147° 51' E) |
| | KM902 | ♀ | JN965111 | JQ290812 | Wangarratta, NSW (36° 21' S, 146° 20' E) |
| | | | | | |
| ***Sarcorohdendorfia*** | | | | | |
| ***alpha**** | KM805 | ♀ | JN964719 | n/a | Lamington NP, Qld (28° 8' S, 153° 7' E) |
| Johnston and Tiegs, 1922 | KM994 | ♂ | JN964718 | JQ290861 | Qld (24° 31' S, 151° 28' E) |
| | | | | | |
| ***bidentata**** | KM039 | ♀ | JN964792 | JQ290554 | Adelaide River, NT (13° 17' S, 131° 11' E) |
| (Lopes, 1953) | KM051 | ♀ | JN964791 | JQ290561 | Katherine, NT (14° 24' S, 132° 20' E) |
| | KM070 | ♀ | JN964790 | JQ290572 | Litchfield NP, NT (13° 12' S, 130° 44' E) |
| | KM073 | ♂ | JN964789 | JQ290573 | Litchfield NP, NT (13° 12' S, 130° 44' E) |
| | KM075 | ♂ | JN964788 | n/a | Litchfield NP, NT (13° 12' S, 130° 44' E) |
| | KM078 | ♂ | JN964787 | n/a | Litchfield NP, NT (13° 12' S, 130° 44' E) |
| | KM079 | ♂ | JN964786 | n/a | Kakadu NP, NT (12° 51' S, 132° 42' E) |

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| | KM080 | ♂ | JN964785 | n/a | Kakadu NP, NT (12° 51' S, 132° 42' E) |
| | KM085 | ♀ | JN964784 | n/a | Kakadu NP, NT (12° 51' S, 132° 42' E) |
| | KM086 | ♀ | JN964783 | JQ290575 | Elsey NP, NT (14° 56' S, 133° 6' E) |
| | KM088 | ♀ | JN964782 | JQ290915 | Kakadu NP, NT (12° 51' S, 132° 42' E) |
| | KM098 | ♀ | JN964781 | JQ290582 | Kakadu NP, NT (12° 25' S, 132° 58' E) |
| | KM110 | ♂ | JN964780 | JQ290589 | Umbrawarra Gorge, NT (13° 57' S, 131° 41' E) |
| | KM112 | ♂ | JN964779 | n/a | Umbrawarra Gorge, NT (13° 57' S, 131° 41' E) |
| | KM125 | ♀ | JN964778 | JQ290592 | Kakadu NP, NT (13° 17' S, 132° 20' E) |
| | KM127 | ♀ | JN964777 | JQ290593 | Kakadu NP, NT (13° 17' S, 132° 20' E) |
| | KM130 | ♂ | JN964776 | n/a | Kakadu NP, NT (13° 17' S, 132° 20' E) |
| | KM151 | ♂ | JN964775 | JQ290684 | Humpty Doo, NT (12° 35' S, 131° 7' E) |
| | KM152 | ♀ | JN964774 | n/a | East Point, NT (12° 24' S, 130° 49' E) |
| | KM162 | ♂ | JN964773 | JQ290911 | East Point, NT (12° 24' S, 130° 49' E) |
| | KM797 | ♀ | JN964772 | JQ290860 | Currawinya NP, Qld, (28° 48' S, 144° 27' E) |
| | KM827 | ♀ | JN964771 | n/a | Qld (19° 41' S, 146° 26' E) |
| | KM844 | ♀ | JN964770 | n/a | Noonbah Station, Qld (24° 8' S, 143° 12' E) |
| | KM850 | ♀ | JN964769 | n/a | Qld (20° 7' S, 146° 37' E) |
| | KM853 | ♂ | JN964768 | n/a | Qld (20° 7' S, 146° 37' E) |
| | KM900 | ♀ | JN964767 | JQ290811 | Katherine, NT (132° 33' S, 14° 36' E) |
| | | | | | |
| ***bifrons**** | KM547 | ♀ | JN964793 | JQ290674 | Great Sandy NP, Qld ( 25° 56' S, 153° 4' E) |
| Walker, 1853 | | | | | |
| | | | | | |
| ***froggatti**** | JW224 | ♂ | GQ254503 | JQ290534 | Balranald, NSW (34° 7' S, 143° 30' E) |
| Taylor, 1917 | JW244 | ♀ | GQ254502 | JQ290546 | Broome, WA (17° 57' S, 122° 14' E) |
| | KM040 | ♀ | JN964857 | JQ290555 | Adelaide River, NT (13° 17' S, 131° 11' E) |
| | KM042 | ♀ | JN964856 | JQ290557 | Kakadu NP, NT (12° 38' S, 132° 34' E) |
| | KM043 | ♀ | JN964855 | n/a | Kakadu NP, NT (12° 38' S, 132° 34' E) |
| | KM044 | ♀ | JN964854 | n/a | Kakadu NP, NT (12° 38' S, 132° 34' E) |
| | KM048 | ♂ | JN964853 | JQ290559 | Katherine, NT (14° 24' S, 132° 20' E) |
| | KM049 | ♂ | JN964852 | n/a | Katherine, NT (14° 24' S, 132° 20' E) |
| | KM053 | ♀ | JN964851 | JQ290563 | Katherine, NT (14° 24' S, 132° 20' E) |
| | KM095 | ♀ | JN964850 | n/a | Kakadu NP, NT (12° 25' S, 132° 58' E) |
| | KM116 | ♂ | JN964849 | n/a | Umbrawarra Gorge, NT (13° 57' S, 131° 41' E) |
| | KM194 | ♀ | JN964848 | JQ290694 | Berry Springs, NT (12° 42' S, 131° 0' E) |
| | KM270 | ♂ | JN964847 | JQ290700 | Eradu Nature Reserve, WA (28° 43' S, 115° 2' E) |
| | KM272 | ♂ | JN964846 | JQ290701 | Eradu Nature Reserve, WA (28° 43' S, 115° 2' E) |
| | KM279 | ♂ | JN964845 | JQ290703 | Chapman River, WA (28° 46' S, 114° 43' E) |
| | KM436 | ♂ | JN964844 | JQ290629 | Williams, WA (33° 1' S, 116° 52' E) |
| | KM508 | ♀ | JN964843 | JQ290831 | Dalby, Qld (27° 11' S, 151° 15' E) |
| | KM514 | ♂ | JN964842 | JQ290659 | Chinchilla, Qld (26° 42' S, 150° 40' E) |
| | KM521 | ♀ | JN964841 | JQ290662 | Jandowae, Qld (26° 42' S, 151° 16' E) |
| | KM522 | ♀ | JN964840 | JQ290835 | Jandowae, Qld (26° 42' S, 151° 16' E) |
| | KM562 | ♂ | JN964839 | n/a | Munduberra, Qld (25° 35' S, 151° 18' E) |
| | KM591 | ♀ | JN964838 | JQ290841 | Biloela, Qld (24° 33' S, 150° 40' E) |
| | KM629 | ♂ | JN964837 | JQ290731 | Clareview, Qld (22° 7' S, 149° 32' E) |
| | KM760 | ♀ | JN964836 | JQ290781 | Currawinya NP, Qld (28° 43' S, 144° 29' E) |
| | KM774 | ♀ | JN964835 | n/a | Currawinya NP, Qld (28° 55' S, 144° 26' E) |
| | KM814 | ♂ | JN964834 | n/a | Currawinya NP, Qld (28° 55' S, 144° 26' E) |
| | KM884 | ♂ | JN964833 | JQ290807 | Culgoa Flood Plain NP, Qld (28° 55' S, 147° 0' E) |
| | KM909 | ♂ | JN964832 | n/a | Kanjini, WA (22° 21' S, 118° 16' E) |
| | | | | | |
| ***furcata**** | JW234 | ♂ | GQ254450 | JQ290540 | Kings Park, WA (31° 57' S, 115° 49' E) |
| Hardy, 1932 | | | | | |
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| ***impatiens**^** | JW49v1 | ♀ | GQ254451 | n/a | Cambewarra, NSW (34° 49' S, 150° 34' E) |
| Walker, 1849 | JW49v2 | ♀ | GQ254472 | JQ290513 | Cambewarra, NSW (34° 49' S, 150° 34' E) |
| | JW188 | ♀ | GQ254471 | JQ290514 | Mt Keira, NSW (34° 25' S, 150° 52' E) |
| | JW194 | ♀ | GQ254468 | n/a | Mt Keira, NSW (34° 25' S, 150° 52' E) |
| | JW201v1 | ♀ | GQ254469 | n/a | Brisbane, Qld (27° 20' S, 152° 58' E) |
| | JW201v2 | ♀ | GQ254466 | JQ290520 | Brisbane, Qld (27° 20' S, 152° 58' E) |
| | JW202 | ♀ | GQ254467 | JQ290521 | The Oaks, NSW (34° 4' S, 150° 34' E) |
| | JW203 | ♂ | GQ254464 | JQ290522 | Coffs Harbour, NSW (30° 18' S, 153° 7' E) |
| | JW204 | ♀ | GQ254470 | n/a | Adelaide, SA (34° 54' S, 138° 40' E) |
| | JW205 | ♂ | GQ254465 | n/a | Adelaide, SA (34° 54' S, 138° 40' E) |
| | JW206v1 | ♀ | GQ254454 | JQ290523 | Coburg , Vic (37° 44' S, 144° 57' E) |
| | JW206v2 | ♂ | GQ254455 | n/a | Coburg, Vic (37° 44' S, 144° 57' E) |
| | JW207v1 | ♀ | GQ254457 | n/a | Bega, NSW (36° 40' S, 149° 49' E) |
| | JW207v2 | ♀ | GQ254456 | JQ290524 | Bega, NSW (36° 40' S, 149° 49' E) |
| | JW220v1 | ♂ | GQ254460 | n/a | Kensington Gardens, SA (34° 55' S, 138° 39' E) |

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| | JW220v2 | ♂ | GQ254458 | n/a | Kensington Gardens, SA (34° 55' S, 138° 39' E) |
| | JW235v1 | ♀ | GQ254463 | JQ290541 | Highton, Vic (38° 11' S, 144° 17' E) |
| | JW235v2 | ♀ | GQ254462 | n/a | Highton, Vic (38° 11' S, 144° 17' E) |
| | JW248v1 | ♀ | GQ254452 | JQ290548 | Moorebank, NSW (33° 58' S, 150° 20' E) |
| | JW248v2 | ♀ | GQ254453 | n/a | Moorebank, NSW (33° 58' S, 150° 20' E) |
| | JW249v1 | ♀ | GQ254459 | JQ290549 | Holsworthy, NSW (33º 58'S, 150º 55'E) |
| | JW249v2 | ♀ | GQ254461 | n/a | Holsworthy, NSW (33º 58'S, 150º 55'E) |
| | KM001 | ♀ | JN964885 | n/a | Healesville, Vic (37° 40' S, 145° 31' E) |
| | KM002 | ♀ | JN964886 | n/a | Healesville, Vic (37° 40' S, 145° 31' E) |
| | KM010 | ♂ | JN964884 | n/a | Healesville, Vic (37° 40' S, 145° 31' E) |
| | KM013 | ♂ | JN964883 | n/a | Oakdale, NSW (34° 4' S, 150° 30' E) |
| | KM014 | ♀ | JN964882 | n/a | Healesville, Vic (37° 41' S, 145° 31' E) |
| | KM019 | ♂ | JN964881 | n/a | AJ Davis Reserve, Vic (37° 43' S, 144° 52' E) |
| | KM021 | ♀ | JN964880 | n/a | Healesville, Vic (37° 41' S, 145° 31' E) |
| | KM024 | ♀ | JN964879 | JQ290553 | Springvale, Vic (37° 57' S, 145° 9' E) |
| | KM025 | ♂ | JN964878 | n/a | Springvale, Vic (37° 57' S, 145° 9' E) |
| | KM494 | ♀ | JN964877 | JQ290647 | Nixon Park, Qld (27° 33' S, 152° 58' E) |
| | KM497 | ♀ | JN964876 | JQ290650 | Ipswich, Qld (27° 34' S, 152° 46' E) |
| | KM503 | ♀ | JN964875 | JQ290652 | Lake Manchester, Qld (27° 32' S, 152° 45' E) |
| | KM599 | ♂ | JN964874 | JQ290718 | Rockhampton, Qld (23° 22' S, 150° 30' E) |
| | KM605 | ♀ | JN964873 | JQ290858 | Emu Park, Qld (23° 15' S, 150° 49' E) |
| | KM608 | ♀ | JN964872 | JQ290720 | Yepoon, Qld (23° 9' S, 150° 45' E) |
| | KM623 | ♀ | JN964871 | JQ290726 | Byfield NP, Qld (22° 49' S, 150° 37' E) |
| | KM671 | ♀ | JN964870 | JQ290751 | Wooroonooran NP, Qld (17° 37' S, 145° 44' E) |
| | KM674 | ♀ | JN964869 | JQ290754 | Millaa Millaa, Qld (17° 29' S, 145° 39' E) |
| | KM695 | ♀ | JN964868 | JQ290763 | Kuranda, Qld (16° 48' S, 145° 37' E) |
| | KM704 | ♀ | JN964867 | JQ290768 | Danbulla NP, Qld (17° 10' S, 145° 39' E) |
| | KM712 | ♂ | JN964866 | JQ290771 | Byfield State Forest, Qld (22° 55' S, 150° 40' E) |
| | KM867 | ♀ | JN964865 | n/a | Canungra, Qld (28° 12' S, 153° 7' E) |
| | KM882 | ♂ | JN964864 | JQ290806 | Yeerongpilly, Qld (27° 31' S, 153° 0' E) |
| | KM910 | ♀ | JN964863 | JQ290815 | Sylvania, NSW (34° 0' S, 151° 5' E) |
| | KM916 | ♂ | JN964862 | JQ290817 | Waurn Ponds, Vic (38° 12' S, 144° 17' E) |
| | KM966 | ♀ | JN964861 | n/a | Dandenong, Vic (37° 59' S, 145° 12' E) |
| | | | | | |
| ***megafilosia* #** | JW247v1 | ♂ | GQ254479 | JQ290547 | Keppel Sands, Qld (23° 13' S, 150° 41' E) |
| Pape, McKillup and | JW247v2 | ♀ | GQ254480 | n/a | Keppel Sands, Qld (23° 13' S, 150° 41' E) |
| McKillup, 2000 | | | | | |
| | | | | | |
| ***meiofilosia* #** | JW250v1 | ♀ | GQ254481 | JQ290517 | Keppel Sands, Qld (23° 13' S, 150° 41' E) |
| Pape, McKillup and | JW250v2 | ♀ | GQ254482 | n/a | Keppel Sands, Qld (23° 13' S, 150° 41' E) |
| McKillup, 2000 | | | | | |
| | | | | | |
| ***omikron* *** | KM221 | ♂ | JN965021 | JQ290639 | John Forrest NP, WA (31° 53' S, 116° 5' E) |
| Johnston and Tiegs, 1921 | KM225 | ♂ | JN965020 | JQ290931 | Bindoon, WA (31° 17' S, 116° 5' E) |
| | KM228 | ♂ | JN965019 | JQ290929 | Bindoon, WA (31° 17' S, 116° 5' E) |
| | KM239 | ♂ | JN965018 | JQ290849 | Bindoon, WA (31° 17' S, 116° 5' E) |
| | KM255 | ♀ | JN965017 | JQ290868 | WA (31° 7' S, 116° 3' E) |
| | KM257 | ♂ | JN965016 | JQ290889 | Moora, WA (30° 38' S, 116° 0' E) |
| | KM259 | ♂ | JN965015 | JQ290886 | Coorow, WA (29° 59' S, 116° 5' E) |
| | KM269 | ♂ | JN965014 | n/a | Eradu Nature Reserve, WA (28° 43' S, 115° 2' E) |
| | KM282 | ♂ | JN965013 | JQ290887 | Chapman River, WA (28° 46' S, 114° 43' E) |
| | KM304 | ♀ | JN965012 | n/a | Dongara, WA (29° 16' S, 114° 55' E) |
| | KM309 | ♂ | JN965011 | n/a | WA (29° 56' S, 114° 59' E) |
| | KM311 | ♀ | JN965010 | JQ290919 | WA (29° 56' S, 114° 59' E) |
| | KM321 | ♂ | JN965009 | JQ290604 | Nambung NP, WA (30° 35' S, 115° 6' E) |
| | KM322 | ♂ | JN965008 | JQ290888 | Orange Springs, WA (30° 59' S, 115° 42' E) |
| | KM325 | ♂ | JN965007 | JQ290885 | Orange Springs, WA (30° 59' S, 115° 42' E) |
| | KM331 | ♂ | JN965006 | JQ290788 | Roleystone, WA (32° 7' S, 116° 3' E) |
| | KM335 | ♀ | JN965005 | JQ290890 | Rockingham, WA (32° 16' S, 115° 43' E) |
| | KM350 | ♂ | JN965004 | JQ290923 | Wellington Dam, WA (33° 19' S, 116° 2' E) |
| | KM354 | ♂ | JN965003 | JQ290935 | Margaret River, WA (33° 47' S, 115° 3' E) |
| | KM356 | ♀ | JN965002 | n/a | Nannup, WA (34° 3' S, 115° 45' E) |
| | KM374 | ♂ | JN965001 | JQ290617 | Stirling Range NP, WA (34° 26' S, 118° 4' E) |
| | KM402 | ♂ | JN965000 | JQ290936 | Broomehill East, WA (33° 52' S, 117° 46' E) |
| | KM429 | ♂ | JN964999 | JQ290922 | Little Parkeyerring Lake, WA (33° 22' S, 117° 21' E) |
| | KM440 | ♀ | JN964998 | JQ290937 | Williams, WA (33° 1' S, 116° 52' E) |
| | KM441 | ♂ | JN964997 | n/a | Hotham River, WA (32° 46' S, 116° 35' E) |
| | KM445 | ♂ | JN964996 | JQ290891 | WA (32° 34' S, 116° 26' E) |
| | KM458 | ♂ | JN964995 | n/a | Wungong Dam, WA (32° 11' S, 116° 3' E) |
| | KM473 | ♀ | JN964994 | JQ290829 | Westdale , WA (32° 13' S, 116° 24' E) |

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| | KM475 | ♂ | JN964993 | JQ290875 | Beverley, WA (32° 10' S, 116° 50' E) |
| | KM476 | ♂ | JN964992 | JQ290892 | York, WA (31° 50' S, 116° 46' E) |
| | KM482 | ♂ | JN964991 | JQ290893 | Mingenew, WA (29° 11' S, 115° 26' E) |
| | KM582 | ♂ | JN965025 | JQ290939 | Eidsvold, Qld (25° 22' S, 151° 8' E) |
| | KM804 | ♂ | JN965024 | n/a | Charles Darwin Reserve, WA (29° 30' S, 117° 3' E) |
| | KM834 | ♀ | JN965023 | JQ290783 | Lochada, WA (29° 11' S, 116° 30' E) |
| | KM848 | ♀ | JN965022 | JQ290804 | Noonbah Station, Qld (24° 8' S, 143° 11' E) |
| | | | | | |
| ***piva* *** | KM622 | ♀ | JN965098 | JQ290796 | Byfield NP, Qld (22° 49' S, 150° 37' E) |
| Roback, 1952 | | | | | |
| | | | | | |
| ***praedatrix* *^** | JW131 + | ♂ | GQ254439 | n/a | Coffs Harbour, NSW (30° 18' S, 153° 7' E) |
| Walker, 1849 | JW197 | ♂ | GQ254438 | JQ290515 | Mt Sampson, Qld (27° 18' S, 152° 50' E) |
| | JW208 | ♀ | GQ254435 | JQ290550 | Urunga, NSW (30° 28' S, 153° 2' E) |
| | JW209v1 | ♀ | GQ254436 | JQ290525 | Lismore, NSW (28° 48' S, 153° 17' E) |
| | JW209v2 + | ♀ | GQ254437 | n/a | Lismore, NSW (28° 48' S, 153° 17' E) |
| | KM041 | ♂ | JN965091 | JQ290556 | Kakadu NP, NT (12° 38' S, 132° 34' E) |
| | KM054 | ♀ | JN965090 | JQ290564 | Katherine, NT (14° 24' S, 132° 20' E) |
| | KM055 | ♀ | JN965089 | JQ290565 | Kakadu NP, NT (12° 54' S, 132° 31' E) |
| | KM056 | ♀ | JN965088 | JQ290566 | Kakadu NP, NT (12° 54' S, 132° 31' E) |
| | KM057 | ♀ | JN965087 | n/a | Kakadu NP, NT (12° 54' S, 132° 31' E) |
| | KM061 | ♂ | JN965086 | n/a | Kakadu NP, NT (12° 54' S, 132° 31' E) |
| | KM066 | ♀ | JN965085 | JQ290570 | Litchfield NP, NT (13° 12' S, 130° 44' E) |
| | KM067 | ♀ | JN965084 | n/a | Litchfield NP, NT (13° 12' S, 130° 44' E) |
| | KM081 | ♀ | JN965083 | JQ290574 | Kakadu NP, NT (12° 51' S, 132° 42' E) |
| | KM082 | ♀ | JN965082 | n/a | Kakadu NP, NT (12° 51' S, 132° 42' E) |
| | KM083 | ♀ | JN965081 | JQ290914 | Kakadu NP, NT (12° 51' S, 132° 42' E) |
| | KM084 | ♀ | JN965080 | n/a | Kakadu NP, NT (12° 51' S, 132° 42' E) |
| | KM087 | ♀ | JN965079 | n/a | Elsey NP, NT (14° 56' S, 133° 6' E) |
| | KM091 | ♀ | JN965078 | JQ290579 | Kakadu NP, NT (12° 25' S, 132° 58' E) |
| | KM092 | ♀ | JN965077 | JQ290580 | Kakadu NP, NT (12° 25' S, 132° 58' E) |
| | KM093 | ♀ | JN965076 | JQ290864 | Kakadu NP, NT (12° 25' S, 132° 58' E) |
| | KM094 | ♀ | JN965075 | JQ290581 | Kakadu NP, NT (12° 25' S, 132° 58' E) |
| | KM106 | ♂ | JN965074 | JQ290585 | Edith Falls, NT (14° 10' S, 132° 11' E) |
| | KM108 | ♀ | JN965073 | JQ290587 | Umbrawarra Gorge, NT (13° 57' S, 131° 41' E) |
| | KM111 | ♂ | JN965072 | n/a | Umbrawarra Gorge, NT (13° 57' S, 131° 41' E) |
| | KM113 | ♂ | JN965071 | n/a | Umbrawarra Gorge, NT (13° 57' S, 131° 41' E) |
| | KM118 | ♀ | JN965070 | n/a | Umbrawarra Gorge, NT (13° 57' S, 131° 41' E) |
| | KM120 | ♀ | JN965069 | JQ290590 | Umbrawarra Gorge, NT (13° 57' S, 131° 41' E) |
| | KM123 | ♀ | JN965068 | JQ290591 | Kakadu NP, NT (13° 17' S, 132° 20' E) |
| | KM124 | ♀ | JN965067 | n/a | Kakadu NP, NT (13° 17' S, 132° 20' E) |
| | KM131 | ♀ | JN965066 | JQ290595 | Darwin, NT (12° 27' S, 130° 50' E) |
| | KM132 | ♀ | JN965065 | JQ290909 | Darwin, NT (12° 27' S, 130° 50' E) |
| | KM133 | ♀ | JN965064 | JQ290676 | Darwin, NT (12° 27' S, 130° 50' E) |
| | KM135 | ♂ | JN965063 | n/a | Darwin, NT (12° 27' S, 130° 50' E) |
| | KM143 | ♀ | JN965062 | JQ290681 | Humpty Doo, NT (12° 35' S, 131° 7' E) |
| | KM144 | ♀ | JN965091 | n/a | Humpty Doo, NT (12° 35' S, 131° 7' E) |
| | KM156 | ♀ | JN965060 | JQ290687 | East Point, NT (12° 24' S, 130° 49' E) |
| | KM157 | ♀ | JN965059 | JQ290688 | East Point, NT (12° 24' S, 130° 49' E) |
| | KM166 | ♀ | JN965058 | JQ290690 | Berry Springs, NT (12° 42' S, 131° 0' E) |
| | KM167 | ♀ | JN965057 | n/a | Berry Springs, NT (12° 42' S, 131° 0' E) |
| | KM172 | ♀ | JN965056 | JQ290692 | Berry Springs, NT (12° 42' S, 131° 0' E) |
| | KM174 | ♀ | JN965055 | n/a | Berry Springs, NT (12° 42' S, 131° 0' E) |
| | KM186 | ♀ | JN965054 | JQ290912 | Berry Springs, NT (12° 42' S, 131° 0' E) |
| | KM495 | ♀ | JN965053 | JQ290648 | Nixon Park, Qld (27° 33' S, 152° 58' E) |
| | KM504 | ♀ | JN965052 | JQ290653 | Lake Manchester, Qld (27° 32' S, 152° 45' E) |
| | KM516 | ♂ | JN965051 | JQ290834 | Jandowae, Qld (26° 42' S, 151° 16' E) |
| | KM520 | ♀ | JN965050 | JQ290661 | Jandowae, Qld (26° 42' S, 151° 16' E) |
| | KM528 | ♀ | JN965049 | JQ290856 | Maidenwell, Qld (26° 49' S, 151° 4' E) |
| | KM529 | ♀ | JN965048 | JQ290664 | Nanango, Qld (26° 26' S, 152° 3' E) |
| | KM535 | ♀ | JN965047 | JQ290667 | Gympie, Qld (26° 5' S, 152° 20' E) |
| | KM537 | ♀ | JN965046 | JQ290669 | Gympie, Qld (26° 11' S, 152° 39' E) |
| | KM541 | ♀ | JN965045 | JQ290671 | Rainbow Beach, Qld (26° 0' S, 153° 2' E) |
| | KM542 | ♀ | JN965044 | JQ290672 | Rainbow Beach, Qld (26° 0' S, 153° 2' E) |
| | KM544 | ♀ | JN965043 | JQ290673 | Poona Beach, Qld (25° 53' S, 153° 5' E) |
| | KM549 | ♀ | JN965042 | JQ290675 | Poona Creek, Qld (25° 45' S, 152° 51' E) |
| | KM553 | ♂ | JN965041 | n/a | Brooweena, Qld (25° 33' S, 152° 27' E) |
| | KM556 | ♀ | JN965040 | JQ290876 | Qld (25° 34' S, 151° 57' E) |
| | KM557 | ♀ | JN965039 | JQ290877 | Munduberra, Qld (25° 35' S, 151° 18' E) |
| | KM565 | ♂ | JN965038 | JQ290857 | Munduberra, Qld (25° 30' S, 151° 17' E) |

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|---|---|---|---|---|---|
| | KM566 | ♂ | JN965037 | JQ290711 | Munduberra, Qld (25° 30' S, 151° 17' E) |
| | KM595 | ♂ | JN965036 | JQ290717 | Catfish Creek, Qld (24° 0' S, 151° 1' E) |
| | KM604 | ♂ | JN965035 | JQ290719 | Emu Park, Qld (23° 15' S, 150° 49' E) |
| | KM609 | ♀ | JN965034 | JQ290721 | Yepoon, Qld (23° 9' S, 150° 45' E) |
| | KM614 | ♀ | JN965033 | JQ290880 | Yepoon, Qld (23° 9' S, 150° 45' E) |
| | KM620 | ♀ | JN965032 | JQ290724 | Byfield NP, Qld (22° 59' S, 150° 41' E) |
| | KM626 | ♂ | JN965031 | JQ290728 | Clareview, Qld (22° 7' S, 149° 32' E) |
| | KM632 | ♂ | JN965030 | JQ290732 | Finch Hatton Gorge, Qld (21° 4' S, 148° 38' E) |
| | KM653 | ♂ | JN965029 | JQ290741 | Magnetic Is., Qld (19° 6' S, 146° 51' E) |
| | KM709 | ♀ | JN965028 | JQ290897 | Byfield State Forest, Qld (22° 55' S, 150° 40' E) |
| | KM880 | ♀ | JN965027 | JQ290907 | Yeerongpilly, Qld (27° 31' S, 153° 0' E) |
| | | | | | |
| ***spinigera** ** | KM258 | ♀ | JN965110 | JQ290825 | Coorow, WA (29° 59' S, 116° 5' E) |
| (Lopes, 1953) | KM260 | ♀ | JN965109 | JQ290869 | WA (28° 33' S, 115° 38' E) |
| | KM265 | ♂ | JN965108 | JQ290785 | Eradu Nature Reserve, WA (28° 43' S, 115° 2' E) |
| | KM273 | ♂ | JN965107 | JQ290702 | Eradu Nature Reserve, WA (28° 43' S, 115° 2' E) |
| | KM289 | ♀ | JN965106 | JQ290786 | Kalbarri NP, WA (27° 52' S, 114° 31' E) |
| | KM293 | ♀ | JN965105 | JQ290787 | Kalbarri NP, WA (27° 41' S, 114° 11' E) |
| | KM295 | ♂ | JN965104 | JQ290871 | Isseka, WA (28° 27' S, 114° 38' E) |
| | KM481 | ♀ | JN965103 | n/a | Mingenew, WA (29° 11' S, 115° 26' E) |
| | KM487 | ♂ | JN965102 | n/a | Mingenew, WA (29° 11' S, 115° 26' E) |
| | KM770 | ♀ | JN965101 | n/a | Arima, Qld (26° 32' S, 142° 30' E) |
| | KM783 | ♀ | JN965100 | n/a | Currawinya NP, Qld (28° 43' S, 144° 29' E) |
| | | | | | |
| ***villisterna** ** | KM058 | ♀ | JN965163 | JQ290567 | Kakadu NP, NT (12° 54' S, 132° 31' E) |
| Salem, 1946 | KM602 | ♂ | JN965162 | JQ290793 | Rockhampton, Qld (23° 22' S, 150° 37' E) |
| | KM650 | ♀ | JN965161 | JQ290800 | Ayr, Qld (19° 34' S, 147° 24' E) |
| | | | | | |
| | | | | | |
| ***zeta** ** | JW223 | ♀ | GQ254434 | JQ290533 | Orbost, Vic (37° 43' S, 148° 27' E) |
| Johnston and Tiegs, 1921 | KM506 | ♂ | JN965170 | JQ290654 | Toowoomba, Qld (27° 32' S, 152° 3' E) |
| | KM509 | ♀ | JN965169 | JQ290832 | Dalby, Qld (27° 11' S, 151° 15' E) |
| | KM548 | ♀ | JN965168 | JQ290837 | Poona Creek, Qld (25° 45' S, 152° 51' E) |
| | KM580 | ♂ | JN965167 | JQ290712 | Eidsvold, Qld (25° 22' S, 151° 8' E) |
| | KM586 | ♀ | JN965166 | JQ290839 | Cania Gorge NP, Qld (24° 42' S, 150° 59' E) |
| | KM984 | ♀ | JN965165 | n/a | Qld (25° 3' S, 148° 13' E) |
| | KM1001 | ♂ | JN965164 | n/a | Qld (25° 16' S, 151° 54' E) |
| | | | | | |
| ***Sarcosolomonia*** | | | | | |
| ***collessi* * sp. nov.** | KM575 | ♀ | JN965171 | JQ290928 | Munduberra, Qld (25° 30' S, 151° 17' E) |
| Meiklejohn, 2012 (*in prep.*) | KM768 | ♂ | JN965173 | n/a | Qld (19° 58' S, 145° 34' E) |
| | KM831 | ♂ | JN965178 | JQ290905 | Qld (19° 41' S, 146° 26' E) |
| | KM865 | ♂ | JN965181 | JQ290933 | Qld (19° 58' S, 145° 34' E) |
| ***Taylorimyia*** | | | | | |
| ***aurifrons** ** | JW218v1 | ♂ | GQ254477 | JQ290530 | Bright, Vic (36° 43' S, 146° 56' E) |
| Macquart, 1846 | JW218v2 | ♂ | GQ254476 | n/a | Bright, Vic (36° 43' S, 146° 56' E) |
| | JW237 | ♂ | GQ254475 | JQ290543 | Holbrook, NSW (35° 42'S, 147° 19'E) |
| | JW240v1 | ♀ | GQ254474 | JQ290544 | Pinaroo, NSW (35° 15' S, 140° 54' E) |
| | JW240v2 | ♂ | GQ254473 | n/a | Pinaroo, NSW (35° 15' S, 140° 54' E) |
| | KM205 | ♂ | JN964937 | JQ290917 | Kalamundra NP, WA (31° 58' S, 116° 3' E) |
| | KM206 | ♂ | JN964936 | n/a | Kalamundra NP, WA (31° 58' S, 116° 3' E) |
| | KM210 | ♀ | JN964935 | JQ290638 | Kalamundra NP, WA (31° 58' S, 116° 3' E) |
| | KM222 | ♂ | JN964934 | JQ290640 | Twin Swamp Reserve, WA (31° 44' S, 116° 1' E) |
| | KM230 | ♂ | JN964933 | JQ290867 | Bindoon, WA (31° 17' S, 116° 5' E) |
| | KM234 | ♂ | JN964932 | JQ290695 | Bindoon, WA (31° 17' S, 116° 5' E) |
| | KM248 | ♂ | JN964931 | JQ290823 | WA (31° 7' S, 116° 3' E) |
| | KM250 | ♂ | JN964930 | JQ290699 | WA (31° 7' S, 116° 3' E) |
| | KM281 | ♂ | JN964929 | JQ290704 | Chapman River, WA (28° 46' S, 114° 43' E) |
| | KM298 | ♂ | JN964928 | JQ290596 | Dongara, WA (29° 16' S, 114° 55' E) |
| | KM310 | ♂ | JN964927 | JQ290598 | WA (29° 56' S, 114° 59' E) |
| | KM312 | ♂ | JN964926 | JQ290599 | WA (30° 15' S, 115° 10' E) |
| | KM313 | ♂ | JN964925 | JQ290600 | WA (30° 15' S, 115° 10' E) |
| | KM315 | ♀ | JN964924 | JQ290601 | WA (30° 15' S, 115° 10' E) |
| | KM320 | ♂ | JN964923 | JQ290603 | Nambung NP, WA (30° 35' S, 115° 6' E) |
| | KM324 | ♂ | JN964922 | JQ290605 | Orange Springs, WA (30° 59' S, 115° 42' E) |
| | KM329 | ♂ | JN964921 | JQ290607 | Roleystone, WA (32° 7' S, 116° 3' E) |
| | KM345 | ♂ | JN964920 | JQ290614 | Serpentine, WA (32° 21' S, 115° 59' E) |
| | KM346 | ♂ | JN964919 | JQ290790 | Wellington Dam, WA (33° 24' S, 115° 58' E) |
| | KM347 | ♂ | JN964918 | JQ290615 | Wellington Dam, WA (33° 19' S, 116° 2' E) |
| | KM349 | ♀ | JN964917 | JQ290708 | Wellington Dam, WA (33° 19' S, 116° 2' E) |

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| | KM358 | ♂ | JN964916 | JQ290709 | Peaceful Bay, WA (34° 57' S, 116° 59' E) |
| | KM359 | ♂ | JN964915 | JQ290710 | Waychonicup NP, WA (34° 50' S, 118° 20' E) |
| | KM360 | ♀ | JN964914 | JQ290852 | Waychonicup NP, WA (34° 50' S, 118° 20' E) |
| | KM363 | ♀ | JN964913 | JQ290853 | Waychonicup NP, WA (34° 50' S, 118° 20' E) |
| | KM364 | ♂ | JN964912 | JQ290616 | Waychonicup NP, WA (34° 50' S, 118° 20' E) |
| | KM368 | ♂ | JN964911 | JQ290618 | Porongurup NP, WA (34° 40' S, 117° 53' E) |
| | KM381 | ♂ | JN964910 | JQ290619 | Stirling Range NP, WA (34° 26' S, 118° 4' E) |
| | KM383 | ♂ | JN964909 | JQ290924 | Stirling Range NP, WA (34° 26' S, 118° 4' E) |
| | KM389 | ♂ | JN964908 | JQ290620 | Stirling Range NP, WA (34° 20' S, 118° 9' E) |
| | KM391 | ♂ | JN964907 | JQ290621 | Gnowangerup, WA (33° 57' S, 118° 6' E) |
| | KM394 | ♂ | JN964906 | JQ290622 | Broomehill East, WA (33° 52' S, 117° 46' E) |
| | KM401 | ♂ | JN964905 | JQ290874 | Broomehill East, WA (33° 52' S, 117° 46' E) |
| | KM406 | ♂ | JN964904 | JQ290623 | Broomehill East, WA (33° 52' S, 117° 46' E) |
| | KM413 | ♂ | JN964903 | JQ290625 | Broomehill East, WA (33° 52' S, 117° 46' E) |
| | KM430 | ♂ | JN964902 | JQ290626 | Little Parkeyerring Lake, WA (33° 22' S, 117° 21' E) |
| | KM431 | ♂ | JN964901 | JQ290627 | Narrogin, WA (32° 56' S, 117° 9' E) |
| | KM432 | ♂ | JN964900 | JQ290630 | Williams, WA (33° 1' S, 116° 52' E) |
| | KM443 | ♂ | JN964899 | JQ290631 | Hotham River, WA (32° 46' S, 116° 35' E) |
| | KM446 | ♂ | JN964898 | JQ290635 | WA (32° 34' S, 116° 26' E) |
| | KM472 | ♀ | JN964897 | JQ290636 | Westdale, WA (32° 13' S, 116° 24' E) |
| | KM474 | ♀ | JN964896 | JQ290637 | Beverley, WA (32° 10' S, 116° 50' E) |
| | KM477 | ♂ | JN964895 | JQ290646 | York, WA (31° 50' S, 116° 46' E) |
| | KM491 | ♀ | JN964894 | JQ290779 | Serpentine, WA (32° 19' S, 116° 2' E) |
| | KM758 | ♀ | JN964893 | JQ290780 | Charles Darwin Reserve, WA (29° 34' S, 116° 59' E) |
| | KM759 | ♀ | JN964892 | JQ290862 | Noonbah Station, Qld (24° 8' S, 143° 12' E) |
| | KM775 | ♀ | JN964891 | JQ290784 | Nth Stradbroke Island, Qld (27° 37' S, 153° 26' E) |
| | KM806 | ♂ | JN964890 | n/a | Noonbah Station, Qld (24° 8' S, 143° 11' E) |
| | KM835 | ♂ | JN964889 | JQ290867 | Lochada, Qld (29° 11' S, 116° 30' E) |
| | KM883 | ♂ | JN964888 | n/a | Gluepot Reserve, SA (33° 45' S, 140° 7' E) |
| | KM985 | ♀ | JN964887 | n/a | Qld (25° 3' S, 148° 13' E) |
| | | | | | |
| **Unplaced to *Sarcophaga* subgenus** | | | | | |
| ***simplex**** | KM601 | ♀ | JN964766 | JQ290842 | Rockhampton, Qld (23° 22' S, 150° 30' E) |
| Lopes, 1967 | KM658 | ♀ | JN964765 | JQ290845 | Paluma NP, Qld (19° 0' S, 146° 16' E) |
| | KM677 | ♂ | JN964764 | JQ290846 | Millaa Millaa, Qld (17° 29' S, 145° 39' E) |
| | | | | | |
| ***SARCOPHAGA UNKNOWNS*** | | | | | |
| ***Sarcorohdendorfia* sp. nov.*** | KM670 | ♂ | JN964860 | JQ290746 | Wooroonooran NP, Qld (17° 37' S, 145° 44' E) |
| (unnamed species near to | KM672 | ♀ | JN964859 | JQ290752 | Wooroonooran NP, Qld (17° 37' S, 145° 44' E) |
| *praedatrix*) | KM680 | ♂ | JN964858 | JQ290747 | Dinner Falls, Qld (17° 25' S, 145° 29' E) |
| | | | | | |
| **Unknown A *** | KM546 | ♀ | JN965176 | JQ290836 | Great Sandy NP, Qld (25° 56' S, 153° 4' E) |
| | KM898 | ♀ | JN965179 | JQ290810 | Kensington Gardens, SA (34° 55' S, 138° 39' E) |
| | | | | | |
| **Unknown B *** | KM689 | ♀ | JN964687 | JQ290940 | Ellis Beach, Qld (16° 40' S, 145° 34' E) |
| | | | | | |
| **Unknown C *** | JW241 + | ♀ | GQ254446 | n/a | Adelaide, SA (34° 54' S, 138° 40' E) |
| | JW246v2 + | ♀ | GQ254448 | n/a | Pinaroo, NSW (35° 15' S, 140° 54' E) |
| | | | | | |
| **Unknown D *** | JW216v2 + | ♀ | GQ254483 | n/a | Yass, NSW (34° 50' S, 148° 54' E) |
| | | | | | |
| ***TRICHARAEA*** Thomson | | | | | |
| ***Tricharaea*** | | | | | |
| ***brevicornis**** | KM750 | ♀ | JN965189 | JQ290903 | Seven Mile Beach, Tas (42° 49' S, 147° 31' E) |
| (Wiedmann, 1830) | KM751 | ♂ | JN965188 | JQ290904 | Seven Mile Beach, Tas (42° 49' S, 147° 31' E) |

**Appendix 2.** The complete list of 110 morphological characters, along with states, scored for sarcophagids in this study. Uninformative characters are denoted with a *, and were excluded only in the parsimony analysis.

### a) Head

1. *Head colouration*: yellow microtrichosity (0); silver/grey microtrichosity (1); sparkling gold microtrichosity (2)
2. *Proclinate fronto-orbital setae*: absent (0); present (1)
3. *Number of frontal setae*: <10 (0); >10 (1)
4. *Gena setulae colour*: only black (0); yellow/white (1); black anteriorly then yellow/white (2)
5. *Postocular setulae colour*: only black (0); yellow/white (1); black dorsally then yellow/white (2)
6. *Parafacial setulae colour*: yellow/white (0); only black (1); yellow superiorly, black inferiorly (2); yellow/black superiorly, yellow inferiorly (3); absent (4)
7. *Vibrissal setulae colour*: supra/sub with black setae only (0); supra/sub with some yellow setae (1); only supra with yellow setae (2); only sub with yellow setae (3)
8. *Arista*: bare (0); setose (1)
9. *Setose on arista*: long setulae (0); short setulae (1)

*10. *Face shape*: width of eye to width of head, no more than 1/2 (0); width of eye to width of head, generally 3/4 (1)

11. *Eye size:* not modified (0); enlarged with reduced genae (1)
12. *Postcranium*: convex (0); flat or concave (1)
13. *Frons (male vs. female)*: male with narrower frons (0); frons equibroad in both sexes (1)
14. *Postocular setae length*: alternating long and short (0); all equal length (1)
15. *Ground colour of palps*: at least partly yellow (0); at most reddish black (1)
16. *Antennal scape relative to lunule*: scape reaching above lunule (0); scape flush with or below lunule (1)
17. *Frontal setae*: rows of parallel or gradually diverging near lunule (0); rows strongly diverging near lunule (1)

### b) Thorax

18. *Presutural dorsocentral setae*: absent (0); present (1)
19. *Presutural acrostichal setae*: absent (0); present (1)
20. *Prescutellar acrostichal setae*: absent (0); present (1)

*21. *Postsutural dorsocentral setae*: absent (0); present (1)

22. *Number of postsutural dorsocentral setae*: one (0); two (1); three (2); four or more (3); n/a (8)
23. *Position of postsutural dorsocentral setae*: evenly positioned (0); on posterior half only (1); n/a (8)
24. *Strength of postsutural dorsocentral setae*: all strongly developed (0); all weakly developed (1); posterior setae strong, anterior setae weaker (2); n/a (8)
25. *Apical scutellar setae*: absent (0); present (1)

26. *Preapical scutellar setae*: one pair only (0); two pairs only (1); three or more pairs (2)
27. *Propleura*: bare (0); setose (1)
28. *Propleura setulae*: more than 10 (0); only a few in centre (1); n/a (8)
29. *Propleura setulae colouration*: yellow/white (0); black (1); yellow/black (2); n/a (8)
30. *Coxopleural streak*: absent (0); present (1)
31. *Thorax colouration*: yellow microtrichosity (0); silver/grey microtrichosity (1); sparkling gold microtrichosity (2)
32. *Hind tibia setulae length*: long (0); short (1)
33. *Ventral preapical seta on hind tibia*: absent (0); present (1)
34. *Setulae colouration on katepisternum*: black (0); yellow/white (1); black and yellow/white (2)
35. *Notopleuron*: with 2 strong (primary) setae (0); with 2 primary and 2 subprimary setae (1)
36. *Prosternum*: bare (0); setose (1)
37. *Metasternum*: bare (0); setose (1)

*38. *Hind coxa, posterior surface*: bare (0); setose (1)

*39. *Male mid femur*: with a ctenidium of normal spines (0); without a ctenidium of normal spines (1)

**c) Wing**

40. *$R_1$ wing vein*: bare (0); setose (1)

*41. *Lower calypter*: rather flat (0); distinctly arching over the halter (1)

**d) Abdomen**

42. *Ground colour terminalia (males-protandrial segment-epandrium-cercus, females-6th abdominal tergite)*: black at most reddish black (0); bright red to orange (1)
43. *Median marginal setae on 3rd abdominal tergite*: absent (0); present (1)
44. *Median marginal setae on 4th abdominal tergite*: absent (0); present (1)
45. *1st/2nd abdominal sternite setulae colouration*: only black (0); with some yellow/white (1)
46. *1st/2nd abdominal sternite setulae length*: long (0); short (1); very long (2)
47. *Median patch of dense setae on male 4th abdominal tergite*: absent (0); present (1)
48. *Male abdominal sternites 3-4*: covered in parts by margins of T3-T4 (0); overlapping margins of T3-T4 (1)
49. *Male abdominal tergite 6*: narrow but well developed (0); reduced and either absent or present as a small sclerite (1)

**e) Male terminalia**

50. *Vesica length*: short (<200 µm) (0); long (>250 µm) (1)
51. *Vesica shape*: straight (0); curved (1); rounded (2); '√' shaped (3); flower like (4)
52. *Vesica width*: narrow (<200 µm) (0); wide (>250 µm) (1)
53. *Vesica bifurcation at distal part*: absent (0); present (1)

54. *Vesica membranous*: no (0); yes (1)
55. *Vesica membrane location:* proximally only (0); distally only (1); entire length (2); anterior margin/surface only (3)
56. *Vesica spines*: absent (0); present (1)
57. *Vesica curvature direction*: inward (0); outward (1)
58. *Connection between vesica and paramere*: membranous with compressions (0); membranous without compressions (1); not membranous (2)
59. *Connection of vesica to distiphallus*: broadly connected (0); by means of narrow, stalk-like connection (1)
60. *Proximal part of vesica*: not elongated (0); drawn out into a pair of divergent processes (1)

*61. *Vesica structure*: not developed (0); developed (1)

62. *Juxta length*: short (<200 µm) (0); long (>250 µm) (1)
63. *Juxta general shape*: straight (0); curved (1); triangular (2); rounded (3)
64. *Juxta spines*: absent (0); present (1)
65. *Juxta setulae*: absent (0); present (1)
66. *Juxta membranous*: no (0); yes (1)
67. *Juxta membrane location*: proximally only (0); distally only (1); entire length (2); anterior margin/surface only (3); posterior margin/surface only (4); proximal and distal (5)
68. *Juxta anteroventral part*: bifurcation present (0); pointed projection only (1); rounded (2)
69. *Juxta width at distal part*: narrow (<200 µm) (0); wide (>250 µm) (1)
70. *Juxta structure*: not developed (0); developed (1)
71. *Positioning of juxta*: simple continuation of the distiphallus (0); distinctly set off from remaining distiphallus (1)
72. *Distal margin of juxta*: entire (0); with a deep median cleft (1)

*73. *Base of juxta*: trilobed (0); not trilobed (1)

74. *Length of lateral styli*: not reaching beyond juxta (0); greatly surpassing the juxta (1)
75. *Width of lateral styli*: narrow and thread like (0); not narrow and thread like (1)
76. *Distal part of lateral styli*: bifurcation absent (0); bifurcation present (1)
77. *Ventral face of lateral styli*: not jaggered (0); jaggered (1)
78. *Structure of styli (lateral and medial)*: 3 conducting styli (0); lateral conducting styli and median non-conducting stylus (1)
79. *Base of lateral styli*: with straight base (0); with base coiled (1)
80. *Base of median styli*: straight (0); bilobed, recurving base (1)

*81. *Lateral styli collapsed*: no (0); yes (1)

*82. *Position and length of the lateral styli*: small and close to the median line (0); not small and close to the median line (1)

83. *Harpes*: not developed (0); developed as a separate sclerite at or near the base of the lateral styli (1)

84. *Cercus setulae length*: short (<300 µm) (0); long (300-500 µm) (1); very long (>500 µm) (2)
85. *Cercus setulae location*: entire length (0); posterior margins (1); dorsal tip only (2); patch at apex (3); dorsal half only (4); patch at apex and dorsal half (5); posterior margins and dorsal half (6)
86. *Cercus spines*: absent (0); present (1)
87. *Cercus spine location*: ventral tip only (0); ventral half only (1); dorsally on prong (2)
88. *Cercus shape*: straight (0); curved inward (1); curved outward (2)
89. *Cercus curvation*: on apical 1/3 (0); on apical ¼ (1); on apical 2/3 (2)
90. *Cercus apex*: enlarged (0); bifurcated (1); pointed (2); hollowed out creating lateral projections (3); pointed and hollowed out (4)
91. *Surstylus setulae location*: entire surface (0); anterior margin only (1); ventral 2/3 (2); ventral 1/3 (3); apex only (4); n/a (5); anterior margin and ventral 2/3 (6)
92. *Surstylus shape*: triangular (0); elongated (1); with hook at apex (2); rounded (3)
93. *Surstylus setulae*: with unmodified setulae (0); with long setulae apically (1)
94. *Proximal margin of surstylus*: unmodified (0); thickened (1)
95. *5$^{th}$ abdominal sternite setulae*: absent (0); present (1)
96. *5$^{th}$ abdominal sternite setulae location*: apex (0); surface (1); inner margin (2); outer margin (3); apex and surface (4); apex, surface and inner margin (5); apex and inner margin (6); apex, surface, inner and outer margins (7)
97. *5$^{th}$ abdominal sternite setulae length*: short (<200 µm) (0); long (>250 µm) (1)
98. *5$^{th}$ abdominal sternite spines*: absent (0); present (1)
99. *5$^{th}$ abdominal sternite spine location*: inner margin (0); apex (1)
100. *5$^{th}$ abdominal sternite shape*: with posterior margin incised (0); with posterior margin truncated (1)
101. *Epiphallus*: distinct (0); absent (1)
102. *Ventral surface below the acrophallus*: unmodified (0); produced into a swollen or lobate structure or vesica (1)
103. *Distal part of phallus*: a single, simple opening (0); with an acrophallus formed from a tripartition of the distal part (1)
104. *Connection between basi- and distiphallus*: continuous (0); with desclerotised/membranous strip (1); with a distinct hinge (1)

**f) Female terminalia**

105. *6$^{th}$ abdominal tergite shape*: entire (0); composed of 2 plates (1)
106. *7$^{th}$ abdominal sternite setulae*: absent (0); present (1)
107. *7$^{th}$ abdominal sternite setae*: absent (0); row of setae on hind margin (1); single seta on both lateral hind margins (2); a few setae on both the lateral hind margins (3)
108. *7$^{th}$ abdominal sternite shape*: broader than other sternites (0); narrower than other sternites (1); concave on hind margin (2); very concave on hind margin (3); tear drop shaped (4)

109. *Length of setae on the hind margin of the 2nd-5th abdominal sternites*: short (span halfway to next sternite) (0); long (span to next sternite) (1); n/a (8)

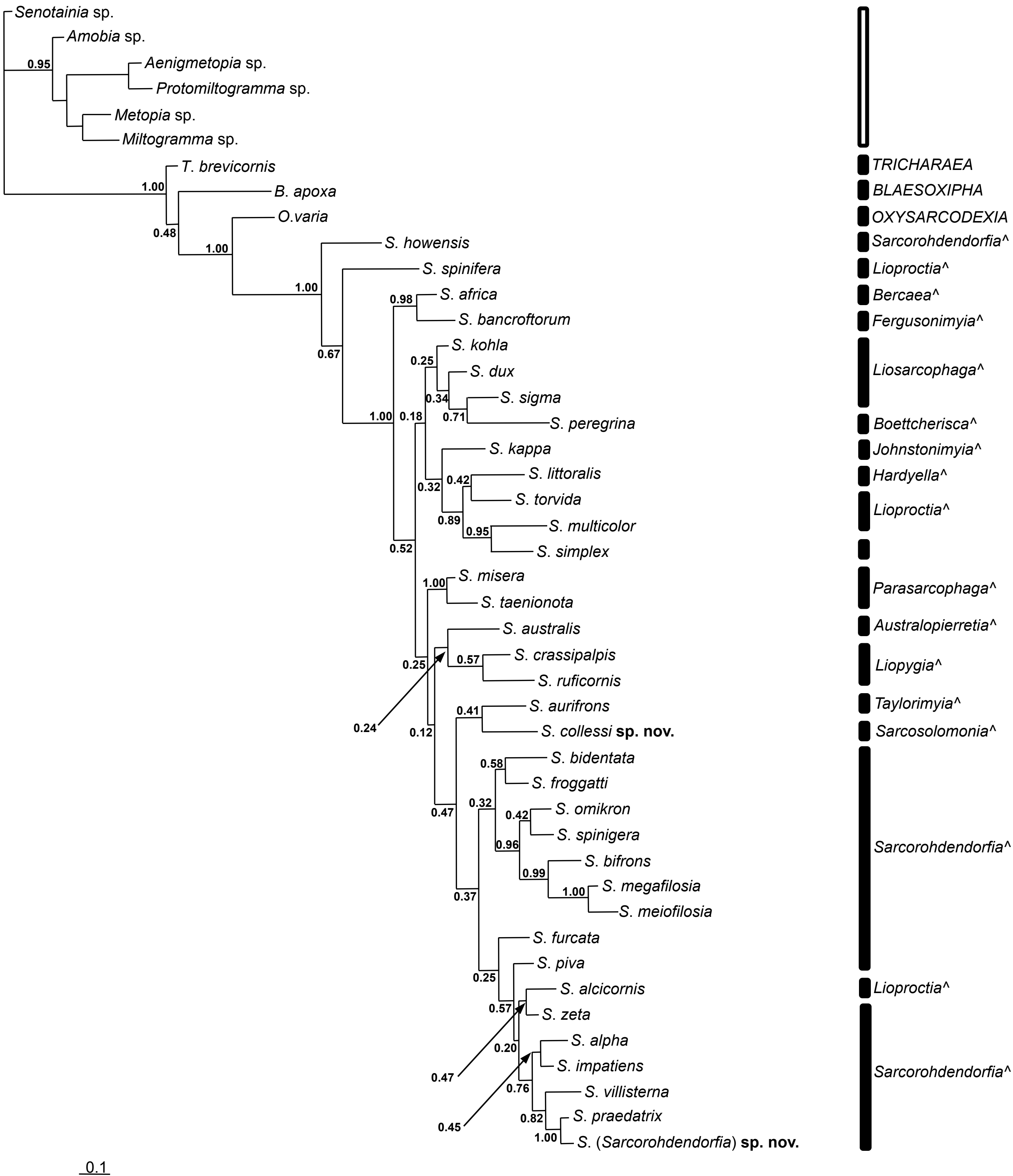
**g) Body length**

110. *Body length*: < 5mm (0); 5-10 mm (1); 10-15 mm (2); >15 mm (3)


**Appendix 3.** Bayesian inference of phylogeny for 39 species from 14 of the 132 *Sarcophaga s.l.* subgenera. GENERA and *subgenera* are given on the right-hand side: white bars indicate Miltogramminae, black bars indicate Sarcophaginae (^ denotes subgenera of *Sarcophaga s.l* ). Numbers given at main branches refer to posterior probability values as a proportion. Morphological species identifications are given for all specimens along with voucher IDs. Outgroups are Miltogramminae specimens: *Miltogramma* Unknown A (KM837) and *Protomiltogramma* Unknown A (KM059). Evolutionary distance divergence scale bar, 0.1.

**a.** Based on CAD sequence data alone. Arrows highlight 'outlier' specimens which have not been resolved with conspecifics.


**Appendix 3b.** Based on COI sequence data alone.

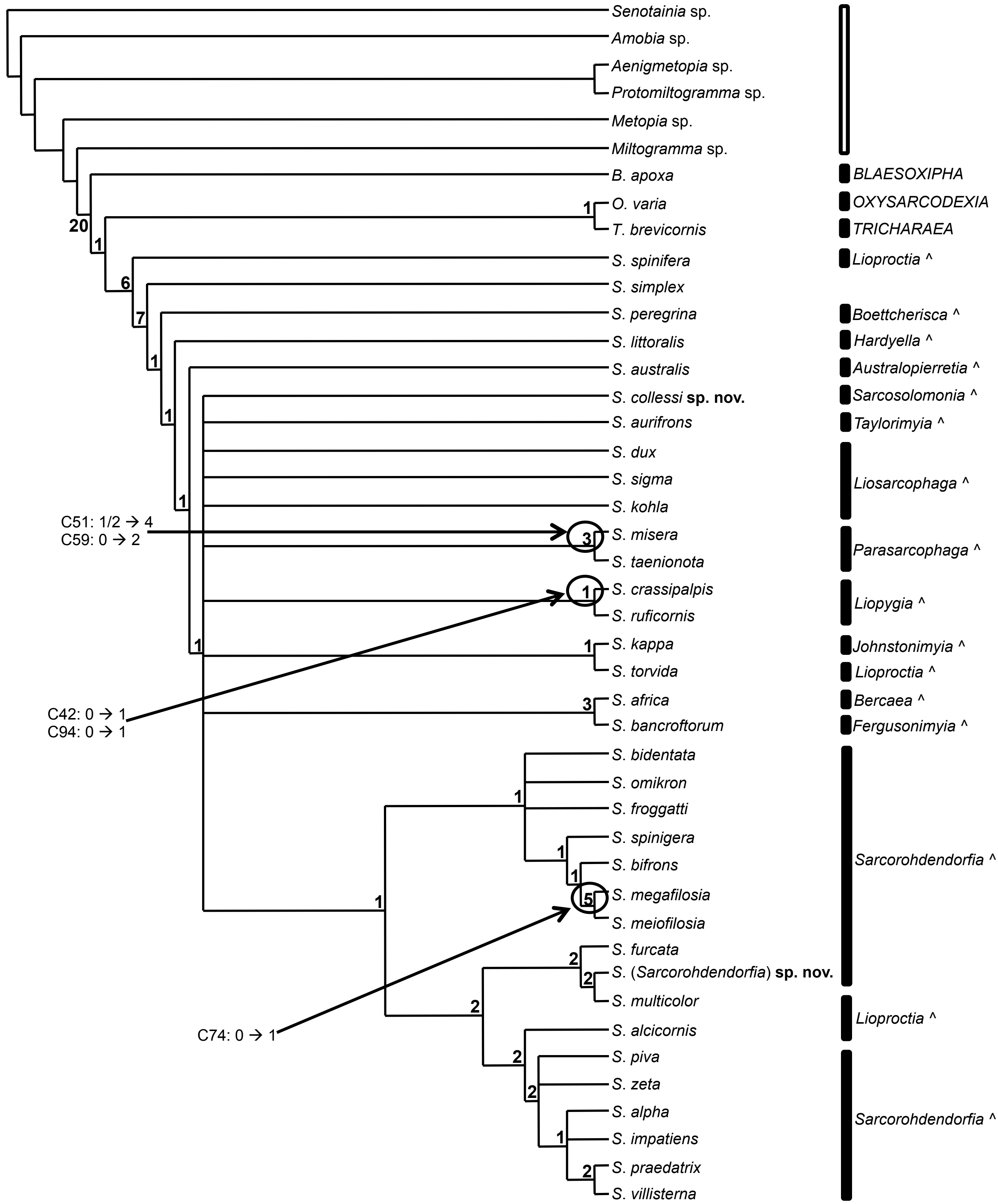



**Appendix 3c.** Based on morphological data alone.


**Appendix 3d.** Based on both CAD and morphological data. Arrows highlight 'outlier' specimens which have not been resolved with conspecifics.


**Appendix 3e.** Based on both COI and morphological data.


**Appendix 3f.** Based on both CAD and COI sequence data.




**Appendix 4.** Parsimony inference of phylogeny for 39 species from 14 of the 132 *Sarcophaga s.l.* subgenera, based on 101 informative morphological characters. *GENERA* and *subgenera* are given on the right-hand side: white bars indicate Miltogramminae, black bars indicate Sarcophaginae (^ denotes subgenera of *Sarcophaga s.l* ). Numbers given at resolved nodes are Bremer support values. Character synapomorphies are given for nodes with circled Bremer values: 'C' denotes character, with the number given adjacent correlated to the characters given in Appendix 1. Outgroups are Miltogramminae specimens: *Miltogramma* Unknown A (KM837) and *Protomiltogramma* Unknown A (KM059).