

## *Gastromermis massei* n. sp. (Nematoda: Mermithidae) a parasite of blackflies *Simulium wolffhuengeli* Enderlein (Simuliidae: Diptera) in Argentina

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Accepted for publication 27 January 1997.

**Summary** – *Gastromermis massei* n. sp. (Nematoda: Mermithidae) is described and illustrated. This species was found parasitizing *Simulium wolffhuengeli* Enderlein (Simuliidae: Diptera) in the Cosquin River in Córdoba, Argentina. It can be distinguished from the other species of the genus by the following characters: number (six) of hypodermal chords, position of the mouth, shape of amphidial pore, absence of vulval flaps, length of spicule, shape of proximal and distal ends of spicule, number of genital papillae, and length of tail appendage of postparasitic juveniles. *G. massei* n. sp. is the sixth species of *Gastromermis* described in Argentina; among Mermithidae, this genus is the most common in the country. A key to the species of *Gastromermis* with six hypodermal chords is proposed.

**Résumé** – *Gastromermis massei* n. sp. (Nematoda: Mermithidae), un parasite de *Simulium wolffhuengeli* Enderlein (Simuliidae: Diptera) en Argentine – *Gastromermis massei* n. sp. (Nematoda: Mermithidae) est décrit et illustré. Cette espèce, parasite de *Simulium wolffhuengeli* Enderlein (Simuliidae: Diptera), a été trouvée dans la rivière Cosquin, à Córdoba, Argentine. Elle peut être séparée des autres espèces du genre par la combinaison des caractères suivants : présence de six cordes hypodermiques, position de l'orifice buccal, forme du pore amphidial, absence de volets vulvaires, longueur et forme des extrémités proximale et distale des spicules, nombre de papilles génitales et longueur de l'appendice caudal chez les individus postparasites. *G. massei* n. sp. est la sixième espèce du genre décrite en Argentine, genre le mieux représenté chez les Mermithidae de ce pays. Une clef d'identification des espèces du genre à six cordes hypodermiques est proposée.

**Key-words** : blackfly host, *Gastromermis massei*, key, mermithid, nematode, taxonomy.

*Gastromermis* is the genus of Mermithidae that has the largest list of species. Up to now, the following species have been described from Argentina: *G. kolleonis* Doucet & Poinar, 1984, *G. fidelis* Doucet, 1982, *G. vaginiferous* Camino, 1985, *G. cordobensis* Camino, 1991, and *G. doloresi* Camino, 1993. The first of these species parasitizes *Chironomus*, the others, *Simulium* (Doucet 1982; Doucet & Poinar, 1984; Camino 1985, 1991, 1993). During 1995 and 1996, mermithid parasites of *Simulium wolffhuengeli* Enderlein were collected from Cosquin river in Bialet Massé (Córdoba, Argentina). These mermithids are here described as a new species of the genus *Gastromermis*.

### Materials and methods

Parasitic juveniles were recovered from larvae of the blackfly *Simulium*. All the specimens were observed alive, then heat-killed in 65 °C water, fixed in TAF for 1 week, and processed to glycerin by the Seinhorst method (Curran & Hominick, 1980). Drawings and measurements were made from fixed specimens. Sec-

tions, made for hypodermal chord observation, and head face view were mounted in glycerin jelly (Anderson, 1958). The embryonic development was observed by hanging drop preparation (B'Chir, 1979).

### *Gastromermis massei* n. sp. (Fig. 1)

#### MEASUREMENTS

*Females* (n=6): Body length =  $19.1 \pm 3.1$  (15.1-22.2) mm. Maximum diameter  $256.4 \pm 48.7$  (208.3-338.0)  $\mu\text{m}$ . Body width at level of: cephalic papillae =  $73.2 \pm 7.0$  (67.5-86.5)  $\mu\text{m}$ ; at nerve ring = 124.4 (106.1-145.4)  $\mu\text{m}$ . Cuticle thickness: at nerve ring = 4.6 (3.7-5.0)  $\mu\text{m}$ ; at mid-body = 2.7 (2.5-3.0)  $\mu\text{m}$ ; at terminus = 3.7 (2.5-6.2)  $\mu\text{m}$ . Amphid pouch =  $17.3$  (12.5-23.6)  $\times$   $10.5$  (7.5-15.7)  $\mu\text{m}$ . Distance from head to nerve ring =  $235.4 \pm 18.7$  (204.4-259.4)  $\mu\text{m}$ . V =  $52.6 \pm 4.1$  (47.9-59.1). Vagina length =  $392.5 \pm 57.9$  (300-460)  $\mu\text{m}$ . Eggs in uterus (n = 5) =  $58.2 \times 42.7$   $\mu\text{m}$ .

*Holotype (female)*: Body length = 19.7 mm. Maximum diameter = 269.2  $\mu$ m. Body width: at cephalic papillae = 75.0  $\mu$ m; at nerve ring = 121.8  $\mu$ m. Distance from head to nerve ring = 204.4  $\mu$ m. Cuticle thickness: at nerve ring = 5  $\mu$ m; at mid-body = 2.5  $\mu$ m; at terminus = 2.5  $\mu$ m. Amphid pouch = 12.5  $\times$  7.5  $\mu$ m. V = 53. Length and width of vagina = 300  $\times$  62.8  $\mu$ m.

*Males (n=11)*: Body length = 13.0  $\pm$  1.6 (10.9-15.6) mm. Maximum diameter = 119.4  $\pm$  13.6 (100.0-145.0)  $\mu$ m. Body width: at cephalic papillae = 57.0  $\pm$  4.6 (50.0-65.0)  $\mu$ m; at nerve ring = 88.6  $\pm$  8.7 (70.7-106.1)  $\mu$ m. Cuticle thickness: at nerve ring = 3.5 (2.0-5.9)  $\mu$ m; at mid body = 3.5 (2.0-5.9)  $\mu$ m; at terminus = 3.5 (2.0-5.9)  $\mu$ m. Amphid pouch = 16.7 (12.5-22.5)  $\times$  11.5 (8.7-15)  $\mu$ m. Distance from head to nerve ring = 215.6 (176.8-247.6)  $\mu$ m. Spicule length = 617.1  $\pm$  37.4 (570.0-680.0)  $\mu$ m. Spicule head width = 20.3  $\pm$  4.1 (15.7-27.5)  $\mu$ m; mid-shaft width = 15.6  $\pm$  3.5 (10.0-23.6)  $\mu$ m. Tail length = 148.4  $\pm$  9.5 (127.5-162.5)  $\mu$ m. Tail width at cloaca = 101.5  $\pm$  6.7 (90.0-110.0)  $\mu$ m.

*Allotype (male)*: Body length = 13.77 mm. Maximum diameter = 115  $\mu$ m. Body width: at cephalic papillae = 60  $\mu$ m; at nerve ring = 90.4  $\mu$ m. Distance from head to nerve ring = 235.8  $\mu$ m. Cuticle thickness at nerve ring = 5  $\mu$ m; at mid-body 5  $\mu$ m; at terminus = 3  $\mu$ m. Amphid pouch = 12.5  $\times$  12.5  $\mu$ m. Spicule length = 580  $\mu$ m; mid-shaft width = 27.5  $\mu$ m.

*Infective-stage juveniles (n=23)*: Body length = 680.7  $\pm$  27.6 (650.0-751.0)  $\mu$ m. Maximum diameter = 5.2  $\pm$  0.5 (4.0-6.0)  $\mu$ m. Distance from head to nerve ring = 67.3  $\pm$  2.5 (63.5-73.0)  $\mu$ m. Body width at nerve ring = 9.4  $\pm$  0.7 (8.0-10.5)  $\mu$ m. Stylet length = 19.8  $\pm$  3.0 (14.0-27.0)  $\mu$ m.

*Postparasitic juveniles females (n=13)*: Body length = 18.4  $\pm$  1.51 (16.3-22.1)  $\mu$ m. Tail appendage length = 105.5  $\pm$  13.4 (80.0-127.5)  $\mu$ m. Tail appendage width = 12.7  $\pm$  3.1 (7.5-20.0)  $\mu$ m; V = 52.2  $\pm$  2.1 (48.5-56.3).

*Posparasitic juveniles males (n=6)*: Body length = 12.5  $\pm$  1.7 (10.0-14.3)  $\mu$ m. Tail appendage length = 99.4  $\pm$  17.4 (75.0-120.0)  $\mu$ m. Tail appendage width = 12.8  $\pm$  2.8 (7.9-15.7)  $\mu$ m. Spicule length = 473.0  $\pm$  23.9 (330.0-600.0)  $\mu$ m.

#### DESCRIPTION

*Adults*: Medium sized nematodes with thin cuticle (2.5-6  $\mu$ m), without criss-cross fibers. Habitus helioid. Rounded head. Six head papillae groups. Six hypodermal chords at mid body. Mouth with slight ventral shift. Vestibulum well developed. Amphids flask-shaped, slightly shifted to dorsal side, without a commissure; anterior portion of amphidial openings pointed and located posterior to circle of cephalic papillae. Live females (adults and juvenile) green in

color; juvenile males, males and preparasitic juveniles, transparent. Oesophagus of uniform width (2.5  $\mu$ m), with strongly cuticularized walls, extending past the middle of the body. Trophosome beginning immediately behind nerve ring and terminating near end of body. Protein platelets occurring in body cavity. Tails of both sexes conoid, ventrally flattened, dorsally convex.

*Female*: Muscular, modified S-shaped or elongated vagina; extending posteriorly, then bending toward the anterior end. Vulval flaps absent.

*Eggs*: Elliptical in shape, unembryonated at oviposition and freely deposited in the substrate; chorion composed of single layer, thin and colorless.

*Males*: Single spicule, longer than twice anus-to-terminus distance; head of the spicule with the dorsal flange extending farther toward the anterior end than the ventral flange; distal portion ventrally bent with a pointed end. Genital papillae variable in number, extending from tail tip to proximal portion of the spicule and arranged in three rows; central row bifurcating around anus and with thirteen-eighteen preanal and ten to twelve postanal papillae; lateral row with ten to twelve preanal and eight to ten postanal papillae.

*Post-parasitic juveniles*: Slightly larger than the adults, but similar in width. Cephalic papillae and amphids reduced. Long and acute tail appendage. Future females developing a clearly visible S-shaped vagina. Future males with developing spicule.

*Infective-stage juveniles*: Head rounded. Thin and long stylet. Stichocytes and penetration glands not clearly differentiated. Intestine ending in a faint anal opening. Filiform tail.

*Parasitic stage*: Variable morphology during development. In future females incipient vagina. In future males presence of the spicule not observed.

#### TYPE HOST AND LOCALITY

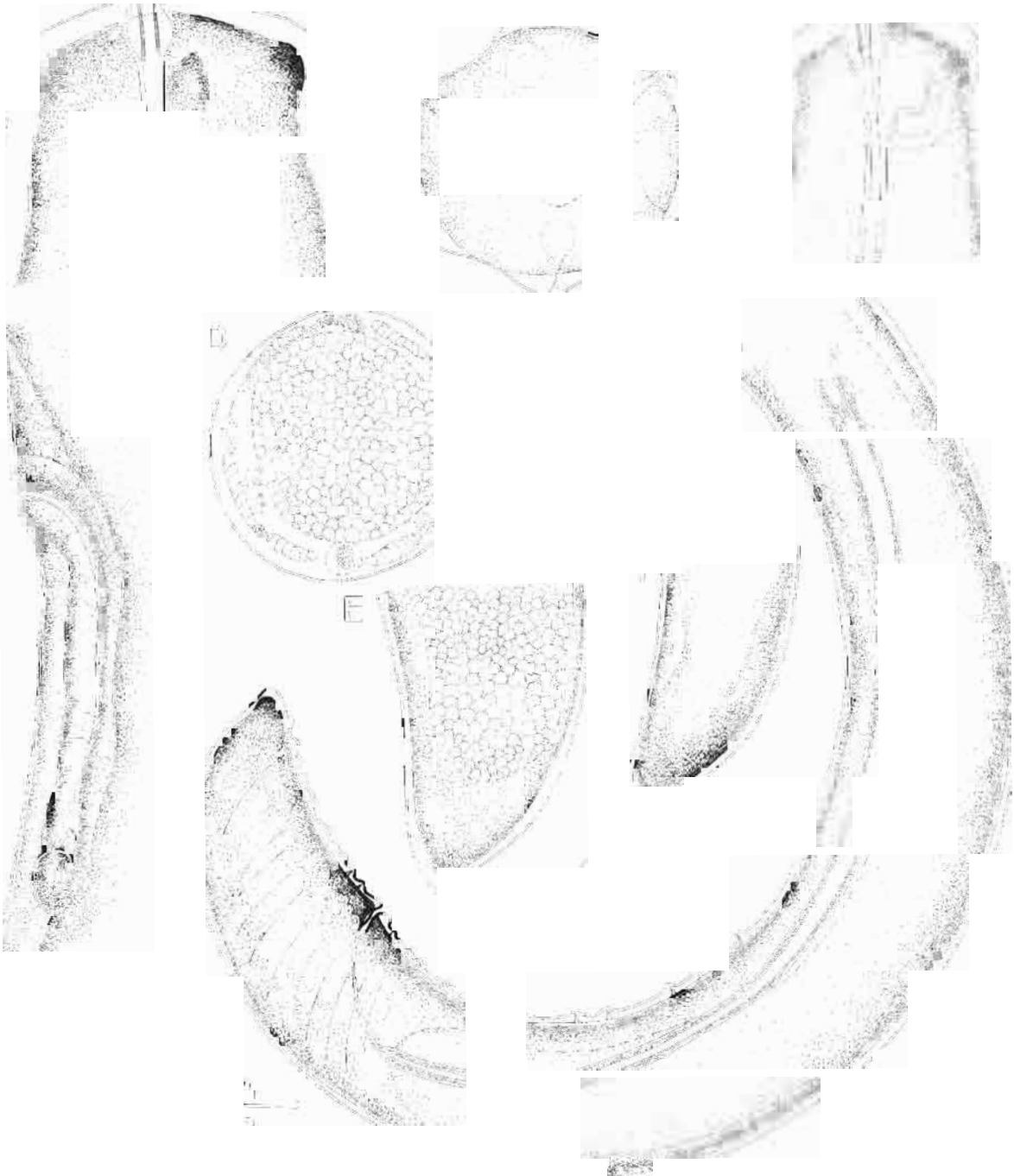
Larvae of *Simulium wolffhuengeli* (Diptera: Simuliidae), collected from Cosquin river, Córdoba, Argentina.

#### TYPE SPECIMENS

Holotype (female) and allotype (male) deposited in the Nematology Collection at the Centro de Zoología Aplicada, Universidad Nacional de Córdoba. Paratypes (one female, one male and three infective-stage juveniles) deposited in Laboratoire de Biologie Parasitaire, Protistologie, Helminthologie, Muséum National d'Histoire Naturelle, Paris, France.

#### DIAGNOSIS AND RELATIONSHIPS

*G. massei* n. sp. is characterized by the presence of the following features: six hypodermal chords, mouth opening slightly shifted to the ventral side, anterior portion of amphidial openings pointed, vulval flaps



absent, medium-sized spicule (617 [570-680]  $\mu\text{m}$ ), spicule head with the dorsal flange extending farther toward the anterior end than the ventral flange, spicule distal part ventrally bent with pointed end, and length of the postparasitic juveniles tail appendage (102.5 [75-127.5]  $\mu\text{m}$ ).

*G. massei* n. sp. belongs to a group of species with six hypodermal chords including: *G. philipponi* Mondet, Poinar & Bernadou, 1977; *G. leberrei* Mondet, Poinar & Bernadou, 1977; *G. cloacachilus* Poinar & Takaoka, 1981; *G. brevispicularis* Vosylyte & Kontrimavichus, 1981; *G. hexachordalis* Vosylyte & Kontrimavichus, 1981; *G. metae* Curran & Hominick, 1981; *G. fidelis* Doucet, 1982; *G. kolleonis* Doucet & Poinar, 1984, and *G. mesostoma* Poinar & Takaoka, 1986. The length of the spicule – 617.1 (570-680)  $\mu\text{m}$  – clearly differentiates *G. massei* n. sp. from the other *Gastromermis* species with six hypodermal chords (see key).

*G. massei* n. sp. is similar to *G. cloacachilus* and *G. fidelis* in having a mouth opening slightly ventrally shifted but it may be differentiated from them by the shape of the amphids and by the longer tail appendage of the postparasitic juveniles (102.5  $\mu\text{m}$  vs 40 and 90  $\mu\text{m}$ , respectively).

The absence of a vulval flap in *G. massei* n. sp. clearly differentiates this species from *G. kolleonis*. It can be distinguished from *G. philipponi* because the postparasitic juveniles of *G. philipponi* have a conical-trunk tail appendage whereas this appendage is fili-form in the new species.

The position of the mouth opening is variable in *G. hexachordalis* and *G. mesostoma*; however, the amphids of both species are larger than those of *G. massei* n. sp.; moreover, the first species differs by having amphids opening at the level of the cephalic papillae and by having a developed mouth cone; the second species has a tail appendage longer than that of *G. massei* n. sp. (110 vs 102.5  $\mu\text{m}$ ).

The number of hypodermal chords was not given in the descriptions of *G. haempeli* Micoletzky, 1923, *G. leptopeos* Mulvey & Nickle, 1978, *G. pachopeos* Mulvey & Nickle, 1978, and *G. phethelos* Mulvey & Nickle, 1978; the new species differs from these species by the length of spicule.

**BIOLOGICAL OBSERVATIONS**

Forty percent of the samples analyzed (n = 80) during the sampling period included free-living nematodes and/or parasitized host. The free-living stages were localized in the sandy bottom of the river, at a depth of 10 to 20 cm. Among the aquatic insect dissected, only the black fly larvae were parasitized at a rate of 8.4 % (n = 190). One nematode per host was found. Eggs completely developed into preparasitic juveniles in 12 to 15 days (at 26 °C).

**REMARKS**

In Argentina, Mermithidae is one of the entomoparasitic nematode families the best represented and, in this family, the genus *Gastromermis* has the greatest number of species. Up to now, five species of the genus have been observed in the country; two of them have six hypodermal chords, the others eight. All species parasitize aquatic Diptera, in particular simuliids and chironomids (Doucet & Doucet, 1996).

A key for the identification of the *Gastromermis* species with six hypodermal chords was prepared using the combination of the characters: length and shape of spicule, mouth opening position, absence of a vulval flap and tail appendage length in postparasitic juveniles. Although all of these characters are taxonomically significant, the length of the spicule is the most relevant (Nickle, 1972; Poinar, 1975; Mulvey & Nickle, 1978). Consequently this character was the basis of the key.

**Key to the species of *Gastromermis* with six hypodermal chords**

- 1. – Spicule very large  $\geq 700 \mu\text{m}$  ..... 2
  - Spicule medium or small-sized  $\leq 700 \mu\text{m}$  ..... 5
- 2. – Mouth cone, membranous tip of the spicule, and cloacal lips present. Spicule length 793-1063  $\mu\text{m}$  .....
  - ..... *G. cloacachilus*
  - Mouth cone, membranous tip of the spicule, and cloacal lips absent. Spicule length varying according to the species ..... 3
- 3. – Spicule length  $\geq 1200 \mu\text{m}$  ..... *G. metae*
  - Spicule length  $\leq 1200 \mu\text{m}$  ..... 4
- 4. – Mouth opening terminal or subterminal.. *G. mesostoma*
  - Mouth opening ventrally displaced ..... *G. leberrei*
- 5. – Spicule medium sized (500-700  $\mu\text{m}$ ) ..... 6
  - Spicule small sized ( $\leq 500 \mu\text{m}$ ) ..... 8
- 6. – Vulvar flap present..... *G. kolleonis*
  - Vulvar flap absent ..... 7
- 7. – Spicule length 527-539  $\mu\text{m}$ , with membranous and acute distal tip; amphidial pore rounded ..... *G. fidelis*
  - Spicule length 570-680  $\mu\text{m}$ , with pointed distal tip; anterior portion of amphidial pore pointed.....
    - ..... *G. massei* n. sp.
- 8. – Vulvar flap present..... *G. brevispicularis*
  - Vulvar flap absent ..... 9
- 9. – Spicule length 360-420  $\mu\text{m}$  ..... *G. hexachordalis*
  - Spicule length 294-336  $\mu\text{m}$  ..... *G. philipponi*

**Acknowledgments**

The authors thank the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), the Consejo de Investigaciones Científicas y Tecnológicas de la Provincia de Córdoba (CONICOR) and the Secretaría de Ciencia y Tecnología (SECYT) for their financial support. We are indebted to Lic. J. Warde for reproducing the drawings.

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