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Psammomermis nitidulensis n. sp. (Nematoda : Mermithidae) from sap beetles (Coleoptera : Nitidulidae) with biological observations and a key to the species of *Psammomermis*(1)

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Summary – *Psammomermis nitidulensis* n. sp. (Nematoda : Mermithidae) is described from three species of sap beetles, *Carpophilus lugubris* Murray, *C. antiquus* Melsh and *Colopterus truncatus* (Randall) (Coleoptera : Nitidulidae) from Mason County, IL, USA. This mermithid differs from previously described members of the genus by the shape of the amphids and their association with the amphidial glands, the length, form and curvature of the spicules and the shape of the *vagina vera* and *vagina uterina*. A key to the species of *Psammomermis* is provided and the generic diagnosis is emended. This mermithid causes up to 80 % parasitism and death of the dusky sap beetle (*C. lugubris*), an important vector of mycotoxigenic fungi. Investigations are under way to determine the possibility of utilizing this mermithid for biological control of this insect pest.

Résumé – Psammomermis nitidulensis *n. sp. (Nematoda : Mermithidae) provenant de trois coléoptères suceurs de sève (Coleoptera : Nitidulidae); observations biologiques et clé des espèces du genre* Psammomermis – *Psammomermis nitidulensis* n. sp. (Nematoda : Mermithidae) est décrit provenant de trois espèces de coléoptères suceurs de sève, *Carpophilus lugubris* Murray, *C. antiquus* Melsh et *Colopterus truncatus* (Randall) (Coleoptera : Nitidulidae), récoltées dans le Comté de Mason, Illinois, États-Unis d'Amérique. Ce Mermithide diffère des autres espèces du genre par la forme des amphides et leur type d'association avec les glandes amphidiales, la longueur et la forme des *vagina vera* et *vagina uterina*. Une clé des espèces du genre *Psammomermis* est proposée et la diagnose en est amendée. Le Mermithide peut provoquer jusqu'à 80 % d'infestation et de mort chez le coléoptère suceur de sève *C. lugubris*, un vecteur important de champignons mycotoxigènes. Des recherches sont en cours concernant l'utilisation possible de ce Mermithide en vue du contrôle biologique de cet insecte nuisible.

Key-words : *Carpophilus*, dusky sap beetle, Nitidulidae, *Psammomermis*, nematodes.

Sap beetles (Coleoptera : Nitidulidae) are mostly small beetles less that 12 mm in length. Members of the family are attracted to fresh, processed and stored fruits and vegetables and some are pests. One member of this family, the dusky sap beetle, *Carpophilus lugubris* Murray, can damage a variety of vegetables, especially corn, where it occurs inside the kernels at the top of the ears. This species is also a significant vector of mycetoxigenic fungi and is known to carry the aflatoxin-producing fungus, *Aspergillus flavus* Link *ex* Fries to corn (Lussenhop & Wicklow, 1990).

A survey of various localities in Illinois, USA was initiated to search for natural enemies of *C. lugubris* and at one of nineteen sites surveyed over a 4-year period, a nematode was found parasitizing up to 80 % of the adults of the dusky sap beetle (Dowd *et al.*, 1995).

The nematode proved to be an undescribed species of *Psammomermis* Polozhentsev, 1941 and is described below and biological observations and a key to the known species of *Psammomermis* are provided.

Materials and methods

Sap beetles were collected from the University of Illinois River Sand Field Experiment Station, Kilbourne, IL, USA from 1991 to 1994. The collection area was planted in corn and soybeans and was surrounded on two sides by oak forest and on the third side by open prairie. The individual insects were collected in PVC pipe traps (Dowd *et al.*, 1992) baited with the pheromone of *C. lugubris*. The traps were examined once or twice a week and any adult sap beetles were removed from the traps and held on pinto bean-based diet in small plastic cups at room temperature and ambient light (Dowd, 1987). When the insects died, they were placed individually in small plastic vials containing 1 %

(1) Names are necessary to report factually on available data; however, the USDA neither grarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

water agar and held until the nematodes emerged. The dead insects were removed after the nematodes had completely emerged and the nematodes were maintained in the agar at room temperature at ambient light and left until they had molted to the adult state. Adult nematodes were heat killed in water (60 °C), fixed in TAF and processed to glycerin.

Psammomermis Polozhentsev, 1941

DIAGNOSIS (Kaiser, 1984; Poinar & Jackson, 1992; emended)

Long, thin terrestrial mermithids ranging in color from white to yellow; postparasitic and adult cuticle usually relatively thin, with faint or no cross-fibers under the light microscope at 20 ×; head rounded to truncate, mouth opening terminal or shifted ventrad; lip papillae absent; six cephalic papillae arranged in one or rarely two planes; two hypodermal pegs may be present in the area of the cephalic papillae, often positioned at the tip of the head (these may be additional cephalic papillae or another type of sensory structure); amphids small to medium in size, from cup to pear to flask- shaped; opening circular to elliptical; eight hypodermal cords at midbody; spicules paired, separate, from medium to long (longer than body width at cloaca); vagina barrow or pear-shaped, muscular, often composed of an outer *vera* portion and an inner *uterina* portion; vaginal canal straight or bent at the junction of *vagina vera* and *vagina uterina;* tail of postparasitic juvenile either entire, with a cuticular appendage or a small knob.

TYPE SPECIES

P. korsakovi Polozhentsev, 1941.

OTHER SPECIES

P. acricephala Ipatjeva & Pimenova, 1985
P. alechini Artyukhovsky & Kharchenko, 1965
P. busuluk Polozhentsev, 1952
P. byssina Rubstov, 1976
P. canterburiensis Poinar & Jackson, 1992
P. communis Popov, 1978
P. conjuncta Kaiser, 1984
P. cornicularis Kaiser, 1984
P. filiformis Kaiser, 1984
P. guffeldi Ipatjeva & Pimenova, 1985
P. ipatjevae Popov, 1978
P. kulagini Polozhentsev, 1941
P. montana Ipatjeva & Pimenova, 1985
P. minor Kaiser, 1984
P. nitidulensis n. sp.
P. oesophaga Artyukhovsky & Lisikova, 1977
P. parvula Rubstov, 1976
P. pologenzevi Popov, 1978
P. sericesthidis Baker, Poinar & Campbell, 1997

***Psammomermis nitidulensis* n. sp.**

(Fig. 1)

MEASUREMENTS

Female (n = 7) : L = 54 (26-85) mm; greatest diameter = 104 (92-113) µm; distance from head to nerve ring = 262 (238-276) µm; length of amphidial pouch = 18 (16-19) µm; diameter of amphidial opening = 3.8 (3.0-4.6) µm; *vagina vera* length = 28 (19-32) µm; *vagina uterina* length = 38 (29-45) µm; V = 52 (49-56); distance from end of trophosome to tail tip = 140 (100-195) µm.

Male (n = 6) : L = 28 (20-42) mm; greatest diameter = 78 (63-113) µm; distance from head to nerve ring = 179 (174-190) mm; length of amphidial pouch = 19 (15-22) µm; diameter of amphidial opening = 3.2 (3.0-3.4) µm; length of spicules = 261 (200-293) µm; greatest width of spicule shaft = 5.6 (4.7-7.3) µm; tail length = 170 (145-181) µm; tail width at cloaca = 67 (60-73) µm; ratio of tail length to tail width at cloaca = 2.6 (2.3-2.8).

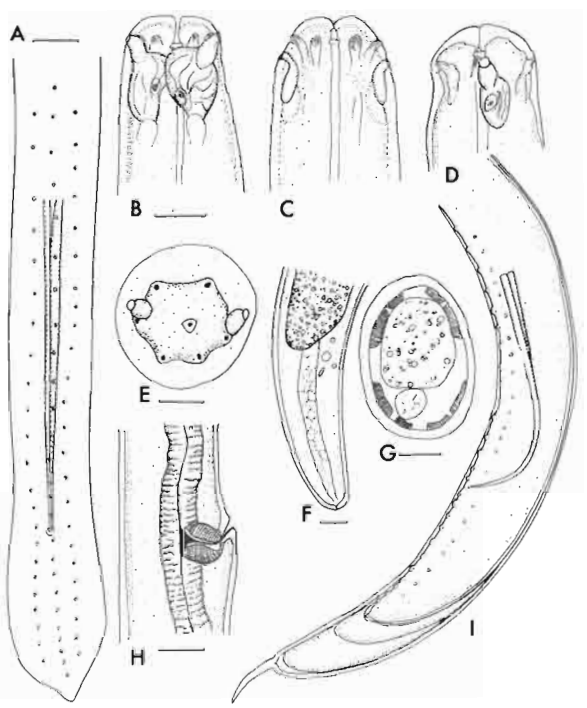

Fig. 1. Psammomermis nitidulensis *n. sp. A : Ventral view of male tail; B : Dorsal view of male head; C : Dorsal view of female head; D : Lateral view of female head; E : En face view of male; F : Lateral view of female tail; G : Cross section of male at mid-body; H : Lateral view of vulvar area; I : Lateral view of male tail with cuticles from last molts present (Scale bars : A, F = 32 µm; B-D = 23 µm; E = 13 µm; G = 19 µm; H-I = 38 µm).*

DESCRIPTION

Adults : Narrow, small, white nematodes with a smooth cuticle. Flat to rounded head. Terminal mouth opening : six cephalic papillae arranged in a single circle, lacking distinct hypodermal cephalic pegs. Amphidial pouches flask to pear-shaped, closely associated with amphidial glands; amphidial openings circular, similar in both sexes; amphids located either slightly posterior or adjacent to the lateral cephalic papillae; eight hypodermal cords at mid-body; *vagina vera* (outermost portion of vagina adjacent to vulva) thin, lined with cuticle, meeting the *vagina uterina* (inner portion of vagina between *vagina vera* and the uterus) at a sharp angle; *vagina uterina* surrounded with strong circular muscles; vulva opening at a sharp angle. Spicules long, paired, separate, with a strong curve in the distal half, yellow, closely appressed in the distal half and tapering to a fine point; genital papillae in three rows; eight to eleven postcloacal papillae and fifteen to seventeen pre-cloacal papillae per row; pre-cloacal papillae extending past base of spicules; tail of both sexes narrowly rounded; small hypodermal peg transversing the cuticle of the tail in both sexes; disc-shaped protein crystals occuring in the body cavity of both sexes.

Postparasitic juveniles (n = 10) : Cephalic papillae and amphids smaller and less conspicuous than in the adults; tail tip with a large cuticular projection, 40-55 µm in length.

An atypical condition occurred where the final molt consisted of not two but three separate cuticles (Fig. 1I). It is not known whether one of the inner cuticles represented an extra molt or whether the cuticles represented the true second, third and fourth molts of the specimen. If the latter, then either the egg molt or the parasitic molt that is typical of mermithids, was surpressed.

No cross fibers were noted in any of the cast cuticles.

TYPE HOST AND LOCALITY

Dusky sap beetle, *Carpophilus lugubris* Murray (Coleoptera : Nitidulidae), collected from the Illinois River Sand Field Experiment Station at Kilbourne, Mason County, IL, USA. Other hosts were the smaller antique sap beetle (*Carpophilus antiquus* Melsh) and *Colopterus truncatus* (Randall), all of which are in the subfamily Carpophilinae of the Nitidulidae.

TYPE SPECIMENS

Holotype (male)(UCDNC # 3319) and allotype (female)(UCDNC # 3320) deposited in the Division of Nematology, University of California, Davis, California. Paratypes in the collection of the Muséum National d'Histoire Naturelle, Paris, and in the senior author's collection.

DIAGNOSIS AND RELATIONSHIPS

Small, narrow nematode with six cephalic papillae, eight hypodermal cords and medium-sized amphids. Both the adult and the postparasitic juvenile cuticles lack cross-fibers. Key characters separating the present species from previously described ones are the ratio and angle of the *vagina vera* to the *vagina uterina*, the amphidial glands in association with the amphidial pouches, the length and shape of the spicules, the length/width ratio of the male tail – a character used by Artyukhovski (1990) for separating members of this genus –, the area covered by the genital papillae (especially pre-cloacal), the terminally placed mouth and the absence of cephalic hypodermal pegs.

BIOLOGICAL OBSERVATIONS AND DISCUSSION

Most of the postparasites of *P. nitidulensis* emerged from their adult beetle hosts within 2 weeks after field collections. The final molt to the adult stage occurred between 2 and 3 months later. Normally there was only a single nematode per host but as many as four nematodes were observed to emerge from a single beetle.

Three separate beetle species can serve as hosts to *P. nitidulensis* n. sp. However almost all of the nematodes emerging from *C. lugubris* were female while nematodes emerging from *C. antiquus* and *C. truncatus* were both male and female. Environmental control of sex in the Mermithidae is well known (Poinar & Hansen, 1984) and it is generally considered that an abundance of nourishment results in female production whereas restricted nourishment (as a result of small hosts or multiple infections in a single host) results in male formation. In the case of *Hexamermis arvalis* Poinar & Gyrisco, 1962, infection of alfalfa weevil larvae resulted in the production of almost all males (Poinar & Gyrisco, 1962). This could have been an indication of a relatively recent host-parasite association. The alfalfa weevil (*Hypera postica* Gyll.) was a recent European introduction into the area where *H. arvalis* normally parasitized plant bugs and noctuid larvae. Parasitizing the alfalfa weevil without a period of adaptation resulted in the formation of males, perhaps because some nutritional unbalance affected female development. The same scenario may occur in the present situation. Just when *C. lugubris* entered North America is not known but since it is primarily a southern and neotropical species (Blackwelder, 1945), introductions could have been less that 100 years ago. In this case, *C. lugubris* would be a recent host without any period of adaptation, resulting in the formation of predominately females. In contrast, the nematode had already established a balance with the smaller native species, *C. antiquus* in which it produced both sexes. It appears that mermithids, as with entomogenous nematode parasites in general, are able to adapt quickly to new hosts in the environment. However, several stages of adaptation are required, including an adjustment to the physiological conditions in the host's hemocoel, avoiding host reactions, completing parasite development and establishing a normal sex ratio. In the present case, parasitism of *C. lugubris* is made possible since the

native host species can provide males. As long as at least one other of the three hosts is present with *C. lugubris*, a normal parasitic cycle should continue. After a period of time, a physiological race of *P. nitidulensis* n. sp. would be expected to arise which would produce both sexes in *C. lugubris*.

Key to the species of *Psammomermis*

The following key separates the present species from previously described members of *Psammomermis*. Diagnostic characters of many of the Russian species were obtained from Artyukhovski (1990). Since basic diagnostic characters (including males) are absent from the description of *P. ipatjevae* Popov 1978, it is omitted from the key.

1 - Head with one or two hypodermal pegs in addition to six cephalic papillae 2
- Head without hypodermal pegs 5

2 - Head with a single dorsal hypodermal peg 3
- Head with a pair of hypodermal pegs 4

3 - Female length over 100 mm; female head flat ..... *P. sericesthidis*
- Female length under 50 mm; female head attenuated ..... *P. acricephala*

4 - Hypodermal pegs in lateral position ..... *P. cornicularis*
- Hypodermal pegs in dorsal position ..... *P. communis*

5 - Spicules short, under 200 µm in length 6
- Spicules greater than 200 µm in length 11

6 - Spicule length less than 75 µm ..... *P. guffeldi*
- Spicule length 75-200 µm 7

7 - Mouth ventral ..... *P. parvula*
- Mouth terminal 8

8 - *Vagina vera* meeting *vagina uterina* at an angle ..... *P. montana*
- *Vagina vera* and *vagina uterina* form a straight or nearly a straight line 9

9 - *Vagina vera* longer than *vagina uterina* *P. canterburiensis*
- *Vagina vera* shorter than *vagina uterina* 10

10 - Spicule length 75-100 µm; adult head not pointed ..... *P. byssina*
- Spicule length 100-200 µm; adult head pointed ..... *P. minor*

11 - Spicule length 200-450 µm 12
- Spicule length > 450 µm 17

12 - Ratio of tail length over tail width 1.5-3.0 13
- Ratio of tail length over tail width 0.5-1.5 .. *P. oesophaga*

13 - Ratio of tail length over tail width 2.3-3.0 14
- Ratio of tail length over tail width 1.5-2.3 15

14 - *Vagina vera* meeting *vagina uterina* at an angle; spicule length < 300 µm ..... *P. nitidulensis* n.sp.
- *Vagina vera* forming a straight line with *vagina uterina;* spicule length > 300 µm ..... *P. alechini*

15 - Spicule length 200-300 µm ..... *P. kulagini*
- Spicule length 300-450 µm 16

16 - *Vagina vera* meeting *vagina uterina* at an angle; spicule curved twice ..... *P. conjuncta*
- *Vagina vera* forming a straight line with *vagina uterina;* spicule curved once ..... *P. korsakovi*

17 - Ratio of tail length over tail width = 1.0 ..... *P. busuluk*
- Ratio of tail length over tail width > 1.5 18

18 - Spicules curved twice ..... *P. pologenzevi*
- Spicules with single curve ..... *P. filiformis*

Discussion

The genus *Psammomermis* is cosmopolitan in distribution, having been recorded from several land masses including New Zealand and Australia. Known hosts are limited to members of the Coleoptera, especially the Scarabaeidae. This is the first report of an infection with members of the family Nitidulidae. The genus *Psammomermis* was erected by Polozhentsev (1941) when he discovered terrestrial mermithids that fit in the aquatic genus *Mesomermis*. Thus, the original diagnostic character separating the two genera was that *Psammomermis* was terrestrial. This ecological distinction met with criticism amongst other workers and Welch (1956) did not recognize the genus since he felt that Polozhentsev was following the teachings of Lysenko and Michurin by erecting *Psammomermis*. As a result *Psammomermis* was and sometimes still is synonymized with *Mesomermis* (Poinar, 1977).

However, a re-evaluation of the characters in the growing number of species showed that members of the genus *Psammomermis* share common characters and that the narrow body shape, form of the amphids, and shape and size of the spicules, differ from the species placed in *Mesomermis*. This is not to say that variation does not exist among and between species of *Psammomermis*. The presence of cephalic hypodermal pegs (function unknown) in some of the species in the genus is one example. As with other mermithid genera, adaptation to new hosts is an important aspect of their evolution and just how different hosts affect character formation in mermithids is a relatively unexplored area.

Some species of *Psammomermis* have been described as possessing an " excretory pore " near the nerve ring. The present species lacks such a pore. In general, mermithids usually lack excretory pores, yet structures resembling excretory pores have been described in species from several genera. Whether this structure is really associated with an excretory system still awaits proof. There is some evidence suggesting that these " excretory pores " are in fact nerve endings that penetrate the cuticle (Poinar, unpubl.). Further studies on these structures are desirable.

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