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Didilia ooglypta n. gen., n. sp. (Tetradonematidae : Mermithoidea : Nematoda), a parasite of phlebotomine sandflies in Afghanistan

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Summary – A new tetradonematid nematode in a laboratory colony of *Phlebotomus papatasi* from Afghanistan is described and named *Didilia ooglypta* n.g., n. sp. This genus differs from related ones by the extreme anterior position of the vulva and presence of a single gonad in both sexes. After eggs of the nematode are ingested by first instar sandfly larvae, they hatch in the intestine and the juveniles migrate to and develop in the haemocoel. Female nematodes in the haemocoel of adult flies lay eggs by pushing their heads through the abdominal intersegmental membranes of the host. An emended description of the family Tetradonematidae is provided.

Résumé – Didilia ooglypta *n. gen., n. sp. (Tetradonematidae : Mermithoidea : Nematoda), parasite de plébotomes en Afghanistan* – Un Tétradonématode nouveau, trouvé dans un élevage de laboratoire de *Phlebotomus papatasi* provenant d'Afghanistan, est décrit et nommé *Didilia ooglypta* n. gen., n. sp. Ce nouveau genre diffère des genres voisins par la position très antérieure de la vulve et la présence d'une seule gonade chez les deux sexes. Après avoir été ingérés par les larves du premier stade, les œufs de ce nématode éclosent dans l'intestin du plébotome, puis les formes juvéniles migrent et se développent dans l'hémocèle. Les femelles, toujours localisées dans l'hémocèle des phlebotomes adultes, passent ensuite leur tête à travers la membrane intersegmentaire abdominale de l'hôte pour pondre leurs œufs.

Key-words : *Didilia ooglypta,* Tetradonematidae, Nematoda, n. sp., n. gen., sandfly, taxonomy, Afghanistan.

The family Tetradonematidae has eight generally accepted species belonging to six genera. The validity of nine other species described by Rubtzov (1966) has been questioned by most workers (see Zervos, 1980). When the family was established, the principal distinguishing morphological character was the presence of four cells, the tetrad, now known to be connected to the oesophageal tube.

The hosts of most tetradonematids are species of Diptera or Coleoptera in which the adult worms are always parasitic. This contrasts with species of the related family Mermithidae, where the last stage juveniles leave the host and mature as free living adults.

Few complete life cycles of tetradonematids have been described. In 1988, we established an unnamed nematode of phlebotomine sandflies from Afghanistan in laboratory-bred *Phlebotomus papatasi* (Scopoli) and, on numerous occasions, have transmitted the worm without difficulty by feeding eggs to larvae of the fly. This has provided abundant material which forms the basis of the present description of the type species of a new genus of the family Tetradonematidae.

Materials and methods

Nematodes were found in the first generation of a colony of *P. papatasi* reared from eggs of adult flies collected in Afghanistan in 1988. Methods of maintaining the nematode in laboratory reared sandflies are given by Killick-Kendrick *et al.* (1989).

Infected sandfly larvae were dissected under a dissecting microscope in Lum's Ringer solution (0.215 g $NaHCO_3$, 0.115 g $CaCl_2 \cdot 2\ H_2O$, 0.09 g KCl and 6.75 g NaCl in 1000 ml distilled water). The nematodes were killed in hot Ringer's or water (60 °C for about 10 seconds) and fixed in TAF. Permanent preparations were made by Seinhorst's glycerin mounting method. Drawings were prepared with both interference and phase contrast illuminations. Living specimens were observed in Ringer's solution with the cover slip sealed with nail varnish. The SEM specimens were killed and fixed by using the same technique as described above. They were then dehydrated in ascending grades of ethanol and critical point dried. After sputter coating with gold, they were examined with a Cambridge S-100 scanning electron microscope. Detailed examinations of the gonads

were made by Geraert's (1972) extruding method. Measurements were taken from drawings of the permanently mounted specimens. Variability is shown by giving minimum and maximum measurements, and the mean ± SD.

Didilia n. gen.

Head homocephalic; no papillae. Stoma small, bearing a strong stylet in both juvenile and adult stages. Four tetrad cells distinct only in immature stages. Vulva lies at the anterior end, close to the mouth. Gonad single in both sexes. Anus absent.

TYPE SPECIES

Didilia ooglypta

*Didilia ooglypta** n. gen., n. sp.
(Figs 1-3)

MEASUREMENTS

Female (*paratypes*, n = 17) : L = 2220-3700 (3113 ± 360.9) µm; diam. at mid-body = 120-170 (141 ± 16.4) µm; stylet = 3.5-6.0 (4.8 ± 0.9) µm; dist. ant. end to vulva = 25-36.8 (32.6 ± 3.9) µm; body diam. at vulva 35.3-55.9 (50.0 ± 6.0) µm; oesoph. tube L = 220.6-411.8 (321.2 ± 53.1) µm; W = 3.1 ± 0.3 µm; vagina L = 48.5-85.3 (69.9 ± 10.2) µm; W (at middle) = 29.4-41.2 (35.6 ± 4.5) µm.

Male (*paratypes*, n = 10) : L = 544.1-900 (701.8 ± 86.9) µm; diam. at mid-body = 50-70.6 (61.6 ± 6.3) µm; stylet = 2.9-4.1 (3.7 ± 0.6) µm; oesoph. tube L = 152.9-252.9 (213.5 ± 27.8) µm; W = 2.8 ± 0.2 µm; dist. ant. end to gonad = 44.1-100 (73.8 ± 16.0) µm; body diam. at level ant. tip of gonad = 45.6-79.4 (57.1 ± 12.0) µm; length of the assumed seminal vesicle = 41.2-73.5 (57.7 ± 9.9) µm; spicule L = 35.3-52.9 (42.0 ± 5.2) µm; W = 3.8-5.3 (4.8 ± 0.5) µm; dist. opening of cloaca to post. end = 14.7-38.2 (30.1 ± 7.0) µm; body diam at level of cloaca = 20.6-32.2 (23.7 ± 3.4) µm.

Holotype (female) L = 3150.9 µm; W = 140.8 µm; dist. ant. end to vulva = 32.1 µm; W at vulva = 53.4 µm; W at mid-body = 36.9 µm.

Allotype (male) L = 1228.7 µm; W = 71.8 µm; W at spicule = 32.8 µm; spicule L = 37.6 µm; W = 5.8 µm.

DESCRIPTION

Adults and immature stages : Medium-sized nematodes. Both ends taper slightly. Head symmetrically round. Mouth exactly terminal. Oral aperture 1.5 to 2 µm in diameter. Stylet distinct, present in all stages. Male appears to have stronger cephalic cuticle than female. Striations around oral aperture are concentric circles, well marked on male, faint on female. No head papillae in either sex. In early stages, the tetrad is seen in the anterior third of the body behind numerous irregularly arranged small cells. The nuclei are large with many dark granules. All four cells have a nucleus with a granular extension which contacts the cell membrane adjacent to the oesophageal tube. When the small cells disappear and the gonad grows, the tetrad moves forward and the oesophageal tube loops. Nuclear extensions of the anterior and posterior pairs can be seen clearly and are in close proximity. The anterior two cells disappear during the nematode's development to adult, quickly followed by the posterior pair. Hence, the tetrad is not present in fully mature adults. The oesophageal tube ends blindly at the anterior end of the intestine. The nerve ring is extremely difficult to see, and was seen only once about 60 µm from the mouth in a young female with her body contents extruded. Two subdorsal and two subventral bands of muscles extend the length of the body. The vulva is controlled by two short retractor muscles. The intestine is long and slender, and occupies the middle third of the body in immature stages. It is in the center of the body cavity when the nematode is young and is pushed dorsally as the gonad develops.

Female : Vulva lies transversely at the anterior end of the body, ventral to the mouth. Posterior to the muscular vagina, which has a long barrel shape, the uterus is composed of two layers of cells. The single ovary occupies most of the body cavity. Eggs are spherical (diam. = 27.7 ± 1.5 µm; n = 21) or oval (26.6 × 21.8 µm; n = 17), dark brown, with a thick shell (3.0 µm) and a distinct surface pattern divided into hexagonal plates about three quarters of which measure 1.1 µm across; the smallest plates are half that size. Grooves between the plates are 0.1 to 0.2 µm in width and 0.3 µm in depth. Each female produces approximately 2000 eggs.

Male : One third the size of a female. Spicule single, arcuate with a sharp tip. Two or three small protuberances on the base can be seen occasionally. Genital papillae absent. Gonad single, a long sac, full of sperm and large granules. A duct, 14.1 µm long connects the gonad to a sac assumed to be a seminal vesicle, 57.6 µm long. Spermatozoa have a round head (diam. = 3.4 ± 0.7 µm), a long tail (15.4 ± 1.2 µm) and a dark spot (1.5 µm in length) near the neck.

TYPE HOST AND LOCALITY

Phlebotomus papatasi (Scopoli) (Diptera : Psychodidae). Kabul, Afghanistan (34° 28′ N, 69° 11′ E).

Additional host : *P. sergenti* Parrot.

DIAGNOSIS

Adults live in the haemocoel of the host; the egg is the only free living stage. Vulva located immediately ventral

* The generic name *Didilia* (fem.) is from a diminutive of Odile in honour of Dr. Odile Bain. The specific name *ooglypta* is from two Greek woords : *oön* (egg) and *glyptos* (carved or sculptured).

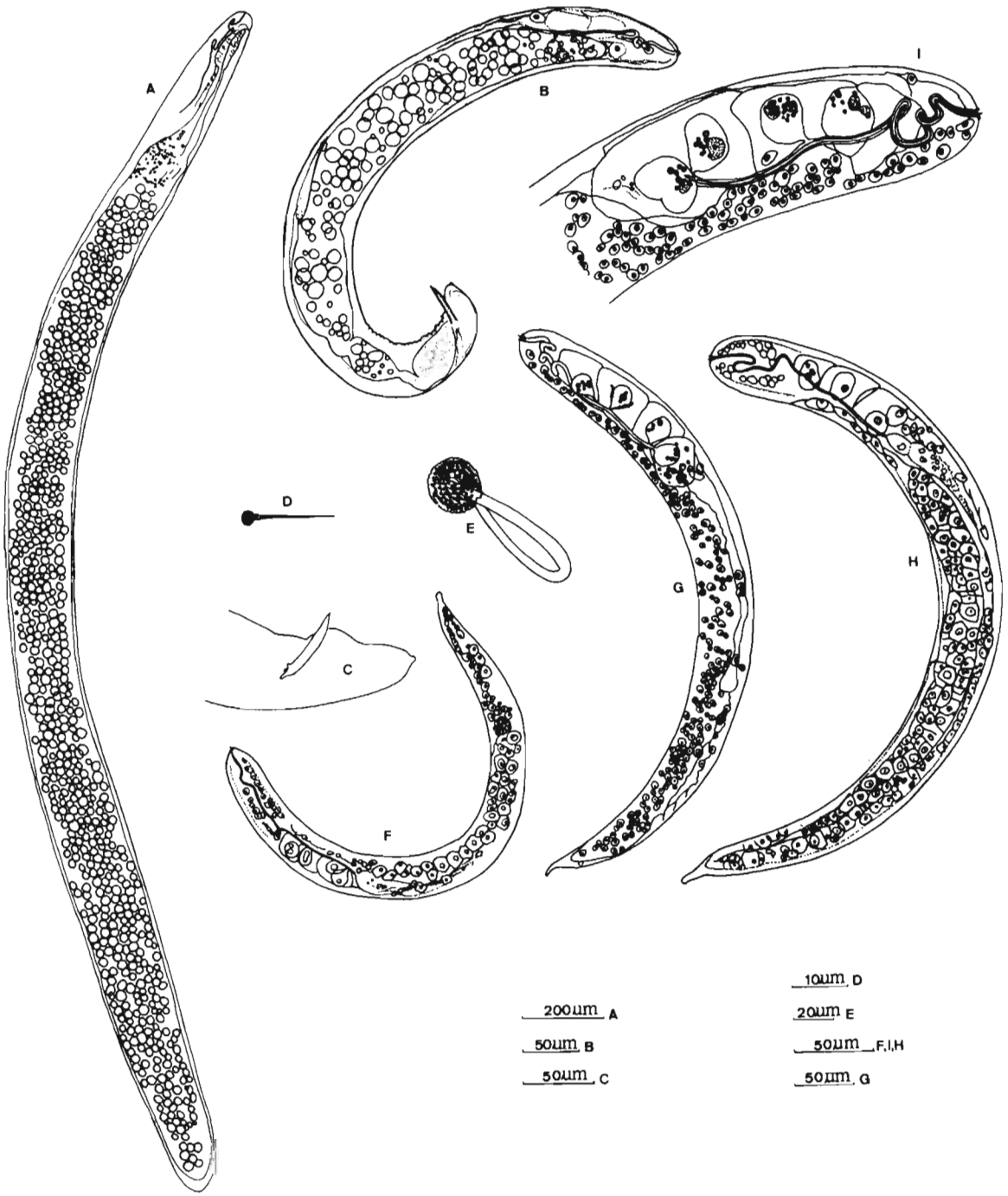

Fig. 1. *Didilia ooglypta* n. gen., n. sp. (lateral views). A : Mature female showing the anteriorly positioned vulva and single gonad; B : Mature male showing single gonad; C : Spicule with three protuberances on base; D : Sperm showing a dark spot near the neck; E : Artificially hatched juvenile; F : Young juvenile; G : Young juvenile showing intestine and nuclear extensions of tetrad cells; H : Young juvenile showing developing gonad; I : Oesophagus region showing tetrad and oesophageal tube.

Fig. 2. *Didilia ooglypta* n. gen., n. sp. (lateral views, except B). A : Adult female showing vagina and oviduct; B : Dorsal view of adult female showing vulva, vagina and oviduct; C : Anterior portion of young female with contents extruded showing nerve ring and bands of muscles; D : Female gonad; E : Anterior tip of a female showing stylet; F : Oviposition through abdominal segments of female sandfly; G : Oviposition through abdominal segments of male sandfly; H : Extruded testis of adult male.

to the mouth, which is unique for tetradonematids described to date. A stylet is found in both sexes and all stages. Spicule single. Gonad single in both sexes. Highly sculptured eggs.

Type specimens

Holotype (female) and allotype (male) deposited in The Natural History Museum, London, England. Two paratypes (male and female) deposited in the Nematology collection, Department of Nematology, University of California, Davis, CA 196516, USA; another two in the Muséum National d'Histoire Naturelle, Paris, France; and another two in Guangdong Entomological Institute, Guangzhou 510260, China.

Life cycle

First instar larvae of the sandfly become infected by

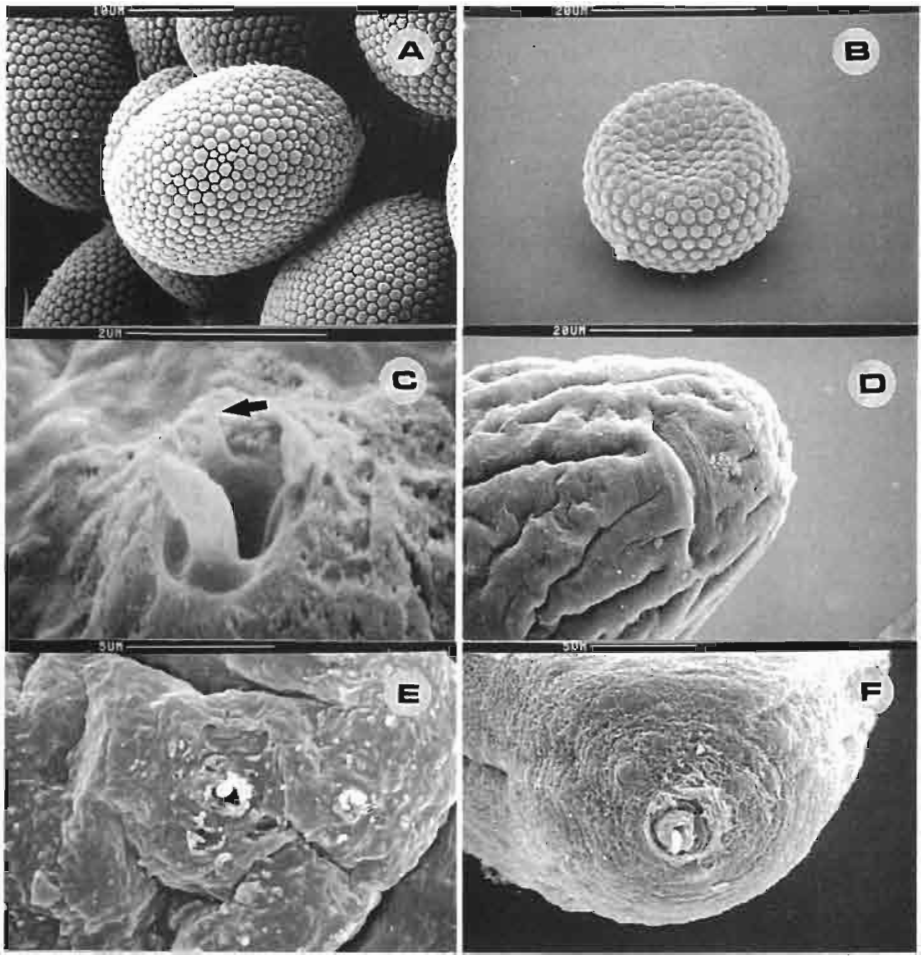

Fig. 3. *Didilia ooglypta* n. gen., n. sp. A : Normal eggs; B : Partially collapsed egg; C : Anterior tip of female showing stylet and tooth (arrowed); D : Anterior portion of female showing vulva; E : Head of female showing distorted cephalic cuticle; F : Head of male showing cephalic cuticle.

ingesting the eggs of *D. ooglypta*. The three later instars are not susceptible. The eggs hatch in the intestine of the larvae and infective stage juveniles penetrate through the gut wall into the haemocoel. The nematodes grow to adults by the time the sandfly larvae reach the third instar. The number of females per host is usually one or two, rarely three or four, whereas males are always more numerous, sometimes exceeding fifty. After mating, the male nematodes shrink and the females expand as they fill with eggs. When the adult fly emerges, one or more female worms can be seen coiled inside the abdominal cavity. By this time, juvenile nematodes have already developed inside the eggs in the uterus.

Within a day after adult fly emergence, the nematodes begin to lay their eggs by poking the anterior tip through the intersegmental abdominal membrane of the fly, usually between segments 8 to 9 of the males and segments 7 to 8 of the females (Fig. 2 F, G). The fly is not killed immediately and is able to resume flying, but dies usually within four days of emergence. When a fly is parasitized by more than one nematode, some of the parasites may not protrude, and the eggs remain in the worm until the host dies.

Each female nematode produces around 2000 eggs. The external genitalia of infected male sandflies fail to rotate and infected females will not take a bloodmeal (Killick-Kendrick *et al.*, 1989).

Discussion

Didilia ooglypta n. gen., n. sp. falls into the family Tetradonematidae because it possesses a typical tetrad and because the adults are parasitic. It differs from all other named species of the family by the anteriorly placed vulva of the female and the single gonad of both

sexes, features which warrant classification in a new genus and revision of the definition of the family.

The egg of *D. ooglypta* n. gen., n. sp., is the only free living stage, and it survives more than two years in humid frass at 27 °C. The stored frass was extracted by using the Whitehead tray method, and no nematodes were recovered. As the frass was then found still to be infective to sandfly larvae, we conclude that the nematode has no free living juveniles. This differs from *Tetradonema plicans* Cobb, 1919 which has a free living infective stage (Hudson, 1975). Moulting of juveniles was not seen and the number of stages of the worm is unknown. Although copulation was never observed, post copulatory changes were obvious. The males were shrunken and large numbers of spermatozoa were found in the females.

The eggs of *D. ooglypta* n. gen., n. sp. are spherical or oval but, when not properly fixed, they have the shape of a mushroom cap (Fig. 3 B). Inadequate fixation perhaps explains the description of the shape of the eggs of *T. plicans* as " mushroom cap " (Cobb, 1919) or " spheres depressed on one side " (Hudson, 1975). Our observations suggest that these descriptions are based on fixation artefacts and the character should be re-examined.

The SEM observations confirmed the morphological details and revealed that the stoma has a sharp tooth opposite the stylet of mature females (Fig. 3 C). This tooth might be used with the stylet to break through the intersegmental membranes when the female pushes her head out to lay eggs. This structure is not seen under the light microscope.

Reports of similar but unidentified nematodes in various species of phlebotomine sandflies suggest that *Didilia* n. gen. or related nematodes are parasites of *P. papatasi* in Pakistan (Lewis, 1967) and Afghanistan (Killick-Kendrick *et al.*, 1989); *P. sergenti* Parrot in Saudi Arabia (Büttiker & Lewis, 1983), Afghanistan (Killick-Kendrick *et al.*, 1989) and Morocco (J.-A. Rioux, pers. comm.); *Sergentomyia clydei* Sinton and *S. christophersi* Sinton in Saudi Arabia (Büttiker & Lewis, 1983); and *S. adleri* Theodor, *S. bedfordi* Newstead and *S. schwetzi* Adler, Theodor & Parrot in Uganda (G. R. Barnley, *in* Büttiker & Lewis, 1983). The lengths of the female worms in *S. clydei* from Pakistan (Lewis, 1967) and in Ugandan flies (Büttiker & Lewis, 1983) are similar to those of *D. ooglypta*. Sizes of the eggs of the parasites of *P. papatasi* and *S. clydei* from Pakistan fall into the range for eggs of *D. ooglypta* n. gen., n. sp. and one of the figures in the paper by Büttiker and Lewis (1983) shows the anteriorly situated vulva of a female worm. The same authors noted that, as with male flies infected with *D. ooglypta* n. gen., n. sp., the external genitalia of the males from Saudi Arabia were not fully rotated. There appear to be no published reports of tetradonematids in phlebotomines of the New World.

Tetradonema solenopsis Nickle, 1988 was described from a fire ant of South America. As the body contents of all the worms were disintegrated, no details of internal structures could be described. It is, therefore, difficult to be sure that the nematode belongs in the genus *Tetradonema*. It is noteworthy that all other tetradonematids are parasites of Diptera or Coleoptera whereas the host of *T. solenopsis* is in Hymenoptera. Further study is required to confirm the taxonomic position of this parasite.

Tetradonematidae Cobb 1919 (emended)

Head round; mouth minute; lips rudimentary or absent; stylet present in all or some stages or absent; oesophagus simple, with tubular lumen, associated with tetrad or its equivalent structure; anus absent; ovaries and testes outstretched, paired or single; spicule without accessories, paired or single; vulva equatorial or at the front extremity; maturing in the host's haemocoel, subsequent generation liberated from host by oviposition of female in the environment, by breakdown of host tissue, or by an escape-form.

Key to the species of the family Tetradonematidae

(Including *Tetradonema solenopsis* sp. inq.)

1. – Four celled tetrad distinct or with two companion cells 2
   – Oesophageal region has no four-celled structure 6
2. – Gonad single, vulva at anterior tip ..... *Didilia ooglypta* n. gen., n. sp.
   – Gonad paired, vulva not at tip 3
3. – Spicule paired ..... *Bispiculum inaequale* Zervos, 1980
   – Spicule single 4
4. – Female less than 1 mm long, first generation parthenogenetic ..... *Heterogonema ovomasculis* Van Waerebeke & Remillet, 1973
   – Female more than 1 mm long, not parthenogenetic ..... genus *Tetradonema* 5
5. – Female less than 2 mm long ..... *T. solenopsis* Nickle & Jouvenaz, 1987
   – Female more than 4 mm long, 2 companion cells visible, some lay eggs underneath the cuticle ..... *T. plicans* Cobb, 1919
6. – Intestinal diverticulum by oesophagus ..... genus *Aproctonema* 7
   – No intestinal diverticulum by oesophagus 8
7. – Female less than 3 mm long, male less than 0.4 mm long ..... *A. chapmani* Nickle, 1969
   – Female more than 4 mm long, male more than 2 mm long ..... *A. entomophagum* Keilin, 1917
8. – Oesophagus associated with three nucleated gland-like cells, spicule biarcuate ..... *Corettrellonema grandispiculosum* Nickle, 1969
   – Oesophagus associated with eight-celled stichosome, spicule arcuate ... *Mermithonema entophilum* Goodey, 1941

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