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Article

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Chromaspirina guanabarensis sp. n. (Nematoda: Desmodoridae) and a new illustrated dichotomous key to *Chromaspirina* species

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Abstract

A new species of *Chromaspirina* is described from Bica Beach, a polluted beach situated at Ilha do Governador, Guanabara Bay on the coast of Rio de Janeiro (Brazil). The new species belongs to the group of species within the genus which bears a poorly developed dorsal tooth. The new species is characterized by large body size, conoid tail shape, small acute dorsal tooth, long slender spicules with sclerotised hooked *capitulum*, and sexual dimorphism of the non-striated part of the tail. A new illustrated dichotomous key to males of species of *Chromaspirina* is proposed.

Key words: marine nematodes, free-living, taxonomy, Guanabara Bay, Bica Beach, Brazil

Resumo

Uma nova espécie de *Chomaspirina* é descrita para a Praia da Bica, uma praia poluída localizada na Ilha do Governador, Baía de Guanabara, na costa do Rio de Janeiro (Brasil). Esta nova espécie pertence ao grupo de espécies que possui um dente dorsal pouco desenvolvido e é caracterizada pelo seu grande tamanho, a forma cônica da cauda, o dente dorsal pequeno e pontiagudo, espícula delgada e longa com *capitulum* esclerotizado e em forma de gancho e o dimorfismo sexual na região não-estriada da cauda. Uma chave dictômica e ilustrada para os machos das espécies do gênero *Chromaspirina* é proposta.

Palavras-chave: nematódeos marinhos, vida-livre, taxonomia, Baía de Guanabara, Praia da Bica, Brasil

Introduction

A new species of the genus *Chromaspirina* was found during the assessment of meiofauna (including nematodes) conducted as part of a larger project on "Biomonitoring of sandy beaches of Guanabara Bay after the oil spill in January 2000" (Michel, 2001). This paper presents a description of the new species. The genus and its constituent species are reviewed, and a new illustrated dichotomous key to valid species of *Chromaspirina* for males is presented.

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Material & methods

Specimens were collected on the coastline of Ilha do Governador, Rio de Janeiro, Praia da Bica (Bica Beach), a coarse sandy beach with sediment of median grain size ranging from 570μm to 2850μm. Sediment samples were taken using a cylindrical corer of 10cm^2 surface area at the upper and low tide levels and were immediately fixed using 4% formaldehyde (Maria, 2006).

In the laboratory, nematode specimens were processed according to the method of Platt & Warwick (1983), and mounted on Cobb slides. Measurements and drawings were done using a LEICA DMLS microscopy provided with a *camera lucida.* Photographs were taken with a digital camera connected to a Leica DMR microscopy.

Type specimens of *Chromaspirina guanabarensis* sp. n. are deposited in the Museu Nacional do Rio de Janeiro (National Museum of Rio de Janeiro) under the identification number MNRJ305 (holotype), MNRJ306, MNRJ307, MNRJ308 and MNRJ309 (paratypes).

The identification is based on the list of valid species of *Chromaspirina* by Gerlach & Rieman (1973) and Muthumbi *et al.* (1995) and published species descriptions. The pictures of the key are adapted from the original descriptions.

Abbreviations used in the text:

a, b, c, c'	body proportions by de Man (1880)
abd	anal body diameter
All.	allotype
amph%	diameter of the amphidial fovea as percentage of the corresponding body diameter
aov	anterior ovary
aw	width amphidial fovea
bd	diameter pharyngeal bulb
bw	maximum body width
cs	length of cephalic sensilla
gub	length of gubernaculum
Hol.	holotype
hw	head width
L	total body length
ph	pharynx length
nr%	nerve ring as percentage of the total pharynx length from the anterior end
Par.	paratype
pov	posterior ovary
spic	length of spicules measured along the arc
t	tail length
tna%	smooth tail portion as percentage of the tail total length
v	distance from anterior end to vulva
V%	position of the vulva as a percentage of the total body length from anterior

Classification of the genus *Chromaspirina* Filipjev, 1918

According to Lorenzen (1994), the genus *Chromaspirina* belongs to the subfamily Spiriniinae, family Desmodoridae, superfamily Desmodoroidea. The superfamily is characterized by the holapomorphy of a single anterior testis in males and two antidromously reflexed ovaries in females. However, no holapomorphy is known for the family Desmodoridae.

Diagnosis of the genus *Chromaspirina* Filipjev, 1918 (emended). Desmodoridae. Spiriniinae. Robust nematodes with round head and conical tail, cuticle finely transversely striated. Anterior sensilla in three separate circles (6+6+4). Amphidial fovea rounded spiral surrounded by striations (with one exception). Cephalic setae at the level of the amphidial fovea. Buccal cavity with a large dorsal tooth and two small ventrosublateral teeth. Pharynx with a pyriform posterior bulb. No secretory-excretory system observed. Precloacal supplements present or absent and when present of different shape: fine tubules, papillae, or thorn-like structures. Spicules of variable shape but with well developed *capitulum* and *velum*. Females didelphic-amphidelphic.

Relationships. *Chromaspirina* resembles *Spirinia* in the shape of the amphidial fovea and tail, but differs in having a large stoma and dorsal tooth, as well as pyriform pharyngeal bulb. *Spirinia* has a small stoma, small dorsal tooth and true rounded terminal bulb.

List of valid species (after Muthumbi *et al.*, 1995, emended and updated)

C. chabaudi Boucher, 1975
C. crinita Gerlach, 1952
C. cylindricollis (Cobb, 1920)
    syn. *Mesodorus cylindricollis* Cobb, 1920
C. dubia Inglis, 1968
C. gerlachi Blome, 1982
C. guanabarensis sp. n.
C. inaurita Wieser & Hopper, 1967
C. indica Gerlach, 1963
C. inglisi Warwick, 1970
C. lunatica Gerlach, 1965
C. madagascarensis Gerlach, 1953
C. modesta Bussau, 1993
C. multipapillata Jayasree & Warwick, 1977
C. okemwai Muthumbi, Verschelde & Vincx, 1995
C. parapontica Luc & De Coninck, 1959
C. parma Ott, 1972
C. pellita Gerlach, 1954
    syn. *C. renaudae* Boucher, 1975
C. pontica Filipjev, 1918
C. thieryi De Coninck, 1943
C. vanreuselae Verschelde & Vincx, 1996

Invalid species
C. amabilis (De Man, 1922) *sp. inq.* of *Bolbolaimus* by Jensen (1978)
C. cobbi (Chitwood, 1938) *sp. inq.* of *Bolbolaimus* by Jensen (1978)
C. denticula (Gerlach, 1953) re-established as *Bolbolaimus* species by Jensen (1978)
C. dimorpha (Hopper, 1961) re-established as *Desmodora* species by Wieser & Hopper (1967)
C. inflexa (Wieser, 1954) re-established as *Desmodora* species by Wieser & Hopper (1967)
C. longisetosa Jensen, 1985 transferred to *Bolbolaimus* by Muthumbi *et al.* (1995)
C. paucispira Stekhoven, 1950 *sp. inq.* by Wieser & Hopper (1967)
C. pellucida (Cobb, 1920) re-established as *Bolbolaimus* species by Jensen (1978)
C. punctata (Cobb, 1920) re-established as *Bolbolaimus* species by Jensen (1978)
C. rabosa (Gerlach, 1956) re-established as *Desmodora* species by Wieser & Hopper (1967)

C. robusta Wieser, 1954 *sp. inq.* by Wieser & Hopper (1967)
C. spinulosa Wieser, 1959 transferred to *Metadesmodora* by Gerlach (1963) and synomyzed by Blome (1982) as *Echinodesmodora spinulosa*

Description

***Chromaspirina guanabarensis* sp. n.**
Figs 1 and 2

Type specimens: all in the Museu Nacional do Rio de Janeiro: holotype male (MNRJ305), two paratype males (MNRJ307), allotype female (MNRJ306), two paratype females (MNRJ308) and three paratype juveniles (MNRJ309).

Type locality: Bica Beach (22°49'37.9"S and 43°11'15.9"W), Guanabara Bay, Rio de Janeiro, Brazil, collected on 08/06/2000 by Dr. André Esteves. Sediment of very coarse to coarse sand with median grain size ranging from 2850μm to 570μm; intertidal zone.

Etymology: The species name refers to the type locality.

Measurements: Table 1.

TABLE 1. Morphometric data for *Chromaspirina guanabarensis* sp. n. (all measurements except ratios expressed in μm).

	Hol ♂	All. ♀	Par. ♂$_{(1)}$	Par. ♂$_{(2)}$	Par. ♀$_{(1)}$	Par. ♀$_{(2)}$	Par. Juv$_{(1)}$	Par. Juv$_{(2)}$	Par. Juv$_{(3)}$
L	2110	2570	2415	2570	2310	1985	865	755	865
cs	4	4	5	5	6	-	4	4	4
hw	25	-	-	-	-	-	17	14	13
aw	8	8	8	9	10	8	7	7	7
amph%	29	30	32	39	34	32	48	42	45
ph	200	224	183	186	215	190	124	109	111
nr%	53	-	-	-	-	-	54	-	54
bd	34	33	32	34	29	30	21	17	20
bw	55	69	50	69	50	66	77	52	62
v	-	1508	-	-	1328	1092	-	-	-
V%	-	59	-	-	57	55	-	-	-
aov	-	272	-	-	105	118	-	-	-
pov	-	310	-	-	108	129	-	-	-
abd	46	43	39	40	45	39	22	22	23
spic	70	-	69	58	-	-	-	-	-
gub	27	-	24	25	-	-	-	-	-
t	121	134	111	133	140	116	93	82	80
tna %	28	17	27	26	19	24	26	27	26
a	39	37	48	45	46	30	32	26	25
b	11	11	13	14	11	10	7.0	7.0	6.2
c	17	19	22	19	16	17	9.3	9.2	8.6
c'	2.6	3.1	2.8	3.3	3.1	2.9	4.2	3.8	3.5

-: not applicable or not seen.

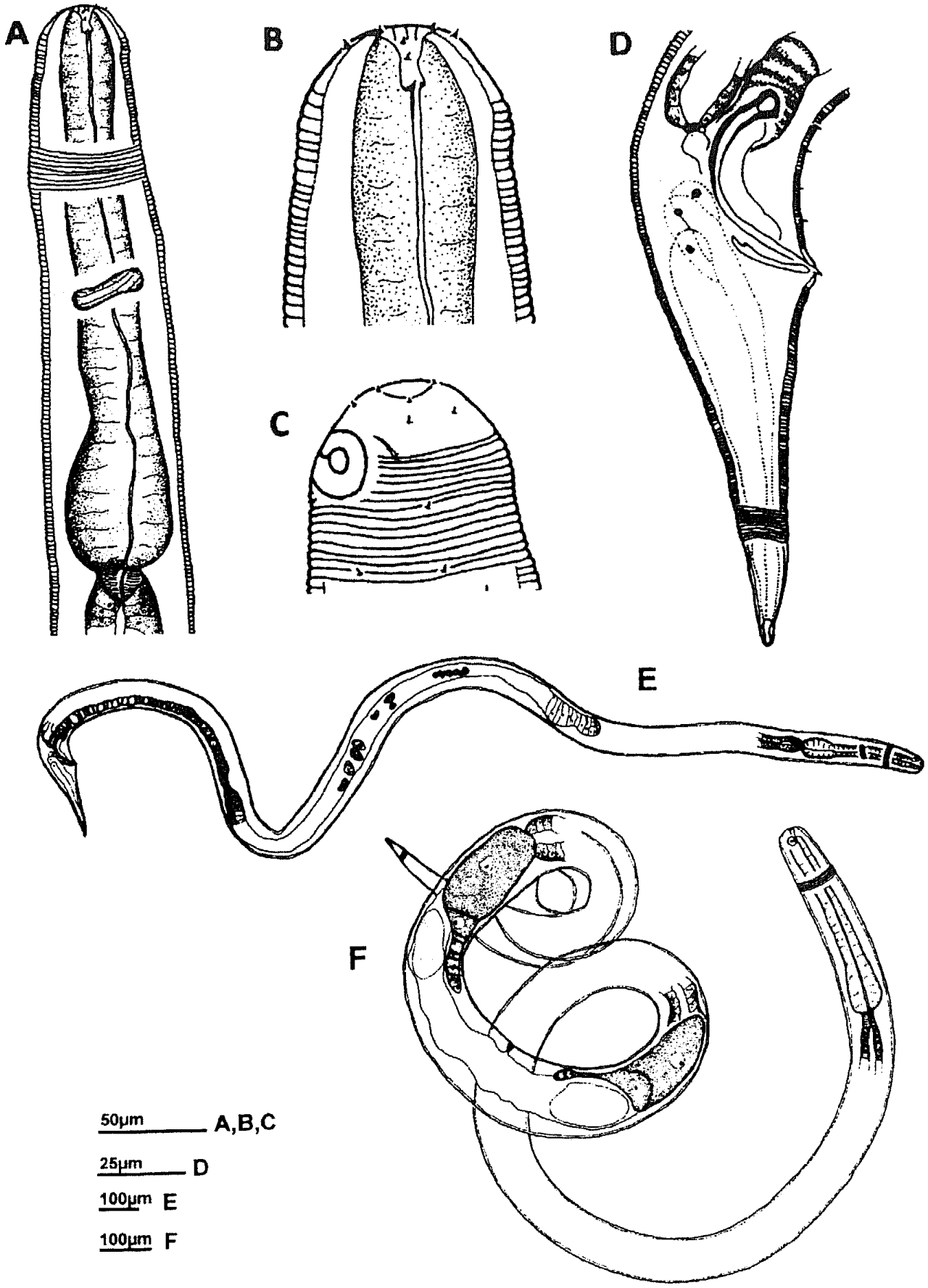

FIGURE 1. *Chromaspirina guanabarensis* sp. n.; A–E: holotype male. A. neck region; B. head region, C. head region; oblique surface view; D. tail region and copulatory apparatus, E. habitus. F: Allotype female, habitus.

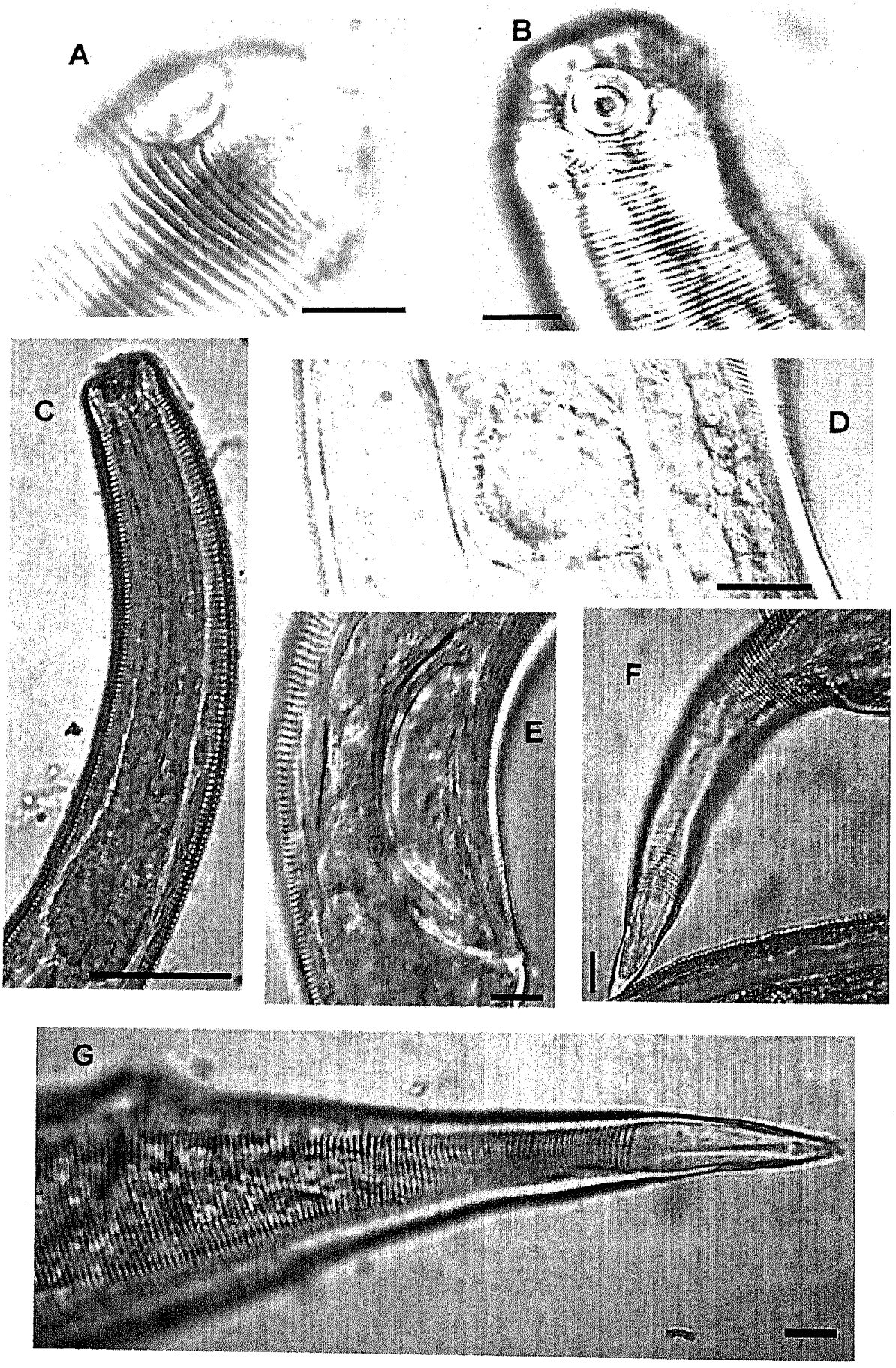

FIGURE 2. Light micrographs of *Chromaspirina guanabarensis* sp. n. Surface view of head region, oblique view showing outer labial papillae (holotype); B. Head region, lateral surface view, showing setiform cephalic setae and wound spiral amphidial fovea (paratype ♂); C. Pharyngeal region (paratype ♂); D. Round sperm cell (holotype); E. Copulatory apparatus (holotype); F. Female tail (paratype); G. Male tail (holotype). Scale bars: 10μm.

Male. Body long and relatively slender. Head blunt, rounded. Tail conical. Cuticle with fine transverse striations starting posterior to the anterior edge of amphidial fovea and without ornamentation. Minute somatic setae arranged in eight longitudinal rows extending from the pharyngeal region to tail region. Head region smooth, with six inner and six outer labial papillae, and four cephalic setae 4–5 µm long, located just anterior to the anteriormost striation of the body cuticle. Amphidial fovea a ventrally wound spiral, largely surrounded by striations; its diameter 29–39% of corresponding body diameter. Buccal cavity large with an acute dorsal tooth and two smaller ventrosublateral teeth. Pharynx anteriorly cylindrical, posteriorly widened to a pyriform bulb. Nerve ring located at about mid-pharynx length. Cardia triangular in cross section, surrounded by intestine. Tail conical with three caudal glands dorsally located, extending up to the cloaca and opening at the tail tip through a spinneret. Terminal part of tail smooth, 26–28% of the total tail length. Reproductive system monorchic with anterior outstretched testis on right side of the intestine. Testis with narrow short germinal zone. Sperm cells round (16–21µm diameter). Spicules equal, slender, ventrally curved with thick sclerotized lamina and hook-shaped *capitulum*; thin *velum* present. Gubernaculum slightly arcuate, trough-shaped, without apophysis. No obvious precloacal supplements present, but equally spaced short ventral setae which could be interpreted as supplements.

Female. Similar to male except for a cylindrical cardia, a shorter smooth tail portion (17–24% of tail length). Reproductive system didelphic-amphidelphic with reflexed ovaries; anterior ovary to the left of intestine, posterior ovary to the right of intestine. Anterior genital branch shorter than posterior. Vulva a simple transverse slit. One large egg (78–82µm long and 38–44µm wide) present in each uterus.

Juvenile. Only second stage juveniles observed; this stage is characterized by its small body size and small primordium, not clearly observed. External morphology similar to the adults.

Diagnosis. *Chromaspirina guanabarensis* sp. n. is characterized by its large size (>2mm); the position of the cephalic setae at the posterior edge of the smooth head region, and the conical tail shape in both sexes. The non-striated part of the tail is shorter in females than in males. Males are characterized by spicules shaped with hooked sclerotized capitula and well sclerotized lamina, together with absence of precloacal supplements but presence of short setiform precloacal sensilla.

Differential diagnosis. *Chromaspirina guanabarensis* sp. n. most closely resembles *C. okemwai* Muthumbi, Verschelde & Vincx, 1995 in having similar dorsal tooth and spicule shape, but differs in having a more evenly tapering tail and more pronounced labial papillae (*C. okemwai* has a tail with a wide anterior part narrowing posteriorly and minute labial sensilla only visible with SEM).

Discussion. The species included in the genus *Chromaspirina* have changed frequently. We consider the genus to have twenty valid species (including the new species). There are two *species inquirendae*, eight species transferred back to their original genus, and two species transferred to another genus.

C. robusta Wieser, 1954 and *C. paucispira* Stekhoven, 1950, known from two juveniles and one female respectively, were regarded as *species inquirendae* by Wieser & Hopper (1967). The genus *Bolbolaimus* was synonymized with *Chromaspirina* by Luc & De Conninck (1959) but re-established as a valid genus by Jensen (1978), based mainly on the female reproductive system with outstretched ovaries, stoma and pharynx structure and cuticle ornamentation. Jensen re-established *C. denticulata* (Gerlach, 1953), *C. pellucida* (Cobb, 1920) and *C. punctata* (Cobb, 1920) within *Bolbolaimus* when rejecting synonymy of this genus with *Chromaspirina;* and regarded two other species *C. amabilis* (De Man, 1922) and *C. cobbi* (Chitwood, 1938) as *species inqueridae* of *Bolbolaimus*.

C. dimorpha (Hopper, 1961), *C. inflexa* (Wieser, 1951) and *C. rabosa* (Gerlach, 1956) have been transferred back to their original genus *Desmodora* mainly because of the amphidial fovea not being surrounded by striations of the body cuticle (Wieser & Hopper, 1967). Among the *Chromaspirina* species, the amphideal fovea is partially or totally surrounded by striations, e. g. *C. pellita* and *C. parma*, respectively. However, one exception occurs in the genus: *C. dubia* possesses striations restricted to the posterior quarter of the body, but other characteristics, such as a large dorsal tooth and a pyriform bulb support its presence within the genus *Chromaspirina*.

C. longisetosa Jensen, 1985 was transferred to the genus *Bolbolaimus* by Muthumbi *et al.* (1995) and *C. spinulosa* Wieser, 1959 was transferred to the genus *Metadesmodora* by Gerlach (1963), but Blome (1982) proposed the new combination *Echinodesmodora spinulosa* when he erected the genus *Echinodesmodora*.

C. inaurita is added to the list of valid *Chromaspirina* species; however it was not recorded by Muthumbi *et al.* (1995). *C. inaurita* possesses several characters (e.g. large stoma, pyriform pharyngeal bulb) indicating affinities with *Chromaspirina*, although it also shows some characteristics of the genus *Spirinia*, e.g. a small dorsal tooth. Within the genus *Chromaspirina* the dorsal tooth may be variable in size, so this character is insufficient to place the species outside *Chromaspirina*.

C. modesta is regarded as valid because the work of Bussau (1993) conforms to the code for zoological nomenclature is a valid publication, and has been recognised world-wide as a reference among the scientific community of marine nematodes researchers.

C. pontica and *C. inglisi* differ from each other only by the presence of fine hairs over the body in the latter. Warwick (1970) suggested that the hair-like ornamentations may be either easily overlooked or considered as epigrowths of unicellular cyanophyceous algae, and if so the species are the same. Muthumbi *et al.* (1995) describing *C. okemwai* also found some fine hair-like structures attached to the cuticle of their specimens, but when the organisms were sonicated and viewed by SEM, the tail region of some animals appeared devoid of the "hairs". The hair-like structures were also present on pre-moult phase of the newly made cuticle (Muthumbi *et al.*, 1995). Therefore, the origin of these structures remains doubtful. We regard *C. pontica* and *C. inglisi* as valid species, but consider that the hypothesis of *C. inglisi* being a junior synonym of *C. pontica* should not be completely disregarded though further investigation is needed.

Four species in the genus have a smaller dorsal tooth when compared with the remaining 16 species. This emphasises the close relationship of this genus with *Spirinia* as suggested by Wieser & Hopper (1967). Further investigation is needed into the relationships *Spirinia* with *Chromaspirina*. *Chromaspirina guanabarensis* sp. n. belongs to the group of species characterized by a poorly developed dorsal tooth. Within this group, the new species is easily discriminated by the absence of sexual dimorphism in the amphidial fovea, distinct labial sensilla and tail shape.

Illustrated identification key to valid species of *Chromaspirina* for males

1 - Cuticle striation starting at head level (Fig. 3) and extending to tail 2
- Cuticle striation restricted to posterior quarter of the body..... *C. dubia* Inglis, 1968 (Fig. 4)

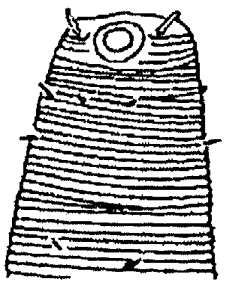

Fig. 3

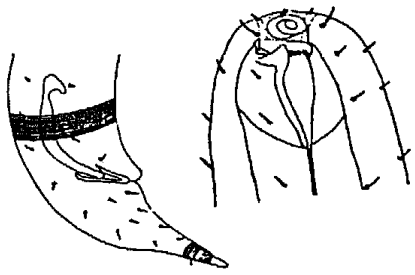

Fig. 4

2 - Buccal cavity with large, strongly sclerotized dorsal tooth (Fig. 5) 3
- Buccal cavity with acute, less sclerotized dorsal tooth (Fig. 6) 17

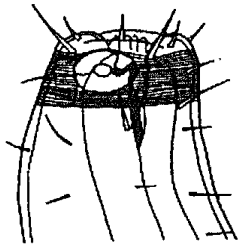

Fig. 5

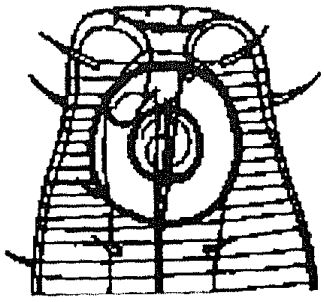

Fig. 6

3 - Pyriform bulb subdivided into two regions by protoplasmatic interruptions (Fig. 7) 4
- Pyriform bulb not subdivided (Fig. 8) 9

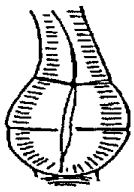

Fig. 7

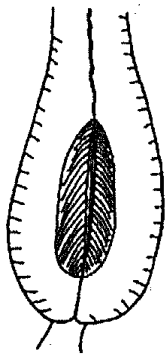

Fig. 8

4 - Amphidial fovea partially surrounded by striations (Fig. 9) 5
- Amphidial fovea completely surrounded by striations (Fig. 10) 7

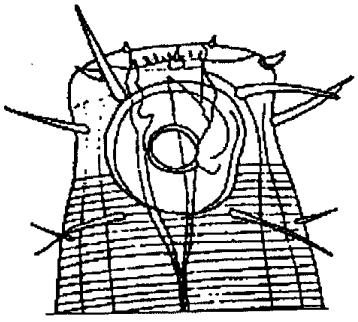

Fig. 9

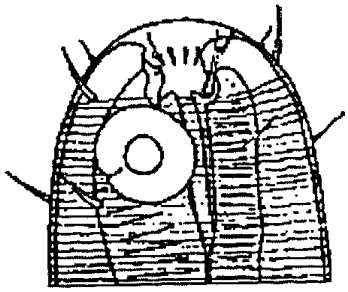

Fig. 10

5 - Precloacal supplements present (Fig. 11) 6
- Precloacal supplements absent ..... *C. pellita* Gerlach, 1954 (Fig. 12)

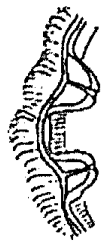

Fig. 11

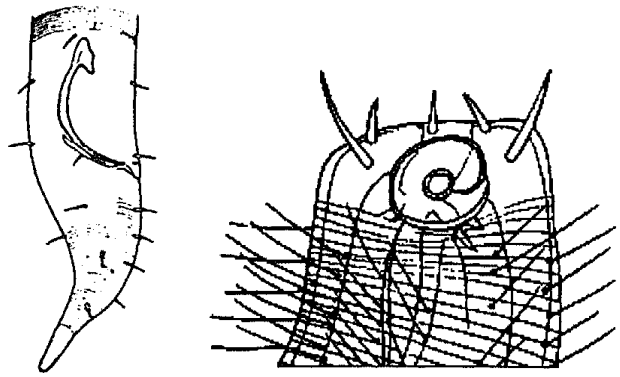

Fig. 12

6 - Precloacal supplements nine, unequally spaced ..... *C. indica* Gerlach, 1963 (Fig. 13)
- Precloacal supplements more than fifty, equally spaced .....C. *multipapillata* Jayasree & Warwick, 1977 (Fig. 14)

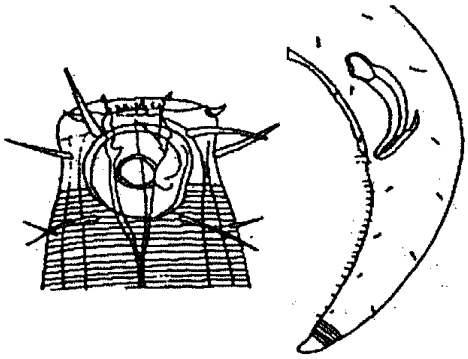

Fig. 13

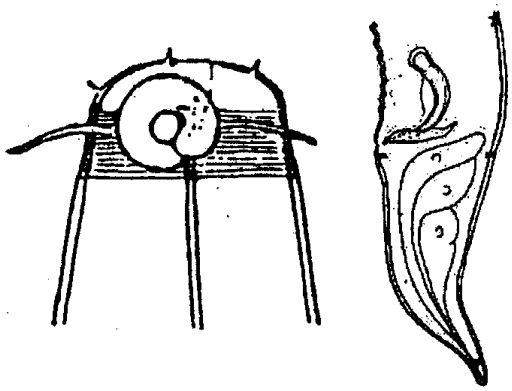

Fig. 14

7 - Amphidial fovea a rounded spiral in both sexes (Fig. 10) 8
- Amphidial fovea a lunar spiral in males, rounded spiral in females (Fig. 15) .....*C. lunatica* Gerlach, 1965 (Fig. 15)

Male Female

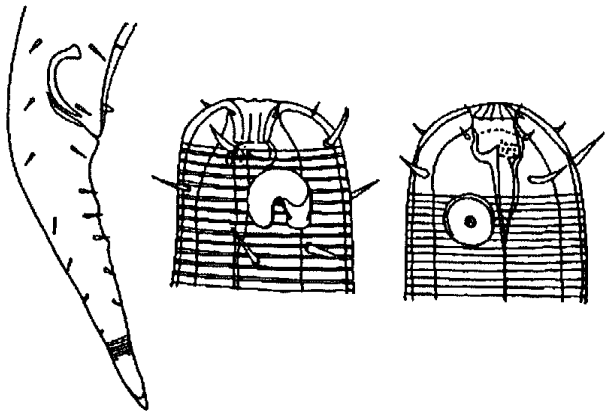

Fig. 15

8 - Cephalic sensilla short papilliform ..... *C. thieryi* De Coninck, 1943 (Fig. 16)
- Cephalic sensilla long, setiform ..... *C. madagascarensis* Gerlach, 1963 (Fig. 17)

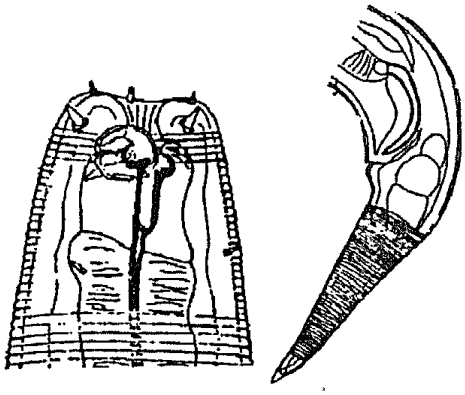

Fig. 16

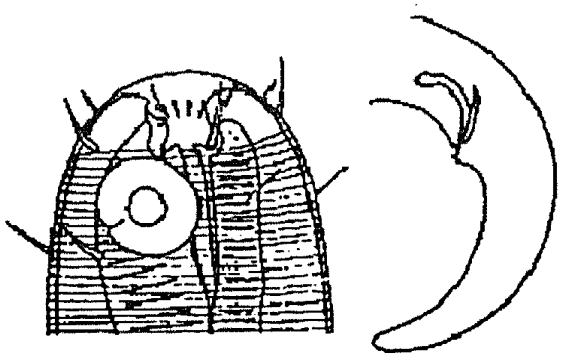

Fig. 17

9 - Cephalic setae length approximately one head diameter ..... *C. crinita* Gerlach, 1952 (Fig. 18)
- Cephalic setae length less than 60% of head diameter (Fig. 5) 10

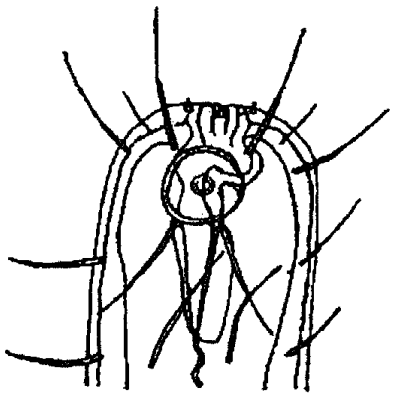

Fig. 18

10 - Supplements absent (Fig. 19) 11
- Supplements present (Fig. 20) 13

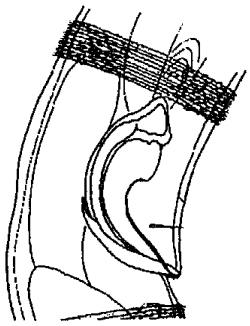

Fig. 19

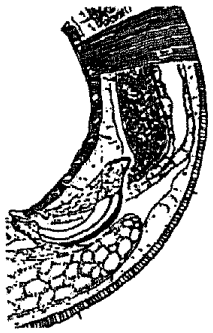

Fig. 20

11 - Cephalic setae 9µm or more in length in males, spicules short (19-20µm long) ..... *C. gerlachi* Blome, 1982 (Fig. 21)
- Cephalic setae 5–6µm long in males and 9–12µm in females, spicules long (more than 45µm) 12

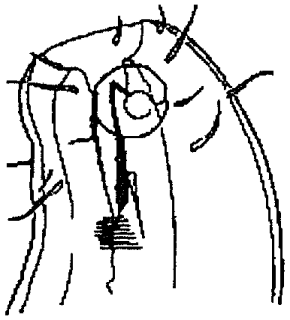

Fig. 21

12 - Body covered by fine hairs (Fig. 22) ..... *C. inglisi* Warwick, 1970 (Fig. 22)
- Body devoid of hairs (Fig. 23) ..... *C. pontica* Filipjev, 1918 (Fig. 23)

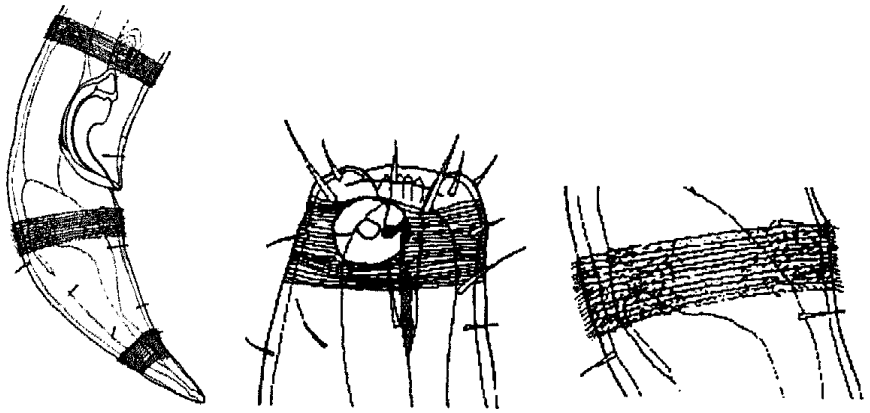

Fig. 22

Fig. 23

13 - Precloacal supplements tubiform or semi-cylindroid (Fig. 24) 14
- Precloacal supplements papilliform or thorn-like (Fig. 25) 16

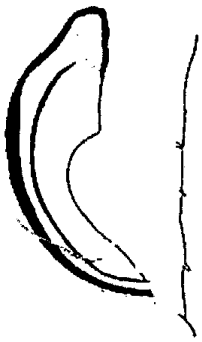

Fig. 24

Fig. 25

14 - Supplements tubiform (Fig. 24) 15
- Supplements semi-cylindroid ..... *C. cylindricollis* Cobb, 1920 (Fig. 26)

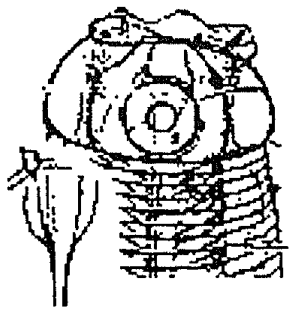

Fig. 26

15 – Amphideal fovea circular in both sexes and less than 25% of corresponding body diameter, tail conical ..... *C. parapontica* Luc & De Connick, 1959 (Fig. 27)
- Amphideal fovea oval and occupying almost all corresponding body diameter in males and circular in females, tail conico-cylindrical ..... *C. modesta* Bussau, 1993 (Fig. 28)

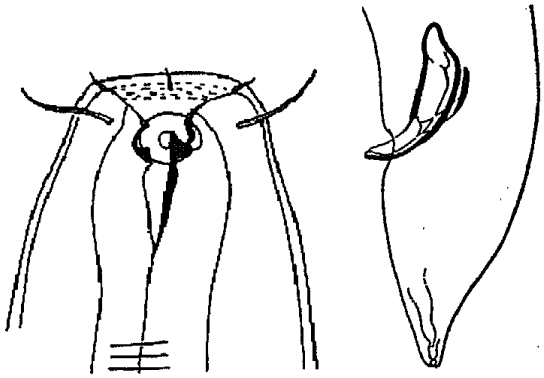

Fig. 27

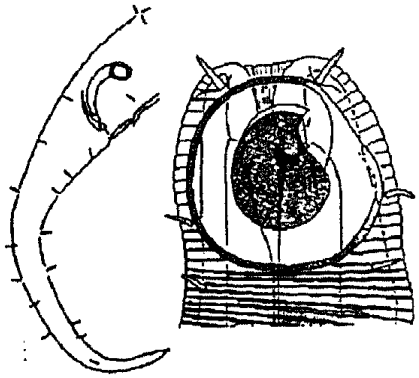

Fig. 28

16 -Supplements twenty-seven to thirty-one, thorn-like (Fig. 25), *capitulum* dorsally pointed, tail cylindrical posteriorly ..... *C. vanreuselae* Verschelde & Vincx, 1996 (Fig. 29)
- Supplements fifty-three to sixty-seven, S-shaped papilliform, *capitulum* round, tail conoid posteriorly ..... *C. chabaudi* Boucher, 1975 (Fig. 30)

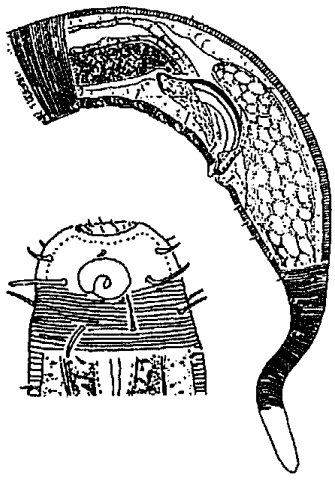

Fig. 29

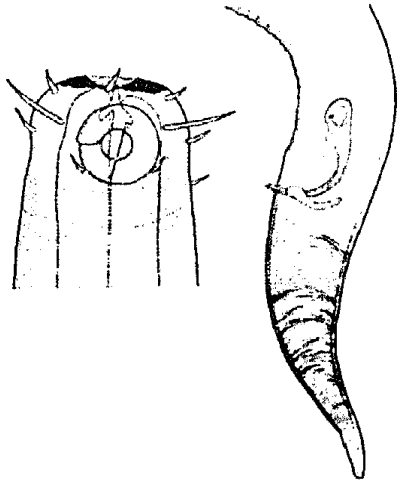

Fig. 30

17 - Sexual dimorphism in shape of amphidial fovea: oval in males, circular in females (Fig. 31) 18
- No sexual dimorphism in shape of amphidial fovea: circular in both sexes (Fig. 3) 19

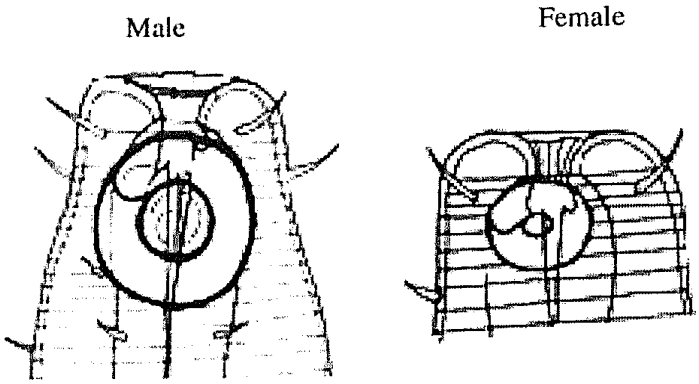

Fig. 31

18 - Precloacal supplements absent ..... *C. parma* Ott, 1972 (Fig. 32)
- One stout precloacal supplement and four papilliform postcloacal supplements accompanied by a pair of setae .....
..... *C. inaurita* Wieser & Hopper, 1967 (Figs. 31, 33)

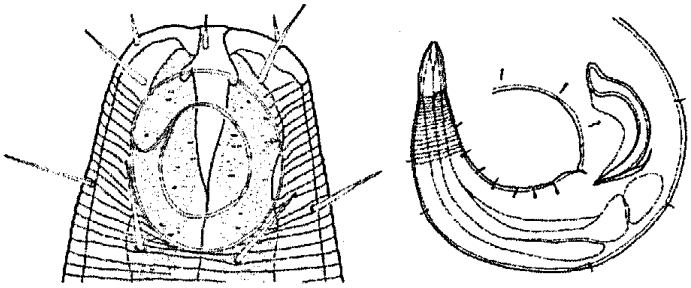

Fig. 32

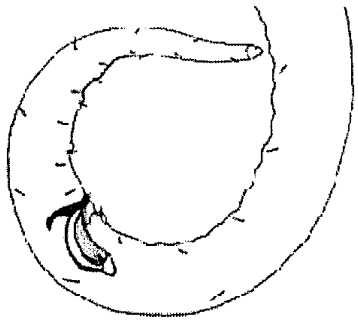

Fig. 33

19 - Labial papillae indistinct (Fig. 3),tail in males rapidly reducing in diameter about 25% of length posterior to cloaca (Fig. 34) ..... *C. okemwai* Muthumbi, Verschelde & Vincx, 1995
- Labial papillae distinct, tail in males evenly tapering ..... *Chromaspirina guanabarensis* sp.n. (Fig. 35)

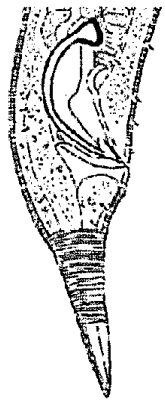

Fig. 34

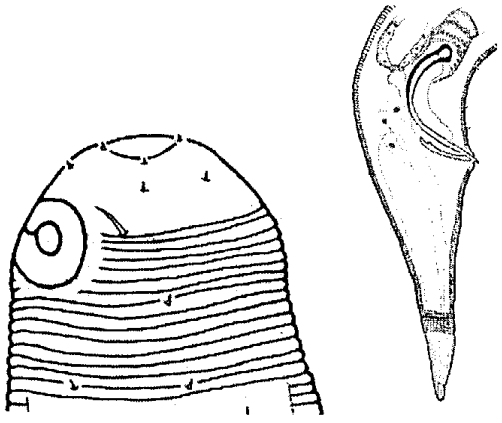

Fig. 35

Acknowledgements

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