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INSECT-PARASITIC NEMATODES RESEMBLING PLANT-PARASITIC FORMS

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INTRODUCTION

Entomo-nematology is a large discipline. Poinar (1975) records 3150 cases of association between nematodes and insects, and there are 1140 entries in Shephard (1974). However, only a small fraction of these nematodes resemble plant-parasitic forms. They are found among Aphelenchida and Tylenchida, and they represent about 450 species distributed among approximately fifty genera.

The most important development phase for the insect-parasitic nematodes takes place in the host hemocoele, while the free-living phase is generally relatively brief. This phase is the only one that can be found in soil samples, and it is the only one that will be treated here. These nematodes have four different types of life cycles

1. Adults free-living, mycetophagous, or plant-parasitic; only the larval stages are associated with insects (Aphelenchida);

2. Pre-adults free-living during a brief period in the soil; the other stages parasitize the hemocoele of insects (Tylenchida and Aphelenchida);

3. Nematodes with a succession of insect-parasitic generations and generations that are either mycetophagous or plant-parasitic, often parthenogenetic (Tylenchida);

4. Nematodes whose whole life cycle is insect ectoparasitic; free-living forms, if existing, are unknown and heterogony would be exceptional (Aphelenchida).

Free-living forms of all four types of nematode associations are most frequently found in decaying substances, where the insect larvae live. The great majority of these free-living nematodes are mycetophagous. They can be found in humus, in decaying materials on the soil (e.g. fruit), in galleries of xylophagous host insects in trees and some other plants, in galls, but rarely in the soil itself or in stems, and never in roots.

The classification of entomo-nematodes has raised many problems. It is generally included within general studies on Aphelenchida and Tylenchida.

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The first family to be proposed was Sphaerulariidae of Lubbock (1861), but it was only in 1951 that the first attempt was made to present all known forms of insect-parasitic Tylenchida (Goodey, 1951). The most recent classifications including insect nematodes are those of Maggenti (1982) and Siddiqi (1986). There are few other available publications on insect-parasitic Aphelenchida (Nickle, 1967; 1970) and Tylenchida (Poinar, 1977).

Most of the data and features used to characterize entomo-nematodes among the other forms in the phylum Nemata are biological features and types of life cycle. Also, most of the few morphological features that are used by the authors relate to parasitic forms. As a consequence, only a few morphological characters are used to describe the free-living forms of insect nematodes. Poinar (1977) uses a total of about 50 characters, but only 27 of these are used for free-living forms, including body shape and color, head, stylet, position of excretory pore, oesophageal bulb and glands, gonads, vulva, spicules, gubernaculum, bursa, and tail. This compares poorly with the list of about three hundred characters that are used for plant-parasitic nematodes.

A few examples of free-living forms of insect-parasitic nematodes that are closest to plant-parasitic forms are given below for each type of association. The criteria the most commonly used for identification and differentiation of these forms are also discussed.

ADULT FREE-LIVING IN THE SOIL; JUVENILES ASSOCIATED WITH INSECTS

This category includes nine genera, all in Aphelenchida: *Bursaphelenchus*, *Cryptaphelenchus*, *Cryptaphelenchoides*, *Ektaphelenchus*, *Rhadinaphelenchus*, *Ruehmaphelenchus*, *Schistonchus*, *Sheraphelenchus*, and *Tylaphelenchus*. The two most interesting genera are discussed below. Most species in the other genera are rare, and their biology is little known.

Bursaphelenchus Fuchs, 1937: Parasitaphelenchidae (40 species)

The juveniles are associated with forest Coleoptera (Cerambycidae, Curculionidae). They are found mostly under the elytra of the host. Several species are economically important because of their association with plant diseases. For example, *B. xylophilus* (Steiner & Buhrer, 1934) Nickle, 1970 (= *B. lignicolus* Mamiya & Kiyohara, 1972) causes the pine wilt disease. *Bursaphelenchus* adults are found in waste in galleries bored by xylophagous insects underneath the bark or in the xylem of trees. They are mycetophagous and can also be found on the outer surface of bark in association with algae and mosses.

The genus is characterized by its large size, posterior location of the vulva, enlarged vulva lips, long postvulval uterine sac, tail of male markedly curved, terminal caudal alae, three pairs of papillae, absence of gubernaculum, and spicules with capitula very characteristic at the species level.

Rhadinaphelenchus Goodey, 1960: Parasitaphelenchidae (1 species)

This genus includes only one species, *R. cocophilus* (Cobb, 1919) Goodey, 1960. Juveniles are found in Malpighian tubules, trachea, intestine, sometimes in hemocoele of the insect hosts. Hosts are Coleoptera, mainly Curculionidae (*Rhynchophorus*, *Rhinostomus*, *Metamasius*), but also Scarabaeidae (*Strategus*). The genus is characterized by a long and slender body (average: 1 mm), stylet with well developed knobs, median oesophageal bulb twice as long as wide, with valve posterior to center, overlapping vulval flap, and very long postvulval uterine sac. The male spicules have prominent

rostra, and curved tail with four pairs of papillae. Adults of *R. cocophilus* are responsible for the well documented "Red-ring" disease of the coconut tree.

PRE-ADULT STAGE FREE-LIVING IN THE SOIL; OTHER STAGES INSECT-ASSOCIATES

These nematodes belong to Allantonematidae (Tylenchida) and Entaphelenchidae (Aphelenchida). The allantonematid life-cycle is the most typical cycle for insect-parasitic nematodes. The fertilized female enters a host, lays eggs, the juveniles hatch and develop until the third or fourth stage, then they leave the host for a brief passage outside the insect host as free-living pre-adults. They become males and females. After mating the males die and the infesting females look for another host.

Howardula Cobb, 1921: Tylenchida, Allantonematidae (16 species)

This genus parasitizes Coleoptera, Diptera and perhaps Mites. Free-living pre-adults can be found in vegetal materials (fruit or vegetables) where the larvae of the insect hosts develop. The free-living pre-adult nematodes do not feed.

The female nematode is medium-sized, from 0.4 to 0.6 mm, with a remarkably long stylet (12 to 24 μm long) with slight knobs or basal thickenings, three well developed oesophageal glands, ovary rudimentary, and small oviduct. The uterus acts as a spermatheca. The male appears to be without a stylet, its caudal alae are rarely present and, when present, it is not well developed.

Metaparasitylenchus Wachek, 1955: Tylenchida, Allantonematidae (12 species)

Free-living forms in this genus have a very short life and are probably non-feeding. They can be found in galleries of xylophagous Coleoptera, Cerambycidae and Mycetophagidae. Impregnated infesting females remain in the cuticle of the fourth stage juvenile. The female has a stylet 12 μm long, with well developed knobs, rudimentary ovary, and no postvulval uterine sac. The male has a shorter stylet, gubernaculum present, and large caudal alae.

Entaphelenchus Wachek, 1955: Aphelenchida, Entaphelenchidae (7 species)

Free-living forms of this genus seem to be non-feeding. They are found in places where the larvae of the insect host develop, for example on dung. The insect-parasitic forms generally are associated with Staphylinidae (Coleoptera).

Free-living males and females have small-sized body, stylet without knobs, median oesophageal bulb with large valve, three oesophageal glands overlapping the intestine. In the female, the ovary is reduced to a few cells. The male has spicules not fused, with a large rose thorn-shaped rostrum, gubernaculum and caudal alae are absent.

In conclusion, the forms in this second category are fairly recognizable and should not be confused with plant-parasitic forms. In this type of life cycle it can be noticed that all free-living forms have prodelphic gonad, ovary with few oocytes, and uterus with sperm. The males are not infesting. Fourteen other genera can be included in this category with about 130 species: *Allantonema, Aphelenchulus, Bradynema, Contortylenchus, Neoparasitylenchus, Parasitylenchoides, Peraphelenchus, Praecocilenchus, Proparasitylenchus, Protylenchus, Roveaphelenchus, Scatonema, Sulphuretylenchus*, and *Thripinema*.

NEMATODES WITH ALTERNANCE OF INSECT-PARASITIC AND NON INSECT-PARASITIC GENERATIONS

The non insect-parasitic forms of these nematodes are mycetophagous or plant-parasitic. They are remarkable because they can alternately have a neotylenchoid form and an allantonematid one. All are in Tylenchida.

Deladenus Thorne, 1949: Tylenchida: Phaenopsitylenchidae (17 species)

The species in this genus have two types of males and two types of females, all present in the environment. Some are associated with Hymenoptera Siricidae (wood wasps). Free-living forms are found either in galleries bored by these insects in *Pinus* and other higher plants, or in galls (*Quercus prinus*).

The neotylenchoid free-living female has small subventral oesophageal glands but a huge dorsal oesophageal gland, ovary well developed, eggs in oviduct, postvulval uterine sac absent. Male is identical to female, with amoeboid sperm 10 to 12 μm in diameter, and tylenchoid gubernaculum.

The pre-adult infesting female has a longer stylet than that of the free-living female, subventral oesophageal glands well developed, ovary not developed, uterus full of small sperms. The male is identical to the free-living form but produces small-sized sperm (diameter: 1 to 2 μm). The mature adult female has body greatly enlarged, and oesophagus degenerate.

Most of the seventeen species in the genus *Deladenus* are known only by their free-living forms. Insect-parasitic forms are known only for *Deladenus canii* Bedding, 1974, *D. imperialis* Bedding, 1974, *D. nevexii* Bedding, 1974, *D. proximus* Bedding, 1974, *D. rudyi* Bedding, 1974, *D. siridicola* Bedding, 1968, and *D. wilsoni* Bedding, 1968.

Fergusobia Currie, 1937: Tylenchida, Neotylenchidae (2 species)

Free-living forms of this genus found in Australia and in India, participate with their hosts (Diptera) in the formation of galls on Eucalyptus and Syzygium where they live.

The free-living forms have body typically swollen, a very rare occurrence among insect-parasitic nematodes, oesophagus corpus with large crescent-like valves, oesophageal glands surrounding the intestine, ovary prodelphic, postvulval uterine sac absent. The females are parthenogenetic.

The insect-infesting female has ovary and oviduct with flexures, vulva a large transverse slit. The male of the insect-parasitic generation has small stylet knobs, spicules paired and stout, gubernaculum absent, tail peloderan and sperm small and round.

Undescribed genus (Laumond, 1970): Tylenchida, Allantonematidae (1 species)

A new genus cited but not described by Laumond (1970) has remarkable taxonomic and phylogenetic features. Like *Fergusobia*, it shows an alternance of insect-parasitic and plant-parasitic generations. The plant-parasitic generation parasitizes Colza, while the insect-parasitic generation lives on *Baris* (Coleoptera, Curculionidae), an insect pest of Colza. The plant-parasitic phase is of neotylenchoid type, the entomo-parasitic one is of allantonematid type.

The plant-parasitic female has eight-sectored labial framework, neotylenchoid stylet 12 to 14 μm long, with basal thickenings, large oesophageal glands, with oesophago-intestinal junction just behind the nerve

ring. The corresponding male produces large sperm (10 μm in diameter). The fourth stage infesting nematode of the insect-parasitic generation has longer stylet (18 to 20 μm), oesophageal glands narrow and elongate, oesophago-intestinal junction at level of the nerve ring, ovary and oviduct little developed, uterus with sperm. The insect-parasitic male is very similar to the plant-parasitic male, except for spicules longer and thinner, and much smaller sperm (1.5 μm).

The life-cycle of this species is very unusual: after *Fergusobia,* this is the second occurrence of a nematode with both complete insect-parasitic and plant-parasitic generations. The other species in this category are mycophagous.

Prothallonema Christie, 1938: Tylenchida, Sphaerulariidae (19 species)

This genus was recently discussed by Siddiqi (1986). One of its species, *P. intermedium* (Christie, 1938) Siddiqi, 1986 well illustrates how difficult it is to analyze a soil sample when free-living forms of entomonematodes are present. Christie discovered two new nematode species in a agar-slant culture, associated with the fungus *Alternaria citri.* The inoculum for this culture had been secured from a decaying citrus fruit. He described them as *Hexatylus intermedius* in Neotylenchidae, and *Prothallonema dubium,* representing a new genus in Allantonematidae.

The first form is typically neotylenchoid: eight-sectored framework, cylindroid corpus, wide basal bulb, quadricolumella with more than four cells in each row, very posterior vulva, postvulval uterine sac absent. The second form is typically allantonematid and was later synonymized with *Howardula:* long stylet (11 to 19 μm) of the infesting female, well developed knobs, long and wide oesophageal glands, immature ovary, very long uterus, with a postvulval uterine sac. There may be two types of males, but difficult to distinguish.

Siddiqi (1986) proposed the hypothesis that the free-living form (*H. intermedius)* can develop over several generations on fungus; then an infesting form (*P. dubium)* would appear. If confirmed, this type of life cycle would be similar to life cycles in *Deladenus* or an undescribed genus discussed by Laumond (1970). Siddiqi (1986) considered that the two names in Christie (1938) apply in fact to the same species in two different forms, and he proposed to combine them under the name *Prothallonema intermedium.*

Some *Stictylus* and *Sphaerulariopsis* species have been placed in the genus *Prothallonema* by Siddiqi (1986), but their insect-parasitic forms remain to be discovered. On the other hand, known insect parasites in the genera *Sphaerularia* and *Tripius* should be placed in the family Sphaerulariidae instead of Allantonematidae.

Heteromorphotylenchus Remillet & Waerebeke, 1978: Tylenchida, Allantonematidae (2 species)

This genus exhibits an alternance of heterosexual and parthenogenetic generations. Unlike previous species in *Deladenus* and *Prothallonema,* the free-living generation is non-feeding and immediately gives a insect-parasitic generation.

The free-living female has a dorsal oesophageal gland bigger than the subventral ones, no postvulval uterine sac, uterus with only one egg at a time. There is only one type of male with small stylet, gubernaculum, and bursa completely enveloping the tail. The pre-adult infesting female has well developed subventral glands and an uterus full of small sperm.

These forms were found in decaying fruit where host larvae develop (Coleoptera, Nitidulidae).

Heterotylenchus Bovien, 1937: Tylenchida, Parasitylenchidae (6 species)

This is the first heterogonic genus that was discovered among entomo-nematodes. It exhibits an alternance of heterosexual and parthenogenetic generations. The free-living form is represented by the male and the pre-adult infesting female.

The pre-adult female has small body (0.5 mm long), well developed stylet with distinct knobs, excretory pore posterior to the nerve ring, and an immature ovary. The male has stylet shorter than that of the female, and caudal alae absent.

These are parasites of Diptera and Coleoptera. The free-living form of *H. aberrans* Bovien, 1937 was found in decomposing onion bulbs associated with larvae of *Delia antiqua* (Meigen). The insect-parasitic forms occupy most of this heterogonic type of life-cycle.

Two other genera belong to this category: *Pareglytylenchus* Slobodianuc, 1984, 1 species, and *Psyllotylenchus* Poinar & Nelson, 1973, 25 species.

NEMATODES PERMANENTLY ECTOPARASITIC ON INSECTS

Only two monospecific genera are known in this very exceptional category, both in Aphelenchida: *Acugutturus* Hunt, 1980, an ectoparasite on cockroaches, and *Noctuidonema* Remillet & Silvain, 1988, an ectoparasite on Lepidoptera.

Noctuidonema and *Acugutturus* accomplish their entire life-cycle on the surface of insect teguments. All stages are plump. They all have a very long and slender stylet (up to 160 μm in adults), an oval bulb with well distinct valves, oesophageal subventral glands not very visible, vulva transverse and prominent.

The male of *Noctuidonema* has spicules with long and thin rostra, no gubernaculum, two pairs of papillae, and peloderan caudal alae. The male of *Acugutturus* has spicules with short rostra, gubernaculum present, two pairs of papillae, and caudal alae absent.

No free-living forms have been found for these two genera. Their existence would involve a heterogonic cycle, unknown among Aphelenchida.

CONCLUSION

The main morphological criteria for identification with dissecting or compound microscope, have been given for the various genera cited as examples of these four categories of associations. These morphological elements are not numerous but, except in a few cases, they allow a good differentiation of genera. Biological data about the type of parasitism may also be necessary for final identification. However, free-living forms of entomo-nematode should easily be recognized as such, because of the strong stylet of the infesting female, very developed oesophageal glands, the small-size ovary, and the uterus full of small sperms.

Obviously, there is still a great deal of work to be done both in taxonomy and biology of insect-parasitic nematodes. Thus, among *Hexatylina* many parasitic forms are unknown. Poinar (1983) estimates that 485,000

species of nematodes are still to be discovered in the world. Certainly a large proportion of these species are likely to be associated with insects, because insects represent 83% of animal species with about one million of currently known species.

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