



## ***Elaeolenchus parthenonema* n. g., n. sp. (Nematoda: Sphaerularioidea: Anandranematidae n. fam.) parasitic in the palm-pollinating weevil *Elaeidobius kamerunicus* Faust, with a phylogenetic synopsis of the Sphaerularioidea Lubbock, 1861**

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### **Abstract**

A new nematode, *Elaeolenchus parthenonema* n. g., n. sp., is described from the palm-pollinating weevil *Elaeidobius kamerunicus* Faust. The new genus is placed in the Anandranematidae n. fam., which, together with the genus *Anandranema* Poinar et al., 1993, is characterised by nematodes having only a single autotokous generation in the insect host. This is the first report of a member of this superfamily reproducing only parthenogenetically. The development of *E. parthenonema* and its effect on the weevil host is discussed, along with a phylogenetic synopsis of the families of the Sphaerularioidea Lubbock 1861. The Beddingiidae n. fam. is proposed for *Beddingia* Blinova & Korenchenko, 1986, comprising the original *Deladenus* parasites of Hymenoptera that possess both free-living and parasitic amphimictic generations in their life-cycles. This family is considered to have the most primitive type of development in the superfamily.

### **Introduction**

The oil palm *Elaeis guineensis* Jacq., indigenous to West Africa, is pollinated by weevils of the genus *Elaeidobius* (Derelominae: Curculionidae) (Figure 3). The adults are attracted to the male inflorescences where they mate and oviposit, and the larvae develop in the gynoecea of the post-anthesis male flowers. Adults carrying pollen on their bodies also visit and pollinate female flowers, but never feed or oviposit on them (Free, 1993). When the oil palm was introduced into SE Asia, where *Elaeidobius* weevils were absent, the plants often had to be hand-pollinated in order to obtain fruit set. In 1981, *E. kamerunicus* was introduced into Malaysia, greatly improving pollination and oil yield of the palm (Free, 1993). It has subsequently been introduced into Papua New Guinea, the Solomon Islands, Sumatra and Thailand (Greathead, 1983).

Recently, an apparent reduction in weevil numbers, accompanied by a decline in fruit set and yield,

was noted in some regions, particularly Sabah where nematodes were associated with the waning weevil populations (Rao & Law, 1998). A nematode parasite belonging to the superfamily Sphaerularioidea Lubbock 1861, was discovered to be an internal parasite of the weevil (Jackson & Bell, 2001). This nematode is presumed to have been introduced with the weevils brought from Africa, since it is highly adapted to its insect host. Although efforts were made to rid the weevil of all associated organisms before importation into Malaysia (Syed et al., 1982), an internal parasite could have escaped notice. The present study was undertaken to determine the systematic placement of this nematode and study its effect on weevil viability.

### **Materials and methods**

Colonies of parasitised weevils were established in the laboratory by adding adult beetles from nematode infected populations to containers with fresh male in-

florescences of *Elaeis guineensis* Jacq. The containers were maintained at room temperature (20-25°C) in a humid atmosphere. At 5 and 10 days, respectively, after the colonies were established, live adult, pupal and larval weevils were sampled from each colony. The prevalence of nematode infections was based on the percentage of infected larvae and pupae in the male florets. Male palm florets were also examined for free-living nematodes by direct observation under the binocular microscope and by extraction of nematodes into White traps.

Microscopic observations and photographs were made on living and dead nematodes with a Nikon Optiphot Microscope. Measurements were made on individuals that had been killed in hot (60°C) water, fixed in TAF and processed to glycerine. All measurements are in micrometres unless otherwise specified.

## Results

Two species of nematodes were found associated with the larvae, pupae and adult weevils in the palm florets. One species was only associated with the insects phoretically, with large numbers of dauer juveniles occurring on the adult beetles, especially under the elytra, in folds of the membranous wings and on the dorsal surface of the abdomen. It was identified as an undescribed species of the family Cylandrocorporidae T. Goodey, 1935 (Diplogasteroidea: Rhabditida) and will be discussed in a separate study. This nematode is microbotrophic and breeds in the decomposing male palm florets, apparently feeding on bacteria and possibly fungi. Members of this family have been reported from decaying plant and animal matter (Goodey, 1963) and the dauer of some species are known to form phoretic associations with bark beetles of the family Scolytidae (see Poinar, 1975).

The second nematode associated with the palm-pollinating weevils, a member of the insect-parasitic superfamily Sphaerularioidea, was found in the haemocoel of weevils collected from laboratory colonies and field populations. It is described below in a new genus and a new family together with the existing genus, *Anandranema* Poinar et al. 1993 which has similar characteristics.

## Tylenchida Filipjev, 1934 Sphaerularioidea Lubbock, 1861

### Anandranematidae n. fam.

#### Diagnosis

Infective stages small, with well-developed stylet and stylet knobs; anus conspicuous; tail pointed; parasitic hermaphrodites or parthenogens elongate and sausage-shaped, at least when young (pleomorphic in aged hermaphrodites of *Anandranema phlebotophaga* Poinar et al., 1993); oviparous or ovoviviparous; cycle homogonic (parasitic generation only – no alternating free-living cycle) and completely autotokous. Type-genus: *Anandranema* Poinar et al., 1993.

#### Comments

This family contains nematodes that reproduce only by autotoky (production of progeny without males) (Poinar & Hansen, 1983) and includes the type-genus *Anandranema* Poinar et al., 1993 and the new genus *Elaeolenchus* described below. The two genera can be distinguished as follows:

1. Females hermaphroditic; excretory pore posterior to nerve-ring; oviparous; parasites of phlebotomine flies (Psychodidae: Diptera) . . . . .  
 . . . . . *Anandranema* Poinar et al., 1993
2. Females parthenogenetic; excretory pore anterior to nerve-ring; ovoviviparous; parasites of derelominid weevils (Curculionidae: Coleoptera) . . . . .  
 . . . . . *Elaeolenchus* n. g.

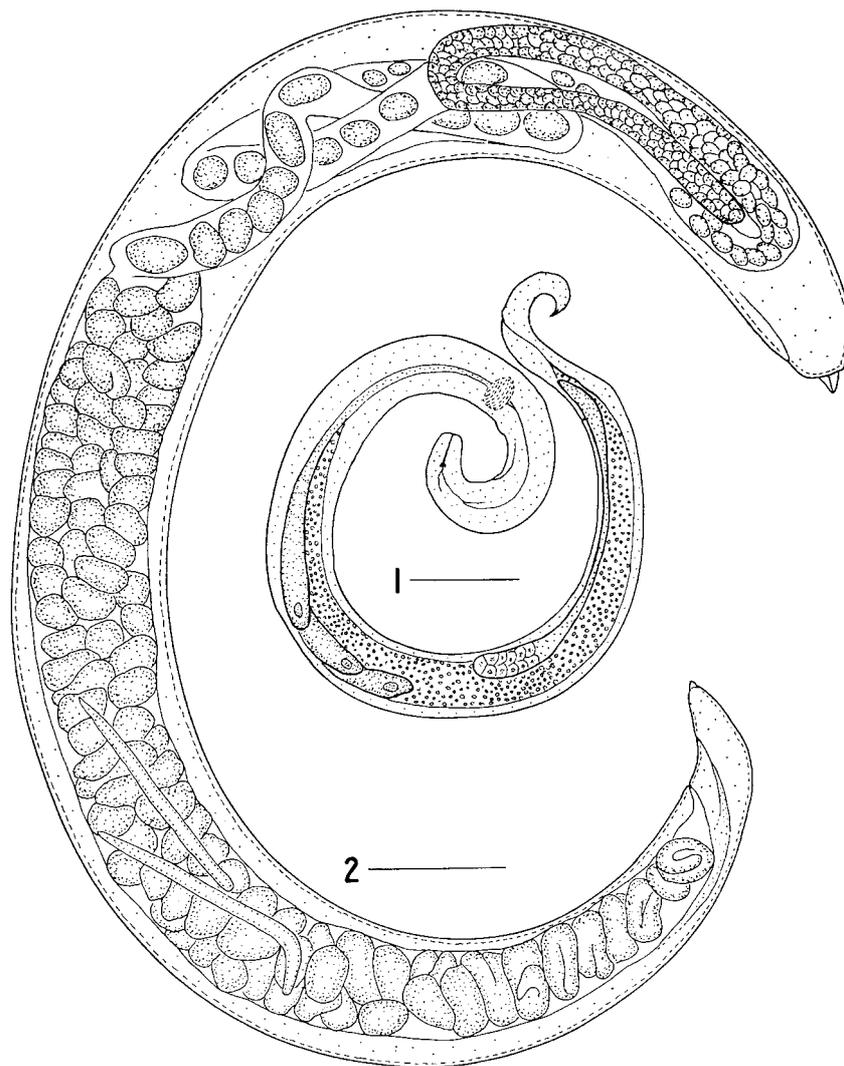
### *Elaeolenchus* n. g.

#### Diagnosis

Sphaerularioidea, Anandranematidae n. fam. Parthenogenetic. Infective stage with well-developed stylet containing minute knobs at base. Excretory pore anterior to nerve-ring. Vulvar and anal openings conspicuous. Parasitic parthenogens sausage-shaped. Body curved ventrally. Ovoviviparous; males absent. Type-species: *E. parthenonema* n. sp.

#### Comment

*Elaeolenchus* is the only member of the Sphaerularioidea reproducing strictly by parthenogenesis.



Figures 1-2. *Elaeolenchus parthenonema* n. sp. 1. Infective stage parthenogen 2. Mature parasitic parthenogen. Scale-bars: 1, 27  $\mu\text{m}$ ; 2, 90  $\mu\text{m}$ .

***Elaeolenchus parthenonema* n. sp.**

*Description* (Figures 1-2, 4-6)

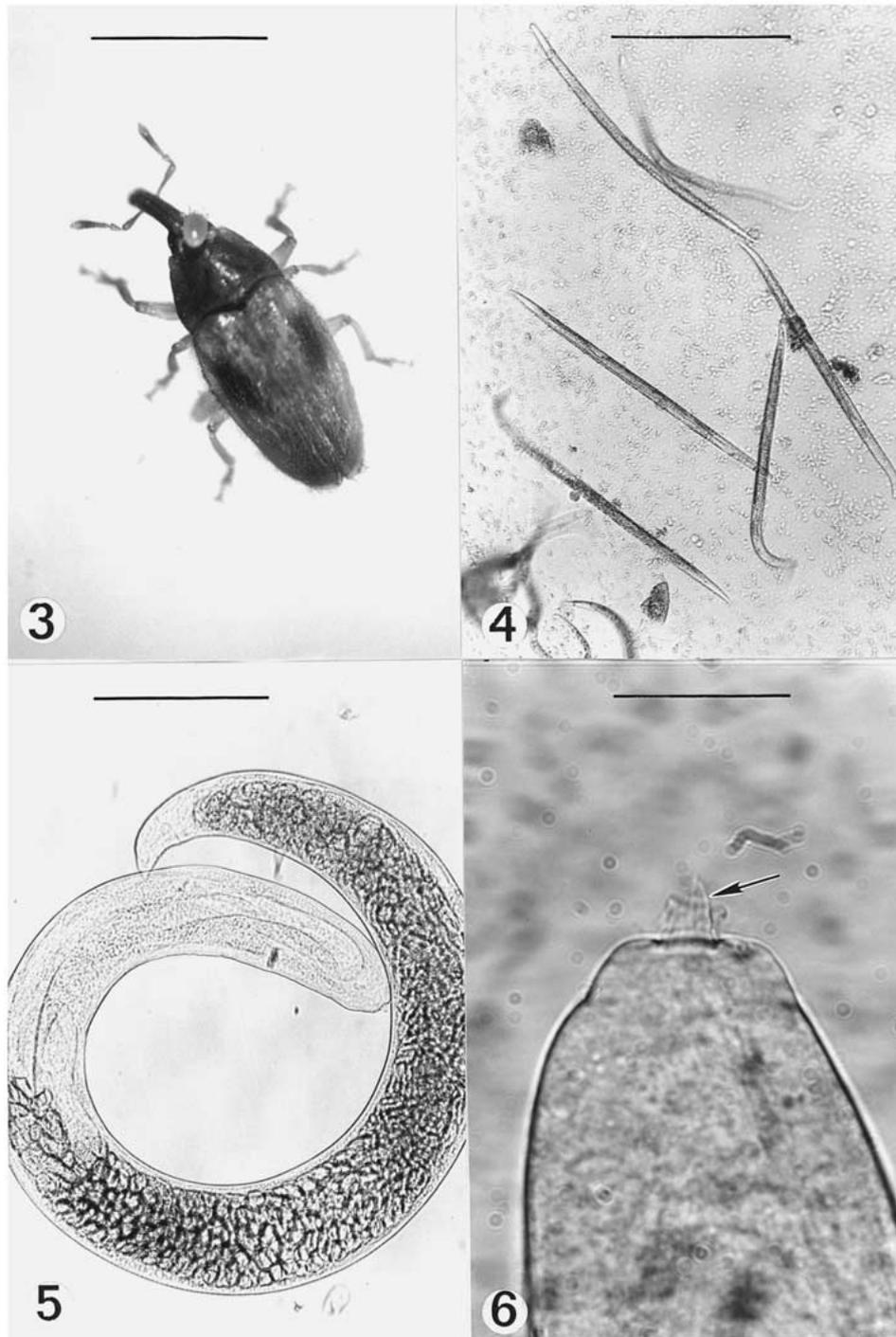
*Measurements*

*Infective stage parthenogen* (n=10). L, 350 (317-378); greatest body width, 13 (12-14); stylet length, 8 (7-10); head to EP, 25 (21-28); head to nerve-ring, 58(54-60); tail length, 20 (14-26); tail tip to vulva, 56 (41-70); % vulva, 84 (81-87).

*Mature parasitic parthenogen* (n=7). L, 1.4 (1.1-1.8) mm (in cases of heavy superparasitism, some individuals only reach 0.5 mm in length); greatest body width, 93 (70-122); stylet length, 9 (8-11); head

to EP, 23 (19-29); tail tip to vulva, 64 (56-70); % vulva, 95 (93- 98); tail length, 29 (24-32); length developing egg, 35 (29- 43); width developing egg, 9 (8-11).

*Infective stage parthenogen* (Figure 1). Small, slender forms, capable of serpentine, swimming movements in water; stylet well-formed, with swollen base and composed of 3 minute knobs; dorsal gland opening immediately posterior to stylet base; subventral gland opening between 1-2 stylet lengths posterior to stylet base; pharyngeal glands well developed, mostly dorsal in position, extending almost to ovary; excretory pore located anterior to nerve-ring and hemizonid;



Figures 3-6. Parasitised adult *Elaeidobius kamerunicus*. Note phoretic mite on head of weevil. 4. Third-stage juveniles of *Elaeolenchus parthenonema* n. sp. from an infected adult weevil. 5. Mature ovoviviparous parasitic parthenogen of *E. parthenonema* removed from the body-cavity of a larva of *Elaeidobius kamerunicus*. 6. Anterior end of mature parthenogen of *E. parthenonema* showing 'nose cone' (arrow) containing the stylet. Scale-bars: 3, 1.9 mm; 4, 190  $\mu$ m; 5, 150  $\mu$ m; 6, 30  $\mu$ m.

hemizonid located just anterior to nerve-ring; vulva posterior; uterus expanded; oviduct narrow; ovary outstretched, containing approximately 10-12 ovules; tail tip straight or curved ventrally; tail tip often with minute mucron or peg varying from 1-6 in length; often enclosed in 1 or 2 moulted cuticles. In rare cases, ova initiate development before parthenogen enters insect host.

*Parasitic parthenogen* (Figures 2, 5-6). Sausage-shaped and curved ventrally throughout development; anterior tip often set off from remainder of body in form of 'head cone' (Figure 6) containing stylet; excretory pore, anus and vulva conspicuous; vulva sometimes with very short flap; ovary elongate, reflexed 1 to several times; tail tip with blunt or pointed peg-like projection.

*Type-host*: *Elaeidobius kamerunicus* Faust (Derelominae: Curculionidae: Coleoptera). It is possible that *E. plagiatus* (Fåhræus), *E. subvittatus* (Faust) and other species of *Elaeidobius* are also hosts of this nematode, since many weevils of this genus visit the flowers of *Elaeis guineensis* in West Africa (Free, 1993).

*Type-locality*: Specimens from Bandar Baru Bangi in Peninsular Malaysia, but the same species is found in *E. kamerunicus* populations throughout Malaysia and Sabah. The nematode population studied here probably originated from Cameroon, Africa.

*Holotype*: (mature parasitic parthenogen) (T-546t) and allotype (infective stage parthenogen) (T-547t) deposited in the USDA Nematode Laboratory, Beltsville, Maryland.

#### *Life-cycle*

The infective stage is the adult parthenogen that emerges from the insect host as a third-stage juvenile (J3) (Figure 4), then moults twice, both moults normally occurring simultaneously. These moults may be retained while the nematode searches for a host, but are normally shed before entering the body-cavity of weevil larvae in the male florets of the oil palm. The infective parthenogens are able to 'swim' in water by rapidly undulating their bodies and rising to the surface of water droplets. This adaptation probably assists them in finding hosts. Female weevils oviposit on male inflorescences and the larvae develop in the carpels of the male flowers. The J3 nematodes exit from adult weevils, mature to the infective stage

adult in the floret and enter the haemocoel of weevil larvae. Growth and egg production is initiated in weevil larvae and continues into the pupal and adult stages at which time the third-stage juveniles emerge from the body of the parthenogen and enter the host haemocoel. The first moult occurs in the egg and the second in the host. Eventually, the third-stage juveniles emerge via the intestine and reproductive system of the adult weevils. No plant-feeding stages or secondary generations could be detected in the palm florets by direct examination and only post-parasitic and infective stages of *E. parthenonema* (together with other nematode species) were found in White traps. The absence of a free-living cycle in *E. parthenonema* is confirmed by the fact that the juvenile stages emerging from the parthenogen inside the host develop into infective stages containing the same type of stylet as the parthenogen, a stylet formed for entry into insect hosts and not for feeding on fungi or higher plants (not a neotylenchid type of stylet). No males of *E. parthenonema* were found in either the White traps or parasitised weevils. Attempts were made to locate sperm in the genital tract of various stages to determine if the autotokous condition was hermaphroditism or parthenogenesis. Although no sperm were located, small, dense, spherical bodies were observed in the developing eggs and oviduct. These dense bodies measured from 0.6 to 1.2  $\mu\text{m}$  in diameter and were quite numerous, often appearing in the middle of the oviduct and extending to the beginning of the uterus. They appear to be some type of storage protein, although the possibility of micro-organisms such as rickettsia could not be ruled out.

#### *Effect on host*

The nematodes occurred in both male and female hosts. In laboratory colonies, 40-70% of weevils were parasitised. Up to 30 parthenogens and their progeny could occur in a single beetle. Such superparasitism often leads to dwarfism of the mature parthenogens. Heavy infections reduced egg production and fat reserves and resulted in smaller weevil larvae, which may have been incapable of completing development to the adult stage. In addition, parasitism appeared to sterilise many female weevils. The effects on male hosts is not known. The adult beetles are frequently covered with phoretic mites that are constantly examining the external surface of the weevils for nematodes (Figure 3). These mites feed on both the phoretic nematodes carried on the adult (and pupal) beetles

Table 1. Life-cycles in families of the Sphaerularioidea (beginning with the most primitive) (for genera in these families; see Remillet & Laumond, 1991 and Siddiqi, 2000).

Family	Insect generation	Plant (or fungal) generation
Beddingiidae n. fam.	single-multiple amphimictic	single-multiple amphimictic
Phaenopsitylenchidae	single amphimictic	single-multiple parthenogenetic
Iotonchiidae	single amphimictic	single-multiple parthenogenetic
Fergusobiidae	single amphimictic	single-multiple parthenogenetic
Parasitylenchidae	single amphimictic followed by second amphimictic or autotokous	absent
Sphaerulariidae	single amphimictic	absent
Allantonematidae	single amphimictic	absent
Anandranematidae n. fam.	single autotokous	absent

as well as the free-living stages (third- and fourth-stage juveniles and infective adult parthenogens) of *E. parthenonema* in the florets. Further investigations are being conducted on this interesting tri-trophic association.

The distribution of *E. parthenonema* outside Malaysia is currently unknown, but the insect is of such importance to the oil palm industry that a survey throughout the areas where *E. kamerunicus* has been introduced for oil palm pollination would be warranted, as well as a study on the overall effects of the nemaode parasite on the weevil's pollinating abilities.

## Discussion

This is the first case of a member of the superfamily Sphaerularioidea undergoing only parthenogenetic development in the host in the absence of a free-living cycle. Assuming that autotoky is a condition derived from apomixis, then Anandranematidae n. fam. would be the most phylogenetically advanced group in the Sphaerularioidea. In their synopsis of the superfamily Sphaerularioidea, Remillet & Laumond (1991) list six families, all with varying types of life-cycles. To this list is added Anandranematidae n. fam. and a new family proposed here, the Beddingiidae n. fam. (based on the species of *Deladenus* from Hymenoptera described by Bedding) (Table 1). In their paper erecting the family Phaenopsitylenchidae, Blinova & Korenchenko (1986) established *Beddingia* Blinova & Korenchenko, 1986 and placed it in this family along with *Phaenopsitylenchus* Blinova & Korenchenko, 1986. Unfortunately, the life-cycles of these two genera differ significantly from a phylogenetic viewpoint. Whereas *Phaenopsitylenchus* has a

parthenogenetic free-living cycle, the corresponding cycle of *Beddingia* is amphimictic. A diagnosis of the family Beddingiidae follows:

## Beddingiidae n. fam.

### Diagnosis

Tylenchida, Sphaerularioidea. Life-cycle involving two separate amphimictic cycles, one free-living with fungi as food source, other with insects of order Hymenoptera as food source. Both cycles can continue indefinitely under controlled conditions. Each cycle has morphologically distinct male populations. Type-genus: *Beddingia* Blinova & Korenchenko, 1986.

### Comments

In the scheme presented here, the Beddingiidae n. fam. is the only family in the Sphaerularioidea to have an amphimictic, free-living generation feeding on fungi which in our scheme would make it the most primitive in the superfamily (Table 1). The nematode *Beddingia barisii* Laumond & Bonifassi in Remillet & Laumond, 1991, must at present be considered a *nomen nudum*, since its description was never published and its true placement not verified.

From the primitive condition of amphimixis in both the free-living and insect parasitic cycles where two morphologically distinct males are involved, the free-living cycle then became autotokous (in these cases, involving parthenogenesis) in three families [Phaenopsitylenchidae Blinova & Korenchenko, 1986, Iotonchiidae Goodey, 1953 and Fergusobiidae Goodey, 1963] with males retained only in the insect cycle.

In the Parasitylenchidae Siddiqui, 1986, the free-living (plant or fungal) generation disappears but a second amphimictic or autotokous generation follows the first amphimictic cycle in the insect. In the Allantonematidae Pereira, 1932 and Sphaerulariidae Lubbock, 1861, the secondary generations in the insect are lost and only a single parasitic amphimictic cycle remains. Finally, in the Anandranematidae n. fam., males completely disappear and only a single autotokous generation exists.

Heterogamy, the alternation of amphimictic and autotokous life-cycles, as occurs in the families Phaenopsitylenchidae, Itonchiidae and Fergusobiidae, presents a special case of adaptation to successive different environments with conservation of adapted genotypes by autotokous reproduction (Poinar & Hansen, 1983). With an autotokous method of reproduction, both *Elaeolenchus* and *Anandranema* had to solve the problem of maintaining genetic variability in the population. Hermaphroditism in *Anandranema* would provide a certain amount of genetic variation, but variability in *Elaeolenchus* would have to be accomplished by mutations or some other presently unknown method.

It is likely that the precursor of *Elaeolenchus* was heterogamic with an amphimictic cycle followed by an autotokous cycle similar to some members of the Sphaerularioidea. Various environmental pressures resulted in the elimination of the plant (or fungal) feeding cycle with only the insect parasitic autotokous cycle remaining. The following factors were probably responsible for the elimination of the plant feeding cycle and the development of autotoky. The first was exposure of the free-living stages to desiccation on the flowers of the oil palm. The second was the constant presence of predatory mites on the flowers. Such pressures would have resulted in adaptations that would have reduced exposure time to a bare minimum. This was accomplished by the double moult from the third stage juvenile to the infective parthenogen, the ability of the infective parthenogen to 'swim' in moisture layers on the floral spikelets of the oil palm and the initiation of parthenogeny that would eliminate the need to find a mate in the environment. Thus, co-evolution not only occurred between *Elaeolenchus* and its weevil hosts, but also between the nematode and its mite predators.

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## References

- Blinova, S.L. & Korenchenko, E.A. (1986) *Phaenopsitylenchus laricis* gen. and sp. n. (Nematoda: Phaenopsitylenchidae fam. n.) a parasite of *Phaenops guttulata* and remarks on the taxonomy of nematodes of the superfamily Sphaerularioidea. *Trudy Gel'mintologicheskoi Laboratorii*, **34**, 14–23 (In Russian).
- Free, J.B. (1993) *Insect pollination of crops*. (2nd Edition). London: Academic Press, 684 pp.
- Goodey, J.B. (1963) *Soil and freshwater nematodes*. London: Methuen & Co. Ltd., 544 pp.
- Greathead, D.J. (1983) The multi-million dollar weevil that pollinates oil palms. *Antenna* **7**, 105–107.
- Jackson, T.A. & Bell, N.L. (2001) *Characterisation and identification of nematodes associated with the palm pollinating weevil, *Elaeidobius kamerunicus**. Unpublished Report for the Malaysian Palm Oil Board. Lincoln, New Zealand; AgResearch, 17 pp.
- Poinar, Jr, G.O. (1975) *Entomogenous nematodes: A manual and host list of insect-nematode associations*. Leiden: E.J. Brill, 317 pp.
- Poinar, Jr, G.O. & Hansen, E. (1983) Sex and reproductive modifications in nematodes. *Helminthological Abstracts, Series B*, **52**, 145–163.
- Poinar, Jr, G.O., Ferro, C., Morales, A. & Tesh, R.B. (1993) *Anandranema phlebotophaga* n. gen., n. sp. (Allantonematidae: Tylenchida), a new nematode parasite of phlebotomine sand flies (Psychodidae: Diptera) with notes on experimental infection of these insects with parasitic rhabditoids. *Fundamental and Applied Nematology*, **16**, 11–16.
- Rao, V. & Law, I.H. (1998) The problem of poor fruit set in parts of East Malaysia. *The Planter*, Kuala Lumpur, **74**, 463–483.
- Remillet, M. & Laumond, C. (1991) Sphaerularioid nematodes of importance in agriculture. In: Nickle, W.R. (Ed.) *Manual of Agricultural Nematology*. New York: Marcel Dekker, pp. 967–1023.
- Siddiqui, M.R. (2000) *Tylenchida parasites of plants and insects*. (Second Edition). Wallingford CABI Publishing, 833 pp.
- Syed, R.A., Law, I.H. & Corley, R.H.V. (1982) Insect pollination of oil palm: Introduction, establishment and pollinating efficiency of *Elaeidobius kamerunicus* in Malaysia. *The Planter*, Kuala Lumpur, **58**, 547–561.