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The nematode genus *Fergusobia* (Nematoda: Neotylenchidae): molecular phylogeny, descriptions of clades and associated galls, host plants and *Fergusonina* fly larvae

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Abstract

Collection data and biological information is presented on the genus *Fergusobia* (Nematoda: Neotylenchidae) from Australasia, India and The Philippines, an emended diagnosis of the genus is presented, and its putative phylogeny is discussed based on molecular and morphological evidence. About 20 clades have been found, and are outlined, including information on nematode and fly morphology; plant host species and distribution. Fly morphology, particularly the structure of the dorsal shield of third stage larvae, is congruent with the clades of nematodes, suggesting coevolution. However, little evidence of coevolution between the fly/nematode association and their host plants is apparent: host-switching appears to have been common, although host-specificity is stringent among most clades. A key to the species and morphospecies of nematodes collected from *Corymbia*, *Angophora*, *Metrosideros*, *Syzygium*, narrow-leaved *Melaleuca*, and *Eucalyptus* is presented.

Key words: Flies, galls, Myrtaceae, phylogeny, taxonomy, clades, dorsal shields, morphology, key, diagnosis

Introduction

Nematodes of the genus *Fergusobia* Currie 1937 (Tylenchida: Neotylenchidae) occur in a mutualistic association with flies of the genus *Fergusonina* Malloch 1924 (Diptera: Fergusoninidae). Evidence from molecular sequencing of flies and nematodes suggests that each genus is monophyletic, and each species of *Fergusonina* is associated with a particular species of *Fergusobia* (Goolsby *et al.* 2000; Giblin-Davis *et al.* 2001; Taylor 2004; Ye *et al.* 2007b). The nematodes are thought to induce galls on host plants via oesophageal secretions produced during feeding (Currie 1937; Giblin-Davis *et al.* 2001). Thus, the flies probably rely on the nematodes for gall induction, and the nematodes rely on the flies for transport from plant to plant and for nutrition of the parasitic female. *Fergusobia* is dicyclic, with a parthenogenetic generation in a plant, followed by an amphimictic generation that begins in a plant and ends with a parasitic, fertilised female in the haemolymph of (female only) flies. Host fly resistance and nematode virulence must be modified in some way in female flies, because they always carry nematodes (Giblin-Davis *et al.* 2003). The parasitic female deposits hundreds of eggs into the haemolymph of the fly, where they hatch and the juveniles move to the fly's oviducts. When the fly deposits her eggs in fresh meristematic tissue, she also deposits juvenile nematodes. The life cycles of the flies and nematodes are described in more detail in Giblin-Davis *et al.* (2004b) and Taylor *et al.* (2005).

This fly/nematode association is found only on Myrtaceous plants (Taylor *et al.* 2005). Galls have been collected from *Angophora, Corymbia, Eucalyptus, Melaleuca,* and *Metrosideros* from the Leptospermoidea, and from *Syzygium* from the Myrtoidea (Taylor *et al.* 2005; Taylor *et al.* 2007). They are also reported from *Leptospermum* (Robin Adair, personal communication) from the Leptospermoidea. More than 70 species of 'eucalypts' (*Angophora, Corymbia* and *Eucalyptus*) and two species of *Syzygium* have been recorded as hosts (Siddiqi 1986a; K.A. Davies, unpub. data). Until recently, it was thought that, in the genus *Melaleuca,* galls were largely confined to the broad-leaved *leucadendra* complex, but recent collection has shown that at least four species of narrow-leaved melaleucas growing on the eastern coast of Australia are hosts (Taylor 2004; R.M. Giblin-Davis & K.A. Davies, unpub. data) bringing the collective total of *Melaleuca* hosts to 12. Molecular sequences from some *Fergusobia* species from the narrow-leaved melaleucas are included in the phylogenies presented in this paper.

Fergusonina/Fergusobia galls have been collected from India, the Philippines, New Guinea, New Zealand and all states of Australia (Siddiqi 1986a, 1994; Taylor *et al.* 2005; Taylor *et al.* 2007). Each specific fly/nematode mutualism appears to induce formation of a particular gall form (Davies & Giblin-Davis 2004), usually on a single host plant species. In most cases, particular gall forms are restricted to particular sites of meristematic tissue, e.g., axial or terminal leaf buds, leaf tissue or flower buds. An exception is the case of the fly/nematode association inducing galls on *Syzygium luehmannii*, where small uni- to trilocular galls are induced in a variety of sites, e.g., on stems, expanded leaf blades, axial and terminal buds, and flower buds (K.A. Davies, unpub. data). Similarly, unilocular galls develop on both stems and leaf blades of developing axial leaf buds on *E. gomphocephala, E. leucoxylon*, and *E. microcarpa* (K.A. Davies and G.S. Taylor, unpub. data). Some plant species are hosts for multiple *Fergusonina/Fergusobia* species associations, e.g., *E. camaldulensis*, from which five different gall forms and fly/nematode mutualisms have been collected (Taylor *et al.* 2005).

Australia is regarded as the centre of radiation for the eucalypts (Pryor & Johnson 1981) and the melaleucas (Barlow 1988), with similar radiations of gall-forming insects (Gullan 1984; Taylor 1990; Blanche 1995) that include the *Fergusonina/Fergusobia* association. Thus, this mutualism represents a model system to examine not only the evolutionary relationship between the nematode and fly mutualists, but also their evolutionary history in comparison with the radiation of their myrtaceous plant hosts.

Possible coevolution in the form of cospeciation between *Fergusobia* and *Fergusonina* mutualists was considered by Giblin-Davis *et al.* (2003) and Taylor *et al.* (2005). Strict cospeciation between nematodes and flies would result in congruence between the fly and nematode phylogenies, while host shifting of nematodes among fly species would result in discordant nematode and fly phylogenies. Because nematode transmission between flies appears to be entirely vertical, that is from mother to offspring via the galls, tight cospeciation is predicted. Preliminary data (Giblin-Davis *et al.* 2003) did support the hypothesis that cospeciation between fly and nematode was relatively strict. The hypothesis was further explored in Ye *et al.* (2007b). In addition, nematode and fly sequence data obtained from individual female flies also provides evidence of at least some cospeciation, although a lack of resolution at deeper nodes in both nematode and fly phylogenies prevents a fully informed analysis of the exact patterns (S. Scheffer *et al.*, unpub. data).

The degree of congruence of the speciation of the particular *Fergusonina/Fergusobia* mutualisms and the radiation of the Myrtaceae, particularly the Leptospermoidea, remains unclear. A lack of congruence between the nematode and/or fly phylogenies and the phylogeny of the Myrtaceae would indicate some degree of plant host switching by the fly/nematode mutualism. We know almost certainly that this has occurred and that strict cospeciation between the nematode/flies and their host plants is unlikely; the repeated occurrences of unrelated nematode/fly pairs on the same host plant species argues for some degree of host shifting. However, there is a fair amount of correspondence of major nematode/fly clades with plant generic and subgeneric classification, raising the possibility of strict cospeciation during recent diversification events. Unfortunately, species level phylogenetics of eucalypts and other Myrtaceae is notoriously difficult due to a lack of informative markers at that level as well as frequent local hybridisation between plant species (*e.g.*, see Ladiges 1997).

The WINC contains about 250 collections of *Fergusonina* flies and about 200 collections of *Fergusobia* nematodes, having diverse morphology, and clearly showing that they are speciose genera. These comprise the largest collection of *Fergusobia* in the world, and probably the largest collection of *Fergusonina* flies. These collections are invaluable because they provide correlated data for a large sample of host species, gall types, fly species and nematode species, mostly from Australia. Earlier collections, such as the collection of *Fergusonina* held by the Australian Museum, Sydney, contain considerable material obtained from light traps (K.A. Davies, unpub. data). While such material is valuable in providing insights into the overall variability within the genus and temporal availability in the field, it provides little specific biological information. Known collections of *Fergusobia/Fergusonina* and/or their galls are listed in Table 1.

TABLE 1. Plant host records for the *Fergusonina* fly/*Fergusobia* nematode association on Myrtaceae.

Host	**Location**	**Reference**
Myrtoidea		
Syzygium cumini	India	Harris (1982), Siddiqi (1986)
S. luehmannii	Queensland	Davies unpub.
Leptospermoidea		
Angophora		
A. apocynifolia	Queensland	Colbran (1964), Australian Museum Collection
A. floribunda	New South Wales	Davies unpub.
A. subvelutina	Queensland	Colbran (1964)
Corymbia		
C. abbreviata	Western Australia	Taylor unpub., Davies unpub.
C. citriodora	Queensland	Taylor unpub.
C. intermedia	Queensland	Colbran (1964), Davies unpub.
C. maculata	New South Wales, Queensland	Currie (1937), Tonnoir (1937), Davies unpub.
C. ptychocarpa	Queensland	Taylor & Davies (2008)
C. tessellaris	Queensland	Colbran (1964), Davies unpub.
C. torreliana	Queensland	Davies unpub.
C. trachyphloia	New South Wales	Davies unpub.
Eucalyptus		
(*Eucalyptus*)		
E. acmenioides	Queensland	Colbran (1964), Davies unpub.
E. amygdalina	Victoria, Tasmania	Currie (1937), Davies unpub.
E. coccifera	Tasmania	Scheffer unpub.

..... continued on the next page

TABLE 1 (continued)

Host	Location	Reference
E. diversifolia	South Australia	Davies unpub., Taylor unpub.
E. haemostoma	New South Wales	Davies unpub.
E. macrorhyncha	New South Wales	Australian Museum Collection, Currie (1937), Davies unpub.
E. obliqua	South Australia, Tasmania	Australian Museum Collection, Davies unpub., Taylor unpub.
E. pauciflora	New South Wales, Tasmania	Currie (1937), Davies unpub., Scheffer unpub.
(*Symphyomyrtus*)		
E. aggregata	Australian Capital Territory	Scheffer unpub.
E. albens	New South Wales, Victoria, South Australia	Currie (1937), Tonnoir (1937), Davies unpub.
E. aromaphloia	South Australia	Davies unpub., Taylor unpub.
E. baueriana	Tasmania	Davies unpub.
E. baxteri	Victoria	Davies unpub.
E. blakelyi	Australian Capital Territory	Currie (1937), Davies unpub., Scheffer unpub.
E. brevifolia	Western Australia	Davies unpub., Taylor unpub.
E. camaldulensis	New South Wales, Victoria, South Australia	Currie (1937), Taylor & Davies (2010), Davies unpub., Taylor unpub., Scheffer unpub.
E. capularis	Western Australia	Davies unpub.
E. coolabah	South Australia	Davies unpub., Taylor unpub.
E. confluens	Western Australia	Davies unpub.
E. cosmophylla	South Australia	Taylor unpub., Davies unpub.
E. crebra	Queensland	Currie (1937), Tonnoir (1937)
E. dalrympleiana	South Australia	Davies unpub.
E. dealbata	New South Wales	Davies unpub., Taylor unpub.
E. deglupta	Papua New Guinea, Philippines	Siddiqi (1994)
E. drepanophylla	Queensland	Colbran (1964)
E. fasciculosa	South Australia	Davies unpub., Taylor unpub.
E. gomphocephala	Western Australia	Davies unpub., Currie (1937)
E. interstans	Queensland, South Australia	Davies unpub., Taylor unpub.
E. johnstonii	Tasmania	Davies unpub.
E. leucoxylon	South Australia, Victoria	Davies unpub., Taylor unpub., Davies & Lloyd (1996)

..... continued on the next page

TABLE 1 (continued)

Host	Location	Reference
E. lesouefi	Western Australia	Davies unpub.
E. mannifera	New South Wales	Scheffer unpub.
E. marginata	Western Australia	Davies unpub., Taylor unpub.
E. melanophloia	Australian Capital Territory	Currie (1937), Tonnoir (1937)
E. melliodora	Australian Capital Territory, New South Wales	Currie (1937), Tonnoir (1937), Davies unpub., Scheffer unpub.
E. microcarpa	South Australia, Victoria	Davies unpub., Taylor unpub.
E. odorata	South Australia	Taylor unpub.
E. ovata	Tasmania	Davies unpub.
E. polyanthemos	Australian Capital Territory	Currie (1937), Tonnoir (1937), Scheffer unpub.
E. populnea	Queensland, New South Wales	Colbran (1964), Davies unpub.
E. porosa	South Australia	Davies unpub., Taylor unpub.
E. pruinosa	Western Australia	Davies unpub., Taylor unpub.
E. robusta	New South Wales	Davies unpub.
E. rudis	Western Australia	Currie (1937), Tonnoir (1937), Davies unpub.
E. siderophloia	Queensland	Davies unpub., Taylor unpub.
E. sideroxylon	Australian Capital Territory, New South Wales	Currie (1937)
E. tereticornis	Queensland, New South Wales, Victoria	Currie (1937), Tonnoir (1937), Davies unpub.
E. viminalis	South Australia, Victoria, New South Wales	Davies unpub., Taylor unpub.
Leptospermum		
Leptospermum sp.	Victoria	Robin Adair pers. com.
Melaleuca		
M. argentea	Northern Territory	Taylor (2004)
M. armillaris	New South Wales	Taylor (2004)
M. cajuputi	Queensland	Davies & Giblin-Davies (2004), Taylor (2004)
M. dealbata	Queensland	Davies & Giblin-Davies (2004), Taylor (2004)
M. decora	Queensland	Davies unpub.
M. fluviatilis	Queensland	Davies & Giblin-Davies (2004), Taylor (2004)
M. leucadendra	Queensland, Western Australia	Davies & Giblin-Davies (2004), Taylor (2004)
M. linariifolia	New South Wales	Davies unpub.

..... *continued on the next page*

TABLE 1 (continued)

Host	Location	Reference
M. nervosa	Queensland, Western Australia	Davies & Giblin-Davies (2004), Taylor (2004)
M. nodosa	New South Wales	Davies unpub.
M. quinquinervia	New South Wales, Queensland	Davies & Giblin-Davies (2004), Taylor (2004)
M. stenostachya	Queensland	Davies & Giblin-Davies (2004), Taylor (2004)
M. viridiflora	Queensland	Davies & Giblin-Davies (2004), Taylor (2004)
Metrosideros		
Mt. excelsa	New Zealand	Taylor *et al.* (2007)

Material collected by Davies (unpub.), Taylor (unpub.) and Scheffer (unpub.) is deposited in the WINC.

This paper presents a summary of ecological, morphological, and taxonomic data currently known for *Fergusobia* from research in the 1920's and 1930's (Currie 1937) and from intensive work during the past 15 years by more than a dozen researchers. The data presented here include new information on gall forms, plant hosts, nematode morphology, larval morphology of associated fly species, as well as analyses of DNA sequence datasets expanded to include samples from recent collections. In some cases, e.g., *F. pohutakawa* Davies 2010 and *F. magna* Siddiqi 1984, morphology alone is diagnostic. However, morphological conservation among nematode species is notorious, and cryptic species within *Fergusobia* and other groups are common (Powers 2004; Ye *et al.* 2007b). These *Fergusobia* species can be separated only by using additional characters such as plant host species, gall form, fly host species, and DNA sequence data. Many *Fergusobia* remain undescribed due to difficulties in obtaining all life stages needed for full descriptions.

The work presented here is incomplete, and continued sampling will no doubt reveal many new forms and associations of the *Fergusobia/Fergusonina* mutualism. However, given the sampling efforts already made and ongoing, and the many undescribed species of nematodes and flies now available, it seems timely to summarize what is now known about the mutualism. The taxonomic status of the genus *Fergusobia* is summarized, and the diagnosis of the genus is emended. General descriptions are provided for representative species of *Fergusobia* nematodes (Figs 1–61), and of the dorsal shield of their associated third-stage *Fergusonina* fly larvae (Figs 62–68) from each of 20 clades elucidated by molecular sequencing and phylogenetic analysis of *Fergusobia* (Ye *et al*. 2007b). Scanning electron micrographs of lateral fields (Figs 67–72) and heads (Figs 73–76) are presented for some forms. Phylogenetic trees for *Fergusobia* inferred from DNA sequence data are shown in Figs 77–79. Gall forms are illustrated in Figs 80–88. A key to species and morphospecies (defined as a group of nematodes having a similar morphological character or set of characters suggesting that it constitutes a species, a proposition requiring testing with additional molecular, biological, etc data) of *Fergusobia*, excluding those from the broad-leaved *Melaleuca leucadendra* complex (see key in Davies & Giblin-Davis 2004), is provided. It is hoped that this paper will establish a useful foundation for future work. Formal descriptions of *Fergusobia* species will be published separately.

Taxonomic status of the genus *Fergusobia* Currie 1937

The name *Fergusobia* was first proposed as a subgenus of the genus *Anguillulina* by Currie in 1937 and raised to generic rank by Christie in 1941. Siddiqi (1986a) reviewed the taxonomic status of the genus, and Davies & Giblin-Davis (2004) provided additional notes.

A chronological history of the genus follows immediately, and a summary of nomenclatorial changes for the genus together with gall type, host plant and species of associated *Fergusonina* fly is presented in Table 4.

Chronological history of the genus Fergusobia

1937 Currie erected *Fergusobia* as a subgenus of *Anguillulina* (described *Anguillulina (Fergusobia) tumifaciens* Currie from 'leafy' leaf bud galls on *E. Stuartiana* (now *E. bridgesiana*), associated with *Fergusonina (Fn.) carteri* Tonn.

1938 Johnston considered *Anguillulina (Fergusobia) tumifaciens* was homonymous with *Anguillina* (now *Anguina*) *tumefaciens* (Cobb 1932) T. Goodey 1933. He proposed replacing the name *A. tumifaciens* with *curriei*.

1941 Christie recognised *Fergusobia* as a genus. He proposed *Fergusobia curriei* (Currie 1937) n. comb. He thus attributed the species name to Currie rather than Johnston.

1955 Wachek proposed *Fergusobia tumifaciens*, recognising the species name given by Currie (1937).

1964 Colbran reported several collections of *Fergusobia*, from various galls and host plants, and placed them all as *F. tumifaciens*.

1968 Fisher and Nickle considered Wachek (1955) in error, that *tumifaciens* was homonymous with *tumefaciens*, and that *F. curriei* was the valid name. They described a nematode from flower bud galls on *E. camaldulensis*, associated with *Fn. tillyardi* Tonnoir, which they believed was actually a re-description of the nematode described by Currie (1937). However, host species and gall types differed.

1986a Siddiqi recognised *F. tumifaciens*, pointing out that the species name was neither a homonym of *A. tumefaciens* (different spelling) nor congeneric with it. Siddiqi re-described *F. tumifaciens*, using the published descriptions of Currie (1937), Fisher & Nickle (1968) and nematodes from galls on *E. camaldulensis* sent to him by Fisher (J.M. Fisher, personal communication). Siddiqi called all these nematodes *F. tumifaciens*, i.e., he effectively synonymised *F. tumifaciens* and *F. curriei*. He also described *F. magna* from 'twig' galls on *Eucalyptus* sp., and *F. jambophila* from flower bud galls on *Syzygium cumini*, associated with *Fn. syzygii* Harris 1982.

1986b Siddiqi transferred *Boleodorus indicus* Jairajpuri, 1962 to *Fergusobia* (until 1986a, the genus *Fergusobia* was monospecific).

1994 Siddiqi described *F. brevicauda* and *F. philippinensis* from flower bud galls on *E. deglupta* from Papua New Guinea and The Philippines, respectively.

1996 Davies and Lloyd described *F. fisheri* from flat leaf galls on *E. leucoxylon*, now known to be associated with an undescribed species of *Fergusonina* (G. S. Taylor, unpub. data).

2004 Davies and Giblin-Davis described *F. quinquenerviae, F. cajuputiae, F. dealbatae, F. leucadendrae, F. nervosae,* and *F. viridiflorae* from shoot bud galls on species in the *Melaleuca leucadendra* broad-leaved paperbark species complex, associated respectively with *Fn. turneri* Taylor, *Fn. purcelli* Taylor, *Fn. makinsoni* Taylor, *Fn. centeri* Taylor, *Fn. goolsbyi* Taylor, and *Fn. burrowsi* Taylor.

2007 Davies (in Taylor *et al*. 2007) described *F. pohutukawa* from unilocular basal stem galls on *Metrosideros excelsa*, associated with *Fn. metrosiderosi* Taylor.

2008 Davies (in Taylor & Davies 2008) described *F. ptychocarpae* from flower bud galls on *C. ptychocarpa*, associated with *Fn. giblindavisi* Taylor.

2010 Re-description of *F. magna* Siddiqi from axial bud 'stem' galls on *C. tessellaris*, associated with an undescribed species of *Fergusonina* (Davies *et al*. 2010).

Comments on the taxonomic status of the genus Fergusobia. The type specimens of *F. tumifaciens* prepared by Currie appear to have been lost. Repeated attempts to re-collect this nematode from its host *E. bridgesiana*, formerly *E. stuartiana*, from near Canberra, have been unsuccessful (K.A. Davies, unpub. data), although galls have been found. It is most likely that the nematodes described by Currie (1937), Fisher &

Nickle (1968) and Siddiqi (1986a) (including material sent to him from axial bud 'stem' galls [J.M. Fisher, personal communication]), were actually three distinct species. The respective nematodes were associated with three different species of *Fergusonina*, from three different gall forms, and from two species of *Eucalyptus* that are not closely related. Disparate DNA sequences of nematodes, respectively from flower bud galls and axial bud 'stem' galls on *E. camaldulensis,* confirm that these nematodes are separate species (Fig. 78). The nematodes from the 'stem' galls (here Morphospecies 10) will be described separately as a new species. Thus, the nematode described by Fisher & Nickle (1968) from flower bud galls from *E. camaldulensis* should be regarded as a valid species, and recognised as *Fergusobia curriei*. It should be noted from the sequence data that there is considerable variation at both the D2/D3 and COI loci for *F. curriei* which may suggest that there is more than one cryptic species.

Problems with the current nomenclature of species of *Fergusobia* have arisen because of a lack of understanding of the biology of the fly/nematode association, in particular, host plant specificity (see Currie 1937). In general, there is considerable similarity in the morphology of many species of *Fergusobia,* and hence it is not surprising that species were wrongly synonymised (Siddiqi 1986a) or mistakenly recorded as '*tumefaciens*' (Colbran 1964). In addition, Currie (1937) recorded several species of *Fergusonina* from more than one species of *Eucalyptus*, on the basis of the form of the dorsal shield of the fly larvae. While the morphology of the dorsal shield of flies is a useful taxonomic tool, some species have similar shields (G.S. Taylor, unpub. data) and it is possible that Currie was dealing with separate species from the different host plant species (Davies & Giblin-Davis 2004). Thus, historically, there has been a tendency to 'lump' potentially new species of *Fergusobia* into those previously described.

Materials and methods

Preparation and measurement of specimens

Fly larvae were collected from dissected galls. Preparation and examination of specimens was described in Taylor (2004).

Nematodes were collected, fixed and processed for mounting on glass slides as described in Davies & Giblin-Davis (2004). Measurements are given in µm. Drawings and measurements were made from material mounted in glycerol using a camera lucida. Body width was measured at mid-length. Body length was measured along the mid-line. Here, *Fergusobia* nematodes less than 350 µm long were described as small in size, 350–450 µm as medium, and greater than 450 µm long as large. Spicules were measured along the mid-line in lateral view. Percentage of the body length occupied by the bursa is the proportion of the body length measured from the tail tip forwards to the point at which the bursa arose, multiplied by 100.

For scanning electron microscopy (SEM), nematodes fixed in formalin were washed in three changes of water purified by reverse osmosis (RO). They were then immersed in a 0.05% solution of Tween 20, and sonicated for 60 seconds, using setting 4 on a GS UP 50 H sonication probe. The detergent was removed from the nematodes by three washes in filtered RO water, and they were post-fixed and stained with 2% aqueous osmium tetroxide. After an hour, the nematodes were washed three times in filtered RO water. They were dehydrated through an ethanol series, with 20–30 min. in each stage (30, 70, 80, 90, 95% ethanol, and then two changes of 100% ethanol). A 1:1 solution of absolute ethanol and hexamethyldisilazane (HMDS) was added, and left for 30 min (Heegaard *et al.* 1986). It was replaced with 100% HMDS, which was allowed to evaporate slowly overnight in a fume cupboard. For mounting of the dry nematodes on a stub, a piece of human hair was placed on a sticky disc on a stub. Nematodes were placed along the hair and perpendicular to it. Stubs were coated with 3 nm of platinum, and viewed using a Philips XL30 Field Emission scanning electron microscope.

Collections of nematodes from which molecular sequences were made were videotaped at the Hubbard Center for Genome Studies, University of New Hampshire, Durham, NH, USA. Images were made using an inverted Olympus microscope connected to a Retiga-1300 digital camera, and IP Lab software. Videotapes of

specimens of *Fergusobia* species can be found at the database for the nematode branch of Assembling the Tree of Life (NemATOL http://nematol.unh.edu), in the UNH HCGS. Videotape numbers for particular species of *Fergusobia* are given in Table 2, together with plant host, locality and gall data for the various voucher numbers shown in the phylogenetic trees, and the voucher numbers for the WINC.

TABLE 2. Information on collections of *Fergusobia* held in the WINC, and sample data for voucher numbers.

Host	*Fergusobia* sp./ morphospecies number	Locality	Gall type	Clade no. from D2/ D3 analysis	Voucher no.	NemaTOL ID	**WINC**
A. floribunda	1	Merriwa, NSW	TLG	12	346	022305KD10-11, 022405KD1	004789
? Angophora sp.	2	Pimpama, QLD	TLG	12	339	030205KD15-17	004374
C. ptychocarpa	*ptychocarpae*	Brisbane, QLD	FBG	11	30	022505KD6-8	004794
C. ptychocarpa	*ptychocarpae*	Brisbane, QLD	FBG	11	52		
C. ptychocarpa	*ptychocarpae*	Cairns, QLD	FBG	11	450		
C. tessellaris	*magna*	Brisbane, QLD	SBG	1	42		004335
C. tessellaris	*magna*	Cardwell, QLD	SBG	1	24, 444	022505KD17	004351
C. tessellaris	*magna*	Ellis Beach, QLD	SBG	1	327	022505KD18	063699
C. tessellaris	*magna*	Hervey Bay, QLD	SBG	1	353		063836
C. tessellaris	*magna*	Cairns, QLD	SBG	1	452		004354
C. tessellaris	*magna*	Mission Beach, QLD	SBG	1	465		004354
Corymbia sp.	3	Woodburn, NSW	PG	14	39	022505KD9-11	004336
E. amygdalina		near Gordon, TAS	TLG	6	276	022405KD2-4	004315
E. camaldulensis	*brittenae*	Kellerberrin, WA	TLG	3	62	021705KD11-12, 030705KD12-13	004286
E. camaldulensis	*brittenae*	Hall's Creek, WA	TLG	3	205	021705KD3-4	004721
E. camaldulensis	*brittenae*	Brachina Gorge, SA	TLG	3	292	021505KD1	004391
E. camaldulensis	*brittenae*	Marla, SA	TLG	3	59		004390
E. camaldulensis	*brittenae*	Goolwa, SA	TLG	3	6, 55		004340
E. camaldulensis	*brittenae*	Bunyeroo Gorge, SA	TLG	3	56, 201		004322
E. camaldulensis	*curriei*	Urrbrae, SA	FBG	4	3		
E. camaldulensis	*curriei*	Verdun, SA	FBG	4	206	022305KD1-2	004309
E. camaldulensis	*curriei*	Adelaide, SA	FBG	4	5		004792
E. camaldulensis	10	Adelaide, SA	SBG	15	54, 311	030305KD10-12, 022405KD11-13	004749, 026047
E. cladocalyx		Meningie, SA	TLG		739		063707
E. cladocalyx		Meningie, SA	PG		740		063704
E. cosmophylla		Mylor, SA	TLG		282	022005KD2-4	004305
E. delegatensis	7	Ben Lomond, TAS	TLG	6	281	022405KD7-9	004319
E. diversifolia	8	Meningie, SA	TLG	6	7, 284	022505KD12-14	004337
E. eugenioides	5	Canberra, ACT	FBG	13	349	022305KD7-9	004312
E. fasciculosa	15	near Strathalbyn, SA	STG		65	012605KD 4-6	004498
E. fibrosa	14	Shute Bay, QLD	FBG	5	330	022505KD1-3	004328
E. gomphocephala	9	Cervantes, WA	PG	7	63	021705KD5-7, 030705KD7-9	004281, 004598
E. leucoxylon	*fisheri*	Meningie, SA	FLG	13	8		063709
E. leucoxylon	16	Adelaide, SA	PG	7	68	021605KD14, 021705KD1-2	004509

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TABLE 2 (continued)

Host	*Fergusobia* sp./ morphospecies number	Locality	Gall type	Clade no. from D2/ D3 analysis	Voucher no.	NemaTOL ID	**WINC**
E. leucoxylon	16	Adelaide, SA	PG	7	314		004773
E. leucoxylon	16	SA	PG	8	71	021605KD7-9	026020
E. leucoxylon	17	Adelaide, SA	ABPG		741		063709
E. macrorhyncha	4	Clare, SA	FBG		4	021505KD4-6, 030705KD1-3	004516
E. macrorhyncha	4	Canberra, ACT	FBG		350	022305KD3-4	004310
E. marginata		Busselton, WA	PG	14	64	021705KD13-14, 030705KD10-11	004717
E. microcarpa		near Strathalbyn, SA	PG	5	70	021605KD10-11	004502
E. microcarpa		Adelaide, SA	FBG	5	1	021705KD15-16	004290, 004878
E. microcarpa	12	near Strathalbyn, SA	FLG	13	67	021505KD10-12	004853
E. obliqua		Myponga, SA	FBG	6	2	021505KD2-3	004515
E. obliqua		Bridgewater, SA	FBG	6	315		026062
E. pauciflora		Bridport, TAS	PG	14	280	022405KD5-6	004317
E. planchoniana	6	Stradbroke Island, QLD	SBG	8	275	021505KD7-9, 030705KD4-6	004263, 004554
E. porosa		near Strathalbyn, SA	FLG		66	021605KD1-3	004270
E. porosa	13	near Strathalbyn, SA	SBG		69	021605KD12-13	004276
E. racemosa		Stradbroke Island, QLD	TLG	6	41		
E. racemosa		Stradbroke Island, QLD	TLG		337	022505KD4-5	004331
E. siderophloia		Brisbane, QLD	FLG	13	25, 26, 27	021805KD4-5	004895, 004898, 004294, 003400
E. siderophloia		Brisbane, QLD	FLG	13	28	021805KD1, 022005KD1	004746
E. tereticornis		Sydney, NSW	TLG	3	401		004362
E. tereticornis		Townsville, QLD	TLG	3	438		004367
E. tereticornis		Tully, QLD	TLG	3	446		004367
E. tereticornis		Brisbane, QLD	PG	9	34	030705KD14	004770, 004308
E. viminalis		Scott Creek, SA	'leafy' SBG		273	030305KD1-2	004368, 004369
E. viminalis		Mt. Crackenback, NSW	'leafy' SBG		347		
Eucalyptus near *acmenioides*		Woodburn, NSW	TLG	12	340	030305KD7-9	004361
Eucalyptus near *nitida*		Zeehan, TAS	SBG	14	286	022405KD10	004728
Eucalyptus sp.		Brisbane, QLD	FLG	6	33	021805KD13-15	004745
Eucalyptus sp.		Woodburn, NSW	TLG	6	36	021805KD6-8	004738
Eucalyptus sp.		Woodburn, NSW	ABG	11	37	021805KD9-11	004298
Eucalyptus sp.		Brisbane, QLD	FLG	13	29	030305KD5-6	004446

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TABLE 2 (continued)

Host	*Fergusobia* sp./ morphospecies number	Locality	Gall type	Clade no. from D2/ D3 analysis	Voucher no.	NemaTOL ID	**WINC**
Eucalyptus sp.		Lake Tinnaroo, QLD	FLG	11	328	022405KD14-15	004325
Eucalyptus sp.		Woodburn, NSW	FLG	11	38		
Eucalyptus sp.		Brisbane, QLD	PG	7	31, 32	021805KD12, 021805KD2-3	004735, 004743
Eucalyptus sp.		Canberra, ACT	PG	13	351	022305KD5-6	003410, 004311
Eucalyptus sp.		Canberra, ACT	PG	13	348		004791
M. argentea		NT	SBG	16	469	030305KD3-4	004377
M. armillaris	18	Kuringai Chase, NSW	ULPG	2	405		004345–7
M. armillaris	18	Tea Gardens/Hawk's Nest, NSW	ULPG	2	410		
M. cajuputi	*cajuputiae*	Daintree, QLD	SBG	16	17, 46		
M. dealbata	*dealbatae*	Cairns, QLD	SBG	16	18	030105KD10-12	022291
M. dealbata	*dealbatae*	Cardwell, QLD	SBG	16	19		004089
M. dealbata	*dealbatae*	Hervey Bay, QLD	SBG	16	352		
M. dealbata	*dealbatae*	Mon Repos, QLD	SBG	16	331		
M. decora		Ingham, QLD	ULPG		462		063692
M. fluviatilis	*quinquenerviae*	Home Hill, QLD	SBG	16	22	030205KD14	022244
M. fluviatilis	*quinquenerviae*	Townsville, QLD	SBG	16	23	030205KD12-13	022252, 022248
M. leucadendra	*leucadendrae*	Cardwell, QLD	SBG	16	461		004352
M. leucadendra	*leucadendrae*	Cairns, QLD	SBG	16	451		004349
M. linariifolia	19	Pt. Macquarie, NSW	ULPG	2	412		004348
M. nervosa	*nervosae*	Mareeba, QLD	SBG	16	49	030205KD6-8	004359
M. nervosa		Mareeba, QLD	PG	17	47	030205KD9	022287
M. nodosa		Tea Gardens/Hawk's Nest, NSW		17	411		
M. quinquenervia	*quinquenerviae*	Maryborough, QLD	SBG	16	12		
M. quinquenervia	*quinquenerviae*	Arrawarra, NSW	SBG	16	341, 414, 415, 416, 418, 419		
M. quinquenervia	*quinquenerviae*	Port Macquarie, NSW	SBG	16	342	022805KD1-2	021994
M. quinquenervia	*quinquenerviae*	Woolgoolga, NSW	SBG	16	420, 421, 422, 423, 424, 426, 427		063735
M. quinquenervia	*quinquenerviae*	Boonoroo, QLD	SBG	16	332		022000
M. quinquenervia	*quinquenerviae*	Boondall Wetlands, QLD	SBG	16	336		022017
M. quinquenervia	*quinquenerviae*	Mudjimba, QLD	SBG	16	334		022006
M. quinquenervia	*quinquenerviae*	Rainbow Beach, QLD	SBG	16	333	030105KD4-5	021979
M. quinquenervia	*quinquenerviae*	Bateau Bay, NSW	SBG	16	343		021997

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TABLE 2 (continued)

Host	*Fergusobia* sp./ morphospecies number	Locality	Gall type	Clade no. from D2/ D3 analysis	Voucher no.	NemaTOL ID	**WINC**
M. quinquenervia	*quinquenerviae*	Eleebana, NSW	SBG	16	406, 407, 408		
M. quinquenervia	*quinquenerviae*	Stradbroke Island, QLD	SBG	16	13, 51, 338		022015
M. quinquenervia	*quinquenerviae*	Pottsville, NSW	SBG	16	335		022029
M. quinquenervia	*quinquenerviae*	Kingscliff, NSW	SBG	16	431, 432, 433		
M. quinquenervia	*quinquenerviae*	Lennox Heads, NSW	SBG	16	428, 429, 430		
M. quinquenervia	*quinquenerviae*	Gainesville, Florida, USA	SBG	16	455, 568, 572		
M. quinquenervia		Yellow Water Holes, QLD	RBG	18	357, 358		063819
M. stenostachya		near Mareeba, QLD	SBG	16	48	030205KD4-5	022282
M. viridiflora	*viridiflorae*	Mackay, QLD	SBG	16	21	030205KD10-11	004360
Metrosideros excelsa	*pohutukawa*	New Zealand	SBG	10	359	022505KD15-16, 030305KD13	004343
Syzygium luehmannii		Tolga, QLD	PG	10	735, 470		063694

Information on host classification and distribution is summarised in Table 3. For *Corymbia* and *Eucalyptus*, this information was taken from Brooker & Kleinig (1999, 2001, 2004); for *Angophora*, from Chippendale (1988); for *Syzygium*, from Harrington & Gadek (2004); for *Melaleuca*, from Holliday (2004) and Craven & Lepschi (1999); and for *Metrosideros* from Wright *et al.* (2000). To avoid repetition, the information is not referenced in the following text.

Molecular analyses

For later amplification and DNA analyses, nematodes collected from galls were stored in 100% ethanol on ice. The DNA samples were prepared as described in Ye *et al.* (2007a, b). The D2/D3 expansion segment of the large subunit (D2/D3 LSU) and near full length fragment of the small subunit (SSU) ribosomal DNA, and a fragment of the mitochondrial cytochrome oxidase gene (COI) were amplified and sequenced using the methods in Ye *et al*. (2007a, b).

Sequences were deposited into the GenBank database under the accession numbers listed in Figures 77, 78 and 79. Some species used in these figures are from our various other sequencing projects (Ye *et al*. 2007a, b). Additional species were obtained from GenBank (associated accession numbers are listed in Figs 77, 78 and 79) for phylogenetic analysis (Perlman *et al*. 2003; Subbotin *et al*. 2006). DNA sequences were aligned by ClustalW (http://workbench.sdsc.edu, Bioinformatics and Computational Biology group, Dept. Bioengineering, UC San Diego, CA). The model of base substitution was evaluated using MODELTEST (Posada & Crandall 1998). The Akaike-supported model, the base frequency, the proportion of invariable sites, and the gamma distribution shape parameters and substitution rates in the Akaike information criterion (AIC) were used in phylogenetic analyses. Bayesian analysis was performed to confirm the tree topology for each gene separately using MrBayes 3.1.0 (Huelsenbeck & Ronquist 2001) running the chain for 1,000,000 generations and setting the "burn-in" at 1,000. We used MCMC (Markov Chain Monte Carlo) methods within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees (Larget & Simon 1999) using 50% majority-rule. The maximum parsimony (MP) method was performed using the heuristic search with stepwise- addition options to determine the most parsimonious tree. Neighbor-joining (NJ) analysis (Saitou & Nei, 1987) was conducted using the HKY85 (Hasagawa *et al*. 1985) distance option. Sites with missing data

or gaps were treated as missing characters for all analyses. The robustness of the parsimony and NJ trees was tested using the bootstrap method (Felsenstein 1985). All bootstrap values are based on 1,000 replicates.

Abbreviations

ABG, axial bud gall; ABPG, axial bud pea gall; ACT, Australian Capital Territory; ANIC, Australian National Insect Collection, Canberra; AS, abdominal segment (nos); FBG, flower bud gall; FL, Florida; FLG, flat leaf gall; GST, Gary Taylor; JRM, Jeff Makinson; KAD, Kerrie Davies; LBG, leaf bud gall; PNG, New Guinea; NR, nerve ring; NSW, New South Wales; NZ, New Zealand; PG, pea gall; QLD, Queensland; RBG, rosette bud gall; RGD, Robin Giblin-Davis; SA, South Australia; SBG, shoot bud gall; SEM, scanning electron microscopy; STG, stylet gall; TAS, Tasmania; TLG, terminal leaf gall; TS, thoracic segment (nos); VIC, Victoria; WA, Western Australia; WINC, Waite Insect and Nematode Collection, Adelaide; WNC, Waite Nematode Collection. Other abbreviations refer to Demanian indices (a, b', c and V).

Results

Emendation of the genus *Fergusobia*

Following morphological examination of new collections in the WINC, the diagnosis of *Fergusobia* presented by Siddiqi (1986a) has been emended. It includes new information on measurements, structure of the cephalic region and lateral fields, form of the male spicules and bursa, and information on the biology of *Fergusobia*.

Genus *Fergusobia*

Anguillulina (*Fergusobia*) Currie, 1937
Fergusobia (Currie, 1937) Christie, 1941

Diagnosis (emended from Siddiqi 1986a): Nematodes that, in a mutualism with *Fergusonina* flies, induce galls on Myrtaceae; gall forms specific to particular fly/nematode associations, and host species specific; two overlapping generations, one parasitic in plant galls on Myrtaceae, the other entomoparasitic in the haemolymph of female flies.

Plant parasitic forms:

1. Parthenogenetic female. Occur in plant galls. Develop from juveniles deposited by adult fly during her oviposition; these apparently induce development of a new plant gall via feeding. Semi-obese, usually dorsally curved when heat-killed but straight in some species. Less than 1 mm long (*ca* 250–700 µm long). Body begins to swell immediately posterior to cephalic region. Cuticle with obscure annules, variously marked with transverse striae, appears to be longitudinally striated when viewed with light microscope. Lateral fields usually obscure when viewed with light microscope, may be absent. With SEM, in most species examined the lateral field comprised diagonal striae (Figs 69–72), but may have three lines (two ridges). Cephalic region may or may not be offset, unstriated; circum-oral area flat, raised or depressed, with 6 or 8 apparent 'sectors' (Figs 73–76). Stylet knobbed, 5–20 µm long. Opening of dorsal oesophageal gland 2–3 µm behind stylet knobs. Oesophagus with fusiform, non-muscular, glandular 'corpus', with pseudovalves. Position of oesophagointestinal junction unclear. Nerve ring just in front of dorsal oesophageal gland. Dorsal oesophageal gland small in some species, large to enormously developed in most (b'= 1.4–8.8), usually with large nucleus with very large nucleolus, and an extension into the 'corpus'; may have anterior diverticulum, posterior reflex or additional lobe. Secretory/excretory pore opens behind nerve ring, often opposite the nucleus of the dorsal oesophageal gland. Hemizonid at or in front of pore opening. Reproductive tract with a single gonad, prodel-

phic, may or may not be reflexed, may extend to nerve ring. Vulva a transverse slit (Fig. 5), at *ca* 75–90% of body length; may be depressed, flat or may have protuberant lips. Vagina anteriorly directed. No post-vulval sac. Uterus extensile in some species; with a quadricolumella; may contain one, two to three or many eggs, and in some species contains juveniles. Oocytes in single or multiple rows. Rectum non-muscular; anus pore-like. Tail conoid, short rounded or longer and more slender. In plant gall, parthenogenetic female deposits eggs that develop into amphimictic generation of nematodes. Juveniles generally resembling female, but smaller.

2. Male. Occurs in plant gall. Semi-obese, usually more or less J-shaped when heat-killed, but may be straight, arcuate or dorsally curved (C-shaped). Usually slightly larger than parthenogenetic female of same species (*ca* 200–600 µm long). Body begins to swell immediately posterior to cephalic region. Cephalic region, cuticle, lateral lines, stylet, oesophagus, nerve ring and secretory/excretory pore as in parthenogenetic female. Dorsal oesophageal gland opening 1–3 µm behind stylet knobs. Dorsal oesophageal gland small to enormously developed, but usually smaller than in parthenogenetic female (b'=1.8–11.6). Reproductive tract with single testis, variable in length, may extend anteriorly past nerve ring, may be reflexed. Testis, seminal vesicle and vas deferens may or may not be clearly differentiated. Spermatocytes in multiple rows, sperm amoeboid. Bursa membranous, smooth or crenate, usually peloderan but may be leptoderan, varying in length and arising near cephalic region in some species, at mid-body length in others, and just anterior to the cloaca in some. Spicules slender to robust, *ca* 10–40 µm long, usually angular but may be arcuate, with a large, more or less offset manubrium and rounded tip with terminal or sub-terminal opening. No gubernaculum observed. Tail usually ventrally curved, often with ventral side concave, but may be straight and conoid; tip usually rounded. No genital papillae. Present in plant galls before infective females appear; occasionally found with infective female nematodes inside mature fly larvae, but never in adult female flies.

3. Infective stage, pre-parasitic female. Occurs in plant gall. May be similar in size but usually slightly larger and slimmer than parthenogenetic female, i.e., with a smaller 'a' ratio; moves more actively than parthenogenetic females or males; *ca* 220–650 µm long. Straight, arcuate, dorsally curved or J-shaped when heat-killed. Cephalic region may or may not be off-set; cephalic framework weak; circum-oral area usually flat. Stylet knobs smaller and less robust than in parthenogenetic females and males. Opening to dorsal oesophageal gland 1–3 µm behind stylet knobs. Prominent nuclei present in epidermis and intestinal wall, particularly noticeable in body behind vulva. Oesophagus as in parthenogenetic females and males. Dorsal oesophageal gland small to large (b'=1.7–13.6). Secretory/excretory pore behind nerve ring, often obscure. Reproductive tract with single gonad, prodelphic. Vulva a transverse slit at 60–85% of body length; may be surrounded by a cuticular plate, be depressed, or have small lips. No post-vulval sac, but uterus may bulge towards tail in inseminated females. Tract hypertrophied to varying degrees, usually reflexed in region of oesophagus. Vagina usually perpendicular to body axis, but may be directed anteriorly. Uterus apparently extensile, may reach oesophageal region, holds large numbers of sperm. Tail short to medium with tip bluntly rounded or almost hemispherical, or rarely longer and conoid. Only found within galls for a relatively short time, with third instar fly larvae. Following insemination, penetrate into haemolymph of mature female fly larva or pupa, where cuticle is ecdysed, stylet is lost and intestine degenerates.

4. Entomoparasitic female. Occurs in haemolymph in abdomen of adult female *Fergusonina* fly. Larger than other stages (*ca* 300–1400 µm long); non-motile. Straight, or slightly dorsally curved when heat-killed; stout. With TEM, seen to be covered with thickened epidermis with thousands of microvilli, through which food is apparently absorbed directly from fly haemolymph. No stylet; oesophagus, intestine and rectum degenerate. Reproductive tract single, greatly hypertrophied, reflexed to coil along body length. Vulva a transverse slit at 70–90% body length. Vagina anteriorly directed or at right angles to body length. Oocytes in multiple rows around a rachis. Eggs deposited in haemolymph of fly; hatch as J2's and move to fly oviduct. Some J2's deposited with fly eggs by fly into fresh host tissue during oviposition by fly; nematodes apparently induce development of a new plant gall via feeding.

TABLE 3. Myrtaceous hosts from which *Fergusobia* species were sequenced: their taxonomy and relationships, and distributions.

Genus	Subgenus	Species	Section	Series/species complex	Distribution
Angophora		*floribunda*			QLD, NSW
Corymbia	*Blakella*	*tessellaris*	*Extensae*		Widespread in eastern QLD, far north-east NSW, south-west PNG
	Corymbia	*ptychocarpa*	*Septentrionales*	*Dorsiventrales*	Scattered, Kimberley Region of WA, east to north-west QLD and down east coast to Cairns
Eucalyptus	*Eucalyptus*	*acmenioides*	*Amentum*		Coastal eastern Australia, Sydney, NSW to Cooktown, QLD
		amygdalina	*Aromatica*	*Insulanae*	TAS
		delegatensis	*Cineraceae*	*Fraxinales*	Sub-alpine NSW and VIC, TAS
		diversifolia	*Longistylus*	*Diversiformae*	Coastal southern Australia
		eugenioides	*Capillulus*	*Pachyphloius*	Eastern NSW north to south-eastern QLD
		macrorhyncha	*Capillulus*	*Pachyphloius*	Great Dividing Range of NSW and VIC, NSW tablelands, near Clare in SA
		marginata	*Eucalyptus*		South-west of WA
		obliqua	*Eucalyptus*	*Eucalyptus*	South-eastern Australia and TAS
		planchoniana	*Insolitae*		NSW north coast and south-east QLD
		pauciflora	*Cineraceae*	*Pauciflorae*	Alpine NSW and VIC, TAS
		racemosa	*Cineraceae*	*Psathroxylon*	Coastal region and hinterland, from Cape Jervis NSW to south eastern QLD
Eucalyptus	*Symphyomyrtus*	*camaldulensis*	*Exsertaria*	*Rostratae*	Along rivers creeks and valleys in mainland Australia, except northern QLD
		cosmophylla	*Incognita*		South-eastern SA
		fasciculosa	*Adnataria*	*Heterophloiae*	South-eastern SA, western VIC
		fibrosa	*Adnataria*	*Siderophloiae*	Coastal, from Bodalla NSW to Rockhampton, QLD; and west to Narrabri, NSW
		gomphocephala	*Bolites*		Coastal south-western WA
		leucoxylon	*Adnataria*	*Melliodorae*	South-eastern SA, western VIC
		microcarpa	*Adnataria*	*Buxeales*	Western NSW, southern QLD, VIC, south-eastern SA
		porosa	*Adnataria*	*Buxeales*	SA, north-west VIC, western and south-western NSW
		siderophloia	*Adnataria*	*Siderophloiae*	Coastal regions and hinterland, from Sydney, NSW to Bundaberg, QLD
		tereticornis	*Exsertaria*	*Erythroxylon*	Coastal, from Bateman's Bay, NSW to PNG
		viminalis	*Maidenaria*	*Viminales*	Wet forests of NSW, VIC and TAS
Melaleuca		*argentea*		*leucadendra*	Kimberley Region of WA east to northern QLD
		armillaris			Coastal, from northern NSW to south eastern VIC and TAS
		cajuputi		*leucadendra*	North-west WA, northern NT, PNG, Indonesia
		dealbata		*leucadendra*	North-west WA, northern NT, Cape Yorke to Bundaberg in QLD, PNG
		fluviatilis		*leucadendra*	Rockhampton district to Cape Yorke, QLD
		leucadendra		*leucadendra*	Tropical north of Australia, Indonesia, PNG
		linariifolia			Coastal, from southern NSW to Gladstone in QLD
		nervosa		*leucadendra*	From Kimberley Region of WA, east to north-eastern QLD
		nodosa			Coastal, from southern QLD to Sydney in NSW, and inland to the tablelands
		quinquenervia		*leucadendra*	Coastal, from Sydney, NSW to Cape York, QLD; PNG, Indonesia and New Caledonia
		stenostachya		*leucadendra*	Borroloola district of NT, east to Cape York, QLD
		viridiflora		*leucadendra*	Dampier Peninsular, WA, east to Maryborough district, QLD
Metrosideros		*excelsa*			North Island of NZ and off-shore islands
Syzygium		*luehmannii*			Coastal rainforests in NSW and QLD

TABLE 4. Historical changes in nomenclature for the genus *Fergusobia* (syn. *Anguillulina*), and gall type, host plant and host fly for each described species.

Genus	Sub-genus	Species	Gall type	Host plant	Host fly	Reference
Anguillulina	*Fergusobia*	*tumifaciens*	LBG	*Eucalyptus Stuartiana* (now *bridgesiana*)	*Fn. carteri* Tonn.	Currie (1937)
Anguillulina	*Fergusobia*	*curriei* Johnston 1938	LBG	*E. bridgesiana*		Johnston (1938)
Fergusobia		*curriei* Christie 1941	LBG	*E. bridgesiana*		Christie (1941)
Fergusobia		*tumifaciens* Wachek 1955	LBG	*E. bridgesiana*		Wachek (1955)
Fergusobia		*curriei* Fisher & Nickle1968	FBG	*E. camaldulensis*	*Fn. tillyardi*	Fisher & Nickle (1968)
Fergusobia		*indicus*(Jairajpuri 1962) Siddiqi 1986	N/A	N/A	N/A	Siddiqi (1986a)
Fergusobia		*jambophila* Siddiqi1986	?FBG	*Syzygium cumini*	*Fn. syzygii* Harris 1982	Siddiqi (1986a)
Fergusobia		*magna* Siddiqi1986	Stem galls	*Eucalyptus* sp.	N/A	Siddiqi (1986a)
Fergusobia		*brevicauda* Siddiqi 1994	FBG	*E. deglupta*	N/A	Siddiqi (1994)
Fergusobia		*philippinensis* Siddiqi 1994	FBG	*E. deglupta*	N/A	Siddiqi (1994)
Fergusobia		*fisheri* Davies & Lloyd 1996	FLG	*E. leucoxylon*	N/A	Davies & Lloyd (1996)
Fergusobia		*quinquenerviae* Davies & Giblin–Davis 2004	SBG	*M. quinquenervia*	*Fn. turneri* Taylor	Davies & Giblin-Davis (2004)
Fergusobia		*cajuputiae* Davies & Giblin–Davis 2004	SBG	*M. cajaputi*	*Fn. purcelli* Taylor	Davies & Giblin-Davis (2004)
Fergusobia		*dealbatae* Davies & Giblin–Davis 2004	SBG	*M. dealbata*	*Fn. makinsoni* Taylor	Davies & Giblin-Davis (2004)
Fergusobia		*leucadendrae* Davies & Giblin–Davis 2004	SBG	*M. leucadendra*	*Fn. centeri* Taylor	Davies & Giblin-Davis (2004)
Fergusobia		*nervosae* Davies & Giblin–Davis 2004	SBG	*M. nervosa*	*Fn. schefferae* Taylor	Davies & Giblin-Davis (2004)
Fergusobia		*viridiflorae* Davies & Giblin–Davis 2004	SBG	*M. viridiflora*	*Fn. burrowsi* Taylor	Davies & Giblin-Davis (2004)
Fergusobia		*pohutakawa* Davies 2007	Uniloc. SBG	*Metrosideros excelsa*	*Fn. metrosiderosae* Taylor	Taylor *et al.* (2007)
Fergusobia		*ptychocarpae* Davies 2008	FBG	*C. ptychocarpa*	*Fn. giblindavisi*	Taylor & Davies (2008)
Fergusobia		*brittenae* Davies 2010	TLG	*E. camaldulensis*	*Fn. lockharti*	Taylor & Davies (2010)

Type species:

Fergusobia tumifaciens (Currie 1937) Wachek 1955

Syn*: Anguilluina (Fergusobia) tumifaciens* Currie 1937*; Anguillulina (Fergusobia) curriei* Johnston 1938*; Fergusobia curriei* (Johnston 1938) Christie 1941*; (nec Anguillulina tumefaciens* Cobb 1932*; Anguina tumefaciens* (Cobb) Filipjev and Schuurmans Stekhoven 1941).

Other species:

Fergusobia curriei Fisher and Nickle 1968; *F. indica* (Jairajpuri 1962) Siddiqi 1986; *F. jambophila* Siddiqi 1986; *F. magna* Siddiqi 1986; *F. brevicauda* Siddiqi 1994; *F. philippinensis* Siddiqi 1994; *F. fisheri* Davies and Lloyd 1996; *F. quinquenerviae* Davies and Giblin-Davis 2004*; F. cajuputiae* Davies and Giblin-Davis 2004*; F. dealbatae* Davies and Giblin-Davis 2004*; F. leucadendrae* Davies and Giblin-Davis 2004*; F. nervosae* Davies and Giblin-Davis 2004*; F. viridiflorae* Davies and Giblin-Davis 2004; *F. pohutukawa* Davies 2007, *F. ptychocarpae* Davies 2008, and *F. brittenae* Davies 2010. Morphospecies, to be described later, have also been collected from *A. floribunda, Corymbia* sp., *E. camaldulensis, E. cosmophylla, E. delegatensis, E. diversifolia, E. eugenioides, E. fasciculosa, E. fibrosa, E. gomphocephala, E. leucoxylon, E. macrorrhyncha, E. microcarpa, E. planchoniana, E. porosa, E. viminalis, Eucalyptus* sp., *M. armillaris, M. decora, M. linariifolia, M. quinquenervia,* and *Syzygium luehmannii* (K.A. Davies, unpub. data).

Gall forms and their relationship to host plant, fly and nematode

In December, 2008, the WNC alone contained nematode material from 63 host plants identified to species, and other material from hosts identifiable only to genus (K.A. Davies, unpub. data, Table 1). This material included at least 20 distinct gall forms, varying in size from TLG's about 30 mm in diameter, to unilocular axial galls a few mm in diameter. Some gall forms, such as the hollow petiole gall on *E. brevifolia*, the basal pea galls on *M. excelsa*, and the 'rosette' galls on *M. quinquenervia*, are cryptic, and difficult to recognise (K.A. Davies, R. Giblin-Davis and G.S. Taylor, unpub. data). In addition, the small cryptic forms are usually more susceptible to parasitism (Currie 1937; K.A. Davies and G.S. Taylor, unpub. data), and contain fewer nematodes and fly larvae than the larger forms.

If gall form reflects the particular meristem selected by a female fly for egg deposition, it could explain the occurrence of similar gall forms in widely disparate clades and on different hosts, and apparent convergence. For example, morphologically identical small axial bud galls appear on *E. leucoxylon*, but are associated with two different forms of fly/nematode mutualism (K.A. Davies, unpub. data). One fly (WINC 004227) has a dorsal shield with forwardly projecting teeth; and the other (WINC 003127, 003156) has a dorsal shield comprising two patches of sclerotised cuticle. Again, similar TLG's have been collected from *Angophora* and *Eucalyptus* sub-genera *Symphyomyrtus* and *Eucalyptus*. There are only subtle differences in the morphology of the galls from the latter (Giblin-Davis *et al*. 2004a). Nematodes from TLG's on *Angophora* and hosts in the *Eucalyptus* subgenus *Symphyomyrtus* are morphologically similar as are those from hosts in the subgenus *Eucalyptus*, but the two groups differ from each other.

Molecular Phylogeny

For molecular analysis, nearly full length of 18S rDNA, 28S rDNA D2/D3 domains and a portion of mitochondrial DNA cytochrome oxidase subunit I were sequenced. The λ^2 test showed that homogeneity of base frequency across taxa was not rejected for any data set. The trees generated by Bayesian, NJ and MP analyses showed no significant conflict in branching order, so only the Bayesian trees are shown.

Of the 3 loci, the mtCOI sequences had the highest rate of change and the most parsimony informative characters, whereas 18S sequences had the lowest rate of change and the least parsimony informative characters. Because sequences of COI have a high level of variation, especially on the 3rd codon position between species, there is weak support for clades at a higher level above species (Ye *et al*. 2007 a, b). In addition, there was no significant match of the clades between COI and D2/D3 (Figs 78, 79) and therefore no numbering of clades is shown on the phylogenetic tree derived from sequences of COI.

Figure 77 represents a phylogenetic tree based on nearly full length sequences of 18S rDNA, Fig. 78 a tree inferred from 28S rDNA D2/D3 domains from a multiple alignment of 983 total characters, and Fig. 79 a tree inferred from a fragment of the mtCOI gene from a multiple alignment of 618 total characters. In general form, these trees are similar to those shown in Ye *et al.* (2007b), but include sequences from nematodes from narrow leafed *Melaleuca* and other species. The composition of the respective clades is discussed below.

It seems that COI is only valuable at the species level or lower and we are still trying to understand species boundaries in *Fergusobia*. A good example is the species boundaries between *Fergusobia quinquenerviae* and the clearly demarcated geographical and COI molecular genotype from *M. fluviatilis*. While these isolates could be differentiated based upon morphology, originally there was insufficient genotypic data to help inform the decision, and the nematodes were placed together (Davies & Giblin-Davis 2004). Now, after sequencing more isolates, it is clear that these isolates do represent separate species. However, the population structure of *F. quinquenerviae* (or the *F. quinquenerviae* species complex) remains unresolved. Because of the lack of resolution, it is unclear how much variation can be categorized as intra- *vs* interspecies variation for D2/D3 or COI. Another example involves the *F. magna* grouping from *C. tessellaris* where there is a well-

supported clade for a morphological and genotypic grouping and both loci suggest the occurrence of two species. However, given a lack of morphological differences (Davies *et al*. 2010) we were not comfortable with this species designation and hypothesised a possible species complex.

Given the data currently available, it is not possible to further discuss intra- *vs* interspecific variability for D2/D3 and COI in *Fergusobia*. In some cases the level of molecular sequence differences was greater in D2/D3 than in COI, and we have no explanation for this. An example is the *fluviatilis* isolate: where voucher specimen nos 35 and 23 are identical to a series of other *F. quinquenerviae* isolates in D2/D3, but where voucher specimens nos 22 and 23 come out as a well-supported separate clade in COI. Additionally, *F. quinquenerviae* voucher specimens nos 341 and 343 are identical in D2/D3, but are different in COI. Contrastingly, *F. quinquenerviae* voucher specimens nos 416 and 422 are different in D2/D3, but identical in COI.

If the number of extant *Fergusobia* species is much larger than that sampled in this work, then the stability of some of the putative clades may be subject to movement as additional taxa are discovered and added. A misinterpretation of molecular phylogeny may occur when too few species are used for inferring phylogeny or a bias exists in the species sampled. In this context, the phylogenetic trees inferred in this work must be considered as part of a working hypothesis about *Fergusobia* relationships.

Nematodes from at least three cryptic gall forms (elongate shoot galls, 'moss' galls, and seedling stem galls, described in Taylor *et al.* 2005) were not included in the molecular analyses in this work (K.A. Davies, unpub. data). Eighty percent of the nematodes included in the phylogenetic trees were collected from gall forms that are larger and easier to recognise. Thus, the nematodes sequenced were strongly biased towards species collected from only seven gall forms, being TLG, FBG, FLG, SBG, axial bud 'stem' galls, unilocular axial bud galls, and leaf and stem PG. This could help to explain observations that, while the composition of the clades remains generally stable in different analyses, their position within the trees varies, i.e., the sequencing so far completed does not represent a wide enough sample of the species within the *Fergusobia*.

Nematode morphology and clades

In previous work (Taylor *et al.* 2003), eight species groups of *Fergusobia* were outlined, based on morphological observations of parthenogenetic females only. This early attempt at defining species-groups did not include the infective females, because they are present within galls for a short period only, and have been missed in many collections. Males were also excluded, because they were then seen as lacking significant morphological differences. Phylogenetic analysis based on molecular sequencing has shown that exclusion of males and infective females could be misleading (Ye *et al.* 2007b), and that the morphology of all plant-parasitic stages should be considered. In addition, it is considered that gall form and plant and fly host species are valid characters for species identification and phylogenetic analyses. When these, and groupings inferred from analyses of D2/D3 sequences are collectively considered, there is new evidence for about 20 clades within *Fergusobia.*

Of the characters used in Taylor *et al.* (2003) to separate species groups of *Fergusobia*, only body shape (and particularly the shape of the posterior part of the body) appears to correlate with the clades inferred from molecular phylogenetic analyses. Size of the oesophageal gland has been discounted as a useful character for most *Fergusobia* species, due to observations that suggest that in males and parthenogenetic females, it decreases in size as the nematode ages (K.A. Davies, unpub. data). While there are no developmental studies to corroborate this, gland length and volume appears to be reduced as the size of the nematode increases. Similarly, males with a fully developed reproductive system appear to have smaller glands than those with a less developed system. The functions of the oesophageal glands remain unclear, but if they have a role in energy storage or secretions enabling parasitism, it is considered that such stores could become depleted over time. However, in some species, e.g., *F. magna*, the gland is consistently smaller than in the majority of species of *Fergusobia*, and in *F. quinquenerviae* and *F. viridiflorae* it is so large that it is reflexed or has an extra lobe. Gross gonad morphology and the form of the uterus appear to be useful for species definition.

Notes on *Fergusobia* species, their associated gall forms and *Fergusonina* fly larvae

In the following notes, for each species of *Fergusobia* sequenced in this work, information is presented on the gall form from which the species was collected, its voucher number and the species of associated *Fergusonina* fly, form of the dorsal shield of the associated fly larvae, and possible genetic relationships of the nematode. Additional information on respective vouchers is given in Table 2. The clades referred to below were inferred from sequences of D2/D3 (Fig. 78), which provided better resolution at the species level than sequences of COI (Ye *et al.* 2007b). Additional information from SSU and COI sequences is given where appropriate. Information on host plant distribution is given in Table 3.

Myrtoideae

Eucalypteae

Genus *Angophora*

A. floribunda (Sm.) Sweet

Fergusobia Morphospecies 1 (voucher specimen no 346); associated with an unknown species of *Fergusonina* (Figs 13, 35, 55). Clade 12 in Fig. 78.

Form of gall. Terminal (occasionally axillary) shoot bud galls (Giblin-Davis *et al.* 2004a; Taylor *et al.* 2005). Broadly ovate, containing *ca* 20 to 100 locules.

Morphology of nematodes. Parthenogenetic female medium size; open C-shape; oesophageal glands large; tail slender, conoid. Infective female medium to large; arcuate to open C-shape; cylindroid; hemispherical tail tip, V *ca* 80%. Male medium to large size; arcuate to J-shape; medium oesophageal glands; angular spicule; bursa 20–55% body length.

Morphology of dorsal shield. No *Fergusonina* fly larval voucher material available, but collection notes state “no dorsal shield” (K.A. Davies, unpub. data).

Possible relationships. 18S, COI and D2/D3 sequences from the *Fergusobia* obtained from galls on both *Angophora* sp. (voucher specimen no 339) and *A. floribunda* track together in the respective Bayesian trees (Ye *et al.* 2007b; Figs 77–79 in this work), strongly supporting a close genetic relationship of these nematodes. In this work, analyses of D2/D3 sequences gave 100% support for Clade 12, comprising nematodes from *A. floribunda*, *Angophora* sp. voucher specimen no 339, and *E*. near *acmenioides* voucher specimen no 340. The nematodes were associated with morphologically similar gall forms, and had morphological similarity except in spicule form. Distributions of both *E. acmenioides* and *Angophora* sp. probably overlap in coastal NSW but *Angophora* is genetically distant to *Eucalyptus*. These findings suggest that a host switch occurred.

Angophora sp.

Fergusobia Morphospecies 2 (voucher specimen no 339), from *Angophora* sp.; associated with an undescribed species of *Fergusonina*. Clade 12 in Fig. 78.

Form of gall. Terminal (occasionally axilliary) shoot bud galls (Giblin-Davis *et al.* 2004a; Taylor *et al.* 2005), collected from Pimpama, south-eastern QLD. Broadly ovate galls, containing *ca* 30 to *ca* 100 locules.

Morphology of nematodes. Parthenogenetic female medium size; variable shape; head retracted; oesophageal gland large; tail arcuate, conoid. Infective female large size; arcuate to open C-shape; body widest in posterior half of body; with hemispherical tail tip. Male medium size; variable shape; oesophageal gland medium; spicule arcuate; bursa 60–89% body length.

Morphology of dorsal shield. (WINC 003104). No shield or spicules.

Possible relationships. See discussion under *A. floribunda*.

Genus *Corymbia*

Section *Extensae*

C. tessellaris F. Muell.

Fergusobia magna (voucher specimens nos 24, 42, 444, 327, 353, 452, 465); associated with an undescribed species of *Fergusonina* (Figs 1, 23, 44). Clade 1 in Fig. 78.

Form of gall. Terminal and axial bud 'stem' galls (various collections from Brisbane north to Cairns) (Giblin-Davis *et al.* 2004a; Fig. 82). Elliptical in shape, vary from less than 0.6 to more than 1.6 cm in diameter. Woody, with high numbers of locules (>100). Locules with lumens 0.8 to 1.12 mm in diameter in mature gall. Vascular bundles regularly spaced around the outer edges of the gall. Differs from other gall types examined histologically in that individual locules lacked the outer perimeter of red-staining cells.

Morphology of nematodes. Parthenogenetic female large, slender; variable to C-shape; small oesophageal gland; long slender conoid tail. Infective female large; slender, arcuate; small oesophageal gland; long slender conoid tail; V 51–62%. Male medium to large; slender, arcuate; small oesophageal gland; large angular spicule with anterior part longer than posterior; slender tail; bursa 30–50% (Siddiqi 1986a; Davies *et al*. 2010).

Morphology of dorsal shield. (WINC 003284, 004382, 003241) (Davies *et al.* 2010). Shield restricted to a broad area of weakly sclerotised spicules along the anterior margin of TS 2.

Possible relationships. Analyses of sequences from both D2/D3 and COI (Figs 78, 79) gave strong support (100%) for Clade 1 of *Fergusobia*. Intraspecific variation in D2/D3 sequences from differing collections suggested that populations of *F. magna* are genetically diverse, and may include a cryptic species (Davies *et al.* 2010). Many locules within the galls coalesce, potentially allowing cross-fertilisation, which could increase genetic diversity if there are multiple fly foundresses (Giblin-Davis *et al.* 2004a).

Section *Septentrionales*

C. ptychocarpa F. Muell.

Fergusobia ptychocarpae Davies 2008 (voucher specimen nos 30, 52, 450); associated with *Fn. giblindavisi* Taylor 2008 (Figs 12, 34, 54). Clade 11 in Fig. 78.

Form of gall. Flower bud galls (Giblin-Davis *et al.* 2004a, Taylor *et al.* 2005). Large (approximately 30 mm in diameter), each with several hundred larvae. Locules appear to originate from anther primordial cells and disc tissue at base of floret. Operculum dehisces, and mature buds frequently crack, allowing flies to escape.

Morphology of nematodes. Parthenogenetic female medium size; open C-shape; oesophageal glands large; tail narrow, conoid, bluntly rounded tip. Infective female medium to large; open C-shape or hooked behind vulva, cylindroid; short broad tail with broadly rounded tip. Male large size; arcuate to J-shape; medium oesophageal glands; angular spicule; tail relatively narrow, tip bluntly rounded; bursa 50–80% body length.

Morphology of dorsal shield. (WINC 003268). Shield restricted to a series of raised sclerotised spicules on the second and third thoracic segments (Taylor & Davies 2008).

Possible relationships. Clade 11 comprised the flower bud galler from *C. ptychocarpa*, and a pea galler and flat leaf gallers from *Eucalyptus* spp. It was not strongly supported by analyses of sequences from D2/D3 (69%) (Fig. 78), and was not inferred from analyses of COI sequences (Fig. 79). However, in Ye *et al.* (2007b) the clade had 100% support in D2/D3 analyses. There are similarities in the shield form (reduced or missing) of the associated *Fergusonina* flies, and while the host plants come from sub-tropical areas of Australia they are genetically disparate. This may be an example of one fly lineage associated with two lineages of nematodes.

Unknown species of *Corymbia*

Fergusobia Morphospecies 3 (voucher specimen no 39); from *Corymbia* sp. associated with an unknown species of *Fergusonina* (Figs 15, 37, 57). Clade 14 in Fig. 78.

Form of gall. Unilocular 'pea' (discrete) leaf galls on tiny leaflets, protruding from both sides of leaf

blade (Taylor *et al.* 2005) (Fig. 87).

Morphology of nematodes. Parthenogenetic female medium size, straight to arcuate shape; oesophageal gland medium; narrow conoid tail with narrowly rounded tip. Infective female small size; straight shape; relatively long slender conoid tail with narrowly rounded tip. Male medium size, almost straight shape; relatively long narrow tail; spicule arcuate; bursa *ca* 80% body length.

Morphology of dorsal shield. (WINC 003283). No shield or spicules.

Possible relationships. Clade 14 comprised *Fergusobia* from *Corymbia* sp., a terminal leaf bud galler from *E.* near *nitida*, and pea gallers from *E. marginata* and *E. pauciflora*. Ye *et al.* (2007b) found strong support for this clade. In this work, analyses of sequences obtained from D2/D3 provided poor support for the clade as a whole (Fig. 78), but 100% bootstrap support for a clade comprising voucher specimens nos 286, 64 and 39. Voucher specimen no 39 was not sequenced for COI, but the other two vouchers grouped together in the phylogenetic tree inferred from it (Fig. 79).

Coming from a different host plant genus, the nematodes from *Corymbia* were from hosts genetically distant to those from *Eucalyptus*. While *E. marginata, E. nitida* and *E. pauciflora* are all classified as belonging to the subgenus *Eucalyptus*, they are placed in separate sections (respectively, *Eucalyptus*, *Aromatica* and *Cineraceae*). Thus, these hosts are not close genetically, and have widely disparate distributions (WA, TAS and eastern Australia). The associated nematodes have similar general form (but different sizes). The fly/nematode gall forms were of two types; and the fly larvae had two forms of dorsal shield. This may be an example of congruence of gall form for the pea gallers, or indicate a fly/nematode switch.

Genus *Eucalyptus*

Sub-Genus *Eucalyptus*

Section *Amentum*

Eucalyptus near *acmenioides* Schauer

Fergusobia voucher specimen no 340; associated with an unknown species of *Fergusonina*. Clade 12 in Fig. 78.

Form of gall. Terminal (occasionally axilliary) shoot bud galls (Giblin-Davis *et al.* 2004a; Taylor *et al.* 2005). Broadly ovate galls, containing *ca* 10 to 100 locules.

Morphology of nematodes. Parthenogenetic female small to medium size; variable shape; oesophageal glands medium; tail relatively narrow, conoid, tip bluntly rounded. Infective femal medium; arcuate to open C-shape; short broad tail with hemispherical tail tip. Male medium size; variable shape; medium oesophageal glands; strong angular spicule; bursa 70–80% body length.

Morphology of dorsal shield. (WINC 004200). Fly larvae not available. Puparia lacking shield. Note that because cyclorraphan fly puparia retain the cuticle of the last molt, the puparia have the characteristics of the L3 larvae, and can be used to determine shield form.

Possible relationships. These nematodes apparently clump (Clade 12) with those sequenced from *Angophora* (respectively, Morphospecies 1 and 2). See discussion under *A. floribunda*.

Section *Aromatica*

Series *Insulanae*

E. amygdalina Labill.

Fergusobia voucher specimen no 276; associated with an unknown species of *Fergusonina*. Clade 6 in Fig. 78.

Form of gall. Terminal leaf bud gall. Similar morphology to that of *E. delegatensis*, but not glaucous.

Morphology of nematodes. Parthenogenetic female medium in size, arcuate to open C-shape; oesophageal gland large; tail short, conoid, relatively slender, bluntly rounded tip. Infective female arcuate to open C-shape, maximum width at mid-length; tail short, broad, tip almost hemispherical, V *ca* 80–90%. Male arcuate shape; tail ventrally concave, tip bluntly rounded; spicules angular; bursa *ca* 60% body length.

Morphology of dorsal shield. (WINC 003463). Shield comprising six transverse ‘bars’ of narrow patches of small spots of sclerotised cuticle (Fig. 62).

Possible relationships. Clade 6 (Fig. 78) comprised *Fergusobia* nematodes from TLGs from *E. amygdalina*, *E. delegatensis*, *E. diversifolia*, *E. racemosa*, and *E.* sp., FLGs from *E.* sp., and FBGs from *E. macrorhyncha* and *E. obliqua*. Ye *et al.* (2007b) found strong support for this clade. In this work, analyses of sequences obtained from D2/D3 provided poor support for Clade 6 as a whole (Fig. 78), but 93% for a grouping comprising voucher specimens nos 276, 281, 284, 33, 36, 7, 41, and 337. With COI, nematodes from TLG's from *E. diversifolia*, *E. delegatensis* and *E. racemosa* grouped together in the phylogenetic tree inferred from the sequences (Fig. 79).

All the host species in this clade are placed in subgenus *Eucalyptus*, but from five different sections, i.e., they are genetically related but not closely. Of the known species, only *E. racemosa* occurs in northern Australia.

E. near *nitida* Hook.

Fergusobia voucher specimen no 286; associated with an unknown species of *Fergusonina*. Clade 14 in Fig. 78.

Form of gall. Shoot bud galls similar to those described from *E. diversifolia*.

Morphology of nematodes. Parthenogenetic female medium size; arcuate in shape; oesophageal gland large; conoid tails. Male medium size; arcuate shape; oesophageal gland medium; spicule moderately angular (at less than 90°), moderately sclerotised; bursa arising at *ca* 50% body length.

Morphology of dorsal shield. No associated fly larval material collected.

Possible relationships. Grouped in Clade 14; see discussion under *E. amygdalina*.

Series *Pachyphloiae*

E. macrorhyncha F. Muell.

Fergusobia Morphospecies 4 (voucher specimens nos 4, 350); associated with *Fn. nicholsoni*. Clade 6 in Fig. 78.

Form of gall. Flower bud galls (Currie 1937, Giblin-Davis *et al.* 2004a, Taylor *et al.* 2005). Galls club-shaped, larger than uninfested flower buds. Locules originate in a proliferation of anther cells, are membrane-bound, and each is attached to floret wall by an anther filament. Filaments in infested buds 2 to 3 times the diameter of those of uninfested buds. Stigma not apparent.

Morphology of nematodes. Parthenogenetic female small to medium in size, arcuate to C-shape; oesophageal gland large; tail short, broadly conoid, broadly rounded tip. Infective female J-shape, maximum body width at mid-length; narrows gradually behind vulva; tail tip bluntly rounded, V *ca* 80%. Male J-shaped; bursa 20–40% body length; spicules angular; tail ventrally concave, short, tip bluntly rounded.

Morphology of dorsal shield. Associated with *Fn. nicholsoni* (WINC 003118). Broad, heavily sclerotised cuticular plates. First a very short, broad sclerotised plate on posterior edge of TS 3, confluent with a broader sclerotised plate on AS 1, and a slightly narrower plate on the anterior margin of AS 2. The 'bar' form of dorsal shield is illustrated in Figs 62 and 63.

Possible relationships. Grouped in Clade 6; see discussion under *E. amygdalina*. From D2/D3 sequencing, there appeared to be little or no genetic drift between populations of Morphospecies 4 collected from disjunct populations of *E. macrorhyncha* in SA and the ACT (W. Ye, unpub. data). However, sequences from COI did suggest drift (Fig. 79).

The shield form of the flies from *E. macrorhyncha* (plates) as compared with others in this clade (bars) suggests that the flies are from different lineages. The shield is similar to that of fly larvae from FBG on *E. obliqua*. Nematodes from *E. macrorhyncha* may belong to a separate clade.

Series *Pachyphloiae*

E. eugenioides Sieber ex Sprengel

Fergusobia Morphospecies 5 (voucher specimen no 349); associated with an unknown species of *Fergusonina*. Clade 13 in Fig. 78.

Form of gall. Flower bud galls (Fig. 83).

Morphology of nematodes. Parthenogenetic female small to medium size; open C to arcuate shape; large oesophageal glands; short conoid tail with bluntly rounded tip. Infective female medium size; arcuate with hooked tail; cylindroid; broad tail with broadly rounded tip; V 83%. Male medium to large; almost straight to J-shape; medium to large oesophageal gland; short tail with bluntly rounded tip; weak angular spicules, bursa *ca* 25%.

Morphology of dorsal shield. (WINC 004221). Shield comprising 3 broad confluent plates of moderately sclerotised cuticle on TS 3, AS 1 and AS 2 respectively; no teeth.

Possible relationships. Clade 13 comprised *Fergusobia* nematodes from FLGs on *E.* spp. voucher specimen nos 29 and 348, *E. microcarpa, E. leucoxylon, E. siderophloia,* and *E. porosa,* and PGs on *E. porosa* and *E*. sp. voucher specimen no 351. Analyses of sequences of D2/D3 in this work (Fig. 78) provided 94% support for this clade, which received 100% support in Ye *et al.* (2007b). Analyses of sequences from COI gave the grouping 80% support (Fig. 79), and it included voucher specimen no 2 from FBG on *E. obliqua,* but not voucher specimen no 349 from FBG on *E. eugenioides*.

While *E. eugenioides* is classified as subgenus *Eucalyptus*, the other host eucalypts in this clade are from subgenus *Symphyomyrtus*, i.e., are genetically disparate. The distribution of *E. eugenioides* overlaps that of *E. microcarpa* and *E. siderophloia*; and distributions of *E. microcarpa, E. leucoxylon* and *E. porosa* also overlap. Host switching between *E. eugenioides* and *microcarpa* and *siderophloia* may have occurred.

The gall form of voucher specimen no 349 from *E. eugenioides* (FBG) differed from that of the other members of this clade (PG and two forms of FLGs) suggesting that the associated flies had evolved different preferences for oviposition sites. If gall form reflects the particular meristem selected by a female fly for egg deposition, this could explain the occurrence of similar gall forms in widely disparate clades, and apparent convergence.

Section *Eucalyptus*

E. marginata Donn. ex Smith

Fergusobia voucher specimen no 64; associated with an undescribed species of *Fergusonina*. Clade 14 in Fig. 78.

Form of gall. Unilocular 'pea' (discrete) leaf galls (Taylor *et al.* 2005) (Fig. 87). Hemispherical, protruding only from the top surface of the leaf blade. Each 5–6 mm in diameter, with a height of 3–5 mm. Neighboring galls do not merge.

Morphology of nematodes. Parthenogenetic female large in size; straight shape; oesophageal gland medium; slender conoid tails. Male large size; arcuate shape; oesophageal gland medium; spicule moderately angular (at less than 90°), moderately sclerotised; bursa arising at *ca* 90% body length.

Morphology of dorsal shield. (WINC 003434, 003438). No dorsal shield.

Possible relationships. Grouped in Clade 14; see discussion under *Corymbia* sp. for comments.

While *E. marginata, E. nitida* and *E. pauciflora* are all classified as belonging to the subgenus *Eucalyptus*, they are placed in separate sections (respectively, *Eucalyptus, Aromatica* and *Cineraceae*). Thus, these hosts are not close genetically, and have widely disparate distributions (WA, TAS and eastern Australia). However, fly shield morphology is similar, and the associated nematodes have similar general form (but different sizes). This suggests that the fly/nematode association developed from a common lineage before the sea incursion that separated WA from eastern Australia from the late Eocene to the mid-Miocene (Nelson, 1981).

Series *Eucalyptus*

E. obliqua L'Herit.

Fergusobia voucher specimens nos 2, 315; associated with an undescribed species of *Fergusonina* (respectively, Figs 31, 70, 32, 71). Clade 6 in Fig. 78.

Form of gall. Flower bud galls, similar in form to those on *E. macrorhyncha* (Giblin-Davis *et al.* 2004a). Locules develop from proliferation of anther cells. Each locule attached to floret wall by an anther filament. Stigma does develop in these galls.

Morphology of nematodes. Parthenogenetic female (voucher specimen no 2, WNC 2019) medium size, open C or C-shape; oesophageal glands large; tail short, conoid, tip narrowly rounded. Infective female J-shape, maximum body width at mid-length or at vulva; tail tip broadly rounded or almost hemispherical. Male J-shaped; spicules angular; tail tip bluntly rounded; bursa 20–40% body length.

Parthenogenetic female (voucher specimen no 315, WNC 2228) as above, except that in these specimens the tail is sub-cylindroid and the tail tip is bluntly rounded.

Morphology of dorsal shield. (WINC 003450, 003470). Shield comprising broad, heavily sclerotised cuticular plates. First a very short, broad sclerotised plate on posterior edge of TS 3, confluent with a broader sclerotised plate on AS 1, and a slightly narrower plate on the anterior margin of AS 2. Shield form similar to that of *Fn. nicholsoni*.

Possible relationships. From sequencing of D2/D3, these nematodes were inferred to belong to Clade 6. Grouped in Clade 6; see discussion under *E. amygdalina* for comments. Sequences from nematodes from FBG on *E. obliqua* appear in two different groupings in the COI analyses (Fig. 79). These samples came from two trees at one site in SA, and may a) represent an artifact, or b) the occurrence of multiple fly foundresses carrying genetically disparate *Fergusobia* nematodes, or c) the presence of two nematode/fly species with similar gall type on the one species of host plant.

Parthenogenetic females sequenced from FBG on *E. obliqua* had two distinct morphotypes, from two respective sites. This suggests that two different lineages of nematodes and flies have utilised one form of meristematic tissue on *E. obliqua*, and emphasises the need for care when collecting fly/nematode galls.

Nematodes from FBG from *E. macrorhyncha* and *obliqua* are morphologically similar. While both host plant species are from subgenus *Eucalyptus*, they are classified in different sections (respectively, *Capillulus* and *Eucalyptus*), i.e., they are not genetically close. Host plant distributions overlap in NSW. Galls on each host species were similar. The associated fly larvae had similar shields with three confluent plates of heavily sclerotised cuticle of which the middle was the widest, and no teeth. This is a possible case of a common ancestral lineage, and host switching. Further investigation is needed to differentiate the two morphotypes of *Fergusobia* collected from these FBG, and to elucidate their relationships.

Section *Insolitae*

E. planchoniana F. Muell.

Fergusobia Morphospecies 6 (voucher specimen no 275); associated with an undescribed species of *Fergusonina* (Figs 9, 31, 52). Clade 8 in Fig. 78.

Form of gall. 'Leafy' leaf bud galls (Taylor *et al.* 2005), consisting of fused gall tissue, the locules held in a single plane by expanding leaf stem and leaf tissue, so that leaf and meristematic stem tissue covers an elongate, chilli-shaped gall, usually with leaf tissue growing beyond it.

Morphology of nematodes. Parthenogenetic female small to medium size, open C-shape; large oesophageal gland; body narrows rapidly behind vulva; short conoid tail with narrowly or bluntly rounded tail tip. Infective female arcuate, cylindroid; hemispherical tail tip. Male medium size, arcuate or J-shape; oesophageal glands medium; angular spicule; bursa arises near secretory/excretory pore.

Morphology of dorsal shield. (WINC 003065). Shield comprising 9 transverse 'bars', the first on TS 2, followed by three broader bars on TS 3 and AS 1–2, then a narrower bar on AS 3. These bars are formed from patches of raised, heavily sclerotised cuticle, dotted with raised sclerotised spicules. The patches are surrounded with areas of raised, sclerotised, sparse spicules. The following bars are progressively reduced to rows of raised sclerotised spicules on AS 4–7.

Possible relationships. Clade 8 comprises these nematodes from *E. planchoniana* and nematodes from pea galls on *E. leucoxylon*. Analyses of sequences from D2/D3 provided weak (77%) support for this clade (Fig. 78). Unfortunately, nematodes from *E. planchoniana* were not sequenced for COI.

Genetically, *E. planchoniana* and *E. leucoxylon* are widely disparate hosts (coming from subgenera *Eucalyptus* and *Symphyomyrtus* respectively), and their geographic ranges do not overlap. Morphology of the galls,

fly larval shields and respective *Fergusobia* is different. Thus, this seems an unlikely clade. Sequences from *E. planchoniana* were not included in the COI tree, where *Fergusobia* sp. voucher specimen no 71 appeared with *Eucalyptus* sp. voucher specimen no 32 (Figure 3 in Ye *et al.* 2007b).

Section *Cineraceae*

Series *Fraxinales*

E. delegatensis R. Baker

Fergusobia Morphospecies 7 (voucher specimen no 281); associated with an undescribed species of *Fergusonina* (Figs 6, 28, 49). Clade 6 in Fig. 78.

Form of gall. Terminal and axial leaf bud galls similar to those from the subgenus *Symphyomyrtus*, but less spheroid, more 'chilli-shaped', multilocular, and often with leaf tissue protruding/growing from them. Glaucous.

Morphology of nematodes. Parthenogenetic female medium to large in size, arcuate to open C-shape; oesophageal glands large; tail short, conoid, bluntly rounded tip. Infective female arcuate, cylindroid; short broad tail, tip almost hemispherical; V 80–90%. Male shape variable; tail tip broadly rounded; spicules strong, angular; bursa arises near secretory/excretory pore.

Morphology of dorsal shield. (WINC 003478-9). Shield comprises 5 transverse 'bars' of raised sclerotised spicules. The first, on TS 3, consists of a short, broad, transverse area of raised sclerotised spicules; the second, is shorter and broader on AS 1; next three progressively reduce in prominence from AS 2–4.

Possible relationships. Grouped in Clade 6; see discussion under *E. amygdalina*.

Series *Pauciflorae*

E. pauciflora Sieber ex Sprengel

Fergusobia voucher specimen no 280; associated with an undescribed species of *Fergusonina*. Clade 14 in Fig. 78.

Form of gall. Unilocular 'pea' (discrete) leaf galls (Taylor *et al.* 2005) (Fig. 87). Hemispherical, protruding only from the top surface of the leaf blade. Neighboring galls do not merge.

*Morphology of nematodes***.** Parthenogenetic female medium to large size; arcuate to straight shape; oesophageal glands relatively small; slender conoid tails. Male medium to large size; arcuate or straight shape; oesophageal glands small or medium; spicule moderately angular (at more than 90°), moderately sclerotised; bursa arising near secretory/excretory pore.

Morphology of dorsal shield. (WINC 003475). Larvae not seen. Pupa has no apparent dorsal shield.

Possible relationships. Grouped in Clade 14; see discussion under *Corymbia* sp.

Series *Psathroxylon*

E. racemosa Cav.

Fergusobia voucher specimens nos 41, 337; associated with an undescribed species of *Fergusonina*. Clade 6 in Fig. 78.

Form of gall. Terminal leaf bud galls (Taylor *et al.* 2005). Similar morphology to that of *E. delegatensis*, but not glaucous.

Morphology of nematodes. Parthenogenetic female medium size, C-shape; oesophageal glands large; tail short, conoid, bluntly rounded tip. Male arcuate to J-shaped; oesophageal glands medium; spicules angular; tail tip bluntly rounded; bursa *ca* 50% body length.

Morphology of dorsal shield. (WINC 003075). No larval material available. Pupal shield comprising 6 broad bars of sclerotised, raised spicules. The first is in the posterior half of TS 2, and the second and third on AS 1 and 2, respectively. The bars of raised spicules become progressively less prominent and sparser on AS 3 and 4.

Possible relationships. Grouped in Clade 6; see discussion under *E. amygdalina*.

The distribution of *E. racemosa* (in the north-east of Australia) is disjunct from that of *E. amygdalina*, *E. delegatensis* and *E. diversifolia* (in the south-east of Australia), all of which have similar galls with flies with

similar ‘barred’ dorsal shields. This suggests that the fly/nematode mutualisms found on these hosts developed before the separation of TAS from the Australian mainland, the lineage was widespread, and that host-switching occurred. These hosts are all classified as subgenus *Eucalyptus*, i.e., have some genetic similarity.

Section *Longistylus*

E. diversifolia Bonpl.

Fergusobia Morphospecies 8 (voucher specimens nos 7, 284); associated with an undescribed species of *Fergusonina*. Clade 6 in Fig. 78.

Form of gall. Terminal and axial leaf bud galls (Giblin-Davis 2004, Taylor *et al.* 2005). Elongate, ‘chilli-shaped’, multilocular. Fresh galls comprised rigid but relatively soft plant tissue. Usually have leaf tissue protruding/growing from the tip. When sectioned and stained, outer layers of cells in the gall stained red, contained scattered oil glands. Matrix comprised vacuolated parenchymal cells. Lumen of locules lined with granular looking hypertrophied cells, 2–6 cell layers deep.

Morphology of nematodes. Parthenogenetic female medium to large in size, variable shape; oesophageal glands large; tail short, conoid, tip bluntly rounded. Infective female large, arcuate shape; maximum width at mid-length; tail short, broad, tip bluntly rounded or almost hemispherical; V 80–90%. Male medium to large size, arcuate to J-shaped; spicule angular; tail tip bluntly rounded; bursa arises near secretory/excretory pore.

Morphology of dorsal shield. (WINC 002985-7, 002992-3). Shield with 8 or 9 ‘bars’ of raised, sclerotised spicules. First bar on TS 2, short, narrow, towards posterior margin; second on TS 3, longer and broader; third on AS 1, longer and broader again. The bars of raised sclerotised spicules decreased in prominence from AS 1–5. Spicules sparse to almost absent on AS 6 and 7.

Possible relationships. Grouped in Clade 6; see discussion under *E. amygdalina* and *E. racemosa*.

Vouchers specimens nos 284 and 7 from *E. diversifolia* appear to be the same genetically with COI sequences, but different with D2/D3 sequences. This may be an artifact, or there may have been multiple fly foundresses for this gall form.

Subgenus *Symphyomyrtus*

Section *Sejunctae*

E. cladocalyx F. Muell.

a) Unilocular leaf ‘pea’ gall

Fergusobia voucher specimen no 740; associated with an unknown species of *Fergusonina*. No sequences for D2/D3.

Form of gall. Unilocular stem and bud galls at Meningie SA; similar in form to those on *E. gomphocephala*.

Morphology of nematodes. Parthenogenetic female small, C-shape; oesophageal gland large; body narrows gradually behind vulva; tail conoid, tip bluntly rounded. Male medium size, J-shape; oesophageal gland medium; tail tip bluntly rounded; spicule angular; bursa *ca* 40% body length.

Morphology of dorsal shield. (WINC 063703). Shield comprises heavily sclerotised plates, with teeth. A heavily sclerotised broad plate on the posterior margin of TS 3, confluent with a broad heavily sclerotised plate on AS 1 and a shorter, heavily sclerotised broad plate on the anterior margin of AS 2. A row of 4–5 distinctive, short, sharply pointed, forward projecting teeth arises from the posterior edge of the plate on AS 1.

Possible relationships. These nematodes were not sequenced for D2/D3. With COI (Fig. 79), they form part of a grouping which included pea gallers from *E. leucoxylon* and *E.* sp. voucher specimen no 32, and axial bud gallers from *E. leucoxylon* voucher specimen no 741. The distributions of the host species overlap, and all are classified as subgenus *Symphyomyrtus*, but their relationships are unclear because *E. cladocalyx* is unplaced. While similar in that the shield is of the ‘plates with teeth’ form, the dorsal shield of these fly larvae is distinctive and suggests that the flies are from a different lineage to that of the *Fergusonina* inducing TLG on other host plants from the subgenus *Symphyomyrtus*, e.g., *E. camaldulensis***.** Morphology of the various fergusobid nematodes is similar, but body size and length of the bursa in the males differ.

b) Terminal leaf bud gall

Fergusobia voucher specimen no 739; associated with an unknown species of *Fergusonina*. No sequences for D2/D3.

Form of gall. Small terminal leaf bud gall, containing 4 or 5 locules.

Morphology of nematodes. Parthenogenetic female small, open C-shape; oesophageal gland medium; body narrows gradually behind vulva; short broad tail, tip broadly rounded. Infective female small size, open C-shape; short broad tail, broadly rounded tip; V *ca* 85%. Male medium size, arcuate to J-shape; oesophageal gland medium; tail tip bluntly rounded; spicule angular; bursa 20–30% body length.

Morphology of dorsal shield. (WINC 063702). Shield comprising two minute patches of sclerotised cuticle, the first made up of a small spot on the posterior margin of TS 3 confluent with a small spot on the anterior margin of AS 1, and the second made up of a band of sclerotised cuticle on the posterior margin of AS 1 confluent with a small spot of sclerotised cuticle on the anterior margin of AS 2. A few sparse sclerotised spicules occur behind the first and second spots respectively.

Possible relationships. These nematodes did not sequence for D2/D3. With COI (Fig. 79), they were inferred to form part of a group which included flat leaf gallers from *E. porosa* and *E. leucoxylon*. All nematodes were associated with fly larvae having shields comprising two sclerotised patches. Morphology of the nematodes was also similar. The distributions of the host species overlap, and all are classified as subgenus *Symphyomyrtus*, but their relationships are unclear because *E. cladocalyx* is unplaced. Host switching could have occurred.

Section *Bolites*

E. gomphocephala DC

Fergusobia Morphospecies 9 (voucher specimen no 63); associated with *Fn. newmani* Tonnoir 1937 (Figs 8, 30, 51). Clade 7 in Fig. 78.

Form of gall. Small pea-like unilocular galls on young stems and leaf buds (Currie 1937; Taylor *et al.* 2005) (Fig. 88). Consist of discrete chambers 2–3 mm in diameter, and about 2 mm in height. Hemispherical, protrude only from one surface of a newly expanded leaf.

Morphology of nematodes. Parthenogenetic female small to medium size, C-shape; oesophageal gland enormous; body narrows rapidly behind vulva; short conoid tail with bluntly rounded tip. Infective female small, variable shape; maximum width at vulva; short broad tail; V 70–85%. Male small, arcuate or J-shaped; oesophageal gland enormous; spicule angular; short tail with broad tip; bursa 20–30% body length.

Morphology of dorsal shield. Fn. newmani (WINC 003426-7). Shield comprises a heavily sclerotised plate on the posterior margin of TS 3, confluent with heavily sclerotised plate on AS 1, and a short broad plate on anterior margin of AS 2. Two long, widely spaced, anterior projecting, recurved, sharp teeth arise from the posterior margin of AS 1.

Possible relationships. Clade 7 (Fig. 78) comprised these *Fergusobia* from *E. gomphocephala* and pea gallers from *E.* sp. voucher specimen no 32 and *E. leucoxylon* and flat leaf gallers from *E. leucoxylon*. Analyses of sequences from D2/D3 gave weak (75%) support for Clade 7 (Fig. 78). Nematodes from this clade are morphologically similar, and are associated with fly larvae with similar forms of dorsal shield.

Except for *E. gomphocephala* (Section *Bolites*), host plants in this clade are genetically similar (Section *Adnataria*), suggesting that host switches could have occurred. *Eucalyptus gomphocephala* has a disjunct distribution from the other host species in this clade. This suggests that the fly/nematode association collected from *E. gomphocephala* developed from a common lineage before the sea incursion that separated WA from eastern Australia from the late Eocene to the mid-Miocene (Nelson 1981).

Section *Exsertaria*

Series *Erythroxylon*

E. tereticornis Smith

(a) Leaf ‘pea’ galls

Fergusobia (voucher specimen no 34), associated with an unknown species of *Fergusonina* (Figs 10, 32). Clade 9 in Fig. 78.

Form of gall. Leaf 'pea' galls *ca* 2–3mm in diameter, comprising a single chamber. Often found at the tip of a young expanding leaf.

Morphology of nematodes. Parthenogenetic female medium size, open C-shape; oesophageal gland large; short conical tail. Male small to medium size, straight with tail arcuate; oesophageal gland medium; tail tip broadly rounded; angular spicule; bursa *ca* 20% body length.

Morphology of dorsal shield. (WINC 003105). No shield or spicules.

Possible relationships. Clade 9 included these nematodes and a pea galler from *E.* sp. voucher specimen no 31. Here, there was little support for the clade from sequences of D2/D3. From sequencing of D2/D3, Ye *et al.* (2007b) obtained strong support (100%) for the clade. In the present work, analyses of sequences of COI also supported the grouping (Fig. 79; 100% support). It was also supported by the lack of a shield in the associated *Fergusonina* fly larvae, and morphological similarity of the nematodes collected.

Eucalyptus sp. (Section *Adnataria*) and *E. tereticornis* (Section *Exsertaria*) are not close genetically, but their distributions overlap (both samples were collected in Brisbane). This suggests that host-switching by the fly/nematode mutualism could have occurred.

(b) Terminal leaf bud gall

Fergusobia (voucher specimens nos 401, 438) associated with an undescribed species of *Fergusonina*. Clade 3 in Fig. 78.

Fergusobia from *E.* sp. near *tereticornis* (voucher specimen no 446) associated with an unknown species of *Fergusonina*. Clade 3 in Fig. 78.

Form of gall. Terminal leaf bud galls on *E. tereticornis*; similar in form to those from *E. camaldulensis* (K.A. Davies, unpub. data).

Morphology of nematodes. Parthenogenetic female of medium size, C-shape; body narrows rapidly behind vulva; large oesophageal gland; conoid tail. Infectives larger than parthenogenetic female, arcuate, cylindroid; short tail with bluntly rounded or hemispherical tip; vulva at 64–80%. Male medium size, straight to J-shape, oesophageal gland medium to large, angular spicule; bursa 12–40%, tail relatively slender with rounded tip or broad with broadly rounded tip.

Morphology of dorsal shield. (WINC 004388). Similar to that of fly larvae from *E. camaldulensis* TLG. Shield comprising three confluent, sclerotised plates extending over three segments (TS 3, AS 1 and AS 2). Surrounded by many sclerotised raised spicules, arranged in whorls. Seven small teeth arise from the middle plate, projecting forward. There is also a row of raised spicules running transversely across the fourth abdominal segment.

Possible relationships. Clade 3 comprised nematodes forming TLG from *E. tereticornis* and *E. camaldulensis*. In this work, the clade was well supported (87%) for D2/D3 sequences, and also in the analyses of Ye *et al*. (2007b) (100%). With COI, the inferred grouping had 97% support.

Based on similar morphology of nematodes, fly larvae and gall form, it is likely that nematodes from several host plant species, all from subgenus *Symphyomyrtus*, belong to this putative clade (K.A. Davies, unpub. data). Genetically, *E. tereticornis* and *E. camaldulensis* are similar (from Section *Exsertaria*). Their distributions overlap. Fly larvae from *E. camaldulensis* and *E. tereticornis* have similar shield types, with sclerotised plates with teeth. Nematodes from galls on each are also of similar morphological type and have similar molecular sequences. Given the genetic similarity and overlapping distributions of *E. camaldulensis* and *E. tereticornis*, this may be a case of coevolution of the fly/nematode mutualism and the host plant.

Collection of both a pea-galling (voucher specimen no 34) and a terminal leaf bud galling (voucher specimen no 438) fly/nematode mutualism from *E. tereticornis*, from different genetic clades, showed that at least two distinct lineages of the mutualism develop on this one host.

Series *Rostratae*

E. camaldulensis Dehnhardt

(a) Terminal and axial shoot bud galls

Fergusobia brittenae Davies 2010 (voucher specimens nos 62, 205, 292, 59, 55); associated with *Fn. lockharti* Tonnoir 1937 (Figs 3, 25, 46). Clade 3 in Fig. 78.

Form of gall. Terminal and axial shoot bud galls (Fig. 84) (Giblin-Davis *et al.* 2004a, Taylor *et al.* 2005). Entire bud becomes a fused spheroidal mass of tissue. Developing leaves entirely fused in some; in others part grows on separately from gall. Ovate, 0.5 to 3 cm in diameter. No distinct demarcation between locule and gall matrix. Locules surrounded by 2–9 cell layers of hypertrophied tissue. Hypertrophy lost in locules without fly/nematodes.

Morphology of nematodes. Parthenogenetic female medium size, C-shape; body narrows rapidly behind vulva; large oesophageal gland; conoid tail. Infective female larger than parthenogenetic, arcuate; cylindroid; short tail with broadly rounded tip; V 70–80%. Male medium size, straight to J-shape; oesophageal gland large; angular spicule; tail variable, slender or broad, with bluntly or broadly rounded tip; bursa 20–40%.

Morphology of dorsal shield. (WINC 004914, 004004, 004019, 003328, 003358, 003360, 003368-70). Shield a medial black sclerotised plate about 0.30–0.35 mm wide, formed by fusion of posterior portion TS 3, AS 1 and anterior portion of AS 2; usually with four, sometimes three and rarely five anterior projecting, stout, sclerotised teeth; raised sclerotised spicules on anterior dorsal margin of TS 1 and dorsally on TS 1 and 2; a transverse series of raised ridges comprising raised sclerotised spicules occur most prominently on AS 1–4, becoming less prominent on AS 5–7. A similar shield, from TLG on *E. cosmophylla*, is shown in Fig. 68.

Possible relationships. Grouped in Clade 3; for discussion see under *E. tereticornis* TLG.

Galls have been collected from *E. camaldulensis* (river red gum) from both var. *camaldulensis* (found in southern QLD, VIC, and the Mount Lofty Ranges, Kangaroo Island, Eyre Peninsular and the South-East Region in SA) and var. *obtusa* (in the Flinders Ranges and northern desert areas of SA, and in the Kimberley and South West regions of WA).

From D2/D3 and COI sequences (Figs 78, 79), there appears to be some genetic drift between *F. brittenae* collected from *E. camaldulensis* in SA and WA (Ye *et al.* 2007b). Based on D2/D3 and COI sequences, voucher specimens nos 205 and 55 (respectively, collected from Hall's Creek in the north-east of WA and Goolwa in SA) are the same, but the position of the other samples (voucher specimens nos 62, 59, 6, 292, and 56; from Kellerberrin, WA, Marla, SA, Goolwa, SA, Brachina Gorge, SA, and Bunyeroo Gorge, SA, respectively) is unresolved and not well supported for D2/D3 (74%). Vouchers 55 and 6 were both collected from the one tree, albeit at different sampling times, suggesting that there may be an artifact in these analyses. The possibility of development of a species complex inducing terminal leaf bud galls on *E. camaldulensis* would not be surprising given its wide distribution, but against this is the finding that voucher specimens nos 205 and 55, from widely geographically separated areas, are genetically the same. More work is needed to show whether or not there is a species complex, or one species, of *F. brittenae* in these galls from *E. camaldulensis*.

(b) Flower bud galls

Fergusobia curriei (voucher specimens nos 3, 5, 206) associated with *Fn. tillyardi* Tonnoir 1937 or an undescribed species of *Fergusonina* (Figs 4, 26, 47). Clade 4 in Fig. 78.

Form of gall. Flower bud galls (Currie 1937, Giblin-Davis *et al.* 2004a, Taylor *et al.* 2005). Galled buds of similar shape but much larger than uninfested buds; operculum lost in more mature galls. Galled tissue appears to be a proliferation of the disc and tissues, either from the base of the stigma or from the stamens. Ovaries appear similar to those of uninfested buds. Anthers not seen, but remains of filaments are present. Locules not membrane-bound. Unfortunately, no specific gall material was kept as vouchers for the samples sequenced here.

Morphology of nematodes. Parthenogenetic female open C-shape, medium size; oesophageal gland enormous; broad conoid tail with bluntly rounded tip. Infective female medium to large size, arcuate to C-shape

with most curvature in posterior region; short tail with almost hemispherical tip; V 75–80%. Male medium to large size, arcuate to just J-shape; large oesophageal gland; tail tip bluntly rounded; angular spicule; bursa *ca* 15% body length.

Morphology of dorsal shield. While no specific fly voucher material was kept for the associated nematode samples sequenced here, all larvae examined from flower bud galls had similar shield morphology (K.A. Davies, unpubl. data). It was also similar to the 'plates with teeth' form commonly collected from TLG on host plants from the subgenus *Symphyomyrtus*. Here, the shield of fly larvae collected from Ambleside, near Adelaide, SA (WINC 003304) is described.

Fergusonina tillyardi. Dorsal shield heavily sclerotised, formed from a plate on AS 1 confluent with a short broad sclerotised plate on anterior margin of AS 2, and a short broad weakly sclerotised transverse band in the middle of AS 2. Seven to eight very long, sharp, forward projecting teeth arise from the posterior margin of AS 1. The middle teeth are broad, long and blade-like, and the outer teeth are narrower and more sharply tipped. There are raised spicules anterior to the shield on TS 3, lateral to it on AS 1 and lateral and posterior to it on AS 2.

Possible relationships. Clade 4, comprising different collections of *Fergusobia* from FBG on *E. camaldulensis*, was well supported (100%) overall, but the placement of the collections within the clade was poorly supported (58%). Genetic analyses of *Fergusonina* flies collected from flower bud galls on *E. camaldulensis* in Adelaide have shown that there are two species associated with these galls (S. Scheffer, unpub. data). However, no material was available for comparisons of gall morphology, fly larval shields or of associated nematodes. An intensive sampling of these gall forms from the South Australian region is planned to further examine this gall form it and the associated flies and nematodes.

Given the morphological similarity of the fly larval shield types and of the nematodes, and coming from the one host, it is not surprising that Clades 3 and 4 of nematodes forming TLG and FBG from *E. camaldulensis* inferred from D2/D3 sequences are genetically close (Fig. 78). This could be a duplication event for these lineages, which in turn suggests that the fly/nematode mutualism evolved with the host plant, either by coevolution or association by descent (common ancestor).

(c) 'Stem' shoot bud galls

Fergusobia Morphospecies 10 (voucher specimens nos 54, 311); associated with an undescribed species of *Fergusonina* (Figs 16, 38, 58). Clade 15 in Fig. 78.

Form of gall. 'Stem' galls (Taylor *et al.* 2005) are multilocular axilliary vegetative bud galls. Rather nodular, warty multilocular galls with three to five locules, lacking a specific shape. Given their axilliary origin, and because their growth causes dehiscence of the leaf, they frequently appear to be developing on stems. Most found during the Australian spring (Head 2008), but occasional galls appear in autumn.

Morphology of nematodes. Parthenogenetic female medium size; shape arcuate to almost straight; distinct conoid tail with broadly rounded tip. Infective female large; arcuate, cylindroid; short broad tail with sub-truncate or hemispherical tip; V 75–85%. Male medium size; arcuate, C to J-shape; tail tip bluntly rounded; spicule angular, not strongly sclerotised; bursa 45–80%.

Morphology of dorsal shield. (WINC 003304, 003314-5, 003317). Shield comprises 9 transverse 'bars' of raised sclerotised spicules. Widest and with most spicules on TS 2, TS 3 and AS 1. Figure 63 illustrates this form of shield, but from larvae collected from a FLG on *E.* sp.

Possible relationships. Clade 15, inferred from sequences of D2/D3, comprises two collections of these nematodes. The clade was placed close to the previous clade of pea gallers (clade 14), but with poor support (Fig. 78). Analyses of sequences from COI (Fig. 79) place the nematodes close to those from *M. linariifolia* (voucher specimen no 412) but also with poor support. Nematode morphology from all three clades was similar, in that all have straight or arcuate parthenogenetic females with extensile uteri. Larval morphology of associated flies from the three clades was also similar, representing varying degrees of development of the

'bar' form of the shield (from no shield to a few spicules to definite bars of spicules; or vice versa, depending on whether the lack of shield is seen as the primitive or derived condition). Sequencing of a larger sample of nematodes from similar gall forms, and associated with flies having similar shield morphology, is needed to clarify the relationships of these clades.

Given the wide distribution of the host *E. camaldulensis*, these mutualisms may have had a common fly/nematode ancestor, and/or host switching may have occurred.

The shoot bud gall is the third gall form collected from *E. camaldulensis* (see above). The morphology of the fly larvae (shield made of bars of sclerotised spicules) and nematodes (straight or arcuate in shape) from the stem galls contrasts with that of the flies (shields comprising medial plates with teeth) and nematodes (C-shaped parthenogenetic females and J-shaped males) from TLG and FBG on the same host. This supports molecular data (Fig. 78) showing that the fly/nematode mutualism from the shoot bud galls on *E. camaldulensis* developed from a different lineage to that of the TLG and FBG.

Section *Maidenaria*

E. viminalis Labill.

Fergusobia Morphospecies 11 (voucher specimens nos 347, 273); associated with an undescribed species of *Fergusonina* (Figs 22, 43, 61). Unplaced with D2/D3.

Form of gall. 'Leafy' shoot bud galls (Fig. 14 in Taylor *et al.* 2005). Galls consist of coalesced gall tissue, the locules held in a single plane by expanding leaf tissue, so that leaf and meristematic stem tissue covers an irregularly shaped gall, usually with leaf tissue growing beyond it.

Morphology of nematodes. Parthenogenetic female small, open C-shape; large oesophageal gland; body narrows rapidly behind vulva; short conoid tail, with bluntly or broadly rounded tail tip. Infective female medium size, C to J-shape; body width greatest at vulva; body strongly curved behind vulva; broadly rounded tail tip. Male medium size, J-shape; oesophageal glands medium to large; angular spicules; short bursa.

Morphology of dorsal shield. (WINC 003119-21) (Fig. 65). Shield comprises 2 patches of heavily sclerotised cuticle, without surrounding raised spicules. The first is a large sclerotised area on the posterior margin of TS 3 confluent with a short broad area on anterior margin of AS 1. Second is a narrow sclerotised patch on posterior margin of AS 1 confluent with a short sclerotised patch on anterior margin of AS 2.

Possible relationships. Eucalyptus viminalis is a common tree in the wet forests of NSW, VIC and TAS. In SA, it is restricted to higher parts of the Mount Lofty Range.

Interestingly, while nematodes from galls collected in NSW and SA appear to be different species from D2/D3 analysis (voucher specimens nos 347 and 273, Fig. 78), whose positions in the inferred tree were not supported, molecular analysis of the associated flies showed that they were the same species (S. Scheffer, unpub. data). It is unclear if the apparent differences in sequences from the nematodes are a) artifact or b) indicate that there has been genetic drift in the nematodes, possibly reflecting geographic distance or multiple foundresses. Further work on the fly/nematode mutualism is needed.

The *Fergusobia/Fergusonina* galls collected from *E. viminalis* had a similar form to that of galls from *E. stuartiana* now *E. bridgesiana* pictured by Currie (1937), and the morphology of the respective associated fly larvae is also similar (see Fig. 11 in Currie 1937). The distributions of both host species overlapped, and both belong to Section *Maidenaria*, but to differing Series (respectively, *Viminales* and *Bridgesiana*). Thus, *Fergusobia* Morphospecies 11 may be close to the type species for the genus, *F. tumefaciens*. While the sketches in Currie (1937) do suggest similarity in body shape of the nematodes from *E. viminalis* and *F. tumefaciens*, re-collection of the latter is needed to assess morphological similarity, and molecular identity.

'Leafy' leaf bud galls also occur on *E. planchoniana*, a host species with a disjunct distribution from that of *E. viminalis*, and lacking close genetic similarity with it (from subgenus *Eucalyptus* and *Symphyomyrtus* respectively). This seems an example of similarity of gall form reflecting the particular meristematic tissue galled.

Section *Adnataria*

E. microcarpa Maiden

(a) Flower bud galls

Fergusobia voucher specimen no 1; associated with an undescribed species of *Fergusonina* (Figs 5, 27, 48). Clade 5 in Fig. 78.

Form of gall. Flower bud galls (Currie 1937, Giblin-Davis *et al.* 2004a, Taylor *et al.* 2005), club-shaped, larger than uninfested flower buds. Locules appear to originate in a proliferation of anther cells, and each is attached to floret wall by an anther filament. Filaments in infested buds have 2–3x the diameter of those in uninfested buds. Locules membrane-bound. Operculum not released.

Morphology of nematodes. Parthenogenetic female open C-shape, medium size; oesophageal gland large; short conoid tail, tip broadly rounded. Male medium to large size, J-shape; oesophageal gland medium; angular spicule; bursa *ca* 20% body length.

Morphology of dorsal shield. (WINC 004878). Shield comprises a heavily sclerotised, broad plate on posterior margin of TS 3, confluent with one on AS 1, and a shorter plate on anterior margin of AS 2. Two short, forward projecting, blunt teeth arise from the posterior margin of AS 1.

Possible relationships. Clade 5 comprised these nematodes from *E. microcarpa* and also those forming PG from *E. microcarpa*, and FBG from *E. fibrosa*. Analyses of sequences from D2/D3 suggested that the nematodes were grouped, but with no support. With COI sequences, grouping of nematodes from *E. fibrosa* and from *C. tessellaris* was strongly supported (Fig. 79). The latter does not make biological sense, as the shield form of the associated flies differs, the respective nematodes have different morphologies, and the gall forms differ; and while the host plants have overlapping distributions in Queensland, genetically they are widely disparate coming from different genera.

The distributions of *E. microcarpa* and *E. fibrosa* overlap; and both are classified in the Section *Adnataria* but from two series (i.e., they have some genetic similarity). The associated fly larvae had similar shield forms, nematode morphology was similar, and the gall forms on the host plants were similar. This suggests that the flies and nematodes respectively come from the same lineages, and that coevolution occurred with the host plants, or there was a host switch.

(b) Flat leaf galls

Fergusobia Morphospecies 12 (voucher specimen no 67); associated with an undescribed species of *Fergusonina*. Clade 13 in Fig. 78.

Form of gall. Flat leaf galls, formed when flies apparently oviposit into the surface of a newly expanding leaf blade (Giblin-Davis *et al.* 2004a, Taylor *et al.* 2005). Galled leaves have rows of oviposition scars on the leaf surface, each with a gall locule developing beneath it. They consist of a single layer of many locules in a more or less mature leaf. All or part of the whole leaf blade is thickened by 5–10 mm, with little expansion of the leaf area.

Morphology of nematodes. Parthenogenetic female small size; open C-shape; large oesophageal glands; short conoid tail with broadly rounded tip. Infective female small to medium size; straight to arcuate or open C-shape; cylindroid; short broad tail; V 80–90%. Male small to large; almost straight to J-shape; medium to large oesophageal glands; short tails varying in width; weak angular spicules; bursa 25–50% body length.

Morphology of dorsal shield. (WINC 004853, 004861-3, 004866-7). Shield comprises two patches of heavily sclerotised cuticle. First is a patch at the posterior margin of TS 3, confluent with a patch at the anterior margin of AS 1, with areas of sparse raised sclerotised spicules anterior and posterior to it. Second occurs at the posterior margin of AS 2 confluent with a patch on the anterior margin of AS 3. The first patch is larger than the second. Figure 64 shows a shield with similar form from larvae from a PG on *E. porosa*.

Possible relationships. Grouped in Clade 13; see discussion under *E. eugenioides.*

The distribution of *E. microcarpa* overlaps those of *E. eugenioides, E. siderophloia, E. leucoxylon* and *E. porosa*. *Eucalyptus porosa* and *E. microcarpa* are closely related, and the nematodes collected from them may have cospeciated with their hosts.

(c) Unilocular axial pea galls

Fergusobia voucher specimen no 70; associated with an undescribed species of *Fergusonina*. Clade 5 in Fig. 78.

Form of gall. Unilocular axial 'pea' gall; spheroid, stalked.

Morphology of nematodes. Parthenogenetic female medium size; open C-shape; large oesophageal gland; short conoid tail with broadly rounded tip. Male small; J-shape; medium oesophageal glands; short broad tails with broadly rounded tip; angular spicules; bursa *ca* 30% body length.

Morphology of dorsal shield. (WINC 004869). Dorsal shield comprising confluent plates of plain, heavily sclerotised cuticle. First, a heavily sclerotised plate on posterior margin of TS 3, confluent with a broader one on AS 1; confluent with a shorter plate on posterior margin of AS 1; confluent with a plate on anterior margin of AS 2. Patches of raised heavily sclerotised spicules occur on central areas of AS 2–5.

Possible relationships. Grouped in Clade 5; see discussion under *E. microcarpa* FBG.

E. porosa F. Muell. ex Miq.

(a) Flat leaf galls

Fergusobia voucher specimen no 66; associated with an unknown species of *Fergusonina*. Clade 13 in Fig. 78.

Form of gall. Flat leaf galls (Giblin-Davis *et al.* 2004a, Taylor *et al.* 2005). As above, for *E. microcarpa*.

Morphology of nematodes. Parthenogenetic female small to medium size; open C-shape; large oesophageal gland; short conoid tail with bluntly rounded tip. Infective female small size; straight to arcuate or open C-shape; cylindroid; short broad tail; V *ca* 85%. Male small size, arcuate to J-shape; large oesophageal gland; short tail; weak angular spicule; bursa 15–30% body length.

Morphology of dorsal shield. (WINC 004416, WINC 004270). Shield comprises patches of sclerotised cuticle; the first a small area on the posterior margin of TS 3, confluent with a smaller, heavily sclerotised patch on the anterior margin of AS 1; second a small sclerotised area on the posterior margin of AS 1 confluent with a smaller sclerotised area on the anterior margin of AS 2. Raised sclerotised spicules occur around and between the patches of sclerotised cuticle. Fig. 66 shows a shield with similar form from FLG on *E. odorata*.

Possible relationships. Grouped in Clade 13; see discussion under *E. eugenioides* and *E. microcarpa.*

(b) Unilocular 'pea' galls on leaflets

Fergusobia Morphospecies 13 (voucher specimen no 69); associated with an unknown species of *Fergusonina*. Clade 13 in Fig. 78.

Form of gall. Unilocular 'pea' galls on developing leaflets within buds. Similar to those described from *E. gomphocephala*.

Morphology of nematodes. Parthenogenetic female small to medium size; open C to C-shape; large oesophageal gland; small conoid tail with rounded tip. Infective female small to medium size; straight to arcuate; cylindroid; tail short with hemispherical tip; V *ca* 85%. Male small to medium; almost straight to arcuate; short arcuate tail with bluntly rounded tip; angular spicules; bursa 15–33% body length.

Morphology of dorsal shield. From *E. porosa* (WINC 003007). Shield comprising a heavily sclerotised broad plate on posterior margin of TS 3, confluent with one on AS 1, and on anterior margin of AS 2. Four anteriorly projecting, sharp teeth arise from posterior margin of AS 1.

Possible relationships. Grouped in Clade 13; see discussion under *E. eugenioides* and *E. microcarpa.*

Series *Siderophloiae*

E. fibrosa F. Muell.

Fergusobia Morphospecies 14 (voucher specimen no 330); associated with an undescribed species of *Fergusonina*. Clade 5 in Fig. 78.

Form of gall. Flower bud galls; club-shaped, larger than uninfested flower buds. Have not been histologically examined.

Morphology of nematodes. Parthenogenetic female C-shape, medium size, enormous oesophageal gland; narrow conoid tail, bluntly rounded tip. Infective female medium to large, arcuate to J-shape; vulva 73–80%; short tail with almost hemispherical tip. Male medium to large size, arcuate to just J-shape; large oesophageal gland, tail tip bluntly rounded; angular spicule; bursa 75–80% body length.

Morphology of dorsal shield. (WINC 004201). Shield extends over three segments, comprises three fused plates. First, a broad sclerotised plate on TS 3, taking up most of its length; second a plate comprising the length of AS 1, with two sub-medial forward projecting hooked teeth and posterior edge developed into a backward projecting ridge; confluent with a third which is a sclerotised plate on the anterior of AS 2. Sclerotised raised spicules are absent.

Possible relationships. Grouped in Clade 5; see discussion under *E. microcarpa*.

E. siderophloia Benth.

Fergusobia voucher specimens nos 25, 26, 27, 28; associated with an undescribed species of *Fergusonina*. Clade 13 in Fig. 78.

Form of gall. Flat leaf galls (Giblin-Davis *et al.* 2004a). Composed of a series of locules along the leaf blade that alternate between those with typical hypertrophied cell layers and others without the hypertrophy and cells like those of the matrix, i.e., the leaf only thickens in the area of the locules. Locules formed in parenchymatous tissue, with no differentiation of cells into palisade and spongy parenchyma. Vascular tissues and oil glands scattered in gall matrix.

Morphology of nematodes. Parthenogenetic female medium size; open C-shape; large oesophageal glands; short, relatively slender conoid tail with bluntly rounded tip. Male medium size, J-shape; medium oesophageal gland; short tail; weak angular spicules; bursa *ca* 30% body length.

Morphology of dorsal shield. (WINC 003410) (Fig. 67). Shield comprises a sclerotised cuticular patch on the posterior margin of TS 3, confluent with a broad sclerotised patch on anterior margin of AS 1. There is a row of raised sclerotised spicules behind this. A second sclerotised patch occurs on the anterior margin of AS 2, with lateral areas of sparse scattered spicules. Also an area of sparse scattered spicules on anterior margin of AS 3.

Possible relationships. Grouped in Clade 13; see discussion under *E. eugenioides*.

Section *Heterophloiae*

E. fasciculosa F. Muell.

Fergusobia Morphospecies 15 (voucher specimen no 65); associated with an undescribed species of *Fergusonina* (Figs 7, 29, 50). Unplaced in Fig. 78.

Form of gall. Stigma gall (Taylor *et al.* 2005). Inconspicuous, with one to three locules. Gall is indicated by a swollen flower stigma, after the shedding of the operculum and dehiscence of stamens. An apparently unique gall form.

Morphology of nematodes. Parthenogenetic female small; arcuate; with short, broadly conoid tail. Infective female open C-shape; hemispherical tail tip. Male J-shape; spicules angular; short peloderan bursa.

Morphology of dorsal shield. (WINC 003028). Shield comprises 3 confluent plates; the first a heavily sclerotised plate on the posterior margin of TS 3 confluent with a broad heavily sclerotised plate on AS 1 and a short, broad, heavily sclerotised plate on the anterior margin of AS 2. Anterior to the plate on TS 3 there is an area of sparse, raised sclerotised spicules. A row of 8–10 short, sclerotised, anterior projecting, sharp teeth arises from the posterior margin of AS 1. A second row of much smaller teeth arises from the anterior margin of AS 2.

Possible relationships. Fly larval shield morphology from these galls is similar to that from nematodes forming TLG and FBG on other known host eucalypts in Subgenus *Symphyomyrtus*, Section *Adnataria*. Morphologically, the nematodes are also similar to those from hosts within the *Adnataria*.

The data suggest there are respective common lineages for the flies and nematodes, with differing gall forms depending on the types of meristem infested. It is not clear if this is an example of coevolution or host-switching.

Series *Melliodorae*

E. leucoxylon F. Muell.

(a) Leaf 'pea' galls

Fergusobia Morphospecies 16 (voucher specimens nos 68, 314); from *E. leucoxylon* associated with an undescribed species of *Fergusonina.* Clade 7 in Fig. 78.

Form of gall. Small, pea-like galls on young leaves. Similar to unilocular (discrete) leaf galls, but differ in that the galls have 1 to 3 hemispherical locules.

Morphology of nematodes. Parthenogenetic female small size, arcuate, open C-shape, oesophageal glands large; body narrows gradually behind vulva; short broad conoid tail, with broadly rounded tip. Infective female small, open C-shape; maximum body width at vulva; short broad tail; V 70–85%. Male small size, J-shape; oesophageal gland enormous; spicule weakly sclerotised, angular; short tail with broad tip; bursa *ca* 10% body length.

Morphology of dorsal shield. (WINC 003162) No larval material. Pupal shield comprises a heavily sclerotised broad plate on posterior margin of TS 3, confluent with one on AS 1, and on anterior margin of AS 2. A raised ridge arises from posterior margin of AS 1, bearing 3 or 4 strongly curved, anterior projecting, sharp teeth.

*Possible relationships***.** Grouped in Clade 7; see discussion under *E. gomphocephala*.

(b) Small bud galls

Fergusobia Morphospecies 17 (voucher specimen no 71); associated with an undescribed species of *Fergusonina*. Clade 8 in Fig. 78.

Form of gall. Small bud galls with 1 to 5 locules on leaf bud (Fig. 85).

Morphology of nematodes. Parthenogenetic female small, open C-shape; large oesophageal gland; tail conoid with a broadly rounded tip. Male small, barely J-shape; tail tip broadly rounded; angular (not heavily sclerotised) spicules; bursa *ca* 20% body length.

Morphology of dorsal shield. (WINC 003156) Shield comprises 2 patches of heavily sclerotised cuticle. First is a large sclerotised depression on the posterior margin of TS 3 confluent with a short broad depression on anterior margin of AS 1. Second is a short, broad sclerotised depression on posterior margin of AS 1 confluent with a short broad sclerotised depression on anterior margin of AS 2. A similar shield, from a unilocular bud gall on *E. viminalis*, is shown in Fig. 65.

Possible relationships. Grouped in Clade 8; see discussion under *E. planchoniana*.

(c) Flat leaf galls

F. fisheri Davies & Lloyd 1996 (voucher specimen no 8) from *E. leucoxylon*; associated with an undescribed species of *Fergusonina* (Figs 14, 36, 56). Clade 13 in Fig. 78.

Form of gall. Flat leaf galls (Giblin-Davis *et al.* 2004a, Taylor *et al.* 2005). As for FLG from *E. microcarpa*; see above.

Morphology of nematodes. Parthenogenetic female small size; open C-shape; large oesophageal gland; short conoid tail with bluntly rounded tip. Infective female small size; arcuate to open C-shape; cylindroid; short broad tail with tip almost hemispherical; V 75–90%. Male small, variable shape; medium to large oesophageal gland; short tail with bluntly rounded tip; angular spicule; bursa ca 20% body length.

Morphology of dorsal shield. (WINC 004895, 004898, 003384, 004893, 003400) Shield comprises two patches of heavily sclerotised cuticle. First, a small sclerotised patch on the posterior margin of TS 3, confluent with a larger patch on anterior margin of AS 1, surrounded by a few sparse raised sclerotised spicules. Second, a smaller sclerotised patch on anterior margin of AS 2, with a few surrounding sclerotised spicules.

Possible relationships. Grouped in Clade 13; see discussion under *E. eugenioides*.

d) Axial bud galls

Fergusobia voucher specimen no 741; associated with an undescribed species of *Fergusonina*. Clade 7 in Fig. 79.

Form of gall. Small axial bud galls with 2 to 5 locules.

Morphology of nematodes. Parthenogenetic female small, C-shape; large oesophageal gland; tail conoid with a narrowly rounded tip. Male small, almost straight shape; tail tip rounded; spicules angular, not heavily sclerotised; bursa *ca* 20–30% body length.

Morphology of dorsal shield. (WINC 063701) Shield comprising a heavily sclerotised broad plate on posterior margin of TS 3, confluent with one on AS 1, and on anterior margin of AS 2. A raised ridge arises from posterior margin of AS 1, bearing 3 or 4 strongly curved, anteriorly projecting, sharp teeth.

Possible relationships. Voucher specimen no 741 did not sequence for D2/D3. With COI, a group (90% support) of these nematodes and other *Fergusobia* from *E. leucoxylon* (voucher specimen no 71), *E. cladocalyx* (voucher specimen no 740) and *E*. sp. (voucher specimen no 32) was inferred. While the fly larvae associated with voucher specimen no 71 had shields of the 'two patches' form, the other fly larvae associated with this clade all had shields of the 'plates with teeth' form. It is possible that one lineage of nematodes has become associated with two lineages of flies.

The three different forms of shield on the fly larvae collected from various leaf bud galls on *E. leucoxylon* is evidence for three different *Fergusobia/Fergusonina* associations on this host. Morphologically, the nematodes forming LBG cannot be separated, and the 3rd stage *Fergusonina* fly larvae are required for initial identification of the particular nematode/fly association. This is further evidence that for the *Fergusobia/ Fergusonina* mutualism, gall form is determined by the particular meristematic tissue targeted by the fly.

Unknown species of *Eucalyptus*

1. From *Eucalyptus* sp.

Fergusobia (voucher specimen no 33); associated with an unknown species of *Fergusonina*. Clade 6 in Fig. 78.

Form of gall. Flat leaf galls (Giblin-Davis *et al.* 2004a, Taylor *et al.* 2005) from eucalypt fitting Subgenus *Eucalyptus*, Section *Capillulus* at Brisbane Forest Park, QLD. Flies apparently oviposit into the surface of a newly expanding leaf blade. Galled leaves show rows of oviposition scars on the leaf surface, each scar with a gall chamber beneath it.

Morphology of nematodes. Parthenogenetic female medium size, open C-shape; oesophageal glands large; tail short, conoid, relatively slender, finely rounded tip. Infective female arcuate, maximum width at vulva; short broad tail with bluntly rounded tip; V *ca* 80%. Male shape variable; spicules angular; tail short, conoid, tip narrowly rounded; bursa arises at or in front of secretory/excretory pore.

Morphology of dorsal shield. (WINC 003373). Shield comprises 9 transverse 'bars' of raised sclerotised spicules, all positioned mid-segment. First is on TS 2; followed by a weak transverse band of raised spicules on TS 3; bars being more prominent on TS 3 and AS 1–2; sclerotised areas becoming shorter and sparser from AS 3–7.

Possible relationships. See discussion under *E. amygdalina*.

2. From *Eucalyptus* sp.

Fergusobia (voucher specimen no 36); associated with an unknown species of Fergusonina. Clade 6 in Fig. 78.

Form of gall. Terminal leaf bud gall from *Eucalyptus* sp. at Woodburn, NSW.

Morphology of nematodes. Parthenogenetic female medium size, open C-shape; oesophageal glands large; tail short, conoid, finely rounded tip. Infective female arcuate to open C-shape, maximum width at mid-length; short broad tail with bluntly rounded tip; V 85–90%. Male arcuate shape; spicules angular; tail short, tip bluntly rounded; bursa arises near secretory/excretory pore.

Morphology of dorsal shield. From *Eucalyptus* sp. Woodburn, NSW. No voucher material.

Possible relationships. See discussion under *E. amygdalina*.

3. From *Eucalyptus* sp. (*?siderophloia* or ?*fibrosa*)

Fergusobia voucher specimen no 32; associated with an undescribed species of *Fergusonina*. Clade 7 in Fig. 78.

Form of gall. Small, discrete, pea-like unilocular galls on young stems and leaf buds (Taylor *et al.* 2005) from an ironbark. Consist of discrete chambers 2–3 mm in diameter, and about 2 mm in height. Hemispherical, protrude only from one surface of a newly expanded leaf. Similar to those from *E. gomphocephala*.

Morphology of nematodes. Parthenogenetic female small size; arcuate, open C-shape; oesophageal gland large; body narrows gradually behind vulva; short conoid tail with rounded tip. Male small size, just J-shape; oesophageal gland enormous; short tail with bluntly rounded tip; spicule angular; bursa crenate, *ca* 35% body length.

Morphology of dorsal shield. (WINC 003405). Shield similar to that from galls on *E. gomphocephala*, with 4 anterior projecting teeth.

Possible relationships. Grouped in Clade 7; see discussion under *E. gomphocephala*.

4. From *Eucalyptu*s sp. (?*siderophloia* or ?*fibrosa*)

Fergusobia voucher specimen no 31, associated with an unknown species of *Fergusonina*. Did not sequence for D2/D3.

Form of gall. Leaf 'pea' (unilocular) galls. About 2–3 mm in diameter. Often found at the tip of a young expanding leaf.

Morphology of nematodes. No parthenogenetic female collected. Infective female medium size; almost straight shape; short broad tail. Male medium size, J-shaped; oesophageal gland medium; spicules angular; bursa *ca* 20% body length.

Morphology of dorsal shield. (WINC 003400). No shield or spicules.

Possible relationships. Grouped in Clade 6; see discussion under *E. tereticornis* PG.

5. From *Eucalyptus* sp.

Fergusobia voucher specimen no 37; associated with an unknown species of *Fergusonina*. Clade 11 in Fig. 78.

Form of gall. Axial bud galls, with 3 locules, collected from Woodburn, NSW.

Morphology of nematodes. Parthenogenetic female small; open C-shape; oesophageal gland large; tail short; broadly conoid, broadly rounded tip. Infective female small; open C-shape, cylindroid; short conoid tail with broadly rounded tip. Male small; J-shape; medium oesophageal gland; spicule arcuate; bursa *ca* 30% body length.

Morphology of dorsal shield. (WINC 003380). No shield or spicules.

Possible relationships. Grouped in Clade 11; see discussion under *C. ptychocarpa*.

6. From *Eucalyptus* sp.

Fergusobia voucher specimen no 328; associated with an unknown species of *Fergusonina*. Clade 11 in Fig. 78.

Form of gall. Flat leaf galls (Giblin-Davis *et al.* 2004a, Taylor *et al.* 2005) collected at Lake Tinaroo, Queensland; consisting of several distinct rows of individual locules, but only the galled part of the leaf expands. Similar to galls collected from *E.* sp. at Woodburn, NSW (voucher specimen no 38) and at Venman Bushland, QLD (voucher specimen no 29).

Morphology of nematodes. Parthenogenetic female not collected. Infective female small; variable shape; short tail with sub-truncate tip. Male medium size; straight or J-shape; medium oesophageal gland; spicule arcuate; bursa *ca* 40% body length.

Morphology of dorsal shield. No larval voucher material available.

Possible relationships. Grouped in Clade 11; see discussion under *C. ptychocarpa*.

7. From *Eucalyptus* sp.

Fergusobia voucher specimen no 38; associated with an unknown species of *Fergusonina*. Clade 11 in Fig. 78.

Form of gall. Flat leaf galls consisting of 2 distinct rows of locules, collected from Woodburn, NSW.

Morphology of nematodes. No nematodes available for examination.

Morphology of dorsal shield. No larval voucher material.

Possible relationships. Grouped in Clade 11; see under *C. ptychocarpa*.

8. From *Eucalyptus* sp.

Fergusobia voucher specimen no 29; associated with an unknown species of *Fergusonina*. Clade 13 in Fig. 78.

Form of gall. Flat leaf gall (Giblin-Davis *et al.* 2004a), collected from Venman Bushland National Park, Brisbane, QLD. Composed of a series of locules along the leaf blade that alternate between those with typical hypertrophied cell layers and others without the hypertrophy and cells like those of the matrix, i.e., the leaf only thickens in the area of the locules. Locules formed in parenchymatous tissue, with no differentiation of cells into palisade and spongy parenchyma. Vascular tissues and oil glands scattered in gall matrix.

Morphology of nematodes. Parthenogenetic female small size; open C-shape; large oesophageal gland; short conoid tail with broadly rounded tip. Infective female and male not collected.

Morphology of dorsal shield. (WINC 003410). Shield of 'two dots' form. Comprising two moderately sclerotised patches, the first an area of sclerotised cuticle on the posterior margin of TS 3 confluent with an area on the anterior margin of AS 1, and the second a small patch on the posterior margin of AS 1 confluent with a large sclerotised area on the anterior margin of AS 2. This is followed by a central area of sparse sclerotised spicules on AS 3. There are also a few sclerotised spicules around the sclerotised patches of cuticle.

Possible relationships. Grouped in Clade 13; see discussion under *E. microcarpa.*

9. From *Eucalyptus* sp.

Fergusobia voucher specimen no 348; associated with an unknown species of *Fergusonina*. Clade 13 in Fig. 78.

Form of gall. Flat leaf galls, collected from the ACT. Only a part (*ca* 30%) of the leaf blade was galled. Area of the leaf blade was unchanged, and, where galled, the leaf thickened to about 3 mm.

Morphology of nematodes. Parthenogenetic female small size; open C-shape; large oesophageal gland large; short conoid tail with bluntly rounded tip. Male small; almost straight to arcuate; tail with broadly rounded tip; spicules angular; bursa *ca* 30% body length.

Morphology of dorsal shield. (voucher specimen no 348). No larval material available.

Possible relationships. Grouped in Clade 13; see discussion under *E. eugenioides*.

10. From *Eucalyptus* sp.

Fergusobia voucher specimen no 351; associated with an unknown species of *Fergusonina*. Clade 13 in Fig. 78.

Form of gall. Axial unilocular pea galls; spheroid, stalked; collected from ACT.

Morphology of nematodes. Parthenogenetic female small to medium size; open C-shape; large oesophageal gland; short conoid tail with broadly rounded tip. Infective female medium size; arcuate or open C-shape; cylindroid or broadest at vulva; short broad tail with almost hemispherical tip; V 85–90%. Male medium to large size; arcuate shape; oesophageal gland large; short tail with broadly rounded tip; spicules angular, bursa *ca* 25% body length.

Morphology of dorsal shield. (WINC 004435). Shield with two square-ish patches of heavily sclerotised cuticle. The first patch comprises a larger area of sclerotised cuticle on the posterior margin of TS 3 confluent with one on the anterior margin of AS 1, and the second a small patch on the posterior margin of AS 1 conflu-

ent with a large sclerotised area on the anterior margin of AS 2. No sclerotised spicules around the patches. No teeth, but the anterior edge of the first patch has a heavily sclerotised, raised, ridge of cuticle.

Possible relationships. Grouped in Clade 13; see discussion under *E. eugenioides*.

Syzygieae

Genus *Syzygium*

S. luehmannii F. Muell.

Fergusobia voucher specimens nos 470, 735; associated with an undescribed species of *Fergusonina* (Figs 11, 33, 53). Clade 10 in Fig. 78.

Form of gall. Usually one, but up to five locules, in pea-like galls developing on leaf blade, leaf mid-rib, leaf and flower buds, or stems (Fig. 80). Similar to those described from *E. gomphocephala*. The multilocular form on stems has a warty appearance and appears to develop in a leaf axis (leaf usually dehisced).

Morphology of nematodes. Parthenogenetic female small; arcuate to open C-shape; medium oesophageal glands; short conoid tail. Infective female small; arcuate in shape; similar shape to parthenogenetic female but slimmer; small oesophageal glands; conoid tail. Male small; almost straight; oesophageal gland large; spicule arcuate, anterior part distinctly longer than posterior; tail conoid, tip bluntly rounded; bursa arises at *ca* 50% of body length.

Morphology of dorsal shield. (WINC 063818). Shield comprising 2 or 3 transverse 'bars' and many spicules; similar to those from *Mel. cajuputi* (Taylor 2004). 'Bars' comprise narrow patches of small spots of black chitin, running across body width.

*Possible relationships***.** Clade 10 comprised *Fergusobia* from *S. luehmannii* and *Met. excelsa*. This clade, inferred from analyses of sequences of D2/D3, was weakly supported in this work (Fig. 78), but received 100% support in the analysis in Ye *et al.* (2007b). Analyses of sequences from COI gave no support for the grouping (Fig. 79). The nematodes from *S. luehmannii* were morphologically similar to *F. nervosae* from *M. nervosa* (K.A. Davies, unpub. data). However, *F. pohutukawa* Davies 2007 was morphologically closest to *F. jambophila* Siddiqi 1986 from flower bud galls on *S. cumini* in India (Taylor *et al*. 2007). Given the additional differences in gall and shield form and disparate host plant distributions (Australia and New Zealand), this seemed an unlikely grouping of *Fergusobia*.

Metrosideroseae

Genus *Metrosideros*

Met. excelsa Banks ex Gaertn.

Fergusobia pohutukawa (voucher specimen no 359); associated with *Fn. metrosiderosi* Taylor 2007. Clade 10 in Fig. 78.

Form of gall. Unilocular basal shoot bud galls, described and illustrated in Taylor *et al.* (2007). Occur in vegetative and inflorescence shoot-buds, usually originating from terminal buds of the previous year's growth. Cause shortening of internodes, and swelling of base of shoot.

Morphology of nematodes. Parthenogenetic female small to medium size, with swollen cuticle; open C-shape; medium oesophageal glands; relatively long conoid tail. Infective female unknown. Male small; almost straight to arcuate, cuticle not swollen; tail bluntly rounded; spicule angular, anterior part longer than posterior; bursa arises near stylet knobs (Taylor *et al.* 2007).

Morphology of dorsal shield. Fn. metrosiderosae (WINC 063746). Shield restricted to a small area with about 20 sparse raised sclerotised spicules towards anterior margin of AS 1, about 50 towards anterior margin of AS 2, and about 20 medially on AS 3 (Taylor *et al.* 2007).

Possible relationships. Grouped in Clade 10; see discussion under *S. luehmannii.*

Three possible evolutionary processes for the occurrence of the *Fn. metrosiderosi*/*F. pohutukawa* association in New Zealand were suggested (Taylor *et al*. 2007). The first two inferred directional long distance dispersal of *Fergusonina*/*Fergusobia* from Australia to New Zealand, with a host-switching event from each of *Eucalyptus* and *Syzygium*, respectively. However, *Syzygium* is genetically closer to *Metrosideros* than to *Eucalyptus* (Wilson *et al.* 2001, 2005; Sytsma *et al*. 2004), so switching to the former may be more likely. The third inferred speciation was via conventional Gondwanan vicariance.

Melaleuceae

Genus *Melaleuca*

1. *M. leucadendra* clade

M. argentea W.V. Fitzg. (voucher specimen no 469); associated with an unknown species of *Fergusonina.* Clade 17 in Fig. 78.

F. cajuputiae (voucher specimen no 46); from *M. cajuputi* Powell associated with *Fn. purcelli*. Clade 17 in Fig. 78.

F. dealbatae (voucher specimens nos 18, 19, 352, 331); from *M. dealbata* S.T. Blake associated with *Fn. makinsoni*. Clade 17 in Fig. 78.

F. quinquenerviae (voucher specimens nos 22, 23); from *M. fluviatilis* Barlow associated with *Fn. turneri*. Clade 16 in Fig. 78.

F. leucadendrae (voucher specimens nos 461, 451); from *M. leucadendra* (L.) L. associated with *Fn. centeri*. Clade 17 in Fig. 78.

F. nervosae (voucher specimen no 49); from *M. nervosa* (Lindl.) Cheel SBG associated with *Fn. schefferae* (Fig. 18). Clade 17 in Fig. 78.

Fergusobia sp., from *M. nervosa* PG (voucher specimen no 47); associated with *Fn. goolsbyi*. Clade 17 in Fig. 78.

F. quinquenerviae SBG (voucher specimens nos 341, 342, 421, 336, 333, 338, 432); from *M. quinquenervia* (Cav.) S.T. Blake associated with *Fn. turneri* (Figs 17, 39, 59). Clade 16 in Fig. 78.

Fergusobia sp., from *M. stenostachya* (voucher specimen no 48); associated with an unknown species of *Fergusonina.* Clade 17 in Fig. 78.

F. viridiflorae from *M. viridiflora* (voucher specimen no 21); associated with *Fn. burrowsi*. Clade 17 in Fig. 78.

Form of gall. Terminal and axial shoot bud galls from *M. quinquenervia* (Fig. 81), *dealbata*, *fluviatilis*, *leucadendra* and *viridiflora* (Giblin-Davis *et al.* 2004a). As in other galls induced by the fly/nematode association, the locules within the galls were lined with hypertrophied cells, each with a granular appearance, and one enlarged nucleus with a large nucleolus. A variant, collected from *M. stenostachya* and described as a 'leaf gall', contained one central locule surrounded by layers of leaf material, formed as a result of extensive leaf folding, back and forth on itself (Giblin-Davis *et al.* 2004a). In this form, eggs and nematodes were apparently deposited between the leaves of a developing bud.

Giblin-Davis *et al.* (2001) described the development of shoot bud galls on *M. quinquenervia*. Flies deposit their eggs into the youngest part of the shoot including apical meristematic cells associated with leaf or inflorescence primordia. The first evidence of bud swelling and primordial leaf fusion occurred 35 days after deposition of eggs and nematodes. Hypertrophied parenchymal cells and nematode juveniles were present at this time. The first parthenogenetic females were seen in galls dissected after 44 days. By 66 days, the fly eggs had hatched, and galls had primordial leaf fusion and proliferation of parenchymal cells with formation of elliptical shaped locules. The locules were lined with callus-like cells.

Morphology of nematodes. Parthenogenetic female small; arcuate to open C-shape; medium to enormous oesophageal gland, with flexure in some species; tail short, conoid, varying from relatively slender to bluntly rounded to broadly rounded tip. Infective female small to medium; arcuate to open C-shape; oesophageal gland medium to large; cylindroid, maximum width at mid-body; tail conoid, with bluntly or broadly rounded tip. Male small to large; arcuate to J-shape; oesophageal gland medium to enormous; spicule variable; bursa variable (30% to arising near head) (Davies & Giblin-Davis 2004).

Morphology of dorsal shield. Associated *Fergusonina* fly larvae described in Taylor (2004). Dorsal shield of fly larvae comprises narrow to broad black sclerotised plates. Morphology ranges from: shield with 5 to 7,

narrow to broad, black sclerotised plates (simplest) to shield with 6 to 7, narrow to broad, black sclerotised plates; with prominent rugose nodules on anterior portion of plates AS 1–2 or 1–3 (most elaborate).

Possible relationships. There was support for Clade 17 of *Fergusobia* associations from broad leaf melaleucas from analyses of sequences of both D2/D3 and COI (Fig. 78), although it was not strong (93%). The clade was also supported by analyses reported for *Fergusobia* in Ye *et al.* (2007b) and for *Fergusonina* in Scheffer *et al.* (2004).

In this clade, nematodes and flies appear to have co-evolved with their host species. There is molecular evidence for cryptic species in both *F. quinquenerviae* and *F. dealbatae* (Ye *et al.* 2007b) (Fig. 78), which is supported by evidence from sequencing of COI for cryptic species in *Fn. turneri*, respectively developing on *M. quinquenervia* and *M. fluviatilis* (Scheffer *et al.* 2004).

Interestingly, *F. quinquenerviae* is commonly collected from SBG on *M. quinquenervia* in the southern part of its range, but not from northern QLD. However, it is frequently collected from similar galls on *M. fluviatilis* in northern QLD (M. Purcell, pers. com., K.A. Davies and RM Giblin-Davis, unpub. data). This was confirmed by sequences from both D2/D3 and COI (Ye *et al.* 2007b, Fig. 11). The lack of *F. quinquenerviae* on *M. quinquenervia* in the north while it flourishes there on *M. fluviatilis* suggests that, over time, something changed with the association on *M quinquenervia* and that a host-switch to *M. fluviatilis* allowed the species to survive. However, why *F. quinquenerviae* does not re-colonise *M. quinquenervia* in the north is unclear.

2. from *M. nervosa*

Fergusobia voucher specimen no 47 (Davies & Giblin-Davis 2004); associated with *Fn. goolsbyi* Taylor 2004 (Fig. 29). Clade 17 in Fig. 78.

Form of gall. 'Basal rosette' (pea) axial stem galls. Giblin-Davis *et al.* (2004a) examined the histology of the galls from *M. nervosa*. Galls form at the base of the stem below an apical bud, and contain 1 to 5 locules. The gall is formed in ground meristem or pith tissue below the bud, and there is no distinct difference between cells of the gall and surrounding plant tissues. As in other gall forms, hypertrophied cells surround the lumen of the locules.

Morphology of nematodes. Only parthenogenetic females collected (Davies and Giblin-Davis, 2004). Small, open C-shape; greatest width in posterior half of body; oesophageal gland large; tail short, conoid, tip rounded.

Morphology of dorsal shield. Associated with *Fn. goolsbyi* (Taylor 2004). Shield comprises rows of raised spicules on TS 1 and TS 2, three broad sclerotised plates (as two confluent areas) on TS 3 and AS 1 and 2. Lacks raised sclerotised nodules.

Possible relationships. Clade 18 comprised these nematodes from *M. nervosa* and also from SPG on *M. nodosa*. Analyses of sequences from D2/D3 provided weak (70%) support for this grouping of nematodes (Fig. 78). With analyses of sequences from COI (Fig. 79), the nematodes grouped together but without support. The gall forms from which the nematodes were collected were similar. Coming from broad-leaved and narrow-leaved groups of *Melaleuca*, the host plants were genetically disparate, and their distributions are unlikely to overlap. While nematodes from *M. nodosa* were not available for comparison, the dorsal shield of the fly larvae was similar to that of *Fn. purcelli* from *M. cajuputi* (Taylor 2004).

3. From *M. quinquenervia* (voucher specimen no 357)

Fergusobia voucher specimen no 357; associated with an unknown species of *Fergusonina* (Figs 19, 40, 60). Unplaced in Fig. 78.

Form of gall. 'Basal rosette' terminal and axial stem galls from *M. quinquenervia*. Superficially at least, 'basal rosette' stem galls on *M. quinquenervia* and *M. nervosa* are similar. Both occured at the base of the stem below the apical bud, and contained 1 to 5 locules. Fly eggs and nematodes were apparently deposited in ground meristem below the apical bud (Taylor, 2004).

Morphology of nematodes. Parthenogenetic female small size, arcuate in shape; broad; oesophageal gland medium; uterus non-extensile; tail short, conoid, bluntly rounded tip. Infective female small, arcuate shape; relatively slender. Male small, arcuate shape; tail short, conoid; broadly rounded tip; spicule angular to arcuate, bent at about 40% of length, with manubrium and shaft longer than blade; bursa *ca* 40–50% body length.

Morphology of dorsal shield. (WINC 063820). Shield comprises a few sparse sclerotised spicules medially on TS 3, followed by a broader band of spicules on posterior margin of TS 3. There is an additional band of spicules medially on AS 1, and a thin indistinct band on the anterior margin of AS 2. In some specimens, this is followed by a very indistinct narrow medial band of spicules on AS 2.

Possible relationships. These nematodes from RBG from *M. quinquenervia* did not group with any other *Fergusobia* species. From analyses of D2/D3 sequences, they placed basal in the tree, but without support (Fig. 78). From COI analyses, they grouped with *Fergusobia* from *M. nodosa* and *Met. excelsa*.

4. From Narrow leafed Melaleucas

(a) Unilocular terminal shoot bud galls, from *M. armillaris* (Sol. ex Gaertn) Smith.

Fergusobia Morphospecies 19 (voucher specimens nos 410, 405) associated with *Fergusonina* sp. (Taylor 2004). Clade 2 in Fig. 78.

Form of gall. Unilocular terminal shoot bud galls; with form resembling a spear head.

Morphology of nematodes. Parthenogenetic female medium, arcuate; large oesophageal gland; short conoid tail; extensile uterus with up to 5 eggs. Infective female small, arcuate, tail conoid with broadly rounded tip; oesophageal gland small. Male small, arcuate, tail barely concave on dorsal side, tip bluntly rounded; relatively large oesophageal gland; angular spicule with rounded manubrium; smooth bursa arises at 60% body length or near head.

Morphology of dorsal shield. (WINC 004407). Collected Sydney (Taylor 2004). Shield comprises bands of raised spicules, widest and most prominent on TS 3 and AS 1 to AS 4 (5 bars), with a narrow dense band between them (4 bars); all bars situated mid-segment.

Possible relationships. Clade 2 comprised *Fergusobia* from similar gall forms on *M. armillaris* and *M. linariifolia*. It was inferred with 100% support from analyses of sequences of D2/D3 (Fig. 78). Its grouping with *F. magna*, however, was not well-supported (62%). From analysis of COI sequences (Fig. 79), *Fergusobia* from *M. armillaris* grouped with those from *M. decora* but with poor support, and those from *M. linariifolia* were grouped with nematodes from *E. camaldulensis* SBG again with poor support.

While the relationship of the two *Melaleuca* hosts is unknown, both belong to the group of narrow-leaved melaleucas, and their distributions overlap. Both fly/nematode associations induce unilocular galls. The associated fly larvae have similar shield forms.

Parthenogenetic females from *F. magna, M. armillaris, M. linariifolia,* and *E. camaldulenis* SBG all have extensile uteri. This is unusual in *Fergusobia* (K.A. Davies, unpub. obs.) and suggests that nematodes with this particular morphology could have a common origin. More sampling is needed to understand the relationships of these nematodes.

(b) from *M. linariifolia* Smith

Fergusobia Morphospecies 20 (voucher specimen no 412), associated with an undescribed species of *Fergusonina*. Clade 2 in Fig. 78.

Form of gall. Unilocular axial bud galls from *M. linariifolia*. Stalked, with form resembling a spear head. See Fig. 86, from *M. decora,* for photograph of a similar gall form.

Morphology of nematodes. Parthenogenetic female medium, arcuate; relatively large oesophageal gland; short conoid tail; extensile uterus with up to 5 eggs. Infective female small, arcuate; oesophageal gland small; tail conoid with broadly rounded tip. Male small, arcuate; relatively large oesophageal gland; tail barely concave on dorsal side; tip bluntly rounded; relatively large oesophageal gland; angular spicule with rounded manubrium; smooth bursa arises at 60% body length or near head.

Morphology of dorsal shield. (WINC 004401). Dorsal shield comprising 6 or 7 transverse bands of raised sclerotised spicules. First a short, broad, band on TS 2; then a broader band on TS 3; followed by prominent transverse ridges of raised sclerotised spicules on AS 1–4. Each of these is situated mid-segment, and that on AS 2 is most prominent. AS 5 has a transverse band of a few raised spicules.

Possible relationships. Grouped in Clade 2; see discussion under *M. armillaris***.**

(c) from *M. nodosa*

Fergusobia voucher specimen no 411; associated with an unknown species of *Fergusonina*. Clade 17 in Fig. 78. See Fig. 18 for drawing of a similar nematode.

Form of gall. Unilocular basal shoot bud galls on *M. nodosa* (voucher specimen no 411). Galls occur at the base of the stem below an apical bud, and prevent expansion of the shoot bud, which appears to be compressed length-wise.

Morphology of nematodes. No nematode morphological voucher material available, only material sequenced.

Morphology of dorsal shield. (WINC 004406). No larval material available. On pupa, shield weakly sclerotised. Comprising three confluent areas of sclerotised cuticle; the first a transverse band on posterior margin of TS 3, the second occupying the full length of AS 1, and the third the anterior margin of AS 2; with an additional band of sclerotised cuticle on the anterior margin of AS 3. A few sclerotised spicules occur on posterior margin of TS 2 and anterior margin of AS 1. Similar to that of *Fn. purcelli* Taylor 2004 from *M. cajuputi* (Taylor 2004).

Possible relationships. Grouped in Clade 17; see discussion under *M. nervosa* BSG.

(d) from *M. decora*

Fergusobia voucher specimen no 462; associated with an unknown species of *Fergusonina*. Did not sequence for D2/D3.

Form of gall. Unilocular axial bud galls from Tea Gardens/Hawks Nest, NSW; similar to those described for *M. linariifolia.*

Morphology of nematodes. Parthenogenetic female small; arcuate shape; relatively slender; oesophageal gland large; tail broadly conoid to sub-cylindrical with broadly rounded tip. Infective female arcuate shape, small; relatively broad; tail short with broadly rounded tip. Male straight to arcuate shape, small; tail conoid with broadly rounded tip; spicule arcuate; bursa *ca* 30–50% body length.

Morphology of dorsal shield. (WINC 004402). Shield comprises a short, broad band of raised sclerotised spicules on TS 2; followed by a short narrow band between TS 2 and 3; a short broad lightly sclerotised band on TS 2 with sparse raised sclerotised spicules laterally; a short narrow patch between TS 3 and AS 1; a long, broader sclerotised patch between AS 1 and 2; a short narrow sclerotised patch mid-AS 2 with sparse raised sclerotised spicules laterally; a short narrow sclerotised patch between AS 2 and 3; a weak sclerotised patch mid-AS 3 with sparse sclerotised spicules laterally; a weak sclerotised spot between AS 3 and 4; and a transverse band of sparse sclerotised raised spicules on AS 4.

*Possible relationships***.** *Fergusobia* from *M. decora* did not sequence for D2/D3, but with COI a group of these nematodes and those from *M. armillaris* was inferred (Fig. 79). While gall forms and shield morphology of the associated fly larvae from the two samples were similar, nematodes from *M. decora* appeared to lack the extensile uterus found in those from *M. armillaris*.

Key to species and morphospecies of *Fergusobia* nematodes from *Corymbia, Angophora, Metrosideros,* narrow-leaved *Melaleuca, Syzygium* and *Eucalyptus*, based on host plant, gall forms, morphology of nematodes, and associated fly species

1. *Fergusobia* from galls on *Corymbia* spp. 2
   Fergusobia from galls on *Angophora* spp. 3
   Fergusobia from galls on *Metrosideros* spp. ..... *F. pohutukawa*

Fergusobia from galls on *Syzygium* spp. ..... *F. jambophila*
Fergusobia from galls on *Eucalyptus* spp. 4
Fergusobia from narrow leaved *Melaleuca* spp. 15
Fergusobia from soil not from gall ..... *F. indica*

2. *Fergusobia* from flower bud galls ..... *F. ptychocarpae*
Fergusobia from axial bud ('stem') galls ..... *F. magna*
Fergusobia from leaf pea galls ..... *F.* morphospecies 3

3. Males with bursa occupying 25–55% body length, angular spicules ..... *F.* morphospecies 1
Males with bursa occupying 60–80% body length, arcuate spicules ..... *F.* morphospecies 2

4. *Fergusobia* from terminal shoot bud galls 5
Fergusobia from 'leafy' leaf bud galls 6
Fergusobia from flower bud galls 8
Fergusobia from leaf pea galls 12
Fergusobia from flat leaf galls 14
Fergusobia from style galls ..... *F.* morphospecies 15
Fergusobia from axial bud ('stem') galls ..... *F.* morphospecies 10
Fergusobia from axial pea galls ..... *F.* morphospecies 16

5. Males with bursa usually arising in anterior third of nematode ..... *F.* morphospecies 8
Males with bursa usually arising at 40–60% of length of nematode ..... *F.* morphospecies 7
Males with bursa usually arising in posterior third of nematode ..... *F. brittenae*

6. Males with bursa arising near excretory pore ..... *F.* morphospecies 6
Males with bursa occupying <50% of body length 7

7. From galls on *E. bridgesiana*; male tails *ca* 40 µm long ..... *F. tumifaciens*
From galls on *E. viminalis*; male tails 20–35 µm long ..... *F.* morphospecies 11

8. Males with bursa occupying 66% of body length ..... *F. brevicauda*
Males with bursa occupying <60% of body length 9

9. Parthenogenetic females with raised circum-oral area, males with bursa occupying >75% of body length ..... *F.* morphospecies 14
Parthenogenetic females with raised circum-oral area, males with bursa occupying *ca* 25% of body length ..... *F.* morphospecies 5
Parthenogenetic females without raised circum-oral area 10

10. Male tail with truncate tip ..... *F. philippinensis*
Male tail with rounded tip 11

11. Males with bursa occupying 15–20% body length ..... *F. curriei*
Males with bursa occupying 25–40% body length ..... *F.* morphospecies 4

12. Males with bursa occupying 30–50% body length ..... *F.* morphospecies 9
Males with bursa occupying <30% body length 13

13. Parthenogenetic female body narrows sharply behind vulva ..... *F.* morphospecies 13
Parthenogenetic female body narrows gradually behind vulva ..... *F.* morphospecies 17

14. Parthenogenetic females with hemizonid 7–8 annules in front of secretory pore ..... *F. fisheri*
Parthenogenetic females with hemizonid 1–4 annules in front of secretory pore ..... *F.* morphospecies 12

15. Males with bursa occupying *ca* 80% body length.....*F.* morphospecies 19
Males with bursa occupying *ca* 60% body length ..... *F.* morphospecies 18.

Descriptions of Species and Morphospecies

Fergusobia brevicauda

Parthenogenetic female open C-shape, 350–410 µm long, cephalic region with circum-oral area flat, enormous oesophageal gland, one to three eggs present in vagina, short conoid tail; male arcuate, with larger tail than female, bursa arising in anterior third of body, spicules angular and heavily sclerotised, tail tip bluntly to broadly rounded; infective female arcuate, vulva at 76–85%, tail with broadly rounded tip (see Fig. 1 in Siddiqi 1994); from flower bud galls on *E. deglupta* at Bulolo in PNG; associated with an unknown species of *Fergusonina;* no information on shield.

Fergusobia brittenae

Parthenogenetic female C-shape, 330–460 µm long, cephalic region cap-like, circum-oral area slightly raised, large oesophageal glands, oviduct with oocytes not in rows, body conoid behind vulva, slender conoid arcuate tail >23 µm with rounded tip (Fig. 3); male almost straight to J-shape, with bursa at 20–40%, angular spicule >20 µm long; tail tip bluntly rounded (Fig. 25); infective female arcuate, ratio a 9–14, vulva at 72–77%, tail tip broadly rounded (Fig. 46); from TLG on *E. camaldulensis,* associated with *Fergusonina lockharti* with larvae having a heavily sclerotised shield with five rake-like teeth.

Fergusobia curriei

Parthenogenetic female open C-shape, 275–370 µm long, cephalic region with circum-oral area flat, oesophageal glands large, no or one egg in uterus, broad conoid tail with rounded tip; male arcuate, longer than 370 µm, bursa 15–20% body length, arcuate tail with bluntly rounded tip; infective female arcuate, cylindroid, vulva at 76–82%, short tail with hemispherical tip (see Figs 1–2 in Fisher & Nickle 1968); from flower bud galls on *E. camaldulensis*; associated with *Fergusonina tillyardi* Tonnoir with elaborate heavily sclerotised shield having rake-like teeth.

Fergusobia fisheri

Parthenogenetic female open C-shape, 230–305 µm long, cephalic region with circum-oral area flat, oesophageal gland large to enormous, oviduct with oocytes not in rows, no or one eggs in uterus, body tapers gradually behind vulva, hemizonid 7–8 annules in front of the secretory/excretory pore, short broad conoid tail with broadly rounded tip; male variable in shape, bursa 25–50% body length, robust angular spicules, short arcuate tail with bluntly rounded tip; infective female arcuate, cylindroid, vulva at 76–88%, short tail with hemispherical tip (see Fig. 1 in Davies & Lloyd 1996); from flat leaf galls on *E. leucoxylon*; associated with an undescribed species of *Fergusonina* having larvae with shield comprising confluent patches of heavily sclerotised cuticle with ‘hourglass’ form.

Fergusobia indica

From soil in India; gall form and host unknown; parthenogenetic females C-shape to spiral, more than 500 µm long, cephalic region dome-like with circum-oral area slightly raised, oesophageal gland relatively small (b’=4), oviduct with multiple oocytes per row, body tapers behind anus, tail slender, arcuate, conoid (see Fig. 2 in Siddiqi 1986a); males and infective female unknown; no data on associated *Fergusonina* flies.

Fergusobia jambophila

Small nematodes, with arcuate to C-shaped parthenogenetic female to 300 µm long, and males 400 µm with arcuate spicules; parthenogenetic females with swollen cuticle, cephalic region with circum-oral area raised, large oesophageal gland, oviduct with one to two oocytes per row, uterus with one egg, conoid tail with bluntly rounded tip; male arcuate, bursa 60–70% body length, spicules arcuate, tail arcuate with rounded tip; infective female unknown (see Fig. 3 in Siddiqi 1986a); from flower bud galls on *Syzygium cuminii*; associated with *Fergusonina syzygii* Harris having shield comprising several patches of sclerotised cuticle with patterns of raised ridges and spicules.

Fergusobia magna

Parthenogenetic female C-shaped with long slender tails, 420–700 µm long, cephalic region with circum-oral area raised, relatively small oesophageal gland (b' 4–9), oviduct with two oocytes per row, relatively long uterus usually with 2 or more eggs and often hatched juveniles, body tapers gradually behind vulva, relatively slender conoid tail with rounded tip; male arcuate or J-shape, bursa 30–45% body length, angular spicules, arcuate tail narrows to form slender terminus with rounded tip; infective female arcuate, with a slender conoid tail and vulva at 50–60% body length; from axial bud ('stem') galls on *C. tessellaris* (see Figs 1–12 in Davies *et al.* 2010); associated with *Fergusonina* sp. larvae with vestigial shield and yellow pupae.

Fergusobia philippinensis

Parthenogenetic female arcuate to C-shaped, 260–390 µm long, cephalic region with circum-oral area flat, oesophageal gland large to enormous, no or one eggs in uterus, body tapers gradually behind vulva, conoid tail with broadly rounded tip; males arcuate, bursa 33% body length; more or less arcuate spicules, short arcuate tail with truncate tip; infective female arcuate, cylindroid, vulva at 70–82%, tail with hemispherical or truncate tip (see Fig. 2 in Siddiqi 1994); from flower bud galls on *E. deglupta* in The Philippines; associated with an unknown species of *Fergusonina;* no information on dorsal shield.

Fergusobia pohutukawa

Parthenogenetic female with swollen cuticle, arcuate, 245–365 µm long, arcuate, cephalic region with circum-oral area raised, oesophageal glands large, oviduct with two oocytes per row, no or one eggs in uterus, body tapers gradually behind vulva, conoid tail with rounded tip that may have mucron; male almost straight to arcuate, bursa arises at level of stylet, angular spicules, slender arcuate tail with bluntly rounded tip; infective female not seen (see Fig. 3 in Taylor *et al.* 2007); from basal shoot bud galls on *M. excelsa*; associated with *Fergusonina metrosiderosi*, having a vestigial shield.

Fergusobia ptychocarpae

Parthenogenetic female C-shape, 320–460 µm long, cephalic region with circum-oral area flat, large oesophageal glands, oviduct with two to four oocytes per row, body tapers gradually behind vulva, no or one egg in uterus, relatively slender tail with bluntly rounded tip; male J-shape, bursa 50–80% body length, spicules angular, tail arcuate with bluntly rounded tip; infective female open C to J shape with body strongly curved behind vulva, cephalic region clearly offset, vulva at 77–89%, tail short with hemispherical tip (see Figs 14–23 in Taylor & Davies 2008); from flower bud galls on *C. ptychocarpa*, associated with *Fergusonina giblin-davisi* with larvae having a vestigial shield and yellow pupae.

Fergusobia tumifaciens

Parthenogenetic female open C-shape, 415 µm long, ratio a 7–8, tail 40 µm long, tip bluntly rounded; male just J-shape, 415 µm long, a ratio 8–9, spicules 21 µm long, angular, tail 40 µm long, with bluntly rounded tip; infective female unknown (see Figs 26–28 in Currie 1937); from 'leafy' leaf bud galls on *E.*

bridgesiana in the Canberra and southern tablelands areas of NSW; associated with *F. carteri* Tonn. with larvae having shield comprising two patches of heavily sclerotised cuticle.

Fergusobia Morphospecies 1

Parthenogenetic female C-shape, 350–470 µm long, cephalic region with circum-oral area flat, large oesophageal glands, oviduct with oocytes not in rows, body tapers gradually behind vulva, no or one egg in uterus, tail arcuate, slender, with bluntly rounded tip (Fig. 13); male arcuate to J-shape, bursa arising at 25–55% of body length, spicules angular, tail curved with bluntly rounded tip (Fig. 35); infective female arcuate, cylindroid, vulva at 77–83%, short tail with hemispherical tip (Fig. 55); from terminal leaf bud galls on *Angophora floribunda*; associated with an undescribed species of *Fergusonina*, no larval shield.

Fergusobia Morphospecies 2

Parthenogenetic female variable shape, 310–430 µm long, cephalic region with circum-oral area flat, oesophageal gland large, oviduct with oocytes not in rows, no or one eggs in uterus, body tapers gradually behind vulva, arcuate conoid tail with bluntly rounded tip; males arcuate to C-shape, bursa crenate, arising at 60–80% of body length, arcuate spicules; arcuate tail >20 µm long with broadly rounded tip; infective female arcuate to C-shape, maximum width in posterior half of body, vulva at 76–85%, short tail with hemispherical tip; from TLG on *Angophora* sp. in south-eastern QLD; associated with an undescribed species of *Fergusonina* with larvae lacking shield.

Fergusobia Morphospecies 3

Almost straight to arcuate nematodes; parthenogenetic female 310–400 µm long, cephalic region with circum-oral area raised, oesophageal glands medium to large, oviduct with two oocytes per row, no to three eggs in uterus, body tapers gradually behind vulva, short conoid tail with bluntly rounded tip (Fig. 15); male almost straight to arcuate, circum-oral area raised, long bursa arising at 90–95% body length, arcuate spicules, conoid tail with bluntly rounded tip (Fig. 37); infective female almost straight, vulva at 80%, conoid tail with bluntly rounded tip (Fig. 57); from pea galls on leaves of *Corymbia* sp.; associated with an undescribed species of *Fergusonina* lacking a shield.

Fergusobia Morphospecies 4

Parthenogenetic female arcuate to C-shape, 290–385 µm long, cephalic region with circum-oral area flat or slightly raised, oesophageal gland large, oviduct with oocytes not in rows, no to three eggs in uterus, body shape variable behind vulva, tail with bluntly rounded tip; male J-shape, bursa 25–40% body length, angular robust spicules, arcuate tail with bluntly rounded tip; infective female arcuate or J-shape, vulva at 77–82%, vulval plate present, tail tip bluntly rounded to hemispherical; from flower bud galls on *E. macrorhyncha*, associated with *F. nicholsoni* Tonnoir with larvae having heavily sclerotised shield with plates lacking teeth.

Fergusobia Morphospecies 5

All adult forms have cephalic regions with circum-oral areas raised; parthenogenetic female arcuate to C-shape, 300–350 µm long, oesophageal gland large, oviduct with oocytes not in rows, no or one eggs in uterus, body tapers gradually behind vulva, short conoid tail with broadly rounded tip; male just J-shape, bursa 25% body length, angular spicule, short arcuate tail with broadly rounded tip; infective female arcuate, most curved behind vulva, vulva at 83%, arcuate tail with broadly rounded to hemispherical tip; from flower bud galls on *E. eugenioides*; associated with undescribed *Fergusonina* with larvae having shield comprising three confluent dots of heavily sclerotised cuticle.

Fergusobia Morphospecies 6

Parthenogenetic female open C-shape, 250–350 µm long, cephalic region with circum-oral area flat, large oesophageal glands, body conoid behind vulva, oviduct with one or two oocytes per row, tail tip bluntly rounded (Fig. 9); male almost straight, with long, crenate bursa arising near secretory/excretory pore, spicule angular, robust, tail conoid, tip bluntly rounded (Fig. 31); infective female arcuate, cylindroid, vulva at 77–83%, tail short with hemispherical tip (Fig. 52); from 'leafy' leaf bud galls on *E. planchoniana*, associated with an undescribed species of *Fergusonina* having larvae with shield comprising seven bars of sclerotised spicule

Fergusobia Morphospecies 7

Male with crenate bursa terminating at level of cloaca; parthenogenetic female open C-shape, 345–530 µm long, cephalic region with circum-oral area flat, oesophageal glands enormous, oviduct with two or three oocytes per row, no or one eggs in uterus, body tapers gradually behind vulva, short broad conoid tail with broadly rounded tip (Fig. 6); males arcuate to C-shape, bursa 50–70% body length, robust angular spicules, short arcuate tail <20 µm long with bluntly rounded tip (Fig. 28); infective female arcuate, cylindroid, vulva at 83–90%, short tail with hemispherical tip (Fig. 49); from apical leaf bud galls on *E. delegatensis*; associated with an undescribed species of *Fergusonina* with shield comprising 8 or 9 bars of sclerotised spicules.

Fergusobia Morphospecies 8

Parthenogenetic female arcuate to C-shape, 300–475 µm long, cephalic region with circum-oral area slightly raised, large oesophageal glands, body tapers gradually behind vulva, oviduct with two oocytes per row, no or one egg in uterus, conoid tail with bluntly rounded tip; males arcuate to J-shape, bursa smooth, arising in front of secretory/excretory pore, angular spicule, tail curved with bluntly rounded tip; infective female arcuate, cylindroid, vulva at 80–86%, short tail with hemispherical tip; from apical leaf bud galls on *E. diversifolia*; associated with an undescribed species of *Fergusonina* with shield comprising 8 or 9 bars sclerotised spicules.

Fergusobia Morphospecies 9

Behind the vulva, body of parthenogenetic female tapers most on ventral side; parthenogenetic female C-shape, 240–330 µm long, cephalic region with circum-oral area slightly raised, large oesophageal gland, oviduct usually with one or two oocytes per row, uterus with one egg, tail short conoid with broadly rounded tip (Fig. 8); male arcuate to J-shaped, bursa 30–50% of body length, spicule small, angular, heavily sclerotised, tail curved with bluntly rounded tip (Fig. 30); infective female arcuate, body width greatest at level of vulva, vulva at 73–81%, short broad tail may have notch at broadly rounded tip (Fig. 51); from leaf and stem pea galls on *E. gomphocephala*; associated with *Fergusonina newmani* Tonnoir having larvae with heavily sclerotised shield with two or three teeth.

Fergusobia Morphospecies 10

Arcuate nematodes, tails sub-cylindroid, cephalic region with circum-oral areas raised; parthenogenetic female arcuate to open C-shape, 300–375 µm long, oesophageal gland enormous, oviduct with oocytes not in rows, no or one egg in uterus, body tapers gradually behind vulva, tail with broadly rounded tip (Fig. 16); male arcuate to J-shape, bursa arising at 45–80% body length, angular spicule (Fig. 38); arcuate tail with bluntly rounded tip; infective female arcuate, cylindroid, vulva at 75–85%, tail with hemispherical tip (Fig. 58); from axial bud (‘stem’) galls on *E. camaldulensis*; associated with undescribed species of *Fergusonina* having larvae with shield comprising 9 reduced bars of sclerotised spicules.

Fergusobia Morphospecies 11

Parthenogenetic female open C to C-shape, 285–405 µm long, cephalic region with circum-oral area slightly raised, large oesophageal gland, oviduct with two oocytes per row, body conoid behind vulva, short tail (Fig. 21); male with bursa 30–50% body length, almost straight to J-shape, spicules slender, angular, tail tip rounded (Fig. 43); infective female arcuate to C-shape with most curvature behind vulva, vulva at 74–82%, tail tip bluntly rounded (Fig. 61); from 'leafy' leaf bud galls on *E. viminalis*; associated with an undescribed species of *Fergusonina* with larvae having shield comprising two patches of heavily sclerotised cuticle.

Fergusobia Morphospecies 12

Parthenogenetic female C-shape, 250–360 µm long, cephalic region with circum-oral area flat, oesophageal glands large to enormous, oviduct with oocytes not in rows, no or one egg in uterus, body tapers gradually behind vulva, tail short conoid with bluntly rounded tip; males arcuate to J-shape, bursa 25–50% body length, angular spicules not heavily sclerotised, short arcuate tail with bluntly rounded tip; infective female with cephalic region barely off-set, arcuate, cylindroid, vulva at 80–87%, short tail with hemispherical tip; from flat leaf galls on *E. microcarpa*; associated with an undescribed species of *Fergusonina* having larvae with shield comprising two patches of heavily sclerotised cuticle.

Fergusobia Morphospecies 13

Parthenogenetic female open C to C-shape, 265–360 µm long, cephalic region with circum-oral area slightly raised, oesophageal gland large to enormous, oviduct with two oocytes per row, no or one egg in uterus, body narrows sharply behind vulva, tail small conoid with rounded tip; male almost straight to arcuate, bursa 15–33% body length, angular spicule not heavily sclerotised, short arcuate tail with bluntly rounded tip; infective female almost straight to arcuate, cylindroid, vulva at 85–86%, short tail with hemispherical tip; from unilocular pea galls on developing leaflets on *E. porosa*; associated with an undescribed species of *Fergusonina* having larvae with shield with 2 patches of sclerotised cuticle.

Fergusobia Morphospecies 14

Parthenogenetic female C-shape, 260–350 µm long, cephalic region with circum-oral area raised, large to enormous oesophageal gland, oviduct with oocytes not in rows, uterus with no or one egg, tail conoid with rounded tip; male arcuate or J-shape, bursa 20–40% body length, spicules robust, angular, tail curved with bluntly rounded tip; infective female arcuate or J-shape, barely offset cephalic region, body cylindroid, vulva at 73–80%, short curved tail with hemispherical tip; from flower bud galls on *E. fibrosa*, associated with an undescribed species of *Fergusonina* with larvae with heavily sclerotised shield with two short, sharp teeth.

Fergusobia Morphospecies 15

Parthenogenetic female open C-shape, with broad conoid tails with broadly rounded tips, 240–285 µm long, cephalic region with circum-oral area raised, oesophageal gland enormous, oviduct with two oocytes per row, body tapers gradually behind vulva, no or one egg in uterus, short tail with bluntly rounded tip (Fig. 7); male < 335 µm, J-shape, bursa 25–45% body length, spicule slender, angular, short tail with bluntly rounded tip (Fig. 29); infective female arcuate or C-shape, cylindroid, vulva at 80–83%, short broad tail with bluntly rounded tip (Fig. 50); from style galls on *E. fasciculosa*; associated with an undescribed species of *Fergusonina* with larvae having a heavily sclerotised shield comprising plates lacking teeth.

Fergusobia Morphospecies 16

Parthenogenetic female arcuate to open C-shape, 221–329 µm long, cephalic region with circum-oral area raised, oesophageal gland enormous, no or one egg in uterus, oviduct with one or two oocytes per row, body tapers gradually behind vulva, hemizonid immediately in front of the secretory/excretory pore, short broad conoid tail with broadly rounded tip; males almost straight to barely J-shape, bursa 10–40% body length,

angular spicule not heavily sclerotised, short arcuate tail with bluntly rounded tip; infective female arcuate, cylindroid, vulva at 80–88%, short tail with broadly rounded to hemispherical tip; from unilocular stalked, axial (pea) galls on *E. leucoxylon*, associated with an undescribed species of *Fergusonina* having larvae with heavily sclerotised shield of cuticular plates with 3 or 4 teeth.

Fergusobia Morphospecies 17

Parthenogenetic female C-shape, 170–383 µm, cephalic region with circum-oral area slightly raised, oesophageal gland enormous, uterus with no or one egg, oviducts with oocytes not in rows, tail conoid small, bluntly rounded tip; male almost straight to just J-shape, bursa 17–30% of body length, spicule slender, angular, not heavily sclerotised, tail with bluntly rounded tip; infective female arcuate, cylindroid or with greatest width at vulva, vulva at 75–83%, tail short with hemispherical tip; from small leaf bud galls with 1–5 locules on *E. leucoxylon*; associated with an undescribed species of *Fergusonina* having larvae with shield comprising two patches of sclerotised cuticle.

Fergusobia Morphospecies 18

Parthenogenetic female almost straight, 250–350 µm long, cephalic region flat, oesophageal gland large, extensile uterus, slender tail with bluntly rounded tip; male 270–350 µm long, arcuate, bursa arises at *ca* 60% body length, spicule arcuate, slender tail with rounded tip; infective female arcuate, cylindroid, vulva at 80%; short broad tail with hemispherical tip; From unilocular tip galls on *M. armillaris*, associated with *Fergusonina* sp. 5 (Taylor 2004) with larvae having bands of raised spicules and an elongate puparium.

Fergusobia Morphospecies 19

Parthenogenetic female almost straight, 270–400 µm long, cuticle swollen in many specimens, cephalic region flat, oesophageal gland relatively small (b'=3.3), extensile uterus with many eggs, short tail with bluntly rounded tip (Fig. 2); male 360–440 µm long, arcuate with most curvature at tail, bursa arises at *ca* 80% body length, spicule widely angular, slender tail with rounded tip (Fig. 24); infective female arcuate, cylindroid, vulva at 80%; short broad tail with broadly rounded tip (Fig. 45); from ULBG on *M. linariifolia;* associated with an undescribed species of *Fergusonina* with larvae having a shield comprising bands of raised sclerotised spicules.

Conclusions

Diversity within the *Fergusobia/Fergusonina* mutualism

Examination of the *Fergusobia* and *Fergusonina* specimens available in the WINC, taken together with the 32 species currently described or being described (Currie 1937; Fisher & Nickle 1968; Siddiqi 1986a, 1994; Davies & Lloyd 1996; Davies & Giblin-Davis 2004, Taylor *et al.* 2007, K.A. Davies unpub. data), plus the large number of known hosts, and the presence of multiple gall types on several host species, confirms that both *Fergusobia* and *Fergusonina* are highly diverse.

While it would be useful to determine what proportion of *Fergusobia* spp. remain unsampled, several factors make such an estimate difficult. The number of potential host plants for the association is extremely large, whether considering all Myrtaceae or just the genera known to serve as hosts (*ca* 800 spp. of *Eucalyptus*; more than 250 spp. of *Melaleuca,* etc). Many myrtaceous species have highly restricted geographic distributions, engage in local hybridization, and are difficult to both find and identify. The geographic range over which the association is recorded is considerable (Australasia, India and The Philippines) and obviously difficult to thoroughly sample, even just within Australia. For most *Fergusobia/Fergusonina* associations, there is marked seasonality to gall presence which requires repeated sampling throughout the year in order to detect even abundant species. Additionally, many *Fergusobia/Fergusonina* mutualisms are rare and/or patchy in distribu-

tion, making the apparent absence of galls insufficient for concluding that a given plant species is not a host. Taken together, these factors prevent a truly informed estimation of *Fergusobia* and *Fergusonina* diversity at this time, other than to say it is higher than previously believed.

In addition to difficulties in thoroughly sampling spacially, temporally, and across plant taxa, it is often difficult to obtain all life stages of nematodes or flies necessary for taxonomic work from a single collection. Often galls of the same type on the same host collected at one time are synchronous in development, and some life stages are missing for either or both nematodes and flies. In addition, there are large guilds of parasites and inquilines that attack both the developing flies as well as the galls themselves (Taylor *et al.* 1996; Taylor *et al.* 2005). In the most extreme case, galls eaten out by lepidopteran inquilines often contain neither nematodes nor fly larvae (K.A. Davies, unpub. data).

Speciation within the *Fergusobia/Fergusonina* mutualism

Available evidence indicates that most *Fergusobia/Fergusonina* associations are specific to host plant tissue ("gall type") and host plant species. In addition, in both the nematode and fly phylogenies, there is clear evidence of some degree of conservation of both host tissue and host taxonomy (Ye *et al*. 2007b; Scheffer *et al*. unpub. data, see also Scheffer *et al*. 2004). This can also be seen in the observations of nematode and larval fly morphology detailed here. Estimation of the degree of cospeciation driving diversification within the nematode fly association or between the associates and the host plants is not currently possible. In addition, the lack of fly and nematode fossils means that independent age estimates for their lineages cannot be made. Without such estimates, it is not possible to test hypotheses regarding plant-insect-nematode co-divergence, as postulated for the fig-wasp symbiosis (Ronsted *et al.* 2005).

From numerous studies of the evolution of resistance in agriculturally important nematodes and insects, it is known that many (pest) populations faced with resistant plant cultivars and/or pesticides are often able to develop sub-populations that can overcome plant resistance or pesticide efficacy. This rapid evolution in response to an environmental challenge is believed to be due to the combination of very strong selection acting on genetically variable pest populations. Presumably, host-switching of fly/nematode pairs onto novel plant hosts involves some sort of similar adaptive process, although we do not currently understand the details. Because most fly/nematode species feed on a single host plant species, host-shifting of the fly/nematode associates between plant species appears to be strongly related to fly/nematode speciation events. It may be that the highly extreme and often unpredictable weather patterns common in Australia cause high variability in availability of suitable oviposition sites on particular host plant species, thus giving rise to strong selection for the ability to form galls and feed on alternative host species.

Future directions in *Fergusobia/Fergusonina* research

The work reported here is obviously incomplete and does not represent a true revision of *Fergusobia*. However, these data and observations represent the most current assessment of the mutualistic relationship between *Fergusobia* and *Fergusonina*. Undoubtedly, continued sampling will reveal many new species of the nematodes and their mutualist flies, new host plants and new gall forms. Future sampling should be targeted to areas that have been inadequately sampled to date, particularly in northern Australia. This region has not been well-sampled to date and may prove especially interesting as *Eucalyptus* and *Melaleuca* may have differentiated in parallel, on the edges of northern rainforests, the former colonizing drier nutrient-poor soils and the latter seasonally flooded areas (Barlow 1988; Pryor & Johnson 1981). The finding of a new type of gall form (hollow petiole galls on *E. brevifolia)* from the Kimberley region of WA (K.A. Davies, unpub. data) is an example of the novel forms and untapped diversity that is no doubt present in many regions. In addition, future sampling should be targeted towards host plant groups comprising fewer plant species, e.g., *Corymbia* or *Angophora*, with the expectation that focused sampling on fewer species could be more thorough and might allow more rigorous testing of the hypotheses about coevolution and cospeciation of the *Fergusobia/Fergusonina* mutualism.

The *Fergusonia/Fergusonina* galling mutualism is a unique association that gives rise to questions at many levels and within many subfields of the biological and chemical sciences. The overriding goal of our work has been to document the basic biology, ecology, taxonomy, and diversity of the mutualism in order to explore coevolutionary patterns across the tritrophic nematode-fly-plant system. With a better understanding of overall evolutionary patterns, we hope to be able to better explore the evolutionary processes that have given rise to the diversity within this phenomenal nematode-fly association.

While this paper is an example of 'incomplete taxonomy' *sensu* Lorenzen (1994), it represents a starting point for further research on *Fergusobia*. In part it is a 'survey of species diversity' (Lorenzen 1994), but its overall aim is to assist in the development of hypotheses about the phylogenetic relationships of the genus.

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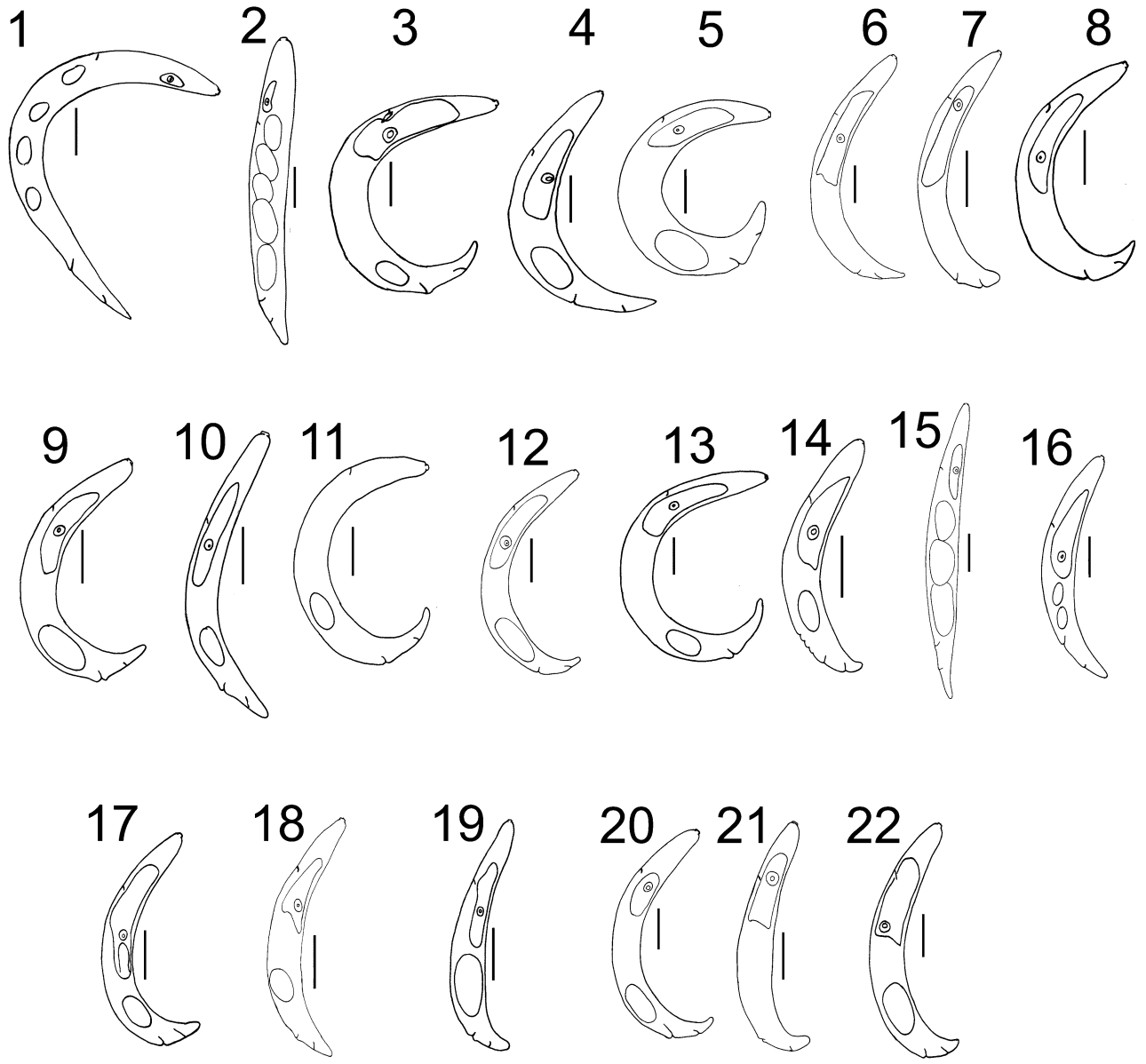
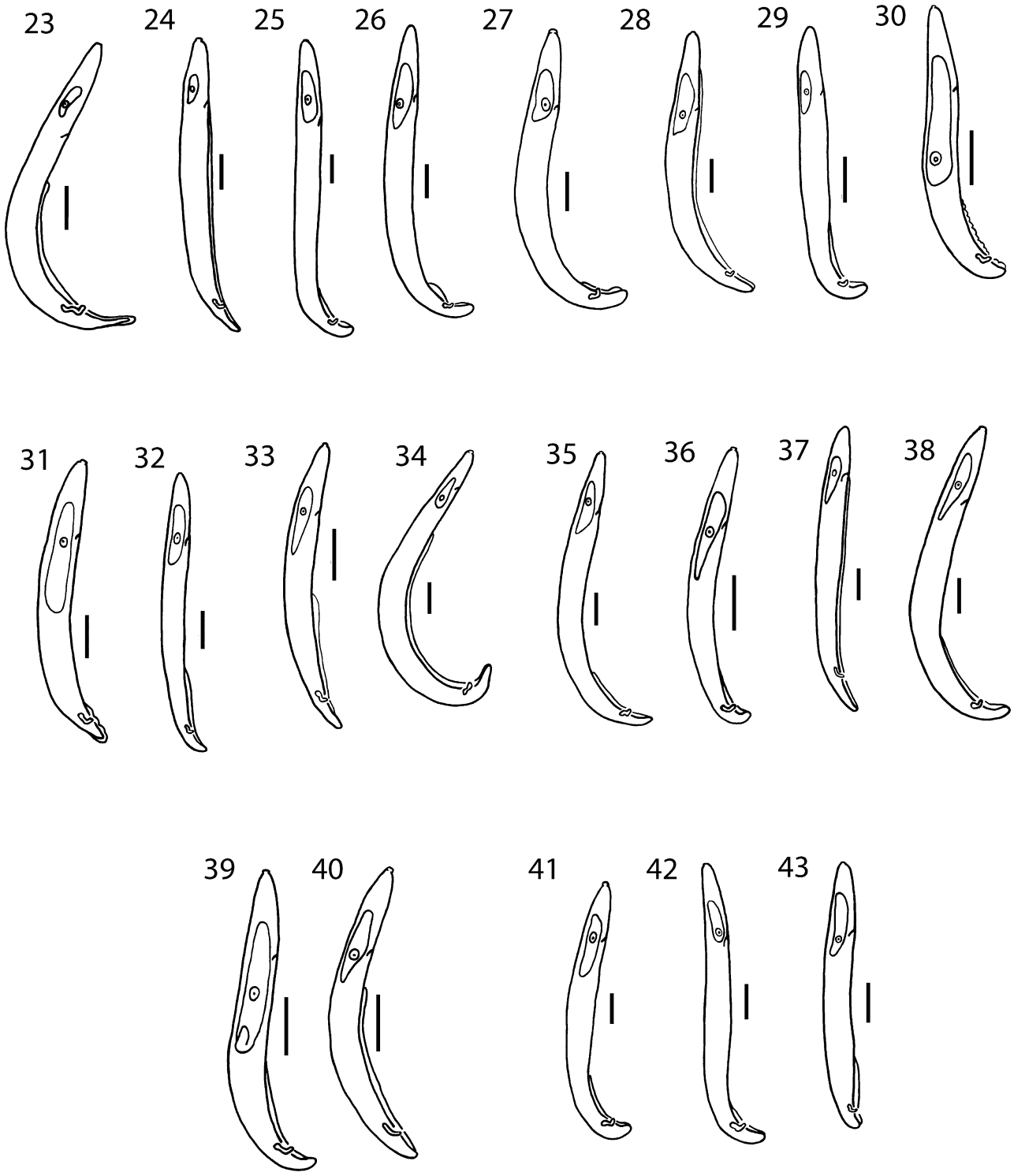
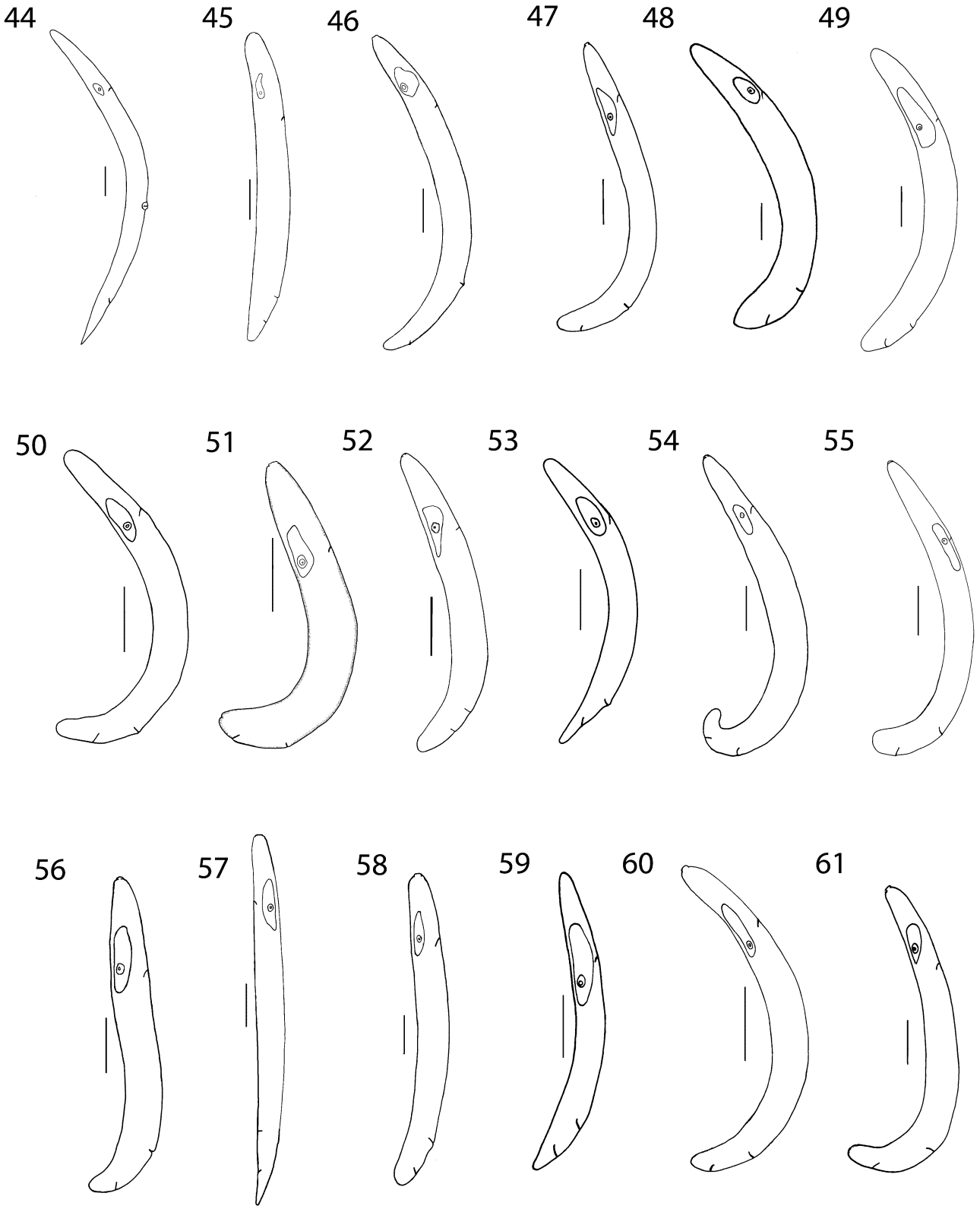


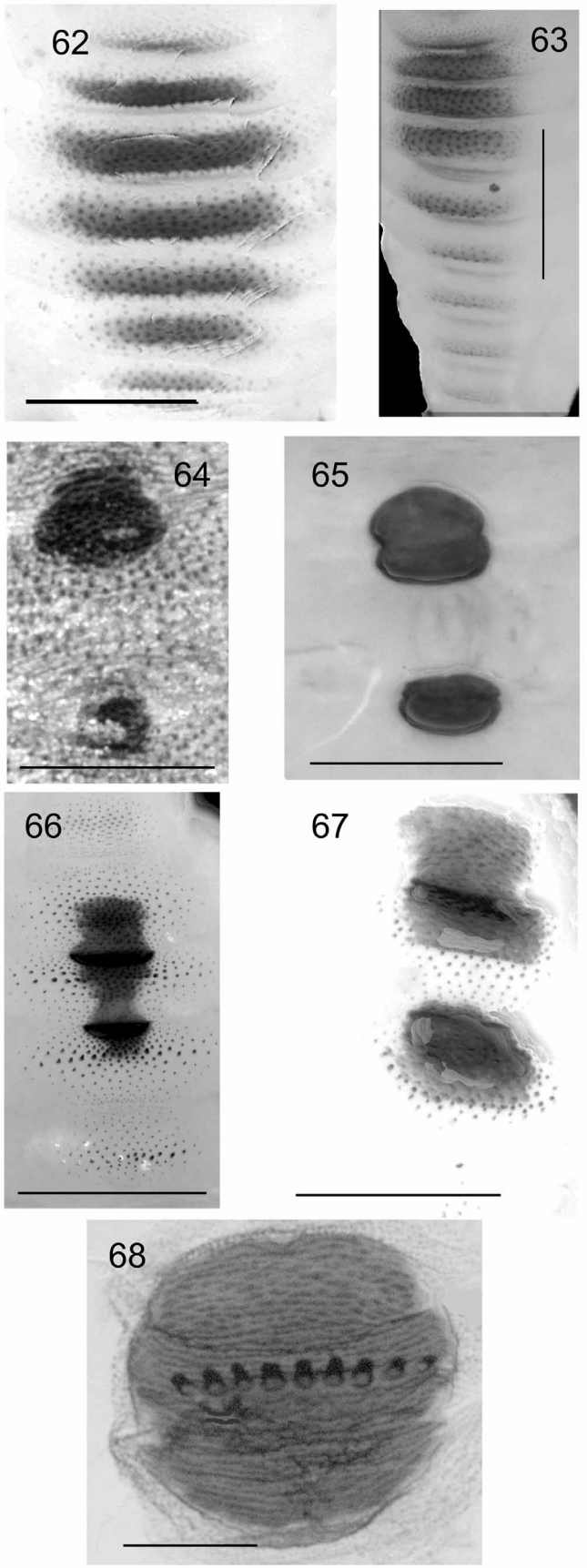
FIGURES 1–22. Habitus drawings of parthenogenetic females from *Fergusobia* clades inferred from sequences of D2/D3. 1: *F. magna* from *C. tessellaris* axial bud 'stem' galls. 2: from *M. linariifolia* SBG. 3: from *E. camaldulensis* TLG. 4: from *E. camaldulensis* FBG. 5: from *E. microcarpa* FBG. 6: from *E. delegatensis* TLG. 7: from *E. fasciculosa* style gall. 8: from *E. gomphocephala* PG. 9: from *E. planchoniana* 'leafy' LBG. 10: from *E. tereticornis* PG. 11: from *S. luehmannii* PG. 12: *F. ptychocarpae* from *C. ptychocarpa* FBG. 13: from *A. floribunda* TLG. 14: *F. fisheri* from *E. leucoxylon* FLG. 15: from *Corymbia* sp. PG. 16: from *E. camaldulensis* axial bud 'stem' galls. 17: *F. quinquenerviae* from *M. quinquenervia* SBG. 18: from *M. nervosa* BSG. 19: from *M. quinquenervia* 'rosette' gall. 20: from *E. obliqua* FBG a. 21: from *E. obliqua* FBG b. 22: from *E. viminalis* 'leafy' LBG. Scale bars = 50µm.



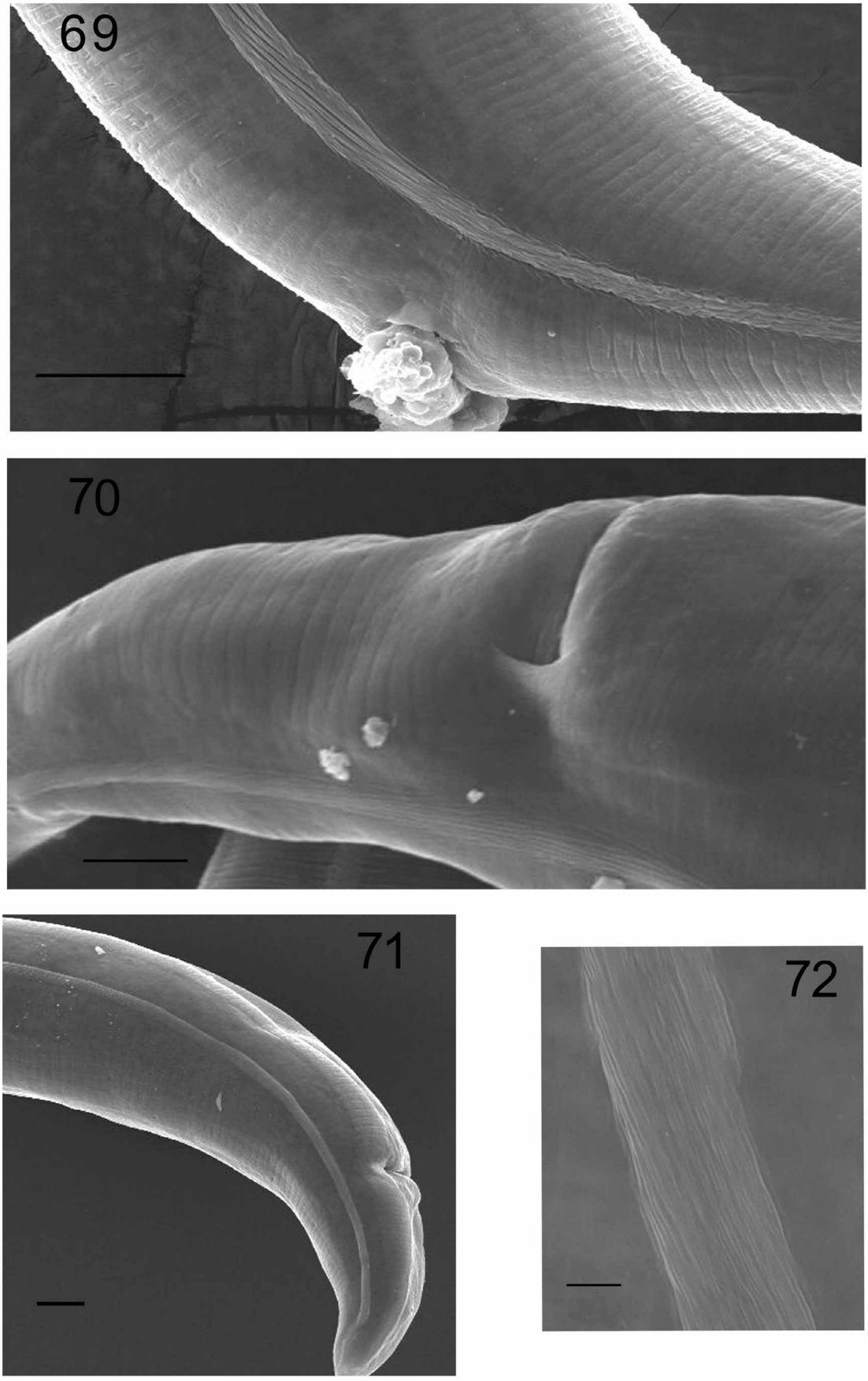
FIGURES 23–43. Habitus drawings of males from *Fergusobia* clades inferred from sequences of D2/D3. 23: *F. magna* from *C. tessellaris* axial bud 'stem' galls. 24: from *M. linariifolia* ULBG. 25: from *E. camaldulensis* TLG. 26: from *E. camaldulensis* FBG. 27: from *E. microcarpa* FBG. 28: from *E. delegatensis* TLG. 29: from *E. fasciculosa* style gall. 30: from *E. gomphocephala* PG. 31: from *E. planchoniana* 'leafy' LBG. 32: from *E. tereticornis* PG. 33: from *S. luehmannii* PG. 34: *F. ptychocarpae* from *C. ptychocarpa* FBG. 35: from *A. floribunda* TLG. 36: *F. fisheri* from *E. leucoxylon* FLG. 37: from *Corymbia* sp. PG. 38: from *E. camaldulensis* axial bud 'stem' galls. 39: *F. quinquenerviae* from *M. quinquenervia* SBG. 40: from *M. quinquenervia* 'rosette' gall. 41: from *E. obliqua* FBGa. 42: from *E. obliqua* FBGb. 43: from *E. viminalis* 'leafy' LBG. Scale bars = 50µm.



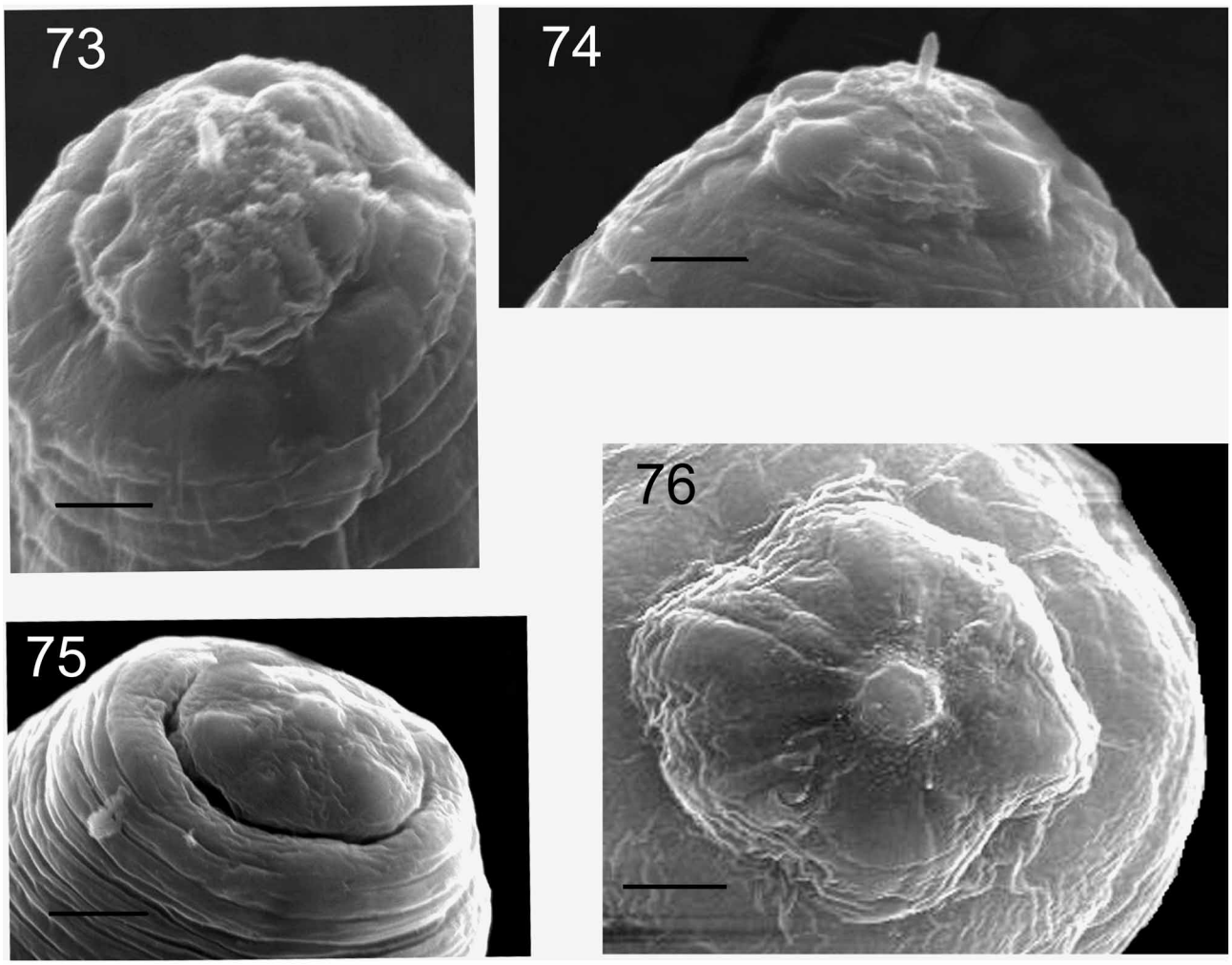
FIGURES 44–61. Habitus drawings of preparasitic infective females from *Fergusobia* clades inferred from sequences of D2/D3. 44: *F. magna* from *C. tessellaris* axial bud 'stem' galls. 45: from *M. linariifolia* 46 from *E. camaldulensis* TLG. 47: from *E. camaldulensis* FBG. 48: from *E. microcarpa* FBG. 49: from *E. delegatensis* TLG. 50: from *E. fasciculosa* style gall. 51: from *E. gomphocephala* PG. 52: from *E planchoniana* 'leafy' LBG. 53: from *S. luehmannii* PG. 54: *F. ptychocarpae* from *C. ptychocarpa* FBG. 55: from *A. floribunda* TLG. 56: *F. fisheri* from *E. leucoxylon* FLG. 57: from *Corymbia* sp. PG. 58: from *E. camaldulensis* axial bud 'stem' galls. 59: *F. quinquenerviae* from *M. quinquenervia* SBG. 60: from *M. quinquenervia* 'rosette' gall. 61: from *E. viminalis* 'leafy' LBG. Scale bars = 50µm.



FIGURES 62–68. Shield forms found on third stage larvae of *Fergusonina* fly species associated with *Fergusobia* nematodes. "Bars" form. 62: from *E. delegatensis* TLG. 63: from *Eucalyptus* sp. FLG. "Dots" form. 64: from *E. porosa* PG. 65: from *E. viminalis* ULBG. 66: from *E. odorata* FLG. 67: from *E. siderophloia* FLG. "Plates with teeth" form. 68: from *E. cosmophylla* TLG. Scale bars 62–67 = 0.02mm; 68= 0.2mm.



FIGURES 69–72. Scanning electron micrographs of lateral fields of parthenogenetic females of *Fergusobia*. 69: from *E. gomphocephala* PG. 70: from *E. tereticornis* TLG. 71: from *E. viminalis* 'leafy' LBG. 72: from *E. gomphocephala* PG. Scale bars 69–71 = 5 µm; 72 = 2 µm.



FIGURES 73–76. Scanning electron micrographs of anterior end of parthenogenetic females of *Fergusobia*. 73: from *E. planchoniana* 'leafy' LBG. 76: from *E. delegatensis* TLG. 77: from *E. tereticornis* TLG. 78: from *E. gomphocephala* PG. Scale bars 2 µm.

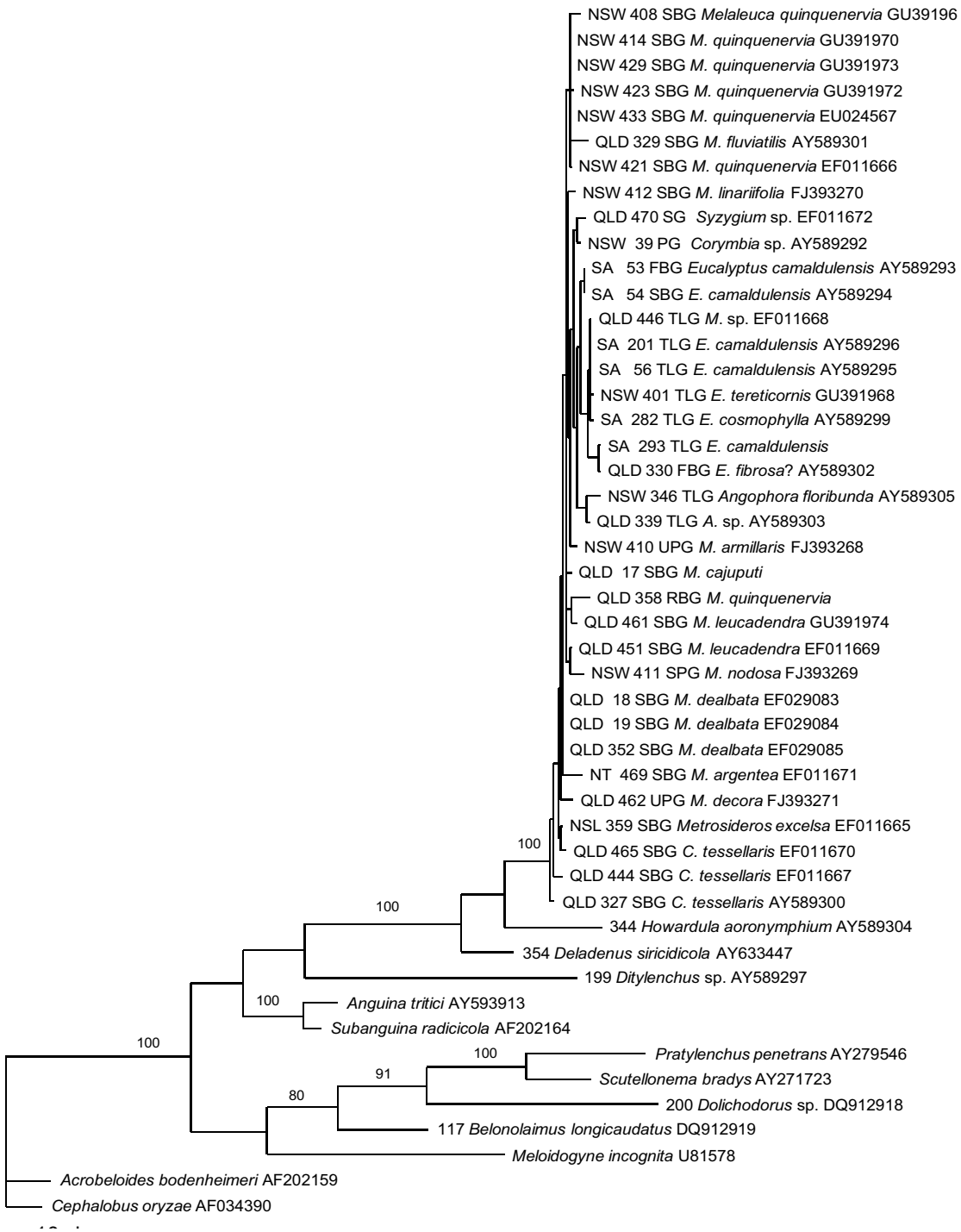


FIGURE 77. 10001st Bayesian tree inferred from 18S under GTR+I+G model (lnL=5233.1992; freqA=0.2521; freqC=0.218; freqG=0.2672; freqT=0.2626; R(a)=1.2514; R(b)=2.5545; R(c)=1.3166; R(d)=0.5886; R(e)=6.225; R(f)=1; Pinva=0.4418; Shape=0.6926). Posterior probability exceeding 50% are given on appropriate clades.

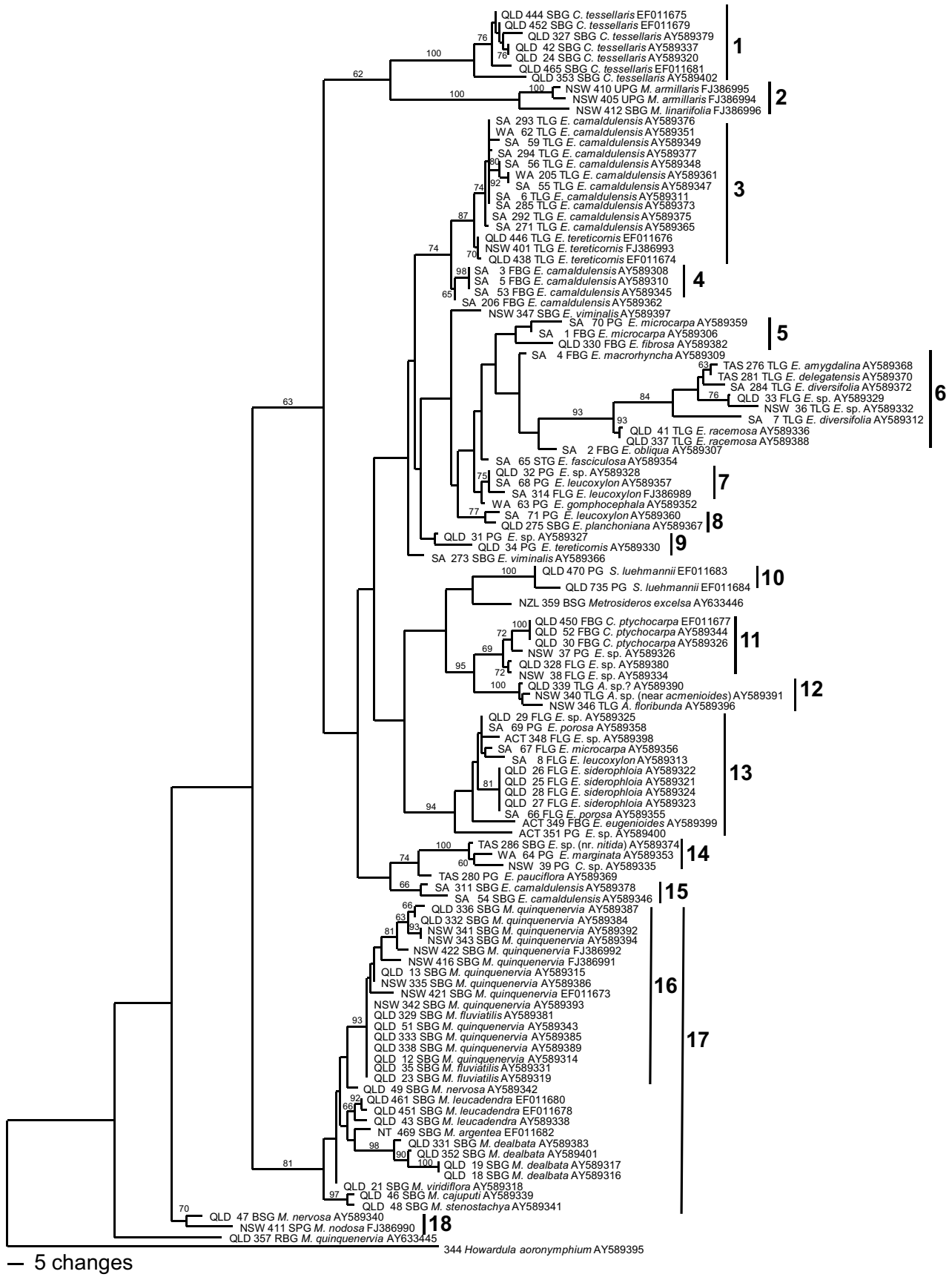


FIGURE 78. 10001st Bayesian tree inferred from D2/D3 under GTR+I+G model (lnL=5776.4028; freqA=0.3077; freqC=0.1381; freqG=0.2167; freqT=0.3375; R(a)=0.5642; R(b)=6.7737; R(c)=1.6055; R(d)=0.5194; R(e)=4.7267; R(f)=1; Pinva=0.3784; Shape=0.5658). Posterior probability exceeding 50% are given on appropriate clades. Clades are numbered 1–18.

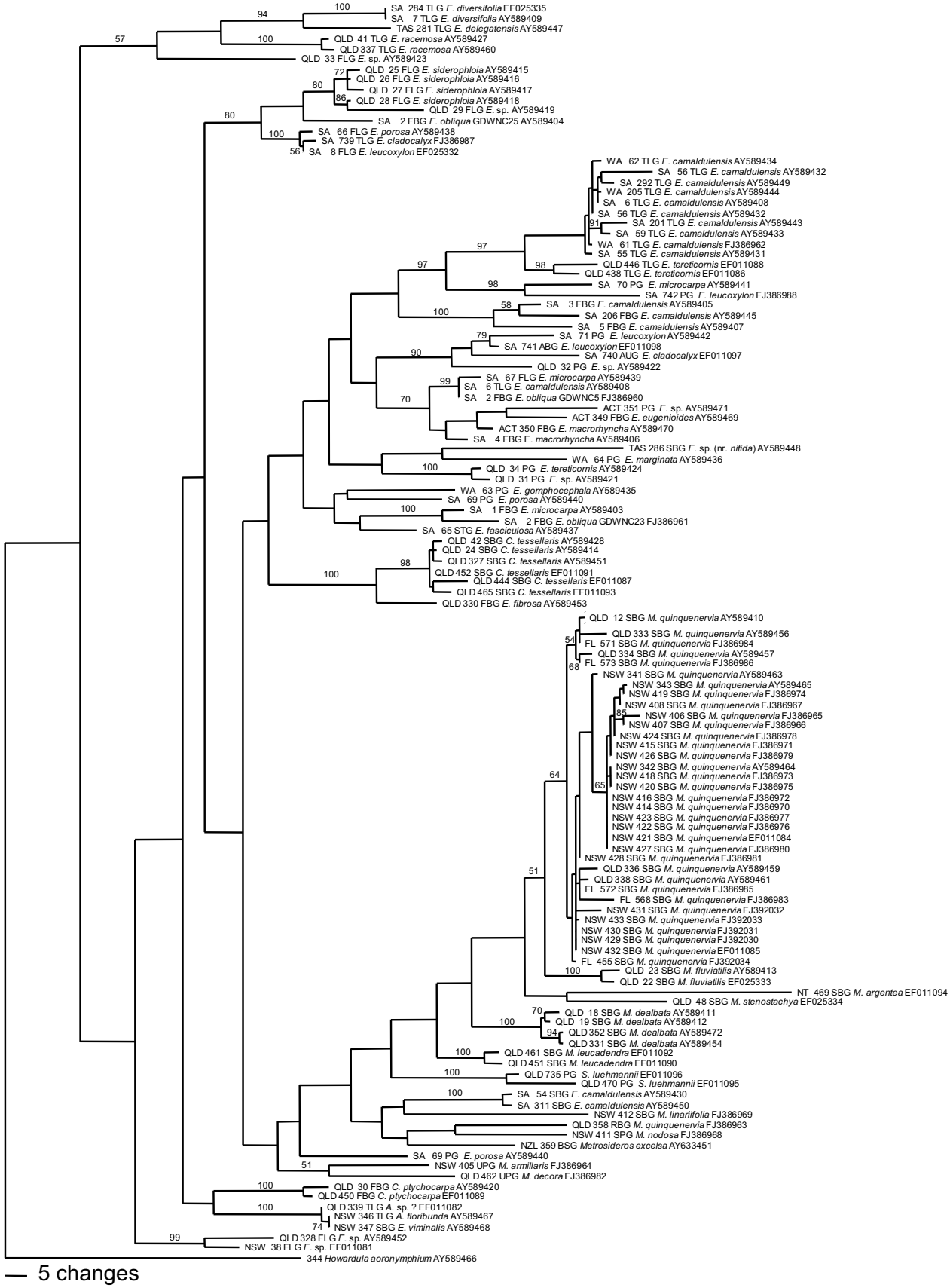
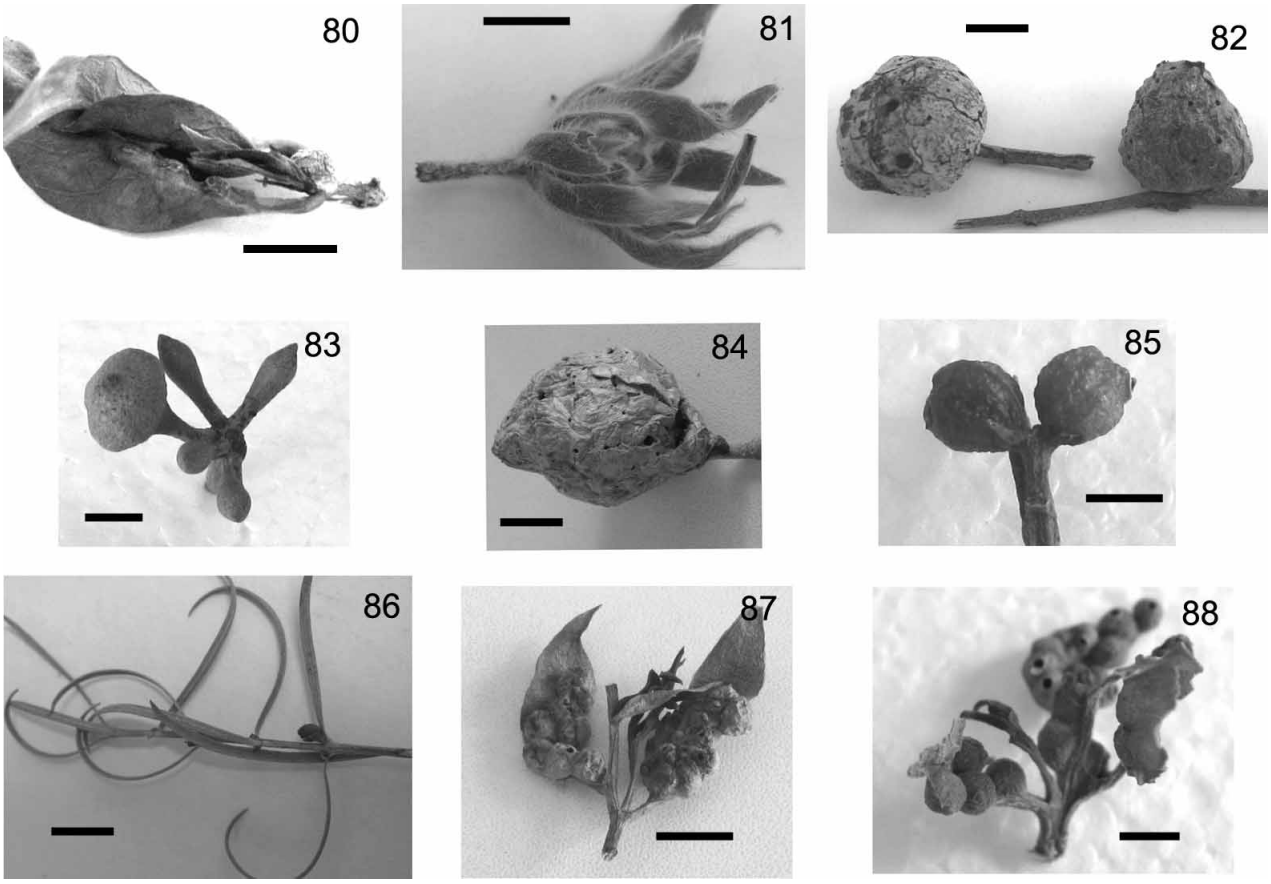


FIGURE 79. 10001st Bayesian tree inferred from COI under TVM+I+G model (lnL=7935.9834; freqA=0.2379; freqC=0.0644; freqG=0.1768; freqT=0.5209; R(a)=2.105; R(b)=8.6246; R(c)=1.5486; R(d)=1.0628; R(e)=8.6246; R(f)=1; Pinva=0.3383; Shape=0.6284). Posterior probability exceeding 50% are given on appropriate clades.



FIGURES 80–88. Gall forms induced by the *Fergusobia/Fergusonina* mutualism. 80: PG on leaf and petiole on *S. luehmannii*. 81: SBG on *M. quinquenervia*. 82: axial bud 'stem' galls on *C. tessellaris*. 83: FBG on *E. eugenioides*. 84: TLG on *E. camaldulensis*. 85: Bud galls on *E. leucoxylon*. 86: Unilocular axial bud gall on *M. decora*. 87: PG on leaf of *E. pauciflora*. 88: PG on leaf of *E. gomphocephala*. Scale bars 80–83 = 5mm; 84 = 1cm; 85–88 = 5mm