Evolution of Parasitism in Insect-transmitted Plant Nematodes[1]

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Abstract: Nematode-insect associations have evolved many times in the phylum Nematoda, but these lineages involve plant parasitism only in the Secernentean orders Aphelenchida and Tylenchida. In the Aphelenchida (Aphelenchoidoidea), *Bursaphelenchus xylophilus* (Pine wood nematode), *B. cocophilus* (Red ring or Coconut palm nematode) (Parasitaphelenchidae), and the many potential host-specific species of *Schistonchus* (fig nematodes) (Aphelenchoididae) nematode-insect interactions probably evolved independently from dauer-forming, mycophagous ancestors that were phoretically transmitted to breeding sites of their insect hosts in plants. Mycophagy probably gave rise to facultative or obligate plant-parasitism because of opportunities due to insect host switches or peculiarities in host behavior. In the Tylenchida, there is one significant radiation of insect-associated plant parasites involving *Fergusobia* nematodes (Fergusobiinae: Neotylenchidae) and *Fergusonina* (Fergusoninidae) flies as mutualists that gall myrtaceous plant buds or leaves. These dicyclic nematodes have different phases that are parasitic in either the insect or the plant hosts. The evolutionary origin of this association is unclear.

Key words: Aphelenchida, *Bursaphelenchus cocophilus,* *Bursaphelenchus xylophilus,* evolution, *Fergusobia* spp., plant-parasitism, *Schistonchus* spp., tritrophic associations, Tylenchida.

Nematodes have independently evolved many commensal (phoretic) and parasitic associations with members of the class Insecta (Giblin, 1987; Poinar, 1975). The greatest diversity of insect-nematode associations occurs in the class Secernentea with known radiations in the orders Rhabditida, Diplogasterida, Tylenchida, Aphelenchida, Oxyurida, Spirurida, Strongylida, Rhigonematida, and Ascarida (Poinar, 1975). In contrast, the Mermithida is the only known order of insect-associated nematodes that occurs in the class Adenophorea. According to a recent investigation establishing a molecular evolutionary framework for the phylum Nematoda using small subunit (SSU) rDNA sequences, plant-parasitism evolved at least twice in each class (i.e., Secernentea and Adenophorea) (Blaxter et al., 1998). Yet, only a very small subset of nematode lineages is known to be involved in tritrophic associations that use both insects and plants as hosts, and these exclusively occur in the Secernentean orders Aphelenchida and Tylenchida (Giblin-Davis, 1993; Giblin-Davis et al., 2002). The purpose of this paper is to introduce the reader to these interesting tritrophic associations and speculate on how they evolved.

Aphelenchoidoid nematode associations with insects and plants: According to Hunt (1993), there are two sub-

families in the order Aphelenchida: the Aphelenchoidea, which comprises two monogeneric families of mycophagous nematodes, and the Aphelenchoidoidea, which consists of six families of mycophagous, plant-parasitic, predatory, insect-phoretic, or parasitic nematodes (i.e., Aphelenchoididae, Seinuridae, Ektaphelenchidae, Acugutturidae, Parasitaphelenchidae, and Entaphelenchidae). Insect- and plant-associated members of the Bursaphelenchinae (Parasitaphelenchidae) include the Pine wood nematode *Bursaphelenchus xylophilus* and the Red ring nematode *B. cocophilus* (Giblin-Davis, 1993). The only other known genus in the Aphelenchoidoidea that is associated with both insects and plants is the potentially large radiation of host-specific species of *Schistonchus* (Aphelenchoididae) (Giblin-Davis, 1993; Giblin-Davis et al., 1995).

The pre-adaptations associated with phoretic transport of Aphelenchoidoid nematodes by insects appear to be critical for subsequent evolution of insect-associated plant-parasitism (Giblin-Davis, 1996). These pre-adaptations include developmental synchrony with the insect transport host in the production of a dispersal stage juvenile (dauer juvenile) or adult female to survive environmental stresses (e.g., starvation and (or) desiccation) before, during, and after transport. Some of these stresses also could have selected for internal phoresy, as in *Schistonchus*, which may have been a precursor to the evolution of insect parasitism.

Insect-associated plant-parasitism in Aphelenchoidoid nematodes probably evolved independently from dauer-forming, mycophagous ancestors that were phoretically transmitted to breeding sites of their insect hosts in plants. Mycophagy probably gave rise to facultative or obligate plant-parasitism because of opportunities due to insect host switches (e.g., *Schistonchus*), peculiarities in host behavior such as maturation feeding of the insect host on healthy plant hosts (e.g., *B. xylophilus*), or attraction of insect hosts to wounds on healthy plant hosts (e.g., *B. cocophilus*). The life histories of *B. xylophilus, B. cocophilus,* and *Schistonchus* are

reviewed here to further investigate the evolution of insect-associated plant-parasitism in the Aphelenchoidoidea.

Pine wood nematode, Bursaphelenchus xylophilus: During primary transmission of the plant-parasitic phase, dauer juveniles (JIV stage) of the Pine wood nematode *B. xylophilus,* which are phoretically associated in the tracheae of their longhorn beetle hosts (usually *Monochamus* spp.) (Cerambycidae: Coleoptera), are transmitted to young twigs of a susceptible healthy conifer host (usually *Pinus* spp.) (Fig. 1). This "maturation feeding" by a newly emerged female beetle is required for reproductive maturity. At this point, the dauer juveniles disembark from their host, perhaps in response to semiochemicals released by the beetle feeding, and enter the wound to infect, feed, and develop on pine parenchymal cells. In susceptible pines under the right conditions this can lead to classical pine wilt disease that results in the death of the tree within a year of infection (Fig. 1) (Giblin-Davis, 1993).

The maturation feeding requirement of the *Monochamus* host probably allowed for the evolution of facultative plant-parasitism in *B. xylophilus* from the ancestral mycophagous life cycle involved in secondary transmission (Giblin-Davis, 1993). As conifers wilt and die, either from pine wilt disease or other causes, they become suitable breeding hosts for *Monochamus,* and this allows for the ancestral secondary transmission pathway (Fig. 1). Dauer juveniles transmigrate out of the tracheal system of their beetle host during oviposition into these dying or dead conifer hosts where they feed, reproduce, and greatly increase their population densities on fungi. The biology of the Pine wood nematode is synchronized with the development of the progeny of the longhorn beetle, with pre-dauer juveniles (JIII) developing as conditions in the host conifer become less suitable. This is usually correlated with the development of late instar larvae or pupation of its *Monochamus* host and results in large aggregations of pre-dauer juveniles around the pupal chambers where they overwinter with the beetle. A large proportion of the pre-dauer juveniles of *B. xylophilus* molt to dauer juveniles at about the time of adult beetle emergence and are attracted to CO_2 produced by newly eclosed adults. These dauer juveniles migrate into the metathoracic spiracles where most of the CO_2 is emitted during beetle respiration.
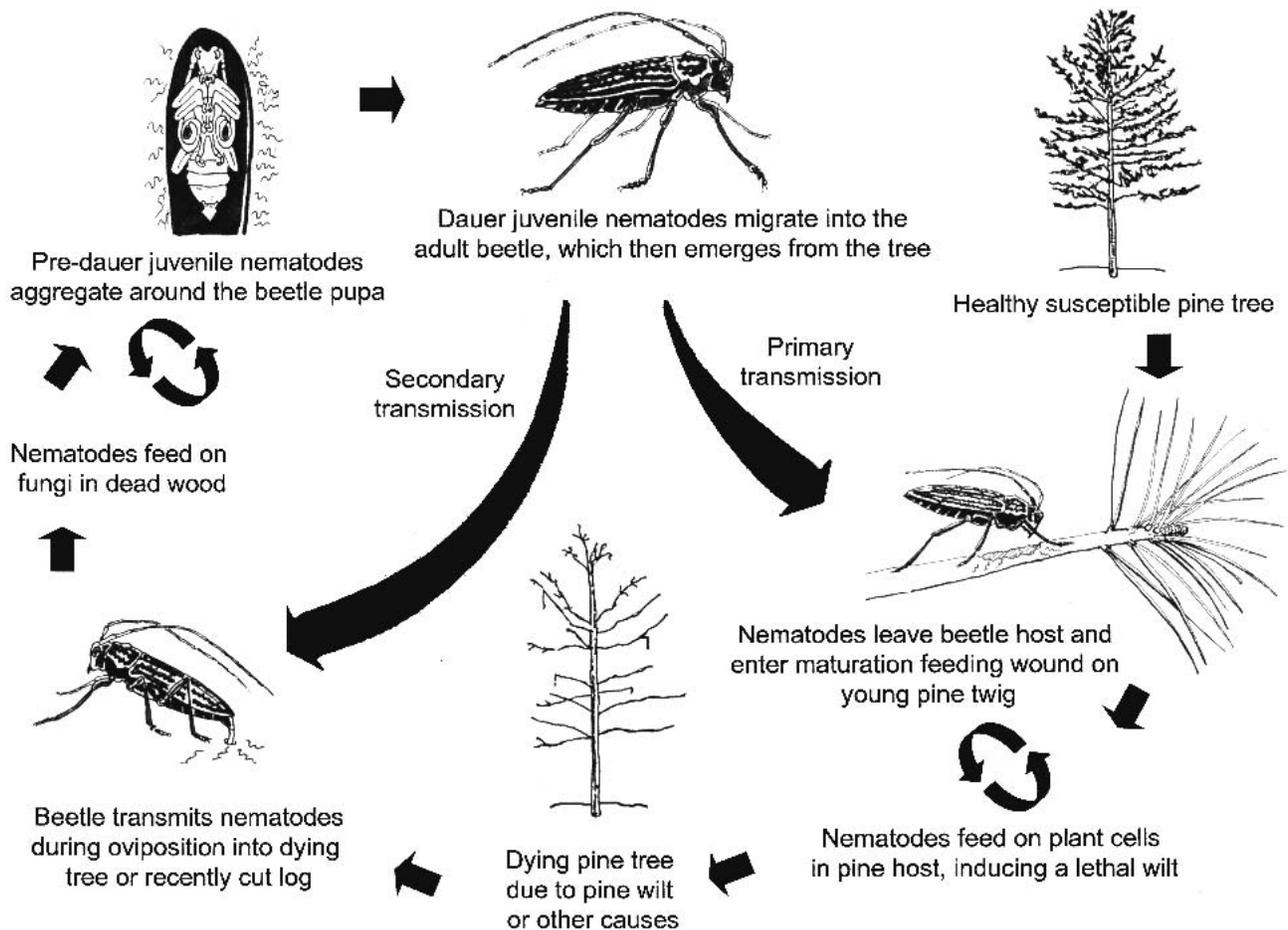

FIG. 1.   Generalized representation of the association between the pine wood nematode, *Bursaphelenchus xylophilus,* its *Monochamus* vector, and a susceptible conifer host.

The beetle host emerges from the long-dead conifer host and starts the life cycle over again, sometimes with as many as 289,000 dauer juveniles (Linit, 1988).

This association probably functions quite well without the plant-parasitic phase. In fact, the *Bursaphelenchus* species most closely related to *B. xylophilus*, such as *B. mucronatus*, *B. fraudulentus*, *B. kolymensis*, and possibly *B. abruptus*, exhibit the ancestral mycophagous and phoretic condition, and morphologically share similarly shaped and distinctive spicules with a cucullus and females with a vulval flap (Giblin-Davis, 1993; Giblin-Davis et al., 1993).

Red ring or Coconut palm nematode, Bursaphelenchus cocophilus: *Bursaphelenchus cocophilus* represents a more derived situation than that observed for *B. xylophilus* because it appears to be an obligate parasite of palms and does not feed on fungi (Giblin-Davis, 1993; Giblin-Davis et al., 1989a). *Bursaphelenchus cocophilus* shares the character of a single preanal papilla in males, helping to justify its congeneric status with *B. xylophilus* (Giblin-Davis et al., 1989b). However, there are arguments about just how closely related it is to other *Bursaphelenchus*. It was only recently transferred into the genus from the monospecific genus *Rhadinaphelenchus* (Baujard, 1989) and not without some debate (Hunt, 1993).

The biology of the Red ring nematode is less well studied than that of *B. xylophilus* but shows many similarities (Giblin-Davis, 1993). Transmission of the dauer juveniles (JIII) of *B. cocophilus* occurs chiefly via a phoretic or parasitic association with the American palm weevil, *Rhynchophorus palmarum* (Curculionidae: Coleoptera). The dauer juveniles of *B. cocophilus* do not exhibit the same level of environmental resistance as those from *B. xylophilus*, suggesting that they may need more protection or even sustenance during their association with the weevil through its metamorphosis. It is probable that tracheal infestations of adults are the main pathway for transport. As with *B. xylophilus*, both male and female insect hosts probably can transmit nematodes horizontally to each other or vertically to their plant hosts through their activities. However, in Red ring disease the main transmission route is probably through oviposition by heavily infested female palm weevils; a single female has been observed with as many as 13,000 dauer juveniles in the region of the ovipositor (Giblin-Davis, 1993).

Palm weevils are attracted to semiochemicals emanating from fresh wounds on healthy palms and use male-produced aggregation pheromones for recruitment of large numbers of insects (Giblin-Davis et al., 1996). This behavior may have allowed for the evolution of obligate plant-parasitism in *B. cocophilus*. Nematodes that are introduced into the palm can disperse and reproduce quickly, feeding as intercellular parasites on thin-walled parenchymal cells. Nematode densities increase dramatically and the damage caused by their feeding induces a symptomatic anthocyanin-rich red ring in stem cross section, which can lead to a lethal wilt in 3 to 7-year-old coconut palms within 2 to 4 months (Fig. 2) (Giblin-Davis, 1993). Dauer juveniles accumulate in the red ring tissue (up to 11,000 per gram of tissue) and associate with larval progeny of palm weevils that breed in the diseased and dying palm. The dauer juveniles are carried through metamorphosis, either in the tracheal system or internally, and some members of the weevil population emerge heavily infested to complete the life cycle.

The evolutionary lines leading to *B. xylophilus* and *B. cocophilus* probably diverged prior to the evolution of insect-associated facultative or obligate plant-parasitism from a phoretically associated, mycophagous ancestor. Based on spicule morphology, *B. cocophilus* appears to be more closely aligned with other phoretically transmitted and mycophagous members of the genus (e.g., the *B. hunti* species-group) than to the *B. xylophilus*-group (Giblin and Kaya, 1983; Giblin-Davis et al., 1989b). Phylogenetic analysis of D3 large subunit rDNA sequences supports a *B. xylophilus* grouping of mycophagous and phoretic forms that include *B. xylophilus*, *B. mucronatus*, and *B. fraudulentus* that are close to, but distinct from, *B. cocophilus* (Fig. 3). In addition, *B. abruptus* does not appear to be as closely related to the *B. xylophilus*-group as previously indicated by morphology (Fig. 3) (Giblin-Davis et al., 1993).

Fig nematodes, Schistonchus spp.: The assignment of *Bursaphelenchus* (Parasitaphelenchidae) and *Schistonchus* (Aphelenchoididae) to separate families in the current classification scheme suggests that insect-associated plant-parasitism in those groups arose independently (Hunt, 1993). Phylogenetic analysis of sequences of the D3 large subunit rDNA from nematodes from these groups is consistent with this hypothesis (Fig. 3).

Species of *Schistonchus* are associated with figs of *Ficus* species and their minute fig wasp pollinators (Agaonidae) (Giblin-Davis et al., 1995, 2003) (Fig. 4). It is presumed by Giblin-Davis et al. (1995) that most, if not all, of the more than 700 monoecious and gynodiecious species of *Ficus* in the world will each have its own unique associated *Schistonchus* species because of the intimate nature of the association.

In the Neotropics, entomophilic females of *Schistonchus* are carried in the hemocoel of the fig wasp and appear to be internal phoretics (Giblin-Davis et al., 1995). *Schistonchus caprifici* from Europe can be carried as eggs, juveniles, and adults in the host, suggesting parasitism (Vovlas et al., 1992). The nematode leaves the wasp shortly after entering the lumen of a phase B syconium (female phase fig; female florets are receptive to pollination) and parasitizes epidermal cells of the pedicels or the syconial wall (*S. aureus* from *F. aurea*) (Center et al., 1999; DeCrappeo and Giblin-Davis, 2001) or the anthers of the fig (*S. laevigatus* from *F. laevigata*) (Center et al., 1999; DeCrappeo and Giblin-
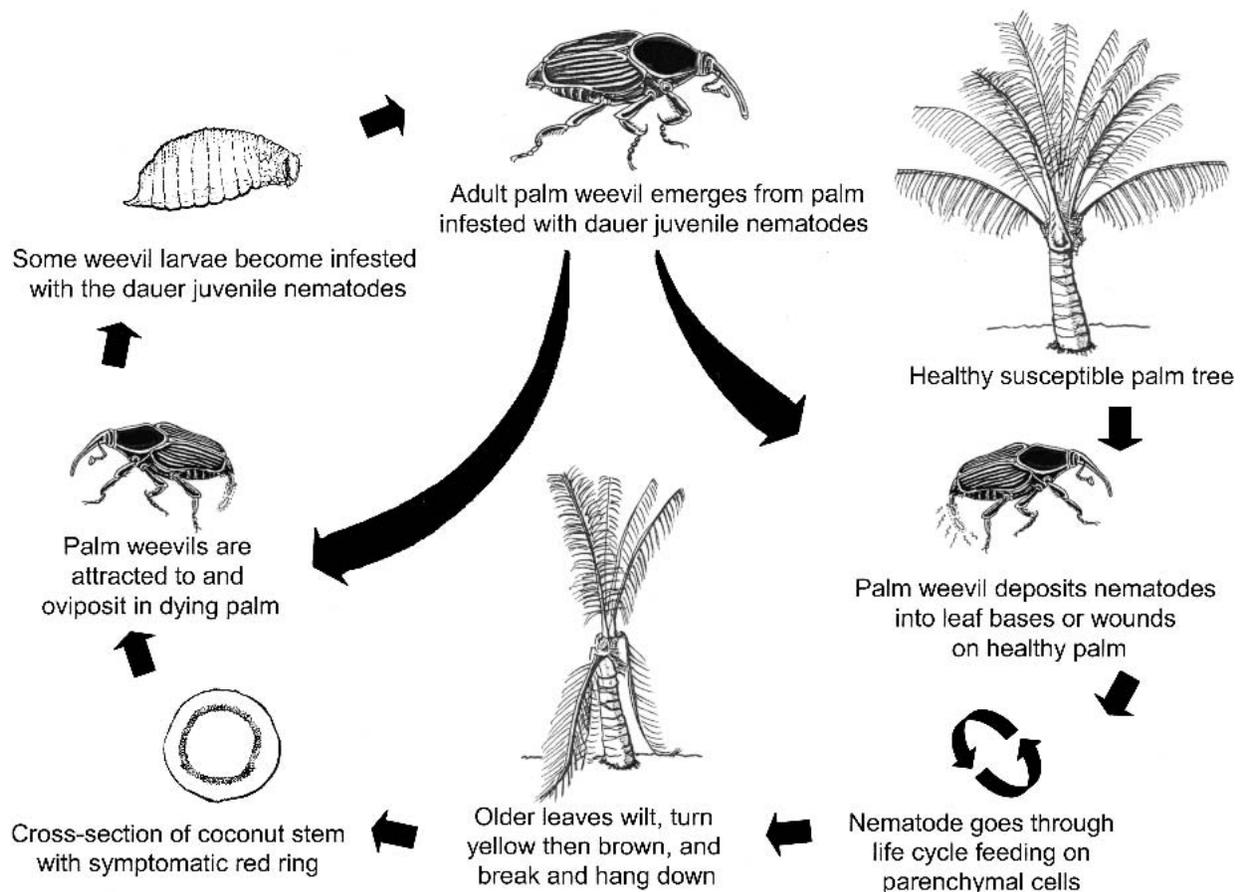
Fig. 2. Generalized representation of the association between the red ring nematode, *Bursaphelenchus cocophilus*, its palm weevil vector, and a coconut palm host.

Davis, 2001; Giblin-Davis et al., 1995), or both (*S. caprifici* from *F. carica*) (Vovlas and Larizza, 1996) (Fig. 4). In *Schistonchus*, plant-parasitism differs from typical Aphelenchoidoid plant-parasitic associations, where nematode feeding leads to cell death and necrosis. In *Schistonchus*, feeding often leads to cell hypertrophy, producing a layer of enlarged uninucleate cells, each with an enlarged nucleus and nucleolus, and granular cytoplasm indicative of increased metabolism (Center et al., 1999; Giblin-Davis et al., 1995). At least one generation of *Schistonchus* develops at the expense of the fig during phase C (interfloral phase fig; where developing fig embryos and wasp larvae and pupae occur). After about 30 days, infested phase D syconia (male phase fig; male florets with mature pollen) contain adult male and female *Schistonchus*, adult male and female wasps, and maturing fig seeds. Entomophilic female nematodes presumably mate and enter the hemocoels of emerging female wasps of the next generation. Wasps collect pollen, exit the syconium through an emergence hole created by the wingless male wasps, and seek out a new phase B syconium for oviposition. In the process, the wasp vertically transmits nematodes to the next generation of figs and fig wasps (Fig. 4) (Giblin-Davis et al., 1995). Amphimictic reproduction is presumed.

Fig wasps in the family Agaonidae belong to the superfamily Chalcidoidea, which is the largest superfamily in the Hymenoptera with estimates of more than 60,000 species (Gordh, 1979). By 1975, however, only two nematode associates had been reported for the entire superfamily, and these were from agaonid fig pollinators (Poinar, 1975). Since then, an additional 17 species of *Schistonchus* and *Parasitodiplogaster* have been reported (DeCrappeo and Giblin-Davis, 2001; Giblin-Davis et al., 1992; Giblin-Davis et al., 1995; Lloyd and Davies, 1997; Poinar, 1975; Poinar and Herre, 1991; Vovlas et al., 1998), but they all have been limited to fig-associated pollinators and inquilines in the Agaonidae. This suggests that the association between the Agaonidae and nematodes is the result of a unique evolutionary history within the Chalcidoidea.

The modern fig fruit (syconium) that functions as a "tomb blossom" is thought to have evolved from an open infructescence to an essentially closed structure to restrict access to over-exploitative beetle or fly pollinators (Berg, 1990). This allowed the ancestors of the present-day agaonid fig wasps to evolve a unique pollination niche as the exploiters were screened out. *Schistonchus* may be a remnant associate of those original pollinators that switched hosts and developed a new
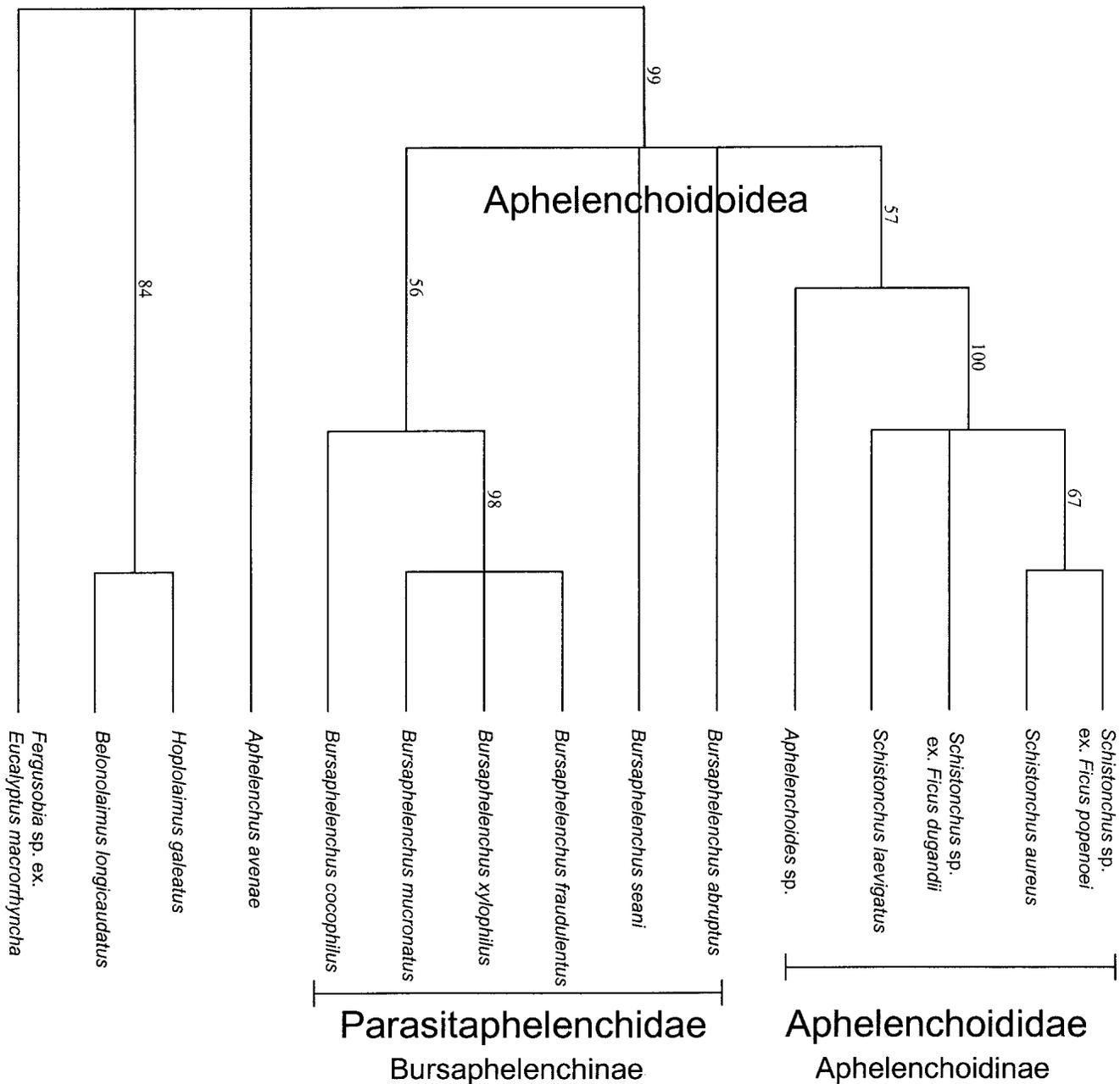
FIG. 3. Aphelenchoidoid relationships inferred from analysis of rRNA coding sequences. In the analysis generating this tree all four taxa at the bottom (i.e., *Aphelenchus avenae, Belonolaimus longicaudatus, Fergusobia* sp., and *Hoplolaimus galeatus*) were defined as outgroups. The tree is generated from an alignment of 227 basepairs of the D3 expansion segment of the lsu rRNA genes. Only unambiguously alignable regions of the sequences were used. The tree was generated by maximum parsimony in PAUP* version 4.0b8 using 1000 bootstrap replicates.

association with fig wasps during the evolution of the syconium (Fig. 5). It is presumed that the *Schistonchus* stem ancestor was a mycophagous and phoretic associate of a beetle because there are no known contemporary Aphelenchids associated with flies. Another possibility is that the pre-agaonid chalcid was already associated with nematodes before the evolution of the syconium. The recent discovery of nematodes associated with an unidentified chalcid wasp in Dominican amber (Poinar, pers. obs.) supports this idea.

Recent molecular work supports the hypothesis that

a large radiation of *Schistonchus* species occurred as a result of the genetic isolation afforded by the tightly coevolved relationships between fig wasp pollinators and figs (Giblin-Davis et al., 2003).

Tylenchid nematode associations with insects and plants; Fergusobia spp.: Fergusobia (Tylenchida: Neotylenchidae: Fergusobiinae) nematodes are involved in what appears to be the only known mutualistic association between nematodes and insects. The insects in this case are flies in the genus *Fergusonina* (Diptera: Fergusoninidae). Together they induce a variety of gall types in young mer-
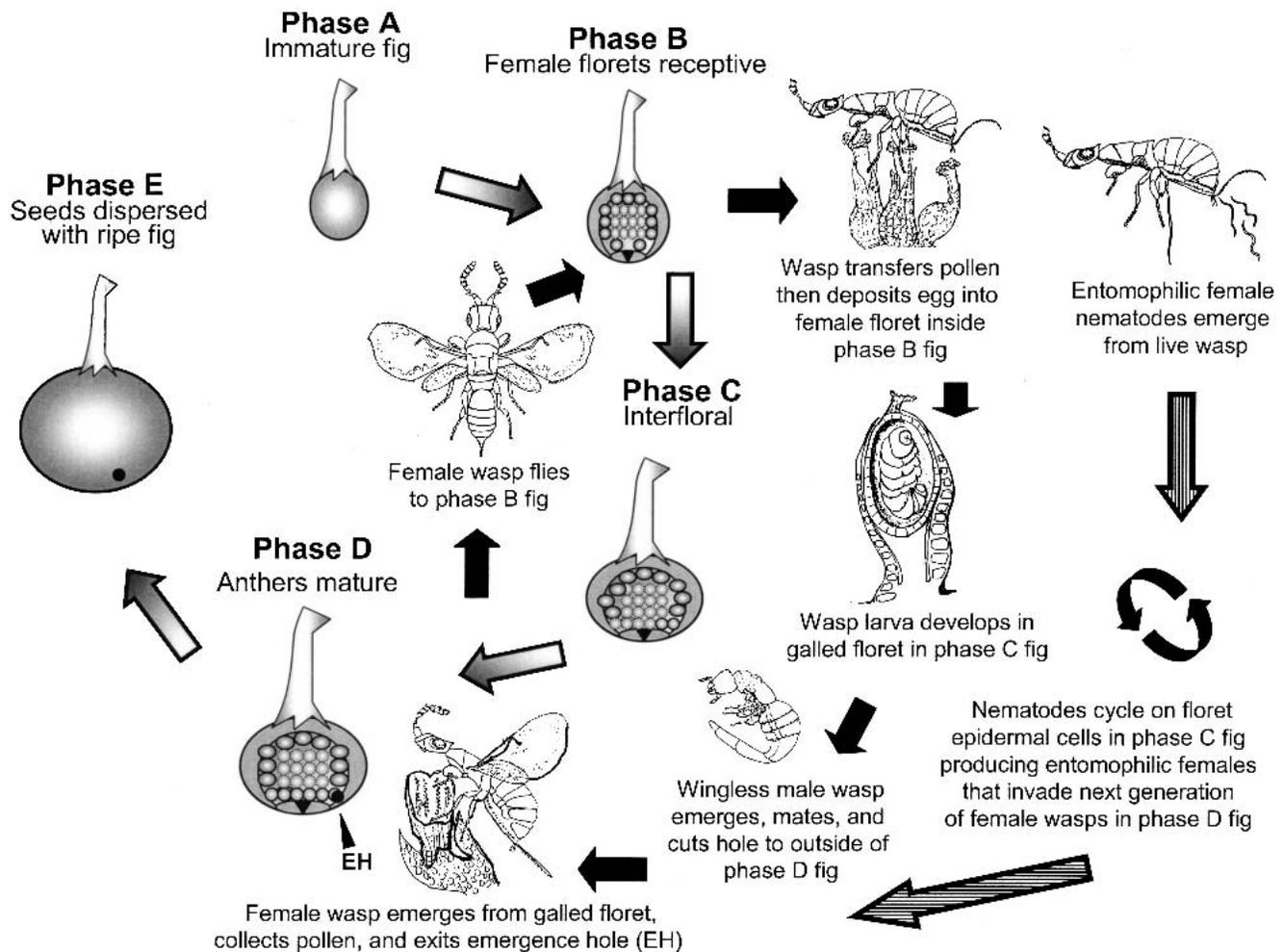
FIG. 4. Generalized representation of the association between the fig nematode, *Schistonchus*, its fig wasp vectors, and *Ficus*.

istematic/apical tissues of myrtaceous hosts, including *Eucalyptus, Corymbia, Angophora, Melaleuca,* and *Syzygium cumini* in Australasia (Giblin-Davis et al., 2003). Currently, approximately 20 *Fergusonina* fly species have been described, but many more are known (Giblin-Davis et al., 2003).

The *Fergusobia/Fergusonina* interaction involves an extremely close relationship between the nematodes and the flies (Fig. 6). The nematode appears to be responsible for gall induction, and the fly for gall maintenance and for dispersal and sustenance of the nematode (Currie, 1937). The female fly deposits its eggs along with juveniles of *Fergusobia* in young stem, leaf, or flower buds, where undifferentiated meristematic tissue is available (Currie, 1937; Giblin-Davis et al., 2001b). As these nematodes feed, and before the fly eggs hatch, a gall begins to form and the nematodes develop into parthenogenetic females (Giblin-Davis et al., 2001b). The fly eggs then hatch, and developing larvae (3 instars) feed on gall tissue. The last instar usually has a unique and species-specific cuticular structure known as the "dorsal shield" that may be used as a scraper to enlarge the chambers within the gall, forming pellets of

plant tissue to be used as food, to anchor the larva within the chamber, to excavate windows for adult emergence, or to help defend against parasitoids (Currie, 1937; Taylor et al., 1996). Currie (1937) observed third instar fly larvae defecating on the dorsal shield where *Fergusobia* nematodes appeared to aggregate and feed. This interesting observation needs to be confirmed. Given the stout nature of the stylet of the parthenogenetic female and the male, it seems more likely that they feed on plant cells, but the pre-parasitic female has a much slighter stylet. Taylor et al. (1996) reported examination of material associated with the spines of the dorsal shield of *F. flavicornis* that comprised "pellets" of plant cells.

Parthenogenetic female *Fergusobia* lay eggs that produce amphimictic male and female nematodes about the time that the fly host molts to the third instar. Inseminated pre-parasitic female nematodes with large loads of sperm are infective only to female third instar fly larvae (Fig. 6) (Currie, 1937; Davies et al., 2001), suggesting that host defense is either relaxed or can be avoided in female flies, or that nematodes in locules with male fly larvae are not infective. Females may pro-
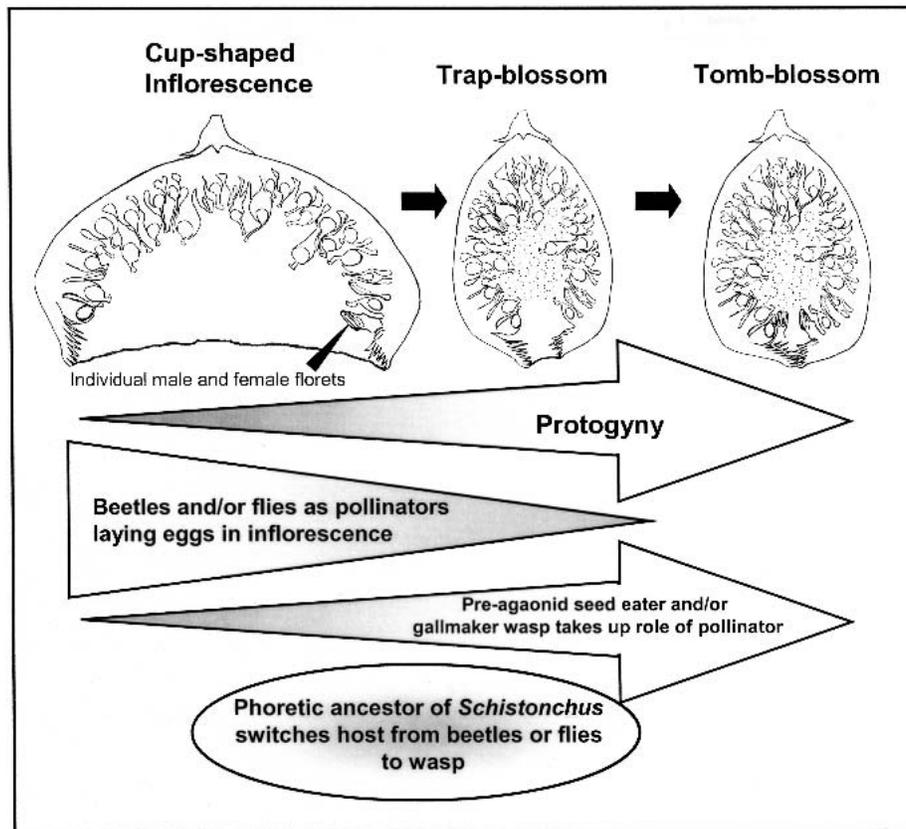
Fig. 5. Possible scenario for the evolution of the *Ficus* syconium (fig) and the mutualistic association with fig wasps and associated *Schistonchus.*

duce pheromones that act as attractants for the nematodes. Pre-parasitic female nematodes develop inside the female fly into a parasitic stage that undergo separation (apolysis) and shedding of the cuticle (ecdysis), including the stylet, without producing a new cuticle. This could be the result of an additional molt without cuticular replacement (Giblin-Davis et al., 2001a). Conversely, it could be the product of a normal molting pattern that ends without cuticular replacement if the pre-parasitic female retains the fourth-stage cuticle until after entry into a host, as apparently happens in *Howardula husseyi.* After the cuticle is shed, the epidermis becomes hypertrophied and the corresponding surface area is increased with large numbers of epidermal microvilli, presumably for more efficient nutrient acquisition (Giblin-Davis et al., 2001a). In some *Fergusobia/ Fergusonina* associations, the parasite load appears to be restricted to two large parasitic females (i.e., *Fergusobia* from *Fergusonina nicholsoni*), whereas in other associations the number of parasites appears to be less regulated (Currie, 1937; Davies et al., 2001). The parasitic females lay eggs into hemolymph in the abdomen of the fly. Eclosed juvenile nematodes move to the oviducts and, following emergence from the gall and dispersal by the fly, are deposited with its eggs into a new bud to begin the next cycle (Fig. 6). All female flies contain nematodes, whereas males have never been ob-

served with nematodes (Currie 1937; Davies et al., 2001; Giblin-Davis et al., 2001b).

Sequence comparisons within *Fergusonina* flies (mtDNA) and *Fergusobia* nematodes (D3 rDNA) from a variety of gall types, hosts, and geographic isolates have shown a high degree of host specificity within the Myrtaceae (Giblin-Davis et al., 2000; Scheffer et al., unpubl. data).

So far, the fossil record has not yielded any clues about how this putative mutualism between *Fergusonina* flies and *Fergusobia* nematodes evolved to parasitize plants together. In most cases, *Eucalyptus* species cannot be identified from pollen or from leaves alone, and fossil fruits are limited. Galls in the Australian fossil record of Myrtaceae are unknown (Christophel, pers. comm.). There is a record of a drosophilid fly parasitized by a nematode from the Allantonematidae (a member of the Sphaerularoidea in the Hexatylina; the same superfamily and suborder, respectively, as *Fergusobia*) in Mid-Tertiary fossil amber from the Dominican Republic (Poinar, 1984). Thus, it is possible that an ancestral *Fergusobia* could have been present at about the time of the radiation of the eucalypts and melaleucas.

The Agromyzidae is the putative sister group of the Fergusoninidae (Scheffer, pers. obs.). However, to date there are no known nematode associates of the Agro-

Female fly deposits its eggs
and nematode juveniles into plant bud

Parasitic female nematodes
produce juveniles that invade
the ovaries of the female fly,
which emerges from gall

Fly eggs

Nematode
juveniles

Apical
meristem

Parthenogenetic
nematode

Fly pupa

Fly larva
(3rd instar)

Fly larva

Parasitic
nematode
develops

Nematodes feed
and develop into
parthenogenetic
females, gall begins
to develop, then fly egg
hatches into first instar
fly larva

Amphimictic generation;
mated infective female nematodes
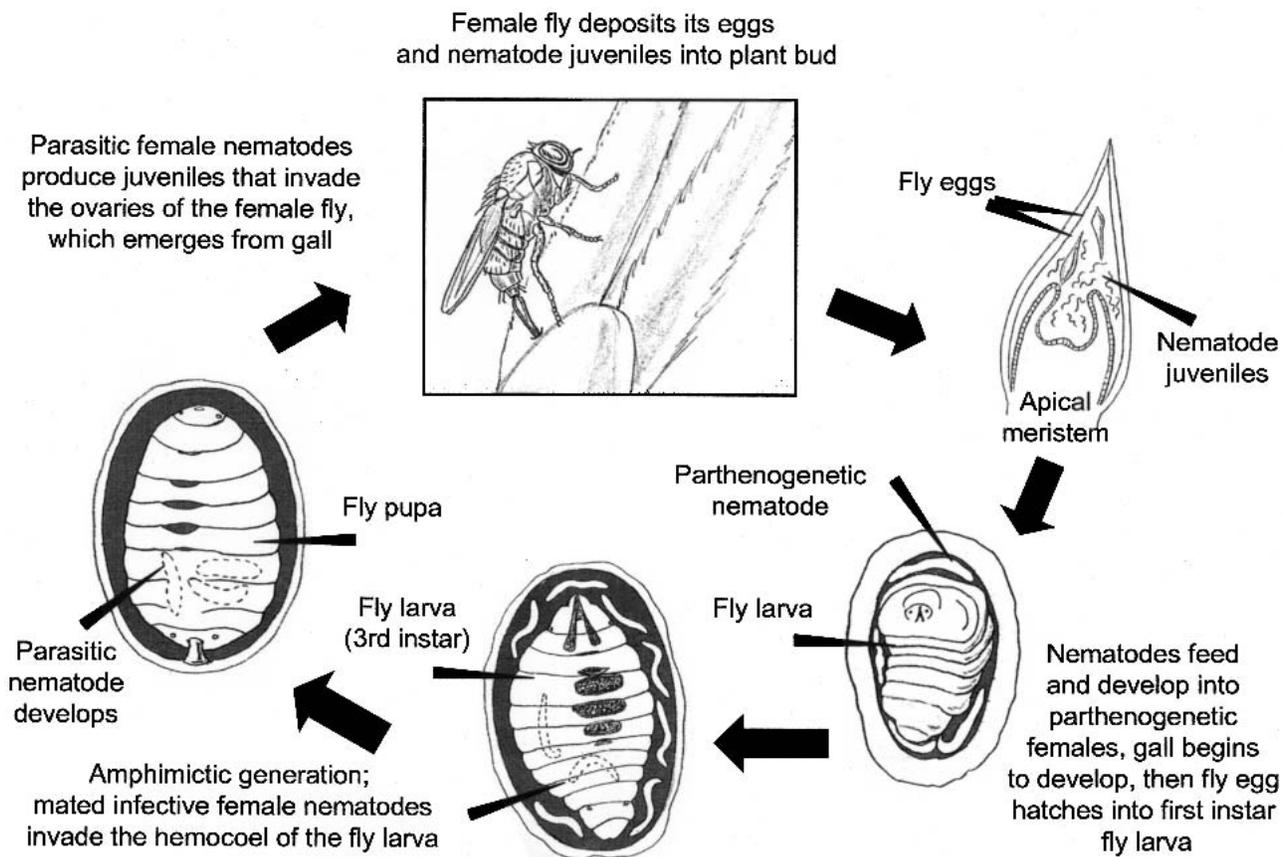invade the hemocoel of the fly larva

FIG. 6.   Generalized representation of the association between a *Fergusobia* nematode, its *Fergusonina* fly host, and a myrtaceous host.

myzidae (Poinar, 1975) and no known insect- and plant-parasitic tylenchid nematodes associated with flies. The closest related associations in contemporary nematodes that parasitize cyclorrhaphan flies (series Schizophora) are *Howardula* species that parasitize species of *Copromyza, Drosophila, Oscinella*, and *Sepsis* (Siddiqi, 2000). However, *Howardula* species have no parthenogenetic phase that is associated with plants or fungi. There are also interesting fly/fungus gall/nematode associations that involve nematoceran fungus gnats, galls on basidiomycetes, and *Iotonchium* species (Iotonchoidea: Hexatylina) (Tsuda and Futai, 2000), but the biology and morphology of these nematodes are very different from the Fergusobiinae and probably represent an example of convergence. Another contemporary example that has parallels to the *Fergusobia/Fergusonina* association but is too different morphologically and biologically to fit as a logical relative is the dicyclic genus *Deladenus*, which has a free-living amphimictic generation that feeds on fungus and an amphimictic generation that parasitizes an insect host (usually *Sirex* spp. woodwasps) (Siricidae: Hymenoptera) (Siddiqi, 2000). These three systems, because of their ease of culture, may prove useful in the study of virulence and in generating hypotheses on how the *Fergusobia/Fergusonina* mutualism could have evolved and is maintained.

Histological sections of early infestations by *Fergusobia* sp. in shoot bud galls of *Melaleuca quinquenervia* suggest that the uninucleate hypertrophied cells produced during gall formation are very similar to those caused by the non-insect associated plant bud gallers in the Anguinoidea (Hexatylina) such as *Anguina agrostis* (Giblin-Davis et al., 2001b). Thus, the potentially speciose radiation of the monogeneric subfamily Fergusobiinae has combined biological and morphological attributes of the Anguinoidea and the Neotylenchoidea, which makes it difficult to place it in a phylogenetic scheme. This suggests that *Fergusobia* represents a potentially large radiation that is a truly unique and ancient monophyletic lineage stemming from a common Hexatylina ancestor.

Hypothetically, *Fergusobia* could have evolved from parasitic nematodes similar to present-day *Howardula* that parasitized the stem ancestor of agromyzid flies and developed a plant-parasitic gall association that radiated with the radiation of the Myrtaceae. Alternatively, these could be relatives of plant-parasitic nematodes similar to present-day anguinids that produced aboveground bud galls that developed an association with an agromyzid stem ancestor. Other possibilities are equally tenable. Phylogenetic hypotheses that are generated from molecular data, such as small subunit (SSU) rDNA sequences from *Fergusobia* along with con-

temporary insect-, fungal-, and plant-parasitic forms in the Hexatylina, are greatly needed.

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