

CSIRO Division of Horticultural Research
Merbein, Victoria, 3505, Australia

OBSERVATIONS ON THE MORPHOLOGY OF BELONOLAIMINAE

by

M. R. SAUER, M. W. BRZESKI⁽¹⁾ and R. N. CHAPMAN

The subfamily Belonolaiminae was redefined by Siddiqi (1970) and Golden (1971), both independently establishing Belonolaimidae. Both included *Belonolaimus* Steiner and *Morulaimus* Sauer in the subfamily, but *Carphodorus* Colbran, included by Golden, was assigned to the Telotylenchinae by Siddiqi. Andrassy (1976) synonymised *Carphodorus* with *Belonolaimus*, without giving reasons. Monteiro and Lordello (1977) added a new genus, *Ibipora*, which they differentiated from *Belonolaimus* by the number of lateral lines, transferring *B. lineatus* Roman to *Ibipora*.

No detailed description of the lip region of *Carphodorus* has been published. The structure of the feeding apparatus in this group is obscure. Apart from the observations of Seinhorst (1971 a), there is little information about the disposition of the oesophageal glands. We have undertaken a comparative morphological study of some of the features of the subfamily.

Materials and methods

Species examined were *Belonolaimus gracilis* Steiner, *Ibipora lineatus*, and *Morulaimus whitei* (Fisher) in glycerine mounts, *Car-*

⁽¹⁾ On temporary leave from Instytut Warzywnictwa, 96-100 Skierniewice, Poland.

phodorus bilineatus Colbran, *M. soldus* Colbran, and *Ibipora lolii* Siviour and McLeod (glycerine mounts and fixed material) and *M. arenicolus* Sauer, *M. geniculatus* Sauer, and *M. sclerus* Sauer collected from local soils. Some populations of *Morulaimus* collected could not be identified to species because of uncertainty about intra specific variability.

Some specimens were examined live by mounting in a solution of 12.5% Ficoll (Pharmacia Fine Chemicals, Uppsala, Sweden) and 0.5% propylene phenoxitol. Nematodes were killed with hot formalin 4% or hot F.P. 4:1 and fixed for a minimum of 48 hours before further processing. Most were processed to glycerine by Seinhorst's (1959) method. Some were heated in lactophenol over a spirit lamp and immediately transferred to glycerine.

Fixed specimens were dehydrated in a graded ethanol/distilled water series, transferred through an amyl acetate/ethanol series, critical point dried using carbon dioxide, mounted on stubs, sputter coated with gold (nominal 50 nm) and examined in a Philips 500 scanning electron microscope at 25 kv accelerating voltage.

External morphology

1. *Lip Region*

In all four genera, the lip region is high, usually set off from the body, dominated by four large submedian lobes. In *Belonolaimus* (Smart *et al.*, 1972) and *Ibipora* (Sher and Bell, 1975) these lobes are separated by deep grooves which interrupt the labial annules. In *Morulaimus* (Fig. 1, a, b, c) the lobes are less distinctly separated by a depression. Golden (1971) described the head of *Carphodorus* as two lobed, the lobes separated by dorsal and ventral grooves. We see it as four lobed (Fig. 1 d). Laterally the lobes are separated by lateral lips and a slight depression. Dorsally and ventrally, the lobes are separated by a combination of a depression, near the base, and a distinct groove near the apex, not a complete groove as in *Belonolaimus* and *Ibipora*.

Belonolaimus and *Ibipora* have a circular labial disc with a distinct rim, usually slightly elevated above the lip lobes. We found *Carphodorus* has a similar circular disc (Fig. 1 d). In *Morulaimus* the disc is thin, elongated laterally, the elongations extending posteriad below the tops of the submedian lobes. Six small pits are visible

around the oral aperture in *Morulaimus* (Fig. 1 b, c) and *Carphodorus* (Fig. 1 d). Sher and Bell (1975) claimed these pits are absent in *I. lineatus*. Figures D, E, F of Smart *et al.* (1972) suggest they may be present in *B. longicaudatus*. Protrusion of the stylet, or processing distortions, often give the impression of a two part labial disc, which we believe is an artefact.

The lateral lips are represented by small lobes which in *Belonolaimus* and *Ibipora* are situated in the deep grooves separating the submedians, near the apex. Amphid openings in these two genera are located on the lateral lobes (Smart *et al.* 1971, Monteiro and Lordello 1977). The SEM shows *Carphodorus* has well developed lateral lobes near the apex, though these were not seen by light microscope. The lateral lobes in *Morulaimus* are inserted at a lower level, as much as half way down the submedian lobes, and usually are visible in glycerine mounts. In *Morulaimus*, labial annules may be interrupted between the submedian lobes; laterally only, or laterally and dorso-ventrally, by incisures or platelets. Amphid apertures of *Carphodorus* and *Morulaimus* are situated between the labial disc and the lateral lobes.

2. Lateral fields

The lateral field of *Belonolaimus* is marked by a single incisure. *Ibipora* and *Morulaimus* show four distinct lines setting off a three band field. Alternate body annules usually cross the outer bands. *Carphodorus* has two distinct lines (Figs C, D, F of Colbran 1965) but the SEM shows a definite three band field perhaps accentuated by processing. The two distinct lines delimit the centre band. The edges of the fields are marked either by a bend of the annules or a very obscure line. In midbody alternate annules extend to the inner band, the others terminate about half way between the edge and the inner band (Fig. 1 e). The same annule configuration was seen in *Ibipora* sp.

Internal morphology

1. Labial framework

Our observations suggest that the hexaradiate framework is lightly sclerotised and inconspicuous in *Belonolaimus*, *Ibipora*, and

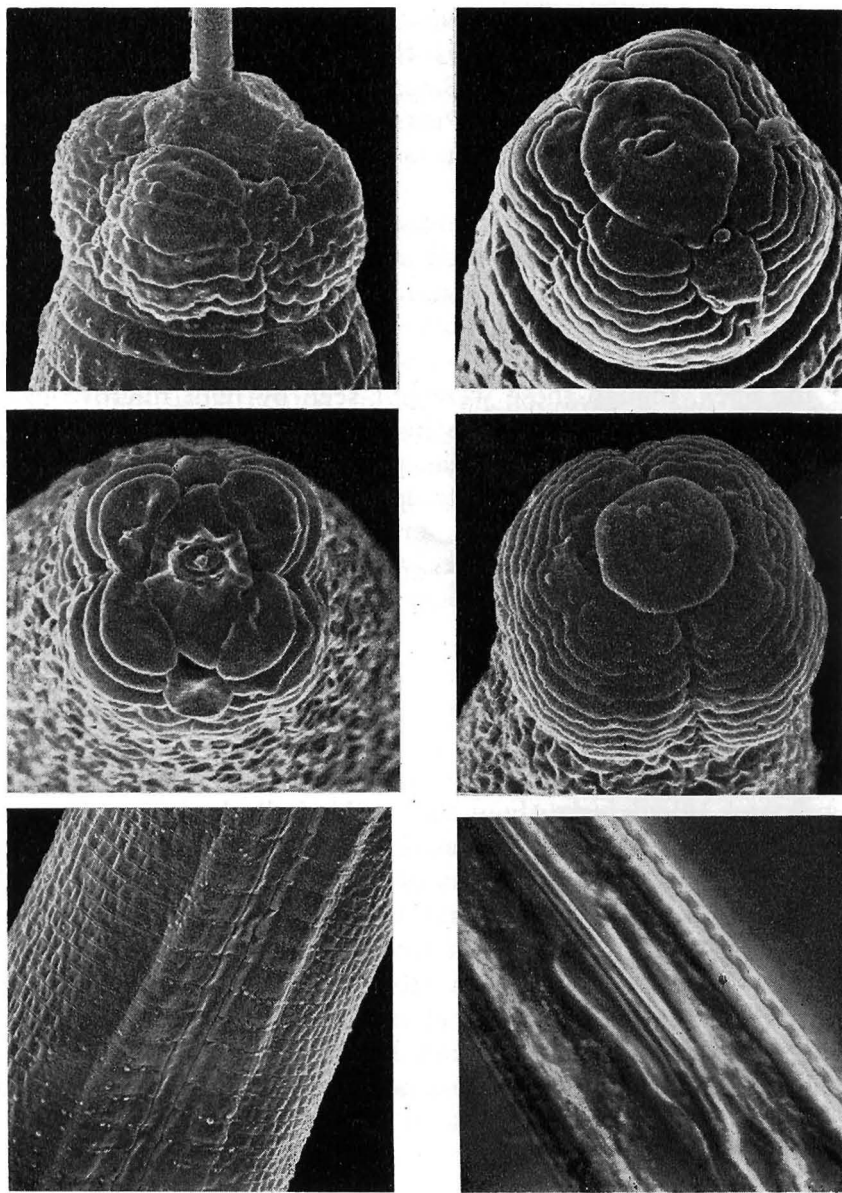


Fig. 1 - Lip regions and lateral fields as seen by scanning electron microscopy: top left: *Morulaimus arenicolus* ♂; top right: *M. sclerus* ♀; middle left: *M. soldus* ♂; middle right: *Carphodorus bilineatus* ♂; bottom left: lateral field of *C. bilineatus* ♂; bottom right: attachment of stylet muscles to stomatal rhabdions, *Ibipora lolii* ♀ (Interference contrast).

some species of *Morulaimus*, though the stomatal rhabdions show some thickening. *M. sclerus* and *M. whitei* have heavy sclerotisation of the framework in the dorsal and ventral sectors above the junction with the basal plate. In some unidentified populations of *Morulaimus* there is obvious thickening at the base of each sector, indicating a wide range of sclerotisation in this genus. The framework in *Carphodorus* is massive, with strong basal thickenings in each sector and rhabdions heavily sclerotised towards the base of the lip region.

2. Feeding apparatus

Belonolaiminae have an elongate stylet, the conus measuring 55-75% of total length. It was found that as total stylet length increases, conus length increases slightly more than shaft length (Table I). Stylet muscles are attached to the stylet knobs and the main bands are attached to slight thickenings of the long slender vestibulum extension. The point of attachment is always closer to the base of the conus than to the basal plate in the species examined (Fig. 1f, Fig. 2). Other thinner muscle bands appear to extend to the outer margins of the basal plate, but ultrastructure studies are needed to confirm this impression. As far as we know, these two types of stylet muscles have not been reported previously for any tylench.

Taxonomy of Belonolaiminae

Based on these studies, we recognize four genera in the subfamily, differentiated by the characters set out in Table II. Andrassy's (1976) synonymy of *Carphodorus* with *Belonolaimus* and Siddiqui's (1970) placement of *Carphodorus* in Telotylenchinae are rejected. We suggest there is a difference between *Ibipora* and *Belonolaimus* in the position of the oesophageal glands. We found the spicules of *I. lineatus* have a notched tip. Probably there are differences within the group in the structure of spicules and gubernaculum, but the material available was inadequate to clarify these details.

We propose an emended diagnosis of the subfamily as follows:

Belonolaiminae: Large nematodes with cylindroid body. No sexual dimorphism in anterior body end. Lip region with usually six or more

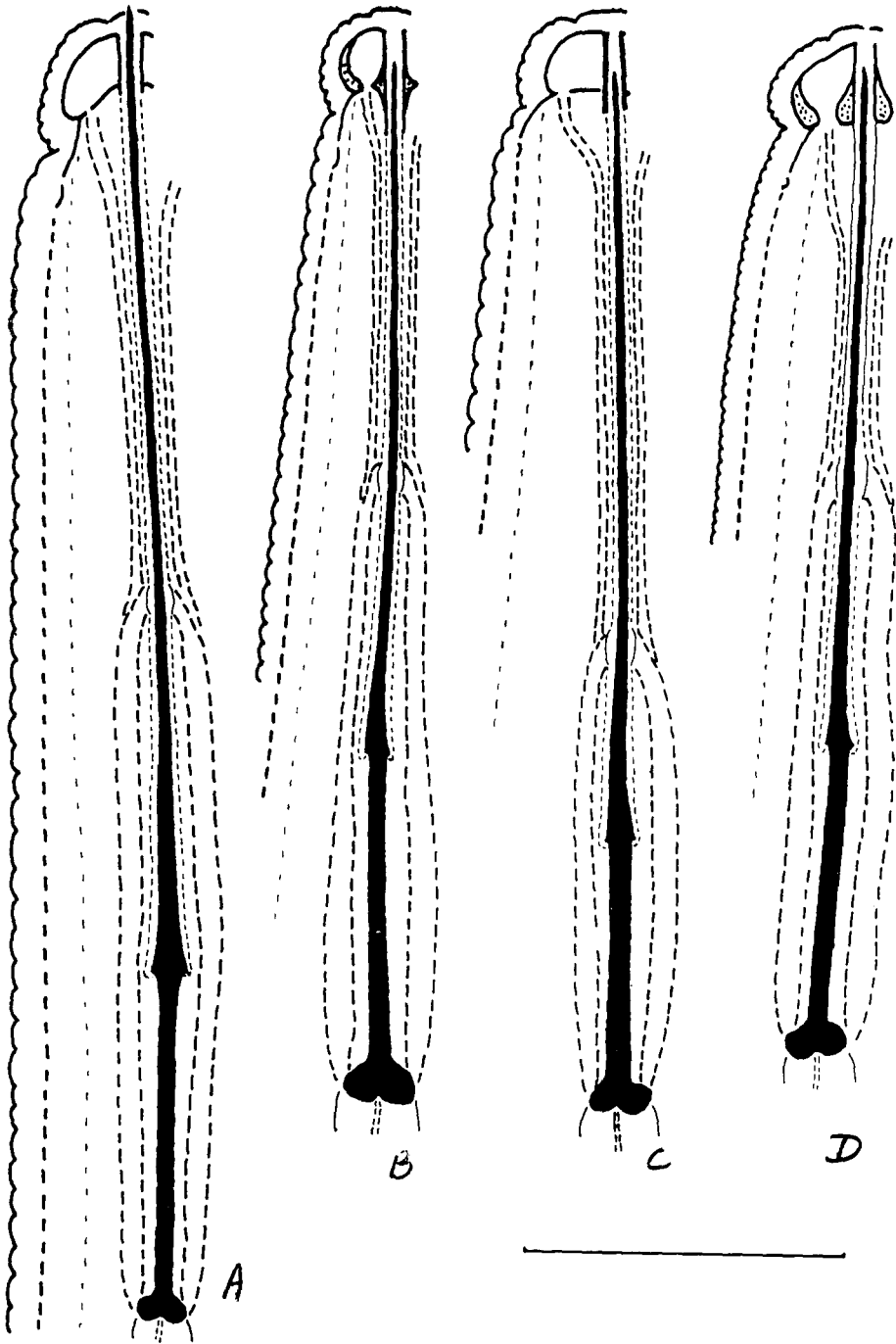


Fig. 2 - Anterior body ends; a. *Belonolaimus gracilis*; b. *Morulaimus sclerus*; c. *Ibipora lineatus*; d. *Carphodorus bilineatus* (Bar represents 30 μm).

Table I - Relation of stylet length to conus length in some *Belonolaiminae*.

Species	n.	Stylet length (μm)		Conus length as percent of stylet length	
		mean	variation	mean	variation
<i>Ibipora jara</i> (σ)	1	66		70	
<i>Morulaimus geniculatus</i>	16	67	62 - 70	61	59 - 64
<i>M. whitei</i>	3	73	68 - 79	67	65 - 69
<i>I. anama</i> (σ)	1	83		72	
<i>Carphodorus bilineatus</i>	6	87	79 - 92	67	62 - 69
<i>M. sclerius</i>	7	91	83 - 97	69	67 - 71
<i>M. arenicolus</i>	11	96	85 - 113	75	64 - 77
<i>I. lineatus</i>	2	98	97 - 99	74	73 - 75
<i>Ibipora sp.</i>	5	116	108 - 121	71	70 - 72
<i>Belonolaimus gracilis</i>	3	121	114 - 126	73	71 - 76

σ : based on published measurements of holotype.

Table II - Differentiating characters of the genera of *Belonolaiminae*.

Characters	Genera			
	<i>Belonolaimus</i>	<i>Carphodorus</i>	M <i>Morulaimus</i>	<i>Ibipora</i>
Labial disc	rounded	rounded	elongated	rounded
Separation of submedian lobes	4 distinct grooves	no distinct grooves	no distinct grooves	4 distinct grooves
Lateral lines	1	2 distinct (see text)	4	4
Amphids	on lateral lips	between lateral lips and labial disc	between lateral lips and labial disc	on lateral lips
Position of dorsal oesophageal gland	ventro-lateral	dorso-lateral	dorso-lateral	lateral
Position of oesophago-intestinal junction	close to median bulb, anterior to glands	opposite posterior half of glands	opposite anterior half of glands	close to median bulb, anterior to glands
Body canals	very distinct, thick	faint, thinner	faint, thinner	very distinct, thick
Spermatheca	offset	axial	offset	offset

striae. Lateral lips reduced. Labial disc present. Deirids not seen, phasmids post anal. Stylet elongate, conus 55-75% of total stylet length. Median oesophageal bulb strongly muscular. Oesophageal lumen displaced. Subventral glands small, dorsal large, elongate. Cardia absent. Body canals distinct. Ovaries paired, crustaformeria in form of quadricolumella, spermatheca axial or slightly offset, epiptygma present. Female tail cylindroid to conoid, with rounded tip. Male tail conical, with caudal alae enveloping tip.

As pointed out by Seinhorst (1971, 1971 a), further studies are required to determine the relationships of such genera as *Telotylenchus*, *Trichotylenchus*, *Telotylenchoides* and *Histotylenchus*, with this subfamily.

We are indebted to R. C. Colbran for specimens of *Carphodorus* and *M. soldus*, R. W. McLeod for specimens of *Ibipora lolii*, and J. M. Fisher for slides of *M. whitei*.

S U M M A R Y

Four genera, *Belonolaimus*, *Carphodorus*, *Morulaimus*, and *Ibipora*, are recognized in the Belonolaiminae. The subfamily diagnosis is emended, the characters differentiating genera are tabulated, and some aspects of the morphology discussed. In these nematodes, the main stylet muscle bands are attached at the stylet knobs and the long vestibulum extension, and there appear to be thinner secondary bands attached at stylet knobs and basal plate.

R I A S S U N T O

Osservazioni sulla morfologia di Belonolaiminae.

La sottofamiglia Belonolaiminae comprende quattro generi: *Belonolaimus*, *Carphodorus*, *Morulaimus* e *Ibipora*. La diagnosi della sottofamiglia è emendata e sono stati tabulati i caratteri differenziali dei quattro generi. In questi nematodi le principali bande muscolari dello stiletto sono attaccate ai nodi basali ed alla lunga estensione del vestibolo; sembra poi che vi siano bande secondarie più sottili attaccate ai nodi basali ed alla piastra basale.

L I T E R A T U R E C I T E D

- ANDRÁSSY I., 1976 - Evolution as a basis for the systematization of nematodes. Pitman Publ. Ltd., London, 288 pp.
- COLBRAN R. C., 1965 - Studies of plant and soil nematodes, 11. *Carphodorus bilineatus* n.g., n.sp. (Nematoda: Dolichodorinae) from eucalypt forest in Queensland. *Qd. J. agric. Sci.*, 22: 481-484.

- GOLDEN A. M., 1971 - Classification of the genera and higher categories of the order Tylenchida (Nematoda). *In: Plant Parasitic Nematodes, Vol. 1, Academic Press, edit. B.M. Zuckerman, W.F. Mai, R.A. Rohde, pp. 191-232.*
- MONTEIRO A. R. and LORDELLO L. G. E., 1977 - Dois novos nematóides encontrados associados à cana-de-açúcar. *Revta Agric. Piracicaba, 52: 5-11.*
- SEINHORST J. W., 1959 - A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. *Nematologica, 4: 67-69.*
- SEINHORST J. W., 1971 - On the genera *Trichotylenchus* and *Telotylenchus*. *Nematologica, 17: 413-416.*
- SEINHORST J. W., 1971 a - The structure of the glandular part of the oesophagus of Tylenchidae. *Nematologica, 17: 431-443.*
- SHER S. A. and BELL A. H., 1975 - Scanning electron micrographs of the anterior region of some species of Tylenchoidea (Tylenchida: Nematoda). *J. Nematol., 7: 69-83.*
- SIDDIQI M. R., 1970 - On plant parasitic nematode genera *Merlinius* gen. n. and *Tylenchorhynchus* Cobb and the classification of the families Dolichodoridae and Belonolaimidae n. rank. *Proc. helm. Soc. Wash, 37: 68-77.*
- SIVIOUR T. R. and MCLEOD R. W., 1979 - Redescription of *Ibipora lolii* (Sivour, 1978) comb. n. (Nematoda: Belonolaimidae) with observations on its host range and pathogenicity. *Nematologica, 25: 487-493.*
- SMART G. C., HARTMAN R. D. and CARLYSLE T. C., 1972 - Labial morphology of *Belonolaimus longicaudatus* as revealed by the scanning electron microscope. *J. Nematol., 4: 216-218.*