Utility of COI, CAD and morphological data for resolving relationships within the genus *Sarcophaga* (sensu lato) (Diptera: Sarcophagidae): a preliminary study

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**Abstract**

Currently there are \(~3,000\) known species of Sarcophagidae (Diptera), which are classified into 173 genera in three subfamilies. Almost 25\% of sarcophagids belong to the genus *Sarcophaga* (sensu lato) however little is known about the validity of, and relationships between the \(~150\) (or more) subgenera of *Sarcophaga* s.l. In this preliminary study, we evaluated the usefulness of three sources of data for resolving relationships between 35 species from 14 *Sarcophaga* s.l. subgenera: the mitochondrial COI barcode region, \(~800\) bp of the nuclear gene CAD, and 110 morphological characters. Bayesian, maximum likelihood (ML) and maximum parsimony (MP) analyses were performed on the combined dataset. Much of the tree was only supported by the Bayesian and ML analyses, with the MP tree poorly resolved. The genus *Sarcophaga* s.l. was resolved as monophyletic in both the Bayesian and ML analyses and strong support was obtained at the species-level. Notably, the only subgenus consistently resolved as monophyletic was *Liopygia*. The monophyly of and relationships between the remaining *Sarcophaga* s.l. subgenera sampled remain questionable. We suggest that future phylogenetic studies on the genus *Sarcophaga* s.l. consider COI,
CAD, and morphological data for analyses. We also advocate the use of additional data and a range of inference strategies to assist with resolving relationships within *Sarcophaga s.l.*

**Keywords**

Sarcophagidae; Diptera; *Sarcophaga (sensu lato)*; phylogeny; molecular; morphological

**1. Introduction**

The Sarcophagidae (flesh flies) are a globally distributed family of 173 genera and ~3,000 species, which are known mainly as carrion-breeder 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, 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 even the still unnamed McAlpine’s fly (Kutty et al., 2010; McAlpine, 1989; Nelson et al., 2012; Pape, 1992; Rognes, 1997).

The largest of the three subfamilies, the Sarcophaginae, comprise ~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 (sensu lato)*. 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., 2001; Zehner et al., 2004); however the circumscription and classification of its ~150 subgenera remain open to discussion. 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 et al. (2010) used 73 morphological characters (including 41 features from the male terminalia) for phylogenetic reconstruction of 72 species of Sarcophaginae, representing 19 genera and 31 *Sarcophaga s.l.* subgenera. Kutty et al. (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
Calypratae, which included 46 sarcophagid species from 28 genera. Kutty et al. (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.

2. Materials and methods

2.1. Molecular data

2.1.1. Taxon Sampling

Sarcophagid specimens used in this study were collected across Australia 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 (Wollongong, NSW, Australia). Each specimen was identified morphologically using the taxonomic literature for the Australian Sarcophagidae (Lopes, 1954, 1959; Lopes and Kano, 1979), but also assisted by examination of curated collection material from the Australian National Insect Collection (ANIC; Canberra, ACT, Australia), Queensland Department of Primary Industries (QDPC; Indooroopilly, Qld, Australia), Queensland Museum (QM; Brisbane, Qld, Australia) and the University of Queensland (UQIC; Brisbane, Qld, Australia - now housed at the QM).

The specimens used to obtain COI and CAD sequences represent 35 species from 14 of the ~150 *Sarcophaga s.l.* subgenera (Supplementary Material Table S.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 five subgenera that are monotypic and restricted to the Australasian/Oceanian zoogeographic regions (except for *Poseidonimyia*, which reaches into southern Indonesia). 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 *Sarcothorodendorfia* and *Sarcosolomonia*.

### 2.1.2. 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.

### 2.1.3. Gene amplification and alignment

The 648 bp COI barcode fragment was amplified for all specimens as outlined by Meiklejohn et al. (2011). All barcode sequences used in this study have been published previously, either in Meiklejohn et al. (2011) or Meiklejohn et al. (2012).

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 *S. (Sarcorohdendorfia) impatiens*. After sequence alignment, the most variable portion of the CPS domain in these species was identified as the initial ~800 bp (or fragment one). Based on this, fragment one of CAD was amplified using the primer combination of 54F (5’-GTNGTNTTYCARACNGGNATGGT-3’) and 405R (5’-GCNGTRTGYTCNGGRTGRAAYTG-3’) (Moulton and Wiegmann, 2004). It is important to highlight that attempts were made to amplify fragment one of CAD from a total of 588 specimens, which represented the COI dataset from Meiklejohn et al. (2012). CAD was successfully amplified and sequenced for 435 specimens, without the need for re-amplification. Amplicons could not be obtained from the remaining specimens, despite several re-amplification attempts. Each 25 µl PCR reaction contained 2 µl of 10X PCR buffer (with 20 mM MgCl2; 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 H2O (negative control). The PCR temperature cycling consisted of an initial denaturation at 94°C for 2 min, followed by: 5 cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 2 min; 7 cycles of 94°C for 30 s, 51°C for 1 min, 72°C for 2 min; 37 cycles of 94°C for 30 s, 45°C for 20 s, 72°C for 2 min 30 s; along with a final elongation for 3 min at 72°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°C for 15 min followed by 80°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 H2O for a final volume of 2.5 µl. Cycling conditions for sequencing reactions consisted of 28 cycles of 96°C for 10 s, 50°C for 5 s and 60°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°C. Sequencing products were sent to the ACRF Biomolecular Resource Facility (Canberra, ACT, Australia) for separation and generation of electropherograms. Individual exon sequences for both COI and CAD were translated into amino acids and separately aligned using ClustalW within MEGA version 4 (Tamura et al., 2007).

2.2. 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 character matrix is shown in Figure 1 and the corresponding character definitions are given as Supplementary Material (Figure S.1). To allow for direct comparison between markers, these characters were scored for the Sarcophaga s.l. species from which COI and CAD 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 for a
particular species were denoted by a ‘?’. Characters where both states were represented
within a given species, were coded as if each specimen possessed both states.

2.3. Phylogenetic analysis
Partitioned Bayesian and maximum likelihood (ML) analyses were conducted using
MRBAYES (Version 3.1.2; Huelsenbeck and Ronquist, 2001) and GARLi (Version 2.0;
Zwickl, 2006), respectively. In addition, an equally-weighted maximum parsimony (MP)
analysis was performed in TNT (Version 1.1; Goloboff et al., 2003a). For all analyses,
miltogrammine 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 Sarcophaginiae
and Sarcophaga s.l. It is important to note that morphological characters were not scored for
each individual specimen listed in Supplementary Material Table S.1. Instead 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).

2.3.1. Bayesian and maximum likelihood analyses
For the molecular data, the Akaike Information Criterion implemented within MRMODELTEST
(Version 2.2; Nylander, 2004) and MODELTEST (Version 3.7; Posada and Crandall, 1998)
were used to determine the most suitable evolutionary model(s) separately for the COI and
CAD data, for the Bayesian and ML analyses, respectively. The data were partitioned by
gene and then further partitioned by codon (first-, second- and third-codon position). The
AIC models selected for the first-, second- and third-codon positions of COI and CAD from
both MRMODELTEST and MODELTEST were, respectively: GTR+I+G, GTR+G, GTR+I+G
(COI); and GTR+I+G, F81+G and GTR+G (CAD). Based on the study by Lewis (2001), the
morphological data were analysed using the discrete model of evolution (Mkv). The Bayesian
analysis was run on the High Performance Computing (HPC) cluster at the University of
Wollongong (Wollongong, NSW, Australia) for 30,000,000 generations, with trees sampled
every 5,000 generations and 27% of trees discarded as burn-in. A partitioned ML analysis of
150 bootstrap replicates was conducted in GARLi, through the University of Oslo Bioportal (available online at https://www.bioportal.uio.no [last accessed 12/01/2013]; Kumar et al., 2009). A majority rule consensus of the 150 ML bootstrapped trees was generated using PAUP* (Version 4.0a125; Swofford, 2001).

2.3.2. Parsimony analysis
A maximum parsimony analysis was conducted using TNT. The data were specified as DNA (COI and CAD) and numbered (morphological), with the morphological characters also treated as unordered. A standard bootstrap analysis was performed with 5,000 replicates, applying traditional searches set with 10 random addition sequences and 10 trees saved per random addition, branch swapping as tree bisection reconnection (TBR), and support values given as frequency differences (GC; for Group present/Contradicted as developed by Goloboff et al., 2003b).

3. Results and discussion
3.1 Marker choice and evaluation
The COI and CAD gene regions of the genus Sarcophaga s.l., along with 110 morphological characters, were all evaluated for phylogenetic signal in this preliminary study. The COI barcoding region was chosen as published studies have deemed it as reliable for species-level identification of Australian sarcophagids (Meiklejohn et al., 2011; 2012; 2013a) and COI 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, 1959), along with a range of supplementary characters from the head, abdomen, thorax and terminalia.

To assess whether the relationships within Sarcophaga s.l. were robust to differences in phylogenetic inference methods, we performed Bayesian, maximum likelihood (ML) and maximum parsimony (MP) analyses. The MP analysis contained numerous polytomies and was poorly resolved even at the species level. For this reason, we examined the preliminary monophyly and relationships of the Sarcophaga s.l. subgenera using only the Bayesian and ML analyses. The overall tree topologies for the Bayesian and ML analyses were similar,
especially at basal and species-levels. The Bayesian topology was considered better resolved, with 78% of nodes having posterior probabilities (PP) of ≥0.90 (fewer than half of the nodes had bootstrap support of ≥90 in the ML analysis). However, this high level of node support is likely to change with the addition of more loci and/or taxa. We mapped the corresponding node supports from the ML analysis onto the Bayesian topology (Figure 2). For completeness, we also mapped the frequency differences from the MP analysis onto Figure 2. The results below relate only to the Bayesian and ML analyses, unless stated otherwise.

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 et al. (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 et al. (2010). The CAD sequences obtained by Kutty et al. (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.

### 3.2. 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 (Figure 2), with monophyly of this subfamily also documented in a range of studies (Giroux et al., 2010; Kutty et al., 2010; Pape, 1996).

### 3.3. Genus Sarcophaga s.l.

The genus *Sarcophaga* s.l. was resolved as monophyletic (Figure 2). This is consistent with several studies that have also resolved *Sarcophaga* s.l as monophyletic, based on both molecular and morphological data (Giroux et al., 2010; Kutty et al., 2010; Wells et al., 2001; 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, with high node support (Figure 2). Zehner et al. (2004) and Wells et al. (2001), who used mitochondrial COI/ND5 and COI genes, respectively, for phylogenetic reconstruction, also resolved *Liopygia* as monophyletic.
The subgenus *Parasarcophaga* was resolved as monophyletic with high node support only in the Bayesian analysis (Figure 2). The subgenus *Lioproctia* consistently emerged as polyphyletic (Figure 2). The second largest *Sarcophaga s.l.* subgenus, *Liosarcophaga*, was resolved in the Bayesian analysis as paraphyletic, disrupted by a single representative from the subgenus *Boettcherisca* (Figure 2).

*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 2). The placement of *alcicornis* among species of *Sarcorohdendorfia* was not surprising, as this species possesses the diagnostic features of *Sarcorohdendorfia*: a setulose proepisternum and a？” shaped vesica (Lopes, 1959). As *alcicornis* was similarly clustered within *Sarcorohdendorfia* (Figure 2), it is plausible to suggest an assignment of *alcicornis* to the subgenus *Sarcorohdendorfia*.

### 3.3.1. *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). This was supported by these species being robustly resolved together in the Bayesian, ML and MP analyses (Figure 2).

### 3.3.2. Classification of unknown species

Two morphologically identical female *Sarcophaga s.l.* specimens could not be reliably identified to the species level, but were included in the taxon set as *Sarcophaga* Unknown A (Supplementary Material Table S.1). Subgeneric classification of *Sarcophaga* Unknown A to *Sarcorohdendorfia* was possible, as both specimens possess a setulose proepisternum and were resolved with other *Sarcorohdendorfia* species (Figure 2). A third female *Sarcophaga s.l.* specimen that was not reliably identified was included as *Sarcophaga* Unknown B, and was resolved with *S. (Hardyella) littoralis* (Figure 2). Given that *Hardyella* is a monotypic subgenus, it is difficult to infer the identity of this Unknown from the analyses. Two new distinct species were also included in the taxon set: *Sarcophaga* (*Sarcorohdendorfia*) sp_A sp. nov. (specimens KM670, KM672 and KM680; to be described in a subsequent publication) and *Sarcophaga* (*Sarcosolomonia*) sp_X sp. nov. (specimens KM575, KM831 and KM865; Meiklejohn et al., 2013b). 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.

3.3.3. Placement of monotypic subgenera

Five monotypic subgenera, known exclusively (or nearly so) from the Australasian/Oceanian region, were included in the taxon set: *Australopierretia, Fergusonimyia, Hardyella, Poseidonimyia* and *Taylorimyia*. None of these monotypic subgenera were placed within another subgenus (Figure 2), providing some evidence that they are correctly classified as monotypic.

3.3.4. *Sarcophaga* (*Fergusonimyia*) bancroftorum

*Fergusonimyia* is a monotypic subgenus of *Sarcophaga s.l.*, with its species *bancroftorum* documented as highly morphologically variable. 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, 1958). Additionally, the 2nd and 3rd antennomere of this species are 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 proepisternum, colour and number of setae of the head, villosity of the hind tibiae, and structure of the male terminalia (Lopes, 1958). Interestingly, these specimens of *bancroftorum* were not resolved as a single species based on the COI barcode approach (Meiklejohn et al. 2012). Despite this, to date, all morphological and molecular variation between *bancroftorum* specimens has been classified as intraspecific variation, with no separation into distinct species or subspecies.

Four male specimens were confidently identified as *bancroftorum* and were included in the taxon set (Supplementary Material Table S.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 clustered together within a clade (Figure 2). 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.
(Supplementary Material Table S.1). Both female specimens, KM842 and KM691, were consistently resolved together within the *bancroftorum* clade (Figure 2).

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 proper taxonomic decisions.

4. Conclusions

The molecular gene regions of COI and CAD, along with morphological characters, should be considered when choosing markers in future more comprehensive studies examining the relationships within the genus *Sarcophaga s.l.*, as they may be important building blocks in larger data matrices. The barcode region of COI and the morphological data facilitated strong support at the species level. Fragment one of CAD facilitated strong support of the Sarcophaginiae and *Sarcophaga s.l.*; however support for nodes at the subgeneric level across all analyses was poor. Resolution and support 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. We suggest that future phylogenetic studies of the genus *Sarcophaga s.l.* continue to combine data sets.

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References


**Figure 1.** Character matrix of morphological characters for species of Australian Sarcophagidae included in this study. ? = missing data, - = inapplicable data.

**Figure 2.** Topology from the Bayesian inference of phylogeny for 35 species from 14 of the ~150 Sarcophaga s.l. subgenera, based on COI, CAD 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: the posterior probability (PP) value as a proportion of one from the Bayesian analysis, bootstrap support from the maximum likelihood (ML) analysis and frequency differences from the maximum parsimony (MP) analysis, respectively. Only in the Bayesian and ML analysis were species robustly resolved, with 1.00 PP and 100 bootstrap support, respectively (values not shown). 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.

**Supplementary Material Table S.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.

**Supplementary Material Figure S.1.** Character definitions for the 110 morphological characters scored for sarcophagids in this study.
Figure 1.

OUTGROUP Miltogramma Unknown A 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Supplementary Material Figure S.1.

a) Head
1. **Head coloration**: yellow microtrichosity (0); silver/grey microtrichosity (1); sparkling gold microtrichosity (2); yellow or silver/grey microtrichosity (3)
2. **Proclinate fronto-orbital setae**: absent (0); present (1); absent or present (2)
3. **Number of frontal setae**: <10 (0); >10 (1); <10 and >10 (2)
4. **Gena setulae color**: only black (0); yellow/white (1); black anteriorly then yellow/white (2); yellow/white or black anteriorly then yellow/white (3); black or black anteriorly then yellow/white (4)
5. **Occiput setulae color**: only black (0); yellow/white (1); black dorsally then yellow/white (2); yellow/white or black dorsally then yellow/white (3); black or black dorsally then yellow/white (4)
6. **Parafacial setulae color**: yellow/white (0); only black (1); yellow superiorly, black inferiorly (2); yellow/black superiorly, yellow inferiorly (3); absent (4); yellow superiorly, black inferiorly or yellow/black superiorly, yellow inferiorly (5); yellow/white or yellow superiorly, black inferiorly (6); yellow/white or yellow/black superiorly, yellow inferiorly (7)
7. **Vibrissal setulae color**: supra/sub with black setulae only (0); supra/sub with some yellow setulae (1); only supra with yellow setulae (2); only sub with yellow setulae (3); supra/sub with black setulae only or supra/sub with some yellow setulae (4); supra/sub with black setulae only or supra/sub with some yellow setulae (5); supra/sub with black setulae only or only sub with yellow setulae (6); supra/sub with some yellow setulae or only sub with yellow setulae (7)
8. **Arista**: bare (0); setulose (1)
9. **Setulae on arista**: long setulae (0); short setulae (1)
10. **Face shape**: width of eye to width of head, no more than 0.5 (0); width of eye to width of head, generally 0.75 (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. **Occiput setae length**: alternating long and short (0); all equal length (1)
15. **Ground color 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 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); absent or present (2)
20. **Prescutellar acrostichal setae**: absent (0); present (1); absent or present (2)
21. **Postsutural dorsocentral setae**: absent (0); present (1)
22. **Number of postsutural dorsocentral setae**: one (0); two (1); three (2); four or more (3); three or four or more (4); one or two (5); one or four or more (6)
23. **Position of postsutural dorsocentral setae**: evenly positioned (0); on posterior half only (1); evenly positioned or on posterior half only (2)
24. **Strength of postsutural dorsocentral setae**: all strongly developed (0); all weakly developed (1); posterior setae strong, anterior setae weaker (2); all strongly developed or posterior setae strong, anterior setae weaker (3)
25. *Apical scutellar setae*: absent (0); present (1); absent or present (2)
26. *Preapical scutellar setae*: one pair only (0); two pairs only (1); three or more pairs (2); n/a (3); one pair only or two pairs only (4)
27. *Proepisternum*: bare (0); setulose (1); bare and setulose (2)
28. *Preepisternal setae*: more than 10 (0); only a few in centre (1)
29. *Proepisternal setae coloration*: yellow/white (0); black (1); yellow/black (2); yellow/white or black (3); yellow/white or yellow/black (4); black or yellow/black (5)
30. *Coxopleural streak*: absent (0); present (1)
31. *Thorax coloration*: yellow microtrichosity (0); silver/grey microtrichosity (1); sparkling gold microtrichosity (2); yellow microtrichosity or silver/grey microtrichosity (3)
32. *Hind tibia setulae length*: long (0); short (1); long and short (2)
33. *Ventral preapical seta on hind tibia*: absent (0); present (1); absent or present (2)
34. *Setae coloration on katepisternum*: black (0); yellow/white (1); yellow/white and black (2); yellow/white and yellow/white and black (3)
35. *Notopleuron*: with 2 primary (strong) setae (0); with 2 primary and 2 subprimary setae (1)
36. *Prosternum*: bare (0); setulose (1)
37. *Metasternum*: bare (0); setulose (1)
38. *Hind coxa, posterior surface*: bare (0); setulose (1)
39. *Male mid femur*: with a ctenidium of normal spines (0); without a ctenidium of normal spines (1)

c) *Wing*
40. *R1 wing vein*: bare (0); setulose (1)
41. *Lower calypter*: rather flat (0); distinctly arching over the halter (1)

d) *Abdomen*
42. *Ground color terminalia (males-prospondial segment-epandrium-cercus, females-6th abdominal tergite)*: black at most reddish black (0); bright red (1)
43. *Median marginal setae on 3rd abdominal tergite*: absent (0); present (1); absent or present (2)
44. *Median marginal setae on 4th abdominal tergite*: absent (0); present (1)
45. *1st/2nd abdominal sternite setae terminations*: only black (0); with some yellow/white (1); black or some yellow/white (2)
46. *1st/2nd abdominal sternite setae terminations*: long (0); short (1); very long (2); long or short (3); very long or very long (4); short or very long (5)
47. *Median patch of dense setae on male 4th abdominal sternite*: 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 or 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. **Harpex**: 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); ventral half only or dorsally on prong (3)
88. **Cercus shape**: straight (0); curved inward (1); curved outward (2)
89. **Cercus curvature**: 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 or 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. **5th abdominal sternite setulae**: absent (0); present (1)
96. **5th 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. **5th abdominal sternite setulae length**: short (<200 µm) (0); long (>250 µm) (1)
98. **5th abdominal sternite spines**: absent (0); present (1)
99. **5th abdominal sternite spine location**: inner margin (0); apex (1)
100. **5th 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 desclerotized/membranous strip (1); with a distinct hinge (1)

**f) Female terminalia**
105. **6th abdominal tergite shape**: entire (0); composed of 2 plates (1)
106. **7th abdominal sternite setulae**: absent (0); present (1)
107. **7th 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. **7th 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); narrower than other sternites or concave on hind margin (5)
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)

**g) Body length**
110. **Body length**: < 5mm (0); 5-10 mm (1); 10-15 mm (2); >15 mm (3)
### Supplementary Material Table S.1.

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<td>Jandowae, Qld (26° 42'S, 151° 16'E)</td>
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<td>KM526</td>
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<td>JQ290663</td>
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<td>JQ290855</td>
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<td>JQ290665</td>
<td>Gympie, Qld (26° 5'S, 152° 20'E)</td>
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<td>KM536</td>
<td>JN65125</td>
<td>JQ290668</td>
<td>Gympie, Qld (26° 5'S, 152° 20'E)</td>
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<td>JN65124</td>
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<td>Gympie, Qld (26° 11'S, 152° 39'E)</td>
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<td>KM563</td>
<td>JN65122</td>
<td>JQ290878</td>
<td>Mundubbera, Qld (25° 30'S, 151° 17'E)</td>
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<td>KM581</td>
<td>JN65121</td>
<td>JQ290713</td>
<td>Eidsvold, Qld (25° 22'S, 151° 8'E)</td>
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<td>KM592</td>
<td>JN65120</td>
<td>JQ290715</td>
<td>Callide Dam, Qld (24° 20'S, 150° 37'E)</td>
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<td>JN65119</td>
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<td>Callide Dam, Qld (24° 20'S, 150° 37'E)</td>
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<td>KM634</td>
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<td>Finch Hatton Gorge, Qld (21° 4'S, 148° 38'E)</td>
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<td>JN65116</td>
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<td>Qld (21° 2'S, 148° 43'E)</td>
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<td>KM637</td>
<td>JN65115</td>
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<td>Qld (21° 2'S, 148° 43'E)</td>
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<td>JN65113</td>
<td>JQ290772</td>
<td>Ingham, Qld (18° 35'S, 146° 9'E)</td>
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<td>KM815</td>
<td>♂</td>
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<td>JQ290801</td>
<td>The Tombs, Qld (25° 4' S, 147° 51' E)</td>
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<td>KM902</td>
<td>♀</td>
<td>JN965111</td>
<td>JQ290812</td>
<td>Wangaratta, NSW (36° 21' S, 146° 20' E)</td>
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**Poseidonimyia**

**simplex**  
(Lopes 1967)  
KM601 | ♀ | JN964766 | JQ290842 | Rockhampton, Qld (23° 22' S, 150° 30' E) |
KM658 | ♀ | JN964765 | JQ290845 | Paluma NP, Qld (19° 0' S, 146° 16' E) |
KM677 | ♀ | JN964764 | JQ290846 | Millaa Millaa, Qld (17° 29' S, 145° 39' E) |

**Sarcorhodendorphia**

**alpha**  
Johnston & Tiegs 1922  
KM994 | ♀ | JN964718 | JQ290861 | Qld (24° 31' S, 151° 28' E) |

**bidentata**  
(Lopes 1953)  
KM039 | ♀ | JN964792 | JQ290554 | Adelaide River, NT (13° 17' S, 131° 11' E) |
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) |
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) |
KM125 | ♀ | JN964778 | JQ290592 | Kakadu NP, NT (13° 17' S, 132° 20' E) |
KM127 | ♀ | JN964777 | JQ290593 | Kakadu NP, NT (13° 17' S, 132° 20' E) |
KM151 | ♀ | JN964775 | JQ290684 | Humpty Doo, NT (12° 35' S, 131° 7' 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) |
KM900 | ♀ | JN964767 | JQ290811 | Katherine, NT (132° 33' S, 14° 36' E) |

**bifrons**  
Walker 1853  
KM547 | ♀ | JN964793 | JQ290674 | Great Sandy NP, Qld (25° 56' S, 153° 4' E) |

**froggatti**  
Taylor 1917  
JW224 | ♀ | GQ254503 | JQ290534 | Balranald, NSW (34° 7' S, 143° 30' E) |
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) |
KM048 | ♀ | JN964853 | JQ290559 | Katherine, NT (14° 24' S, 132° 20' E) |
KM053 | ♀ | JN964851 | JQ290563 | Katherine, NT (14° 24' S, 132° 20' 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) |
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) |
KM884 | ♀ | JN964833 | JQ290807 | Culgoa Flood Plain NP, Qld (28° 55' S, 147° 0' E) |

**furcata**  
Hardy 1932  
JW234 | ♀ | GQ254450 | JQ290540 | Kings Park, WA (31° 57' S, 115° 49' E) |

**impatiens**  
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) |
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 | Coff's Harbour, NSW (30° 18' S, 153° 7' E) |
JW206v1 | ♀ | GQ254454 | JQ290523 | Coburg, Vic (37° 44' S, 144° 57' E) |
JW207v2 | ♀ | GQ254456 | JQ290524 | Bega, NSW (36° 40' S, 149° 49' E) |
JW235v1 | ♀ | GQ254463 | JQ290541 | Highton, Vic (38° 11' S, 144° 17' E) |
JW248v1 | ♀ | GQ254452 | JQ290548 | Moorebank, NSW (33° 58' S, 150° 20' E) |
Walker 1849
praedatrix
Roback 1952
piva
Johnston & Tiegs 1921
omikron
McKillup 2000
megafilosia
JW247v1 ♀ GQ254479 JW290547 Keppel Sands, Qld (23° 13' S, 150° 41' E)
Pape, McKillup & McKillup 2000
meiofilosia
JW250v1 ♀ GQ254481 JW290517 Keppel Sands, Qld (23° 13' S, 150° 41' E)
Pape, McKillup & McKillup 2000
omikron
KM221 ♀ JN965021 JQ290639 John Forrest NP, WA (31° 53' S, 116° 5' E)
Johnston & 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)
KM282 ♀ JN965013 JQ290887 Chapman River, WA (28° 46' S, 114° 43' 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)
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 Parkeeryerring Lake, WA (33° 22' S, 117° 21' E)
KM440 ♀ JN964998 JQ290937 Williams, WA (33° 1' S, 116° 52' E)
KM445 ♀ JN964996 JQ290891 WA (32° 34' S, 116° 26' E)
KM473 ♀ JN964994 JQ290829 Westdale, WA (32° 13' S, 116° 24' E)
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 Minganew, WA (29° 11' S, 115° 26' E)
KM582 ♀ JN965025 JQ290939 Eidsvold, Qld (25° 22' S, 151° 8' 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
JW197 ♀ GQ254438 JW290515 Mt Sampson, Qld (27° 18' S, 152° 50' E)
Walker 1849
JW208 ♀ GQ254435 JW290550 Urunga, NSW (30° 28' S, 153° 2' E)
JW209v1 ♀ GQ254436 JW290525 Lismore, NSW (28° 48' S, 153° 17' E)
KM041 ♀ JN965091 JQ290556 Kakadu NP, NT (12° 38' S, 132° 34' E)
zeta
Johnston & Tiegas 1921
JW223 ♂ GQ254434 JQ290533 Orboast, Vic (37° 43' S, 148° 27' E)
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)

sp. A sp. nov
KM670 ♂ JN964860 JQ290746 Wooroonooran NP, Qld (17° 37' S, 145° 44' E)
KM672 ♂ JN964859 JQ290752 Wooroonooran NP, Qld (17° 37' S, 145° 44' E)
KM680 ♂ JN964858 JQ290747 Dinner Falls, Qld (17° 25' S, 145° 29' E)

Sarcosolomonia
sp. X sp. nov
KM575 ♂ JN965171 JQ290928 Munduberra, Qld (25° 30' S, 151° 17' E)
KM831 ♂ JN965178 JQ290905 Qld (19° 41' S, 146° 26' E)
KM865 ♂ JN965181 JQ290933 Qld (19° 58' S, 145° 34' E)

Taylorimyia
aurifrons
Macquart 1846
JW218v1 ♂ GQ254477 JQ290530 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)
KM205 ♂ JN964937 JQ290917 Kalamunda NP, WA (31° 58' S, 116° 3' E)
KM210 ♂ JN964935 JQ290638 Kalamunda 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° 50' 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)
KM358 ♂ JN964916 JQ290709 Peaceful Bay, WA (34° 57' S, 116° 59' E)
KM359 ♂ JN964915 JQ290710 Waychinicup NP, WA (34° 50' S, 118° 20' E)
KM360 ♂ JN964914 JQ290852 Waychinicup NP, WA (34° 50' S, 118° 20' E)
KM363 ♂ JN964913 JQ290853 Waychinicup NP, WA (34° 50' S, 118° 20' E)
KM364 ♂ JN964912 JQ290616 Waychinicup 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)
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<td>Serpentine, WA (32° 19' S, 116° 2' E)</td>
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<td>Charles Darwin Reserve, WA (29° 34' S, 116° 59' E)</td>
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<td>JQ290784</td>
<td>Nth Stradbroke Island, Qld (27° 37' S, 153° 26' E)</td>
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<td>KM835</td>
<td>♂</td>
<td>JN964889</td>
<td>JQ290867</td>
<td>Lochada, Qld (29° 11' S, 116° 30' E)</td>
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**SARCOPHAGA UNKNOWNS**

**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) |

**TRICHARAEA** Thomson

**Tricharaea brevicornis**
- KM750 | ♀ | JN965189 | JQ290903 | Seven Mile Beach, Tas (42° 49' S, 147° 31' E) |
- KM751 | ♂ | JN965188 | JQ290904 | Seven Mile Beach, Tas (42° 49' S, 147° 31' E) |

(Wiedemann 1830)