Phylogenetics of Australasian gall flies (Diptera: Fergusoninidae): evolutionary patterns
of host-shifting and gall morphology

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Abstract

This study investigated host-specificity and phylogenetic relationships in Australian galling flies, *Fergusonina* Malloch (Diptera: Fergusoninidae), in order to assess diversity and explore the evolutionary history of host plant affiliation and gall morphology. A DNA barcoding approach using COI data from 203 *Fergusonina* specimens from 5 gall types on 56 host plant species indicated 85 presumptive fly species. These exhibited a high degree of host specificity; of the 40 species with multiple representatives, each fed only on a single host genus, 29 (72.5%) were strictly monophagous, and 11 (27.5%) were reared from multiple closely related hosts. COI variation within species was not correlated with either sample size or geographic distance. However variation was greater within oligophagous species, consistent with expectations of the initial stages of host-associated divergence during speciation. Phylogenetic analysis using both nuclear and mitochondrial genes revealed host genus-restricted clades but also clear evidence of multiple colonizations of both host plant genus and host species. With the exception of unilocular peagalls, evolution of gall type was somewhat constrained, but to a lesser degree than host plant association. Unilocular peagalls arose more often than any other gall type, were primarily located at the tips of the phylogeny, and did not form clades comprising more than a few species. For ecological reasons, species of this gall type are predicted to harbor substantially less genetic variation than others, possibly reducing evolutionary flexibility resulting in reduced diversification in unilocular gallers.

Keywords: *Fergusonina*, multigene phylogeny, host specificity, galling insects, Myrtaceae

Running Title: Diversity and Phylogeny in *Fergusonina* Flies

Introduction

The fields of ecology and evolution have the common goals of documenting biodiversity and illuminating the associated ecological patterns and evolutionary history. Plant-feeding insects are a particularly diverse assemblage and often exhibit conspicuous and biologically important ecological characteristics, including host shifts, which potentially affect patterns of diversification (e.g., Mitter et al. 1991; Farrell 1998; Winkler et al. 2009). Comparison of phylogenies from analyses of groups of phytophagous insects and their sister groups has shown that plant-feeding is significantly associated with increased diversity (Mitter et al. 1988).

Host specialization is a common characteristic of plant-feeding insects and appears to be linked to diversity and speciation in these organisms (Janz et al. 2006; but see Novotny et al. 2002). Assessing the degree of host specialization of insect species is an important first step in understanding the ecology and evolution of diet breadth, particularly in the face of abundant seemingly suitable empty niches (Hairston et al. 1960; Strong et al. 1984; Walker and Valentine 1984). To address questions of host specialization, it is necessary to define or delimit the species being studied. Much of the world's biodiversity is currently undescribed, particularly for small, morphologically similar organisms, which includes insects (Purvis and Hector 2000). Unfortunately, defining species is not straightforward; many different criteria and modifications have been proposed over the last 50 years (reviewed in de Queiroz, 2007). It has been suggested that difficulties with fixed species definitions come from the very nature of speciation, that it is often a temporally extended process with different characters evolving at different rates over time (de Queiroz 1998; Dres and Mallet 2002). It has even been argued that "species", in the sense of valid taxonomic and/or evolutionary entities, do not exist (Mishler 2010).

Approaches and methods with the goal of determining species limits have expanded rapidly since the advent of molecular systematics, which has allowed both the

detection of morphologically cryptic species as well as the assessment of biodiversity in understudied groups (Avisé 1994; Scheffer and Wiegmann 2000; Condon et al. 2008). Initially, species delineation was accomplished using a variety of molecular markers (Avisé 1994), which could vary depending on the laboratory or taxonomy of the organism being studied. Mitochondrial cytochrome c oxidase subunit I (COI) sequences have proved to be generally useful for species-level analyses in animals (Avisé 1994; Scheffer and Wiegmann 2000; Scheffer 2000; Hebert et al. 2003; Meyer and Paulay 2005), although there is abundant empirical and theoretical evidence that COI sequences may not track species evolution and diversification (Funk and Omland 2004; Rubinoff et al. 2006; Song et al. 2008). Nevertheless, COI sequences are widely used to represent a standardized and somewhat universal DNA barcoding identification system for animals, in which sequences are generated for comparison to provide a framework for species identification as well as for large-scale biodiversity assessments (Hebert et al. 2003; Tautz et al. 2003; Janzen et al. 2005; Meyer and Paulay 2005; Pons et al. 2006).

Although a number of methods for species delimitation using DNA barcode data have been developed, a recent study has concluded that a number of such methods do not perform substantially better than the Neighbor-Joining (NJ) 2.0% divergence cutoff approach used in the early days of species delimitation using mitochondrial COI data (Collins et al. 2012). This should not be surprising, as any system based on a single-marker can be expected to fail a certain amount of time given the vagaries of molecular evolution, differing taxon-specific biologies, and numerous species concepts. There is broad consensus that using data from multiple sources, such as additional genetic data, ecological traits, or newly detected morphological traits, is preferred in delimitation of species (Pons et al. 2006; Dowton et al. 2014; Yeates et al. 2011). Unfortunately, this is not always possible, particularly in the initial studies of diversity in poorly known groups.

In this study we analyzed COI sequence data from an extensive collection of gall-forming flies in the monotypic genus *Fergusonina* Malloch (Diptera: Fergusoninidae) in order to delineate presumptive species (hereafter “species”) and determine their host plant affiliations. Associations between genetic variation and the ecological characters of host specificity and geographic distribution are explored. In the second portion of this study, we conducted a phylogenetic analysis of the delineated species using multiple gene

regions, mitochondrial and nuclear, to explore the evolution of host associations, host specialization, and gall type in these flies.

Study Organisms

Fergusonina are small morphologically similar flies that feed within galls on various tissues of plants including leaf, shoot bud, and flower buds. All known plant hosts are within the family Myrtaceae (Currie 1937; Tonnoir 1937; Nelson et al. 2014). Galls are formed in conjunction with *Fergusobia* nematodes (Nematoda: Neotylenchidae) in an obligate mutualism, whereby the nematodes apparently facilitate gall-formation, while the female flies provide dispersal to new gall sites (for details see: Currie 1937; Giblin-Davis et al. 2003, 2004; Davies et al. 2010; Scheffer et al. 2013; Nelson et al. 2014). To date, *Fergusonina/Fergusobia* galls have been found on seven plant genera of Myrtaceae (Table 1), most notably, eucalypt (*Eucalyptus*, *Corymbia*, *Angophora*) and paperbark (*Melaleuca*) trees, as well as *Leptospermum*, *Metrosideros*, and *Syzygium*. The mutualism has been found almost exclusively in Australia, with four additional species-pairs known from India, New Guinea, New Zealand, and the Philippines (Harris 1982; Siddiqi 1986, 1994; Taylor et al. 2005; Taylor et al. 2007; Nelson et al. 2014).

From the cumulative morphological and molecular evidence available to date, most fly/nematode associations appear to induce gall formation on a specific host tissue and on a specific host species, although some instances of feeding on multiple hosts are known (Giblin-Davis et al. 2004; Davies and Giblin-Davis 2004; Nelson et al. 2014; Purcell et al. 2016). For example, some widespread eucalypts serve as hosts for multiple pairs of *Fergusonina/Fergusobia* mutualists (Taylor et al. 2005; Nelson et al. 2014; Purcell et al. 2016). However, a considerable number of fly and nematode species are undescribed (the authors, pers. obs.), making complete and comprehensive fly/nematodes/host associations difficult.

Since the initial revision by Tonnoir (1937), most taxonomic work on *Fergusonina* has been descriptions of one or a few species (e.g., Harris 1982; Taylor et al. 2007; Nelson et al. 2011a, 2011b, 2012), with the exception of Taylor (2004), in which eight species from broad-leaved paperbarks (*Melaleuca leucandendra* group) were described. In this latter study, each fly species was shown to feed on a single host plant

species, with the exception of *Fergusonina turneri* Taylor, which appears to feed on both *M. quinquenervia* and *M. fluviatilis*. Molecular analysis of COI/COII sequence data using a 2% cutoff from 53 members of the broad-leafed paperbark-feeding clade corroborated Taylor's morphological results for seven of the eight species. The sole exception was that the molecular data split *F. turneri* into three host-specific groups: two differing by 3% on *M. fluviatilis* and one on *M. quinquenervia* differing by 4.6–5.1% to the *M. fluviatilis* groups (Scheffer et al. 2004). Given the morphological similarities of closely related species in the Fergusoninidae, it is not clear whether *F. turneri* represents an oligophagous species feeding on two hosts or a set of monophagous cryptic species. Therefore, this nominal species cannot be used to evaluate the validity of a 2% delineation cutoff. In any case, all available morphological and molecular data indicate a high level of fly-nematode fidelity and host-plant specificity (Scheffer et al. 2004; Nelson et al. 2014; Purcell et al. 2016).

Taxonomic research on *Fergusobia*, the nematode mutualists, is ongoing. More than 40 nematode species have been described, generally based on both morphological and molecular findings (Currie 1937; Fisher and Nickle 1968; Siddiqi 1986, 1994; Davies and Lloyd 1996; Davies and Giblin-Davis 2004; Taylor et al. 2007; Taylor and Davies 2008, 2010; Davies *et al.* 2010a, 2012a, b, 2013a, b, 2014a, b, c, d, e). Molecular phylogenetic analysis of *Fergusobia* has found considerable specificity and constraint in host use and gall type (Ye et al. 2007).

This study uses DNA-barcoding, in conjunction with a three-gene phylogenetic approach, to provide an overall assessment of diversity, host specificity, and evolutionary history in *Fergusonina* flies.

2. Materials and Methods

2.1. Sampling

All specimens used in this study were dissected or reared from galls on myrtaceous plant species between 1998 and 2013 (Table 1). Identification of host plant species was accomplished using a variety of taxonomic keys including Brooker and Kleinig (2006), Nicolle (1997, 2006), and Euclid (Brooker et al. 2006). Our samples include specimens from galls on all seven Myrtaceae genera known to host fergusoninids

(Table 1). In some cases, identifications of *Eucalyptus* collections were not possible due to the lack of flowers, fruits, or mature leaves, and these species are referred to as “*E. sp.*”. *Eucalyptus pauciflora* s.l., “snow gum”, has a number of subspecies, most with very restricted ranges within the wider range of *E. pauciflora* (Brooker and Kleinig 2006; Nicolle 2006). The high elevation *E. niphophila* is considered a subspecies of *E. pauciflora* by some authors (Nicolle 2006) and a distinct species by others (Brooker and Kleinig 2006). In our overall assessment of fergusoninid host specificity, we recognize *E. niphophila* at the rank of species and consider all of the other subspecies to represent *E. pauciflora* s.s.

Galls were classified into broad types based on a modification of the scheme discussed in Nelson et al. (2014) (Table 2). Specifically, galls may be unilocular with a single chamber containing a fly larva or multilocular having as many as several hundred chambers or locules. Multilocular galls most typically involve shoot bud galls, flower bud galls, or expanded leaf blade galls. Unilocular galls often occur in leaf/shoot axils, either sessile or stalked, and resemble a “pea”, here referred to as a unilocular peagalls. Unilocular galls also occur on leaf blades either singly or sometimes in a connected row but because these galls could be considered unilocular or multilocular we simply refer to them without locular designation as leaf peagalls (Table 2). We classified galls collected from *Syzygium luehmannii* as “mix of galls” because these were small unilocular galls on leaves, stems, and buds, which were not separated during rearing.

2.2. DNA Extraction, PCR, and Sequencing

Adult, pupal, or larval fergusoninid specimens were stored in 95% ethanol at -80 C until use. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA). We amplified and sequenced portions of mitochondrial cytochrome c oxidase subunit I (COI), including the DNA barcode region. We used a 665 bp section of COI from 203 specimens to investigate species limits and 1455 bp, nearly the entire COI region, in the phylogenetic analysis. In addition, we sequenced 799 bp of carbamoyl phosphate synthetase domain (CAD, *rudimentary*) (Moulton and Wiegmann 2004; Scheffer et al. 2007) and 746 bp of phosphogluconate dehydrogenase (PGD) (Winkler et al. 2009). Primers for both PCR amplification and DNA sequencing are indicated in

Table 3. Those marked with an asterix are the “standard” primers we used to collect sequence data from all specimens. In cases where additional coverage was deemed necessary, we used additional primers for sequencing in a trial and error approach (Table 3). Variation in ease of data collection was most likely due to sample stage (e.g., small larva vs. adult) and varying collection and storage conditions prior to molecular work.

PCR amplifications for all three gene regions were carried out using Mastercycler Gradient thermocyclers (Eppendorf Scientific, Inc., Westbury, NY, USA) with a touchdown amplification program: initial denaturation at 92 °C for 2 min, followed by 2 touchdown cycles with annealing temperatures from 58 to 46°C (10s at 92°C, 10s at 58–46°C, 2min at 72°C), 29 cycles of 10s at 92°C, 10s at 45 °C, 2 min at 72 °C, and a final extension step for 10 min at 72°C. Sequencing reactions were carried out using BigDye Sequencing kits (Applied Biosystems, Foster City, CA) and the products were fractionated using ABI377 or ABI3130XL Automated DNA Sequencers (Applied Biosystems). Contigs for each gene from each individual specimen were assembled using the software package Sequencher (Gene Codes, Ann Arbor, MI). Consensus sequences for each gene region were aligned using MUSCLE (Edgar 2004) and then inspected using Se-Al (Rambaut 2002). Alignments were inspected by eye. The COI alignment contained no indels; for PGD we removed a 46 bp region comprising an intron; the CAD alignment contained several deletions uniformly within the ingroup as compared to the outgroups, and these were not removed. Sequences for the three genes were concatenated using Geneious 6.1.3 (Drummond et al. 2009). Outgroups used in the phylogenetic analyses were dipterans in families hypothesized to be related to fergusoninids (Wiegmann et al. 20011): *Australimyza* sp. (Australimyzidae), *Clusia lateralis* (Clusiidae), and *Xenasteia shalam* (Xenastiidae). Outgroup sequences KC177416, KC177428, and KC177433 were retrieved from GenBank; all other sequences have been deposited in GenBank with accession numbers as follows: COI [KY378134-KY378337], CAD [KY378060-KY378133], PGD [KY378338-KY378401].

2.3. Analyses

Species Delineation

For species delineation, we used a 665 bp region of the 3' end of COI from all specimens. We chose this region of COI because we have previously found it to be suitably variable for species delimitation in fergusoninids and because its use is consistent with our previous work (Scheffer et al. 2004). We calculated pairwise distances between the sequences using the HKY nucleotide substitution model (Collins et al. 2012). We used the PAUP plug-in within Geneious to construct a Neighbor-Joining tree with 1000 bootstrap pseudoreplicates. Specimens having pairwise distances equal to or less than 2.0 were lumped as a presumptive species. These groups were investigated for monophyly and bootstrap support by visual inspection of the Neighbor-Joining tree.

Host Associations and Gall Type

Host specificity was assessed for those species for which more than one specimen was present in our dataset. We tested whether monophagous and oligophagous species differed in intraspecific (defined at 2%) genetic variability using a t-test on average pairwise distance data. To investigate the relationship of sample size and geographic distance on levels of intraspecific variability, Pearson correlation coefficients were calculated and tested for significance. Correlations were performed first with the combined monophagous and oligophagous dataset and then for each monophagous and oligophagous dataset separately. Geographic distance of samples was considered as the approximate distance (km) between the two most geographically distant samples of any particular species and was estimated using Google Earth (<https://www.google.com/earth/>). Contingency tables were constructed to explore possible associations between dietary specialization and both host genus and gall type using Chi-Square tests of independence (<http://www.socscistatistics.com>).

Phylogenetics and Evolution of Host Use

Phylogenetic analysis of *Fergusonina* was performed using a 2999 bp concatenated alignment of COI, CAD, and PGD sequences from one representative of each “species” delineated in the COI analysis (see results below). Bayesian and maximum likelihood were used to construct phylogenetic trees. Preliminary analysis of each gene separately found substantial congruence (not shown), and the three genes were

concatenated for subsequent analyses. Using PartitionFinder2 (Lanfear et al. 2016), analyses were partitioned by gene and assessed for most suitable evolutionary model. In the subsequent analyses, the GTR+I+G model of nucleotide was used for each gene partition. For the Bayesian analysis, we used MrBayes 3.2 on the CSIRO Burnett supercomputer cluster. Analyses were run for 40 million generations to guarantee convergence. Maximum likelihood analysis was performed in the online CIPRES portal using the RAxML Blackbox tool (Miller et al. 2009). Likelihood branch support was also assessed using 1000 rapid bootstraps (Stamatakis et al. 2008) in RAxML v.8.2.0 (Stamatakis 2008), using a general time reversible model with a gamma model of rate heterogeneity, and data partitioned by gene.

Ancestral states for host genus and gall type were reconstructed using parsimony on the MrBayes 50% majority rule consensus tree using Mesquite v.3.03 (Maddison and Maddison 2015). In order to map characters on a tree that was not fully resolved, a single polytomy was treated as a hard polytomy by Mesquite. Parsimony statistics (consistency index (CI), retention index (RI), and number of steps) for both host genus and gall type separately were also computed in Mesquite in order to compare phylogenetic signal.

3. Results

3.1. Species limits and host-use patterns

The entire dataset included 203 fly specimens from 56 identified host species, and five gall types collected from sites throughout Australia (Tables 1 and 4, Figure 1). The dataset for assessment of species limits within *Fergusonina* comprised COI sequences (665 bp COI) from all specimens. Analysis of this dataset resulted in pairwise distances between specimens ranging from 0 to 23%. Application of a monophyly plus 2.0% maximum dissimilarity threshold resulted in delimitation of 85 presumptive species (Table 4), 43 of which were represented by more than one individual in the NJ analysis (Figure 2). In addition to being monophyletic, 42 had bootstrap support at 90% or above (37 at 100%) (Figure 2). With a single exception, all species having multiple representatives formed only a single gall type. The exception was Sp #57, which was reared from both a shoot bud gall and a flat leaf gall (Table 4). These specimens were both reared from unidentified *Eucalyptus* species and differed by only 0.3%.

There were 40 presumptive species of *Fergusonina* represented by multiple individuals from known hosts (i.e., when “*Eucalyptus* sp.” are excluded). Twenty-nine (72.5%) were monophagous and 11 (27.5%) were oligophagous, having been reared from two or more plant species. As many as four closely related host plant species were fed upon by a single species of *Fergusonina* (Table 5). In all cases, the shared host species were congeneric, and for *Eucalyptus*-feeders, the shared hosts also belonged to the same *Eucalyptus* subgenus (Table 5).

Oligophagous species harbored significantly more genetic variation, as measured by average intraspecific pairwise COI distances, than did monophagous species ($t=-2.93$, $p<0.006$). These intraspecific pairwise distance estimates were not significantly correlated with sample size, either in the entire dataset or by level of specialization (Figure 3a, overall $r=0.20$, $n=40$, $p>0.20$; monophagous only $r=0.35$, $n=29$, $p>0.06$; oligophagous only $r=-0.23$, $n=11$, $p>0.40$). Similarly, the pairwise distances were not significantly correlated with the maximum intraspecific geographic distance of samples, either when using the full dataset or when the data set was subdivided by degree of dietary specialization (Figure 3b, overall $r=0.12$, $n=40$, $p>0.20$; monophagous $r=0.23$, $n=29$, $p>0.20$; oligophagous $r=-0.27$, $n=11$, $p>0.40$).

The genera *Corymbia*, *Eucalyptus* and *Melaleuca* each contained at least one oligophagous species, with *Eucalyptus* recorded as host to 9 (82% of all species recorded as oligophagous) (Table 6a). A test of association to determine whether oligophagy occurred significantly more frequently in *Eucalyptus* than the other genera (combined) was marginally significant (Table 6a, Chi-sq=4.40529, $df=1$, $p=0.044$).

Among the oligophagous species of *Fergusonina*, shoot bud galls were the most common gall type, with 7 (63%) species forming this gall type. Leaf peggalls, flower bud galls, and unilocular peggalls comprise the remainder (Table 5). There was no association of host specificity and gall type (Table 6b, Chi-sq=0.1813, $df=1$, $p=0.670$).

Individual host plant species served as host to as many as eight species of *Fergusonina*, although the majority (67%) were host to only a single species (Table 7). Different species of *Fergusonina* feeding on the same plant host species often formed galls concurrently on the same plant individual. In most cases, the galls were of different types and in different tissues, although in several instances galls of different species on

the same host plant were of the same type and sometimes indistinguishable (Table 7). It was often possible to recognize the different species of *Fergusonina* dissected from these indistinguishable galls by their larval morphology (in third instar), and also by differences in the morphologies of the associated *Fergusobia* nematodes (when diagnosable stages are present).

3.2 Phylogenetics and host use evolution

Maximum likelihood and Bayesian analysis of the concatenated COI, CAD, and PGD dataset found the monophyletic *Fergusonina* to be highly divergent from the outgroups. Within the Fergusoninidae, considerable conservation of host-use was apparent (CI=0.67, RI=0.88, steps=9; Figures 4, 5). Most major clades of *Fergusonina* were reared from only a single host plant genus (Figure 5), and in all of the 25 cases of “sister” taxa (regardless of pairwise distance), both species shared the same host plant genus or subgenus (Figure 4).

There was a deep split between the few samples reared from *Syzygium* and the other plant hosts. Most notably, 41 (89%) out of the 46 species of *Fergusonina* reared from eucalypts were found within two large clades restricted to *Eucalyptus*. Although these had little bootstrap support, they contained well-supported *Eucalyptus*-feeding subclades. Fergusoninids on the two subgenera of *Eucalyptus* included in this study, *Eucalyptus* and *Symphyomyrtus*, also exhibited patterns of host-restriction. The 13 specimens from the subgenus *Eucalyptus* formed two well-supported clades (excluding two fly specimens from undetermined *Eucalyptus* spp.), one sister to a clade of *Symphyomyrtus* gallers and one nested within a clade of *Symphyomyrtus* gallers (Figure 5). *Melaleuca*-feeding fergusoninids formed two distinct host-associated clades: one well-supported clade on the monophyletic broad-leaved *Melaleuca* group, and a second small clade from three narrow-leaved species of *Melaleuca* (Figures 5).

In the ancestral state reconstruction of host plant affiliation, the identity of the ancestral host was ambiguous, being either *Eucalyptus* or *Syzygium* (Figure 5). Subsequent nodes were clearly associated with *Eucalyptus* (Subgenus *Symphyomyrtus*). All remaining fergusoninids formed a large clade associated with a mix of host genera, but the polytomy at the base of this clade precludes ancestral host reconstruction.

However, within the clade, host-associated lineages are present. Association with *Melaleuca* appears to have evolved once, with a subsequent divergence into a primarily broad-leaf *Melaleuca* clade (one narrow-leaf exception) and a lineage comprised of a narrow-leaf *Melaleuca* clade sister to a clade of six species exhibiting unusually diverse feeding habits: four gall types on three host genera (Figure 5).

Host shifts to *Corymbia*-feeding likely evolved three times (Figure 5). Approximately half of the eight or so species that feed on *Corymbia* formed a highly supported monophyletic clade, while the remaining species were present in two well-supported clades each of which contained species from more than one host genus. These two small “mixed” clades, one of which was sister to the narrow-leaved melaleucas, above, were the only well-supported clades to involve shifts between host plant genera (Figure 5).

Oligophagous *Fergusonina* species were distributed across the phylogeny, but were significantly overrepresented in the C+D clade (Figure 5). Approximately 55% (6 of 11) of the oligophagous species were present in this clade of 17 species. In contrast, only 5 of the remaining 67 species in the phylogeny were oligophagous, a highly significant difference in distribution of oligophagy across the phylogeny (Table 6c, Chi-sq=19.01, df=1, $p < 0.0001$; Figure 5).

The evolution of gall type exhibited a moderate degree of conservation on the *Fergusonina* phylogeny (CI=0.25, RI=0.66, steps=16), although less than did host affiliation (Figure 5). All gall types appear to have evolved at least twice, with unilocular peagalls having arisen as many as seven times (Figure 5). Most species of *Fergusonina* were embedded within clades of various sizes consisting of the same gall type. In 23 of 25 cases of supposed sister taxa (regardless of distance), both species shared the same gall type (Figure 5).

There appeared to be no relationship between shifts to new plant genera and the evolution of a new gall type (Figure 5). Of 68 resolved nodes in the phylogeny, ancestral reconstructions indicated that only two nodes involved both a shift to a new genus and the acquisition of a new gall type (black dots in Figure 5). However, in both cases, the shift in the fly and the shift in the nematode were not in the same direction. At the upper node, the shift in host affiliation involved a host shift from *Melaleuca* to *Corymbia* on

branch A. From the same node there was a shift in gall type, from shoot bud galls to unilocular peggalls on branch B (Figure 5). Although the two shifts in host affiliation and gall types occurred from the same node, the shifts occurred in different lineages (A vs. B branches) and did not correspond with each other. The second set of black dots indicates another case of apparently associated changes in host affiliation (*Eucalyptus* subgenus *Symphyomyrtus* to subgenus *Eucalyptus*) and changes in gall type. As in the previous case, careful inspection showed that the changes did not occur on the same branches (C vs. D) and, therefore, did not correspond (Figure 5).

The clade containing *Melaleuca* may contain two additional co-associated shifts in plant host and gall type, but without resolution of the basal polytomy and reconstruction of the characters, the status of potential nodes was unclear (Figure 5).

Discussion

In recent years, morphological and ecological investigations of fergusoninid flies and their nematode mutualists have made clear that substantial diversity awaits discovery in these unusual organisms (Taylor 2005; Taylor et al. 2007; Scheffer et al. 2004; Ye et al. 2007; Davies et al. 2010, 2016; Nelson et al. 2011a, 2011b, 2012, 2014; Purcell et al. 2013, 2016). Our present analysis of COI sequence data from 203 *Fergusonina* specimens representing five gall types and seven host genera has resulted in the delimitation of 85 putative species, approximately 19 of which correspond to one of the 38 named *Fergusonina* species (Table 7, see Nelson et al. 2014).

As is typical of most endophagous phytophagous insects, we found fergusoninids to be quite host-specific. Of the 43 presumptive species represented in our study by more than one individual, 100% fed only on a single genus, while 73% were strictly monophagous, feeding on a single host plant species. However, this is undoubtedly an underestimate of monophagy. First, two of 11 oligophagous species are classified here as oligophagous depending on whether *E. niphophila* is considered distinct from *E. pauciflora*. If these were each considered to represent a single host species, the reclassification of two oligophagous species of *Fergusonina* into four monophagous species would increase the estimate of monophagy from 73% to 83%. Second, our estimate of specialization does not consider the 42 species represented in this study by

only a single fly specimen. Most specimens in this study were collected in the presence of many potential host plant species. This is particularly true for samples from *Eucalyptus*, which is an extremely widespread genus of over 700 species, most of which are found in sympatry with other species of *Eucalyptus*. Simply put, *Fergusonina* species have substantial ecological opportunity for oligophagous associations, and researchers in the field have ample opportunity for collecting them on more than one host. We suggest that host-plant specialization of fergusoninids is considerably greater than appears here.

In contrast to general expectations, intraspecific genetic variation was not correlated with either sample size (ranging from $n=2$ to 15) or geographic distance of samples (ranging from 0 to 3000 km) in any of the analyses; this is true whether using the entire dataset or subdivisions based on host specificity (Figure 4). This indicates, that in terms of sampling, our 2% COI distance criterion was largely robust to variation in sample size and geographic distance, such that it was reasonably suitable for delimitation of putative species. However, without multiple lines of evidence, the accuracy of our COI approach to species delimitation remains to be rigorously tested.

Although there appeared to be no significant relationship between genetic variation and either sample size or geographic distance, genetic variation did differ with host specialization. Oligophagous species harbored significantly more genetic variation than monophagous species ($t=-2.93$, $p<0.006$; see values in Table 4). Within phytophagous insects, it is not uncommon for what appear to be oligophagous species to represent diverging or recently diverged cryptic species (e.g., Fox and Morrow 1981; Blair et al. 2005; Scheffer and Hawthorne 2007; Nosil 2012). Genetic adaptation to different host plants could lead to what appears to be increased intraspecific genetic variation when both host-associated populations are regarded as a single species. Our results are consistent with a scenario involving increased genetic variation arising from processes of host plant associated divergence within oligophagous species. Interestingly, oligophagous species were disproportionately represented in clade C+D (Figure 5), indicating a propensity for either acquiring and/or maintaining this habit within this clade. Further exploration of oligophagy within *Fergusonina*, along with the genetics and ecology of associated host-use characters, is likely to shed light on processes of diversification and uncover substantial cryptic diversity.

Phylogenetics

Phylogenetic analysis found the *Syzygium* feeders to be sister to the clade encompassing all of the other fergusoninids (Figure 5). To date, flies from *Syzygium* in Australia have only been found from a single location on the Atherton Tablelands of the Wet Tropics in Far North Queensland, despite considerable search efforts targeting *Syzygium* in other locations within Australia. An additional fergusoninid, *F. syzygii*, was previously described from *Syzygium* in India but no specimens were available for this study.

Syzygium is widespread in southeastern Asia, and it is likely that additional fergusoninid species will be found on this genus in this region. Whether additional *Fergusonina* on *Syzygium* are affiliated with those in the current tree forming the sister group to all the other *Fergusonina* is unknown, but has important implications for our understanding of the origin, early host use, and diversification of these flies.

The evolutionary history of host-use by fergusoninids on the other genera of Myrtaceae is one of considerable genus-level conservatism; most species of *Fergusonina* belonged to large host restricted clades (Figures 5, 6), indicating that once a host genus or subgenus is colonized, the descendant species typically remain on hosts within that genus or subgenus. However, with the exception of *Leptospermum* galls (n=2) fergusoninids were not found to be strictly monophyletic on any host genera or *Eucalyptus* subgenera, indicating some degree of colonization and host switching at the level of plant genus.

Feeding on *Eucalyptus* appears to be the ancestral state for the main lineage of *Fergusonina* (Figure 5). A single host shift from *Eucalyptus* to other genera in an large unresolved polytomy is likely to represent one of only two host shifts away from *Eucalyptus*. The second shift involves only a single species (FC-79 in Figure 5) and is from *Eucalyptus* to *Corymbia*. All species of *Fergusonina* feeding on the *Eucalyptus* subgenus *Eucalyptus* formed three distinct clades (Figure 5, yellow boxes), one imbedded within and one sister to clades feeding on the subgenus *Symphyomyrtus*, with the third encompassing the clade containing the shift from *Eucalyptus* to *Corymbia* (Figure 5).

Fergusonina feeding on *Melaleuca* also exhibit an evolutionary history of host-conservation. In the host plant reconstruction, the fergusoninids feeding on *Melaleuca* came out in a single clade within which a shift to *Corymbia* occurred (Figure 5, node A-

B) resulting in two *Melaleuca* subclades. The largest fed almost exclusively on the monophyletic broad-leaved paperbark group with the single exception of one species on *M. nodosa*, a narrow-leaved form. The second smaller clade (n=3) was monophyletic on narrow-leaved *Melaleuca* species (Figures 4, 5). Despite the small number of *Corymbia*-feeding samples in this study, the reconstruction indicated that this host genus has been colonized at least three times (Figure 5).

The high degree of plant host conservation of the majority of *Fergusonina* clades suggests that host shifts during speciation in *Fergusonina* occur under considerable evolutionary constraint. The notion of evolutionary constraints has a long history in evolutionary biology and encompasses various conditions of genetic variation, efficacy of selection, and evolutionary stasis (reviewed by Futuyma 2010; Futuyma and Agrawal 2009). Heritable genetic variation is necessary for evolutionary change (Falconer and Mackey 1989); an absence of such variation in a particular character constrains evolution in that character. For example, an insect species may lack the genetic variation necessary for evolutionary change in a character, such as the ability to feed on plant A, but possess the genetic variation needed to shift to plant B. Over evolutionary time, a host shift by this species to plant B would be more likely than to plant A (Futuyma et al. 1993, 1995; Scheffer 1999; Uesugi 2008). This is simultaneously a constraint on evolutionary change to host A as well as a preferential shift to host B.

That host constraints and preferential host shifting are a large component of host-use evolution in the fergusoninids is apparent not only in the high level of host genus conservation in the phylogeny, but also by the observation that none of the 25 sister lineages identified in the phylogeny involve a shift to a new host genus. Some of the splits between these sister lineages are quite deep and probably do not represent recent speciation events; that there appear to be no host genus shifts even at these older nodes reinforces the conclusion that host shifts at the genus level are rare. More dramatically, the fact that no fergusoninids have successfully shifted to plants beyond the Myrtaceae serves to highlight their propensity for evolutionary constraint in host affiliation.

In this study, 18 of the 64 plant species served as host to more than one fergusoninid species (Table 5). In most cases, the fergusoninids sharing a host produced different types of gall and were rarely sister species, indicating repeated recolonization of

host plant species (Figure 5). This is in contrast to some other galling insects (Cook et al. 2002; Joy and Crespi 2007) that appear to radiate into different gall niches following colonization of a single host species. The greatest number of *Fergusonina*/*Fergusobia* species has been found on the most widespread host plant species – *E. camaldulensis* (4 species, 3 gall types) and *E. pauciflora* (6 species, 3 gall types) (Table 5). These host species have broad geographic ranges, thus presenting more opportunity for colonizations by fergusoninids. However, such widespread distributions also made it more likely that we encountered and sampled these plants. Whether the level of diversity in fergusoninids and associated nematodes seen on these host plants reflects a general pattern remains to be seen.

The evolution of gall type in fergusoninids showed varying degrees of evolutionary constraint across the fly phylogeny (Figure 6). In only two of 25 cases of “sister” species was there a difference in gall type, indicating that shifts are not especially frequent. Several large clades of flies reared from a single gall type can be seen in the phylogeny (Figure 5). Character reconstruction of gall type across the phylogeny suggests that essentially all shifts in gall type involved shifts from shoot bud galls, the sole exception being a leaf peggaller arising from a flower bud galler (FC-113, FC-173). All five of our defined gall types have evolved multiple times (Figure 5). In fact, the six species forming the clade designated as A (Figure 5) exhibit four of the five gall types.

Although the evolution of gall type appears to be somewhat labile, with multiple instances of all gall types, future sub-classification of gall types may indicate less lability, in the sense of less convergence in gall type, than appears here (see Purcell et al. 2016). For example, the site of galling within flower buds differs in which tissue is actually galled, e.g., ovaries vs. stamens (Giblin-Davis et al. 2004), but this type of fine-scale data were not collected for all galls in the study so we treated all flower bud types as one category. Subdivision of the leaf peggaller (one vs. several locules) category and the unilocular peggaller (axillary vs. stalked) might also prove useful. It could be that, in some cases, what appears to be multiple origins of the same gall type across the phylogeny may instead represent the evolution of novel gall sub-types following recolonization of broad categories of host tissues for oviposition. In *Fergusonina*, it seems that oviposition typically takes place while the host tissue is in the early bud stage (Giblin-Davis et al.

2004). Although buds may ultimately give rise to different tissues, it may be that at the time of oviposition, the buds for different host tissues represent fairly similar targets for female flies, setting the stage for an evolutionary shift in gall type. Analysis of the microfeatures of galls and their reconstruction across *Fergusonina*, *Fergusobia*, and host plant phylogenies could greatly impact our current view of gall type lability in this mutualism.

An additional feature of *Fergusonina* possibly relevant to the evolution of gall type is interspecific variation in a morphological character known as the larval dorsal shield. This is a conspicuous structure on the fly larvae of sclerotized (hardened) bumps, ridges, plates, and, in some cases, dramatic hooks of unknown function and not present in any other Diptera (Currie 1937, Taylor et al. 2005; Taylor and Davies 2010; Purcell et al. 2016). The presence of thick mucus near the dorsal shield, often associated with nematodes, suggests a possible secretory role (Currie 1937; Davies et al. 2016; David Merritt, pers. obs.). In addition, the dorsal shield may be used to anchor the fly larva within the gall cavity while feeding or to scrape the internal wall of the gall chamber to enlarge it and/or to stimulate the proliferation of fresh gall tissue on which the larva and/or nematodes feed (Taylor et al. 2005; Taylor and Davies 2010). However, the dorsal shields of some fergusoninid species seemingly lack characters useful for scraping. A recent study has shown that individual clades of fergusoninids share the same dorsal shield morphology (Purcell et al. 2016), indicating that additional exploration of all aspects of the dorsal shield is important to understanding *Fergusonina/Fergusobia* biology and host plant interactions.

For the Fergusoninidae, the intimate and obligate relationship between the flies and their associated nematodes undoubtedly adds a layer of complexity to the mechanisms involved with shifting onto new host species or new host tissues (gall types) in this mutualism. Unlike all other phytophagous insects, female fergusoninids carry nematodes that are deposited during each fly oviposition. Gall formation on a new host necessarily requires survival of both the fly larvae and the *Fergusobia* nematodes as galls lacking either one of the mutualists have not been found (nor yet generated in the lab).

Obviously, the initial stage of a host range expansion or a host shift in *Fergusonina/Fergusobia* depends on the behavior of the female fly as she determines

which novel host plants and/or host tissues are used for oviposition. Because the nematodes are responsible for gall formation, they are critical to the success of the initial stages of a host shift; the nematodes must be able to survive and perform on the new host. If the nematodes die or perform poorly, the offspring of the female fly will also die or perform poorly. The outcome of these processes, i.e., whether a shift occurs or not, depends to a large extent on the levels of genetic variation for these traits within both the fly and nematode populations.

In the case of the *Fergusobia* nematodes, which essentially have no “free-living” stage, effective population sizes are likely to vary depending on the locularity of the galls (Nelson et al. 2014). Large multilocular galls can have multiple, conspecific, fly foundresses (Purcell et al. 2015), probably allowing mating among nematodes deposited by multiple female flies into a single gall. Such panmixia of conspecific nematodes should maintain some degree of genetic variation within nematode species in these larger galls. In contrast, nematodes within unilocular galls, those having an isolated, single chamber, must be restricted to extreme inbreeding via mating between siblings multiple generations. In addition, a large portion of the nematode population in unilocular galls is lost each generation when they are deposited along with a *Fergusonina* egg destined to be male (adult male flies do not carry nematodes, Scheffer et al. 2014). These *Fergusobia* are predicted to harbor low levels of genetic variation causing limited evolutionary flexibility. Due to the critical role played by *Fergusobia* nematodes in gall formation (and probably other functions), the genetic effects of long-term inbreeding may reduce the ability of the entire mutualism to withstand environmental or other challenges, thus leading to increased extinction and possibly constraining speciation.

The highly inbred mating system of unilocular galling nematodes may cause their genetic structure to be very similar to that of asexual species. A classic case of the apparent effect of lack of genetic variation on evolutionary flexibility is that of asexual organisms. On theoretical grounds, the origin of asexuality is often considered to be advantageous (Williams 1975; Maynard Smith 1978), and asexuality can be found in many animal lineages (Bell 1982; Schwander and Crespi 2009). However, asexuality may also have a cost in that genetic variation is lost, deleterious mutations accumulate, and population-wide recombination does not produce new genotypes (Maynard Smith

1978). These consequences may prevent a species from moving into new resources and adapting to new environmental challenges. This implies that although asexuality may arise not uncommonly, the lineages do not persist and/or form large clades (but see Schwander and Crespi 2009). Under this scenario, asexuality should be most often associated with recent shallow lineages at the tips of the phylogenies, but not with older and larger clades.

Phylogenetic analysis of *Fergusonina* shows that the evolutionary history of unilocular peagall formation is similar to that of asexuality. On the fergusoninid phylogeny, unilocular peagalls show a pattern consistent with repeated origins and reduced diversification: there are seven independent origins of unilocular peagallers with no more than three extant species in any clade. In contrast, flowerbud gallers appear to have originated five times, with one of the clades containing eight species (plus a shift to a leaf peagall), while the other major gall types have evolved three times or fewer. The seven lineages of unilocular peagallers are found throughout the phylogeny, indicating that this gall type has arisen in multiple major lineages (Figures 4, 5). Why this gall type has arisen more frequently than others is a puzzle. Genetic variation aside, it seems as though the small size of this gall type would make the occupants more susceptible to a variety of ecological challenges such as increased parasitism by hymenopterans, less protection from dramatic changes in climate, and substantially smaller population sizes (for review of advantages, see Price et al. 1983; Stone and Schönrogge 2003). However, at this point we know very little of the ecological and genetic aspects of Fergusobia. Further phylogenetic and population genetic investigations of both *Fergusonina* and *Fergusobia* mutualists will be necessary to address the possible impact of gall-associated life history differences in diversification in this mutualism.

Currently, we know little regarding the initial shift or origin of *Fergusonina* and *Fergusobia* on Myrtaceae; neither contemporary *Fergusonina* nor their nematode mutualists, *Fergusobia*, appear to have close relatives that might shed light on ancestral hosts and feeding modes, and the mutualistic association itself is unique (Giblin-Davies et al. 2003, 2004; Wiegmann et al. 2011; Nelson et al. 2014). In general, galling insects are disproportionately overrepresented in xeric habitats, where galls serve to provide constant humidity, in addition to protection from natural enemies and nutrition

(Fernandez and Price 1992; Price et al. 1998). Australia has become increasingly xeric over the past 20 million years (Byrne et al. 2008), possibly contributing to the radiation of *Fergusonina*/*Fergusobia* (Nelson et al. 2014). Additional work comparing history of the Australian climate and biota along with timing and topologies of the *Fergusonina*, *Fergusobia*, and Myrtaceae host plant diversifications is underway and expected to shed light on diversification of this mutualism.

Conclusion

Our study has focused on evolutionary history of *Fergusonina* and its association with host plants in the Myrtaceae. We found that diversity and patterns of host-associated radiations in this group primarily reflect plant taxon-constrained host shifting such that host shifts associated with speciation most often involved closely related plants. This pattern was not only seen in the conservation of host genus and subgenus in the phylogeny, but is also consistent with the observation that fergusoninids have not successfully colonized any other plant family nor even most genera within the Myrtaceae. Sister species (based on current phylogeny) of *Fergusonina* fed only on congeneric hosts, and the hosts of oligophagous species were also closely related plant species. The oligophagous species contained more genetic variation than the specialists, consistent with oligophagy as an early stage of host-constrained and host-associated speciation.

The evolution of gall type also exhibited some evolutionary constraint across the fly phylogeny, but to a lesser degree than host plant genus. Unilocular peggalls originated more times than any other gall type, but were found primarily at the tips of the phylogeny. It may be that the evolution of unilocular gall type is advantageous in some unknown way, but that reduced genetic variation in the *Fergusobia* nematodes associated with this gall type, impairs evolutionary flexibility, speciation, and/or persistence.

Fergusonina and *Fergusobia* are part of a more complex network of interactions involving parasitic relationships between the mutualism and the host plants. In contrast to the flies feeding on the host plants, for many gall forms the *Fergusobia* nematodes are vertically transmitted across fly generations, thus raising the expectation of cospeciation between at least some lineages of *Fergusonina* and *Fergusobia*. Further investigation of the phylogenetics of these mutualists, as well as their host plants, will shed light on the

roles played by cospeciation, host-shifting, and evolutionary constraint in determining current patterns.

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Figure Legends

Figure 1. Collecting locations in Australia. Each dot represents a location where samples were collected. Colors indicate genera of hosts from which samples were collected.

Figure 2. Neighbor-Joining consensus of 665 bp of COI for species delimitation. Species for which more than one individual is in the study are shown in blue with plant host(s) shown. Light blue denotes monophagous species, dark blue indicates oligophagous species, and a black circle indicates a singleton representative. Bootstrap values shown below branches of monophagous and oligophagous species. Height of triangles reflects sample size.

Figure 3. Relationship between average pairwise distance and a) sample size of each species, and b) maximum geographic distance within each species. Both datasets analyzed three ways: 1) All species in dataset, 2) Monophagous species only, 3) Oligophagous species only. Bonferroni correction for multiple (six) tests requires significance criterion of $p < 0.05$ be reduced to $p < 0.008$.

Figure 4. Bayesian analysis of relationships among *Fergusonina* species. Posterior probabilities above 0.95 indicated above branches while likelihood bootstrap values indicated below. Colors designate genus affiliations, while gall type is shown as cartoon representations (see Table 2). Red circles indicate nodes with descendants categorized here as “sister” species (see Methods).

Figure 5. Character state reconstruction of host plant affiliation (on left) and gall type (on right) using the topology of the Bayesian tree that is presented in Figure 4. Code names of oligophagous species indicated by blue rectangles. *Eucalyptus* subgenus *Eucalyptus* contained in yellowish boxes. Remaining *Eucalyptus* belong to subgenus *Symphytomyrtus*. In this figure, unidentified *Eucalyptus* species were drawn as belonging to the subgenus of their sister species or clade. Black branch in the Plant Host Genus tree on the left indicates an ambiguity???? in the host reconstruction. Clades A, B, C, D, and black circles denote regions of the trees discussed in the text (Results, Discussion).

References

- Avice, J.C., 1994. *Phylogeography: The History and Formation of Species*. Harvard Univ. Press, Harvard, MA.
- Bell, G., 1982. *The Masterpiece of Nature. The Evolution and Genetics of Sexuality*. University of California Press, Berkeley, California.
- Blair, C.P., Abrahamson, W.G., Jackman, J.A., Tyrrel, L., 2005. Cryptic speciation and host-race formation in a purportedly generalist tumbling flower beetle. *Evol.* 59: 304-316.

Brooker, M.I.H., Kleinig D.A., 2006. Field Guide to Eucalypts, Vol. 1: South-Eastern Australia, 3rd ed. Blooming Books, Melbourne, Australia.

Brooker, M.I.H., Slee, A.V., Connors, J.R., 2006. EUCLID Eucalypts of southern Australia, 3rd edition, CD. CSIRO Publishing, Collingwood, Australia.

Byrne, M., Yeates, D. K., Joseph, L., Kearney, M., Bowler, J., Williams, M.A.J., Cooper, S., Donnellan, S.C., Keogh, J.S., Leys, R., Melville, J., Murphy, D.J., Porch, N., Wyrwoll, K.-H., 2008. Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota. *Mol. Ecol.* 17: 4398–417.

Collins, R.A., Boykin, L.M., Cruickshank, R.H., Armstrong, K.F., 2012. Barcoding's next top model: an evaluation of nucleotide substitution models for specimen identification. *Methods in Ecology and Evolution* 3: 457-465. doi: 10.1111/j.2041-210X.2011.00176.x

Condon, M.A., Scheffer, S.J., Lewis, M.L., Swensen, S.M., 2008. Hidden neotropical diversity: greater than sum of its parts. *Sci.* 320: 928-931. doi: 10.1126/science.1155832

Cook, J.M., Rokas, A., Pagel, M., Stone, G.N., 2002. Evolutionary shifts between host oak sections and host-plant organs in *Andricus* gallwasps. *Evol.* 56: 1821-1830.

Currie, G.A., 1937. Galls on *Eucalyptus* trees. A new type of association between flies and nematodes. *Proc. Linn. Soc. New South Wales* 62: 147-174.

Davies, K.A., Giblin-Davis, R.M., 2004. The biology and associations of *Fergusobia* (Nematoda) from the *Melaleuca leucadendra*-complex in eastern Australia. *Invert. Syst.* 18: 291-319.

Davies, K.A., Lloyd, J., 1996. Nematodes associated with Diptera in South Australia: a new species of *Fergusobia* Currie and a new record of *Syrphonema* Laumond & Lyon. *Trans. R. Soc. S. Australia* 120: 13-20.

Davies, K.A., Ye, W., Giblin-Davis, R., Taylor, G.S., Thomas, W.K., 2010. Nematodes from galls on Myrtaceae. I. *Fergusobia/Fergusonina* galls on *Corymbia* spp., with redescription of *F. magna* and notes on its phylogenetic relationships. *Zootaxa* 2634: 25-40.

Davies, K.A., Ye, W., Giblin-Davis, R., Taylor, G.S., Thomas, W.K., 2012a. Nematodes from galls on Myrtaceae. II. *Fergusobia/Fergusonina* from small axillary bud ('stem') and leaf ('pea') galls in Australia, with descriptions of two new species. *Zootaxa* 3415: 1-22.

Davies, K.A., Ye, W., Giblin-Davis, R., Taylor, G.S., Thomas, W.K., 2012b. Nematodes from galls on Myrtaceae. III. *Fergusobia* from flower bud and stigma galls on *Eucalyptus*, with descriptions of four new species. *Zootaxa* 3532: 1-36.

Davies, K.A., Ye, W., Giblin-Davis, R., Taylor, G.S., Thomas, W.K., 2013a. Nematodes from galls on Myrtaceae. IV. *Fergusobia* from flat leaf galls on *Eucalyptus* and *Corymbia*, with descriptions of two new species. *Zootaxa* 3741: 151-171.

Davies, K.A., Ye, W., Giblin-Davis, R., Taylor, G.S., Thomas, W.K., 2013b. Nematodes from galls on Myrtaceae. V. *Fergusobia* from large multilocular shoot bud galls from *Angophora* and *Eucalyptus* in Australia, with descriptions of five new species. *Zootaxa* 3741: 101-140.

Davies, K.A., Giblin-Davis, R.M., Ye, W., Taylor, G.S., Hodda, M., Thomas, W.K., 2014a. Nematodes from galls on Myrtaceae. VII. *Fergusobia* from 'leafy' leaf bud galls, with re-description of *Fergusobia tumifaciens* (Currie 1937) Wachek 1955 and descriptions of two new species. *Zootaxa* 3856: 529-554.

Davies, K.A., Taylor, G.S., Nelson, L.A., Yeates, D., Giblin-Davis, R.M., 2014b. Nematodes from galls on Myrtaceae. VI. *Fergusobia* from galls on *Angophora* in Australia, with description of *F. colbrani* n. sp. and key. *Zootaxa* 3856: 326-348.

Davies, K.A., Bartholomaeus, F., Giblin-Davis, R.M., Ye, W., Taylor, G.S., Thomas, W.K., 2014c. Nematodes from galls on Myrtaceae. VIII. *Fergusobia* from small galls on shoot buds, with descriptions of four new species. *Zootaxa* 3857: 1-40.

Davies, K.A., Ye, W., Giblin-Davis, R., Taylor, G.S., Thomas, W.K., 2014d. Nematodes from galls on Myrtaceae. IX. *Fergusobia rosettae* n. sp. on *Melaleuca quinquenervia* and *F. tolgaensis* n. sp. on *Syzygium luehmanna*, from Queensland. *Zootaxa* 3889: 214-236.

Davies, K.A., Bartholomaeus, F., Giblin-Davis, R.M., Ye, W., Taylor, G.S., Thomas, W.K., 2014e. Nematodes from galls on Myrtaceae. X. *Fergusobia* from galls on narrow-leaved *Melaleuca* spp. in Australia, with descriptions of three new species. *Zootaxa* 3889: 237-258.

Davies, K., Ye, W., Giblin-Davis, R., Taylor, G., Scheffer, S., Thomas, W., 2010a. The nematode genus *Fergusobia* (Nematoda: Neotylenchidae): molecular phylogeny, descriptions of clades and associated galls, host plants and *Fergusonina* fly larvae. *Zootaxa* 2633: 1-66.

De Queiroz, K. 1998. The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations, in: Howard, D.J., Berlocher, S.H. (Eds.) *Endless Forms: Species and Speciation*, Oxford Univ. Press, Oxford, UK.

De Queiroz, K., 2007. Species Concepts and Species Delimitation. *Syst. Biol.* 56: 879-886. doi: 10.1080/10635150701701083

Dowton, M., Meiklejohn, K., Cameron, S.L., Wallman, J., 2014. A preliminary framework for DNA barcoding, incorporating the multispecies coalescent. *Systematic Biology*, 63: 639-644.

Drès, M., Mallet, J., 2002. Host races in plant-feeding insects and their importance in sympatric speciation. *Phil. Trans. R. Soc. B Biol. Sci.* 357: 471-492.

Drummond, A. J., Ashton, B., Cheung, M., Heled, J., Kearse, M., Moir, R., Wilson, A., 2009. Geneious R7.

Drummond AJ, Rambaut A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* 7:214.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**, 1792–1797.

Falconer, D.S., Mackay, T.F.C., 1996. Introduction to Quantitative Genetics, 4th edition. Pearson, 480pp.

Farrell, B.D., 1998. “Inordinate fondness” explained: why are there so many beetles? *Sci.* 281: 555-559.

Fernandez, G.W., Price, P.W., 1992. The adaptive significance of insect gall distribution: survivorship of species in xeric and mesic habitats. *Oecol.* 90: 14-20.

Fisher, J.M., Nickle, W.R., 1968. On the classification and life history of *Fergusobia curriei* (Sphaerulariidae: Nematoda). *Proc. Helminth. Soc. Wash.* 35: 40-46.

Fox, L.R., Morrow, P.A., 1981. Specialization: species property or local phenomenon? *Sci.* 211: 887-893.

Funk, D.J., Omland, K.E., 2004. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Ann. Rev. Ecol. Evol.* 34: 397-423.

Futuyma, D.J., 2010. Evolutionary constraint and ecological consequences. *Evol.* 64: 1865-1884.

Futuyma, D.J., Agrawal, A.A., 2009. Macroevolution and the biological diversity of plants and herbivores. *PNAS* 106: 18054-18061.

Futuyma, D.J., Keese, M.C., Funk, D.J., 1995. Genetic constraints on macroevolution: the evolution of host affiliation in the leaf beetle genus *Ophraella*. *Evol.* 49: 797-809.

Futuyma, D.J., Keese, M.C., Scheffer, S.J., 1993. Genetic constraints and the evolution of insect-plant associations: responses of *Ophraella communa* (Coleoptera: Chrysomelidae). *Evol.* 47: 888-905.

Giblin-Davis, R.M., Center, B.J., Davies, K.A., Purcell, M.F., Scheffer, S.J., Taylor, G.S., Goolsby, J., Center, T.D., 2004. Histological comparisons of *Fergusobia/Fergusonina*-induced galls on different myrtaceous hosts. *J. Nematol.* 36: 249-262.

Giblin-Davis, R.M., Makinson, J., Center, B.J., Davies, K.A., Purcell, M., Taylor, G.S., Scheffer, S.J., Goolsby, J., Center, T.D., 2001. *Fergusobia/Fergusonina*-induced shoot bud gall development on *Melaleuca quinquenervia*. *J. Nematol.* 33: 239-247.

Giblin-Davis, R.M., Scheffer, S.J., Davies, K.A., Taylor, G.S., Curole, J., Center, T.D., Goolsby, J., Thomas, W.K., 2003. Coevolution between *Fergusobia* and *Fergusonina* mutualists. *Nematol. Monogr. Persp.* 2: 1-11.

Hairston, N.N., Smith, F.E., Slobodkin, L.B., 1960. Community structure, population control, and competition. *Am. Nat.* 94: 421-425.

Harris, K.M., 1982. First record of Fergusoninidae (Diptera: Schizophora) outside Australia: a new species of *Fergusonina* on *Syzygium* in India. *Syst. Entomol.* 7: 211-216.

Hartl, D.L., Clark, G.C., 1997. Principles of Population Genetics, 3rd ed. Sinauer Associates, Sunderland, MA.

Hebert P. D. N., Cywinska A., Ball S. L., DeWaard J. R., 2003. Biological identifications through DNA barcodes. *Proc. R. Soc. B* 270:313-321.

Janz, N., Nylin, S., Wahlberg, N., 2006. Diversity begets diversity: host expansions and the diversification of plant-feeding insects. *BMC Evol. Biol.* 6. doi:10.1186/1471-2148-6-4.

Janz, N., Nylin, S., 2008. The Oscillation Hypothesis of Host-Plant Range and Speciation.

Janzen, D.H., Hajibabaei, M., Burns, J.M., Hallwachs, W., Remigio, E., Hebert, P.D., 2005. Wedding biodiversity inventory of a large and complex Lepidoptera fauna with DNA barcoding. *Phil. Trans. R. Soc. B Biol. Sci.* 360: 1835-1845.

Joy, J.B., Crespi, B.J., 2007. Adaptive radiation of gall-inducing insects within a single host-plant species. *Evol.* 61: 784-795.

- Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., Calcott, B., 2016. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Mol. Biol. Evol.* DOI: [dx.doi.org/10.1093/molbev/msw260](https://doi.org/10.1093/molbev/msw260)
- Maddison, W.P., Maddison, D.R., 2015. Mesquite: A modular system for evolutionary analysis. Version 3.03. <http://mesquiteproject.org>
- Maynard Smith, J., 1978. *The Evolution of Sex*. Cambridge University Press, Cambridge, UK. 236 pp.
- Mishler, B.D., 2010. Species are not uniquely real biological entities. 'Contemp. Debates Philos. Biol.' (Eds F Ayala, R Arp) pp. 110–122. Wiley-Blackwell.
- Mitter, C., Farrell, B., Futuyma, D.J., 1991. Phylogenetic studies of insect-plant interactions: insights into the genesis of diversity. *TREE* 6: 290-293.
- Mitter, C., Farrell, B., Wiegmann, B., 1988. The phylogenetic study of adaptive zones: has phytophagy promoted insect diversification? *Am. Nat.* 132:107-128.
- Meyer, C.P., Paulay, G., 2005. DNA barcoding: error rates based on comprehensive sampling. *PLOS Biol.* <http://dx.doi.org/10.1371/journal.pbio.0030422>
- Moulton, J. K., Wiegmann B.M., 2004. Evolution and phylogenetic utility of CAD (rudimentary) among Mesozoic-aged eremoneuran Diptera (Insecta). *Mol. Phylogenet. Evol.* 31: 363-378.
- Nelson, L.A., Davies, K.A., Scheffer, S.J., Taylor, G.S., Purcell, M.F., Giblin- Davis, R.M., Thornhill, A.H., Yeates, D.K., 2014. An emerging example of tritrophic coevolution between flies (Diptera: Fergusoninidae) and nematodes (Nematoda: Neotylenchidae) on Myrtaceae host plants. *Biol. J. Linn. Soc.* 111: 699-718.
- Nelson, L. A., Scheffer, S. J., Yeates, D. K., 2011a. Two new species of sympatric *Fergusonina* Malloch flies (Diptera: Fergusoninidae) from bud galls on high-elevation snow gums (*Eucalyptus pauciflora* Sieb. ex Spreng. complex) in the Australian Alps. *Australian Journal of Entomology*, 50: 356–364.
- Nelson, L. A., Scheffer, S. J., Yeates, D. K., 2011b. Three new species of *Fergusonina* Malloch gall-flies (Diptera: Fergusoninidae) from terminal leaf bud galls on *Eucalyptus* (Myrtaceae) in south-eastern Australia. *Zootaxa* 3112: 36-48
- Nelson, L. A., Scheffer, S. J., Yeates, D. K., 2012. Species diversity of *Fergusonina* Malloch gall flies (Diptera: Fergusoninidae) forming leaf bud galls on snow gum (*Eucalyptus pauciflora* Sieb. ex Spreng. complex), with a description of a new species from Tasmania. *Insect Systematics & Evolution* 43: 147-160.

Newman, D., Pilson, D., 1997. Increased probability of extinction due to decreased genetic effective population size: experimental populations of *Clarkia pulchella*. *Evol.* 51: 354-362.

Nicolle, D., 1997. *Eucalypts of South Australia*. Published by D. Nicolle Pub., Morphett Vale, S.A., Australia, 208 pp.

Nicolle, D., 2006. *Eucalypts of Victoria and Tasmania*. Bloomings Books, Melbourne, Australia. 310 pp.

Nieminen, M., Singer, M.C., Fortelius, W., Schops K., Hanski, I., 2001. Experimental confirmation that inbreeding depression increases extinction risk in butterfly populations. *Am Nat* 157:237–244

Novotny, V., Basset, Y., Miller, S.E., Weiblen, G.D., Bremer, B., Cizek, L. and Drozd, P., 2002. Low host specificity of herbivorous insects in a tropical forest. *Nature* 416: 841-844.

Pons, J., Barraclough, T.G., Gomez-Zurita, J., Cardoso, A., Duran, D.P., Hazell, S., Kamoun, S., Sumlin, W.D. and Vogler, A.P., 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology*, 55: 595-609.

Price, P.W., Fernandes, G.W., Lara, A.C.F., Brawn, J., Barrios, H., Wright, M.G., Ribero, S.P., Rothcliff, N., 1998. Global patterns in local number of insect galling species. *J. Biogeog.* 25: 581-591.

Price, Peter W., G. Wilson Fernandes, and Gwendolyn L. Waring. "Adaptive nature of insect galls." *Environmental Entomology* 16.1 (1987): 15-24.

Purcell, M.F., Rowell, D.M., Yeates, D.K., 2015. Are the shoot bud galls of *Fergusonina* Malloch, 1924 (Diptera: Fergusoninidae) founded by multiple mothers? *Austral. Entomol.* 54: 286-291.

Purcell, M.F., Nelson, L.A., Thornhill, A.H., Yeates, D.K., 2013. Two new species of *Fergusonina* Malloch gall fly (Diptera: Fergusoninidae) from leaf bud galls on black sallee (*Eucalyptus stellulata*) and manna gum (*Eucalyptus viminalis*). *Austral. Entomol.* 52, 363–370. doi:10.1111/aen.12044.

Purcell, M.F., Wallenius, T.C., Yeates, D.K., Rowell, D.M., 2016. Larval dorsal shield morphology is highly correlated with gall type in the enigmatic gall-forming fly, *Fergusonina* Malloch (Diptera : Fergusoninidae). *Austral. J. Zool.*

Purvis A., Hector, A., 2000. Getting the measure of biodiversity. *Nature* 405: 212-219.

Rambaut A (2002) Sequence Alignment Editor (Se-AL) v2.0. <http://tree.bio.ed.ac.uk/software/seal/>.

Regier, J.C., 2008. Protocols, Concepts, and Reagents for preparing DNA sequencing templates. Version 12/4/08. www.umbi.umd.edu/users/jcrlab/PCR_primers.pdf.

Rubinoff, D., Cameron, S. and Will, K., 2006. A genomic perspective on the shortcomings of mitochondrial DNA for “barcoding” identification. *J. Heredity*, 97: 581-594.

Scheffer, S.J., 1999. Genetic variation and the potential for host range evolution in the leafmining fly *Amauromyza flavifrons* (Diptera: Agromyzidae). *Ann. Ent. Soc. Am.* 92: 742-747.

Scheffer, S.J., 2000. Molecular evidence of cryptic species within the *Liriomyza huidobrensis* (Diptera: Agromyzidae). *J. Econ. Entomol.* 93:1146-1151.

Scheffer, S.J., Giblin-Davis, R.M., Taylor, G.S., Davies, K.A., Purcell, M., Lewis, M.L., Goolsby, J., Center, T.D., 2004. Phylogenetic relationships, species limits, and host specificity of gall-forming *Fergusonina* flies (Diptera: Fergusoninidae) feeding on *Melaleuca* (Myrtaceae). *Ann. Ent. Soc. Am.* 97: 1216-1221.

Scheffer, S.J., Hawthorne, D.J., 2007. Molecular evidence of host-associated genetic divergence in the holly leafminer *Phytomyza glabricola* (Diptera: Agromyzidae): apparent discordance among marker systems. *Molec. Ecol.* 16: 2627–2637.
<http://doi.org/10.1111/j.1365-294X.2007.03303.x>

Scheffer, S.J., Nelson, L.A., Davies, K.A., Lewis, M.L., Giblin-Davis, R.M., Taylor, G.S., Yeates, D.K., 2013. Sex-limited association of *Fergusobia* nematodes with female *Fergusonina* flies in a unique Australasian mutualism (Nematoda: Neotylenchidae; Diptera: Fergusoninidae). *Austr. J. Entomol.* 52: 125-128. doi:10.1111/aen.12013

Scheffer, S.J., Wiegmann, B.M., 2000. Molecular phylogenetics of the holly leafminers (Diptera: Agromyzidae: Phytomyza): species limits, speciation, and dietary specialization. *Mol. Phylogenet. Evol.* 17: 244-255.

Scheffer, S.J., Winkler, I.S., Wiegmann, B.M., 2007. Phylogenetic relationships within the leaf-mining flies (Diptera: Agromyzidae) inferred from sequence data from multiple genes. *Mol. Phylogenet. Evol.* 42: 756-775.

Schwander, T., Crespi, B.J., 2009. Twigs on the tree of life? Neutral and selective models for integrating macroevolutionary patterns with microevolutionary processes in the analysis of asexuality. *Molec. Ecol.*, 18: 28–42. doi:10.1111/j.1365-294X.2008.03992.x

Scott, M.J., Kriticou, D., Robinson, A.S., 1993. Isolation of cDNAs encoding 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase from the Mediterranean fruit fly *Ceratitidis capitata*: correlating genetic and physical maps of chromosome 5. *Insect Mol. Biol.* 1: 213-222.

- Siddiqi, M.R., 1986. A review of the genus *Fergusobia* Currie (Hexatyline) with descriptions of *F. jambophila* n. sp. and *F. magna* n. sp., in: Swarup, G., Dasgupta, D.R., (Eds.) Plant Parasitic Nematodes of India, Problems and Progress. New Delhi, India. pp. 264-278.
- Siddiqi, M.R., 1994. *Fergusobia brevicauda*, sp.n. and *F. philippinensis*, sp.n. (Nematoda: Hexatyline) from *Eucalyptus deglupta*. Proceedings of the Second Afro-Asian Nematology Symposium, 18–22 December 1994. Afro-Asian Society of Nematologists, Cairo, Egypt, 96–100.
- Simon, C., F. Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* 87: 651-701.
- Song, H., Buhay, J.E., Whiting, M.F. and Crandall, K.A., 2008. Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. *PNAS* 105: 13486-13491.
- Stamatakis, A., 2008. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312-1313.
- Stamatakis, A., Hoover, P. and Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web servers. *Syst. Biol.* 57:758-771.
- Stone, Graham N., and Karsten Schönrogge. "The adaptive significance of insect gall morphology." *Trends in Ecology & Evolution* 18.10 (2003): 512-522.
- Strong, D.R., Lawton, J.H., Southwood, S.R., 1984. *Insects on Plants: Community Patterns and Mechanisms*. Harvard Univ. Press, Cambridge, MA, 313 pp.
- Tautz, D., Arctander, P., Minelli, A., Thomas, R.H., Vogler, A.P., 2003. A plea for DNA taxonomy. *Trends Ecol. Evol.* 18: 70-74.
- Taylor, G.S., 2004. Revision of *Fergusonina* Malloch gall flies (Diptera: Fergusoninidae) from *Melaleuca* (Myrtaceae). *Invert. Syst.* 18: 251–290.
- Taylor, G.S., Davies, K.A., 2008. New species of gall flies (Diptera: Fergusoninidae) and an associated nematode (Tylenchida: Neotylenchidae) from flower bud galls on *Corymbia* (Myrtaceae). *Austr. J. Entomol.* 47: 336–349.
- Taylor, G.S., Davies, K.A., 2010. The gall fly, *Fergusonina lockharti* Tonnoir (Diptera: Fergusoninidae) and description of its associated nematode, *Fergusobia brittenae* sp. nov. (Tylenchida: Neotylenchidae). *J. Nat. Hist.* 44: 927–957.
- Taylor, G.S., Davies, K., Martin, N., Crosby, T., 2007. First record of *Fergusonina* (Diptera: Fergusoninidae) and associated *Fergusobia* (Tylenchida: Neotylenchidae)

forming galls on *Metrosideros* (Myrtaceae) from New Zealand. Syst. Entomol. 32: 548-557.

Taylor, G.S., Head, E., Davies, K.A., 2005. Gall flies (Diptera: Fergusoninidae) on Myrtaceae: a mutualistic association between flies and nematodes, in: Rahman, A., Schaefer, C.W., Withers, T.M. (Eds.) *Biology, Ecology and Evolution of Gall-Inducing Arthropods*, Vol. 2, Science Publishers, New Hampshire, U.S., pp. 643-671.

Tonnoir, A.L., 1937. Revision of the genus *Fergusonina* Mall. Proc. Linn. Soc. NSW 62: 126-146.

Uesugi, A., 2008. Evolution of host avoidance in a leafmining fly, *Amauromyza flavifrons*. Entomol. Exp. Appl. 128:398–402.

Walker, T.D., Valentine, J.W., 1984. Equilibrium models of evolutionary species diversity and the number of empty niches. Am. Nat. 124: 887-899.

Wiegmann, B.M., Trautwein, M.D., Winkler, I.S., Barr, N.B., Kim, J., Lambkin, C., Bertone, M.A., Cassel, B.K., Bayless, K.M., Heimberg, A.M., Wheeler, B.M., Peterson, K.J., Pape, T., Sinclair, B.J., Skevington, J.S., Blagoderov, V., Caravas, J., Kutty, S.N., Schmidt-Ott, U., Kampmeier, G.E., Thompson, F.C., Grimaldi, D.A., Beckenbach, A.T., Courtney, G.W., Friedrich M., Meier, R., Yeates, D.K., 2011. Episodic radiations in the fly tree of life. PNAS 108: 5690–5695.

Williams, G.C., 1975. Sex and Evolution. Princeton Univ. Press, Princeton, NJ.

Winkler, I.S., Rung, A., Scheffer, S.J., 2010. Hennig's orphans revisited: testing morphological hypotheses in the "Opomyzoidea" (Diptera: Schizophora). Mol. Phylog. Evol. 54: 746-762.

Winkler, I. S., Scheffer, S. J., Mitter, C., 2009. Molecular phylogeny and systematics of leaf-mining flies (Diptera: Agromyzidae): delimitation of *Phytomyza* Fallen sensu lato and included species groups, with new insights on morphological and host-use evolution. Syst. Ent. 34: 260–292. <http://doi.org/10.1111/j.1365-3113.2008.00462.x>

Ye, W., Giblin-Davis, R.M., Davies, K.A., Purcell, M., Scheffer, S.J., Taylor, G.S., Center, T.D., Morris, K., Thomas, W.K., 2007. Molecular phylogenetics and the evolution of host plant associations in the nematode genus *Fergusobia* (Tylenchida: Fergusobiinae). Mol. Phyl. Evol. 45: 123-141.

Yeates, D.K., Seago, A., Nelson, L., Cameron, S.L., Joseph, L., Trueman, J.W.H., 2011. Integrative taxonomy, or iterative taxonomy? Syst. Ent. 36: 209-217

Figure 3. Relationships between average intraspecific pairwise distance and a) sample size of each species; and b) maximum geographic distance of samples within each species. Bonferroni correction for multiple tests requires significance criterion of $p < 0.05$ to be reduced to $p < 0.008$.

Table 1. Collection information for each specimen. When present in the figures, text, other tables, and GenBank, the code number will be preceded by “FC-“ or “FCV-“. Host genus abbreviations are as follows: *A*=*Angophora*, *C*=*Corymbia*, *E*=*Eucalyptus*, *M*=*Melaleuca*, *Metr*=*Metrosideros*, *L*=*Leptospermum*, *S*=*Syzygium*. Further information on Gall type can be found in Table 2. All specimens were collected from Australia with the exception of samples from *Metrosideros excelsa* in New Zealand. Australian state abbreviations used here are: ACT, Australian National Territory; NSW, New South Wales; QLD, Queensland; SA, South Australia; TAS, Tasmania; VIC, Victoria; WA, Western Australia.

Code #	Host Plant	Gall Type	Date	Collection Location
18	<i>E. diversifolia</i>	M-Shoot Bud	04/1999	SA, Meninge
19	<i>E. leucoxydon</i>	M-Flat Leaf	04/1999	SA, Meninge
20	<i>M. quinquenervia</i>	M-Shoot Bud	07/1999	QLD, N. Stradbroke Island
21	<i>M. fluviatilis</i>	M-Shoot Bud	08/1999	QLD, Home Hill
22	<i>M. leucandendra</i>	M-Shoot Bud	08/1999	QLD, Cairns
23	<i>M. dealbata</i>	M-Shoot Bud	08/1999	QLD, Cairns
24	<i>M. cajuputi</i>	M-Shoot Bud	08/1999	QLD, Daintree Lagoon
25	<i>M. cajuputi</i>	M-Shoot Bud	08/1999	QLD, Daintree Lagoon
26	<i>C. ptychocarpa</i>	M-Flower Bud	08/1999	QLD, Daintree Lagoon
27	<i>C. ptychocarpa</i>	M-Flower Bud	08/1999	QLD, Sherwood
29	<i>E. siderophloia</i>	M-Flat Leaf	07/1999	QLD, Taringa, Brisbane
30	<i>M. dealbata</i>	M-Shoot Bud	08/1999	QLD, Cairns
31	<i>M. nervosa</i>	U-Peagall	08/1999	QLD, Mareeba
33	<i>M. leucandendra</i>	M-Shoot Bud	08/1999	QLD, Cairns
34	<i>M. dealbata</i>	M-Shoot Bud	08/1999	QLD, Cairns
35	<i>M. cajuputi</i>	M-Shoot Bud	08/1999	QLD, Daintree Lagoon
36	<i>C. ptychocarpa</i>	M-Flower Bud	08/1999	QLD, Sherwood
37	<i>E. racemosa</i>	M-Shoot Bud	07/1999	QLD, N. Stradbroke Island
38	<i>M. quinquenervia</i>	M-Shoot Bud	08/1999	NSW, Woodburn
39	<i>E. siderophloia</i>	M-Flat Leaf	07/1999	QLD, Brisbane
40	<i>M. dealbata</i>	M-Shoot Bud	06/1999	QLD, Cardwell
41	<i>M. nervosa</i>	U-Peagall	08/1999	QLD, Mareeba
42	<i>M. cajuputi</i>	M-Shoot Bud	08/1999	QLD, Daintree Ferry Turn
43	<i>M. fluviatilis</i>	M-Shoot Bud	06/1999	QLD, Home Hill
45	<i>M. quinquenervia</i>	M-Shoot Bud	06/1999	QLD, N. Stradbroke Island
46	<i>M. dealbata</i>	M-Shoot Bud	05/1999	QLD, Cardwell
47	<i>M. fluviatilis</i>	M-Shoot Bud	06/1999	QLD, Townsville, Ross River Dam
48	<i>C. ptychocarpa</i>	M-Flower Bud	1999	QLD, Indooroopilly

52	<i>M. leucandendra</i>	M-Shoot Bud	1999	QLD, Linear Park
53	<i>M. cajuputi</i>	M-Shoot Bud	08/1999	QLD, Daintree Lagoon
55	<i>M. fluviatilis</i>	M-Shoot Bud	1999	QLD, Townsville, Ross River Dam
61	<i>E. camaldulensis</i>	M-Shoot Bud	06/1998	SA, Bunyeroo Gorge
64	<i>E. macrorhyncha</i>	M-Flower	1999	SA, Spring Gully
67	<i>M. stenostachya</i>	M-Shoot Bud	08/1999	QLD, Mareeba
68	<i>M. viridiflora</i>	M-Shoot Bud	1999	QLD, MacKey
70	<i>E. diversifolia</i>	M-Shoot Bud	04/1999	SA, Meningie
71	<i>E. macrorhyncha</i>	M-Flower Bud	1999	SA, Spring Gully
73	<i>E. intertexta</i>	M-Shoot Bud	1999	SA, Bunyeroo Gorge
74	<i>E. leucoxylon</i>	M-Flat Leaf	04/1999	SA, Meningie
75	<i>M. fluviatilis</i>	M-Shoot Bud	06/1999	QLD, Townsville, Ross River Dam
76	<i>M. nervosa</i>	M-Shoot Bud	08/1999	QLD, Mareeba
77	<i>M. stenostachya</i>	M-Shoot Bud	08/1999	QLD, Mareeba
78	<i>E. microcarpa</i>	M-Flower Bud	04/1999	SA, Urrbrae
79	<i>Corymbia</i>	Leaf Peagall	07/1999	NSW, Woodburn
80	<i>C. maculata</i>	Leaf Peagall	07/1999	QLD, Indooroopilly
81	<i>M. viridiflora</i>	M-Shoot Bud	05/1999	QLD, nr. Mackay
83	<i>E. obliqua</i>	M-Flower Bud	04/1999	SA, Myponga
84	<i>M. nervosa</i>	M-Shoot Bud	1999	QLD, Gilmore Rd.
85	<i>E. leucoxylon</i>	M-Flat Leaf	1999	SA, Meningie
88	<i>E. macrorhyncha</i>	M-Flower Bud	04/1999	SA, nr. Clare
89	<i>E. siderophloia</i>	M-Flat Leaf	07/1999	QLD, Indooroopilly
91	<i>M. cajuputi</i>	M-Shoot Bud	05/1999	QLD, nr. Daintree Ferry
92	<i>M. fluviatilis</i>	M-Shoot Bud	06/1999	QLD, Home Hill
93	<i>M. fluviatilis</i>	M-Shoot Bud	06/1999	QLD, Townsville, Ross River Dam
94	<i>M. leucadendra</i>	M-Shoot Bud	05/1999	QLD, Cardwell
95	<i>M. leucadendra</i>	M-Shoot Bud	05/1999	QLD, Cairns
98	<i>Eucalyptus sp.</i>	M-Flat Leaf	07/1999	QLD, Brisbane Forest
99	<i>E. siderophloia?</i>	Leaf Peagall	07/1999	QLD, Indooroopilly
100	<i>C. tessellaris?</i>	M-Shoot Bud	05/1999	QLD, Cardwell
101	<i>M. quinquenervia</i>	M-Shoot Bud	09/2000	QLD, Kennedy Natl. Park
102	<i>E. pruinosa</i>	M-Flower Bud	05/2001	WA, Tarara Bar, Carlton Hill Station, WA, Kununurra-Wyndham Rd, Halls
104	<i>C. abbreviata</i>	M-Flower Bud	06/2001	Creek turnoff
105	<i>E. camaldulensis</i>	M-Flower Bud	05/2001	SA, Verdun
107	<i>E. marginata</i>	Leaf Peagall	10/2000	WA, nr. Busselton

108	<i>M. stenostachya</i>	M-Shoot Bud	12/2000	QLD, Luster Creek, nr. Cairns
109	<i>M. stenostachya</i>	M-Shoot Bud	12/2000	QLD, Luster Creek, nr. Cairns
110	<i>M. fluviatilis</i>	M-Shoot Bud	09/2000	QLD, Townsville
113	<i>E. gomphocephala</i>	Leaf Peagall	10/2000	WA, Cervantes Caravan Park
114	<i>E. cosmophylla</i>	M-Shoot Bud	12/2000	SA, Kangaroo Island
118	<i>E. camaldulensis</i>	M-Shoot Bud	10/2000	WA, Kellerberin
120	<i>E. leucoxylon</i>	M-Flat Leaf	11/1999	SA, Hazelwood Park
121	<i>E. viminalis</i>	M-Shoot Bud	09/1999	SA, Cleland Conservation Park
122	<i>M. quinquenervia</i>	M-Shoot Bud	06/2001	QLD, Edmund Kennedy Natl. Park
123	<i>M. quinquenervia</i>	M-Flower Bud	06/2001	QLD, Edmund Kennedy Natl. Park
124	<i>M. fluviatilis</i>	M-Shoot Bud	06/2001	QLD, Home Hill
127	<i>M. quinquenervia</i>	M-Shoot Bud	09/2000	QLD, Edmund Kennedy Natl. Park
131	<i>Met. excelsa</i>	M-Shoot Bud	03/2004	Karekare Beach, North Island, New Zealand
132	<i>Met. excelsa</i>	M-Shoot Bud	03/2004	Karekare Beach, North Island, New Zealand
134	<i>E. tereticornis</i>	M-Shoot Bud	07/2004	NSW, Sydney
135	<i>M. armillaris</i>	U-Peagall	07/2004	NSW, Hawk's Nest
136	<i>M. nodosa</i>	U-Peagall	07/2004	NSW, Hawk's Nest
137	<i>M. linariifolia</i>	U-Peagall	07/2004	NSW, Port Macquarie
139	<i>S. luehmannii</i>	Mix of galls	07/2004	QLD, Tolga
140	<i>M. decora</i>	U-Peagall	07/2004	QLD, Ingham
142	<i>E. porosa</i>	M-Flat Leaf	09/2003	SA, nr. Wool Bay
143	<i>E. blakelyi</i>	M-Shoot Bud	01/2004	ACT, Canberra
144	<i>A. floribunda</i>	M-Shoot Bud	01/2004	NSW, nr. Mudgee
145	<i>Eucalyptus sp.</i>	M-Flat Leaf	01/2004	ACT, Cotter Rd.
146	<i>E. viminalis?</i>	M-Shoot Bud	01/2004	NSW, nr. Mt. Crackenback
147	<i>E. pauciflora</i>	Leaf Peagall	11/2002	TAS, nr. Bridport
148	<i>E. amygdalina</i>	M-Shoot Bud	11/2002	TAS, nr. Mt Cameron
152	<i>M. quinquenervia</i>	M-Shoot Bud	07/2004	NSW, Ararawarra
169	<i>E. tereticornis</i>	M-Shoot Bud	07/2004	NSW, Sydney
170	<i>M. linariifolia</i>	U-Peagall	07/2004	NSW, Port Macquarie
171	<i>C. tessellaris</i>	M-Shoot Bud	07/2004	QLD, Cardwell
172	<i>E. robusta</i>	M-Shoot Bud	04/2002	NSW, Cogarah Bay
173	<i>E. microcarpa</i>	Leaf Peagall	08/2001	SA, Urrbrae, Waite Arboretum
177	<i>E. cosmophylla</i>	M-Shoot Bud	12/2000	SA, Kangaroo Island
180	<i>C. ptychocarpa</i>	M-Flower Bud	07/2004	QLD, Cairns

183	<i>M. quinquenervia</i>	M-Shoot Bud	07/2004	QLD, Edmund Kennedy Natl. Park
184	<i>M. argentea</i>	M-Shoot Bud	08/2004	NT, 30km ESE Darwin, Elizabeth River
186	<i>E. baxteri</i>	M-Flower Bud	11/2000	VIC, Grampians
188	<i>E. microcarpa?</i>	U-Peagall	09/1999	SA, Robertstown
189	<i>E. odorata?</i>	M-Flat Leaf	09/1999	SA, nr. Nuriootpa
190	<i>E. odorata?</i>	M-Flat Leaf	09/1999	SA, nr. Nuriootpa
194	<i>E. haemastoma</i>	M-Shoot Bud	01/2007	NSW, Kuringai
196	<i>S. luehmannii</i>	Mix of galls	07/2004	QLD, Tolga
		M-Shoot Bud		Karekare Beach, North Island, New Zealand
198	<i>M. excelsa</i>		03/2004	
199	<i>E. tereticornis</i>	M-Shoot Bud	07/2004	NSW, Sydney
200	<i>S. luehmannii</i>	Mix of galls	07/2004	QLD, Tolga
201	<i>C. ptychocarpa</i>	M-Flower Bud	07/2004	QLD, Cairns
203	<i>M. quinquenervia</i>	M-Shoot Bud	07/2004	NSW, Kingscliff
204	<i>E. camaldulensis</i>	M-Shoot Bud	01/2002	SA, Erindale
500	<i>E. pauciflora</i>	M-Shoot Bud	09/2007	NSW, Braidwood
503	<i>E. pauciflora</i>	M-Shoot Bud	09/2007	NSW, Captain's Flat Rd
506	<i>E. pauciflora</i>	M-Shoot Bud	09/2007	ACT, Edmondson Rest Area
508	<i>E. pauciflora</i>	M-Flower Bud	10/2007	ACT, Edmondson Rest Area
509	<i>E. pauciflora</i>	M-Shoot Bud	09/2007	NSW, Pt Lookout
510	<i>E. pauciflora</i>	M-Shoot Bud	09/2007	NSW, Pt Lookout
511	<i>E. pauciflora</i>	Leaf Peagall	09/2007	NSW, Pt Lookout
512	<i>E. pauciflora</i>	M-Shoot Bud	09/2007	NSW, Namadgi Natl. Park
515	<i>E. niphophila</i>	M-Shoot Bud	11/2007	ACT, Mt. Gingera
521	<i>E. niphophila</i>	M-Shoot Bud	09/2007	VIC, Mt. Buffalo
522	<i>E. niphophila</i>	M-Shoot Bud	09/2007	VIC, Mt. Buffalo
523	<i>E. niphophila</i>	M-Shoot Bud	09/2007	VIC, Mt. Buffalo
526	<i>E. niphophila</i>	M-Shoot Bud	09/2007	VIC, Mt. Hotham
529	<i>E. pauciflora</i>	M-Shoot Bud	09/2007	VIC, Dinner Plains
535	<i>E. pauciflora</i>	M-Flower Bud	10/2007	TAS, Rd C526 (in eastern TAS)
539	<i>E. pauciflora</i>	M-Shoot Bud	10/2007	TAS, Great Lake
540	<i>E. pauciflora</i>	M-Flower Bud	10/2007	TAS, Rd C526 (in eastern TAS)
541	<i>E. coccifera</i>	M-Shoot Bud	10/2007	TAS, Mt. Wellington
542	<i>E. coccifera</i>	Leaf Peagall	10/2007	TAS, Mt. Wellington
543	<i>E. coccifera</i>	M-Shoot Bud	10/2007	TAS, Mt. Field
544	<i>E. stellulata</i>	M-Shoot Bud	11/2007	ACT, Mt. Gingera
580	<i>E. niphophila</i>	M-Shoot Bud	09/2007	VIC, Falls Creek Village

586	<i>E. niphophila</i>	M-Shoot Bud	09/2007	VIC, Mt. Hotham Village
602	<i>E. coccifera</i>	M-Shoot Bud	10/2007	TAS, Mt. Wellington
603	<i>E. coccifera</i>	M-Shoot Bud	10/2007	TAS, Mt. Wellington
606	<i>E. coccifera</i>	M-Shoot Bud	10/2007	TAS, Mt. Field
608	<i>E. coccifera</i>	M-Shoot Bud	10/2007	TAS, Mt. Field
609	<i>E. pauciflora</i>	M-Shoot Bud	10/2007	TAS, Great Lake
610	<i>E. pauciflora</i>	M-Shoot Bud	10/2007	TAS, Great Lake
618	<i>E. pauciflora</i>	M-Shoot Bud	11/2007	ACT, Mt. Gingera
619	<i>E. pauciflora</i>	M-Shoot Bud	11/2007	ACT, Mt. Gingera
632	<i>E. pauciflora</i>	M-Shoot Bud	11/2007	ACT, Mt. Gingera
633	<i>E. pauciflora</i>	M-Shoot Bud	11/2007	ACT, Mt. Gingera
649	<i>E. pauciflora</i>	M-Shoot Bud	06/2007	NSW, Perisher Ski Area, Eyre T-Bar
652	<i>E. pauciflora</i>	M-Shoot Bud	09/2007	NSW, Mt Koziosco, Dead Horse Gap
659	<i>E. pauciflora</i>	M-Shoot Bud	09/2007	VIC, Falls Creek Village
710	<i>E. pauciflora</i>	M-Shoot Bud	10/2007	TAS, nr. Avoca
711	<i>E. pauciflora</i>	M-Shoot Bud	10/2007	TAS, nr. Avoca
716	<i>E. pauciflora</i>	Leaf Peagall	10/2007	TAS, east of Bridport
718	<i>E. pauciflora</i>	M-Flower Bud	09/2007	NSW, Kings Highway, nr. Bungendore
719	<i>Eucalyptus sp.</i>	M-Flower Bud	09/2007	NSW, Goulburn
720	<i>E. pauciflora</i>	M-Flower Bud	09/2007	NSW, Edmonston Rest Area
721	<i>E. pauciflora</i>	M-Flower Bud	09/2007	NSW, Edmonston Rest Area
724	<i>E. niphophila</i>	M-Flower Bud	09/2007	VIC, Mt. Buffalo
725	<i>E. niphophila</i>	M-Flower Bud	09/2007	VIC, Mt. Buffalo
730	<i>M. decora</i>	U-Peagall	08/2007	QLD, Ingham
731	<i>C. tessellaris</i>	M-Shoot Bud	08/2007	QLD, Mission Beach
732	<i>M. dealbata</i>	M-Shoot Bud	08/2007	QLD, Ingham
733	<i>E. acmenoides</i>	M-Shoot Bud	08/2007	QLD, nr. Cardwell
734	<i>S. luehmannii</i>	Mix of Galls	08/2007	QLD, Tolga
				QLD, Edmund Kennedy Natl. Park,
735	<i>C. tessellaris</i>	M-Shoot Bud	08/2007	Cardwell
736	<i>E. camaldulensis</i>	M-Shoot Bud	09/2007	SA, Stonyfell
737	<i>E. obliqua</i>	M-Shoot Bud	09/2007	SA, Stirling
738	<i>E. viminalis</i>	U-Peagall	09/2007	SA, Arbury Park, Stirling
739	<i>E. viminalis</i>	U-Peagall	09/2007	SA, Arbury Park, Stirling
740	<i>E. camaldulensis</i>	M-Shoot Bud	09/2007	SA, Stonyfell, Adelaide
741	<i>E. siderophloia</i>	M-Flat Leaf	02/2008	ACT, Black Mt. Peninsula
742	<i>E. melliodora</i>	M-Flat Leaf	12/2007	ACT, Canberra, Black Mt. Peninsula

743	<i>E. melliodora</i>	U-Peagall	12/2007	ACT, Canberra, Black Mt. Peninsula
745	<i>E. polyanthemus?</i>	M-Flat Leaf	12/2007	ACT, Canberra, Pearce
746	<i>Eucalyptus sp.</i>	M-Flower Bud	09/2007	NSW, nr. Goulburn
748	<i>Eucalyptus sp.</i>	U-Peagall	09/2007	NSW, Ben Lomond
749	<i>Eucalyptus sp.</i>	U-Peagall	09/2007	NSW, Namigi National Park
750	<i>E. niphophila</i>	M-Flower Bud	09/2007	NSW, Bullocks Flat
751	<i>E. stellulata</i>	M-Flower Bud	10/2007	ACT, Canberra, Jerrabomberra
753	<i>E. mannifera</i>	U-Peagall	12/2007	ACT, Canberra, ANU
754	<i>E. leucoxydon</i>	M-Flat Leaf	12/2007	ACT, Canberra
755	<i>E. leucoxydon</i>	M-Flat Leaf	12/2007	ACT, Canberra, ANU
756	<i>Eucalyptus sp.</i>	M-Shoot Bud	07/2007	ACT, Canberra, Black Mountain
757	<i>E. tereticornis</i>	M-Flower Bud	07/2008	QLD, Sarina
758	<i>E. tereticornis</i>	M-Flower Bud	07/2008	QLD, Sarina
759	<i>M. linariifolia</i>	U-Peagall	07/2008	QLD, Mackay
760	<i>C. torelliana</i>	M-Shoot Bud	07/2008	QLD, Kairi
761	<i>C. citriodora</i>	M-Shoot Bud	05/2008	NSW, Woondum
762	<i>C. maculata</i>	M-Shoot Bud	07/2008	NSW, Forster
763	<i>C. citriodora</i>	M-Shoot Bud	07/2008	QLD, Woondum
764	<i>Metr. excelsa</i>	M-Shoot Bud	11/2004	New Zealand, Karekare Beach
771	<i>C. tessellaris</i>	M-Shoot Bud	05/2002	QLD, Hervey Bay
773	<i>Eucalyptus sp.</i>	M-Flat Leaf	05/2002	QLD, Lake Tinnaroo
774	<i>C. tessellaris</i>	M-Shoot Bud	05/2002	QLD, Ingham
775	<i>E. dealbata</i>	M-Shoot Bud	11/2007	NSW, Breeza
777	<i>Eucalyptus sp.</i>	M-Shoot Bud	07/1999	NSW, Woodburn
778	<i>Eucalyptus sp.</i>	U-Peagall	07/1999	NSW, Woodburn
779	<i>Eucalyptus sp.</i>	M-Flat Leaf	07/1999	NSW, Woodburn
780	<i>E. tereticornis</i>	Leaf Peagall	1999	QLD, Indooroopilly
910	<i>E. largiflorens</i>	M-Flat Leaf	07/2011	NSW, Kincheaga National Park
911	<i>E. largiflorens</i>	M-Flat Leaf	07/2011	NSW, Kincheaga National Park
912	<i>L. laevigatum</i>	U-PG	07/2012	VIC, nr. Frankston
913	<i>L. laevigatum</i>	U-PG	07/2012	VIC, nr. Frankston
914	<i>L. madidum</i>	M-Shoot Bud	07/2012	QLD, nr. Injiinoo
915	<i>E. diversifolia</i>	M-Shoot Bud	02/2011	SA, nr. Meningie
916	<i>E. diversifolia</i>	M-Shoot Bud	02/2011	SA, nr. Meningie

Table 2. Gall classification scheme used in this study (modified from Nelson et al. 2014).
Multilocular galls indicated by “M”; unilocular galls indicated by “U.”

Gall	Gall Type	Locules	Plant Tissue	Notes
M-Shoot Bud	M	shoot bud	Spherical, teardrop, or cigar-shaped, involving apical, axillary, or leaf buds
M-Flat Leaf	M	leaf blade	Hypertrophied or puffy leaf blades, with locules arranged internally in tight rows
M-Flower Bud	M	flower bud	Hypertrophied flower buds, locules located in various tissues, including stamens, anthers, top of stigmata, base of stigmata
U-Peagall	U	axillary bud	Pea-shaped in leaf axil, sessile or stalked
Leaf Peagall	U-M	leaf blade	Pea-shaped on leaf surface, one to several in a row

Table 3. Primers used for PCR amplification (P) and DNA sequencing (S). Most commonly used primers indicated with an asterix. Remaining primers used to obtain additional sequencing reads as needed.

Primer	Direction	Use	Sequence	Reference
COI				
TY-J-1461*	F	P, S	TTTACARTTTACCGCCTATTRTCAGCCA	Winkler et al. 2009
LCO1490	F	P, S	GGTCAACAAATCATAAAGATATTGG	Folmer et al. 1994
C1-N-1958	R	S	CGTATATTAATAATTGTTGTAATAA	Scheffer and Wiegmann 2000
C1-N-2004	R	S	CAAATARNGGTATTCGA	this project
C1-J-2183*	F	S	CAACATTTATTTTGATTTTTTGG	Simon et al. 1994
C1-N-2191	R	S	CCCGGTAAAATTTAAATATAAACTTC	Simon et al. 1994
HCO2198	R	S	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. 1994
C1-J-2441 mod	F	S	CCTACAGGAATTTAAATTTTGTGATTAGC	Scheffer et al. 2004
C1-N-2413*	R	S	TCARCTRAAAATTTTAATTCCTGT	Winkler et al. 2009
C1-N-2508	R	S	CTCCAGTTAATCCTCCAAGTGTAAAT	Scheffer et al. 2004
TL2-N-3014 *	R	P, S	TCCATTGCACTAATCTGCCATATTA	Simon et al. 1994
CAD				
54F *	F	P, S	GTNGTNTTYCARACNGGNATGGT	Moulton and Wiegmann 2004
AG-360AR	R	S	CCATGATTYTGTGARGTCAT	Scheffer et al. 2007
AG-360BR	R	S	CCRTGRTTYTGTGAYGTCAT	Scheffer et al. 2007
405R *	R	P	GCNGTRTGYTCNGGRTGRAAYTG	Moulton and Wiegmann 2004
PGD				
2F *	F	P, S	ATHGARTAYGGNGAYATGCA	Regier 2008
2.5R *	R	P, S	ATRC AACNCCRCGCCACAT	Winkler et al. 2009

Table 4. Fergusoninid species (as defined in methods) arranged by alphabetized host plant name(s). “Species” number is an arbitrary designation based upon the order of appearance in this table. N is number of specimens in the species limits analysis. PWD% refers to the average pairwise differences within each presumptive species represented by multiple individuals. Host plants may be listed multiple times for “oligophagous” species, and are followed by specimen codes. Gall Type indicates the type of gall from which specimens in any presumptive species were reared. “Species” number presented here is used throughout this article, including the Tables and Figures. Abbreviations for genera are as follows: A=*Angophora*, C=*Corymbia*, E=*Eucalyptus*, L=*Leptospermum*, Met=*Metrosideros*, M=*Melaleuca*, S=*Syzygium*.

“Species” No.	N	PWD (%)	Host Plants and Fly Specimen codes	Gall Type
Sp #1	1	--	<i>A. floribunda</i> : FC-144	M-Shoot Bud
Sp #2	1	--	<i>C. abbreviata</i> : FC-104	M-Flower Bud
Sp #3	3	0.8	<i>C. citriodora</i> : FC-762 <i>C. variegata</i> : FC-761, FC-763	M-Shoot Bud
Sp #4	1	--	<i>C. citriodora</i> : FC-80	Leaf Peagall
Sp #5	6	0.3	<i>C. ptychocarpa</i> : FC-26, FC-27, FC-36, FC-48, FCV-180, FCV-201	M-Flower Bud
Sp #6	5	1.4	<i>C. tessellaris</i> : FC-100, FC-731, FC-171, FC-771, FC-774	M-Shoot Bud
Sp #7	1	--	<i>C. tessellaris</i> : FC-735	M-Shoot Bud
Sp #8	1	--	<i>C. torrelliana</i> : FC-760	U-Stem
Sp #9	1	--	<i>Corymbia</i> sp.: FC-79	Leaf Peagall
Sp #10	1	--	<i>E. acmenioides</i> : FC-733	M-Shoot Bud
Sp #11	3	2.0	<i>E. amygdalina</i> : FCV-148 <i>E. coccifera</i> : FC-603 <i>E. stellulata</i> : FC-544	M-Shoot Bud
Sp #12	1	--	<i>E. baxteri</i> : FC-186	M-Flower Bud
Sp #13	6	0.4	<i>E. blakelyi</i> : FC-143 <i>E. camaldulensis</i> : FC-61, FC-73, FC-118, FCV-204	M-Shoot Bud

			<i>E. dealbata</i> : FC-775	
Sp #14	1	--	<i>E. camaldulensis</i> : FC-736	M-Shoot Bud
Sp #15	1	--	<i>E. camaldulensis</i> : FC-740	M-Shoot Bud
Sp #16	1	--	<i>E. camaldulensis</i> : FC-105	M-Flower Bud
Sp #17	15	0.7	<i>E. coccifera</i> : FC-541, FC-543, FC-602, FC-606, FC-608	M-Shoot Bud
			<i>E. niphophila</i> : FC-521, FC-522, FC-526, FC-586	
			<i>E. pauciflora</i> : FC-515, FC-539, FC-609, FC-610, FC-618, FC-619	
Sp #18	5	0.8	<i>E. cosmophylla</i> : FCV-114, FC-177	M-Shoot Bud
			<i>E. tereticornis</i> : FC-134, FC-169, FCV-199	
Sp #19	1	--	<i>E. gomphocephala</i> : FC-113	Leaf Peagall
Sp #20	2	1.2	<i>E. haemastoma</i> : FCV-194	M-Shoot Bud
			<i>E. racemosa</i> : FC-37	
Sp #21	2	0.0	<i>E. largiflorens</i> : FC-910, FC-911	M-Flat Leaf
Sp #22	2	0.5	<i>E. leucoxylon</i> : FC-755	M-Flat Leaf
			<i>E. nr. siderophloia</i> : FC-741	
Sp #23	5	0.5	<i>E. leucoxylon</i> : FC-19, FC-74, FC-85	M-Flat Leaf
			<i>E. obliqua</i> : FC-120	
			<i>E. porosa</i> : FC-142	
Sp #24	3	1.5	<i>E. leucoxylon</i> : FC-754	M-Flat Leaf
			<i>E. melliodora</i> : FC-742	
			<i>E. sp.</i> : FC-145	
Sp #25	3	0.3	<i>E. macrorrhyncha</i> : FC-64, FC-71, FC-88	M-Flower Bud
Sp #26	1	--	<i>E. mannifera</i> : FC-753	U-Peagall
Sp #27	1	--	<i>E. marginata</i> : FC-107	Leaf Peagall
Sp #28	1	--	<i>E. microcarpa</i> : FC-173	Leaf Peagall
Sp #29	1	--	<i>E. microcarpa</i> : FC-78	M-Flower Bud
Sp #30	1	--	<i>E. microcarpa</i> : FC-188	U-Peagall
Sp #31	5	0.5	<i>E. niphophila</i> : FC-724, FC-725 FC-750	M-Flower Bud
			<i>E. pauciflora</i> : FC-535, FC-540	
Sp #32	8	1.5	<i>E. niphophila</i> : FC-523, FC-580 BT, FC-649, FC-652, FC-659	M-Shoot Bud
			<i>E. pauciflora</i> : FC-529, FC-632, FC-633	

Sp #33	1	--	<i>E. obliqua</i> : FC-737	M-Shoot Bud
Sp #34	1	--	<i>E. obliqua</i> : FC-83	M-Flower Bud
Sp #35	2	0.0	<i>E. odorata</i> : FC-189, FC-190	M-Flat Leaf
Sp #36	2	0.6	<i>E. pauciflora</i> : FC-710, FC-711	M-Shoot Bud
Sp #37	5	0.8	<i>E. pauciflora</i> : FC-503, FC-506, FC-509, FC-510, FC-512	M-Shoot Bud
Sp #38	2	0.3	<i>E. pauciflora</i> : FC-508, FC-718	M-Flower Bud
Sp #39	2	0.8	<i>E. pauciflora</i> : FC-721, FC-720	M-Flower Bud
Sp #40	3	0.2	<i>E. pauciflora</i> : FC-147, FC-511, FC-716	Leaf Peagall
Sp #41	1	--	<i>E. pruinosa</i> : FC-102	M-Flower Bud
Sp #42	1	--	<i>E. robusta</i> : FC-172	M-Shoot Bud
Sp #43	2	0.0	<i>E. siderophloia</i> : FC-29, FC-39	M-Flat Leaf
Sp #44	1	--	<i>E. siderophloia</i> : FC-89	M-Flat Leaf
Sp #45	1	--	<i>E. stellulata</i> : FC-751	M-Flower Bud
Sp #46	2	0.1	<i>E. tereticornis</i> : FC-757, FC-758	M-Flower Bud
Sp #47	1	--	<i>E. tereticornis</i> : FC-780	Leaf Peagall
Sp #48	1		<i>E. melliodora</i> : FC-743	U-Peagall
Sp #49	1	--	<i>E. viminalis</i> : FC-739	U-Peagall
Sp #50	1	--	<i>E. viminalis</i> : FC-738	U-Peagall
Sp #51	2	0.5	<i>E. viminalis</i> : FC-121, FC-146	M-Shoot Bud
Sp #52	1	--	<i>Eucalyptus sp.</i> : FC-773	M-Flat Leaf
Sp #53	1	--	<i>Eucalyptus sp.</i> : FC-778	U-Peagall
Sp #54	1	--	<i>Eucalyptus sp.</i> : FC-748	U-Peagall
Sp #55	2	0.0	<i>Eucalyptus sp.</i> : FC-719, FC-746	M-Flower Bud
Sp #56	1		<i>Eucalyptus sp.</i> : FC-756	M-Shoot Bud
Sp #57	2	0.3	<i>Eucalyptus sp.</i> : FC-98, FC-777	M-Shoot Bud, M-Flat Leaf
Sp #58	1	--	<i>L. madidum</i> : FC-914	M-Shoot Bud
Sp #59	2	0.0	<i>L. laevigatum</i> : FC-912, FC-913	U-Peagall
Sp #60	4	0.2	<i>Met. excelsa</i> : FC-131, FC-132, FCV-198, FC-764	M-Shoot Bud
Sp #61	1	--	<i>M. argentea</i> : FC-184	M-Shoot Bud
Sp #62	1	--	<i>M. armillaris</i> : FC-135	U-Peagall

Sp #63	6	0.3	<i>M. cajuputi</i> : FC-24, FC-25, FC-35, FC-42, FC-53, FC-91	M-Shoot Bud
Sp #64	6	0.8	<i>M. dealbata</i> : FC-23, FC-30, FC-34, FC-40, FC-46, FC-732	M-Shoot Bud
Sp #65	3	0.6	<i>M. decora</i> : FC-140, FC-730	U-Peagall
			<i>M. linariifolia</i> : FC-759	
Sp #66	4	0.0	<i>M. fluviatilis</i> : FC-47, FC-55, FC-75, FC-93	M-Shoot Bud
Sp #67	5	0.0	<i>M. fluviatilis</i> : FC-21, FC-43, FC-92, FC-110, FCV-124	M-Shoot Bud
Sp #68	5	0.5	<i>M. leucandendra</i> : FC-22, FC-33, FC-52, FC-94, FC-95	M-Shoot Bud
Sp #69	2	0.2	<i>M. linariifolia</i> : FC-137, FC-170	U-Peagall
Sp #70	2	0.9	<i>M. nervosa</i> : FC-76, FC-84	M-Shoot Bud
Sp #71	2	0.0	<i>M. nervosa</i> : FC-31, FC-41	M-Shoot Bud
Sp #72	1	--	<i>M. nodosa</i> : FC-136	U-Peagall
Sp #73	4	0.7	<i>M. quinquenervia</i> : FC-101, FC-122, FC-127, FC-183	M-Shoot Bud
Sp #74	5	1.8	<i>M. quinquenervia</i> : FC-20, FC-38, FC-45, FC-152, FCV-203	M-Shoot Bud
Sp #75	1	--	<i>M. quinquenervia</i> FCV-123	M-Flower Bud
Sp #76	4	0.2	<i>M. stenostachya</i> : FC-67, FC-77, FC-108, FC-109	M-Shoot Bud
Sp #77	2	0.0	<i>M. viridiflora</i> : FC-68, FC-81	M-Shoot Bud
Sp #78	1	--	<i>S. luehmannii</i> : FC-734	Mix of Galls
Sp #79	3	0.0	<i>S. luehmannii</i> : FC-139, FCV-196, FCV-200	Mix of Galls
Sp #80	4	1.1	<i>E. diversifolia</i> : FC-18, FC-70, FC-915, FC-916	M-Shoot Bud
Sp #81	1	--	<i>Eucalyptus sp.</i> : FC-779	M-Flat Leaf
Sp #82	1	--	<i>Eucalyptus sp.</i> : FC-749	U-Peagall
Sp #83	1	--	<i>Eucalyptus sp.</i> : FC-99	Leaf Peagall
Sp #84	1	--	<i>E. polyanthemos</i> : FC-745	M-Flat Leaf
Sp. #85	1	--	<i>E. coccifera</i> : FC-542	Leaf Peagall

Table 5. Host species, Gall Type, and maximum pairwise distance of eleven presumptive fergusoninid species using a monophyly + 2% pairwise distance criterion (see also Fig. 2, dark blue clusters). Species identification number as given in Table 4.

Species No.	Host Plant (Fly Specimens)	<i>Eucalyptus</i> Subgenus	Max. PW Distance	Gall	Locations
Sp. #65	<i>M. decora</i> , FC-730, FC-140 <i>M. linariifolia</i> , FC-759	--	0.6	U-PG U-PG	QLD
Sp. #3	<i>C. citriodora</i> , FC-761, FC-763 <i>C. maculata</i> , FC-762	--	0.8	Shoot Bud Shoot Bud	NSW
Sp. #32	<i>E. pauciflora</i> , FC-529, FC-632, FC-633 <i>E. niphophila</i> FC-523, FC-580, FC-649, FC-652, FC-659	<i>Eucalyptus</i> <i>Eucalyptus</i>	1.5	Shoot Bud Shoot Bud	NSW, VIC
Sp. #20	<i>E. haemastoma</i> , FCV-194 <i>E. racemosa</i> , FC-37	<i>Eucalyptus</i> <i>Eucalyptus</i>	1.2	Shoot Bud Shoot Bud	NSW, QLD
Sp. #17	<i>E. niphophila</i> , FC-521, FC-522, FC-526, FC-586 <i>E. coccifera</i> , FC-541, FC-543, FC-602, FC-606, FC-608 <i>E. pauciflora</i> , FC-515, FC-539, FC-609, FC-610, FC-618, FC-619	<i>Eucalyptus</i> <i>Eucalyptus</i> <i>Eucalyptus</i>	1.7	Shoot Bud Shoot Bud Shoot Bud	ACT, TAS, VIC
Sp. #11	<i>E. amygdalina</i> , FCV-148 <i>E. stellulata</i> , FC-544 <i>E. coccifera</i> , FC-603	<i>Eucalyptus</i> <i>Eucalyptus</i> <i>Eucalyptus</i>	1.9	Shoot Bud Shoot Bud Shoot Bud	ACT, TAS
Sp. #31	<i>E. pauciflora</i> , FC-535, FC-540 <i>E. niphophila</i> , FC-724, FC-725, FC-750	<i>Eucalyptus</i> <i>Eucalyptus</i>	0.5	Flower Bud Flower Bud	NSW, TAS, VIC
Sp. #23	<i>E. leucoxylon</i> , FC-19, FC-74, FC-85, FC-120 <i>E. porosa</i> , FC-142	<i>Symphyomyrtus</i> <i>Symphyomyrtus</i>	0.5	Flat Leaf Flat Leaf	SA
Sp. #24	<i>E. leucoxylon</i> , FC-754 <i>E. melliodora</i> , FC-742 <i>Eucalyptus</i> sp., FC-145	<i>Symphyomyrtus</i> <i>Symphyomyrtus</i> --	1.5	Flat Leaf Flat Leaf Flat Leaf	ACT
Sp. #13	<i>E. blakelyi</i> , FC-143 <i>E. camaldulensis</i> , FC-118, FCV-204 <i>E. dealbata</i> , FC-775 <i>E. intertexta</i> , FC-61, FC-73	<i>Symphyomyrtus</i> <i>Symphyomyrtus</i> <i>Symphyomyrtus</i> <i>Symphyomyrtus</i>	0.8	Shoot Bud Shoot Bud Shoot Bud Shoot Bud	ACT, NSW, SA
Sp. #18	<i>E. cosmophylla</i> , FC-114, FCV-177 <i>E. tereticornis</i> , FC-134, FC-169, FCV-199	<i>Symphyomyrtus</i> <i>Symphyomyrtus</i>	0.8	Shoot Bud Shoot Bud	SA

Table 6. Contingency test of independence between dietary specialization (monophagous vs. oligophagous) and a) host genus (*Eucalyptus* vs. Other Genera); b) gall type (Shoot Bud Galls vs. Other Gall Types); and c) phylogenetic placement (Clade C+D vs Other Clades; see text, Figure 5). Only *Fergusonina* species having more than one representative are included.

a) Host genus and dietary specialization. Chi-sq=4.0529, df=1, p=0.044*.

Contrast	Monophagous	Oligophagous	Total
<i>Eucalyptus</i>	15	9	24
Other Genera	17	2	19
	32	11	43

b) Gall type and dietary specialization. Chi-sq 0.1813, df=1, p=0.670.

Contrast	Monophagous	Oligophagous	Total
Shoot Bud Galls	17	7	24
Other Gall Types	7	4	11
Total	24	11	35

c) Phylogenetic placement and dietary specialization. Chi-sq=19.01, df=1, p<0.0001***.

Contrast	Monophagous	Oligophagous	Total
Clade C+D	5	6	11
Other Clades	68	5	73
Total	73	11	84

Table 7. The plant host species and their *Fergusonina* species used in this study. The fly species numbers used throughout this study are in the second column (defined in Table 4) . Gall type, the samples size of fly specimens on that host species, and their specimen codes are shown. For host plants fed upon by “oligophagous” fergusoninid species, the additional host(s) and the code numbers of specimens from these are given. *Fergusonina* species name given for described species; “*F. n. sp.*” indicates that flies from that gall type and plant host (often from the same collection) have been determined to be new; “*F. unknown*” is given for those species having undetermined status.

Host Plant	Fly Sp. #	Gall Type	N	<i>Fergusonina</i> Specimens	Additional Hosts	<i>Fergusonina</i> species
<i>Angophora</i>						
<i>A. floribunda</i>	1	M-Shoot Bud	1	FC-144	--	<i>F. n. sp.</i> (Davies et al. 2013a, pg.133)
					--	
<i>Corymbia</i>						
<i>C. abbreviata</i>	2	M-Flower Bud	1	FC-104	--	<i>F. thomasi</i> Taylor
<i>C. maculata</i>	3	M-Shoot Bud	2	FC-762, FC-761	<i>C. citriodora</i> : FC-763	<i>F. biseta</i> Tonnoir
	4	Leaf Peagall	1	FC-80	--	<i>F. unknown</i>
<i>C. ptychocarpa</i>	5	M-Flower Bud	6	FCV-201, FCV-180, FC-27, FC-26, FC-36, FC-48	--	<i>F. gibbindavisi</i> Taylor
<i>C. tessellaris</i>	6	M-Shoot Bud	5	FC-100 , FC-731, FC-771, FC-774, FC-171	--	<i>F. n. sp.</i> (Davies et al. 2010a, pg. 22)
	7	M-Shoot Bud	1	FC-735	--	<i>F. n. sp.</i> (Davies et al. 2010a, pg. 22)
<i>C. torelliana</i>	8	U-Stem	1	FC-760	--	<i>F. unknown</i>
<i>C. citriodora</i>	1	M-Shoot Bud	1	FC-763	<i>C. maculata</i> : FC-762, FC-761	<i>F. unknown</i>

<i>Corymbia sp.</i>	9	Leaf Peagall	1	FC-79	--	<i>F. unknown</i>
<i>Eucalyptus</i>						
<i>E. acmenioides</i>	10	M-Shoot Bud	1	FC-733	--	<i>F. n. sp.</i> (Davies et al. 2010b, pg. 23; Davies et al. 2013a, pg. 133)
<i>E. amygdalina</i>	11	M-Shoot Bud	1	FC-148	<i>E. stellulata</i> : FC-544, <i>E. coccifera</i> : FC-603	<i>F. n. sp.</i> (Davies et al. 2010b, pg. 23)
<i>E. baxteri</i>	12	M-Flower Bud	1	FC-186	--	<i>F. n. sp.</i> (Davies et al. 2012b, pg. 29)
<i>E. blakelyi</i>	13	M-Shoot Bud	1	FC-143	<i>E. camaldulensis</i> : FCV-204, FC-118, FC-61, FC-73; <i>E. dealbata</i> : FC-776, <i>E. blakelyi</i> : FC-143	<i>F. n. sp.</i> (Davies et al., 2013b, pg.133)
<i>E. camaldulensis</i>	13	M-Shoot Bud	2	FCV-204, FC-61, FC-73	<i>E. dealbata</i> : FC-776	<i>F. n. sp.</i> (Davies et al. 2013a, pg. 133)
	14	M-Shoot Bud	1	FC-736	--	<i>F. lockharti</i> Tonnoir
	15	M-Shoot Bud	1	FC-740	--	<i>F. lockharti</i> Tonnoir
	16	M-Flower Bud	1	FC-105	--	<i>F. tillyardi</i> Tonnoir
<i>E. coccifera</i>	85	Leaf Peagall	1	FC-542	--	<i>F. unknown</i>
	17	M-Shoot Bud	5	FC-541, FC-543, FC-602, FC-606, FC-608	<i>E. niphophila</i> : FC-586, FC-521, FC-526, FC-522; <i>E. pauciflora</i> : FC-515, FC-618, FC-619, FC-539, FC-610, FC-609	<i>F. taylori</i> Nelson and Yeates
	11	M-Shoot Bud	1	FC-603	<i>E. amygdalina</i> : FCV-148; <i>E.</i>	<i>F. unknown</i>

<i>E. cosmophylla</i>	18	M-Shoot Bud	2	FCV-114, FC-177	<i>stellulata</i> : FC-544 <i>E. tereticornis</i> : FC-169, FC-134, FCV-199	<i>F. n. sp.</i> Davies et al. 2013a, pg. 133
<i>E. dealbata</i>	13	M-Shoot Bud	1	FC-776	<i>E. blakelyi</i> : FC-143; <i>E. camaldulensis</i> : FCV-204, FC-118, FC-61, FC-73,	<i>F. n. sp.</i> (Davies et al. 2013a, pg. 133)
<i>E. diversifolia</i>	80	M-Shoot Bud	4	FC-18, FC-70, FC-915, FC-916	--	<i>F. n. sp.</i> (Davies et al. 2013a, pg. 133)
<i>E. gomphocephala</i>	19	Leaf Peagall	1	FC-113	--	<i>F. newmani</i> Tonnoir
<i>E. haemastoma</i>	20	M-Shoot Bud	1	FCV-194	<i>E. racemosa</i> : FC-37	<i>F. n. sp.</i> (Davies et al. 2013a, pg. 133)
<i>E. largiflorens</i>	21	M-Flat Leaf	2	FC-910, FC-911	--	<i>F. unknown</i>
<i>E. leucoxylon</i>	22	M-Flat Leaf	1	FC-755	--	<i>F. n. sp.</i> (Davies and Lloyd 1996, pg. 17)
	23	M-Flat Leaf	3	FC-19, FC-74, FC-85	<i>E. obliqua</i> : FC-120, <i>E. porosa</i> : FC-142	<i>F. unknown</i>
	24	M-Flat Leaf	1	FC-754	<i>E. melliodora</i> : FC-742	<i>F. unknown</i>
<i>E. macrorrhyncha</i>	25	M-Flower Bud	3	FC-64, FC-71, FC-88	--	<i>F. nicholsoni</i> Tonnoir
<i>E. mannifera</i>	26	U-Peagall	1	FC-753	--	<i>F. unknown</i>
<i>E. marginata</i>	27	Leaf Peagall	1	FC-107	--	<i>F. n. sp.</i> (Davies et al. 2010b, pg. 24)
<i>E. melliodora</i>	48	U-Peagall	1	FC-743	--	<i>F. unknown</i>
<i>E. melliodora</i>	24	M-Flat Leaf	1	FC-742	<i>E. leucoxylon</i> : FC-754	<i>F. unknown</i>
<i>E. microcarpa</i>	28	Leaf Peagall	1	FC-173	--	<i>F. n. sp.</i> (Davies et al. 2014c, pg. 168)
	29	M-Flower Bud	1	FC-78	--	<i>F. n. sp.</i> (Davies et al. 2012 b, pg. 30)
	30	U-Peagall	1	FC-188	--	<i>F. n. sp.</i> (Davies et al.

<i>E. niphophila</i>	31	M-Flower Bud	3	FC-724, FC-725, FC-750,	<i>E. pauciflora</i> : FC-535, FC-540	2010b, pg. 35) <i>F. unknown</i>
	32	M-Shoot Bud	5	FC-523, FC-580, FC-652, FC-659,	<i>E. pauciflora</i> : FC-529, FC-632, FC-633	<i>F. daviesae</i> Nelson and Yeates
	17	M-Shoot Bud	5	FC-515, FC-521, FC-586, FC-526, FC-522,	<i>E. coccifera</i> : FC-608, FC-602, FC-543, FC-606, FC-541; <i>E. pauciflora</i> : FC-515, FC-618, FC-619, FC-539, FC-610, FC-609	<i>F. taylori</i> Nelson and Yeates
<i>E. obliqua</i>	33	M-Shoot Bud	1	FC-737	--	<i>F. n. sp.</i> (Davies et al. 2013a, pg. 133)
	23	M-Flat Leaf	1	FC-120	<i>E. leucoxylon</i> : FC-19, FC-85, FC-74; <i>E. porosa</i> : FC-142	<i>F. unknown</i>
	34	M-Flower Bud	1	FC-83	--	<i>F. n. sp.</i> (Davies et al. 2010b, pg. 26)
<i>E. odorata</i>	35	M-Flat Leaf	2	FC-189, FC-190	--	<i>F. n. sp.</i> (Davies et al. 2013b, pg. 168)
<i>E. pauciflora</i>	17	M-Shoot Bud	6	FC-515, FC-618, FC-619, FC-539, FC-610, FC-609	<i>E. coccifera</i> : FC-608, FC-602, FC-543, FC-606, FC-541; <i>E. niphophila</i> : FC-515, FC-521, FC-586, FC-526, FC-522	<i>F. taylori</i> Nelson and Yeates
	37	M-Shoot Bud	2	FC-500, FC-503, FC-506, FC-509, FC-510, FC-512	--	<i>F. omlandi</i> Nelson and Yeates
	32	M-Shoot Bud	3	FC-529, FC-632, FC-633	<i>E. niphophila</i> : FC-523, FC-580, FC-	<i>F. daviesae</i> Nelson and Yeates

					652, FC-659	
	36	M-Shoot Bud	2	FC-710, FC-711	--	<i>F. tasmaniensis</i> Nelson and Yeates
	38	M-Flower Bud	2	FC-508, FC-718	--	<i>F. unknown</i>
	39	M-Flower Bud	2	FC-721, FC-720	--	<i>F. unknown</i>
	31	M-Flower Bud	2	FC-535, FC-540	<i>E. niphophila</i> : FC-724, FC-725 FC-750,	<i>F. unknown</i>
	40	Leaf Peagall	3	FC-716, FC-147, FC-511	--	<i>F. unknown</i>
<i>E. polyanthemos</i>	85	M-Flat Leaf	1	FC-745	--	<i>F. n. sp.</i> (Davies <i>et al.</i> 2013b, pg. 168)
<i>E. porosa</i>	23	M-Flat Leaf	1	FC-142	<i>E. leucoxylon</i> : FC-19, FC-85, FC-74; <i>E. obliqua</i> : FC-120	<i>F. n. sp.</i> (Davies <i>et al.</i> 2013a, pg. 168)
<i>E. pruinosa</i>	41	M-Flower Bud	1	FC-102	--	<i>F. n. sp.</i> (Davies <i>et al.</i> 2012 b, pg. 29)
<i>E. racemosa</i>	20	M-Shoot Bud	1	FC-37	<i>E. haemastoma</i> : FCV-194	<i>F. n. sp.</i> (Davies <i>et al.</i> , 2013a, pg. 133)
<i>E. robusta</i>	42	M-Shoot Bud	1	FC-172	--	
<i>E. siderophloia</i>	43	M-Flat Leaf	1	FC-29	--	<i>F. n. sp.</i> (Davies <i>et al.</i> , 2013b, pg. 168)
	44	M-Flat Leaf	1	FC-89	--	<i>F. n. sp.</i> (Davies <i>et al.</i> , 2013b, pg. 168)
<i>E. stellulata</i>	11	M-Shoot Bud	1	FC-544	<i>E. coccifera</i> : FC-603; <i>E. amygdalina</i> : FCV-148	<i>F. unknown</i>
	45	M-Flower Bud	1	FC-751	--	<i>F. unknown</i>
<i>E. tereticornis</i>	18	M-Shoot Bud	3	FC-169, FC-134, FCV-199	<i>E. cosmophylla</i> : FCV-114, FC-177	<i>F. n. sp.</i> (Davies <i>et al.</i> , 2013a, pg. 134)
	46	M-Flower Bud	2	FC-757, FC-758	--	<i>F. unknown</i>
	47	Leaf Peagall	1	FC-780	--	<i>F. unknown</i>
<i>E. viminalis</i>	51	M-Shoot Bud	2	FC-121, FC-146	--	<i>F. n. sp.</i> (Davies <i>et al.</i> , 2014a, pg. 552)

	50	U-Peagall	1	FC-738	--	<i>F. unknown</i>
	49	U-Peagall	1	FC-739	--	<i>F. n. sp. (Davies et al., 2014c, pg. 138)</i>
<i>Eucalyptus sp.</i>	52	M-Flat Leaf	1	FC-773	--	<i>F. unknown</i>
<i>Eucalyptus sp.</i>	53	U-Peagall	1	FC-778	--	<i>F. unknown</i>
<i>Eucalyptus sp.</i>	54	U-Peagall	1	FC-748	--	<i>F. unknown</i>
<i>Eucalyptus sp.</i>	55	M-Flower Bud	2	FC-719, FC-746	--	<i>F. unknown</i>
<i>Eucalyptus sp.</i>	56	M-Shoot Bud	1	FC-756	--	<i>F. unknown</i>
<i>Eucalyptus sp.</i>	57	M-Shoot Bud, M-Flat Leaf	1	FC-777, FC-98	--	<i>F. unknown</i>
<i>Eucalyptus sp.</i>	81	M-FLG	1	FC-779	--	<i>F. unknown</i>
<i>Eucalyptus sp.</i>	82	U-Peagall		FC-749	--	<i>F. unknown</i>
<i>Eucalyptus sp.</i>	83	Leaf Peagall	1	FC-99	--	<i>F. unknown</i>
<i>Eucalyptus sp.</i>	84	M-FLG	1	FC-745	--	<i>F. unknown</i>
<i>Leptospermum</i>						
<i>L. madidum</i>	58	M-Shoot Bud	1	FC-914	--	<i>F. n. sp., Taylor in prep.</i>
<i>L. laevigatum</i>	59	U-Peagall		FC-912, FC-913	--	<i>F. n. sp., Taylor in prep.</i>
<i>Metrosideros</i>						
<i>Met. excelsa</i>	60	M-Shoot Bud	4	FC-131, FC-132, FCV-198, FC-764	--	<i>F. metrosiderosi</i> Taylor
<i>Melaleuca</i>						
<i>M. argentea</i>	61	M-Shoot Bud	1	FC-184	--	<i>F. unknown</i>
<i>M. armillaris</i>	62	U-Peagall		FC-135	--	<i>F. n. sp. Taylor 2004</i>
<i>M. cajuputi</i>	63	M-Shoot Bud	6	FC-24, FC-25, FC-35, FC-42, FC-53, FC-91	--	<i>F. purcelli</i> Taylor
<i>M. dealbata</i>	64	M-Shoot Bud	6	FC-23, FC-30, FC-34, FC-40, FC-46, FC-732	--	<i>F. makinsoni</i> Taylor 2004

<i>M. decora</i>	65	U-Peagall	2	FC-730, FC-140	--	<i>F. n. sp.</i> (Davies <i>et al.</i> , 2014, pg. 254)	1.
<i>M. fluviatilis</i>	66	M-Shoot Bud	4	FC-47, FC-55, FC-75, FC-93	--	Currently [<i>F. turneri</i> Taylor 2004]	
	67	M-Shoot Bud	5	FC-21, FC-43, FC-92, FC-110, FCV-124	--	Currently [<i>F. turneri</i> Taylor 2004]	
<i>M. leucandendra</i>	68	M-Shoot Bud	5	FC-22, FC-33, FC-52, FC-95, FC-94	--	<i>F. centeri</i> Taylor 2004	
<i>M. linariifolia</i>	65	U-Peagall	1	FC-759,	<i>E. decora</i> : FC-140, FC-730	<i>F. n. sp.</i> (Davies <i>et al.</i> , 2014, pg. 254)	
	69	U-Peagall	2	FC-137, FC-170	--	<i>F. n. sp.</i> (Davies <i>et al.</i> , 2014, pg. 254)	
<i>M. nervosa</i>	70	M-Shoot Bud	2	FC-76, FC-84	--	<i>F. schefferae</i> Taylor 2004	
	71	M-Shoot Bud	2	FC-31, FC-41	--	<i>F. goolsbyi</i> Taylor 2004	
<i>M. nodosa</i>	72	U-Peagall	1	FC-136	--	<i>F. n. sp.</i> (Davies <i>et al.</i> , 2014, pg. 254)	
<i>M. quinquenervia</i>	73	M-Shoot Bud	4	FC-101, FC-122, FC-127, FC-183	--	<i>F. unknown</i>	
	74	M-Shoot Bud	6	FC-38, FC-45, FC-20, FC-152, FCV-203	--	<i>F. turneri</i> Taylor 2004	
	75	M-Flower Bud		FCV-123	--	<i>F. unknown</i>	
<i>M. stenostachya</i>	76	M-Shoot Bud	4	FC-67, FC-77, FC-108, FC-109	--	<i>F. n. sp.</i> Taylor 2004 p. 257	
<i>M. viridiflora</i>	77	M-Shoot Bud		FC-68, FC-81	--	<i>F. burrowsi</i> Taylor 2004	
<i>Syzygium</i>							
<i>S. luehmannii</i>	78	Mix of Galls		FC-734	--	<i>F. n. sp.</i> (Davies <i>et al.</i> , 2014d, pg. 231)	
	79	Mix of Galls		FC-139, FCV-196, FCV-200	--	<i>F. n. sp.</i> (Davies <i>et al.</i> , 2014d, pg. 231)	