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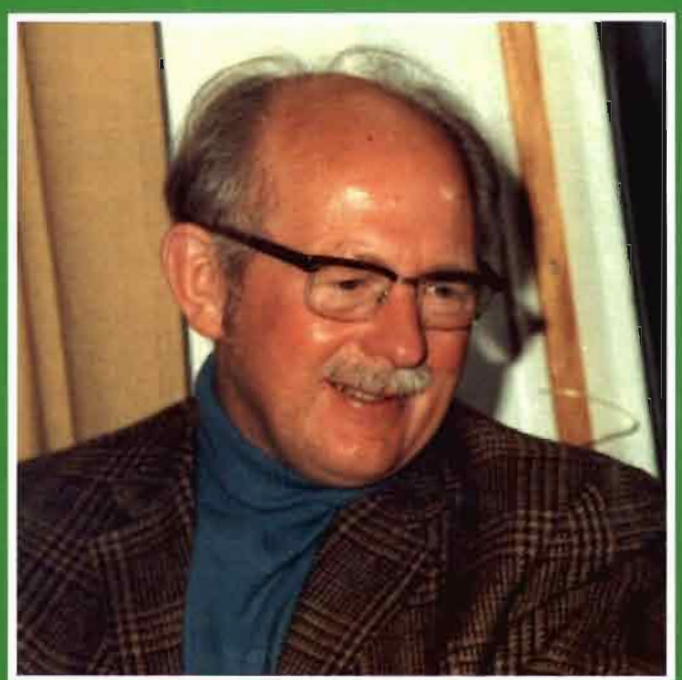
Fundamental and Applied NEMATOLOGY

formerly **REVUE DE NÉMATOLOGIE**

Issue dedicated to Jan Willem Seinhorst



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Jan Willem Seinhorst
(20 August 1918 – 7 April 1997)

The present issue is dedicated to the memory of Jan Willem Seinhorst, one of the most eminent nematologists of the century whose actions and concepts inaugurated a new era in the science of nematology.

The Editor wishes to thank the fellow nematologists who participated in this hommage for sending the manuscripts published in the following pages.

Pierre Baujard
Editor

In Memoriam

Dr Jan Willem Seinhorst

August 20, 1918 – April 7, 1997

Jan Willem Seinhorst was born a farmer's son in Ruurlo, Gelderland, on August 20, 1918. Being a bright child at school, the whole community joined forces to make it possible for him to further his studies. However, he had to study agriculture instead of comparative linguistics, which was his favourite subject. As a result he became a nematologist, a fact he never really regretted. He graduated from the Agricultural University Wageningen in 1941, but he was forced to interrupt his scientific career as continuing would have meant collaborating with the enemy.

From 1945 until 1950, he worked on his thesis on *Ditylenchus dipsaci* at the Agricultural University. In 1949, he was hired as a nematologist at the newly founded Institute for Plant Protection, at Wageningen. He became head of the Nematology Department in 1952, a function he held until his retirement in 1983.

During these years, he made extensive visits to a large number of countries. The most important ones, his 'early travels', included trips to SE Australia in 1954-1955, the USA in 1957 and 1962, Ivory Coast in 1958, the Scandinavian countries in 1964, and Eastern Europe (namely Poland) in 1970. Of course, there were many more trips, mostly to European countries (UK, Germany, Italy, etc.), but they are too numerous to mention here.

He received the title of Doctor Honoris Causa at the Agricultural University of Sweden (1983) and was named Honorary Member of the European Society of Nematologists (1983) and of the Society of Nematologists (1984).

He was a member of the editorial committees of *Nematologica* and *Nematologia mediterranea*. Until his death, he participated in the Scientific Counsel of the Istituto di Nematologia Agraria in Bari, Italy.

Dr. Seinhorst was one of the few nematologists who made a tremendous impact on the science of nematology. His achievements covered large areas of the field, including taxonomy, morphology, methodology, and nematode control. However, his major achievement was the quantification of nematology with the development of population dynamics and yield loss relationships - in short the building of a framework for coherent theory development on the interaction of nematodes and plant growth. He can be hailed as one of the most prominent nematologists from around the world who promoted Nematology from an obscure branch of biology to an adult science.

Dr. Seinhorst made numerous friends during his travels, conferences and international meetings on virus-vector, root-knot, and cyst nematodes. A continuous stream of scientists passed through his laboratory, followed his courses, and enjoyed his hospitality. Everybody was always welcome in his home in Ede and was pampered by his wife Jopie Seinhorst.

Dr. Seinhorst died of heart failure on Monday, April 7, 1997, around one o'clock in the morning at his home in Ede, The Netherlands. He had been at work all Sunday and Sunday evening on one of the manuscripts he was preparing for publication. He was just turning in, after reassuring his wife "I have checked all calculations; they are in order". He was 78 years old.

Those who knew him well knew that Dr. Seinhorst never really retired. He just moved his data to his study at home and continued his research. In fact, the number of papers he published per year actually increased after he was freed from the tedious duties associated with the position of department head. In his latter years, he was persuaded by colleagues to use a computer, a technology he adopted when he was over 70 years old. He wrote almost more than 140 research papers and book chapters and left ten unfinished manuscripts and enough material for twenty more. There was still a lot of valuable information to analyse and publish, theories to be improved. He was in a constant hurry to accomplish these goals. Two papers were accepted for publication shortly after his death, one of which is being published in the present issue of *Fundamental and applied Nematology*. His scientific heritage (data and manuscripts) was left to C.H. Schomaker and T.H. Been at the IPO-DLO, who will try to complete some of his work.

His nematologist colleagues will all miss him very much, his friendship, his counsel and the intellectual conversations on a wide range of topics, which were so characteristic of him.

from C.H. Schomaker & T.H. Been
DLO Research Institute for Plant Protection (IDO-DLO), P.O. Box 9060, 6700 GW Wageningen, The Netherlands.

Jan Willem Seinhorst, or Descartes as a nematologist. A reflection

When going through Seinhorst's most significant publications and reading the excellent article on the 'Seinhorst Research Programme' (Schomaker & Been, 1998) published in the present issue, it becomes evident that the intellectual process of this nematologist followed closely the principles proposed by René Descartes in his *Discours de la méthode*, first published in 1637, at Leiden, a town not very far from the Dutch Province of Gelderland where 'Wim' Seinhorst was born, lived, and died.

To support such a statement, it is necessary to quote the 'principles' of Descartes, a French philosopher who may not be familiar to all nematologists. Descartes was the first philosopher to reject the 'argument of authority' that prevailed before him and to propose a new and logical way of reasoning. In short, he did not give credit to the assumptions of previous philosophers - a category which, at the time, included naturalists - but he only considered what he could himself apprehend as real or true.

The main principles stated in his *Discours de la méthode pour bien conduire sa raison et chercher la vérité dans les sciences* (transl.: Discourse on the method for controlling one's reasoning and finding the truth in sciences) were the following:

First Principle - "*Le premier* [principe] *était de ne recevoir aucune chose pour vraie que je ne la connusse évidemment pour telle, c'est-à-dire d'éviter soigneusement la précipitation et la prévention, et de ne comprendre rien de plus en mes jugements que ce qui se présenterait si clairement et si distinctement à mon esprit que je n'eusse aucune occasion de le mettre en doute*" (transl.: The first principle was to accept nothing as true unless I knew it as such, *i.e.*, to carefully avoid excessive haste and prejudice, and to include nothing more in my judgments than what appeared so clearly and so distinctly in my mind that I would have no reason to question it).

This means that the observer can only admit as true the things or facts he observes himself and that he must reject any *a priori* statement concerning such observations. This is in perfect accordance with Seinhorst's opinion: he considered earlier observations to be subject to review/revision, particularly if they led to insufficiently founded theories.

Descartes also wrote: "*Je rejetais presque pour faux tout ce qui n'était pas vraisemblable*" (transl.: I almost rejected as false anything that was not plausible). This parallels Seinhorst's qualification of insufficiently founded theories as 'fairy tales'.

Second Principle - "*Le second* [principe] *est de diviser chacune des difficultés que j'examinerais en autant de parcelles qu'il serait requis pour les mieux résoudre*" (transl.: The second principle is to divide each of the difficulties I would examine in as many parts as were necessary to better solve them).

This means that the various elements of the problem to be solved must be clearly separated and considered one after the other in a logical sequence. This could be applied in particular to the works of Seinhorst concerning methods in nematology. The aim of Seinhorst's researches was to evaluate the impact of nematode parasitism on selected crops in order to evaluate the cost efficiency of various control methods. The first difficulty was the lack of precision of the methods used at that time to extract nematodes from soil or plants, which made the evaluation of populations very haphazard. All of these methods, perhaps with the exception of the Fenwick can, resembled cookbook recipes more than reliable laboratory procedures. Consequently, Seinhorst developed the elutriator and the mistifier for extracting nematodes from soil and plant tissues, respectively. These devices eliminate the subjective factor and produced reliable results when series of extraction are made. Once these methodological problems were solved, it became possible to evaluate soil and root populations of nematodes better and with sufficient precision, a basic requirement for all subsequent laboratory or field experiments. Seinhorst also perfected the counting tray and, for taxonomy, fixing and mounting procedures.

Third Principle - "*Le troisième* [principe] *est de conduire par ordre mes pensées en commençant par les objets les plus simples et les plus aisés à connaître, pour monter ensuite comme par degrés jusque à la connaissance des plus composés*" (transl.: The third principle was to order my thoughts by starting with the simplest and easiest to know objects, then to proceed, as by successive steps, toward the knowledge of the most complex ones).

This is reflected by the logical approach of the experimental work of Seinhorst. Laboratory observations must precede the pot tests, which are glasshouse experiments that themselves precede the microplots/field trials. The latter type of observations, made in natural sites similar to farmers fields, can be better understood if their various factors have been previously observed in selected simpler laboratory experiments. They also permit to evalu-

ate the weight of the different factors that play a role in the natural process of nematode infestation in the field.

Fourth and last Principle - "*...faire partout des dénombrements si entiers et des revues si générales que je fusse assuré de ne rien omettre*" (transl.: ... to always do enumerations so complete and reviews so general that I would be certain of not omitting anything).

This means that, at any time during the experimentation, all of the factors that concur with the results observed are checked to make sure that they all have been taken into consideration for the interpretation of these results and the formulation of subsequent theories. This is evident in all of Seinhorst's works, as explained at length in the article on 'Seinhorst Research Programme'.

One of the great merits of Seinhorst was that he was able - due to the accuracy of the experimental methods he used - to quantify the results and so to propose mathematical laws for interpretating and predicting the effects of plant-parasitic nematodes on plant growth. Seinhorst was a master in this kind of mathematics and this part of his work recalls Descartes' statement: "*Je me plaisais surtout aux mathématiques à cause de la certitude et de l'évidence de leurs raisonnements*" (transl.: I mostly enjoyed mathematics because of the certainty and the obviousness of their lines of reasoning.)

Co-authorship in Seinhorst's publications also recalls another statement of Descartes: "*Souvent il n'y a pas tant de perfection dans les ouvrages composés de plusieurs pièces et faits de la main de divers maîtres qu'en ceux auquel un seul a travaillé*" (transl.: Often, there is less perfection in the works composed of several parts, made by the hand of various masters, than in those due to the work of a single person).

The list of Seinhorst's publications (not given here) includes 129 published articles or book chapters*. Among those, only 43 - exactly a third - have been published with one (30), two (11), or three (2) co-authors. This represents a low percentage of co-authorship compared to the usual practice in nematological publications.

It should be noted that, with the exception of some recent articles written in cooperation with T.H. Been and C.H. Schomaker, all of the most significant publications, *i.e.*, those reporting experiments and/or interpreting the laws of nematode population dynamics or the relationships between plant and nematodes, have been written by Seinhorst alone.

In conclusion, it seems evident that Seinhorst can be qualified as a Cartesian nematologist. Perhaps, as he was inclined to the study of philosophy, did he read the *Discours...*; however, many people read philosophical works without being influenced by them. The similarities between Descartes and Seinhorst could also be interpreted as a convergence between two thinkers following close, if not similar, intellectual processes.

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Michel Luc
Muséum National d'Histoire Naturelle,
Laboratoire de Biologie Parasitaire, Protistologie,
Helminthologie,
61, rue Buffon
75005 Paris, France.

* From a temporary list kindly communicated by T.H. Been and C.H. Shomaker; who are fully acknowledged. These close colleagues of Seinhorst intend to publish a complete list, including numerous papers considered as 'grey' literature.

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Forum article

THE SEINHORST RESEARCH PROGRAM

Corrie H. SCHOMAKER and Thomas H. BEEN

DLO Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, The Netherlands.

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Summary – We propose the 'Seinhorst Research Program', derived from Seinhorst's empirical philosophy. All theories of the 'Seinhorst Research Program' are developed by searching for recurring regularities (patterns) in a collection of observations, named 'the empirical base'. To prevent 'ghost theories from sloppy data', all assumptions underlying the empirical base are carefully described in theories with respect to methodology and technology, including statistics. The patterns to be recognised are summarised by mathematical equations, which must be connected with biological processes to bridge the gap between 'normal' language and mathematical language for the description of biological theories. Often, the patterns result from more than one biological process. If so, the basic patterns are disentangled from one another using a method of pattern analysis. The procedure is best carried out when only a limited number of more or less congruent patterns are involved. Therefore, attention must be given to the choice of the hierarchic level and the complexity of the investigated system. Investigations proceed from simple experimental systems to complex natural systems at a hierarchic level that is neither so high that manifesting processes are very dissimilar nor so low that one runs the risk of describing processes irrelevant for the purpose of the investigation. In the 'Seinhorst Research Program', this purpose is finding methods for improvement of financial returns of host crops attacked by plant-parasitic nematodes through calculating risks of nematode population development and subsequent yield reduction. Pattern analysis yields theories about causes of phenomena observed at the investigated hierarchic level and about properties of processes at the nearest lower hierarchic level. Predictions at the next higher hierarchic level are made by synthesising several patterns in (stochastic) simulation models. Synthesis is also applied to compound patterns of processes in simple experimental systems, with the objective of explaining complicated patterns in complex systems. © Orstom/Elsevier, Paris

Résumé – *Le Programme de Recherche Seinhorstien* – Nous proposons un " Programme de Recherche Seinhorstien " à partir de la philosophie empirique de Seinhorst. Toutes les théories de ce program sont développées grâce à la recherche de régularités récurrentes (modèles) dans une série d'observations appelée " base empirique ". Pour éviter les " théories fantômes fondées sur des données flasques ", toutes les assertions soutenant cette base empirique sont soigneusement décrites en tant que théories prenant en compte la méthodologie et les techniques, dont les statistiques. Les modèles retenus sont résumés par des équations mathématiques qui doivent être reliées à des processus biologiques afin de combler le fossé entre les théories biologiques exprimées dans les langages " normal " et mathématique. Les modèles se révèlent être souvent le résultat de plus d'un processus biologique. Si c'est le cas, les modèles de base sont démêlés en utilisant une méthode d'analyse des modèles. Cette procédure est accomplie dans les meilleurs conditions si elle ne concerne qu'un nombre limité de modèles plus ou moins congruents. L'attention doit donc être portée sur le choix du niveau hiérarchique et de la complexité du système étudié. Les recherches procèdent à partir de systèmes expérimentaux simples jusqu'à des systèmes naturels complexes et ce à un niveau hiérarchique tel que celui-ci ne soit ni assez élevé pour que les processus révélés ne soient très dissemblables, ni assez bas pour ne pas courir le risque de décrire des processus ne correspondant pas au but de la recherche. Dans le " Programme de Recherche Seinhorstien " ce but est de mettre au point des méthodes permettant une amélioration du rapport financier provenant de plantes hôtes attaquées par des nématodes en calculant les risques concernant l'accroissement des populations de nématodes et de la diminution correspondante des récoltes. L'analyse des modèles conduit à des théories concernant les causes des processus au niveau hiérarchique inférieur le plus proche. Les prédictions concernant le niveau hiérarchique supérieur le plus proche sont réalisées par la synthèse de plusieurs modèles en modèles de simulation stochastique. Cette synthèse est également appliquée aux modèles complexes de processus dans des systèmes expérimentaux simples, et ce dans le but d'expliquer les modèles compliqués existant dans les systèmes complexes. © Orstom/Elsevier, Paris

Keywords: analysis, comprehensive models, empirical base, empirical cycle, empirical philosophy, methodology, nematology, patterns, research program, stochastics, synthesis.

In this paper, we try to describe Seinhorst's empirical philosophy in some detail. He has never put his ideas about this subject into writing, probably because he considered them to be part of a classic philosophy developed and sufficiently described by others. To some extent this may be true but, first, not all interpretations of the classical empirical philosophy are equally satisfactory (Koyré, 1997) and, second, comments of fellow-nematologists on his work suggests that the nature of this philosophy and the way Seinhorst interpreted it in every-day nematological practice might not be quite clear to everybody.

Seinhorst's personal interests (natural sciences, philosophy, modelling) as well as his ideas on what

should be the true purpose of science played an important role in his work. These ideas began to take shape in his 'underground period' during the last year of the Second World War, when he was 26 years old and had ample opportunity for reflection and studies in philosophy, natural sciences, theology, and linguistics.

In his personal diary for this period, he formulated his opinions on the ultimate purpose of the (natural) sciences: "Not extended factual knowledge, which can be such a nuisance in ambitious schoolmasters, but the deeper understanding, the possibility of a view over an unknown landscape must be the purpose of all work. Therefore careful examination of the work of the great scientists is also an instigating part of the study" (Personal diary Seinhorst, March 10, 1945; translated from Dutch by the authors). On the role of philosophy in natural sciences he wrote: "I am more interested in natural sciences than in philosophy. But without philosophy understanding is impossible. To me natural sciences and all that consists of separate observations, including art, is a passion. Philosophy is a duty, a necessity and an ambition" (Personal diary Seinhorst, March 12th, 1945).

These considerations, combined with a set of special conditions at the beginning of his career, were to become the backbone of his work.

Conditions

Apart from his personal interests, the research themes and the empirical philosophy which form part and parcel of the 'Seinhorst Research Program' are also logical consequences of a number of circumstances and conditions at the time of the foundation of this program in the early fifties. Many of these conditions are still valid today. They were described by Seinhorst (1996) as follows:

Purpose of research - Mission of the IPO

At the time of its foundation in 1949, the mission of the Research Institute for Plant Protection (IPO-DLO), commonly known as IPO, was formulated as "Finding methods, by means of scientific research on pests and diseases in crops, to improve the economic returns of these crops". Seinhorst, responsible for the nematological research at IPO, interpreted the IPO mission for his discipline as follows: providing information to weight the costs of control against its benefits and find the optimal balance in individual cases.

The tender age of nematology as a quantitative natural science

When Seinhorst began his research at IPO, almost all scientific tools for accomplishing the IPO mission were lacking. This was even the case for stem, potato cyst, and beet cyst nematodes, that is, for species that were generally considered as harmful ones. There were no quantitative methods for measuring yield reduction by nematode populations in crops. Although the existence of a negative correlation between nematode density at the time of planting of a crop and its expected yield was generally accepted, no mathematical function was available to describe this correlation accurately. Yield reductions by nematodes were prevented by reducing nematode density before planting by means of crop rotation or soil fumigation and by growing resistant varieties, but the true effects of these control measures and the causes of these effects were largely unknown. The lack of knowledge of quantitative relations between nematodes and plants was closely connected to an almost complete lack of reliable methods for quantifying numbers of nematodes in soil samples and in plant parts. Although cysts could be extracted from the soil with Fenwick's (1940) can, no reliable methods were available to estimate numbers of eggs within cysts. Free-living nematodes were separated from soil by Baermann's (1917) funnel, which is only suitable for small soil samples, or by Cobb's (1918) sieving and decanting method, both methods of unknown efficiency and accuracy (Seinhorst, 1988).

Prejudices and fantasies

The lack of quantitative knowledge on plant/nematode relations gave ample room for fantasies about causes of yield reduction by nematodes and about nematode control. Even today the situation has not much improved because of the reluctance of most nematologists to be involved in quantitative research.

Reductionism

In The Netherlands, this situation was mainly due to the fact that quantitative nematology was not included in the education of students. Internationally, there is a shift of nematological research to low hierarchic levels (*e.g.*, molecular level) and a tendency to interpret low level results as causes of phenomena at a higher level. The same tendency has been observed in other natural sciences. In physics, it was called 'reductionism' by Lagendijk (1989). Reductionism assumes that all biological processes follow the same laws and that all phenomena can be explained by studying only the building stones at a low hierarchic level. True reductionists consider that the translation of genetic or molecular information to processes in space and time, resulting in an adult organism, is superfluous and they think that theories on nematode/plant interactions and organisation patterns that exist within and between the intermediate hierarchic levels from low (molecular) to high (farmer's field) are irrelevant. However, new concepts became manifest

at higher hierarchic levels. These concepts must be consistent with those at the lower hierarchic levels, but they cannot be deduced from them (Kooijman, 1987; Lagendijk, 1989): "More is different" (Anderson *et al.*, 1988). Just as the question of whether or not quarks are locked-in is irrelevant to a brain surgeon (Lagendijk, 1989), the point of protein-based similarity dendrograms for pathogens is irrelevant to breeders and farmers.

Yield reduction

Seinhorst (1986*b*) noted most causes attributed to growth reduction by nematodes in the literature as "myths and fairytales". He mentioned obstruction of plant vessels causing wilting (Oostenbrink, 1950), withdrawal of nutrients, mechanical damage to root tissue resulting in a hampered uptake of water and minerals, and decreased shoot-root ratio causing insufficient mineral uptake (Trudgill *et al.*, 1975*a*,*b*,*c*; Evans *et al.*, 1975; Trudgill, 1980; Trudgill & Cotes, 1983).

Control measures

Beliefs and ideals on the ultimate solution for nematode problems range from frequent applications of both fumigant and non-fumigant nematicides (Mulder, 1979; Mulder *et al.*, 1979) - which were wrongly supposed to have favourable cost/benefit ratios -, to the use of late-maturing potato cultivars (Trudgill *et al.*, 1990; Haverkort *et al.*, 1992) - which were wrongly presumed to be more tolerant than early cultivars - and to a balanced bio-diverse (agro)-ecosystem - which was supposed to suppress harmful organisms (Sikora, 1992). The true nature - structural or functional - of such an equilibrium, or homeostasis, in which many biologists tend to believe, is still controversial. As 'normal' ecosystems are characterised by large structural fluctuations, there seems to be more reasons to believe in a functional - with respect to food chains - homeostasis (Odum & Biever, 1984) than in a structural one - with respect to numbers of species - (Rosenzweig & McArthur, 1963). However, the hypothesis of homeostasis is basically disputable because reliable quantitative methods, based on identification and distribution patterns of all relevant organisms, to describe 'biodiversity' or 'equilibrated' ecosystems are conspicuous by their absence.

Research themes

Because of these conditions, which to some extent still persist nowadays (especially for *Meloidogyne*, *Pratylenchus*, and *Trichodorus* species), the 'Seinhorst Research Program' on plant-parasitic nematodes consisted of the following four themes (Seinhorst, 1996):

METHODOLOGY

Methods for the estimation of numbers of the various nematode species in plant and soil samples and other experimental methods, with known accuracy, both for research and extension purposes, including:

- Methods for extraction of nematodes from plant and soil samples with known efficiency.
- Identification methods, including fixation techniques and microscopy.
- Nematode distribution patterns at different scales, varying from centimetres to several metres in farmers' fields.
- Methods to identify and quantify sources of variance.

YIELD REDUCTION

General relation between nematode densities at the time of planting and relative yield (yield of plants with nematodes as a proportion of yield in absence of nematodes, all other conditions being identical).

POPULATION DYNAMICS

General relation between nematode densities in soil or plant samples at successive observation dates during the vegetation period of the host plants (for instance at planting and at any time afterwards, such as after ripening of the plants). This relation must take into account the degree of plant growth reduction by the nematodes.

CONTROL MEASURES

Relations between control measures (nematicides, biological control, resistant cultivars, crop rotation) and nematode population dynamics and crop growth.

A proper integration of control measures and farmers' practices requires integration of all relations in an operational model, which implies that they must be available as mathematical equations. The model must predict, within adequate and specified limits, the consequences of control measures against nematodes in an individual field. These control measures must be taken, at the latest, at the time of planting of the host crop to be protected, but generally much earlier. Such a requirement makes inclusion in the model of most external conditions during crop growth useless. Therefore, deterministic models with their high dependence on external conditions can at best predict average effects in large areas. They are unfit to predict nematological phenomena and their consequences on an individual farmer's field. As a consequence, Seinhorst chose to develop stochastic equations with few parameters, the distribution functions of the latter to be estimated by detailed research.

Empirical philosophy

To exclude as far as possible preconceived ideas and biased conclusions on causes of nematological phenomena, both from the literature and from sloppy empirical methods, and to make all nematological equations and theories consistent, including those influencing the empirical base, such as methodology, technology, chemistry, mathematics, and statistics, Seinhorst applied the Newtonian empirical philosophy, excellently described by Cohen and Westfall (1995), in a consistent manner. According to this philosophy, complex natural (here nematological) situations are reduced to mathematical simplicity by studying the properties of a mathematical analogue. The methods of analysis and synthesis are applied in the order described by Newton, the former always preceding the latter, to be sure that relevant principles are assumed. "Analysis proceeds from effects to their causes and from particular causes to more general ones, guided by mathematical properties of recurring regularities (patterns). Synthesis consists in explaining phenomena from their discovered causes, which are then regarded as principles, thus confirming the explanations" (Newton interpreted by Cohen, 1995). The philosophy further includes correspondence rules to link the results of mathematical analysis to nematological theories in 'normal' language. These rules are: *i*) the mathematical analogue should describe biological processes; *ii*) the variables and parameters in the mathematical analogue should have clear nematological interpretations.

We shall use the empirical cycle as a model to clarify each and every step during investigation in the 'Seinhorst Research Program' and to reveal all external theories used. In some respects, the present empirical cycle differs from those described by others, for instance by Zadoks (1978) or by Campbell and Madden (1990). The reasons why will be explained.

To avoid confusion, some terms used in this paper, such as hypothesis, theory, model, accuracy, precision, etc., that do not have an unequivocal meaning for all scientists, are explained in a Glossary.

Glossary

Accuracy	The closeness of a sample estimate to its true value.
Analysis	"... consists in making experiments and observations and in drawing general conclusions from them by induction, and admitting of no objections against conclusions, but such as are taken from experiments and other certain truths. For hypothesis are not to be regarded in experimental philosophy." Analysis enables us to "proceed from effects to their causes" (Newton in Querie 31 of the Opticks, as interpreted by Cohen, 1995).
Anomaly	A manifest phenomenon in a system that is not explained by the theory with respect to that system.
Deduction	Inference - only by logical rules - of hypotheses or new theories from fundamental theories.
Deterministic model	Model in which parameters are considered to be true constants.
Empirical base	Collection of observations that are free from theories, except methodological theories.
Empirical cycle	Reconstruction of working methods in observations and theory building in science.
Empirical philosophy	Philosophy with respect to scientific working methods, especially those with respect to observations and theory building.
Falsification	Elimination of theories or parts of theories that are contradicted by recurring patterns in the empirical base.
Hierarchic levels	Order in the organisation of a system from low (molecule) to high (ecosystem).
Hypothesis	General statement about causes attributed to phenomena, insufficiently supported by a model.

Glossary (cont.)

Induction	Way of reasoning that derives general causes of phenomena from recurring regularities in an empirical base.
Model	Empirical base, pattern, theory, and a set of rules to connect them mutually.
Parameter	Biologically relevant quantity in an equation that determines its outcome over a certain range of values of the independent variable.
Pattern	Regularity, recurring in the empirical base about natural phenomena manifest in a certain system, from which causes are induced by mathematical analysis and predictions about future phenomena in that same system are deduced. Mathematical analogue of a theory.
Philosophy	In this paper philosophy is used as described by Wittgenstein (1918) in his Tractatus 4.112. "The purpose of philosophy is the logic clarification of thoughts. Philosophy is not a science but an occupation. A philosophical work consists basically of elucidations. The results of philosophy do not consist of 'philosophical propositions' but of clarification of propositions. Philosophy should clarify and demarcate thoughts that otherwise would be troubled and vague." (This choice of definition does not imply that in our opinion there is no justification for philosophy as a science. S & B).
Precision	The repeatability or variability of a sample estimate.
Process	Spatial and/or temporal changes in phenomena.
Reductionism	The concept that phenomena at any hierarchic level can be explained by studying the phenomena at the lowest level.
Research program	Lakatos (1978): A set of logical coherent theories named after its founder and used to explain natural phenomena. The theories consist of methodological and fundamental theories and new theories. Falsification of theories in case of anomalies is done only if it brings scientific progress. Apart from the theories, the program also includes: • An empirical philosophy • Methodology (methods and instruments) • Directions for further development of the program. New theories must be logical consistent with the fundamental theories that represent the core of the research program.
Stochastic model	Model in which parameters are considered to vary under influence of changing known and unknown environmental factors.
Synthesis	or 'composition' "*..consists in assuming the causes discovered and established as principles, and by them explaining the phenomena proceeding from them, and proving the explanations.*" (Newton in query 31 of the Optics, as interpreted by Cohen, 1995).
System	Specific surroundings in which observations are made. These surroundings can vary from simple (experimental systems) to complex (natural systems).
Theory	General pronouncements (in 'normal' language) about causes of phenomena in a certain system, sufficiently supported by a model, from which future phenomena in that same system are predicted. Natural analogue of a pattern.

Empirical cycle

The empirical cycle (Fig. 1) is divided into four empirical sub-cycles, covering the following subjects:

- Methodological models.
- Fundamental models.
- Compound models.
- Models of causes.

At the beginning of a new investigation, when hardly any quantitative information is available, the sequence indicated above is followed, the development of methodological theories with respect to

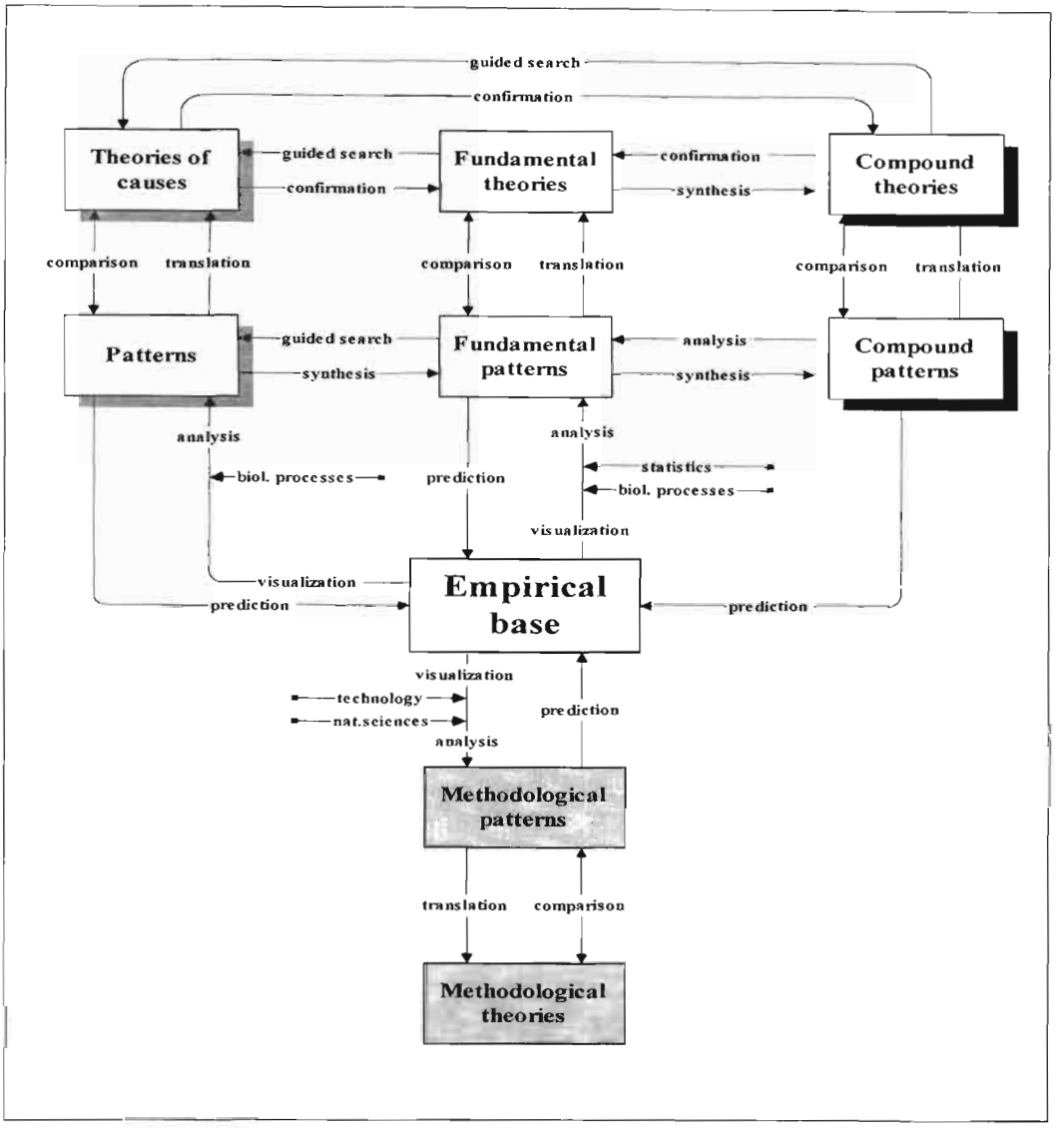

Fig. 1. *Model of the empirical cycle of the 'Seinhorst Research Program', divided in four empirical sub-cycles. Patterns and theories are placed in different box types for each sub-cycle, as indicated below.*
Grey rectangle: Methodological models; White rectangle: Fundamental nematological models; White and black rectangles superposed: Compound nematological models; White and grey rectangle superposed: Models of causes; Empirical base: All sub-cycles originate from and return to the empirical base. ■→ *Imported external information (statistics, biological processes etc.);* → *Processes leading from one (intermediate) result to another (analysis, synthesis, etc.)*

nematological observations preceding that of fundamental nematological theories and theories deduced from these fundamental theories. Later on, the theories in the cycles are considered as jigsaw puzzles. Whenever a piece becomes available, often because of research-questions on agricultural problems, sometimes by coincidence, it is fitted in. When a pattern appears in the puzzle, it can be used in three ways, first to improve calculations on risks of unwanted phenomena in farmers' fields, second to discover the causes (at a lower hierarchic level) to these phenomena, guided by the newly discovered properties of these causes, and third to develop new ways of control (prevention or counteraction) based on these causes, for instance by manipulating plant properties through biotechnology programs.

New nematological theories can only be derived from fundamental nematological theories and their mathematical analogues. In all sub-cycles, the following steps are taken from observation to theory and back.

EMPIRICAL BASE (OBSERVATIONS)

Assumptions

All empirical knowledge goes ultimately back to an empirical base, with records of details of experiments and observations. As this base serves as an impartial arbiter in accepting or rejecting theories, it should be independent of these theories. The requirement of independence also applies to the observation language. Thus, replacement of one theory by another would be of no consequence for the observation terminology or the truth of the basic conclusions (Koningsveld, 1976). It may be feasible to make observations not loaded with nematological theories, but it is impossible to perform theory-free observations when theories from other disciplines are needed to do any observations at all. Examples are physical or statistical theories needed if microscopy or counting problems play a role in the observations and in the basic conclusions drawn from them. Generally speaking, all methods and measuring instruments used to obtain the facts on which theories are to be based contain their own theories and assumptions that will influence the conclusions from observations if these theories should be changed (Popper, 1968). To handle these biases as carefully as possible, all necessary 'external' theories are formulated in a separate methodological empirical sub-cycle (Fig. 1) and are carefully checked for their consistency with fundamental nematological theories during the progress of the research process. Observations from the literature, either on methodology or nematology, are handled in the same way. Nematological observations are added to the empirical base only when the theories underlying the methods are recognised as fundamental in their own disciplines and are consistent with the fundamental nematological theories. If not, the observations cannot be used in the research program discussed here.

The requirement of 'theory-free' observations does not mean that nematological theories cannot be used to decide which observations are relevant and which are not (Koningsveld, 1976). For instance, when nematode/plant relations are studied, one can decide to make observations at a specific range of nematode densities because one knows from the theories that these are relevant to the pattern. In such a case, a nematological theory is used to do observations that will remain valid if the nematological theory should be changed.

Systems studied

In the 'Seinhorst Research Program', the investigations proceed, in general, from simple experimental systems (pot experiments) to complex natural systems (farmer's fields) so that all fundamental processes can be identified without being masked by each other or by secondary effects. Patterns found in simplified experimental systems are compared with those in natural systems (individual farmers' fields) to find out whether the experimental system has the necessary level of complexity to attribute causes, judged as sufficient and true to explain effects in the experimental system, to effects in the natural system. The hierarchic level at which the investigations begin is chosen so that all manifest processes at that level are relevant to predictions at the chosen explanatory level (farmers' fields), without ignoring essential patterns in the relations between the hierarchic levels. The investigations proceed from higher to lower hierarchic levels to answer further questions with respect to the causes of phenomena that appear at the explanatory level. Example 1 illustrates this procedure for Seinhorst's theory on growth reduction by nematodes.

PATTERNS

Sawyer (1955) defines patterns as any kind of regularities that can be recognised by the mind. Sawyer's thesis "A recurring pattern is significant" is adopted in the 'Seinhorst Research Program'. It is therefore assumed that a repeatedly manifesting pattern always has a (here biological) meaning which should be investigated. The significance of the pattern needs no other proof than its recurrence and its biological meaning. The ultimate aim of pattern analysis is twofold:

• Recognition and identification of the biological process(es) that belong to the pattern(s) found. The number of biological processes is higher than the number of patterns. So, the same pattern can be attributed to several biological processes. Therefore,

Example 1. "Growth reduction"

> Yield reductions at small and medium nematode densities are caused by a constant reduction of plant growth, desynchronising plants with and without nematodes (Seinhorst, 1986*b*). From Seinhorst's fundamental theory it can be deduced that this phenomenon can be involved only if nematodes affect growth for a short period (at most 24 hours). Questions about the physiological or biochemical mechanisms involved must be investigated at a lower hierarchic level.

the connection of a biological process with a pattern is also dictated by the nature of the investigated subject.

• Mathematical description of the pattern with minimum differences between observation and theory, in such a manner that variables and parameters have clear biological interpretations.

Visualisation

To trace and recognise regularities in the empirical base in the form of mathematical patterns, the results of observations are visualised graphically, mostly in two-dimensional graphs, but sometimes in three-dimensional graphs when patterns behind patterns are important to attribute causes to phenomena. For instance, this was the case in Seinhorst's relation between relative yield and pre-plant nematode density P (Example 2).

Recurring aberrations from the expected pattern in an empirical base, for instance non-monotonic change in the pattern for population dynamics (Example 3; Fig. 3) or in dose-response relations (Example 7; Fig. 5), or discontinuities often indicate that more than one process became manifest.

Biological processes

In the 'Seinhorst Research Program', patterns are condensed into one or more mathematical equations describing biological processes. The equation parameters must have clear biological meanings so that theory building (transfer of mathematical properties to nematological theories formulated in normal language and vice versa) is possible. The number of parameters in these equations is as small as possible but sufficient to explain the effects of the full range of the independent variable under investigation. "As a rule, no more causes of effects are admitted than is sufficient for their explanation and the same causes are assigned, as far as possible, to the same effects" (Newton interpreted by Cohen, 1995).

To recognise and describe patterns in the empirical base under investigation, one must be familiar with the mathematical patterns belonging to the biological processes discovered up to now and with all underlying assumptions. Pattern analysis opens the possibility to trace biological processes that have not yet been described.

Statistics

In the 'Seinhorst Research Program' statistical methods are developed with the same caution as nematological theories, but nematological theories always come first, before statistics. Statistical theories hardly ever used during pattern analysis as they usually demand drastic additional assumptions, which, later, when more data are available, are often found to be conflicting with the observations. Moreover, information from the usual regression analysis, such as the amount of variance explained or the confidence intervals of parameters, is irrelevant for the explanation of biological patterns.

The aims for pattern analysis in the 'Seinhorst Research Program' do not comply with the common practice of many biologists and nematologists who only try to find the best fitting equation by assuming that the higher the value for R^2, the better the equation describes the relationship. Some nematologists, *e.g.*, Elston *et al.* (1991) and Mulder (1994), even apply this procedure without any restriction to field data on relationships between nematode density P and yield obtained with unknown, but non-constant (as sample sizes were small and not adjusted to nematode density) estimation errors on both the abscissa and the ordinate, with no observations at $P = 0$, and with variables other than nematode density determining yield. Such use degenerates statistics into a ritual (Slob, 1986) instead of a purposeful occupation to develop theories about sources of variation that are consistent with the other (biological and methodological) theories. These statistical theories are mostly developed at the later stages of theory building, when the empirical base is sufficient to determine the distributions' functions of variables and parameters for both the experimental and the natural systems. A recent statistical theory, consistent with nematological theory, is that for infestation foci (Schomaker & Been, unpubl.).

The following quotations of Seinhorst in 'Comments to comments of referees' or in letters to colleagues illustrate the misunderstandings occurring between nematologists with very different opinions on

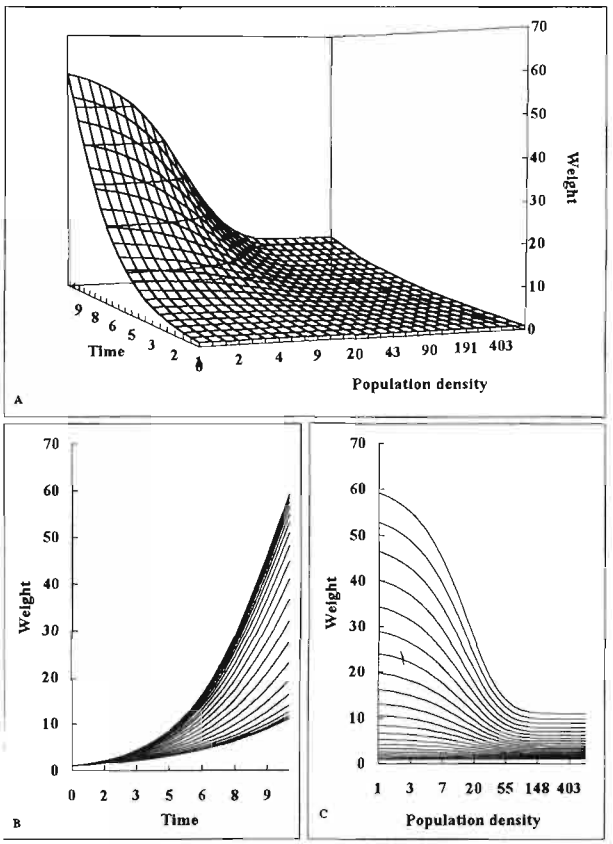

Fig. 2. *Surface plots of the three-dimensional model showing the relation between total weight, Y, of annual plants and relative nematode density, P/T, as cross sections at right angles with the time axis, t, of growth functions of plants at different nematode densities $Y(r_P,t)$ and without nematodes, $Y(r_0,t)$. A: At 230° rotation, which shows the relation between (Y,P/T) and (Y,t); B: At 0° rotation, which shows the relation between Y and t at different relative nematode densities P/T; C: At 270° rotation, which shows the relation between Y and relative population density P/T. (All rotations are clockwise. The growth rates of plants of the same weight without nematodes (r_0) and at nematode densities P (r_P) are related by Equation 3. $r_P/r_0 = k + (1-k).z^{P-T}$ for $P > T$).*

the purpose of statistics: "Models cannot be derived from the data with the help of statistical methods. A straightforward multiple linear regression analysis would almost certainly have led us astray by finding a chaotic set of slopes and would at best tell us that the treatments had one or perhaps two ... effects, which of course is apparent without formal statistical analysis" and "If science would have proceeded as he [the referee, taking pattern analysis for speculation. S & B] seems to suggest it should, it would have stopped developing before Newton. If anything is 'speculative' the 'Principia' is, deriving the weight of the sun, the moon and the planets from what can be seen by simply looking at the sky at night, a simple hypothesis, based on nothing and a lot of mathematics."

Most patterns in biology, including nematology, are non-linear, as dictated by the nature of the investigated subject. Best fits are found by least square calculations of all observations, using numerical trial and error, which is most easily - but not necessarily - done by computer, especially when compound effects are involved. Commercial computer programs for numerical analysis must be regarded with some mistrust because of their ability to produce nonsense estimates in the case of wrong starting values, unsuitable step values, a too small number of iterations or wrong additional assumptions, for instance about the nature and the constancy of variance.

In Example 4, the mathematical analogue of Seinhorst's theory on growth reduction of plants attacked by small and medium densities of nematodes is summarised. The theory is more fully described by Seinhorst (1986*b*) and Schomaker *et al.* (1995). It can be transcribed in 'normal' language because the correspondence rules required in the 'Seinhorst Research Program' are obeyed.

Compounds patterns

Often, a pattern cannot be explained by a single process but only by a combination of two or more processes. In such a case, analysis of the pattern makes it possible to split it into separate patterns, each belonging to separate biological processes. Then, patterns are regrouped by synthesis and compared with patterns from new observations. The choice of the hierarchical level at which observations are made is crucial for the success of this procedure of alternate analysis and synthesis. It should not be too high, because manifest but overlapping processes would become too numerous and too different from each other to be separated. Nor should it be too low, as then one is at risk to describe biological processes that are irrelevant for the purpose of the investigation. The analysis and synthesis of compound comprehensive models on population dynamics, hatching processes, and dose-response relations into separate processes is illustrated in Examples 5, 6, and 7. Full descriptions of these models are given by Seinhorst (1993), Schomaker and Been (1998), and Been and Schomaker (unpubl.).

In the methodological empirical sub-cycle, alternate analysis and synthesis are also applied to compound variance from different sources to make more efficient experimental schemes or practical tests for resistance and tolerance (getting more information from less work) or to choose tests with an optimal cost/uncertainty ratio for extension purposes. In the latter case,

Example 2. "Growth reduction"

Seinhorst (1998) gives the relations in pot and micro-plot experiments between P/T of various nematode species and $y' = (y-m)/(1-m)$ where y and m are the relative dry weights of different hosts at $P/T > 1$ and $P/T = \infty$, respectively, several months after planting. T is the highest density that causes no noticeable yield reduction. The relation between P/T and y' is in close accordance with

$$y' = 0.95^{P/T-1} \quad (1)$$

in all 36 experiments but this equation does not describe an underlying process. The cause of reduction in plant weights by nematodes - a constant growth retardation ("the same happens later", Seinhorst 1986*b*) - is revealed only when these relations are represented as cross sections at right angles with the time axis of growth functions of plants at different nematode densities P (Fig. 2).

Example 3. "Population dynamics"

The pattern belonging to Seinhorst's extended population dynamic model for nematodes with one generation per vegetation period (P_f) is visualised in Fig. 3. The non-monotonic change in its pattern indicates that we are dealing with a compound pattern, the result of two or more underlying patterns. The relevant questions at this stage are: "How many patterns can be distinguished?" and "To which processes are they referring?" These questions can only be answered properly by a mathematical analysis of the pattern.

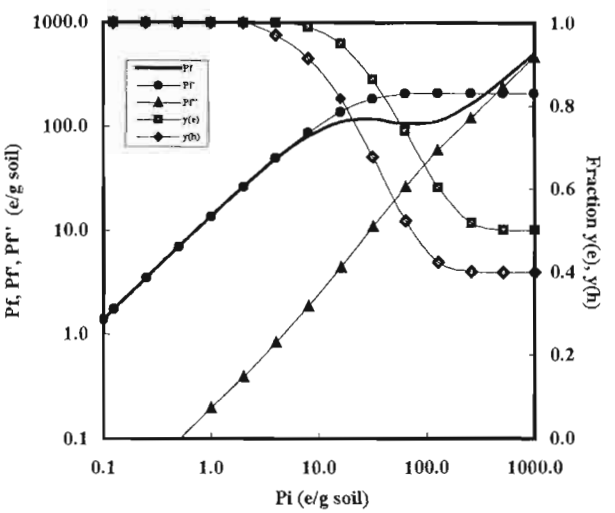

Fig. 3. *The compound model Pf, represented by the heavy line, for population dynamics of tylenchid nematodes with one generation per growing season. It consists of four non-linear equations. Three of them, ●Pf', ◇y_h and □y_e, are multiplicative and one, ▲Pf'', is additive. ◇y_h and □y_e represent fractions; ●Pf' and ▲Pf'' eggs per gram of soil (e/g).*

the financial consequences of the uncertainty in predictions by a test, due to a certain amount of variance, are weighted against the costs of the test to find the optimum.

Examples of the use of compound pattern analysis to identify and quantify sources of variance using parametric statistics (not to be confused with ANOVA or Multivariate Analysis) are presented in a posthumous paper by Seinhorst *et al.* (unpubl.) on tests for partial resistance to potato cultivars for potato cyst nematodes and in a paper by Been and Schomaker (unpubl.) on effects of pesticides on hatching behaviour of potato cyst nematodes.

Another possible use of compound patterns (and theories) are simulation models that synthesize several fundamental (simplified) patterns at a given hierarchic level into a comprehensive model generating predictions and new theories about relevant nematological scenarios at the same and higher hierarchic levels. An instance of a stochastic simulation model for advisory purposes is described in Example 8.

PATTERNS TO CAUSES

Fundamental nematological patterns make it possible to derive the properties of processes at a lower hierarchic level. These properties can be used to trace these processes and the patterns belonging to them. This type of research has never been done at IPO, but we are planning to change that. Elsewhere in this paper, under 'Causes at low hierarchic levels' in

Example 4. "Growth reduction"

The mathematical analogue of Seinhorst's theory on growth of plants affected by small and medium nematode densities (Seinhorst, 1986*b*; Schomaker *et al.*, 1995) shows that:

r_p / r_0	$= t_0/t_p$	for $Y_0 = Y_p$	(2)
	$= k + (1-k).z^{P-T}$	for $P > T$	(3)
	$= 1$	for $P \leq T$ and	
	$= k$	for $P \rightarrow \infty$	
z^T	$= 0.95$		(4)

As a consequence

y	$= m + (1-m).0.95^{P/T-1}$	for $P > T$	(5)
	$= 1$	for $P \leq T$ and	
	$= m$	for $P \rightarrow \infty$	

The parameters m, k, T, r_P, r_P and z have a clear biological meaning.

Y_0	g	weight of whole plants or parts of plants without nematodes.
Yp	g	weight of whole plants or parts of plants at nematode density P
y	-	relative plant weight Y_P/Y_0
t_0; t_p	day	time needed for plants respectively without and with nematodes to reach the same weight Y.
r_p/r_0	-	relative growth rate
m	-	minimum relative plant weight.
k	-	minimum relative growth rate.
T	e/g	largest nematode density not affecting the relative growth rate and relative plant weight
r_P	g/day	growth rate of plants at nematode density P
r_0	g/day	growth rate of plants without nematodes
z	-	the degree to which plants can prevent growth and weight reduction by nematodes.

Equation (3) describes the relevant nematological process at play when nematodes compete for effect on plant growth.

'Future Research' and in Examples 1 and 9, suggestions are given about research proposals to this effect.

THEORIES

Pattern translation

In many biological studies, the stage of theory building is never reached because the conditions (see PATTERNS) enabling translation of the mathematical equations into a theory in 'normal language' are not fulfilled. General conclusions from the observations are then reduced to reports of alternative 'facts' that usually are evaluated statistically in H_0 *vs* H_1 tests (Zadoks, 1978). Campbell and Madden (1990) call these types of models 'correlative models' which, contrary to 'explanatory models', give no information on the causes of phenomena (Fig. 6). In explanatory models, the phenomena are deduced from a theory or concept, which appears out of nowhere or comes - in an unspecified manner - from the descriptive model. The main objection against the working method proposed by Campbell and Madden (1990) is that it

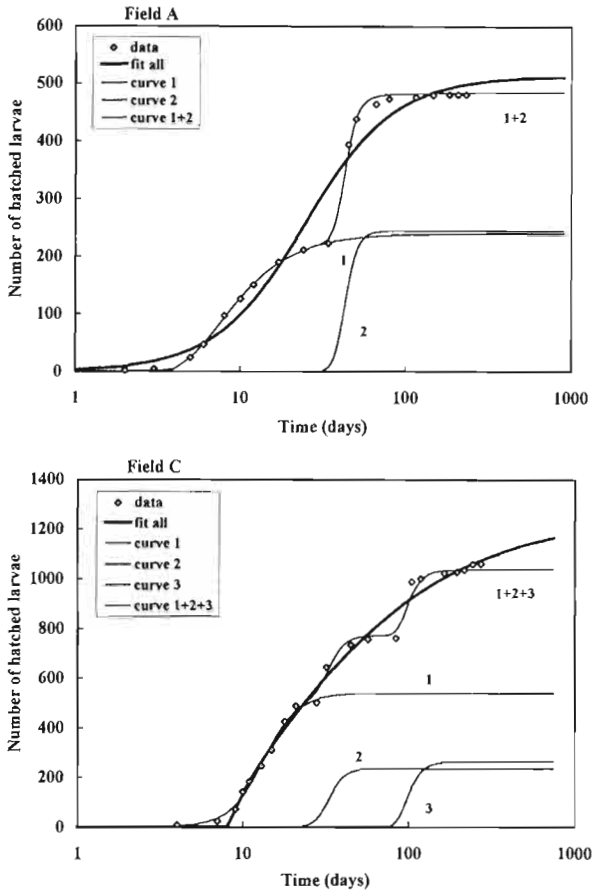

Fig. 4. *Compound hatching curves of field populations of nematodes, consisting of two (field A) and three (field C) superimposed hatching curves. Original data (◇), overall fit to a log-logistic equation using all data, fit of separate curves and a compound fit using two and three log-logistic equations, respectively.*

leaves too much room for preconceived ideas, which, if the model contains many parameters, can explain observation satisfactorily in hindsight (by adjusting the parameters), but need not be true and sufficient causes of the phenomena. Descriptive models have the disadvantage that - although some facts may become plausible - they hardly contribute to theory building as causes of the observed phenomena are not revealed. Unfortunately they have found a widespread use in the 'Deterministic Dynamic Simulation Models', at the cost of theory-building. Seinhorst wrote about this subject: "They confuse dynamic models with *ad hoc* equations for statistical operations, thus reducing scientific work to plain fact reporting, not understanding that 'a fact without a theory is a ship without a sail' ".

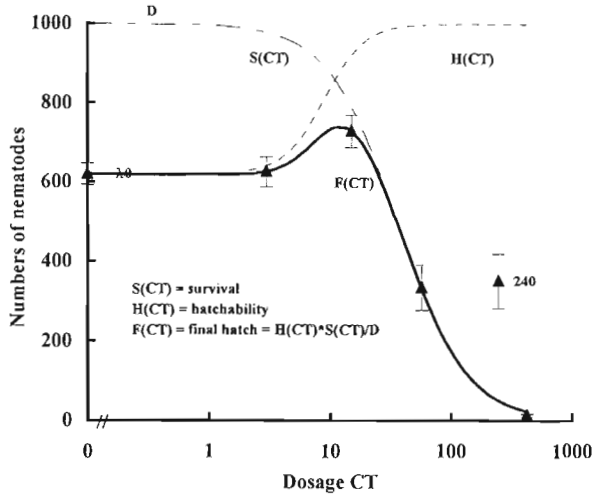

Fig. 5. *Dosage/response relations for the (z)-isomer of 1,3-dichloropropene, expressed in numbers of nematodes. The number of hatched nematodes F(CT) is explained as a product of hatchable nematodes H(CT) and surviving nematodes S(CT), both dependent on dosage CT. There were 1000 (D) nematodes per hatching cup in the test. The symbols (▲) with bars indicate the averages of five × five observations (except for CT = 0 with ten × five replications) with standard errors.*

The correspondence rules in the 'Seinhorst Research Program' make it possible to transfer the conclusions from the mathematical analogue to the corresponding 'natural' analogue in 'normal' language. Here, 'normal' means that the language is understandable and useful for those who are not involved in the 'Seinhorst Research Program'. Therefore, jargon and technical terms are avoided as much as possible. If technical terms are inevitable, they are explained in 'normal' language.

In Examples 9 and 10, Seinhorst's theories on growth reduction of annual crops attacked by nematodes and on population dynamics of nematodes with one generation per year (for instance *Globodera rostochiensis, G. pallida, Heterodera avenae, H. schachtii, Meloidogyne naasi*) are summarised. For more details, the reader is referred to Seinhorst (1986*a*, *b*, 1993, 1998) and Schomaker *et al.* (1995).

Comparison

The correspondence rules in the 'Seinhorst Research Program' make it possible to compare new and current theories. Patterns from simple experimental systems are compared with patterns from more complex systems, and the theories on causes of effects are compared in both systems.

Falsification

These comparisons usually give rise to extensions and/or modifications of the original theory. Not every

Example 5. "Population dynamics"

The pattern for population dynamics of potato cyst nematodes in a complex system (the field) is a compound one. It could be disentengled into four non-linear patterns only after three of these patterns were identified separately in simple experimental systems (plastic containers where plant roots were allowed to occupy the same space as under field conditions). This was done by repeated comparisons of the population dynamic patterns in complex and simple systems. These four patterns are:

1. The relation between P_i, the density of potato cyst nematodes at planting, and P'_f at harvest, (the number of cysts produced per unit haulm weight times the maximum number of eggs per cyst). It describes the way nematodes compete for a feeding site in the roots.

$$P'_f = M\times(1 - e^{-\alpha}) \quad (6)$$

$$\alpha = a\times P_i / M$$

a - maximum multiplication rate

M - maximum number of eggs per gram of soil

2. The relation between P_i and the relative number of eggs per cyst (y_e), describing the way the feeding cells are damaged by the penetration of large numbers of nematodes.

$$y_e = m_e + (1-m_e)\times 0.9^{\gamma} \quad \text{for } P_i > T_e \quad (7)$$

$$\gamma = P_i / / T_e - 1$$

$$y_e = 1 \quad \text{for } P_i \leq T_e$$

m_e - relative minimum number of eggs per cyst

T_e - largest nematode density P_i at which the number of eggs per cyst is not reduced

3. The relation between P_i and relative haulm dry weight (y_h), describing the competition between nematodes for effect on size of the total food source (the latter estimated by haulm dry weight).

$$y_h = m_h + (1-m_h)\times 0.9^{\lambda} \quad \text{for } P_i > T_h \quad (8)$$

λ - $P_i / T_h - 1$

y_h - 1 for $P_i \leq T_h$

m_h - minimum relative haulm weight

T_h - largest nematode density Pi that does not reduce haulm weight

4. The relation between P''_f and P_i in the part of the tilth where no roots are present, which depends on 'normal' root growth, the reduction of root growth by the nematodes, and the fraction of nematode eggs that do not hatch in spring.

$$P''_f \quad b_1\times(1-s\times y_h)\times P_i \quad (9)$$

b - fraction of unhatched eggs

s - proportion of soil with roots present in the tilth

The four patterns compound the extended population dynamic pattern of original Fig. 11.2:

$$P_f \quad s\times y_h\times y_e\times P'_f + P''_f \quad (10)$$

$y_h\times y_e\times M$ is also called M' or hybrid M, for instance in a paper about the partial resistance of eleven potato cultivars (Seinhorst *et al.* 1995).

Example 6. "Hatching curves"

Compound hatching curves of nematodes from farmer's fields (Been & Schomaker, unpub.) are shown in Fig. 4. They indicate that the population consists of three sub-populations with different hatching properties, caused by nematicide treatments in previous years.

Example 7. "Dose-response relations"

Compound response curves of nematodes to doses of nematicides (Schomaker & Been, 1998) are shown in Fig. 5. They indicate two stimulating and one reducing effects at different ranges of doses of a nematicide, the reducing effect and at least one of the stimulating effects being independent, meaning that they act on different nematode receptors.

aberrant pattern leads to changes in the theory. Four conditions must be satisfied before a theory is replaced by another:

- The aberrant pattern must come from observations based on sound methodological theories described in the methodological empirical cycle.
- The aberrant pattern must be a recurring one.
- The aberrant pattern must reveal new processes or clarify already known processes.
- The new theory connected with the aberrant pattern must be consistent with the fundamental theories in the 'Seinhorst Research Program'.

All conditions for falsification, except perhaps the second condition cited, agree well with those described by Lakatos (1978). An 'old' theory is only replaced by a 'new' one if it brings scientific progress. The 'new' theory must be able to explain and predict all phenomena that were satisfactorily explained and predicted by the 'old' theory and it must also explain and predict new phenomena.

Confirmation

Comparisons between new and old patterns from observations and the theories connected to these patterns are repeated whenever additions to the empirical base give cause. If the same process is involved, combining a large number of observations enables an improved separation between 'signal' and 'noise' and results in a more complete and clear emergence of the patterns, thus confirming the theories on the causes of the manifesting phenomena. Example 11 illustrates this for the pattern of equation (1): $y' = 0.95^{\alpha}$.

The fundamental theories are also confirmed if new theories from compound fundamental patterns, or 'theories of causes' derived from the properties indicated by the 'fundamental theories', in turn successfully predict observations in the empirical base.

Working thus from high hierarchic levels to lower ones, applied, fundamental physiological, and molecular biological research can meet and formulate together coherent, consistent theories. This approach has the advantage that every new theory at a low hierarchic level that is relevant to the explanatory level can be directly implemented.

Deduction

When theory building has progressed so far that the theory on the explanatory system agrees satisfactorily with the patterns derived from the observations of simple and more complex systems, unobserved effects of nematodes in host plants can be deduced from the general causes in the theory, which now are considered as principles. For instance general relationships of non-studied or newly discovered nematode species, such as *Meloidogyne chitwoodi* or *M. fallax*, can be predicted from relationships known from fifteen experiments with other *Meloidogyne* species and their hosts (Example 12. Growth reduction).

Future research

In the very near future, the authors and some of their Dutch colleagues will be involved in a research program (not in the sense proposed by Lakatos, 1978), financed by the Dutch government, on population dynamics and distribution patterns of *Pratylenchus penetrans*, *Meloidogyne chitwoodi*, and *Trichodorus* spp. and growth reduction caused by these species in some relevant host plants. An attempt will be made to describe distribution patterns of viruses transmitted by trichodorid nematodes. This approach could later be used with other plant viruses. The part of this program under our responsibility will be done as described in this paper and summarised in Fig. 1. Methodological theories will be developed before nematological theories, all theories will be derived from mathematical properties of patterns describing biological processes. Analysis will always come first, then alternate with synthesis. We will also apply Seinhorst's empirical philosophy to integrate our know-

Example 8. "A stochastic simulation model"

A stochastic dynamic simulation model for potato cyst nematodes was compounded from theories about growth reduction and population dynamics in partially resistant and non-host crops to serve as the basis for an advisory system, enabling farmers to choose agricultural scenarios with maximum financial returns (Been *et al.*, 1995). The extended equation on population dynamics contains ten parameters; too many to be useful in an advisory system. For those values of P_i where potatoes can be grown with acceptable yield reductions ($P_i/T < 100$), the relation between P_i and P_f can be simplified to:

$$P_f = \varphi \times M'(1 - e^{-\alpha}) \qquad (11)$$

in which

$a = a \times P_i / M'$

φ - degree of susceptibility of the potato cultivar for the nematode population

a - maximum multiplication rate

M' - hybrid maximum population density ($y_h \times y_e \times M$, see Example 5) which does not differ much from M under the given constraints ($P_i/T < 100$)

M - maximum egg density per gram of soil.

The population dynamics under non-hosts is given by

$$Pf'' = P_i \times (b_1) \times (b_2)^{n-1} \qquad (12)$$

b_1 - fraction of unhatched nematodes during the first year of a non-host crop.

b_2 - fraction of unhatched nematodes during the second and next years of a non-host crop

n - number of years with a non-host crop

The fraction b_1 in the first year of cultivation of a non-host crop is smaller than the fraction b_2 in the following years. Schomaker and Been (unpubl.) found on twenty experimental fields an average fraction of 30-40% unhatched nematodes in the first year after a potato crop and of 65% in the second year.

The value of the maximum multiplication rate, a, varies strongly from year to year and from field to field, while the variation of M is more limited as M is closely connected with the size of the food source (estimated by dry haulm weight). The large variation of the maximum multiplication rate, a, makes the population dynamics and subsequent yield losses in susceptible potato crops too unpredictable to recommend a fixed rotation, with or without chemical control. Control measures must then be based on nematode numbers in samples using methods with known accuracy and precision. However, the probabilities of densities P_f and their subsequent yield reductions in years following partially resistant potato crops can be predicated from equations (5), (11), and (12) and the probabilities of all possible combination of a and M, based on their distribution functions.

ledge and that of colleagues from various research stations in The Netherlands into a limited number of consistent theories and hypotheses. Later in the program, our attention will turn to the subjects discussed below.

Connection with other research programs

In 'Deterministic Dynamic Simulation Models', applied to potato cyst nematodes in potatoes by several authors (Ward *et al.*, 1985; Schans, 1993; Van Oijen *et al.*, 1995*a*, *b*) and to *Tylenchorhynchus dubius* in *Lolium perenne* by Den Toom (1989, 1990), the approach is the opposite of Seinhorst's. Synthesis goes before analysis, which undermines and questions the relevance, truth, and sufficiency of the assumed principles (biological processes, equations describing these processes, and external conditions influencing them) to explain the nematological phenomena and their consequences in farmers' fields. The situation is exacerbated as these models represent not just one mathematical equation but numerous equations with dozens of parameters. Both the equations and the parameters can be adjusted flexibly to the observations. Consequently, these deterministic simulation

Example 9. "Growth reduction"

Relevant small and medium ($P/T < 100$) nematode densities cause growth retardation of their host plants, resulting in a decrease in weight of whole plants and plant parts. At any time during the growing period, the growth rate r_P of plants at a certain nematode density P is always the same percentage of the growth rate r_0 of (younger) plants with the same weight but without nematodes. Understanding the system demands a comparison between plants of the same weight, but of different age (Seinhorst, 1979, 1986*a*, *b*; Schomaker *et al.*, 1995). A value of k larger than zero - k is seldom smaller than 0.5 - implies that the mechanism causing growth reduction at small and medium densities is unable to stop growth completely (this remains true at large and very large nematode densities). T is the maximum density up to which growth reduction is prevented or counteracted by a yet unknown mechanism. The factor z^x implies that nematodes are randomly distributed over the surface of root tips and that, if areas where nematodes directly exert their effects overlap, the total effect in the overlapping area is the same as the effect exerted by one nematode ($1 + 1 = 1$). It also implies that the average number of nematodes penetrating the roots per unit of time and per unit of volume of soil is constant. The small scale distribution of nematodes through the soil is regular enough to make this plausible. The total growth reducing effect of all nematodes at a certain density is proportional to the total volume of these area's. Host plants of any species of tylenchid nematodes are able to prevent growth reduction by these nematodes to the same degree z^T (= 0.95), which indicates that properties of a large group of nematodes and plants vary little in this respect. The growth reduction by one nematode, one day after its penetration of the root, is negligible. Investigation at a lower hierarchic level into the fundamental cause of growth reduction by tylenchid nematodes - guided by the properties described in the theory - can lead to new control approaches. Theories about these fundamental causes must be consistent with the other theories to be accepted in the 'Seinhorst Research Program'.

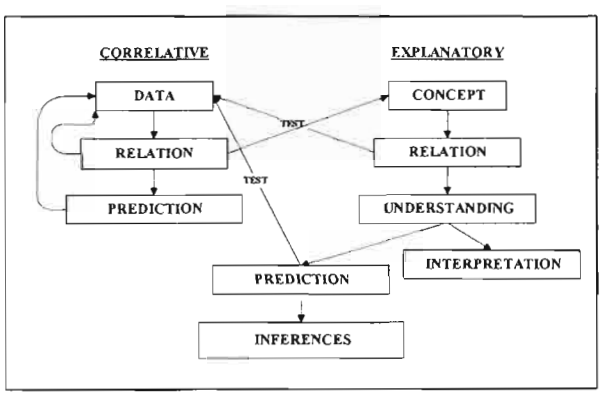

Fig. 6. *The empirical cycle according to Campbell & Madden (1990). This figure discriminates in some respects between empirical or correlative models and explanatory or mechanistic models. Concepts for explanatory models are derived from descriptive ones, but the correspondence rules are absent.*

models can predict almost any phenomenon, albeit only *after the fact.* Therefore, and because the mathematical equations are often purely descriptive (see also Example 13), the 'Deterministic Dynamic Simulation Models' in their present quality do not contribute much to theory building.

The assumptions leading to the 'Dynamic Energy Budget' (DEB) model, which tries to "capture the diversity of the energetics of the different species into one model with different parameter values and to build theories for the parameter values" (Kooijman, 1993), interpreted for potato cyst nematodes by Van Haren *et al.* (1993), proved not to be sufficient and true causes of population dynamics of these nematodes at the explanatory level. The causes are insufficient as they ignore the 'all or nothing' principle in population dynamics of potato cyst nematodes. The causes are non-true causes because they attribute differences in relative susceptibility only to differences in egg numbers produced per female and ascribe decrease in plant weight to withdrawal of food by the nematodes (Seinhorst, 1986*a*, *b*, 1993).

The 'Deterministic Dynamic Simulation Models' as interpreted and applied by various authors (Ward *et al.*, 1985; Den Toom, 1989, 1990; Schans, 1993; Van Oijen *et al.*, 1995*a*, *b*) and the 'Dynamic Energy Budget Model' as interpreted and applied by Van Haren *et al.* (1993) are trying to deduce effects from assumed causes, but these causes are derived from a jumble of loose facts reported in the literature, many of them questionable with respect to their relevance and validity.

Example 10. "Population dynamics"

Equation (6)

$$P'_f = M(1 - e^{-a})$$

The occurrence of discrete, random events in space and/or time, such as the random encounters of nematodes and plant roots, are described by the Poisson distribution. The first term in this distribution function, the likelihood for a plant root to escape nematode attack (zero encounters), is given by e^{α}. The probability of one or more encounters is given by $1- e^{\alpha}$. In its strictest interpretation, the presence of the factor $1-e^{\alpha}$ suggests that plant roots can be imagined as a cylindrical surface divided into equal compartments, that are per cross section randomly penetrated by juvenile nematodes, the cross sections moving up along the cylinder as time goes on. The juveniles can settle in only one compartment at the same time. If they could settle in more than one compartment, this would result in overlapping territories and a decrease in eggs per settled nematode which is in contradiction with the observed patterns. Only one juvenile per compartment can survive. The size of the compartments depends on the place of the root in the root system and the growing conditions of the plant, but not on the density of the surviving juveniles. Juveniles trying to settle in an engaged compartment will remain unsuccessful and eventually die from starvation. In other words, juveniles that successfully enter a root possess a territory that is inaccessible to others. This mechanism prevents females from decreasing in size because of competition for food and, at high densities or in case of coincidental clustering, remain too small to become adults and reproduce.

Equation (7)

$$y_e = m_e + (1-m_e)\times 0.9^{\gamma} \qquad \text{for } P_i > T_e$$

At higher densities P_i, the numbers of eggs per cyst are decreasing, but not because of competition for space between the growing females. The appropriateness and the form of Equation (7) suggest that the quality of the territory degenerates because of random overlapping areas of damaged root tissue, from which these territories are to be developed. (see also Example 8 'growth reduction'). The factor 0.9^{γ} implies that the territories can still reach their maximum quality by using undamaged root cells if they are less than 10% damaged.

Equation (8)

$$y_h = m_h + (1-m_h)\times 0.95^{\gamma} \qquad \text{for } P_i > T_h$$

This equation describes the relative size of the food source, estimated by relative haulm dry weight. Its theory is described under Example 8 'growth reduction'.

Equation (9)

$$P''_f = b_2\times (1-s\times y_h)\times P_i$$

In case of cyst nematodes, the theory must be extended by a term for the number of nematodes per unit of soil that did not hatch spontaneously or under influence of the root system. This number of nematodes is determined by:

- The proportion, s, of soil with roots at nematode densities smaller than T_h. The proportion of nematodes stimulated to hatch in the presence of plant roots is proportional to the relative size of the root system.
- The relative size of the root system at nematode density P_i, described by y_h (Equation 8). The nematode density per unit root weight does not change because of this reduction.
- The fraction b of unhatched nematodes.

Van Oijen *et al.* (1995*a*) carry off the palm as they managed to incorporate only wrong assumptions on plant-nematode relations into the LINTUL crop growth model (Spitters & Schapendonk, 1990) and still obtained, in hindsight and thanks to a convenient number of adjustable parameters and - because of their arbitrariness - adjustable mathematical equations describing nematode-plant relations, some resemblance between simulations and observations in one experiment. Their approach is described in Example 13 as an illustration of 'incomprehensive' and incomprehensible modelling.

The drawbacks of the 'Deterministic Dynamic Simulation Models' could be eliminated if these

Example 11. "Growth reduction"

The existence of a tolerance limit T and its constancy for a given nematode/plant combination under almost all conditions (except short days conditions combined with high light intensity) is often met with doubts or even disbelief. However, Seinhorst did not start his modelling of yield reductions with the concept of a tolerance limit (the fewer parameters the better) but was forced to add this parameter because it gave a better description of the patterns found. The analysis of 29 nematode/plant combinations in Seinhorst's (1998) paper, elsewhere in this edition, is bound to end all doubts. The combination of such a large number of observations reduced variance and clarified the pattern to such an extent that the existence and constancy of T (z^T =0.95) for all nematode/plant combinations) becomes abundantly clear.

Example 12. "Growth reduction"

Seinhorst (1998) proved that the relation between P/T and y', based on his theory about growth reduction by nematodes, closely agree with $y' = z^{P-T}$ (Eq. 1), with $z^T = 0.95$ for all investigated 29 combinations of nematodes and plants. From his theory, growth reduction can be deduced for host plants and tylenchid nematode species other than those investigated, for instance, growth reduction of host plants of the newly discovered nematode species *Meloidogyne chitwoodi* and *M. fallax*.

Example 13. "Growth reduction"

The assumptions on plant growth reducing effects of potato cyst nematodes, conveniently chosen by Van Oijen *et al.* (1995*a*) from a multitude of reports in the literature, are:
1. Accelerated leaf senescence
2. Root death
3. Allocation of assimilates in favour of roots
4. Decreased Light Use Efficiency

but all are demonstrably untrue for (economically important) small and medium nematode densities (Been & Schomaker, 1986; Seinhorst, 1986*b*; Schomaker *et al.*, 1995).
The validity of the model and its predictive ability at the explanatory level (farmer's field) was checked by comparing simulated results with observations in a field experiment (without control) with two cultivars on fumigated and non-fumigated plots. The same observations (biased by questionable methodological methods, especially with respect to the estimation of nematode densities) were used to describe the nematode-plant relations and to estimate the parameters in the model. Aberrant observations in a second field experiment (Van Oijen *et al.*, 1995*b*) were ignored. Differences in growth between the two cultivars could not be explained by the model as it attributes one (linear) mechanism of growth reduction to all nematode densities. Seinhorst's theory on growth reduction by nematodes, confirmed for 29 different nematode/plant combinations in 36 experiments, explain the observations in both experiments well as it discriminates between two mechanism of growth reduction: one operating at small and medium nematode densities ($P/T < 100$) and one at large ($P/T > 100$) nematode densities.

models paid more attention to empirical methodology and if synthesis alternated with pattern analysis of phenomena at the explanatory level to trace and describe processes and causes of relevant processes. Arbitrary equations must be replaced by comprehensive equations describing biological processes. Neither the 'Deterministic Dynamic Simulation Models' nor the 'Dynamic Energy Budget Model' are necessarily incompatible with Seinhorst's models. It is obvious that models inducing causes from their effects, guided by (mathematical) properties of the latter, are useful to models deducing effects from principles and *vice versa*. The two types of models could meet half-way on the hierarchic ladder if the 'believers' in the various models (including the authors of this paper) were able to overcome their inclination to worship the one and only true 'model' and to strive for more consistency of experimental methods and biological theories between different schools of thought.

Causes at low hierarchical levels

Patterns and theories in the 'Seinhorst Research Program' indicate properties of nematological processes at lower hierarchic levels. In Example 1, the causes at a lower hierarchic level of the 'first mechanism of growth reduction' at small and medium nematode densities are biochemical or physiological processes and their properties are indicated by the theory that an individual nematode affects plant growth only during a limited period. From Seinhorst's (1998) data, it is confirmed that this 'first mechanism' applies to many - if not all - plant-parasitic nematodes and their hosts. To find new, broadly applicable, nematological control strategies, these processes should be investigated under the guidance of their properties derived from the mathematical patterns. Other processes considered suitable for investigation in Seinhorst's theory on growth reduction at small and medium nematode densities are the mechanism that neutralises or inhibits the effects of small nematode densities and the mechanism inducing differences in plant growth reducing effects between the first and the second generation (Seinhorst 1986*b*, 1995, 1998). The causes of the 'second mechanism of growth reduction', which becomes noticeable at $P/T > 100$ (Seinhorst, 1998) and is accompanied by diagnostic characteristics (Seinhorst, 1986*b*), and the relation of this mechanism with plant age should be investigated as well.

In population dynamics, much work at molecular and genetic level has already been done, but unfortunately it is not or little (only by establishing a multiplication factor) related to quantitative nematological work at higher hierarchic levels. Therefore, this research cannot be used to make predictions on population dynamics and noxiousness of nematode populations, nor to establish similarities in relevant agricultural properties between nematode populations. For the sake of consistency in theory building, the relationship between the research at higher and lower hierarchic levels must be established in the years to come.

Causes of distribution patterns

A new simulation model would be that of distribution patterns of sedentary nematodes, based on distribution patterns of potato cyst nematodes. The small and medium scale distribution patterns of potato cyst nematodes have been extensively described (Seinhorst, 1988; Been & Schomaker, 1996; Schomaker & Been, unpubl.). The small scale distribution of these and other nematodes was well described by the 'Negative Binomial Distribution' (Seinhorst, 1988). For potato cyst nematodes, the dispersing forces causing small and medium scale patterns proved to be constant and independent of time, place (external conditions), and population density (Schomaker & Been, unpubl.). As the mobility of potato cyst nematodes is only a few centimetres per year - which is negligible - their distribution patterns depend on their population dynamics and on the activities of farmers, who disperse cysts within and between fields and horizontally and vertically through the soil with their machinery - including soil fumigation equipment. It would be relatively simple to use vector analysis of the dispersal forces to explain the distribution patterns of these nematodes. More complex distribution patterns of other nematodes could then be deduced by means of simulation models of the vectors using sub-models of the population dynamics and mobility of these nematodes. The relevant external conditions for mobility should not be suggested by preconceived ideas, *e.g.*, on effects of organic matter or biodiversity, but by mathematical properties of distribution patterns of nematodes under natural conditions, following the empirical cycle (Fig. 1) in a consequent manner. If the presence or absence of micro-organisms and their metabolic products are critical for nematode mobility then the distribution patterns of these micro-organisms must be studied too, for instance by DNA extraction from soils (Van Elsas *et al.*, 1997), and related to that of the nematode species under study and to (small and medium scale) geographic patterns.

Growth reduction by root nematodes in perennial plants

During the first year after planting, effects of nematode attack on perennial plants can be investigated in the same way as on annual plants. We cannot yet answer the question of whether a reduction in growth and productivity should be expected in the second and following years, depending on nematode densities at planting, especially at low densities. It is known from studies on *Radopholus similis* on citrus that nematodes spread from older to new roots at the periphery, thus rapidly increasing in numbers. The same tendency to rapidly increase in numbers and to migrate is observed for stem nematodes in red clover and lucerne - here via moist surfaces of plant leaves. We know that nematodes do not generally cause specific disease symptoms (see Examples 1, 2, 4, 9, 11, and 12 on growth reduction) and that annuals are more tolerant to second and later generations of nematodes than to the first generation present at planting (Seinhorst, 1995). Therefore, it is by no means certain that the presence of large nematode numbers in old orchards will cause substantial reductions in productivity. An increase in productivity, in some but not all cases, after treatment with non-fumigant nematicides is not proof of nematode damage if the effect of these chemicals on the yield of trees without nematodes under the same conditions is not known.

To investigate growth reduction of perennial plants by increasingly large nematode populations, patterns of weight of the whole plant and its fruits must be

studied at a sufficiently wide range of nematode densities and at regular time intervals. To produce a clear pattern, a simple system with external conditions as constant as possible must be studied first. Later, more complex systems can be studied and their patterns compared with the patterns from the simple system.

Fungi and insects

Extension of the 'Research Program' with research on infestation focus development and distribution patterns of fungi and insects living in the soil is worth consideration. The model for foci developed by Van den Bosch (1990) has similarities with our model. It contains a gamma density function for the relative number of biological entities produced per time unit and an exponential function for the spatial distribution. For nematodes, the spatial distribution function is the same as in the model of Van den Bosch (1990). The parameters of the function must be estimated for each separate species. For nematodes, we are planning (see Future Research) to deduce a comprehensive temporal model from nematode dispersal forces and population dynamics. We might do the same for other organisms.

Potato cyst nematodes

Of all plant-parasitic nematodes, the methodological and nematological theories in the 'Seinhorst Research Program' are the most advanced for potato cyst nematodes. The reason is that potato cyst nematodes are economically important and relatively simple research objects because they have only one generation per year and they are easily manageable. Seinhorst (1998) has demonstrated that many causes of phenomena at the explanatory level, especially those with respect to growth reduction, apply to all nematodes. Therefore, we want to choose potato cyst nematodes and their hosts as model organisms to induce theories on the causes of different aspects of growth reduction, distribution patterns, and molecular aspects of population dynamics. From these theories and supplementary observations, similar theories can be deduced for other nematodes.

Final considerations

Seinhorst and the authors of this paper worked together in close collaboration for almost thirteen years. To us, his pupils, Seinhorst's approach to science is a sensible one, adopted naturally. We cannot imagine working in a different manner. During these years of co-operation, the research program was developed further and our influence gradually increased. At the present stage, it is difficult to discriminate between Seinhorst's and our own contribution in the development of the research program during the last decade. Either contribution is reflected in both Seinhorst's and our publications, but we always discussed and criticised each others' work and we wrote some papers together. Our contribution consists mainly in the development of computer programs for analyses and in the synthesis or composition part of the program. This synthesis resulted in simulation models for both distribution patterns of nematodes in farmers' fields (from which sampling methods can be developed) and expert systems for potato cyst nematodes (from which control scenarios can be chosen). Much time and energy were invested in the implementation of scientific results in agricultural practice, *e.g.*, Seinhorst's concept of partial resistance of potato cultivars to pathotypes of *Globodera pallida*, and in the introduction of new sampling methods for the detection of small foci in fields of ware and seed potatoes. This made it possible to reduce nematicide use and improve financial returns.

During the period of our co-operation with Seinhorst, we had many discussions on methods, theories, and philosophy, not because we disagreed but because a relatively small 'Research Program' is 'subject to offence' (Lakatos, 1978) and must develop strategies for its defence. We feel we succeeded in establishing and defending the 'Seinhorst Research Program' so that, in our opinion, it has a fair position and good prospects in comparison with other 'Research Programs'. If the foregoing paragraphs gave the impression that we reject all concepts on which other 'Research Programs' are based, then this image wants nuance. We mainly object against the way these concepts were interpreted and applied for nematode/plant relations, but we know from experience that any 'Research Program' can be put in an unfavourable light if amateurs tamper with its models (Anon., 1991). Therefore, we will not hesitate to adopt approaches or theories from other 'Research Programs' if patterns from observations should guide us in that direction.

Many foreign colleagues co-operated with Seinhorst for long periods. Without suggesting to be complete, we want to mention here our Scandinavian colleagues who appointed Seinhorst as an Honorary Doctor at the Agricultural University of Uppsala and our Italian colleagues from the Istituto di Nematologia Agraria in Bari, where Seinhorst was a consultant for many years, who made a large contribution to the confirmation of Seinhorst's theories (Seinhorst, 1998). They all greatly influenced the course the 'Seinhorst Research Program' has taken in the past, and we hope that they and other colleagues will continue to do so in the future.

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The common relation between population density and plant weight in pot and microplot experiments with various nematode plant combinations

Jan Willem SEINHORST†

Middelberglaan 6, 6711 JH, Ede, The Netherlands.

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Summary – In 31 published and five unpublished experiments, curves according to the equation $y = m + (1 - m)0.95^{P/T-1}$ for $P > T$ and $y = 1$ for $P \leq T$ (Equation 3) were fitted to the relation between P and y, where P is the density at planting of various nematode species and y is the plant weight (expressed as a proportion of plant weight at nematode densities $P \leq T$) of various plant species at the end of the experiment. To compare basic patterns in these experiments, data sets were normalized with respect to m and T. Relative plant weights y were transformed to $y' = (y - m)/(1 - m)$ and nematode density scales were divided by T. Values of P/T were allocated to classes with regular intervals on a logarithmic scale. Averages of all y' values (Y') per class plotted against antilog of average log relative nematode density per class fitted almost exactly to a curve according to the equation $y' = 0.95^{P/T-1}$ for $1<P/T<100$ and $y' = 1$ for $P/T \leq 1$. It is concluded that Equation (3) for the relation between nematode density at the time of sowing or planting and relative plant weight during the first year after sowing or planting applies to a wide range if not to all combinations of nematode and plant species. This confirms Seinhorst's theory about plant growth reduction by nematodes for all these combinations. In only one of fifteen experiments with *Meloidogyne* species on host plants could an effect of population increase on relative plant weight be derived from the data. © Orstom/Elsevier, Paris

Résumé – ***Relation générale entre densité de la population et poids des plantes lors d'expériences en pots et en micro-parcelles concernant différentes combinaisons nématode / plante*** – Se fondant sur les résultats de 36 expériences (dont cinq non publiées) il a été établi des courbes correspondant à l'équation $y = m + (1 - m)0,95^{P/T-1}$ pour $P > T$ et $y = 1$, pour $P \leq T$ (équation 3), courbes ajustées à la relation entre densité à la plantation (P) de différentes espèces de nématodes et le poids de différentes espèces de plantes (y) - exprimé comme la proportion du poids aux densités $P \leq T$ - à la fin de l'expérience. Pour pouvoir comparer les schémas de base de ces expériences, les séries de données ont été normalisées en ce qui concerne m et T. Le poids relatif des plantes, y, a été transformé en $y' = (y - m) (1 - m)$ et les échelles des densités de nématodes divisées par T. Les valeurs de P/T ont été réparties en classes sur une échelle logarithmique. Les moyennes (Y') de l'ensemble des valeurs de y' pour chaque classe ont été alignées contre l'antilog du logarithme moyen de la densité des nématodes par classe ; ces moyennes s'ajustent exactement à une courbe d'équation $y' = 0,95^{P/T-1}$ pour $1 < P/T < 100$ et $y' = 1$ pour $P/T \leq 1$. Il en est conclu que l'Équation 3 concernant la relation entre densité des nématodes au moment du semis (ou de la plantation) et le poids relatif des plantes pendant la première année de croissance peut s'appliquer à un grand nombre sinon à la totalité des associations entre nématode et plante. Cela confirme, pour toutes ces combinaisons, la théorie de Seinhorst concernant la diminution de croissance des plantes sous l'influence des nématodes. Dans une seule des quinze expériences relatives à des espèces de *Meloidogyne*, un effet de l'augmentation de la population sur le poids de la plante a pu être mis en évidence à partir de ces données. © Orstom/Elsevier, Paris

Keywords : first generation, growth reduction, *Meloidogyne* spp, modelling, reproduction, retardation, second generation, tylenchid nematodes, yield reduction.

According to Seinhorst's (1979, 1995*b*) growth model, the growth rates for plants of the same weight (and therefore of different age) at nematode densities P (r_p) and without nematodes (r_0) are related as follows:

$$\begin{aligned} r_p/r_0 &= k + (1 - k)z^{P-T} && \text{for } P > T \\ r_p/r_0 &= 1 && \text{for } P \leq T \text{ and} \\ r_p/r_0 &= k && \text{for } P \to \infty \end{aligned} \qquad (1)$$

assuming that at a given nematode density P, r_p/r_0 is constant throughout the growing period. The value of k is independent of nematode density P and of time after planting, but may vary between experiments. T is the tolerance limit, the largest density P that does not cause growth reduction, and z is a constant smaller than 1. Cross sections orthogonal on the time axis of a three-dimensional model (Schomaker *et al.*, 1995) describing, at different values of k, the relation between total plant weight, nematode density, and time after planting are, from some weeks after planting, in close agreement with Equation (2),

$$y = m + (1 - m)z^{P-T} \quad \text{for } P > T \text{ and}$$
$$y = 1 \quad \text{for } P \leq T \tag{2}$$

in which z and m are constants smaller than 1. In most, if not all experiments, z^T could be considered to be 0.95. Then Equation (2) becomes

$$y = m + (1 - m)0.95^{P/T-1} \quad \text{for } P > T \text{ and}$$
$$y = 1 \quad \text{for } P \leq T \tag{3}$$

Recurrence in experiments of the basic pattern described in Equations (2) and (3) confirm Seinhorst's theory on growth reduction by nematodes, summarized by Seinhorst (1995*b*) as: 'The same happens later'. To compare these basic patterns in experiments with different nematode/plant combinations independently of the parameter values, the data from these experiments must be normalized with respect to the parameters m and T, which have different values for the various combinations.

This is done by transformation of Equation (2) to

$$y' = \frac{y - m}{1 - m} = z^{P-T} \quad \text{for } P > T \text{ and}$$
$$y' = 1 \quad \text{for } P \leq T \tag{4}$$

Then, the value of y' at a relative nematode density $(P/T)_j$ ($j = 0$ to ∞) in n experiments ($i = 1$ to n) is the same in all experiments to which Equation (4) applies. Relative plant weights Y_{ij} at a given relative nematode density $(P/T)_j$ in the i^{th} of n experiments, estimated by fitting a curve according to Equations (2) or (3) to the observed plant weights, can be considered as estimates of y' at that density. Moreover, if the relative minimum plant weight m_i was determined, Y_{ij} can be transformed to

$$Y'_{ij} = \frac{(y_{ij} - m_i)}{(1 - m_i)} \tag{5}$$

which is an estimate of y' at relative nematode density $(P/T)_j$. The n transformed relative plant weights Y'_{ij} at a certain nematode density in all experiments are then estimates of the same y' at the same relative density $(P/T)_j$ and can be treated as replicates in a single experiment. Therefore, their average

$$\overline{Y}_j = \frac{1}{n} \sum_{j=1}^{n} Y'_{ij} \tag{6}$$

is also an estimate of y' at relative density $(P/T)_j$. If the distribution of the transformed relative plant weights Y'_{ij} at each density can be treated as a normal distribution, the standard deviation of $\overline{Y}_j$ is $n^{-0.5}$ times that of the transformed relative plant weights.

The number n of transformed relative plant weights does not have to be the same at all relative nematode densities P/T (as they would be for non-transformed relative plant weights), because all these relative plant weights represent estimates of z^{P-T} with the same value of z or $0.95^{P/T-1}$. Also, in general, the individual values of P/T in the ranges of relative nematode densities will not be the same in different experiments. To compare the results in a single graph, the values of P/T were divided into nematode density classes with the same class limits in the different experiments and a small ratio between these limits. The relation between log relative nematode density and plant weight within each nematode density class can then be treated as being linear. Instead of averages of Y'_{ij} per nematode density $(P/T)_j$ averages per relative density class j are calculated according to Equation (6). Not all experiments may be represented in every class, because the density intervals are larger than the class width and the lengths of the density ranges may be different. However, as above, differences in numbers of data per density class do not affect the quality of their averages as estimate of the theoretical value of y' for each of the classes. Average relative nematode densities per nematode density class is calculated as:

$$\overline{\left(\frac{P}{T}\right)}_j = \text{antilog } \frac{1}{r} \sum_{j=1}^{n} \left(\frac{P}{T} i\right)_j \tag{7}$$

with $(P_i/T_i)_j$ = the value of P/T from the i^{th} experiment belonging in density class j and r_j = the number of observations in this class. With a class width of 1 to 1.4, as used below, the difference between the geometric mean according to Equation (6) and the arithmetic mean is very small.

If the deviations per nematode density class of the relation between the average relative nematode densities and average relative plant weights from Equation (4) are distributed randomly, then Equation (4) describes the relation between nematode density and relative plant weight accurately for most or all of the nematodes and plant species in the experiments, the results of which are summarised according to Equations (5) and (6). For Equation (4) to represent the actual relationship between relative nematode density and relative plant weight, the average relative plant weights per class should not deviate significantly from that according to Equation (4) in any class.

Materials and methods

To investigate whether Equation (4) accurately describes the relation between the population density at sowing or planting and the relative plant weight at some time after sowing or planting, data were used from 31 published and five unpublished experiments with *Pratylenchus penetrans* on *Daucus carota, Digitalis*

purpurea, Vicia faba, and apple seedlings. The five unpublished experiments were made in 10 cm wide, 30 cm deep cylindrical pots with 2 kg soil. To obtain twelve densities ranging from 0.1 to 200 eggs/g soil in the experiment with *D. purpurea*, twelve lots of partially sterilised sandy soil were spread out, inoculated separately by spraying with a suspension of the required number of nematodes, then mixed gently. Each lot of inoculated soil was then divided into five replicates of 2 kg and five replicates of non-inoculated partial sterilized soil were used as controls. All replicates were transferred to 2.5-dm^3 pots in random order. For the experiments with *D. carota* and *V. faba*, 20 kg of partially sterilised soil was inoculated with a large number of nematodes, as described above. Then 10 kg of this inoculated soil was mixed with 10 kg non-inoculated partially sterilised soil. Half of the 20 kg mixture was mixed with another 10 kg of partially sterilised soil and so on until eleven nematode densities were obtained. Each lot of 10 kg inoculated soil was divided into ten portions of 1 kg, and each portion was mixed with 1 kg non-inoculated partially sterilised soil. The 100 portions of 2 kg thus obtained were transferred to 2.5-dm^3 pots in complete random order. Per nematode density five pots were sown with carrots and five pots with broad beans. After the seeds germinated, the number of seedlings was reduced to one per pot. For the published experiments, the reader is referred to the original articles for the methods used.

Partially sterilized soil was obtained by steaming soil to kill all pathogens present, after which the soil was allowed to 'recover' (enabling fungi to grow on the dead organic material) before nematodes were applied. Without this recovery period, the nematodes added to the soil would not have survived.

The data from all experiments were transformed according to Equation (5) and presented in Figs 1, 2, 3, and 5. Tables 1, 2, 3, and 4, associated to these figures, give the details on nematode species; plant species; estimated values of tolerance limits T, assuming that $z^T = 0.95$, and relative minimum plant weights m in curves according to Equations (2) and (3); and references to the original publications. For almost all published experiments, estimates of T and m were given in the original publications. These estimates are also mentioned in Tables 1, 2, 3 and 4. Before combining all results, estimates of T and m these experiments were ckecked and in some cases slightly adjusted. If T and m were not given in the original

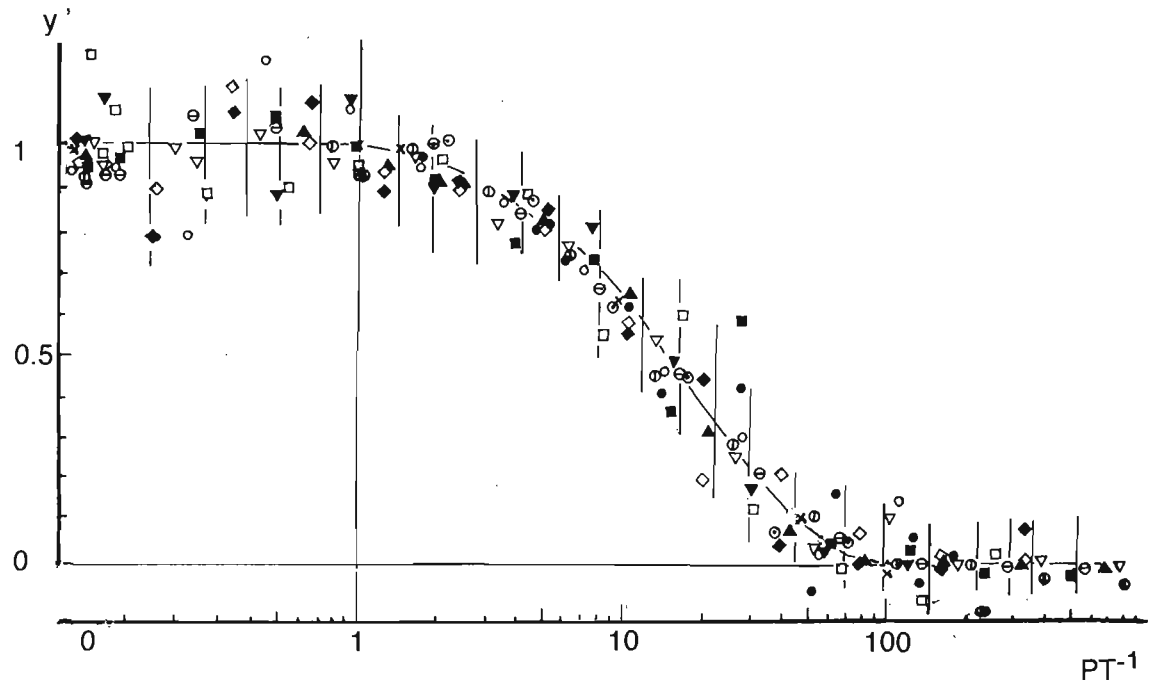

Fig. 1. *Relations between nematode density at sowing or planting* P/T_j *and relative plant weight* Y_{ij}'. *Curve according to Equation (4). References to symbols and associated values of parameters* T *and* m *are summarized in Table 1. In the graph* Y_{ij} *and* P/T_j *are indicated as* y' *and* PT^{-1} *respectively.*

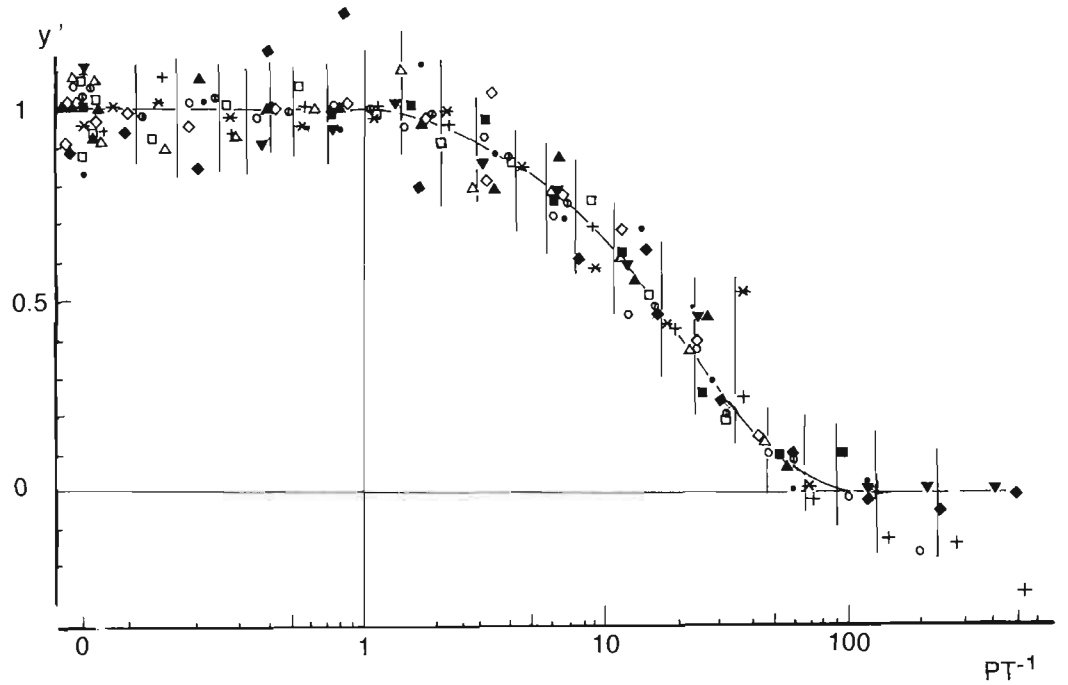

Fig. 2. *As Fig. 1. References to symbols and associated values of parameters* T *and* m *in Table 2.*

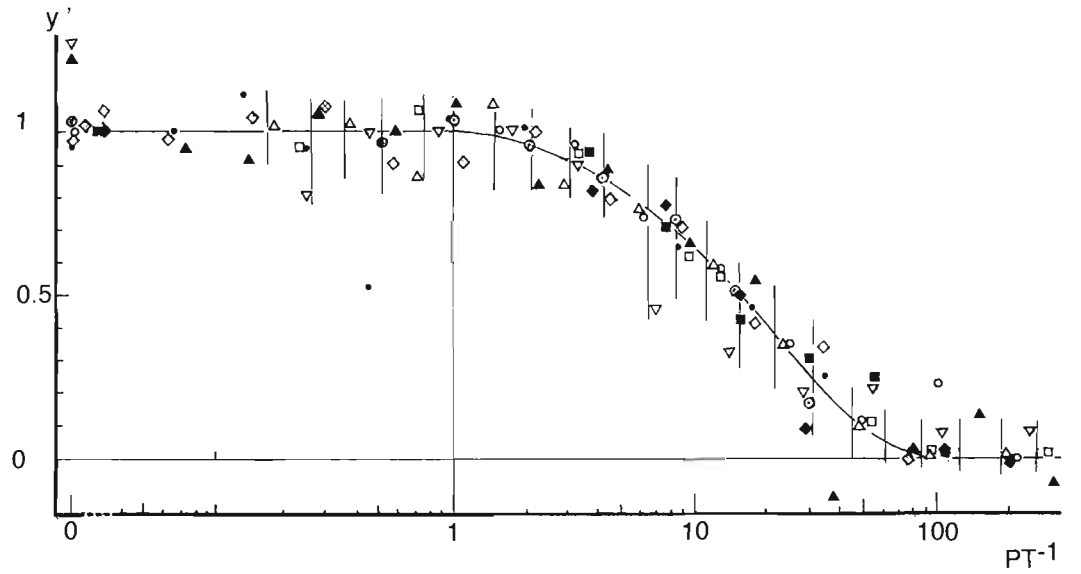

Fig. 3. *As Fig. 1. References to symbols and associated values of parameters* T *and* m *in Table 3.*

article they were estimated for the first time. No predetermined scheme was followed for the distribution of the different experiments among the first three graphs. The nematode density scales in the graphs were divided into classes, starting from $P_{ij}/T_i = 1$ with further class limits 1, 1.41, 2, 2.82 etc. (ratio between

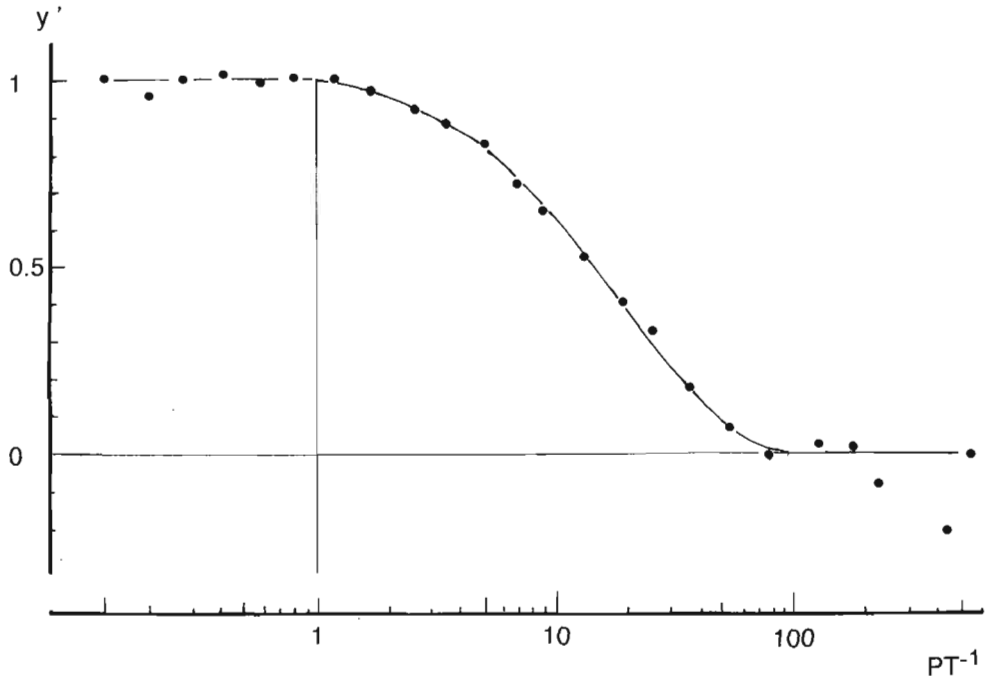

Fig. 4. *Relation between average* (P/T)j (j = *density class) and relative plant weights per experiment* Y'_{ij}, *transformed according to Equation (6). In the graph* $\bar{y}_j$ *and* P/T_j *are indicated as* y' *and* PT^{-1} *respectively.*

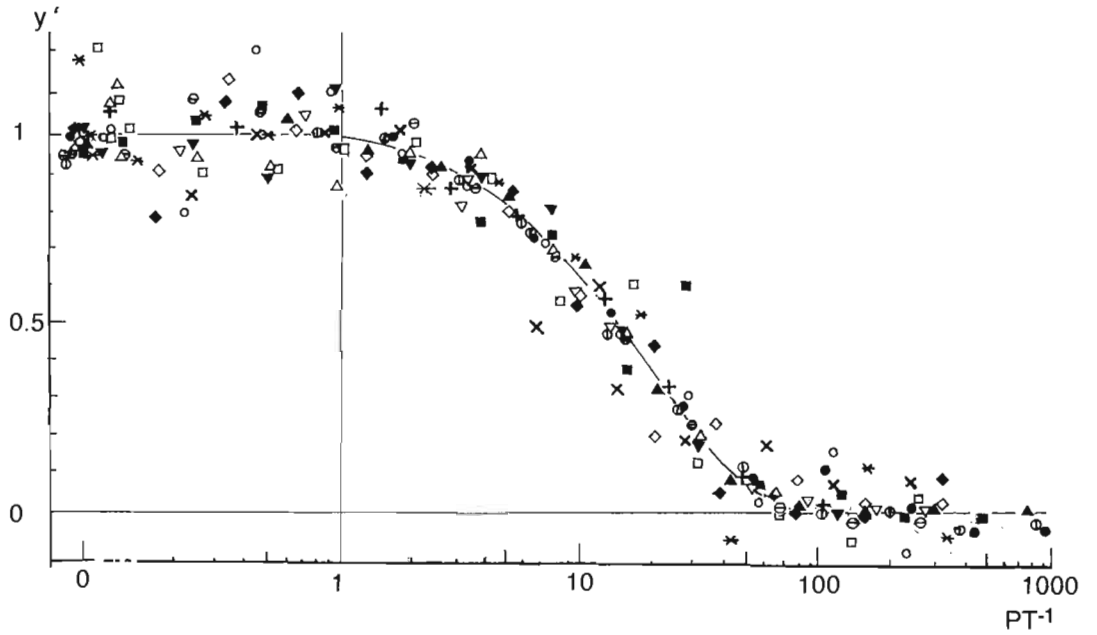

Fig. 5. *Relation between initial density of eggs and second stage juveniles of* Meloidogyne *species* P/T_i *and the relative weight* Y_{ij} *of different plant species some time after sowing or planting. Curve according to Equation (4). References to symbols and associated values of parameters* T *and* m *are summarized in Table 4. In the graph* Y_{ij} *and* P/T_j *are indicated as* y' *and* PT^{-1} *respectively.*

Table 1. *Details of experiments, visualised in Fig. 1, on the relation between nematode density at planting and plant weight at the end of the experiment: nematode plant species; original and new estimates of the tolerance limit* T *and minimum yield* m *according to Equation (3), and the author(s) of the experiments.*

Symbol	Nematode species	Plant species, cultivar	Original publication: *T* nem./g soil	m	Reference	*T* nem./g soil	m
●	*Heterodera avenae*	*Triticum sativum*	-	-	Meagher & Brown (1974)	0.3	0
○	*Meloidogyne incognita*	*Beta vulgaris*	1.1	0.1	Di Vito *et al.* (1981)	1.1	0.1
▽	*Xiphinema index*	*Vitis vinifera*	0.17	0.05	Di Vito *et al.* (1985)	0.15	0.05
▲	*M. incognita*	*Cucumus melo*	0.19	0	Di Vito *et al.* (1983)	0.19	0
▼	"	*Nicotiana tabacum*	2	0	"	2	0
□	"	*Helianthus annuus*	1.85	0.25	Sasanelli & Di Vito (1992)	1.85	0.25
■	"	*Brassica oleracea*	0.5	0.05	"	0.5	0.05
◇	"	*Capsicum annuum* (susceptible)	0.74	0.1	Di Vito (1986)	0.74	0.1
◆	"	*Capsicum annuum* (resistant)	0.74	0.4	"	0.74	0.4
⊕	*M. javanica*	*Oryza sativa*	0.26	0	Di Vito *et al.* (1996)	0.18	0.04
⊖	"	*Oryza glaberrima*	2.68	0	"	2.2	0.035
×	*Tylenchorhynchus dubius*	*Lolium perenne*			Den Toom (1988)	1.6	0.6
⊙	*Longidorus elongatus*	*Fragaria vesca*	0.2[1])	0.3	Seinhorst (1966)	0.09[2])	0.3

1) For $z^T = 0.9$ 2) For $z^T = 0.95$

Table 2. *Details of experiments, visualised in Fig. 2, on the relation between nematode density at planting and plant weight at the end of the experiment: nematode plant species; original and new estimates of the tolerance limit* T *and minimum yield* m *according to Equation (3), and the author(s) of the experiments.*

Symbol	Nematode species	Plant species, cultivar	Original publication: *T* nem./g soil	m	Reference	*T* nem./g soil	m
●	*Heterodera goettingiana*	*Pisum sativum*	3.5	0.3	Di Vito *et al.* (1978)	3.4	0.28
○	*H. avenae*	*Avena sativa*	1.4	0.27	Seinhorst (1981)	1.4	0.27
⊕	"	"	0.85	0.05	"	0.85	0.05
⋆	"	"	0.35	0.6	"	0.35	0.6
+	*Tylenchorhynchus dubius*	*Lolium perenne*	1.8	0.18	"	1.6	0.22
□	*Pratylenchus penetrans*	*Vicia faba*	-	-	unpublished	6.2	0.43
◇	"	*Daucus carota*	-	-	"	1.4	0.49
△	"	*Digitalis purpurea*	-	-	"	5.6	0.16
▲	"	*Malus*	-	-	"	1.5	0.44
■	*H. ciceri*	*Cicer arietinum*	1.34	(av.) 0.26	Greco *et al.* (1993)	1.3	0.16
▼	*H. carotae*	*Daucus carota*	0.8	0	Greco & Brandonisio (1980)	0.7	0
◆	*H. trifolii*	*Trifolium repens*	0.7	0.12	Seinhorst (1981)	0.7	0.12

Table 3. *Details of experiments, visualised in Fig. 3, on the relation between nematode density at planting and plant weight at the end of the experiment: nematode plant species; original and new estimates of the tolerance limit* T *and minimum yield* m *according to Equation (3), and the author(s) of the experiments.*

Symbol	Nematode species	Plant species, cultivar	Original publication			*T* nem./g	m
			T nem./g soil	m	Reference	soil	
●	*Heterodera avenae*	*Avena sativa*	1	0.6	Greco & Brandonisio (1987)	1	0.6
○	*Meloidogyne incognita*	*Solanum melongena*	0.054	0.1	Di Vito *et al.* (1986)	0.054	0.05
△	"	*Coffea arabica*	2.09	0.4	Vovlas & Di Vito (1991)	1.4	0.4
▽	*M. javanica*	"	1.34	0.4	"	1.15	0.4
▲	*M. incognita*	*Lycopersicum esculentum (resistant)*	0.55	0.7	Di Vito *et al.* (1991)	0.5	0.7
□	*M. artiella*	*Cicer arietinum*	0.13	0.1	Di Vito & Greco (1988a)	0.13	0.1
■	*H. trifolii*	*Trifolium repens*	-	-	Hidding *et al.* (1963)	0.8	0
♦	*M. hapla*	"	-	-	"	0.08	0.4
◇	*Pratylenchus penetrans*	*Vicia faba*	-	-	(unpubl.)	1.3	0.43
⊙	*M. javanica*	*Helianthus annuus*	0.45	0	Di Vito *et al.* (1996)	0.25	0.43

Table 4. *Details of experiments, visualised in Fig. 5, on the relation between nematode density at planting and plant weight at the end of the experiment: nematode plant species; original and new estimates of the tolerance limit* T *and minimum yield* m *according to Equation (3), and the author(s) of the experiments.*

Symbol	Nematode species	Plant species, cultivar	Original publication			*T* nem./g	m
			T nem./g soil	m	References	soil	
●	*Meloidogyne incognita*	*Solanum melongena*	0.054	0.05	Di Vito *et al.* (1986)	0.054	0.05
○	"	*Beta vulgaris*	1.1	0.1	Di Vito *et al.* (1981)	1.1	0.1
△	"	*Lycopersicum esculentum* (susceptible)	4	0	"	4	0
▲	"	*Cucumus melo*	0.19	0	Di Vito *et al.* (1983)	0.19	0
▼	"	*Nicotiana tabacum*	2	0	"	2	0
□	"	*Helianthus annuus*	1.85	0.25	Sasanelli & Di Vito (1992)	1.85	0.25
■	"	*Brassica oleracea*	0.5	0.05	"	0.5	0.05
◇	"	*Capsicum annuum* (susceptible)	0.74	0.1	Di Vito (1986)	0.74	0.1
♦	"	*C. annuum* (resistant)	0.74	0.4	"	0.74	0.4
+	"	*Coffea arabica*	2.09	0.4	Vovlas & Di Vito (1991)	1.4	0.4
×	*M. javanica*	"	1.34	0.4	"	1.15	0.4
⊕	"	*Oryza sativa*	0.26	0	Di Vito *et al.* (1996)	0.18	0.04
⊖	"	*Oryza glaberrima*	2.68	0	"	2.2	0.04
*	*M. incognita*	*Lycopersicum esculentum* (resistant)	0.55	0.7	Di Vito *et al.* (1991)	0.5	0.7
▽	*M. artiella*	*Cicer arietinum*	0.13	0.1	Di Vito & Greco (1988)	0.13	0.02

the upper and lower class limits $2^{0.5}$). The same was also done for densities $P_{ij}/T_i < 1$. Average values per nematode density class j of relative plant weights Y_{ij}, in the different experiments according to Equation (6) and of antilog's of average log $(P_i/T_i)_j = (P/T)_j$ (Equation (7)) were calculated and plotted in Fig. 4.

Results and conclusions

Fig. 4 demonstrates an excellent fit of the relation between actual values of Y_j' and average $(P/T)_j$ to the values of y' at these nematode densities according to $y' = 0.95^{P/T-1}$ (Equations [3] and [4]) for average $(P/T)_j = 1$ to 100 and $y' = 1$ for average $(P/T)_j \leq 1$. The poorer fit for average $(P/T)_j > 100$ is probably partly due to Seinhorst's (1981) 'second mechanism of growth reduction' which refers to mechanical damage to the root system at high population densities, the general relation of which is not known. It may, therefore, be concluded that Equations (2), (3), and (4) give a proper description, during the first growing season after sowing or planting, of the relation between relative weight and population densities from 0 to 100 T for a wide range of nematode and plant species (Figs 1, 2, 3), thus confirming Seinhorst's (1979, 1986) theory about growth reduction for these nematode and plant species. Apparently, deviations of actual relative plant weights in separate experiments from those according to Equation (3) with properly estimated values of T and m are generally due to experimental error, although some deviations at population densities > 30 T are most probably caused by Seinhorst's 'second mechanism of growth reduction'.

The confirmation of the constancy of the value of $z^T = 0.95$ emphasizes the need to investigate the mechanism by which the plant counteracts the effect on growth of nematode densities up to T.

Experiments with *Meloidogyne* species

A good fit of a curve according to Equation (4) to the relation between initial nematode density and relative plant weights was also obtained for almost all experiments with *Meloidogyne* species (Fig. 5, data and references in Table 4). A deviation of the relation between initial nematode density and total plant weight several months after planting from the relation estimated according to Equation (4), due to a rapid increase of population density of these species, was described on theoretical grounds by Seinhorst (1995*b*) and illustrated in his Fig. 4. This deviation could only be derived from the data in one experiment, *viz.*, the description by Di Vito *et al.* (1991) of the relation between initial density of second stage juveniles of *M. incognita* and relative weights of the fruits of susceptible tomatoes. An alternative interpretation of the data is given in Fig. 6. The interpretation by a single curve according to Equation (3) in Fig. 1 of Di Vito *et al.* (1991) is replaced by one, that is the product of a reduction by :

1) The initial nematode population according to Equation (3) with $T_1 = 0.43$ second stage juveniles/g soil and $m_1 = 0.7$, as for the resistant tomatoes, with no or only a small second generation.

2) The second generation produced by this initial population, again according to Equation (3) but with T_2 at the initial density of approximately 0.065 second stage juveniles/g soil and $m_2 = 0.85$ (Seinhorst 1995*a*, *b*)

3) The 'second mechanism of growth reduction' (Seinhorst, 1981) at $P > 4$ second stage juveniles/g soil ($62T_2$).

The curve drawn in Fig. 6 for the reduction in point 3) is arbitrary because no general relation between nematode density and the effect of the 'second mechanism' is known. The large values of m possibly resulted from the age of the plants at the start of the experiment, which can be interpreted as a 'delay of attack' resulting in an increase of m (Seinhorst, 1995*a*). The first two reductions of fruit yield (both due to the 'first mechanism of growth reduction' which causes growth retardation) are proportional to the reduction of total plant weight. Most probably the largest densities of the first generation already reduced the relative plant weight to less than 0.7 by the 'second mechanism of growth reduction' and plants died off after attack by the second generation. The new interpretation of the data results in a much smaller estimated experimental error than the original interpretation. An acceptable fit is still obtained for $m_2 = 0.9$ and $m_1 = 0.5$, therefore assuming that m_1 is smaller than for resistant plants. The economic differ-

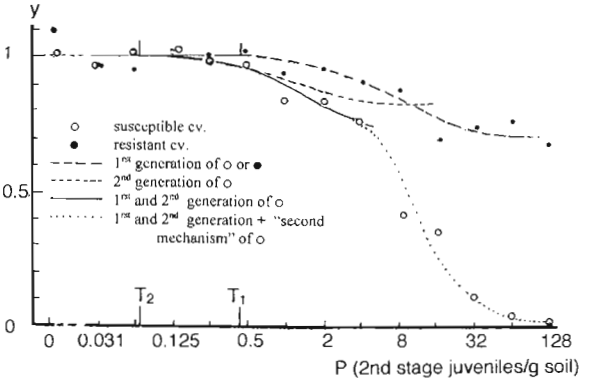

Fig. 6. *Re-interpretation of data from experiments on the relation between initial population density of 2nd stage juveniles of* Meloidogyne incognita *and fruit yield of susceptible and resistant tomato plants by Di Vito* et al. *(1991).* y = *fruit weight divided by estimated fruit weight in the absence of nematodes. Curves are according to Equation (3), except the (arbitrary) dotted line.*

ence between the interpretation by Di Vito *et al.* (1991) and the interpretation of Fig. 6 is a crop loss of 5% at $P = 0.43$ second stage juveniles/g soil according to the latter and no loss according to the former interpretation.

The pattern described above could be expected to fit also to the results of the experiments with *M. incognita* on susceptible and resistant tomatoes of Di Vito *et al.* (1983). However, there are too few observations at very small initial egg densities to estimate the weight of susceptible tomatoes in the absence of nematodes. If there was any effect of the second and later generations on relative plant weight of the susceptible tomatoes it cannot be distinguished from experimental error and would have resulted in a value of m of at least 0.9.

The four relative weights at zero to one egg/g soil of susceptible tomatoes in the experiment of Di Vito *et al.* (1981) varied from 0.8 to 1.2 and did not supply any information on possible effects of the second and later generations on plant weight.

Contrary to what might be expected, root knot nematodes did not affect plant weight in the experiments of Table 4, and Figs 5 and 7 in the same way as

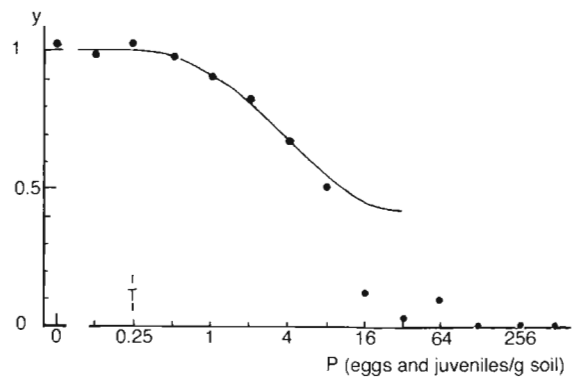

Fig. 7. *Re-interpretation of data from experiments on the relation between initial population density of 2nd stage juveniles of* Meloidogyne javanica *and top weight of sun flower (*Helianthus annuus *by Di Vito* et al., *1996).* y = *top weight divided by estimated top weight in the absence of nematodes. Curve according to Equation (3).*

in Fig. 6. The data fit closely to a relation according to Equation (4). There is no indication of an effect of a second generation with a much smaller value of T than for a first generation and a fairly large value of m (0.7 to 0.9). Moreover, $T = 0.2$ or more eggs/ g soil in eleven of the fifteen experiments with susceptible plants and, therefore, about the same as for resistant plants without a sizeable second generation. The only possible explanation is that, by the time reproduction had increased population density considerably, the plants had become insensitive to the new nematodes, presumably because for these nematodes, m increased until it was too close to 1. Seinhorst's (1995*a*, *b*) experiments with oat cyst nematode on oats show that a very small m for a first generation attacking shortly after sowing can go together with a very large m for a later generation (or after a considerable delay of the start of the first attack). This might have occurred in the experiments mentioned in Table 4 and Fig. 5. If also, as with the tomatoes, m for the growth reduction caused by the 'first mechanism' was large, then only the 'second mechanism' (reducing the capacity of the plant to take up water, with as a result increase of dry matter content and ultimately wilting and death; Seinhorst, 1981) remains as a cause of small plant weights. However, then the relation between nematode density and plant weight can be expected to depart from the relation according to Equations (2), (3), and (4), which is not the case in the experiments summarized in Table 4. Apart from the experiment of Fig. 6, an effect of the 'second mechanism' could only be derived from the relation between initial egg density of *M. javanica* and the total plant weight of sunflower (*Helianthus annuus*) in an experiment by Di Vito *et al.* (1996). In Fig. 7 the data from this experiment were re-interpreted, resulting in $T = 0.25$ eggs and juveniles/g soil and $m = 0.43$ for the reduction of plant weight by the 'first mechanism' according to Equations (2), (3), and (4) with an additional reduction of plant weight by the 'second mechanism' at densities > eight eggs and juveniles/g soil, whereas there is no evidence of weight reduction by the second and later generations. Experimental error is considerably smaller according to the new interpretation than according to the original one, fitting a single curve according to Equation (3) to the data.

It seems improbable, that there is either no effect of the 'second mechanism' or that all reduction at more than about four eggs/g soil is due to it without departure of the relation between initial nematode density and plant weight from one according to Equations (2), (3), and (4). A third mechanism that could affect the relation between nematode density and plant weight is growth stimulation by small nematode densities. However, it could hardly be expected to exceed a few percent weight increase, too little to compensate a sizeable weight decrease by a second generation. Average relative plant weights per class of nematode densities, calculated as for Figs 1 to 3, did not reveal any effect of growth stimulation.

Although the relation between root knot nematode density and plant weight in the different experiments is not understood, the available information could be considered sufficient for agronomic purposes. This means that one may assume that the results of these

experiments are reproducible - apart from a correction for incapacitation in some experiments of a large percentage of the inoculum by the hypochloride treatments (Di Vito *et al.*, 1986, 1991) - and that these results will also apply in the field. But then it is assumed without explicit proof, that, from about three weeks after sowing or planting, plants (except egg plant; see Table 4) become insensitive to growth reduction by the 'first mechanism' resulting from attack by root knot juveniles of the second and later generations but remain sensitive to such growth reduction by juveniles of the original population still present in the soil. This is the paradox derived from the data of an experiment with repeated inoculation with *Heterodera avenae* eggs of soil with oat plants (Seinhorst, 1995*b*). The problem is certainly worth further investigation for both theoretical and practical reasons (effect of delay of attack that is not explained by the increased size of the plant).

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Observations on *Protodorylaimus dalmassoi* (Loof, 1985) Andrássy, 1988 (Nematoda: Dorylaimoidea)

Reyes PEÑA SANTIAGO*, Domingo JIMÉNEZ GUIRADO**, and Joaquín ABOLAFIA*

**Departamento de Biología Animal, Vegetal y Ecología, Universidad de Jaén, Paraje "Las Lagunillas" s/n, Edificio nº 5, 23071-Jaén, Spain, and **Departamento de Biología Animal (Sección Zoología), Facultad de Ciencias, Universidad de Córdoba, Avenida San Alberto Magno s/n, 14004-Córdoba, Spain.*

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Summary – *Protodorylaimus dalmassoi* (Loof, 1985) Andrássy, 1988 is described and illustrated from material collected in two localities of peninsular Spain. Its relationships with species of the genera *Prodorylaimus* Andrássy, 1959 and *Oxydiroides* Altherr, 1972 is briefly discussed and its inclusion in *Protodorylaimus* Andrássy, 1988 is supported. © Orstom/Elsevier, Paris

Résumé – ***Observations sur* Protodorylaimus dalmassoi *(Loof, 1985) Andrássy, 1988 (Nematoda: Dorylaimoidea)*** *Protodorylaimus dalmassoi* (Loof, 1985) Andrássy, 1988 est décrit et figuré à partir de materiel collecté dans deux localités d'Espagne péninsulaire. Ses relations avec les espèces des genres *Prodorylaimus* Andrássy, 1959 et *Oxydiroides* Altherr, 1972 sont brièvement discutées et son appartenance au genre *Protodorylaimus* Andrássy, 1988 confirmée. © Orstom/Elsevier, Paris

Keywords : nematode, *Oxydiroides, Prodorylaimus, Protodorylaimus dalmassoi,* taxonomy.

Loof (1985) described *Prodorylaimus dalmassoi*, an atypical species of the genus, from pastures in Bois de Vergnes, France. Later, the taxonomic position of this species became a subject of controversy: Andrássy (1988) proposed it as a type species of the new genus *Protodorylaimus*, Jiménez Guirado (1990) studied a few specimens from southern Spain and transferred the species to *Oxydiroides* Altherr, 1972, and, very recently, Loof (1996) included it in a key for females of the genus *Prodorylaimus* but without questioning the validity of *Protodorylaimus*.

One of the authors (DJG) collected additional material of the species in a locality from the Cantabria region, northern Iberian Peninsula. The study of the Spanish specimens has allowed us to complete the available information on the species and to discuss its taxonomy.

Nematodes were extracted from soils or sediments by Baermann's method or Flegg's technique somewhat modified, killed by heat, fixed in 4% formaldehyde, and mounted in anhydrous glycerin according to Seinhorst (1962) or Siddiqi (1964) techniques.

Protodorylaimus dalmassoi (Loof, 1985) Andrássy, 1988

(Figs 1, 2)

MEASUREMENTS

See Table 1.

DESCRIPTION

Female: Very large and slender nematodes, 4.0-5.6 mm long. Body cylindrical, tapering towards both extremities but more so towards the posterior end. Habitus variable after fixation, more curved ventrad in the posterior body region. Outer cuticle layer thin throughout the body and with very fine transverse striations. Inner cuticle layer wider than the outer one and somewhat thickened on tail. Lateral chord occupying one-third of the midbody diameter, its margins irregular at level of the vagina. Lateral pores obscure. Lip region rounded, continuous with body or offset by a very weak depression, 2.0-2.3 times as wide as high and about one-fourth of the body diameter at neck base. Labial and cephalic papillae well visible but hardly noticeable on the head outline. Cheilostoma cylindrical, 2.7 times as long as wide; its walls somewhat thickened and weakly sclerotized in the perioral area. Odontostyle rather short, as long (0.9-1.0 times) as the lip region width, and 6-7 times as long as wide; aperture about two-fifths (42-43%) of the total neck length. Odontophore rod-like, a little longer than the odontostyle. Guiding ring apparently double. Pharynx consisting of a slender but muscular anterior part expending gradually into the basal bulb. Pharyngeal bulb cylindrical, 12-14 times as long as wide and occupying about two-fifths of the body diameter at neck base and 49-58% of the total neck length. Pharyngeal gland nuclei and outlets often well visible in the four specimens examined: DO = 49-52%, S_1N =

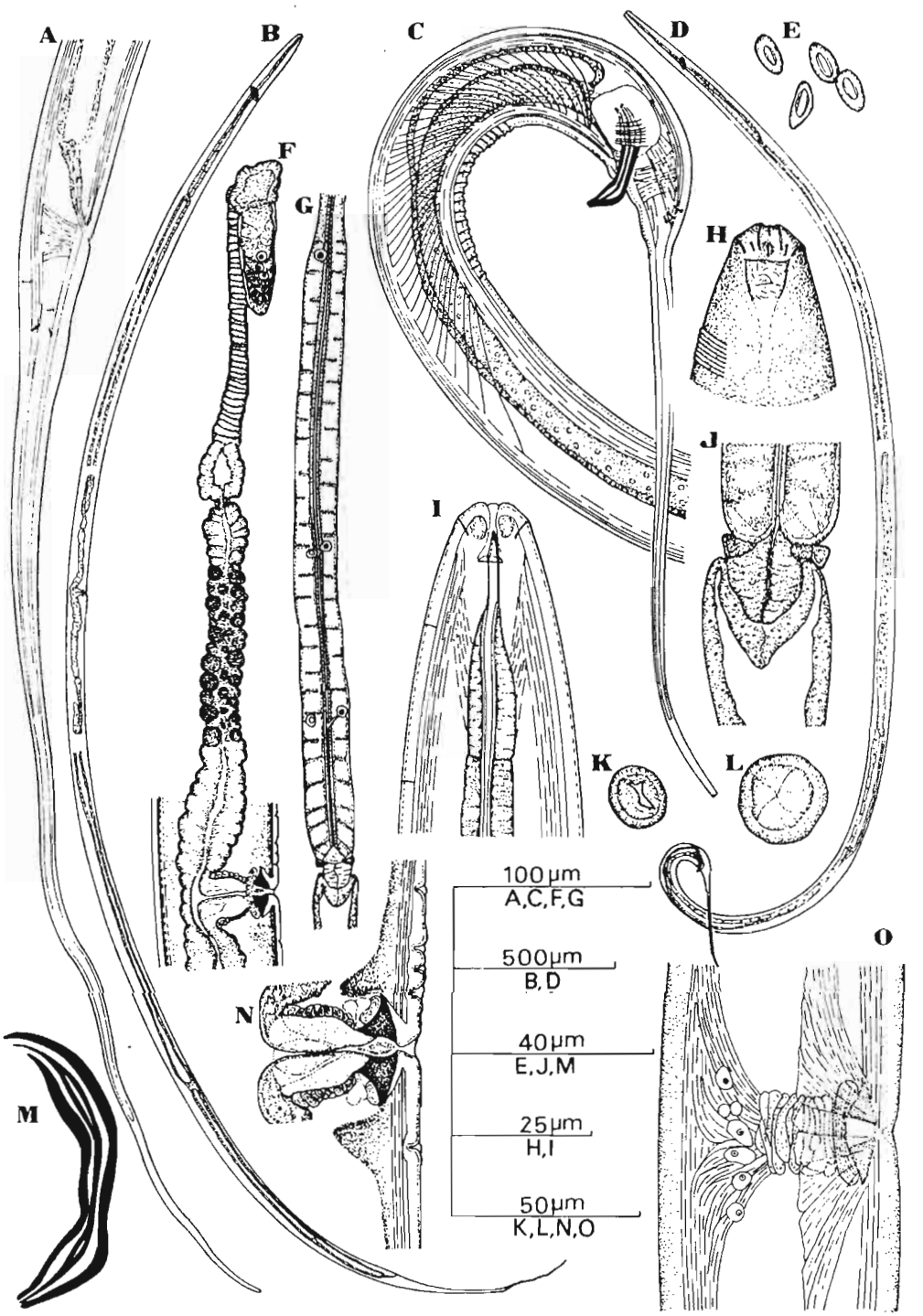

Fig. 1. Protodorylaimus dalmassoi *(Loof, 1985) Andrássy, 1988. A: Female tail; B: Entire female; C: Male posterior body region; D: Entire male; E: Spermatozoa; F: Female anterior genital branch; G: Pharyngeal bulb; H: Lip region in surface view; I: Same in median view; J: Pharyngeal bulb base and cardia; K: Vulva in ventral view; L: Vagina in ventral view; M: Spicule; N: Vagina in lateral view; O: Same in submedian lateral view.*

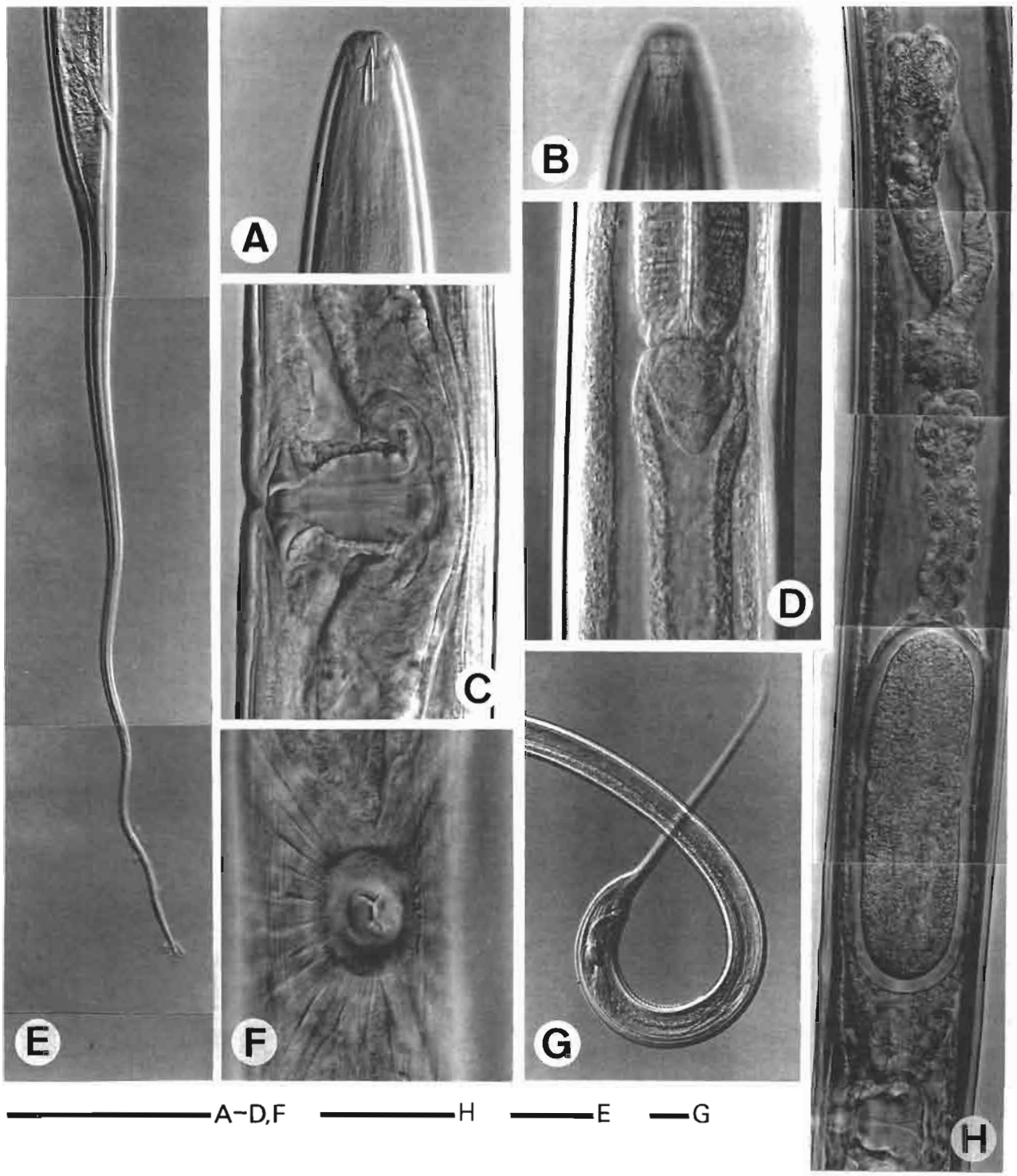

Fig. 2. Protodorylaimus dalmassoi *(Loof, 1985) Andrássy, 1988. A: Lip region in median view; B: Same in surface view; C: Vagina; D: Cardia; E: Female tail; F: Vulva in ventral view; G: Male posterior body region; H: Female anterior genital branch (Scale bars: 50 µm).*

Table 1. *Measurements and diagnostic features of* Protodorylaimus dalmassoi *(Loof, 1985) Andrássy, 1988 (All measurements in μm except L in mm).*

	Vejo stream Vega de Liébana Cantabria, Spain	Yeguas river Cardeña Córdoba, Spain		Valdeseñor stream Saldaña Palencia, Spain (Jiménez Guirado, 1990)	Pasture Bois de Vergnes France (Loof, 1985)		
	Female	Female	Male	Female	Female (Holotype)	Female	Males
n	10	4	1	2	1	4	2
L	4.9±0.4 (3.9-5.5)	(4.3-5.4)	4.1	(5.01-5.04)	4.7	(4.03-4.7)	4.5, 4.7
a	82.4±7.8 (69.5-92.7)	(58.3-74.5)	68.6	(70.4-72.5)	80.0	(73.0-81.0)	65.0, 80.0
b	7.9±0.4 (6.9-8.5)	(7.0-8.8)	7.1	(7.7-7.8)	7.9	(6.8-7.9)	7.8, 8.1
c	9.5±0.7 (8.6-11.4)	(7.0-9.7)	12.6	(7.5-8.0)	9.4	(9.4-14.1)	10.8, 13.9
c'	14.7±1.3 (11.7-16.1)	(11.3-17.6)	9.8	(15.3-16.8)	14.3	(12.2-14.3)	8.5, 10.1
V	40.2±1.8 (38.0-43.4)	(34.6-38.0)	-	(35.7-36.6)	38.0	(38.0-41.0)	-
G1/T1	8.3±0.5 (7.9-9.6)	(10.0-13.0)	?	9.7			
G2/T2	9.2±0.4 (8.7-10.1)	(11.0-13.9)	?	11.0			
Lip region:							
– diam.	(10.5-14.5)	15.0	12.5	15.0			
– height	(4.0-5.5)	?	4.0				
Amphid aperture	(6.3-8.4)	?	8.0				
Odontostyle	13.8±0.6 (11.5-13.5)	(12.5-13.5)	2.1	(13.5-14.5)	12.0	(11.0-12.0)	11.0, 12.0
Odontophore	15.7±1.5 (13.0-18.0)	?	14.0	?	14.0	(13.0-15.0)	14.0
Guiding ring	(8.0-10.5)	?	9.0	?			
Nerve ring-ant. end	184±8.3 (174-203)	?	149	?			
Neck length	601±30.6 (551-640)	?	561	?			
Pharyngeal bulb length	315±24.5 (273-353)	?	346	?			
Cardia: width	(12.0-17.0)	?	?	?			
length	(8.0-19.5)	?	?	?			

End of Table 1 next page.

Table 1. (continued)

Cuticle: head	(2.0-3.5)	(2.5-3.5)	3.5	2.5			
– midbody	(2.0-5.0)	?	3.5	?			
– anus	(3.0-7.5)	?	4.0	?			
Body diam.: neck base	(54.0-64.0)	?	?	?			
– midbody	(55.0-71.0)	?	60.0	?			
– anus	36.0	(40.0-42.0)	33.5	(17.5-18.0)			
Lateral chord	(11.0-24.0)	?	14.5	?			
Ant. ovary/testes	99.9±16.5 (74.0-123)	?	?	?			
Ant. genital branch	368±89.2 (138-440)	?	?	?			
Post. ovary/testes	98.2±19.1 (77.0-129)	?	?	?			
Post. genital branch	388±89.6 (368-486)	?	?	?			
Vagina: length	(29.0-40.5)	?	-	?			
Vulva-ant. end	1986±167 (1628-2202)	(954-1494)	-	(1788-1844)			
Prerectum	867±118 (648-1063)	(691-815)	918	(716-740)			
Rectum/cloaca	43.0±2.5 (38.0-46.0)	(34.5-39.7)	46.5	(40.0-41.0)			
Tail	520±52.6 (411-574)	(450-736)	327	(625-664)	504	(404-504)	317, 442
Spicules	-	-	73.5	-		-	76.0, 78.0
Lat. guid. piece	-	-	13.5	-		-	
Ventr. suppl	-	-	20	-		-	21, 22
Spermatozoa	-	-	9.0	-			
Tail hyal. part	(92.0-145)	?	?	?			

73-77%, S_2N = 86-88%, DO-S_1N = 23-27%, DO-S_2N = 34-38%; S_1N-S_2N = 11-14%. Nerve ring located at one-third (29-33%) of the total neck length. Junction between pharyngeal bulb and cardia surrounded by a weak ring-like structure surrounding the bulb base. Cardia rounded conoid, as long as wide; intestine tissue involving it somewhat behind the base of the pharyngeal bulb and forming a rounded-conoid to conoid extension 12-16 µm long (combined length with the cardia 22-30 µm) projecting into the intestine lumen. Three to five separated cell masses (probably pseudocoelomocytes) well visible at different sites along the body. Genital system didelphic-amphidelphic. Ovaries relatively short, not reaching the oviduct-uterus junction; oocytes not very numerous, first in two rows, then in a single row. Oviduct joining the ovary subterminally and consisting of a slender part with prismatic cells and a moderately developed *pars dilatata*. Conspicuous sphincter present between oviduct and uterus. Uterus a relatively wide tube, 2.8-4.1 times the corresponding body diameter long; its portion adjacent to the sphincter somewhat dilated (*pars dilatata uteri*), followed by an intermediate portion with a rather creased wall and narrow lumen, and by distal portion with less creased wall and clear lumen. Vagina extending inwards 2/3 - 3/4 of the corresponding body diameter; *pars distalis vaginae* 18-22 × 24-30 µm or 1.3-1.4 times as long as wide; its walls slightly sigmoid, convergent proximally and divergent distally, and surrounded by weak musculature; *pars refringens vaginae* with two well separated peculiar-shaped (triangular) sclerotizations measuring 6 × 8 µm and with a combined width of 26-28 µm; *pars proximalis vaginae* 6 µm long. Several cells visible in submedian view in subdorsal position at level of the vagina. Vulva longitudinal. Cuticle with abundant irregularities near the

vulva. Uterine egg 44 × 140 µm (3.2 times as long as wide). Prerectum very long, 22.5-24 times the anal body diameter. Rectum a little longer (1.2-1.3 times) than the anal body diameter. Tail long, filiform, tapering first rather abruptly and then very gradually to the finely rounded tip; hyaline terminal part 88-92 µm. Two pairs of caudal pores, one subdorsal, the other lateral.

Male: General morphology similar to female but with posterior part of the body strongly curved ventrad and odontostyle slightly longer (1.2 times) than the lip region diameter. Prerectum 27.1 anal body diameters long. Genital system diorchic with opposed testes. In addition to the adanal pair, twenty contiguous ventromedian supplements present beginning outside the spicules range. Spicules protruding (n=1), somewhat curved ventrad and about 1.5 anal body diameter long. Lateral guiding pieces not observed. Tail and caudal pores as in female.

REMARKS

P. dalmassoi has been collected in two localities in northern Spain: Vejo stream, Vega de Liébana, Cantabria, and Valdeseñor stream, Saldaña, province of Palencia; and one site in southern Spain: Yeguas river, Cardeña, province of Córdoba.

The material studied is very similar to the type population, with only small differences (odontostyle slightly longer, irregularities of the cuticle near the vulva) which are interpreted as intraspecific variability.

Loof (1985) described this species and recently (1996) included it in the species list of *Prodorylaimus*. This genus, as understood by Loof (1996), is quite heterogeneous and it certainly includes several morphological patterns whose evolutionary origin could not possibly be a very recent common ancestor. In this sense, it seems evident that *P. dalmassoi* is an atypical species within the genus: its very slender body, peculiar lip region (resembling other taxa as *Dorylaimoides* or some leptonchid and belondirid genera), very short odontostyle, and excessively long prerectum constitute a series of peculiar traits which could well belong to one of those morphological patterns. On the other hand, *P. dalmassoi* shares (*cf.* Jiménez Guirado, 1990) some important features with species of *Oxydiroides* (very slender body, shape of the lip region, short odontostyle), but it differs from them in other characters with higher taxonomic value (longer pharyngeal bulb, heavily sclerotized vagina, very long prerectum, and male supplements numerous and contiguous). In our opinion, *P. dalmassoi* represents a morphological pattern well differentiated from the species of both *Prodorylaimus* and *Oxydiroides* and it must be classified in a separate genus. Therefore, we support Andrássy's (1988) proposal of the new genus *Protodorylaimus* to accommodate this species (as type species of the genus) together with *Protodorylaimus kazakhstanicus* (Sagitov, 1973) Andrássy, 1988.

Acknowledgments

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The *Xiphinema americanum*-group (Nematoda: Longidoridae). 2. Observations on *Xiphinema brevicollum* Lordello & da Costa, 1961 and comments on the group

Michel LUC*, August COOMANS**, Pieter A.A. LOOF*** and Pierre BAUJARD*

** Muséum National d'Histoire Naturelle, Laboratoire de Biologie Parasitaire, Protistologie, Helminthologie, 61 rue Buffon, 75005 Paris, France,*

*** Instituut voor Dierkunde, University of Ghent, Ledeganckstraat 35, Gent 9000, Belgium, and*

**** Department of Nematology, Agricultural University, P.O. Box 8123, 6700 ES Wageningen, The Netherlands.*

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Summary – This article begins with a critical review of the article of Lamberti *et al.* (1992) with descriptions of *X. brevicollum*, *X. diffusum*, and three new species (*X. parvum*, *X. pseudoguirani*, *X. taylori*), all closely related to each other. After studying type specimens of these five species and paratypes of *X. incognitum* and *X. sheri*, the present authors propose *X. diffusum*, *X. incognitum*, *X. parvum*, *X. pseudoguirani*, *X. sheri*, and *X. taylori* as junior synonyms of *X. brevicollum*. The second part of the article includes a detailed characterization of the *Xiphinema americanum*-group and a list of its species. *X. pachydermum*, *X. brevisicum*, *X. longistilum*, *X. mesostilum*, and *X. microstilum* are excluded from the *X. americanum*-group. © Orstom/Elsevier, Paris

Résumé – ***Le groupe* Xiphinema americanum *(Nematoda: Longidoridae). 2. Observations sur* Xiphinema brevicollum *Lordello & da Costa, 1961 et commentaires sur le groupe*** – La première partie de cet article est consacrée à un examen critique de la publication de Lamberti *et al.* (1992) traitant de *X. brevicollum* et *X. diffusum* et décrivant trois nouvelles espèces (*X. parvum*, *X. pseudoguirani*, *X. taylori*), toutes très proches les unes des autres. Les observations faites sur des spécimens types de ces cinq espèces et sur des paratypes de *X. incognitum* and *X. sheri*, amènent les auteurs à proposer *X. diffusum*, *X. incognitum*, *X. parvum*, *X. pseudoguirani*, *X. sheri* et *X. taylori* comme synonymes mineurs de *X. brevicollum*. Dans la seconde partie, les auteurs caractérisent le groupe *Xiphinema americanum* et donnent la liste des espèces s'y rapportant. Des arguments sont fournis pour exclure *X. pachydermum*, *X. brevisicum*, *X. longistilum*, *X. mesostilum* et *X. microstilum* du groupe *X. americanum*. © Orstom/Elsevier, Paris

Keywords: Longidoridae, nematode, *Xiphinema americanum*-group, *X. brevicollum*.

Until 1979, what was called for the first time by Tarjan (1969) the *Xiphinema americanum*-group contained only eight nominal species, the four most frequently recorded being: *i*) *X. americanum*: world-wide, mostly temperate, *ii*) *X. brevicollum* *: world-wide, mostly tropical, *iii*) *X. opisthohysterum*: tropical, mostly in India, East Asia, and *iv*) *X. pachtaicum*, often cited under the name of its junior synonym, *X. mediterraneum*: North-West Asia and Mediterranean region.

The other four species, more rarely reported, were two species from India (*X. inaequale*, *X. lambertii*), one from Southern Europe (*X. rivesi*) and one from Mauritius (*X. silvaticum*).

* The specific epithete is modified from *brevicolle* (= short neck) to *brevicollum* to conform to Latin grammar. *Collum*, *i*, neuter substantive must remain as such; *'colle'* does not exist in Latin.

The four main species were easily differentiated from each other, which made the situation deceptively simple. Actually it was not very satisfactory and several nematologists noted the extreme variability recorded in *X. americanum* and suspected that it might include several species (Lima, 1965, 1968; Tarjan, 1969, 1973; Heyns, 1974). After studying a large number of populations from world-wide origin, Lamberti and Bleve-Zacheo (1979) restricted the definition of *X. americanum sensu stricto* and described fifteen new species to accomodate the variability observed in *X. americanum sensu lato*. This publication represented considerable work, and all the specialists in longidorid taxonomy should consider such a task with respect. However, the new situation soon showed itself to be less satisfactory than expected. No key was included in the 1979 article to identify the 23 * species in the group. Later, partial keys were published

by Lamberti and Agostinelli (in Anon., 1984) and Ebsary *et al.* (1989) dealing with six and seventeen species, respectively. A key to all (39) species pertaining to the group was finally proposed by Lamberti and Carone (1992); this key has been discussed in detail earlier (Loof *et al.*, 1993). However, the diagnoses of the species described by Lamberti and Bleve-Zacheo (1979) are inappropriate, trying to unravel the relationships they described between the different species is like trying to get out of the Hampton Court maze, and the illustrations are too restricted (only one drawing of head and one of tail for each species) to give information on the intraspecific variability.

Consequently, controversies exist concerning the nature of the 'true' *X. americanum* and specific identification is very difficult in the group which creates conflicts in published identification. As an example, three populations from Peru were identified as *X. floridae, X. peruvianum,* and *X. inaequale* by Lamberti *et al.* (1987) but the first two were identified as *X. californicum* and the third one as *X. rivesi* by Alkemade and Loof (1990).

So, it is evident that in its present state the *X. americanum*-group is composed of a number of species of which the accurate determination remains ambiguous, if not impossible.

As is often the case when specific identification within a genus is difficult, several nematologists chose to describe as new species the populations that could not be easily attributed to one of the described species. This is certainly the case for several species described after the publication of Lamberti and Bleve-Zacheo (1979).

To clarify the relationships of the species in the group, various authors used different methods, based either on mathematical analysis of measured data or on molecular techniques. Both approaches are briefly discussed below.

Lamberti and Ciancio (1993) published a hierarchical cluster analysis of morphometrics concerning 49 populations pertaining to the 39 species they recognized in the group. This analysis resulted in a dendrogram of similarity leading the authors to divide the *X. americanum*-group into five subgroups: *X. brevicollum*-subgroup, *X. americanum*-subgroup, *X. taylori*-subgroup, *X. pachtaicum*-subgroup, and *X. lambertii*-subgroup. Examinating this dendrogram, and more specifically the species represented by more than one population, one can note that some populations are truly close to each other (average distance between cluster [a.d.cl.] less than 0.3) for some species (*X. californicum*, two populations; *X. pacificum*, two populations), but that others are farther apart (*X. sheri*: a.d.cl. = 0.42; *X. diffusum*: a.d.cl. = 0.45; *X. thornei*: a.d.cl. = 0.6; *X. americanum* : a.d.cl. = 0.7). The widest scattering of populations is seen in *X. brevicollum* (a.d.cl. = about 1) for which two of the three populations are placed in the *X. brevicollum*-subgroup and the third one in the *X. americanum*-subgroup, a rather surprising statement. There is no doubt that if more numerous populations had been used, the validity of these subgroups would have been more seriously affected.

This attempt clearly demonstrates that the taxonomic situation in the *X. americanum*-group, *i.e.*, the relationship between the species cannot be established univocally using a metric approach only.

Molecular techniques, and specifically restriction fragment length polymorphism and internal transcriber spacers of ribosomal DNA, have been applied to clarify the relationships between some species of the group (Vrain *et al.*, 1992; Vrain, 1993). The populations studied originated from Canada and USA and were attributed to *X. americanum*, *X. bricolense*, *X. pacificum*, and *X. rivesi*. In some cases, 'mixed populations' of two of these species were present. The dendrogram of similarity resulting from this study does not shed much light on the relationships between the species studied. Considering only the true, *i.e.*, monospecific, populations, it can be observed that the seven populations of *X. americanum* are distributed over the whole dendrogram, occupying its two extreme lines. The four populations of *X. rivesi* form two groups of two populations each, separated by three populations of *X. americanum*. Finally, *X. bricolense* and *X. pacificum*, each represented by a single population, are both situated in between *X. americanum* populations.

Therefore, it can be said that the above-mentioned molecular techniques are able to separate populations of related species pertaining to the *X. americanum*-group, but not the species themselves. These techniques have proven their value in other groups, and their potential should not be underestimated provided they are used as a complementary approach, not a substitute to 'classical' approaches.

For these reasons, the authors decided to return to basics, *i.e.*, look at type specimens of closely related species, and see how they correspond or differ. This has been for many years the basis of systematics not without results. According to this pragmatic approach, those species in the group having no or very little significant differences would be placed into a single species.

* One of these species (*X. variabile* Heyns, 1966) was later (Loof & Luc, 1990) excluded from the *X. americanum*-group; however, Brown and Halbrendt (1997) due to overall resemblance consider that species to belong in the group, but the exclusion is maintained here. *X. silvaticum* was not included by Lamberti and Bleve-Zacheo (1979), although the reference to its description was given in the reference list.

Besides general remarks on the *X. americanum*-group and a revised definition of the group, the present article constitutes a limited application of this pragmatic approach, dealing only with *X. brevicollum* and some related species. But it also analyses the system that resulted in the increase in number of species to comply with two *a priori* opinions: *i*) the limited distribution of the majority of species; *ii*) the relative stability of the majority of the morphometric characters.

The present article is mostly based on the publication by Lamberti *et al.* (1992) in which *X. brevicollum* is redescribed from topotypes and compared to *X. diffusum* and three new species close to these two species were described.

Through the courtesy of Dr L.C.C.B. Ferraz, we were able to obtain topotypes of *X. brevicollum*. They are studied here, as well as paratypes of the other species treated by Lamberti *et al.* (1992).

X. brevicollum and related species

Comment on the publication of Lamberti *et al.* (1992) on *X. brevicollum* and related species

In that publication, the authors redescribed *X. brevicollum* from a topotype population and studied 25 populations pertaining to or related to that species. They separated these populations into *X. brevicollum*, *X. diffusum* and three new species: *X. parvum*, *X. pseudoguirani*, and *X. taylori*.

Geographical distribution of species

Lamberti's school puts a particularly strong emphasis on the geographical distribution of species, even using it as a character to separate some of them.

This is particularly true in the cited paper. Whereas *X. diffusum* is recognized as a cosmopolitan species, *X. brevicollum* is said to be restricted to Brazil and (doubtfully) Peru. *X. taylori* is said to be an European species and "it is likely that all previous records of *X. brevicollum* from Italy and other European countries should be referred to this species". This constitutes a rather surprising *a priori* statement. *X. parvum* and *X. pseudoguirani* * do not count as they are represented in the cited paper by one small population each, from Jamaica and Madagascar, respectively. The importance attached to geographical distribution is so great that a population from Viçosa, Brazil (pop. X) is said to pertain to *X. brevicollum* although in the dendrogram (Fig. 1 from Lamberti *et al.*, 1992) it is situated exactly on the same line as pop. M of *X. diffusum*

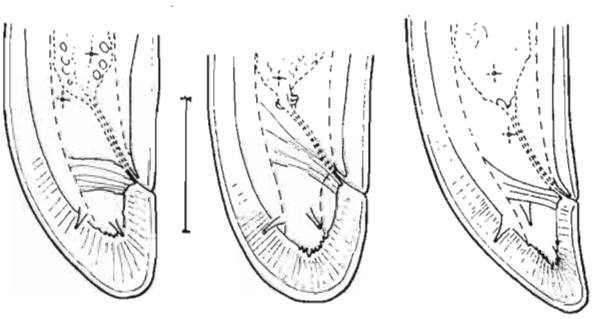

Fig 1. *Variability of tail shape in* Xiphinema silvaticum *(Bar = 30 µm; from Luc & Williams, 1978)*

(from Mataven Otai, Easter Island). Similarly, pop. O, placed in the dendrogram between populations X (*X. brevicollum*), M (*X. diffusum*) and L (*X. diffusum*) is said to pertain to *X. taylori* because it was sampled in Bulgaria. Reasons given in the text are that lip region and tail shapes prevent populations X and O from being considered as *X. diffusum*. Perhaps this is true, but such an argument casts doubts on the validity of the dendrogram, based on measured characters.

The world distribution of *Xiphinema* species is far from being fully investigated, but many species appear to have a wide distribution, and cases in which the distribution apparently does not follow any logic are not rare: why is *X. italiae* Meyl, 1953, apparently a Mediterranean species most often associated with vine, found in the 'bush' in South Africa or under natural cover on Aldabra Atoll? Why is *X. hygrophilum* Southey & Luc, 1974, present in Ivory Coast and Congo (Brazzaville) *, also found on Anjouan (one of the Comoros Islands) and on vegetation close to a spring in Israel? These are extreme examples, but several species are common to West Africa and the Caribbean area, to Pacific islands and South America, to the USA and Japan, etc. It would be very surprising if many species of the *X. americanum*-group had, on the contrary, a restricted area of distribution.

Intraspecific variability

A second common practice of Lamberti's school is to neglect intraspecific variability, at least concerning lip area and (particularly) tail shape (variability was considered for measured characters). As a consequence, only one drawing of a tail and one of an anterior end was published for each species. It would be very surprising if, contrary to the great majority of *Xiphinema* species, no intraspecific variability existed in the *X. americanum*-group. For example, the tail of

* Populations identified as *X. pseudoguirani* have also been recorded from Aldabra, Seychelles and Papua New Guinea (Heyns & Coomans, 1983, 1994; Hutsebaut *et al.*, 1987).

* New record.

X. silvaticum was described and illustrated as rather variable in shape (Fig. 1). Such variability is most probably not exceptional. Outline of lip area is generally less variable than tail shape within a species (Alkemade & Loof,1990). But even if this is true, it would still be very difficult to appreciate differences between species described as having lip areas 'more expanded', 'less expanded', 'more rounded', 'less rounded' etc. It is difficult to avoid subjectivity in defining such lip shapes with precision. In fact, only three categories can be easily and clearly recognized for the lip area: *i*) perfectly continuous with the rest of the body; *ii*) button-like, *i.e.*, separated from the rest of the body by a distinct constriction; *iii*) intermediate shapes. In the last category, differences are very difficult to appreciate and they need to be substantiated by several detailed drawings or photographs.

We believe that these two working practices make it possible to better understand the conclusion reached by Lamberti *et al.* (1992).

Diagnoses

The specific diagnosis is of primary importance, as it is the basis of the definition of the species, by stating the character(s) or the combination of characters that makes the species unique in the genus. Therefore, the diagnosis must leave no doubt concerning the differences from the closest species, usually reported in the 'relationships' part of the description of a new species.

The characters used in the diagnoses of the five species discussed (*X. brevicollum*, *X. diffusum*, *X. parvum*, *X. pseudoguirani* and *X. taylori*) are: L, odontostyle length, V, female genital branches (two), lip region shape, and tail shape. The character 'female genital branches' is irrelevant, as the females of all the species of the *X. americanum*-group have two similarly developed genital branches (interspecific differences exist in structure of the female genital system, but they are not taken into consideration by Lamberti's school). These six characters are reported in Table 1. For each species, data from diagnoses are given on the upper line and the corresponding figures reported for various populations studied in detail in the discussed article are on the lower line between square brackets. The diagram (Fig. 2) repeats the population data in a more intuitive manner. In both cases, only extremes for all populations of the same species have been considered and the ratio c' is given, as it represents quite accurately the degree of tail elongation.

Validity of the considered species

From these data it is evident that:

– L can only be used to separate *X. parvum* from the other species;

– odontostyle length can only be used to separate *X. pseudoguirani* from the other species, but not from some populations of *X. brevicollum*;

– V can be used to separate *X. taylori* from *X. pseudoguirani* and most populations of *X. parvum*; but it cannot separate *X. brevicollum* from the other four species;

– lip region shape is said to be 'slightly set off' for *X. diffusum* and 'set off' for the other four species: this does not help very much;

– tail is said to be 'short-conical' for *X. brevicollum*, *X. diffusum*, and *X. pseudoguirani*; 'conical-elongate' for *X. parvum* and 'broad' for *X. taylori*. Values of the ratio c' confirm that the first three species are similar to each other and that *X. parvum* is different, but it fails to separate *X. taylori* from the first three species:

Table 1. *Data taken into consideration by Lamberti* et al. *(1992) in the diagnoses of* Xiphinema brevicollum, X. diffusum, X. parvum, X. taylori, *and* X. pseudoguirani.

	X. brevicollum	*X. diffusum*	*X. parvum*	*X. taylori*	*X. pseudoguirani*
L (mm)	*ca* 2*	1.7-1.8	1.6	2.3	1.9
	[1.8-2.3]**	[1.6-2.0]	[1.4-1.6]	[1.8-2.5]	[1.8-2.0]
Odontostyle (µm)	*ca* 100	85-87	93	94-95	111
	[83-109.5]	[82-96]	[89-98]	[86.5-100.5]	[107-117]
V	mid-body	mid-body	53	mid-body	54.5
	[47-55]	[47-53]	[51-56]	[48-52]	[53.5-56]
Genital branches	equal	equal	equal	equal	equal
Lip region	set-off	slightly set-off	set-off	set-off	set-off
Tail	short-conical	short-conical	conical elongate	broad	short conical
[c']	[0.9-1.1]	[0.8-1.1]	[1-1.4]	[0.8-1.1]	[0.7-0.8]

* data given in the diagnosis itself.
** [data given in the text].

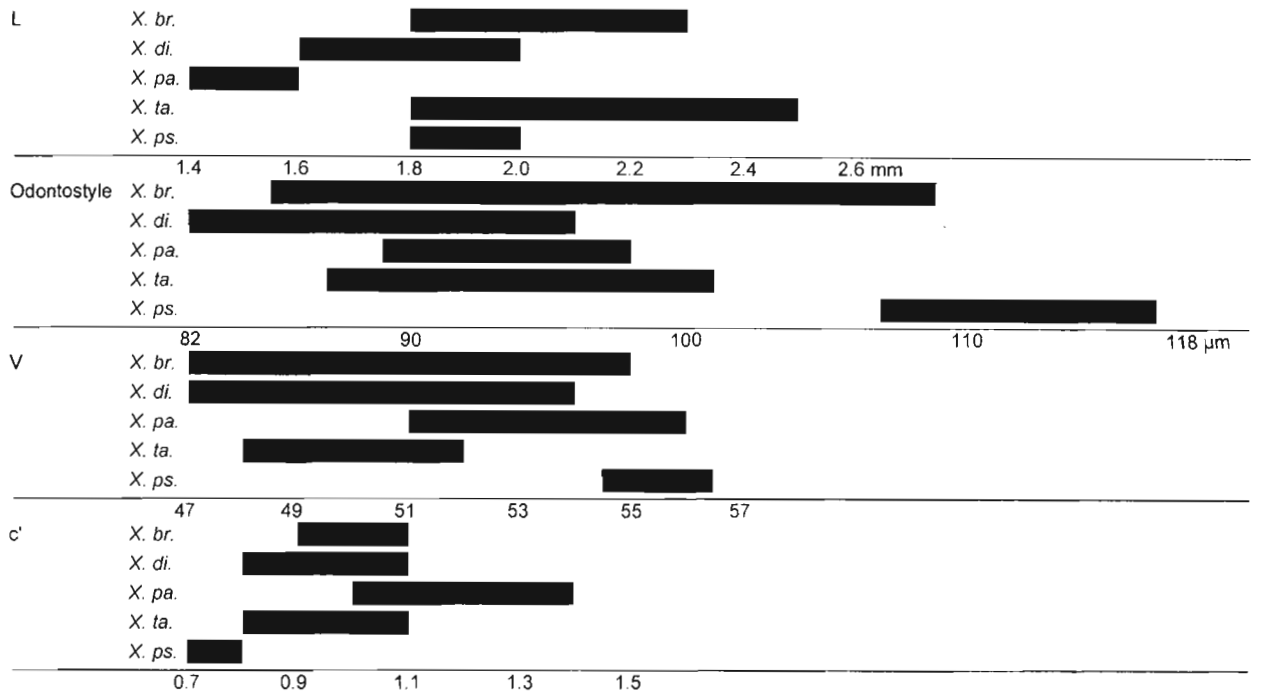

Fig. 2. *Metric characters used in the diagnoses of* Xiphinema brevicollum *(X. br.),* X. diffusum *(X. di.),* X. parvum *(X. pa.),* X. taylori *(X. ta.) and* X. pseudoguirani *(X. ps.) (data from Lamberti* et al., *1992).*

c' is exactly the same in *X. diffusum* and *X. taylori* (0.8-1.1), but a little lower in *X. pseudoguirani* (0.7-0.8). However, in short-tailed species, c' often varies depending on the way the tail is measured and here, subjectivity may be an important factor. Drawings and/or photographs are essential for comparison. Flattening may also modify c' considerably. Consequently, c' is useful only to a limited extent in short tailed species.

The decision concerning the placement of population X in *X. brevicollum* and population O in *X. taylori* makes it difficult to understand Fig. 2 of Lamberti *et al.* (1992), reproduced here as Fig. 3A, representing the "scatterplot of 25 populations of [the five] *Xiphinema* species on the first and second principal axis". In the original diagram, plain lines separate four groupings of letters, each of them representing a population. No explanation is given concerning these groupings. They have to be interpreted as follows: one contains only *X. parvum* (pop. A), a second group includes two populations of *X. brevicollum* (V, W) and one of *X. pseudoguirani* (pop. Y), the third group includes six populations of *X. taylori* (P, Q, R, S, T, U) and the fourth group includes the thirteen populations of *X. diffusum* (B-N), one population of *X. taylori* (O), and one population of *X. brevicollum* (pop. X).

To comply with the placement of pop. O in *X. taylori* and pop. X in *X. brevicollum*, the groupings limits have to be modified as in Fig. 3B.

In Fig. 3B, the areas representing the distributions of populations of *X. brevicollum*, *X. diffusum,* and *X. taylori* look like intricate jigsaw puzzle pieces. The logical conclusion is to consider that they all pertain to a single species; in particular, pop. O (*X. taylori*) and pop. L (*X. diffusum*) appear closer to each other than to other populations of the respective species of reference. It must be noted that the plotting used the mean values of each population. If the values referring to each specimen had been plotted, the graph would certainly show a single cloud.

Remarks and preliminary conclusion

From all these observations, it can be stated that *X. brevicollum*, *X. diffusum* and *X. taylori* have identical morphological characters and very similar, if not identical, measurements. Apart from the lip area, said to be 'slightly set-off' in *X. taylori*, and 'set-off' in *X. brevicollum* and *X. diffusum*, these three species are remarkably similar. In particular, tail shape is identical. Moreover, when the diagram resulting from the principal component analysis produced by Lamberti *et al.* (1992), is analysed as in Fig. 3B, far from making it possible to differentiate *X. brevicollum*, *X. diffu-*

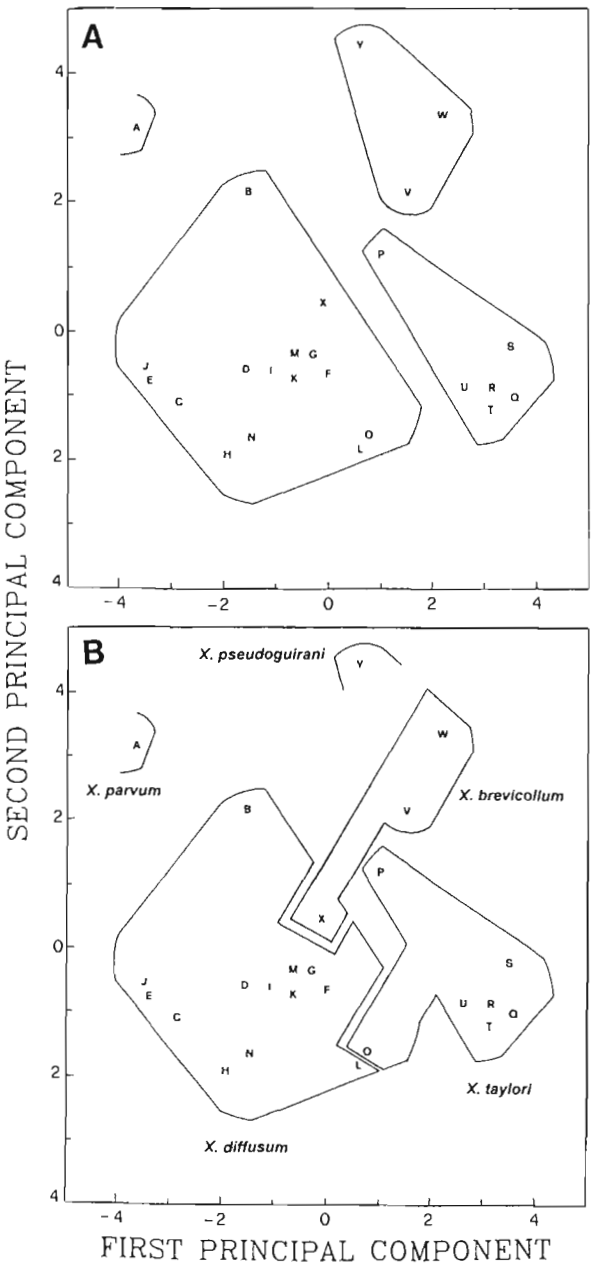

Fig. 3. *Scatterplot of 25 populations (indicated by letters) of* Xiphinema brevicollum, X. diffusum, X. parvum, X. taylori *and* X. pseudoguirani *on the first and second principal components. A: As illustrated in the article of Lamberti* et al. *(1992); B: As corrected according to the text of the same publication.*

sum, and *X. taylori*, it actually supports the conspecific identity of these species.

It seems impossible to clearly separate these species, except by using the 'geographical factor', but the wide distribution accepted for *X. diffusum* weakens the validity of such a 'character'.

Furthermore, it should be stressed that although males do occur occasionally and seem to be functional, all these forms normally reproduce through parthenogenesis (no sperm found in females) which means that each population represents a clone or possibly a mixture of several clones. One could agree that geographically more widely separated populations may differ more from each other than populations that occur in adjacent areas, but there is no genetic evidence for this and the morphological evidence can be severely criticized as mentioned above. Even if there were morphological differences, it would be very difficult to draw boundaries between populational differences and specific differences. A mixture of clones in a variable habitat may present larger variations than observed among single clones from different localities. This was clearly illustrated for *X. elongatum* Schuurmans Stekhoven & Teunissen, 1938, also a parthenogenetic species, in the following way: when studying *Xiphinema* species associated with sugar cane in Mauritius, Williams and Luc (1977) observed two forms of *X. elongatum*. If the authors had not taken into consideration the intraspecific variation, they could have proposed these two forms as two different species. An extensive study by Luc and Southey (1980) on 22 populations of *X. elongatum* from various places in the world showed that they present some geographical variation. Two groups could roughly be recognized: one comprising populations from West Africa and one population from Mauritius (pop. 17), the other comprising populations from East Africa, Madagascar, Mauritius (pops 15 and 16), the Pacific region, and one population from Nigeria. Note that the Mauritian pops 16 and 17 occurred together in the same locality thus forming a metapopulation. From these observations, it is evident that the 'geographical factor' cannot be used to separate species, as proven by the presence of a Mauritian form in the West Africa group, and conversely of a Nigerian form in the East Africa-Pacific group.

Parthenogenetic species can more easily colonize new areas (a single specimen is all it takes). Therefore, they are often more widespread than amphimictic species. All this makes a single widespread parthenogenetic species with some variation in its morphology - and probably also in its genome - more likely than several species with some slight differences in morphometrics.

The case of *X. parvum* and *X. pseudoguirani* is somewhat different. The data from Table 1 and Fig. 2 indicate that the other four species are separated from *X. parvum* (by its shorter body) and from *X. pseudoguirani* (by its longer odontostyle). However, if we consider (Fig. 3) the mean values of the measured

Table 2. *Morphometrics of* Xiphinema brevicollum *and* X. diffusum *(all measurements in µm, except L in mm).*

	X. brevicollum (topotypes; original)					*X. diffusum*		
						Paratypes (orig.)	Type pop. Lamberti & Bleve-Zacheo (1979)	Other pops*
	J1	J2	J3	J4	Female	Females	Females	Females
n	4	6	3	7	25	9	10	66
L	(0.67-0.70)	(0.80-0.95)	(1.08-1.16)	(1.36-1.60)	1.92±0.122 (1.7-2.16)	1.75 (1.56-1.85)	1.7 (1.6-1.8)	(1.3-1.9)
a	(36-40)	(35-41)	(40-42)	(40-45)	46.1±1.72 (44-51)	46.2 (42-51)	47 (46-51)	(30-57)
b	(3.5-4.2)	(4.0-5.4)	(4.2-4.9)	(4.3-5.4)	5.92±0.37 (5.1-6.7)	6.4 (5.1-9.0)	6.9 (5.3-8.9)	(4.2-7.5)
c	(21-22)	(26-32)	(34-42)	(50-59)	76.9±5.64 (67.6-89.9)	70 (62-76)	72 (63-84)	(48-89)
c'	(2.6-2.8)	(1.8-2.2)	(1.4-1.9)	(1.1-1.3)	0.96±0.06 (0.89-1.10)	1.0 (0.9-1.1)	0.9 (0.8-1.1)	(0.7-1.2)
V	–	–	–	–	53±1.9 (50-55)	50 (49-51)	50 (47-52)	(49-57)
Lip reg. diam.	(7.2-7.7)	(8.0-9.0)	(8.8-11.0)	(9.9-11.0)	11.5 (11-13)	11 (11-12)	11 (10-12)	(9.5-13)
Od. style	(42-48)	(48-63)	(67-69)	(82-88)	101±6.14 (89-110)	92.5 (90-95)	87 (84-89)	(71-99)
Od. phore	(34-37)	(35-40)	(41-44)	(46-52)	59±3.43 (50-64)	52.5 (50-55)	50 (48-51)	(50-55)
Stylet	(79-83)	(84-103)	(109-113)	(124-139)	159±8.05 (144-173)	145 (140-149)	–	–
Repl. od. style	(58-62)	(67-70)	(81-88)	(92-113)	–	–	–	–
Guid. ring	(37-40)	(42-51)	(57-60)	(66-76)	86.0±4.23 (77-92)	69 (65-72)	62 (60-64)	(60-95)
Tail	(31-32)	(30-36)	(28-33)	(25-31)	26.0±1.71 (23-28)	25 (22-28)	24 (21-28)	(18-33)
Body diam. anus level	(11-12)	(13-16)	(17-19	(20-25)	26 (22-29)	26 (24-29)	25 (23-28)	(20-31)

* In this column are recorded the overall extreme values of each item given by Lamberti and Bleve-Zacheo (1979) for nine populations they considered as *X. diffusum*: two populations from Malawi (n = 10 and 6), one from Transvaal, South African (n = 8), two from Ivory Coast (n = 8 and 4), one from Gambia (n = 6), one from Sri-Lanka (n = 5), one from Florida, USA (n = 10), and one from Jamaica (n = 10).

characters reported for several populations of the five species (extracted from Table II in Lamberti *et al.*,1992), the separations are no longer obvious: mean body lengths of populations of *X diffusum* are very close to that of *X. parvum*, thus forming a continuum, and mean value of odontostyle length is only 3 µm higher in *X. pseudoguirani* than in the extreme value for means of *X. brevicollum*. It should also be noted that only one population each was measured for *X. parvum* and *X. pseudoguirani* in the analysed article, each represented by a low number of specimens (eleven and four, respectively). Populations certainly exist, perhaps reported in the literature, with intermediate values. For example, Loof and Sharma (1979) reported from Brazil a population of *X. brevicollum* with odontostyle length is 90-110 µm. Heyns and Coomans (1994) later reported a specimen of *X. pseudoguirani* from Mahé, Seychelles, with an odontostyle 101 µm long. These two reports fill the gap between *X. brevicollum* and *X. pseudoguirani* for this character.

Table 3. "Ovejector" length in the species studied.

Species	n	Ovejector length (in µm)
*X. brevicollum***	5	61.5 (53-77)
*X. diffusum**	2	70, 84
*X. incognitum**	1	76
*X. taylori**	1	43
*X. sheri**	1	44
*X. parvum**	2	34, 36
X. pseudoguirani		
– pop. Madagascar*	2	27, 32
– pop. Aldabra	2	32, 45
– pop. Seychelles	3	43, 48, 50

* paratypes.
** topotypes.

To sum up, from the analysis of the publication of Lamberti *et al.*(1992), we can legitimately suspect that *X. diffusum* and probably *X. taylori*, *X. parvum*, and *X. pseudoguirani* are identical to *X. brevicollum*. The second part of this article presents some date supporting this opinion.

Observations on type specimens of the species studied

Type specimens have been examined for each of the five species mentioned above: paratypes for *X. diffusum*, *X. parvum*, *X. pseudoguirani* and *X. taylori*, and topotypes for *X. brevicollum*. It did not seem necessary to give complete redescriptions of the populations examined, but rather to focus on the main characters used for the differentiation of species in the group.

As discussed above, measured characters cannot be used for separating the five species. This was confirmed by measurements on type specimens of *X. brevicollum* and *X. diffusum* as given in Table 2. This Table also includes measurements by Lamberti and Bleve-Zacheo (1979) for the type population of *X. diffusum* and (last column at right) the extreme values for the nine other populations of *X. diffusum* combined, as reported in the same article. From these data, it is obvious that *X. diffusum* presents a large variability in several characters, including those considered as discriminant between species, namely odontostyle length and ratio V. Such a variability cannot be ignored when comparing species with each other.

Head end profiles (Fig. 4) are similar in the five species and can be defined as flat-rounded, separated from the rest of the body by a shallow depression. Photographs of seventeen females from topotype

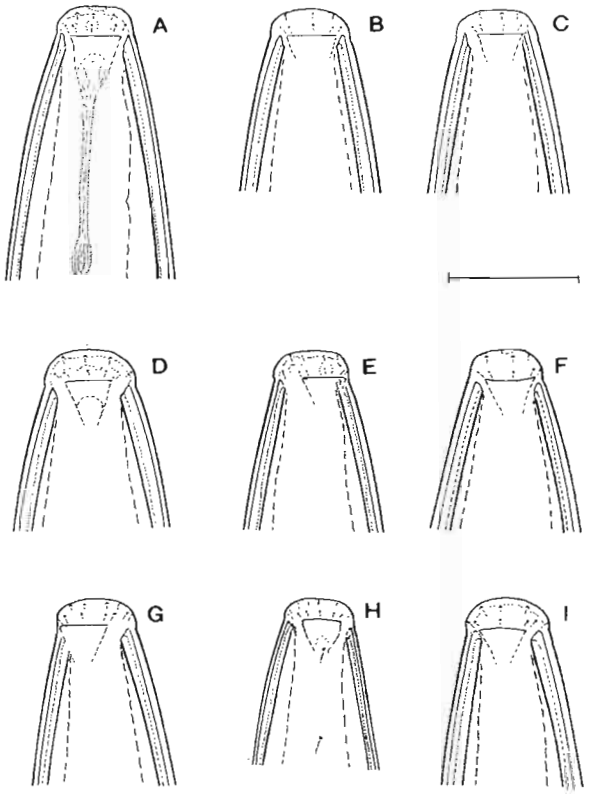

Fig. 4. *Head ends of females. A:* Xiphinema brevicollum*; B,C:* X. diffusum*; D:* X. taylori*; E :* X. pseudoguirani*; F-G:* X. sheri*; H:* X. parvum*; I:* X. incognitum *(all drawings from paratypes except A, from topotypes. Bar = 20 µm).*

population of *X. brevicollum* (Fig. 5A-Q) demonstrate the constancy of the labial profile, at least among a single population. Figs 4 and 5 show that, while the profiles are similar in all the species, the labial diameter seems narrower in *X. pseudoguirani* (Figs 4H; 5R) and larger in *X. taylori* (Figs 4D; 5S, T). However, these differences are small compared to the wide variations of the labial diameter (9.5-13 µm) in various populations of *X. diffusum* (Table 2, last column). Actually, the labial profile appears to be one of the most constant characters at population and, probably, species level. The amphidial slits have about the same length for each species, and they are always situated at the level of the depression.

In all species, the two female genital branches (Figs 6, 7) are of the same length and include short uteri acting as a poorly defined ovejector; each uterus is connected to the oviduct by a small and often indistinct sphincter. Differences in the overall length of the genital branches, as represented in Fig. 6, are related mostly to the various stages of developement/maturity

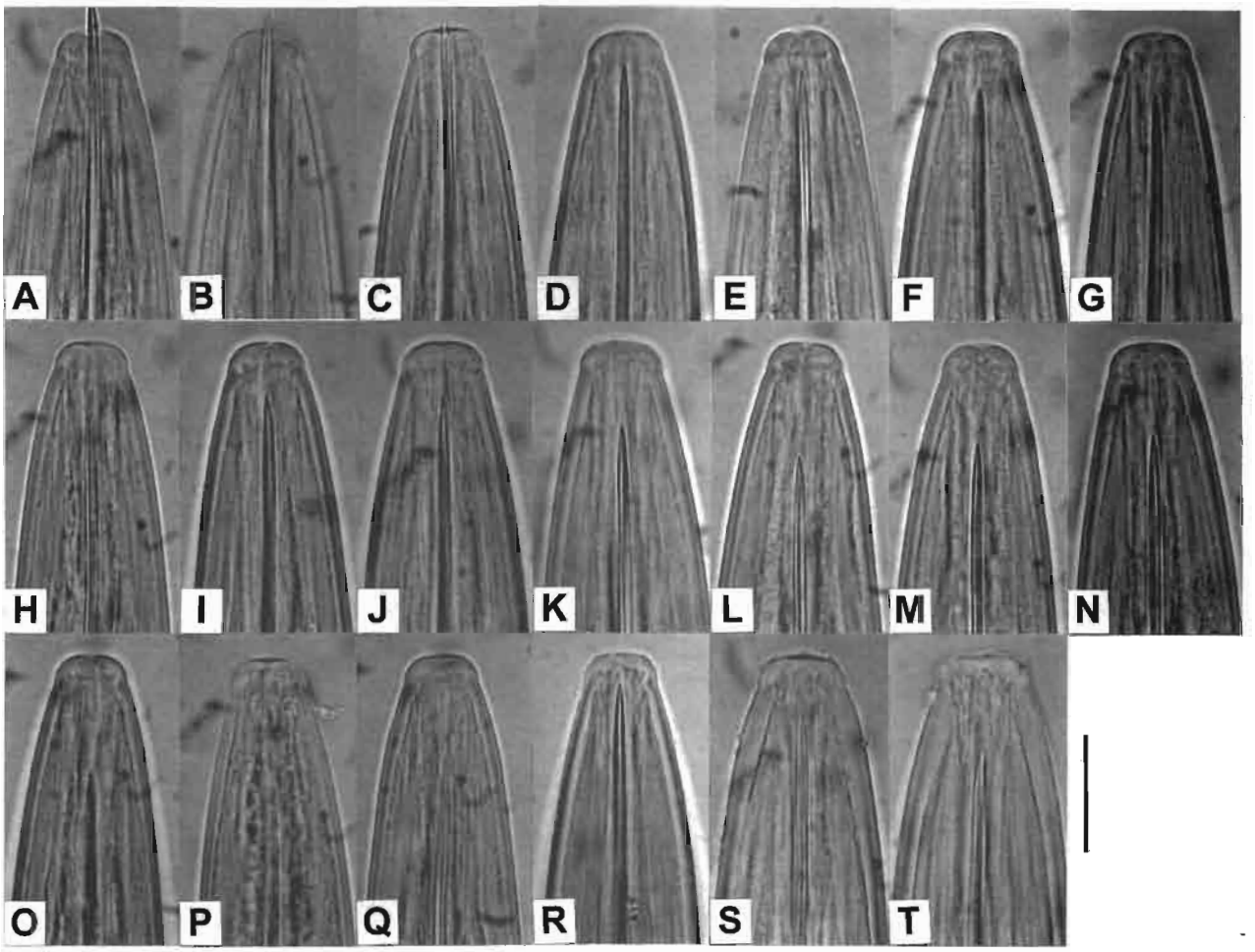

Fig. 5. *Head ends of female. A-Q:* Xiphinema brevicollum*; R:* X. pseudoguirani; *S, T:* X. taylori *(Photographs from topotypes [A-Q] and paratypes. Bar = 20 µm).*

and they do not differentiate the species. However, some differences in length of the uterine part ('ovejector') are observed. From the values reported in Table 3, several groups can be described: *i) X. diffusum* and *X. incognitum* with a long 'ovejector' (70-84 µm); *ii) X. taylori*, *X. sheri*, *X. parvum* and *X. pseudoguirani* with a short 'ovejector' (27-50 µm); and *iii) X. brevicollum* with an intermediate 'ovejector' (53-77 µm). However, the low number of specimens examined for that structure and the nearly continuous values from 27 to 84 µm – with a gap of only 3 µm between the second group and *X. brevicollum* – demonstrate that, at least in this case, the length of the 'ovejector' may not constitute a valid specific character. In addition, this structure is difficult to observe and measure accurately, which precludes its use for determination.

Tails of all of these species have similar profiles (Fig. 8): conical-rounded, with dorsal curvature more important, and with ventral profile continuous with that of the precaudal part of the body; extremity rounded. As usual, some individual variations do exist within some populations, as illustrated for *X. brevicollum* (Figs 8A-C; 9A-Q). In Fig. 9, seventeen photographs of female tails from a topotype population of *X. brevicollum* are given. Although the general profile of the tail appears constant, some variability does exist, essentially in the tail terminal part which is more or less pointed. The terminal profile varies from a rather large curve (Fig. 9B, D, G) to a distinctly narrower curve (Fig. 9Q), all intermediate profiles being

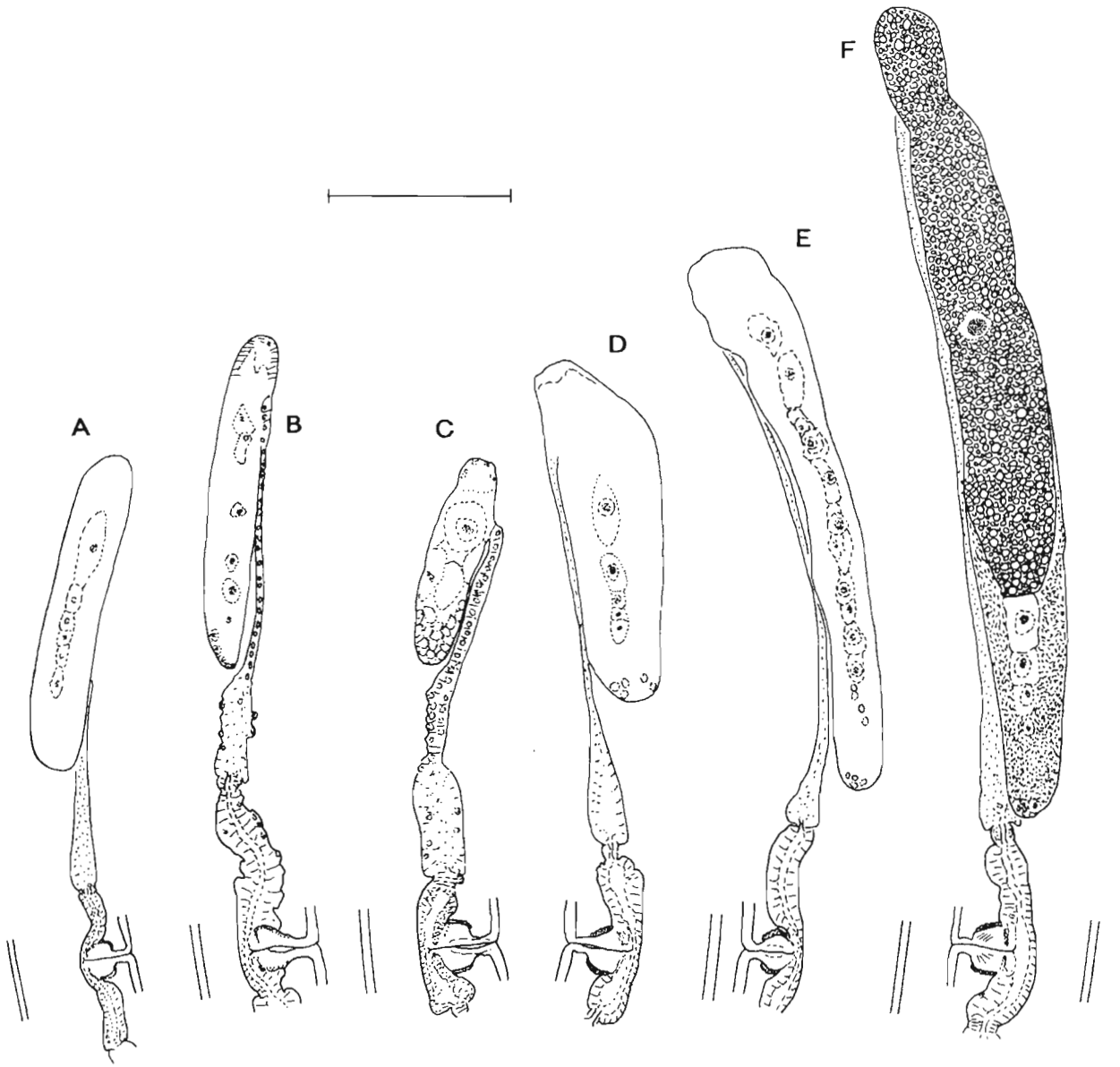

Fig. 6. *Female reproductive system. A :* X. parvum*; B:* X. diffusum*; C:* X. pseudoguirani*; D :* X. sheri*; E :* X. incognitum*; F:* X. brevicollum *(All drawings from paratypes, except F from topotype. Bar = 50 µm).*

present. Photographs of tails of *X. taylori* paratypes (Fig. 9S, T) confirm the similarity in structure and profile with tails of *X. brevicollum*. The tail of *X. pseudoguirani* paratype (Fig. 9R), although shorter, also conforms to the general profile defined above. It is evident that sharper differences also exist among populations. The tail of *X. parvum* is slightly smaller and relatively more elongated than in the other species (Fig. 8). But such minor variations in size do not affect the definition of tail profile.

Observation on some other species

Xiphinema sheri *Lamberti & Bleve-Zacheo, 1979*

When originally described, this species was compared only to *X. brevicollum* and the following characters were given as diagnostic: *i*) smaller size (1.6-1.9

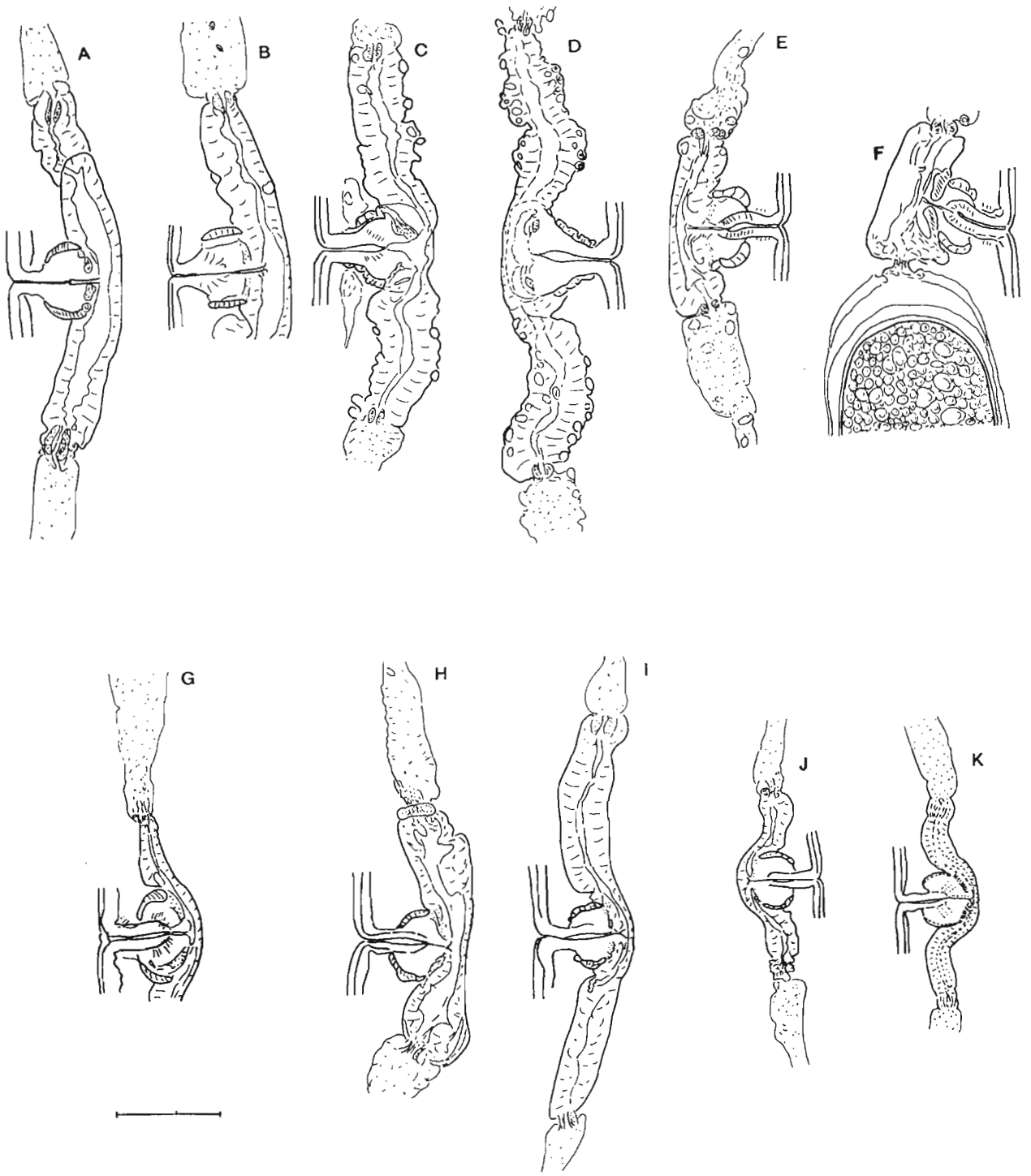

Fig. *7. Vulva, vagina, and ovejector region. A, B :* X. brevicollum*; C, D :* X. diffusum*; E, F:* X. pseudoguirani*; G :* X. taylori*; H :* X. sheri*; I:* X. incognitum*; J, K:* X. parvum *(All drawings from paratypes, except A, B from topotypes. Bar = 20 μm).*

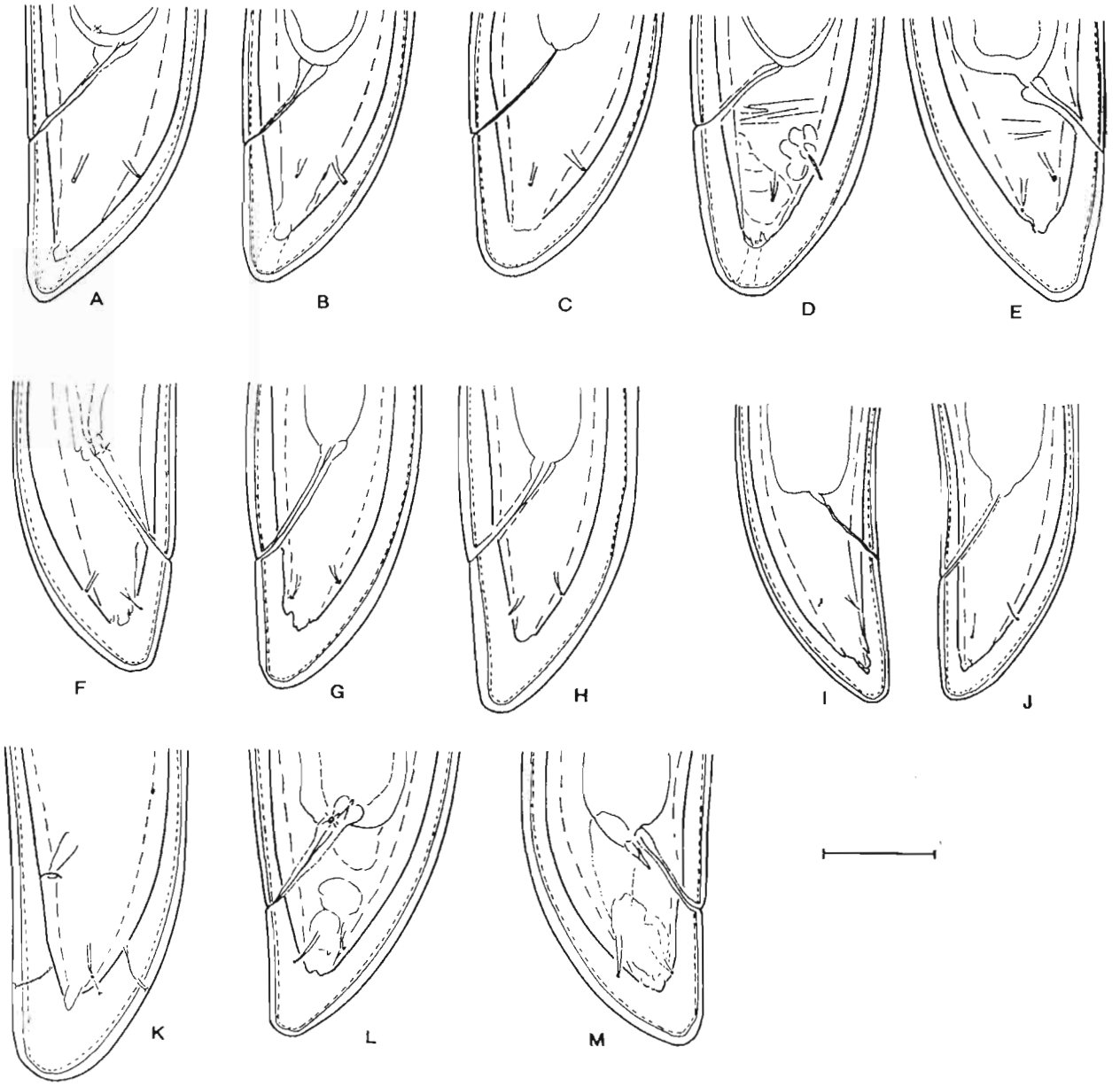

Fig. 8. *Female tails. A - C:* X. brevicollum*; D, E :* X . taylori*; F:* X. pseudoguirani*; G, H :* X. diffusum*; I, J:* X. parvum*; K:* X. incognitum *(subventral position); L, M:* X. sheri *(All drawings from paratypes, except A - C from topotypes. Bar = 20 µm).*

vs 2.0-2.6 mm), *ii*) lower c value (51-77 *vs* 69-115), *iii*) lip region less expanded, *iv*) longer odontostyle (97-112 *vs* 86-100 µm), and *v*) vulva posterior (V = 51-56 *vs* 49-53).

Measurement taken on the holotype and one female paratype are as follows:

Holotype. L = 1.67 mm; a = 37; b = 5.1; c = 69; c' = 0.9; tail = 24 µm; V = 54; lip reg. diam. = 11 µm; odontostyle = 111 µm; odontophore = 55 µm; stylet = 166 µm; guiding ring = 52 µm.

Paratype. L = 1.77 mm; a = 40; b = 5.2; c = 80; c' = 0.8; tail = 22 µm; V = 55; lip reg. diam. = 11 µm; odontostyle = 113 µm (?); odontophore = 57 µm; stylet = 170 µm; guiding ring = 54 µm.

These morphometric data are not significantly different from the corresponding data in the topotype

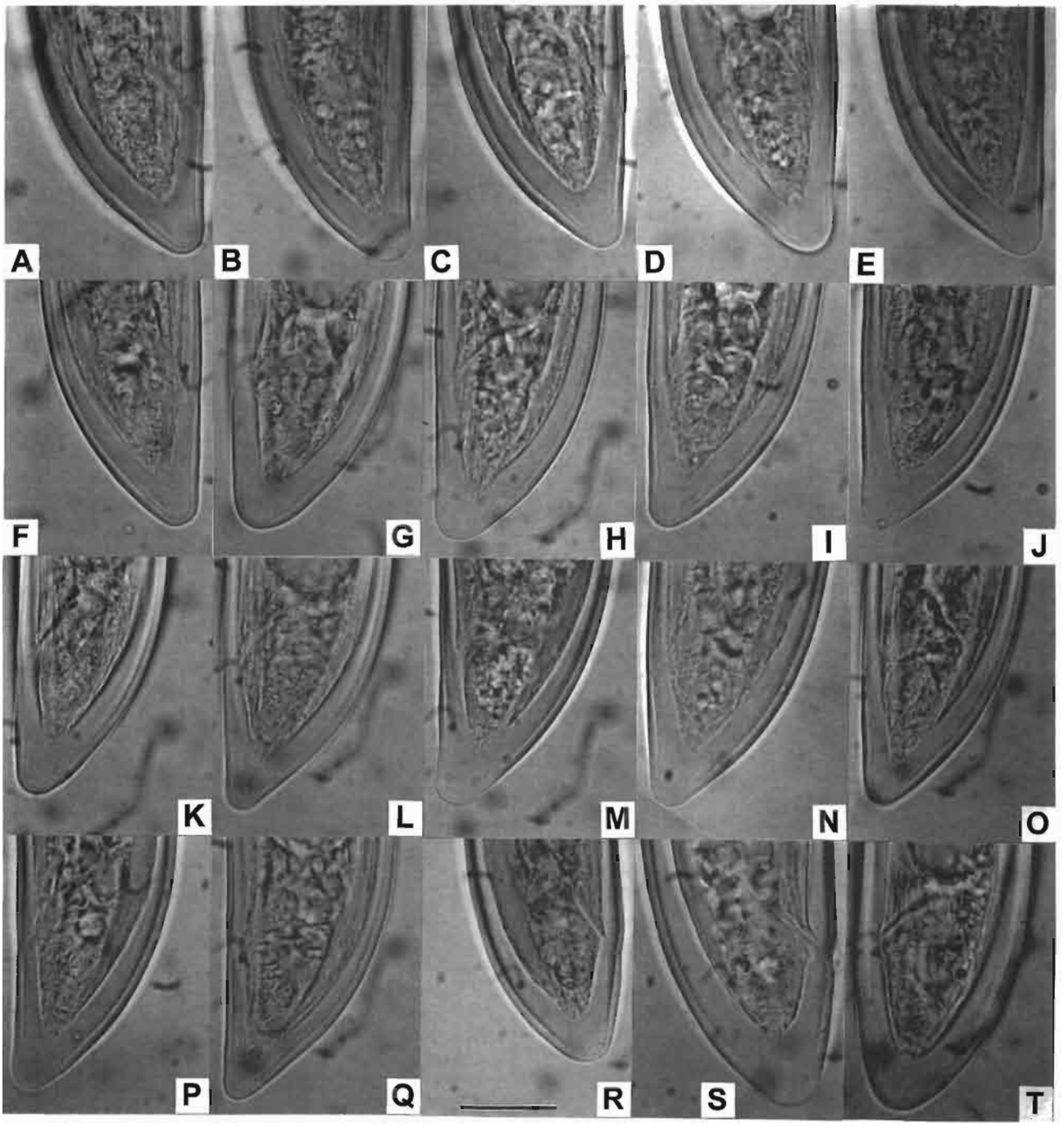

Fig. 9. *Female tails. A-Q:* Xiphinema brevicollum*; R:* X. pseudoguirani*; S, T:* X. taylori *(Photographs from topotypes [A-Q] and paratypes. Bar = 20 µm).*

population of *X. brevicollum* (Table 2). The profile of the lip region (Fig. 4A, F, G), the structure of the genital tract (Figs 6D, F; 7A, B, H), and the tail profile (Fig. 8A-C, L, M) are quite similar. Thus, no consistent differences could be found between *X. sheri* and *X. brevicollum*.

Xiphinema incognitum *Lamberti & Bleve-Zacheo, 1979*

In the original description, this species was compared only to *X. diffusum* and the following differences were noted: *i*) cuticule finely striated *vs* smooth, *ii*) lip region less expanded, and *iii*) tail more elongate (c' = 0.9-1.3).

Measurements taken on two paratype females are as follows:

L = 1.96, 2.08 mm; a = 45, 51; b = 6.0, 6.6; c = 63, 68; c' = 1.0, 1.1; V = 48, 53; tail = 29, 33 µm; lip reg. diam. = 11 µm; odontostyle = 95, 98 µm; odontophore = 53, 54 µm; stylet = 149, 151 µm; guid. ring = 73, 78 µm (note that in the original description, odontostyle length of paratypes was 82-93 µm).

No unique feature in cuticular surface structure could be detected on the specimens examined and the cuticle of all species of *Xiphinema* is finely striated. Moreover, no significant differences are observed concerning the lip area profile (Fig. 4I), the female reproductive system (Figs 6E; 7I), or the tail profile (Fig. 8K). The 'ovejector' is rather long but comparable to that observed in some *X. brevicollum* specimens. The tail of *X. incognitum* appears slightly more rounded, but this is due to the subventral position of the specimen on the slide. So, no consistant differences could be found between *X. incognitum* and *X. brevicollum*.

Conclusion

The conclusion of the present study is that no constant or sufficiently documented differences have been observed between the seven species examined, hence they are considered as pertaining to the same taxon. Consequently, *X. diffusum* Lamberti & Bleve-Zacheo, 1979, *X. incognitum* Lamberti & Bleve-Zacheo, 1979, *X. parvum* Lamberti, Ciancio, Agostinelli & Coiro, 1992, *X. pseudoguirani* Lamberti, Ciancio, Agostinelli & Coiro, 1992, *X. sheri* Lamberti & Bleve-Zacheo, 1979, and *X. taylori* Lamberti, Ciancio, Agostinelli & Coiro, 1992 are proposed as junior synonyms of *X. brevicollum* Lordello & da Costa, 1961.

The present action does not exclude the possibility that future examination of other species pertaining to the *X. americanum*-group may result in proposing the synonymization of some of them with *X. brevicollum*. This is the reason why, at the present state of this re-appraisal of the *X. americanum*-group it appears difficult and not particularly useful to produce an emended diagnosis of *X. brevicollum*.

The *Xiphinema americanum*-group

SPECIES PERTAINING TO THE GROUP

Resulting from the above synonymizations, the *X. americanum*-group now includes 34 valid species and two *species inquirendae*.

Valid species

X. americanum Cobb,1913
= *Tylencholaimus americanus* (Cobb, 1913) Micoletzky, 1922
X. bacaniboia Orton Williams,1984
X. brevicollum Lordello & da Costa,1961
= *X. saopaoloense* Khan & Ahmad,1975
= *X. americanum apud* Carvalho,1955, 1962
= *X. diffusum* Lamberti & Bleve-Zacheo, 1979 (n. syn.)
= *X. incognitum* Lamberti & Bleve-Zacheo, 1979 (n. syn.)
= *X. parvum* Lamberti, Ciancio, Agostinelli & Coiro, 1992 (n. syn.)
= *X. pseudoguirani* Lamberti, Ciancio, Agostinelli & Coiro, 1992 (n. syn.)
= *X. guirani apud* Lamberti & Bleve-Zacheo, 1979
= *X. sheri* Lamberti & Bleve-Zacheo, 1979 (n. syn.)
= *X. taylori* Lamberti, Ciancio, Agostinelli & Coiro, 1992 (n. syn.)
X. bricolense Ebsary, Vrain & Graham,1989
X. californicum Lamberti & Bleve-Zacheo,1979
X. citricolum Lamberti & Bleve-Zacheo,1979
X. duriense Lamberti, Lemos, Agostinelli & d'Addabbo, 1993
X. floridae Lamberti & Bleve-Zacheo,1979
X. fortuitum Roca, Lamberti & Agostinelli,1988
X. franci Heyns & Coomans, 1994
X. georgianum Lamberti & Bleve-Zacheo,1979
X. inaequale Khan & Ahmad,1977, *nom. nov. pro*
X. neoamericanum Khan & Ahmad,1975, junior homonym of *X. neoamericanum* Saxena, Chabbra & Joshi, 1973
X. incertum Lamberti, Choleva & Agostinelli,1983
X. intermedium Lamberti & Bleve-Zacheo,1979
X. kosaigudense Quraishi & Das, 1984
X. laevistriatum Lamberti & Bleve-Zacheo,1979
X. lambertii Bajaj & Jairajpuri,1977
X. luci Lamberti & Bleve-Zacheo,1979
X. madeirense Brown, Faria, Lamberti, Halbrendt, Agostinelli & Jones, 1993
X. occiduum Ebsary, Potter & Allen,1984
X. opisthohysterum Siddiqi,1961
X. oxycaudatum Lamberti & Bleve-Zacheo,1979
X. pachtaicum (Tulaganov,1938) Kirjanova,1951
= *Longidorus pachtaicus* Tulaganov,1938
= *X. mediterraneum* Martelli & Lamberti,1967
= *X. neoelongatum* Bajaj & Jairajpuri, 1977

X. pacificum Ebsary, Vrain & Graham,1989
X. paramonovi Romanenko, 1981
= *X. paramericanum* Romanenko, 1973, *nomen nudum*
X. peruvianum Lamberti & Bleve-Zacheo, 1979
X. rivesi Dalmasso, 1969
X. santos Lamberti, Lemos, Agostinelli & d'Addabbo, 1993
X. silvaticum Luc & Williams,1978
X. simile Lamberti, Choleva & Agostinelli, 1983
X. tarjanense Lamberti & Bleve-Zacheo,1979
X. tenuicutis Lamberti & Bleve-Zacheo,1979
X. thornei Lamberti & Golden,1986
X. utahense Lamberti & Bleve-Zacheo,1979

Species inquirendae

X. neoamericanum Saxena, Chabbra & Joshi, 1973
X. sharmai Luc, Loof & Brown, 1985, *nom. nov. pro*
X. indicum Sharma & Saxena, 1981, *nec X. indicum* Siddiqi, 1959.

Remarks

X. pachydermum Sturhan,1983 was excluded from the group by Loof and Luc (1990) but included by Lamberti and Carone (1992). *X. pachydermum* together with four species described from Portugal by Lamberti *et al.* (1994), *i.e.*, *X. brevisicum*, *X. longistilum*, *X. mesostilum*, and *X. microstilum*, were placed by these authors in the *X. americanum*-group, and Brown and Halbrendt (1997) accepted this move. The present authors disagree with it for the following reasons. It is true that these five species share some characters considered as specific of the *X. americanum*-group, *i.e.*, relatively short body length, coiled habitus, posterior position of the vulva, and backward position of the posteriormost ventromedian male papilla. However, they differ by several characters considered as more important for the characterization of the group: *i*) males common and reproduction apparently amphimictic, *ii*) no symbionts in the oocytes, *iii*) uterus unipartite, of medium length and distinct from the ovejector, *iv*) oviduct with normal structure. Note that these data were confirmed by observations made by the authors on paratypes of the five mentioned species. Consequently, it seems preferable to consider these five species as constituting a complex of species close to, but distinct from, the *X. americanum*-group. This complex, the *X. pachydermum*-group, links the species placed in the *X. americanum*-group with other species of the genus. This is confirmed by the fact that *X. mesostilum* seems to be intermediate between the *X. americanum*-group species and the *X. pachydermum*-group: its female reproductive system is similar to that of the other *X. pachydermum*-group species except for the presence of symbionts in the ovaries. These symbionts, however, are less numerous and arranged in parallel strands in the wall of the ovaries, which possess large oocytes (Coomans, unpubl.). Males are common in *X. mesostilum*.

X. sharmai is tentatively placed in the *X. americanum*-group despite its great body length (2.53-3.20 mm). For further details, see Luc *et al.* (1985).

CHARACTERS OF THE *XIPHINEMA AMERICANUM*-GROUP

The characters used to define the *X. americanum*-group are the following (amended from Loof and Luc,1990):
– habitus usually in close C-shape or spiral,
– body small, under 2.2 mm (exceptions: *X. bacaniboia,* up to 3 mm; *X. sharmai sp. inq.*, up to 3.2 mm),
– stylet robust, its length rarely exceeding 150 µm (exceptions: *X. bacaniboia*, 270-290 µm; *X. silvaticum*, 187-204 µm),
– tubular part of pharynx relatively wider than in other species of *Xiphinema* and gradually expanding into the bulb; as a consequence the bulb is less distinctly offset,
– nuclei in the pharyngeal bulb occupying positions different from those in other species of *Xiphinem*a: DN further from DO, SN further backward,
– V generally 50 or more,
– female genital branches equally developed, generally short; undifferentiated uterus of medium length, usually not clearly demarcated from an ovejector; slender part of the oviduct not clearly demarcated from the poorly developed *pars dilatata*,
– symbionts present in the intestinal cells of juveniles and occasionally in adults; always in the ovaries,
– tail short (c' under 2.5), broadly rounded, conoid-rounded or regularly conical to slightly subdigitate,
– males very rare or unknown; females devoid of sperm,
– males with five or more medioventral supplements, the posteriormost usually lying within the spicule range,
– three or four juvenile stages.

It must be stressed that the most striking character of the group is the very particular structure of the female genital system, mainly the poor differentiation of the gonoduct and the presence of symbionts in the ovaries. This was the reason why *X. bacaniboia*, although presenting characters unusual in the group – great body and stylet lengths – was included (Coomans & Luc, 1998). Similarly *X. pachydermum* Sturhan, 1984 and four related species (the *X. pachydermum*-group) are excluded from the *X. americanum*-group because of their 'normal' female genital system (see above).

Acknowledgements

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Description of two new *Longidoroides* species (Nematoda: Dorylaimida) from South Africa, with a note on *L. strelitziae* (Heyns, 1966)

Juan HEYNS

Department of Zoology, Rand Afrikaans University, P. O. Box 524, Auckland Park, 2006 South Africa.

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Summary – Two new *Longidoroides* species are described from South Africa, and compared with *L. strelitziae,* which they closely resemble. *L. seinhorsti* n. sp. is distinguished by a unique amphid structure, while *L. jacobsi* n. sp. is characterised by a very small amphid aperture, slender body, truncate lip region, and short odontostyle. Jacobs and Heyns (1982) previously identified specimens of this species as *L. strelitziae.* Some comments are made on *L. strelitziae* after the type specimens of this species were examined. Comments are also made on the status of genera in the Longidoridae. © Orstom/Elsevier, Paris

Résumé – ***Description de deux nouvelles espèces de* Longidoroides *(Nematoda: Dorylaimida) d'Afrique du Sud et note sur* L. strelitziae *(Heyns, 1966)*** – Deux nouvelles espèces de *Longidoroides* d'Afrique du Sud sont décrites et comparées à *L. strelitziae* dont elles sont très proches. *L. seinhorsti* n. sp. est caractérisé par la structure unique des amphides, alors que *L. jacobsi* n. sp. est caractérisé par une ouverture amphidiale réduite, un corps étroit, une région labiale tronquée et un odontostyle court. Jacobs et Heyns (1982) avaient prélablement identifié les spécimens de cette espèce comme *L. strelitziae*. Des commentaires sont faits sur *L. strelitziae* dont des spécimens types ont été examinés ainsi que sur le statut des genres de Longidoridae. © Orstom/Elsevier, Paris

Keywords : Nematoda, Longidoridae, *L. jacobsi* n. sp, *L. seinhorsti,* n. sp., *Longidoroides strelitziae.*

Longidoroides strelitziae (Heyns, 1966) Khan, Chawla & Saha, 1978 was described from Port St. Johns in the Transkei, Eastern Cape Province, South Africa. In 1982 Jacobs and Heyns gave a short description of six specimens collected in sugar cane fields in two nearby localities in northern Kwa-Zulu-Natal. These specimens were identified as *L. strelitziae* in spite of several morphological differences. Recently, the author examined two new *Longidoroides* populations resembling *L. strelitziae*. The first one is a large population from sugar cane near Mtubatuba, in the same area as the populations of Jacobs and Heyns. SEM photographs of a specimen from this population were previously published by Swart and Heyns (1987*a*, *b*) under the name *L. strelitziae*. A second, smaller population was collected near Christiana in the North West Province. Although both of these populations resembled *L. strelitziae*, they could not be assigned to this species with certainty. This prompted a re-examination of the type specimens and of the specimens studied by Jacobs and Heyns (1982). This led to the conclusion that these specimens are conspecific with the Mtubatuba population, which is now regarded as a distinct species and is herein described as *Longidoroides jacobsi* n. sp. The population from Christiana is likewise described as a new species under the name *Longidoroides seinhorsti* n. sp.

Longidoroides strelitziae (Heyns, 1966) Khan, Chawla & Saha, 1978

= *Longidorus strelitziae* Heyns, 1966
= *Paralongidorus strelitziae* (Heyns, 1966) Aboul-Eid, 1970
(Fig. 1 A-C; Table 1)

The original description of this species was based on several specimens collected around the roots of *Strelitzia nicolae* in virgin sandy soil near the beach opposite 'Bird rock' beyond Third Beach at Port St. Johns in the Transkei, Eastern Cape Province, in January and December 1964. Unfortunately, the holotype and paratypes which were in the nematode collection of the Plant Protection Research Institute in Pretoria were borrowed in the early 1970's and never returned, and are now considered lost. Two paratypes which were deposited in the Rothamsted collection, one male and one female (slides 168/19/1 and 168/19/2 respectively) are in near perfect condition, and their examination confirmed the accuracy of the original description and illustrations as presented by Heyns (1966).

COMMENTS

The Rothamsted specimens are both very flattened. A comparison of the a-ratios calculated with non-corrected as well as corrected body diameters (using the

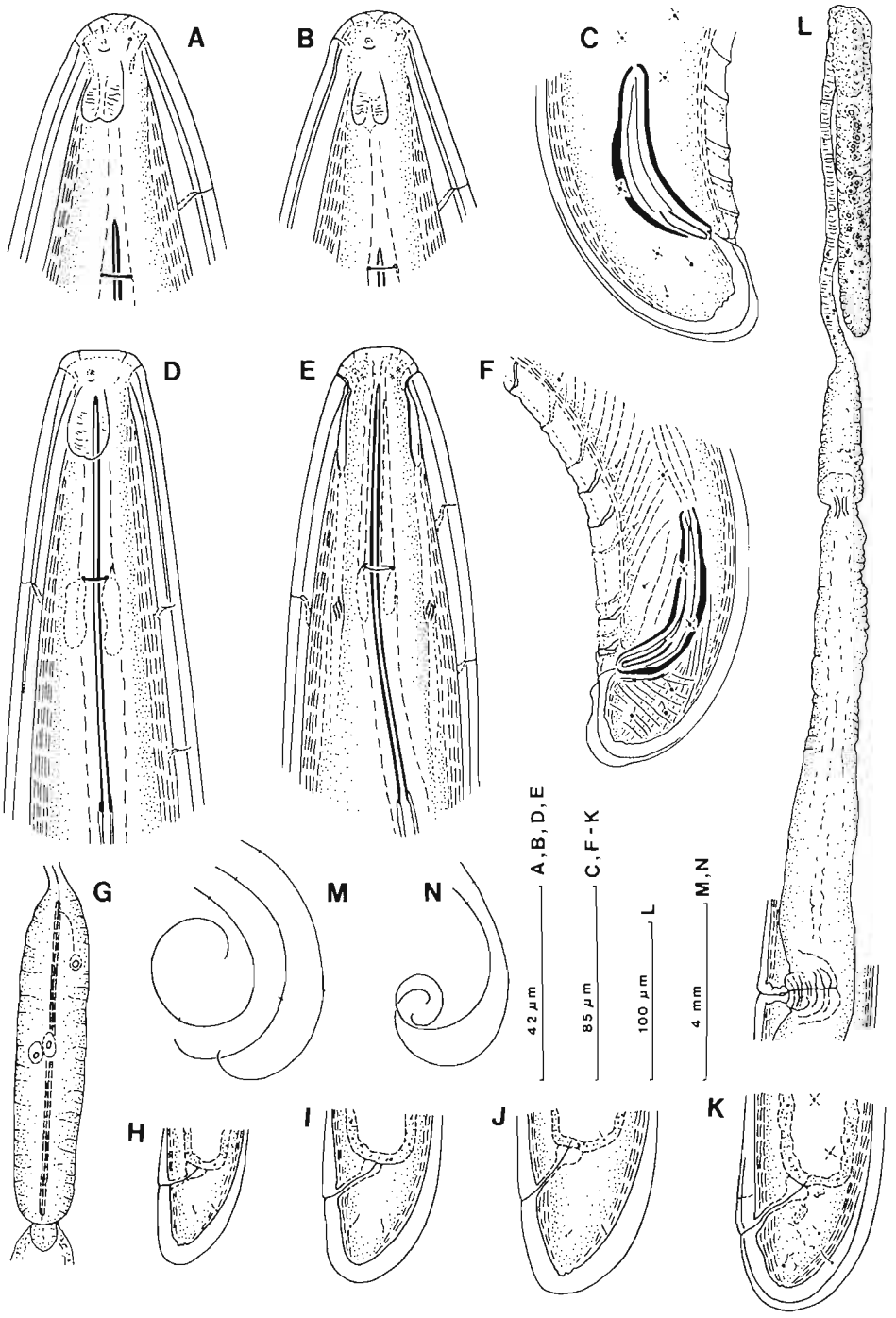

Fig. 1. Longidoroides strelitziae. *A: Head of paratype female; B, C: Head and tail of paratype male* — Longidoroides jacobsi *n.sp. D, E: Head, lateral and dorso-ventral views, respectively; F. Male tail; G: Basal bulb; H: Tail of J3; I, J: Tail of J4; K: Female tail; L: Anterior branch of reproductive system; M, N: Body posture of female and male, respectively.*

Table 1. *Morphometric data of* Longidoroides strelitziae *Heyns, 1966 and* L. seinhorsti *n. sp. (all measurements in µm, except L in mm.).*

	L. strelitziae				*L. seinhorsti* n. sp.				
	Female		Male		J3	J4	Female		Male
	Acc. to Heyns (1966)	Paratype*	Acc. to Heyns (1966)	Paratype*			Holotype	Paratypes	Paratype
n	4		2		2	9		3	1
L	5.48-7.46	5.83	4.98-5.50	5.37	3.30 ; 3.56	4.90 (4.11-5.96)	6.7	5.56-9.08	7.22
a	54-67	53/83**	54-66	53/77**	65 ; 61	78 (72-89)	72	66-89	102
b	9.4-13.0	13.1	10.2-14.9	10.5	6.9 ; 10.2	10.7 (9.0-12.3)	12.6	12.9-16.5	16
c	148-161	182	119-179	149	90 ; 108	131 (105-148)	168	150-216	134
c'	0.5-0.6	0.5	0.6-0.7	0.65	1.01 ; 0.85	0.83 (0.69-1.00)	0.71	0.70-0.79	1.06
V	48-50.5	50	-	-	-	-	50.1	49.9-51.2	-
Tail length	27-30	32	28-34	36	36.5 ; 33	37.6 (33-41)	40	37-45	54
Lip region diam.	18-19	19	?	19	11.5 ; 11	14.1 (13-15)	16	16-17	16
Odontostyle	117-131	127	123	120	85 ; 80	93.3 (88.5-99)	97	105-115	102
Repl. od.style	-	-	-	-	92 ; 94	104 (99-111)	-	-	-
Odontophore	70-82	82	70	103	61 ; 56	71 (68-77)	75	67-89	70
Stylet	191-210	209	193	223	146 : 136	164 (158-171)	172	174-204	172
Guiding ring to front end	55-65	56		56	32.5 ; 33	37.2 (33-40)	43	41-48.5	46
Greatest body diam.	-	110/70**	-	102/70**	51 ; 58	59 (54-67)	93	84-102	71
Anal body diam.	-	67	-	55	36 ; 39	45.3 (39-51)	56	52-60	51
Rectum length	37	37	-	-	-	-	29	33-34	-
Spicule length	-	-	88-91	96	-	-	-	-	83
Lateral guiding pieces	-	-	20	26	-	-	-	-	23

*Borrowed from Rothamsted Experiment Station
**Uncorrected and corrected values

correction formula of Geraert, 1961) with the a-ratios in Heyns (1966) indicates that the specimens were already quite flattened at the time of the original description. This should be kept in mind when comparing the morphometrics of the three species in Tables 1 and 2, since not only a-ratio, but also c and c' are affected by flattening, as shown by Heyns (1983).

The number and arrangement of supplements in the Rothamsted male paratype are rather similar to those illustrated by Heyns (1966, Figs 28-29) and

described as "a more or less equidistant ventromedian series of twelve or thirteen, diverging into two lines posteriorly so that there are three pairs in the region of the spicules, the last of which may be regarded as the adanal pair." In the Rothamsted specimen, there are ten ventromedian supplements followed by eight off-centre, staggered supplements, becoming more closely approximated posteriad so that the final two form an adanal pair.

In Table 1, the original measurements given by Heyns (1966) are compared with data obtained from the Rothamsted paratypes. Additional measurements, not previously given, are the following: amphid aperture 3 µm long, constituting 15.2-15.8% of the lip region diameter as measured at the level of the outer circlet of labial papillae. Distance from amphid aperture to base of bilobed fovea 15.5 - 18.5 µm.

Longidoroides seinhorsti n.sp.
(Figs 2D, E; 3A-S)

MEASUREMENTS

See Table 1

DESCRIPTION

Adults: Cuticle 4-5.5 µm thick on anterior part of neck, 4.5-6 µm around midbody, 12.1 (9.5-14) µm dorsally on female tail, only 6 µm dorsally on male tail, and 12.8 (11-15) µm around tail terminus (=h) in both sexes. Body pores indistinct except on front part of neck, arrangement typically longidoroid, with seventeen to twenty lateral, five to seven dorsal, and nine to eleven ventral pores in neck region. Lip region evenly rounded and confluent with body. Amphid aperture crescent- shaped, 4-4.5 µm long, or 25.0-28.1% of lip region diameter at level of outer circlet of labial papillae. A funnel-shaped structure present posterior to the aperture, internally in the fovea, with the stem of the funnel projecting between the two lobes of the fovea. Distance from aperture to base of fovea 14-16 µm. External wall of fovea thickened, darkly colored (sclerotized?), conspicuous in dorso-ventral view; this sclerotization apparently encircling the neck at the level of the foveae. Posterior one-fourth of fovea differentiated into a separate cavity. Stylet typical for the genus; base of odontophore difficult to observe. Pharyngeal bulb 149 (135-156) × 27.8 (26-29) µm. Gland nuclei mostly indistinct. Cardia hemispherical.

Female: Relaxed body strongly ventrally arcuate, posture varying from open C-shape to more than one full circle. Tail bluntly rounded to hemispherical, bearing two pairs of caudal papillae. Reproductive system typical; vulva transverse; vagina reaching to middle of body diameter; without clearly demarcated ovejector; uterus a 337 (330-345) µm long broad duct without *pars dilatata*, separated from oviduct by well-developed sphincter. Reflexed ovary 239 (200-287) µm long.

Male: Body of single male specimen strongly ventrally arcuate and describing more than two full circles. Tail conoid, dorsally convex, ventrally concave, with outer as well as inner layers of cuticle thickened around the terminus. Two pairs of caudal papillae near middle of tail, and a third pair at the level of the cloaca. Spicules 83 µm long (measured along curved median line). Lateral guiding pieces 23 µm long, exceptionally broad and heavily sclerotized. Supplements with rather prominent papillae. Number and arrangement of supplements as follows: nine ventromedian ones, followed by five off-centre, staggered ones, plus one adanal pair, making a total of sixteen, more or less equidistant supplements.

Juveniles: Only J3 and J4 found. Habitus, especially in J3, much less ventrally arcuate than in adults, otherwise similar to female in general appearance.

TYPE SPECIMENS

Holotype female, two paratype females and one paratype male in the collection of the Biosystematics Division, ARC-Plant Protection Research Institute, Pretoria, slides 31798-31801.

TYPE LOCALITY AND HABITAT

Soil among reeds and grasses on the bank of the Vaal River, Rob Ferreira Resort, Christiana, North-West Province, South Africa. Collected by E. van den Berg, 3 September 1990.

DIAGNOSIS AND RELATIONSHIPS

L. seinhorsti n. sp. is characterized by the unique structure of the amphid.

The new species differs from the very similar *L. strelitziae* in odontostyle length (97-115 *vs* 117-131 µm), position of the guiding ring (41-48.5 *vs* 55-65 µm from front end), diameter of lip region (16-17 *vs* 18-19 µm), and length of the amphid aperture (4-4.5 *vs* 3 µm, or 25-28 *vs* 15.2 - 15.8% of lip region diameter).

The new species differs from *L. jacobsi* n. sp. in odontostyle length (97-115 *vs* 83 -104 µm), a-ratio (66-102 *vs* 93* - 171 µm; *based on non-corrected body diameter of a flattened specimen), shape of lip region (rounded *vs* flattened), and length of amphid aperture (4-4.5 *vs* 1.5 µm or 25-28 *vs* 8.8% of lip region diameter).

L. seinhorsti n. sp. differs from both the above mentioned species in the length of the fovea, which is only 14-16 µm compared with 15.5 - 18.5 µm in *L. strelitziae* and 17-20 µm in *L. jacobsi* n. sp.

***Longidoroides jacobsi* * n. sp.**
= *L. strelitziae apud* Jacobs & Heyns, 1982,
apud Swart & Heyns, 1987
(Figs 1D-N; 2A-C)

MEASUREMENTS

See Table 2

DESCRIPTION

Adults: Cuticle thickness: in anterior part of neck: 5.6 (5-6) µm in female and 6.3 (6-7) µm male; at mid-body: 4-5 µm in both sexes; on dorsal side of tail: 11.8 (10-13) µm in female and 7.3 (6-8) µm in male; and around tail tip (=h): 12.7 (11-15) µm in both sexes. Lateral body pores mostly distinct, ventral pores indistinct except the first few behind the lip region being quite conspicuous. Arrangement of pores typical, with three or four dorsal, nine to eleven ventral, and fifteen to eighteen lateral pores in neck region. Lateral chord exceptionally narrow, and lateral pores over greater part of body practically in a single line. Lip region rather truncate, continuous with body. Amphid aperture close behind outer lateral labial papilla, very small, only 1.5 µm long, which is only 8.5-8.8% of lip region diameter at level of outer circlet of labial papillae. Distance from aperture to weakly bilobed base of fovea 18.3 (17-20) µm. Stylet typical for the genus, odontostyle base without collar, and odontophore base, as seen with SEM, with three longitudinal ridges delimiting the three sinuses. Pharyngeal bulb 150 (137-170) × 29.7 (27-33) µm. Gland nuclei and their outlets situated as follows: DO = 6.2 (5.1-9.3); DN = 19.9 (16.4-21. 1). DO - DN = 12.7 (10.5-14.5); LSN = 50.4 (45.4-53.1); RSN = 52.3 (49.3-55.3); SO = 87.3 (82.9-90.0). Cardia prominent, heart-shaped.

Female: Relaxed body posture ventrally arcuate, ranging from open C-shape to one full circle. Tail bluntly rounded, with two or three pairs of caudal papillae, the third pair at or near the level of the anus. Reproductive system typical: vulva transverse; vagina reaching half body diameter; without individualized ovejector; uterus 253 (190-300) µm long, without differentiated *pars dilatata*, but separated from oviduct by well-developed sphincter; reflexed ovary 297 (270-330) µm long.

Male: Posterior part of body more strongly arcuate than in female. Tail conoid, dorsally convex, ventrally concave; outer layer of the cuticle thickened around the bluntly rounded terminus. Three pairs of caudal papillae, one of which situated at or near the level of the cloaca. Spicules 80-90 µm long (measured along the curved median line). Lateral guiding pieces 21-27 µm long. Supplements varying from 17 to 23 in number, including the adanal ones. Only some of them, notably the more anterior ones, actually midventral, the rest being irregularly arranged either to the left or right of the midventral line, and posteriorly forming one, two, or three pairs, the last of which is adanal (see Fig. 2.7 in Jacobs & Heyns, 1982). Seven or eight prominent subventral papillae in the region of the supplements.

Juveniles: Only J3 and J4 found. Similar to female in general appearance, except smaller and less strongly ventrally arcuate.

TYPE SPECIMENS

Holotype female (slide 1736) and seven female and seven male paratypes in the nematode collection of the Rand Afrikaans University, Johannesburg, slides 1731-1741. One female and one male paratype each deposited in the collections of the Instituut voor Dierkunde, University of Ghent, Belgium, and Muséum National d'Histoire Naturelle, Paris, France.

TYPE LOCALITY AND HABITAT

Sugar cane field next to the Msiduzi River on the estate of Kirko & Co., south of Mtubatuba in northern KwaZulu-Natal. Collected J. Heyns, 26 July 1985.

Also found in sugar cane fields in the Hluhluwe area, northern KwaZulu-Natal. For details see Jacobs and Heyns (1982) under *L. strelitziae*.

DIAGNOSIS AND RELATIONSHIPS

Longidoroides jacobsi n. sp. is distinguished by its very small amphid aperture, flattened lip region, slender body, and short odontostyle.

It is rather closely related to two species, *L. strelitziae* and *L. seinhorsti* n. sp., from which it differs in being more slender (a=93-137 *vs* about 80 in *L. strelitziae* and 66-102 in *L. seinhorsti* n. sp.), in having more truncate *vs* rounded lip region, slightly more posterior vulva (V = 51-56 *vs* 48-50.5 in *L. strelitziae* and 50-51 in *L. seinhorsti* n. sp.), shorter odontostyle (84-97 *vs* 117-131 µm in *L. strelitziae* and 97-115 µm in *L. seinhorsti* n. sp.), very small amphid aperture (1.5 *vs* 3 µm in *L. strelitziae* and 4-4.5 µm in *L. seinhorsti* n. sp.), and longer fovea (17-20 *vs* 15.5-18.3 µm in *L. strelitziae* and 14-16 µm in *L. seinhorsti* n.sp).

COMMENTS

Four of the six specimens described by Jacobs and Heyns (1982) under the name *L. strelitziae* were available for study. This confirmed the conspecificity of these specimens with the Mtubatuba population. Although the short description and illustrations in Jacobs and Heyns (1982) were quite accurate, one fault has to be pointed out, viz. the amphid aperture

* This species is named after Dr. P.J.F. Jacobs in recognition of his valuable work on South African longidorids.

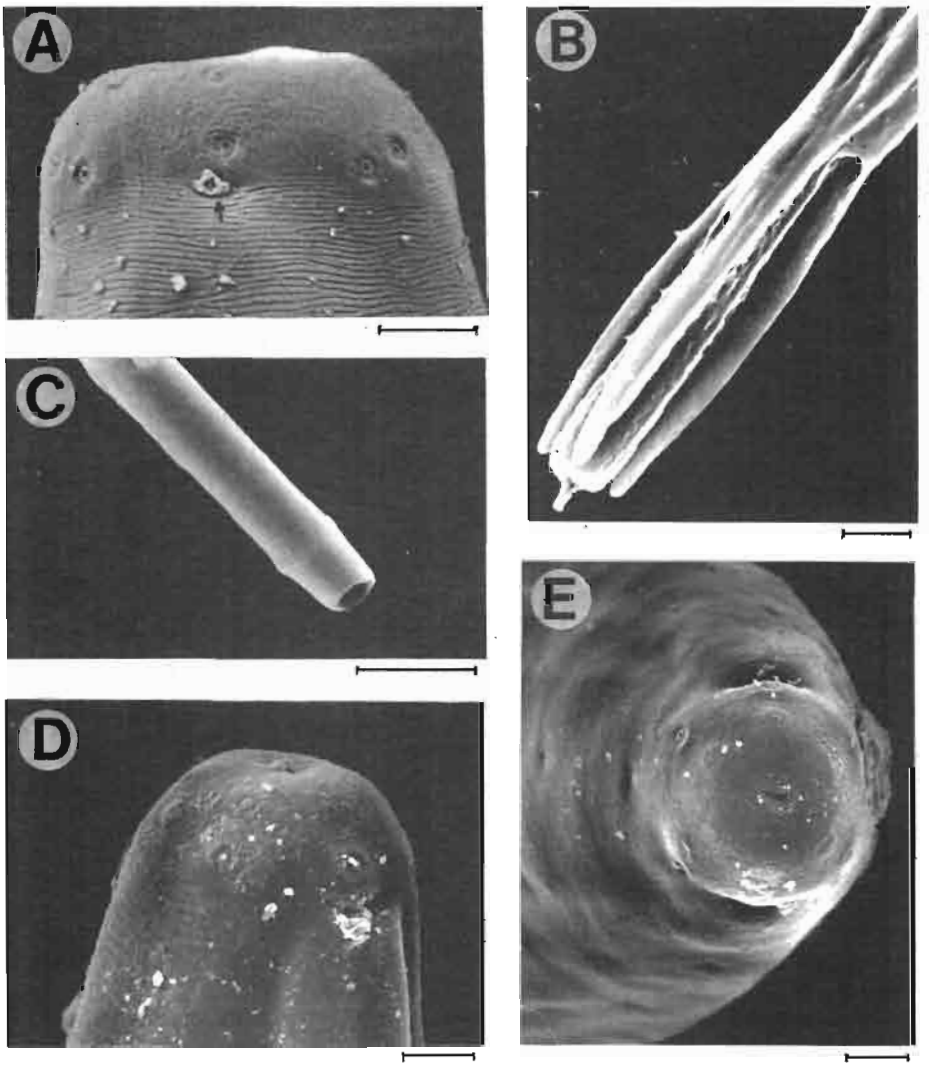

Fig. 2. Longidoroides jacobsi *n. sp. A: Lateral view of head (arrow points towards amphid aperture covered by debris); B: Base of odontophore; C: Odontostyle base* — Longidoroides seinhorsti *n.sp. D: Sublateral view of head; E: En face view of head (Scale bars = 4 µm).*

in their Fig. 2.2 which is shown in the wrong position due to a misinterpretation.

REMARKS

It is interesting to note that, while amphid structure is one of the main generic characters employed in the Longidoridae *sensu* Coomans, 1996, it is also an important diagnostic character differentiating the three species considered in this paper. Such variation in amphid structure within otherwise quite similar (=closely related?) species must obviously be taken into account when evaluating the validity of the genera. This validity has recently been the subject of some difference of opinion, as evidenced by the synonymization first of *Siddiqia* with *Paralongidorus* by Luc and Doucet (1984), followed by synonymization of *Longidoroides* also with *Paralongidorus* by Siddiqi *et al.* (1993). At the same time, however, Hunt (1993) considered *Longidoroides* as valid, while he regarded *Siddiqia* as a subgenus of *Paralongidorus.* Coomans (1985, 1996) endorsed the synonymization of *Siddiqia* with *Paralongidorus,* but agreed with Hunt in regarding

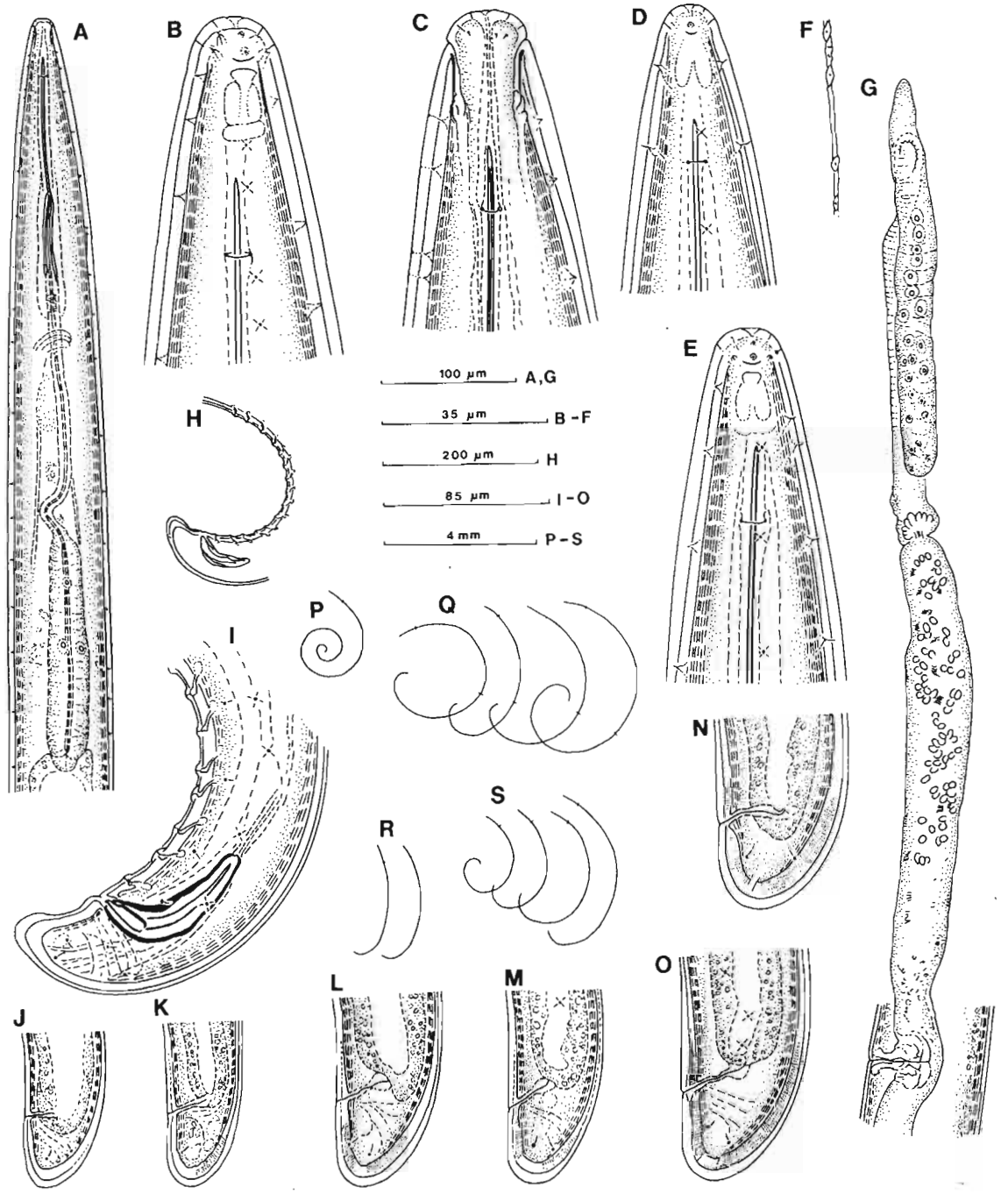

Fig. 3. Longidoroides seinhorsti *n.sp. A: Anterior body region; B, C: Head, lateral and dorso-ventral views; D, E: Head, J3 and J4, respectively; F: Lateral chord on front part of neck; G: Anterior branch of female reproductive system; H: Male posterior end showing supplements; I: Male tail; J, K: Tail of J3; L, M: Tail of J4; N, O: Female tail; P, Q: Body posture of male and female, respectively; R, S: Body posture of J3 and J4, respectively.*

Table 2. *Morphometric data of* Longidoroides jacobsi *n. sp. (all measurements in µm, except L in mm).*

	J3	J4	Female			Male	
			Holotype	Paratypes	Acc. to Jacobs & Heyns (1982)	Paratypes	Acc. to Jacobs & Heyns (1982)
n	5	15		10	3	9	3
L	3.55 (3.04-4.05)	5.32 (4.04-6.44)	17.9	7.72 (6.38-9.26)	6.19-8.39	7.55 (6.09-8.62)	7.96-9.28
a	70 (60-79)	85 (79-102)	100	106 (93-125)	135-138*	113 (97-137)	137-171*
b	11.2 (8.7-13.1)	12.5 (11.5-14.6)	14.1	15.1 (12.7-16.8)	12.8-12.9	15.5 (10.5-18.9)	16.1-18.7
c	106 (91-123)	154 (116-192)	266	245 (181-298)	252-276	203 (142-233)	230-330
c'	0.78 (0.61-0.92)	0.68 (0.59-0.74)	0.6	0.67 (0.55-0.98)	0.6-0.7	0.83 (0.69-0.90)	0.7-0.8
V	-	-	56.2	52.9 (50.9-56.3)	52.5-54.7	-	-
Tail	31.8 (27-34)	32.9 (29-39)	27	31.9 (27-40)	25-30	37.6 (35-43)	28-34
Lip region diam.	12.4 (12-13)	15.7 (14-17)	18.5	17.4 (16-18.5)	20-21	17.8 (17-19)	19-20**
Odontostyle	64.6 (63-67)	78.7 (70-86)	92	90.7 (87-97)	83-102	90.9 (84-94)	89-104
Repl. od.style	74.5 (72-80.5)	89.5 (85.5-100)	-	-	-	-	-
Odontophore	53 (48-59)	64 (56-72)	71	73 (65-79)	53-63	78.2 (75-83)	53-56
Stylet	118 (111-126)	143 (136-151)	163	164 (156-169)	-	169 (159-176)	-
Guiding ring to front end	37.1 (33-41)	41.5 (40.5-51)	50	50.7 (46-60)	47-56	50.4 (48-54)	51-54
Greatest body diam.	47.5 (47-48)	62.5 (53-72)	72	70 (65-75)	-	67 (54-75)	-
Anal body diam.	37.5 (36-43)	47.5 (44-54)	45	47.4 (41-51)	-	48.8 (44-54)	-
Rectum length	21.8 (18-26)	31.6 (26-35)	37	33.7 (34-40)	-	-	-
Spicule	-	-	-	-	-	85.4 (80-90)	73-81
Lateral guiding pieces	-	-	-	-	-	23.6 (21-27)	20-28

*Based on corrected body diameter
**In Jacobs and Heyns (1982) misprinted as 19-30

Longidoroides as valid, even though in his 1996 paper he lists it more tentatively as '*Longidoroides*' and suggests that a reappraisal of the distinguishing characters is necessary, a view with which I concur. Such a study may well show that the most logical solution is to recognize only one genus, *Longidorus*, with four sub-

genera: *Longidorus, Paralongidorus, Siddiqia* and *Longidoroides*. In the mean time, I prefer to recognise *Longidoroides* as a separate genus and to retain *L. strelitziae*, together with the two new species described herein, in *Longidoroides* rather than in *Paralongidorus*.

Acknowledgment

The author would like to thank Dr A. Swart for providing the SEM photomicrographs, and Dr E. van den Berg of the ARC-PPRI in Pretoria and Prof. Brian Kerry of the Rothamsted Experiment Station for the loan of types and other specimens.

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On the ultrastructure of the cuticle of Trichodoridae Thorne, 1935 (Nematoda: Enoplia)

Wilfrida DECRAEMER * and Walter ROBERTSON **

* *Koninklijk Belgisch Instituut voor Natuurwetenschappen, Vautierstraat 29, 1000 Brussel, Belgium, and ** Nematology Department, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK.*

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Summary – The fine structure of the body cuticle was studied in *Trichodorus primitivus, T. similis, T. hooperi, T. viruliferus, Paratrichodorus pachydermus,* and *P. nanus.* The ultrastructure of the cuticle was mostly similar to recent observations on four species from western Africa. Some intraspecific differences were seen in the thickness of layers 4 and 6. The ultrastructure of the body cuticle in the region of the retracted spicules in *P. pachydermus* is clearly different from that in *T. primitivus.* This result confirms the diagnostic value of presence *vs* absence of caudal alae for differentiating the didelphic genera. © Orstom/Elsevier, Paris

Résumé – ***Ultrastructure de la cuticule des Trichodoridae Thorne, 1935 (Nematoda: Enoplia)*** - L'ultrastructure de la cuticule est décrite chez *Trichodorus primitivus, T. similis, T. hooperi, T. viruliferus, Paratrichodorus pachydermus* et *P. nanus.* La cuticule présente une structure largement semblable à celle décrite récemment chez quatre espèces d'Afrique occidentale mais, selon les espèces, une différence dans l'épaisseur des couches 4 et 6 est observée. La structure de la région des spicules est nettement différente chez *P. pachydermus* et chez *T. primitivus,* confirmant ainsi la valeur diagnostique du caractère présence ou absence d'ailes caudales pour différencier les genres didelphes. © Orstom/Elsevier, Paris

Keywords : Cuticle, nematode, *Paratrichodorus, Trichodorus,* ultrastructure.

The family Trichodoridae Thorne, 1935 is a small family with two didelphic genera (*Trichodorus* Cobb, 1893 and *Paratrichodorus* Siddiqi, 1974) and two monodelphic genera (*Allotrichodorus* Rodriguez-Montessoro, Sher & Siddiqi, 1978 and *Monotrichodorus* Andrássy, 1976). Although the most important diagnostic generic features are well described, overlap between the genera may occur, which makes identification to genus level difficult, especially with female specimens (Decraemer & Baujard, 1998). In males, presence *vs* absence of caudal alae differentiates *Paratrichodorus* from *Trichodorus* and *Allotrichodorus* from *Monotrichodorus*, but, here again, exceptions are known. The present study is part of a project that aims at substantiating a phylogenetic approach to the higher classification of the Trichodoridae. Currently, the families Trichodoridae and Diphtherophoridae constitute the order Triplonchida (see Siddiqi, 1983*a*), which is classified under Enoplia and is related to the order Tripylida (see Siddiqi, 1983*b*).

For a better understanding of the trichodorid genera and their phylogenetic relationships, the most important diagnostic features at genus level were reviewed from ultrastructure observations of the body cuticle. Until recently, only one illustrated description of the ultrastructure of the body cuticle in the family Trichodoridae had been published (*Paratrichodorus allius* in Raski *et al.*, 1969), plus a non-illustrated description of the fine structure of *P. minor* (Hirumi *et al.*, 1969). A study on the ultrastructure of the somatic musculature of *P. porosus* (Allen, 1957) Siddiqi, 1974 by Bird (1970) included a TEM picture of the somatic musculature showing part of the body cuticle. Within the scope of the present project, the ultrastructure of the body cuticle was studied in males of six species belonging to the two didelphic genera: *Paratrichodorus pachydermus* (Seinhorst, 1954) Siddiqi, 1974, *P. nanus* (Allen, 1957) Siddiqi, 1974, *Trichodorus primitivus* (de Man, 1880) Micoletzky, 1922, *T. similis* Seinhorst, 1963, *T. viruliferus* Hooper, 1963, and *T. hooperi* Loof, 1973. Apart from the last one, all of these species are known to be natural vectors of economically important virus diseases of crops in Europe, and several are of world-wide significance.

In parallel with our research, Mounport *et al.* (1997) studied the ultrastructure of the body cuticle at mid-body level of females of *P. minor* (Colbran, 1956) Siddiqi, 1974, *P. nanus* (Allen, 1957) Siddiqi, 1974, *P. rhodesiensis* (Siddiqi & Brown, 1965) Siddiqi, 1974, and *T. eburneus* De Waele & Carbonell, 1983. These species all occur in the semi-arid region of western Africa and the specimens studied were reared under laboratory conditions.

Material and methods

For TEM observations, specimens were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 1 h, washed three times in buffer, then postfixed in

1% osmium tetroxide in phosphate buffer for 30 min. Nematodes were rinsed in buffer then embedded in 1% distilled water agar. Agar blocks containing single nematodes were dehydrated in a graded ethanol series and the ethanol replaced by propylene oxide. Specimens were infiltrated for 3 h in Emix epoxy resin at 37°C then placed in flat embedding moulds to be polymerised at 70°C. Specimens were sectioned at a Reichert Ultracut microtome and sections collected on plastic coated grids. These were stained with uranyl acetate and lead citrate before being examined in a JEOL 1200 electron microscope.

Transverse and/or longitudinal sections were made at the levels of the pharyngeal region and the copulatory apparatus and tail.

The fine structure of the body cuticle was studied at the level of the caudal alae in *P. pachydermus* and compared to the ultrastructure of the body cuticle at the same level in *T. primitivus* and *T. hooperi*. In addition, light-microscopic studies of series of transverse sections from the posterior body region of *T. similis* and *P. pachydermus* were compared.

Results

All species showed the same basic structure characterised by *i*) a trilaminate external layer, *ii*) a fine granular layer, and *iii*) an electron-dense or electron-lucent layer with granulations; all three layers are also present in other taxa. Below are three inner layers (4 to 6). Layer 6 consists of three multilaminate units with the outer unit showing a marked banding. This layer of the body cuticle seems to be characteristic of trichodorids, and, according to all known data, is quite different from the structure of the body cuticle in Enoplia and Chromadoria.

Fine structure of the body cuticle

The fine structure of the body cuticle in the species studied largely agreed with the descriptions by Mounport *et al.* (1997) and consisted of six layers; however, layer 5 was not always clearly differentiated. Some intraspecific differences were observed in the thickness of layers 4 and 6. *T. hooperi* (Fig. 2A), *P. nanus* (not illustrated), and *P. pachydermus* each possess a thick layer 4, representing more than 50% of the cuticle thickness, and a multilaminate layer 6, representing 30% of the total cuticle thickness. These data are comparable with the recent observation by Mounport *et al.* (1997). In *P. pachydermus*, the banding of layer 6 in both the anterior and the posterior body regions was not always visible (Fig. 2C, D). Conversely, the pharyngeal (Fig. 1B) and spicule (Fig. 1D) regions of *T. similis* and the spicule region of *T. primitivus* (Fig. 1A) and *T. viruliferus* (Fig. 1C) all possess a thin and amorphous layer 4 and a multilaminate basal layer 6 representing up to 75% of the cuticle thickness. No differences were observed between pharyngeal region and spicule region. The peculiar type (different from hemidesmosomes) of junctions between cuticle and somatic muscle cells that was described by Mounport *et al.* (1997) was observed (Fig. 1A), as well as intracuticular canals mainly in layers 4 and 6.

Caudal alae

One of the most important diagnostic features in males of the didelphic genera *Paratrichodorus/Trichodorus* and the monodelphic genera *Allotrichodorus/Monotrichodorus* is presence *vs* absence of caudal alae, respectively. Caudal alae are present in all species of *Paratrichodorus*, although narrow and non-functional (*P. pachydermus*: Fig. 3A, B); in some species they are weakly developed and hardly recognisable on SEM pictures, for example *P. grandis* Rodriguez-Montessoro & Bell, 1978 (Fig. 3D, courtesy Dr. J. Baldwin, TEM Micrograph Collection Riverside) and *P. rhodesiensis* (Fig. 3C, courtesy P. Baujard, SEM Micrograph Collection, ORSTOM). In *Allotrichodorus*, caudal alae are present in various degrees of development, they are obvious but narrow in *A. brasiliensis* (Fig. 4E), and they are hardly developed in *A. longispiculis* Rashid, De Waele & Coomans, 1986. In *Trichodorus*, caudal alae are generally absent (*T. similis*: Fig. 4A; *T. primitivus*: Fig. 4B), but they are distinctly present in two species (*T. cylindricus* Hooper, 1962 and *T. paracedarus* Xu & Decraemer, 1995: Fig. 4D). Several *Trichodorus* species have 'rudimentary caudal alae', for example *T. sparsus* Szcygiel, 1968 (see Loof, 1973), *T. elefjohnsoni* Bernard, 1992, *T. nanjingensis* Liu & Cheng, 1990 (in Decraemer & Cheng, 1994), and *T. vandenbergae* De Waele & Kilian, 1992 (Fig. 4C); these were also described in *Monotrichodorus sacchari* Baujard & Germani, 1985 (see Decraemer, 1986). The 'rudimentary caudal alae' are possibly induced by temporary muscular activity in *T. elefjohnsoni* (Bernard, 1992). The fine structure of caudal alae in *Paratrichodorus* was compared with the fine structure of the body cuticle in *Trichodorus* species. Also, a comparison was made using light-microscopy observations of transverse sections of the body cuticle in the region of retracted spicules and tail in representative species of the two didelphic genera.

P. pachydermus

Light-microscopy observations of male specimens of *P. pachydermus* revealed well developed caudal alae which protrude lateroventrally from the body (Fig. 5A-C). A transverse section through the cloacal opening showed two well developed flaps of the anterior cloacal lip (Fig. 5B). Just posterior to the posterior cloacal lip (Fig. 5C), two fine subventral canals

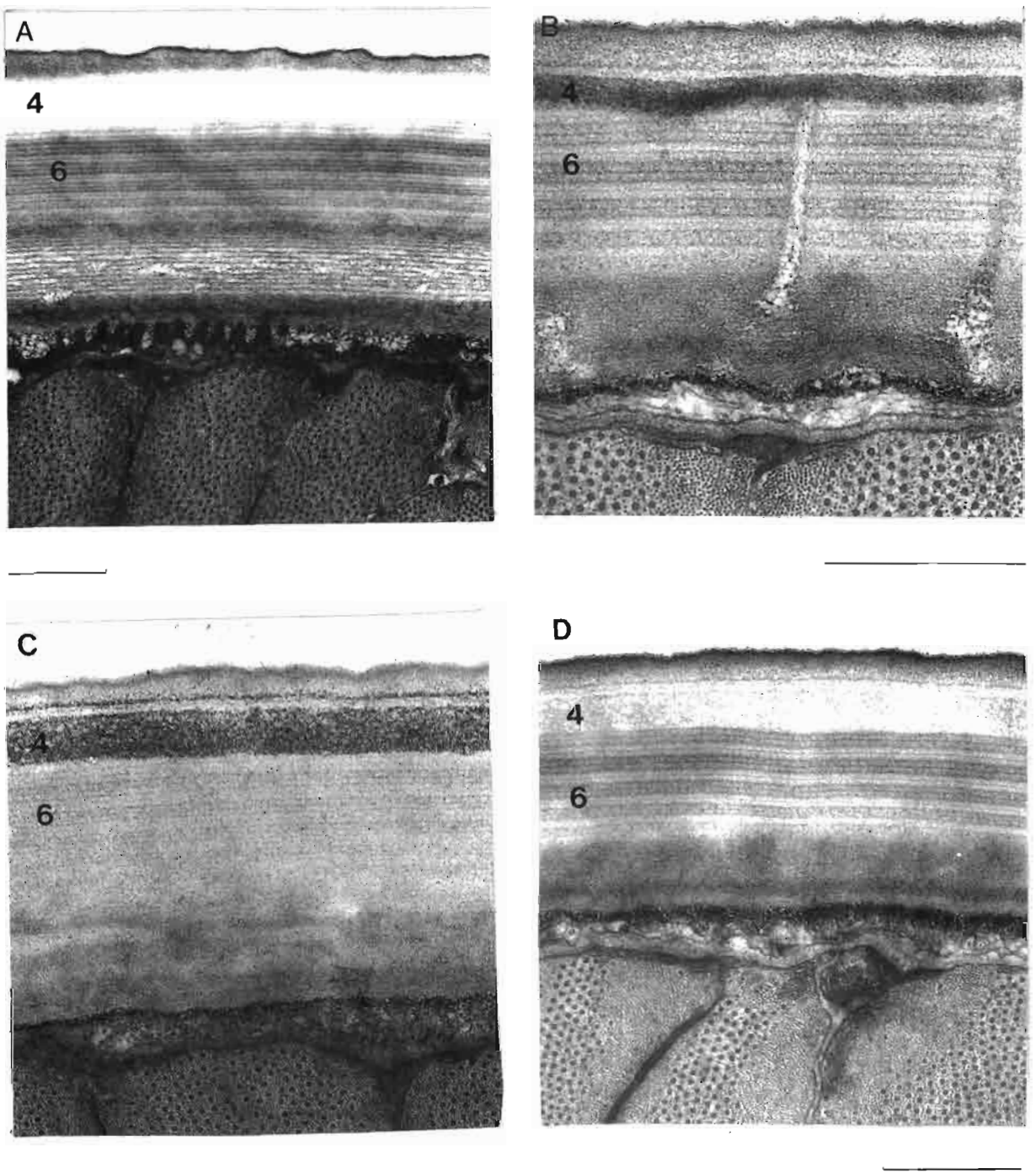

Fig. 1. *Ultrastructure of the body cuticle in males; dorsal wall in transverse sections, with indication of layers 4 and 6, at level of retracted spicules (A, C, D) and in the pharyngeal region (B). A:* Trichodorus primitivus*; B:* T. similis*; C:* T. viruliferus*; D:* T. similis. *(Scale bar = 0.5 μm).*

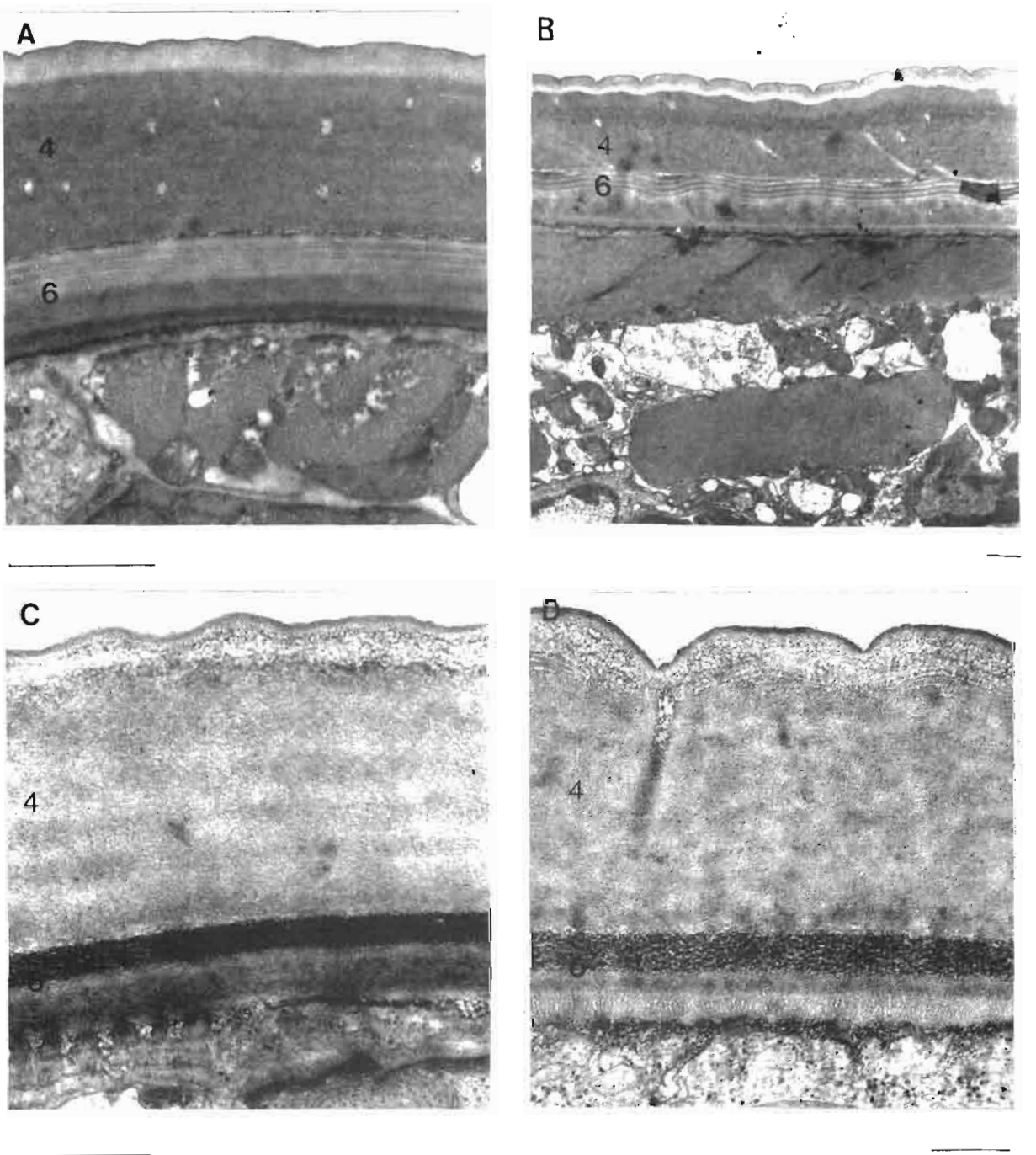

Fig. 2. *Ultrastructure of the body cuticle in males with indication of layers 4 and 6. A, B:* Trichodorus hooperi. *spicule region, dorsal wall in transverse and longitudinal section, respectively; C, D:* Paratrichodorus pachydermus, *dorsal wall in transverse section in the spicule region and longitudinal section in the pharyngeal region (Scale bar = 0.5 µm).*

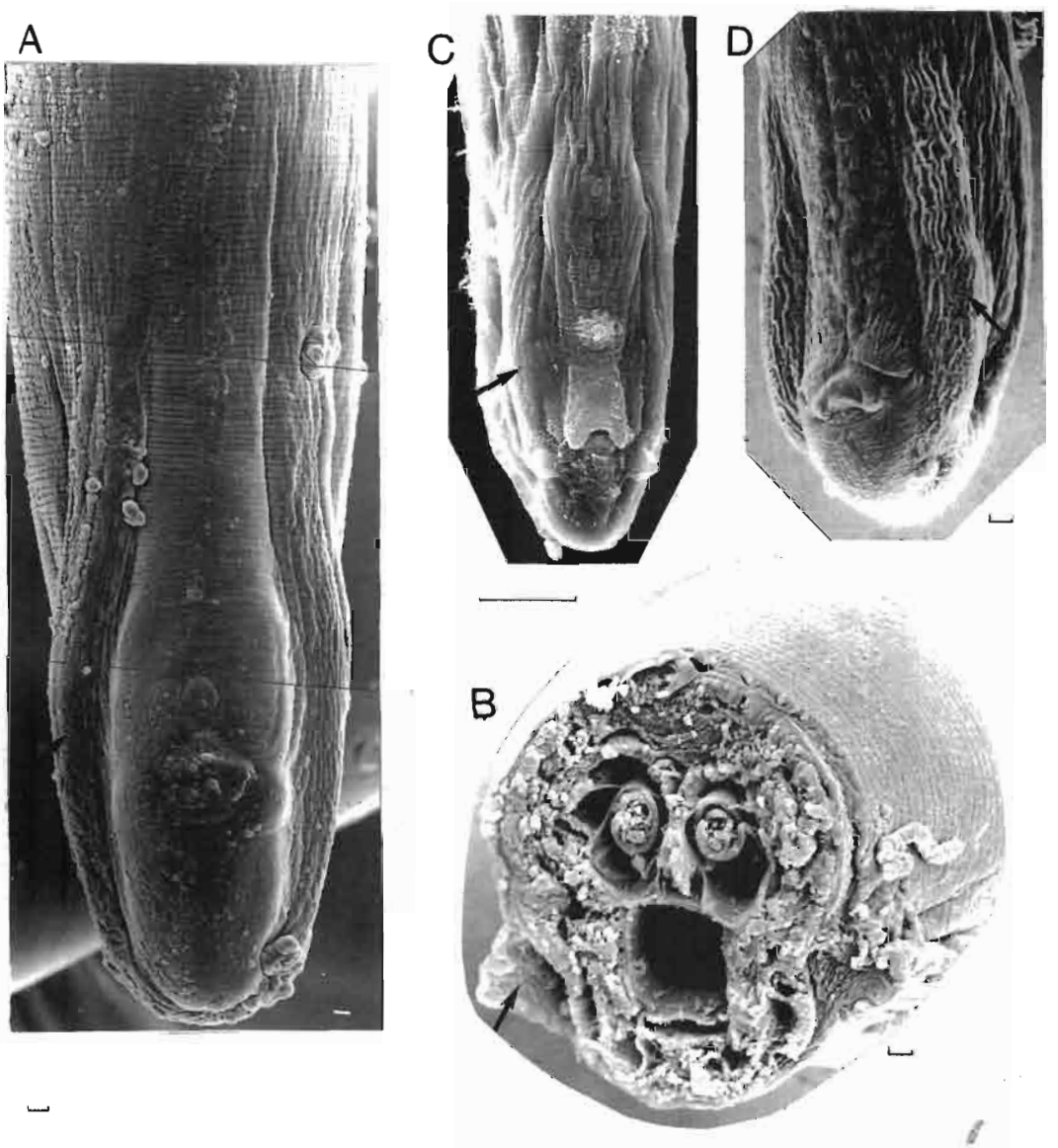

Fig. 3. *SEM observations of caudal alae region in* Paratrichodorus *males, ventral view. A, B:* P. pachydermus, *ventral view and transverse section at level of retracted spicules, respectively; C:* P. rhodesiensis *(courtesy P. Baujard); D:* P. grandis *(courtesy J. Baldwin) (Arrow indicates caudal alae. Scale bars = 1 µm in A-B, D; 10 µm in C).*

each leading to a pore were observed in the transverse optical section. This agrees with the observation by Sturhan (1985) who rejected earlier descriptions of these structures as a pair of minute postcloacal papillae (Seinhorst, 1954; Allen, 1957; Kuiper & Loof, 1962; Baujard, 1980). Transverse sections of this posterior body region show a clear demarcation of the ventral field. Baujard and Germani (1985) described the demarcation of the lateral fields by two incisures in *Monotrichodorus sacchari.* A differentiation of the lateral fields could not be confirmed by light-microscopy nor by TEM in any of the species discussed in present paper. A study of their fine structure, based on a series of transverse sections from the level of the retracted spicules to a subterminal point, showed that the caudal alae were formed lateroventrally by an additional layer of dense tissue within the inner unit of layer 6. This layer appears as a clearly distinguished

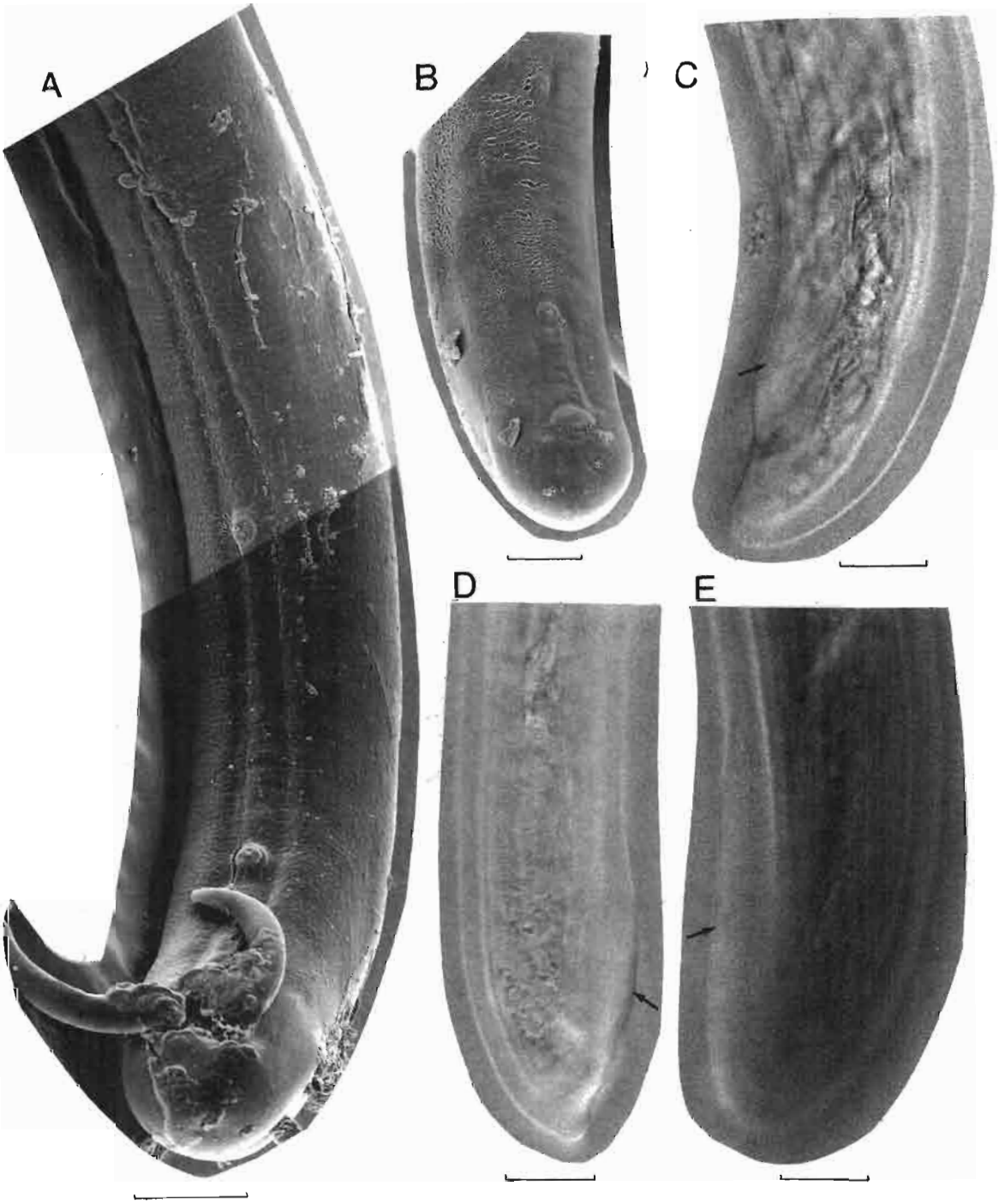

Fig. 4. *SEM (A, B) and LM observations (C-E) of posterior body region in* Trichodorus *males. A:* T. similis*; B:* T. primitivus*; C:* T. vandenbergae *with rudimentary caudal alae (arrow); D:* T. paracedarus *with caudal alae (arrow); E:* Allotrichodorus brasiliensis *with caudal alae (arrow) (Scale bar = 10 μm).*

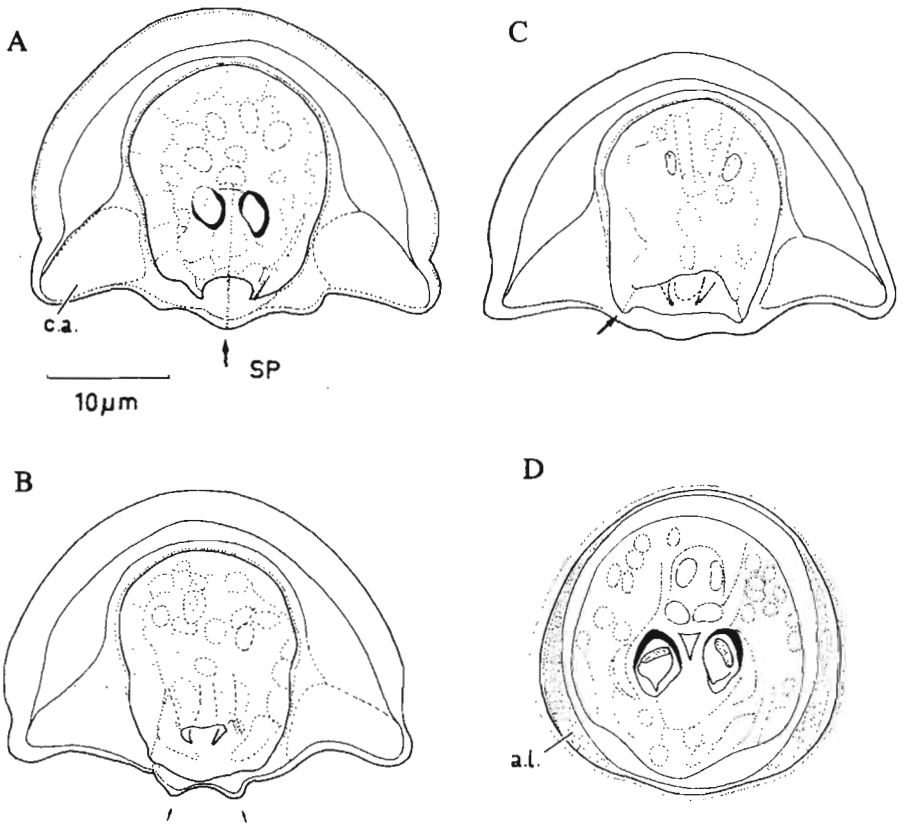

Fig. 5. *Light-microscopic observations of transverse sections of* Paratrichodorus pachydermus *(A-C) and* Trichodorus similis *(D). A: At level of posteriormost precloacal supplement; B: At level of anterior cloacal lip (arrow points to bifid anterior cloacal lip); C: At level of subventral pores (see arrow) just posterior to cloacal opening; D: At level of distal part of retracted spicules (a.l. = additional layer; c.a. = caudal alae; SP = precloacal supplement).*

oval portion which fills the protruding caudal alae. Mid-ventrally, at the level of the ventral field, layer 6 with a wide outer unit forms 75% of the body cuticle (Fig. 7B).

T. similis, T. primitivus and *T. hooperi*

Light-microscopy observations of series of transverse sections of several male specimens of *T. similis* revealed the presence of a largely lateral to lateroventral thickening of the body wall together with a more or less pronounced dorsoventrally flattening of the body, probably resulting from muscular activity (Fig. 5D).

TEM microphotographs of transverse sections of male specimens of *T. primitivus* (Fig. 6) in the region of retracted spicules and in the tail revealed a narrow electron-dense band at the inner base of the outer unit of the multilaminate cuticle layer 6 and its connection with the mid-unit of layer 6. This electron-dense band, or layer, gradually enlarges from laterodorsal to a maximum width lateroventrally on the body, then narrows to a thin band subventrally and ventrally. At the level of the ventral field, the body cuticle is similar to that in *P. pachydermus*, with the outer unit (= with obvious banding) of layer 6 as main component.

In the fine structure of *T. hooperi*, there was some difference in the position of the cuticular thickening between layers 4 and 5 in the spicule and tail region. Midventrally, layer 6 forms 50% of the body cuticle (Fig. 7B, C).

Discussion

The present study of the fine structure of the body cuticle of five species belonging to the two didelphic genera in Trichodoridae supports and extends the recent observations by Mounport *et al.* (1997). Our results confirmed the unique character of the cuticle structure in Trichodoridae, a feature that separates this family from the Dorylaimina.

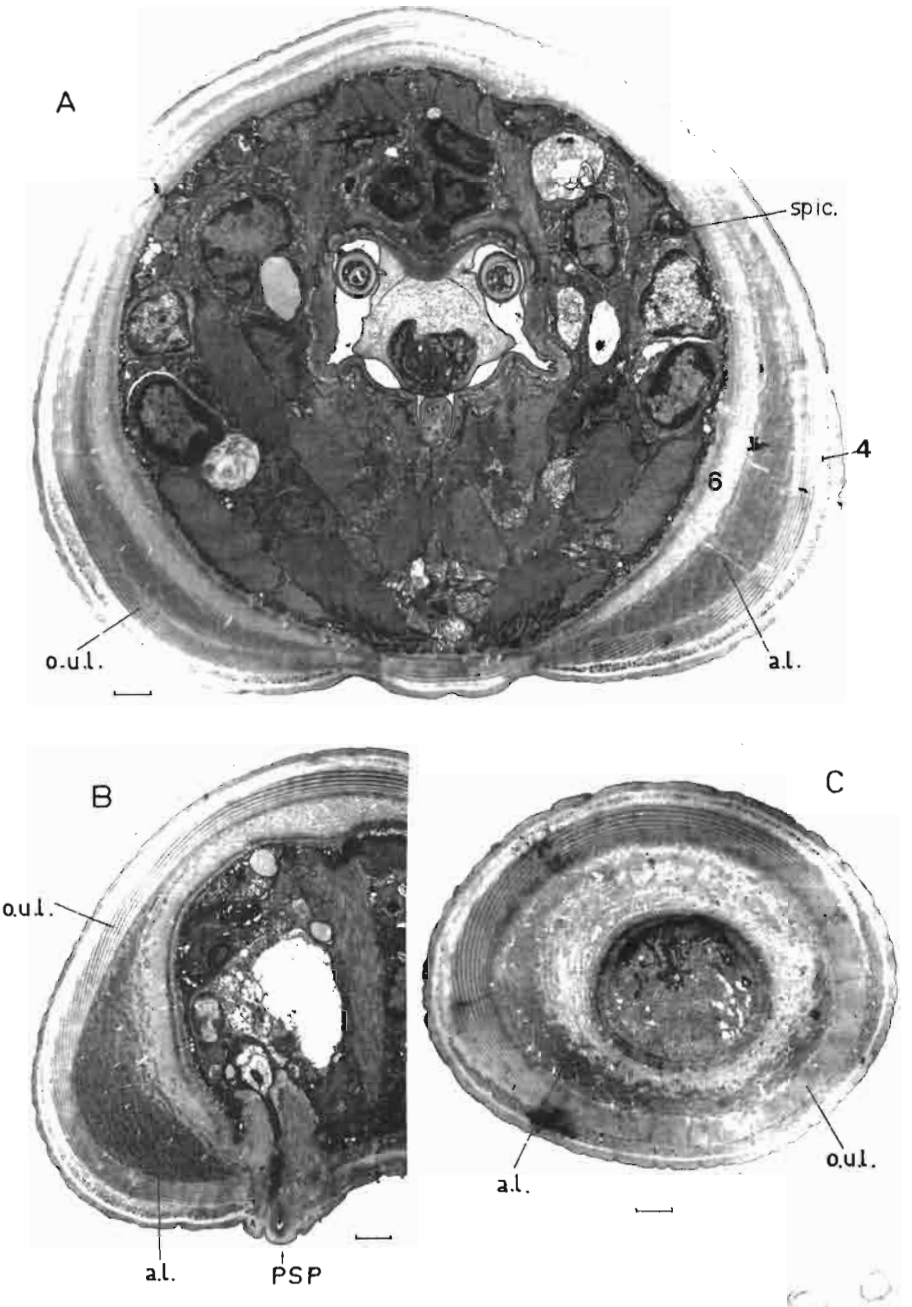

Fig. 6. *Ultrastructure of body cuticle in posterior body region of* Trichodorus primitivus *males. A: Spicule region; B: At level of a postcloacal papilla; C: Subterminal on tail (Scale bar = 1 µm; with indication of layers 4 and 6, a.l. = additional layer; o.u.l. = outer unit of layer 6; PSP = postcloacal supplement; spic. = spicule).*

There are some marked differences in the fine structure of the body cuticle in the region of retracted spicules of specimens belonging to either *Paratrichodorus* or *Trichodorus*. This result confirms the diagnostic value of the feature presence *vs* absence of caudal alae, for differentiating the didelphic genera.

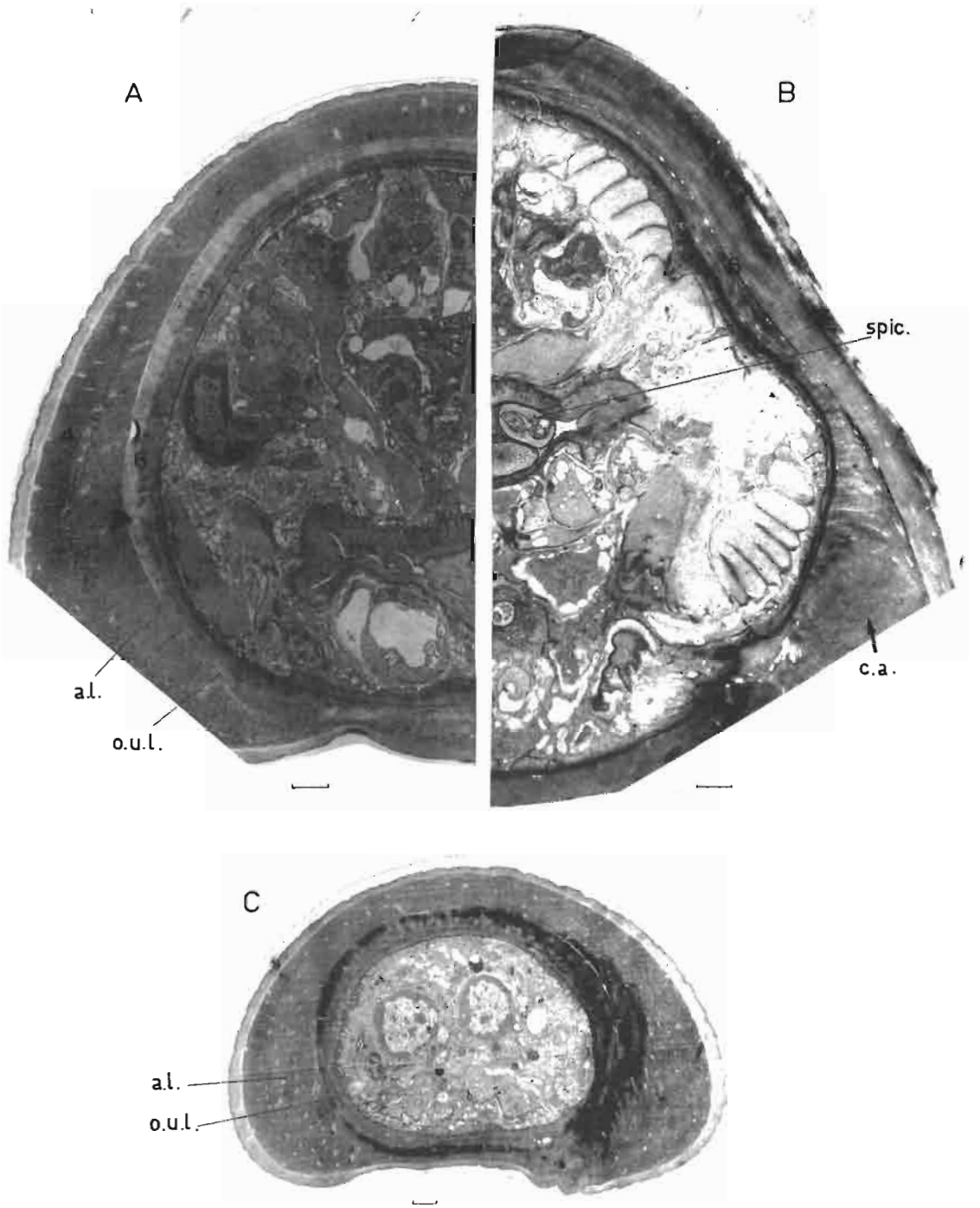

Fig. 7. *Ultrastructure of body cuticle in males at level of caudal alae and tail of* Trichodorus hooperi *(A, C) and* Paratrichodorus pachydermus *(B). A, B: Spicule region; C: At level of a postcloacal papilla (Scale bar = 1 µm; with indication of layers 4 and 6; a.l. = additional layer; c.a. = caudal alae; o.u.l. = outer unit of layer 6; spic. = spicule).*

In the *Trichodorus* species studied, the thickening of the body cuticle is restricted to the region of the retracted spicules and tail, *i.e.*, the posterior body region which shows a more or less pronounced dorso-ventral flattening of the body. Information was not obtained on the nature of the substance forming the 'rudimentary caudal alae'. The enlargement of the body wall is possibly induced by muscular activity; however, the amount of additional tissue seems rather large as compared with a hypothetical reconstruction of a non-flattened transverse section from the more anterior body regions.

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Resistance against the potato cyst nematode *Globodera pallida* systemically induced by the rhizobacteria *Agrobacterium radiobacter* (G12) and *Bacillus sphaericus* (B43)

Katja HASKY-GÜNTHER, Sabine HOFFMANN-HERGARTEN and Richard A. SIKORA

Universität Bonn, Institut für Pflanzenkrankheiten, Phytomedizin in Bodenökosystemen, Nussallee 9, 53115 Bonn, Germany.

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Summary – *Bacillus sphaericus* strain B43 and *Agrobacterium radiobacter* strain G12, selected from the potato rhizosphere, significantly reduced *Globodera pallida* juvenile penetration into potato roots. To better understand the basis of antagonism, the mode-of-action of the bacterial antagonists was investigated. The ability of the rhizobacteria to stimulate induced systemic resistance against the nematode was demonstrated in split-root-trials, where the root system of a single potato plant was spatially divided into two separate parts. Induced systemic resistance in the split-root system was caused by live and heat-killed bacterial cells of both bacteria strains. Culture filtrates of B43 had the same effect but G12-culture filtrates did not. Therefore, it was not possible to demonstrate the presence on the leaf surface of a basipetal systemic mechanism affecting the root system.

Résumé – ***Résistance au nématode à kyste de la pomme de terre* Globodera pallida *induite de façon systémique par les rhizobactéries* Agrobacterium radiobacter *(G12) et* Bacillus sphaericus *(B43)*** – *Bacillus sphaericus* souche B43 et *Agrobacterium radiobacter* souche G12 provenant de la rhizosphère de pomme de terre diminuent significativement la pénétration des juvéniles de *Globodera pallida* dans les racines de pomme de terre. Pour mieux comprendre les bases de cet antagonisme, le mode d'action de ces bactéries antagonistes a été étudié. La capacité des rhizobactéries à stimuler une résistance induite au nématode a été démontrée grâce à un dispositif de division du système radiculaire dans lequel la masse radiculaire d'un seul plant de pomme de terre est séparée physiquement en deux parties. La résistance induite de façon systémique dans un tel dispositif est provoquée par l'une et l'autre bactéries, tant vivantes que tuées par la chaleur. Les filtrats de culture de B43 provoquent le même phénomène, mais non ceux de G12. L'existence d'un mécanisme systémique affectant basipétalement le système radiculaire à partir des feuilles n'a pu être démontrée.

Keywords : *Agrobacterium radiobacter*, antagonistic rhizobacteria, *Bacillus sphaericus, Globodera pallida*, systemic resistance, nematodes, split-root system.

Public concern over pesticide use and limited availability of such products have heightened interest in biological control of plant parasitic nematodes. Since the rhizosphere provides the first line of defence for roots against nematode attack, it is generally considered that rhizosphere bacteria are ideal biocontrol agents. Their ability to multiply and spread in the rhizosphere environment, to colonize potential infection-sites on the root and possibly to act by direct contact with the parasites are characteristics that make them useful agents for nematode management. Initial investigations on antagonistic rhizobacteria against plant-parasitic nematodes include works by Zavaleta-Meija and Van Gundy (1982), Oostendorp and Sikora (1986), and Racke and Sikora (1992). Rhizobacteria with antagonistic potential against potato and sugarbeet cyst nematodes and root knot nematodes were selected, which reduce early infestation processes. The pale potato cyst nematode *Globodera pallida* is of particular interest to nematology as a target for biocontrol, because of a lack of useful crop resistance and acceptable chemical control methods.

Two bacterial strains, *Agrobacterium radiobacter* (G12) and *Bacillus sphaericus* (B43), were shown to reduce penetration of the potato cyst nematode *G. pallida* in greenhouse and field experiments by up to 30% - 40% (Racke & Sikora, 1992). Similar results were obtained by Hackenberg (1993). To understand and improve the effectiveness of this biological control system, it is important to determine the underlying mode-of-action. Competition for nutrients supplied by root exudates probably occurs in most interactions between bacteria and pathogens on the root surface (Elad & Baker, 1985; Elad & Chet, 1987). This factor is involved to some (small) degree in the biocontrol of fungal pathogens by bacteria. In the pathosystem rhizobacteria/plant parasitic nematode, completely different mechanisms are at play. Many rhizobacteria produce antibiotics, siderophores, HCN, and other toxic compounds which could be involved in biologi-

cal control. The ability of bacteria to envelop or bind to root-surface lectins possibly interfere with nematode-host-recognition and, therefore, penetration (Oostendorp & Sikora, 1990). In addition, these authors also demonstrated a reduction or alteration of root exudate hatch stimulation after incubation with several bacterial isolates.

Recent studies have shown that some rhizobacteria are also able to induce systemic resistance in plant to microbial pathogens (Wei *et al.*, 1991). Induced resistance can be triggered by different inductors. Various cell structures, abiotic compounds such as isonicotinic acid, salicylic acid and their derivatives, and extracellular structures of micro-organisms and their metabolites are known as elicitors of induced resistance in different systems (White, 1979; Smith & Metreaux, 1991; Schönbeck *et al.*, 1993; Kessmann *et al.*, 1994). The purpose of this study was to determine, whether the rhizobacteria strain *A. radiobacter* G12 and *B. sphaericus* B43 or their metabolites are able to incite systemically induced resistance in potato plants toward the potato cyst nematode *G. pallida*.

Material and methods

General techniques

Nematodes

G. pallida was originally obtained from a field population and was maintained continually on potato plants. Nematodes for inoculum production were multiplied in 3 dm^3 pots in the greenhouse on the potato cv. Hansa in infested soil containing 1500 eggs and juveniles/100 g soil. After 3 months, shoots of the plants were discarded and the soil, containing newly formed cysts, was stored for at least 9 months to overcome diapause. Cysts were extracted by a wet sieve decantation technique (modified by Ayoub, 1980) and separated from organic material by $MgSO_4$-flotation procedures. Suspensions of eggs and juveniles were separated from the cyst wall debris in a tissue homogenizer.

Bacteria

Both rhizobacteria strains, *A. radiobacter* (G12) and *B. sphaericus* (B43) were maintained for long-term storage at -80°C in Microbank (Pro-Lab Diagnostic). Cultures to be used for testing were transferred to liquid culture media. *B. sphaericus* was cultured in Tryptic Soy Broth (3%, pH 7.2), *A. radiobacter* in King's B Medium (pH 5.8) (King *et al.*, 1954) on a rotary shaker at 24°C for 24 h in an incubator. After incubation the bacterial suspension was centrifuged at 5400 *g* and the pelleted bacterial cells resuspended in sterile 25% concentrated Ringer-solution (Merck). Optical cell density of the bacterial suspensions was adjusted with a spectral photometer to $OD_{560} = 2.0$, which corresponds to 1.8×10^{10} cfu ml^{-1} (B43) and 1.2×10^{10} cfu ml^{-1} (G12). Potato roots were inoculated by pipetting 2.5 ml of a bacterial suspension onto the soil surface. Controls were treated with Ringer-solution.

Rifampicin-resistant mutants of both strains were selected by streaking *B. sphaericus* on Tryptic Soy Agar (TSA) and *A. radiobacter* on King's B Agar (KB) supplemented with 100 and 200 ppm Rifampicin, respectively. Then, mutants were multiplied in 100 or 200 ppm Rifampicin supplemented liquid media. The inoculum preparation and inoculation techniques were the same as for the wild strains outlined above.

Culture filtrates used for inducing experiments were obtained from the liquid culture media after removal of bacteria cell by filtration through a series of nitrocellulose filters down to a final pore size of 0.2 µm. Heat-killed bacteria cells were produced by autoclaving bacteria 20 min at 120°C at 1.2×10^5 Pa pressure.

Experimental evaluation

Nematode penetration was determined 21 days after nematode inoculation when the nematodes have reached the J3 or J4 developmental stage. Both parts of the split-root system were cut off separately and rinsed with tap water. Fresh root weight was determined after blotting off free water. Nematode penetration rates were determined by boiling roots in 0.1% lactic acid fuchsin. Stained roots were then homogenized in an Ultra-Turax (IKA-Werk) and the number of juveniles in the roots were counted under a stereomicroscope.

Experimental design

Split-root system

Potato plants were grown in a three-pot-system with each pot measuring 8 cm in diameter (Fig.1). The potato seed was put in the upper pot and its roots grew through two openings in the bottom of the upper pot and spread to the lower two pots. This system makes it easy to separate the root system into two fractions. Our goal was to prevent movement of inoculated bacteria from one root fraction into the other. The efficacy of the system for preventing bacteria movement from one root fraction to the other was tested using antibiotic-resistant mutants of both bacteria strains.

There were six or seven replications for each experiment. Potatoes cv. Hansa were grown in sterile fine quartz sand. Plants were 2 to 4 weeks old at the onset of the experiments. Depending on the time of year, the plants were cultivated either in climatic chambers at 21°C or in the greenhouse.

Rifampicin resistant mutants of *B. sphaericus* and *A. radiobacter* (10^{10} cfu) were applied onto one side of the split-root-system. After 3 and 16 days of incuba-

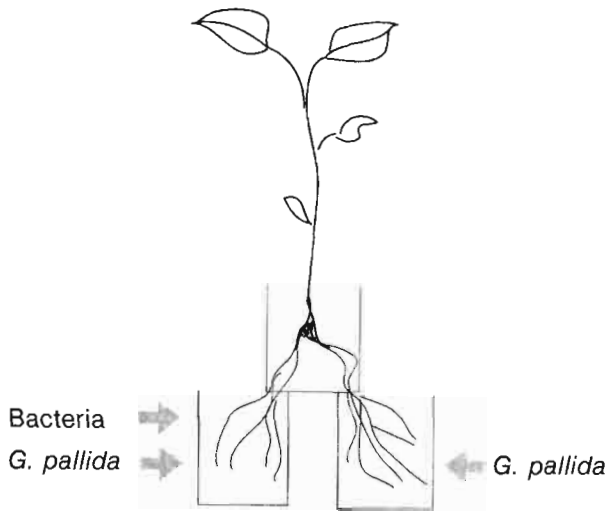

Fig. 1. *Potato plant grown in a three-pot split-root system where roots are separated in two fractions (Bacteria were applied to one root fraction; after 24 h incubation, nematodes were inoculated to both sides of the root system to examine systemic induced effects of the bacteria against* Globodera pallida*).*

tion at 21°C in the climate chamber or the greenhouse, the presence of the mutants in the rhizosphere and rhizoplane was determined in both segments of the root system. The roots in each lower pot were cut and sand particles were carefully washed away with tap water. The roots were then thoroughly washed in saline buffer. The resulting solution was spread over TSA containing 100 ppm Rifampicin for *B. sphaericus* and KB Agar with 200 ppm Rifampicin for *A. radiobacter*. No movement of bacteria to the untreated section of the root system was detected in repeated tests.

Resistance induction – roots

In the first experiment, both sides of the root system were inoculated with juveniles to determine whether control occurred when *i*) the nematodes were in direct contact with the bacteria and *ii*) on the bacteria-free fraction of the root system, which would indicate induced resistance.

A 2.5 ml solution of viable bacteria cells containing either 1.8×10^{10} *B. sphaericus*/ml or 1.2×10^{10} *A. radiobacter*/ml was applied to one side of the split-root system. After 24 h incubation, a suspension containing 1500 nematode eggs and juveniles was inoculated to both sides of the root system. The nematode inoculum was pipetted onto the sand near the roots in the lower pots. Control plants were treated with 25% concentrated Ringer-solution, which was also used to resuspend the bacteria. Previous experiments have demonstrated that there was no difference between water- and Ringer-treatment concerning the penetration of nematodes into the roots. Plants were incubated in the growth chamber or the greenhouse at 22°C with 16 h illumination until evaluation.

In the second experiment, the bacteria were applied to one side of the root system. After 24 h incubation, a suspension containing 1500 nematode eggs and juveniles was applied only to the bacteria-free side of the split-root system. The experiment was designed to exclude bacteria from possibly entering the root through wounds produced by nematode penetration.

Resistance induction – leaves

Induced systemic resistance in plants has been demonstrated in many plant-pathogen systems. Very often elicitor-induced resistance appeared after leaf inoculation (Guedes *et al.*, 1980). In the present case, leaf inoculation with bacteria would be easier to use than root inoculation for field experiments. To determine whether *B. sphaericus* or *A. radiobacter* have the ability to induce resistance in roots after leaf application, bacterial suspensions or bacteria culture filtrates were spread onto the upper and lower side of the leaves of potato plants with a fine paint brush. To promote dispersion and adhesion of the bacteria to the leaf surface, 0.1% methylcellulose was added to the bacterial suspension. Control plants were treated with sterile water or Ringer solution. After application of bacteria onto the leaves, transparent polyethylene bags were placed over the plants to maintain high humidity and improve conditions for micro-organism survival. Two days later, 30 cysts of *G. pallida* were inserted into the soil by placing the cysts between 100 µm mesh gauze sandwiched in a slide frame. Cysts were chosen as the inoculum because they provide a higher rate of penetration than egg and juvenile suspensions.

Inducing agents

An attempt was made to determine which bacterial component can or cannot elicit resistance. Viable cells, heat-killed cells and culture filtrates of both bacteria strains were examined for their ability to induce systemic resistance toward *G. pallida*.

A 2.5 ml solution of each component was applied in separate treatments to one side of a split root. One day later, 30 cysts of *G. pallida* in slide frames were inserted into the soil on the untreated side of the root system.

Results

RESISTANCE INDUCTION – ROOTS

Experiment 1

• *B. sphaericus* : The application of the bacterial strain to one side of a split-root system followed by

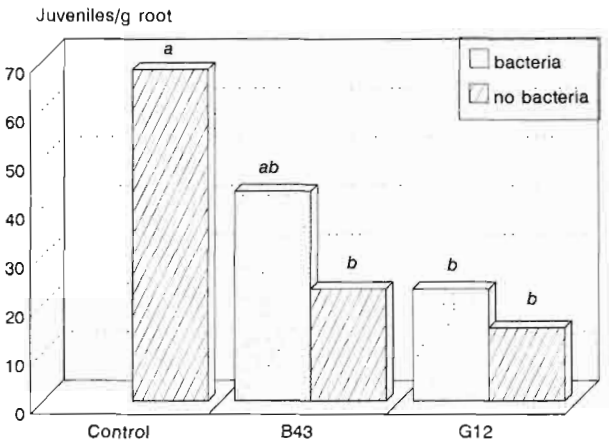

Fig. 2. Penetration of Globodera pallida *into the bacteria treated and into the untreated root section in a split root system. The bacteria used were* Bacillus sphaericus *(B43) and* Agrobacterium radiobacter *(G12) (Mean values with different letters are significantly different; Duncan's Multiple Range Test; $P \leq 0.05$; $n = 7$).*

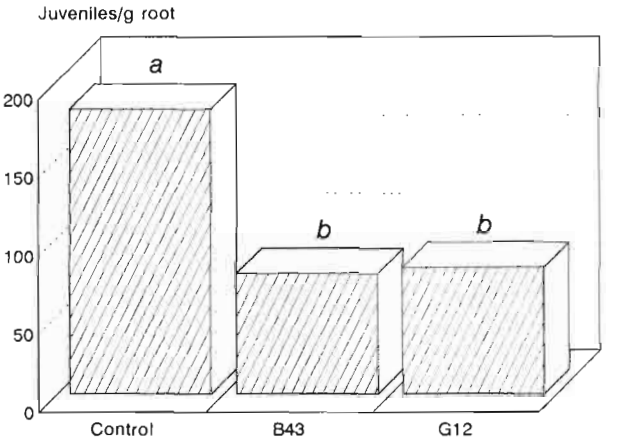

Fig. 3. Number of juveniles of Globodera pallida *which had invaded the roots of the untreated side of a split root system. The other side was inoculated with* Bacillus sphaericus *(B43) and* Agrobacterium radiobacter *(G12). (Mean values with different letters are significantly different; Duncan's Multiple Range Test; $P \leq 0.01$; $n = 7$).*

inoculation of *G. pallida* to both parts of the split-root system resulted in significant reductions of nematode penetration into both root sections (Fig. 2). Nematode penetration was reduced by about 37% on the side of the root system where both *B. sphaericus* and *G. pallida* were present. It was reduced by 67% in the bacteria-free side of the split-root system.

• *A. radiobacter* : The application of *A. radiobacter* to one half of the split-root system caused a significant decrease of 67% in nematode penetration (compared to control) on the side of the root system where both the bacteria and the nematodes were present. Penetration on the bacteria-free side of the split-root system was reduced by about 78% when compared to absolute control.

Experiment 2

The *Bacillus sphaericus* treatment caused a 58% decrease in nematode penetration in the bacteria-free side. *A. radiobacter* caused a 55% decrease (Fig. 3). No effects on fresh root weights were detected in either experiments.

Resistance induction - leaves

The bacteria strains and their culture filtrates exhibited no basipetal activity in inducing resistance to nematode root infestation after inoculation onto the leaf surface. On the contrary, leaf treatment with culture filtrates of both bacteria led to nonsignificant increases in nematode penetration (Table 1).

Inducing agents

The treatment of split-root systems with living or dead bacteria cells and culture filtrates of *B. sphaericus* and *A. radiobacter* led to different induced resistance reactions toward *G. pallida*. Inoculation of viable cells of *B. sphaericus* or *A. radiobacter* resulted in a significant reduction in nematode penetration: 77% with *B. sphaericus* and 59 % with *A. radiobacter*. With both bacteria, heat-killed cells produced the same effect as living cells (Fig. 4).

The culture filtrate of the two bacteria had very different effects on the expression of induced resistance. The culture filtrate of *B. sphaericus* applied to one side of the split-root system induced an 84% reduction in nematode penetration per gram of root in the untreated side. In contrast, culture filtrates of *A. radiobacter* did not cause any systemic induced resistance against the nematodes in the split-root tests.

Discussion

Previous studies have shown that specific rhizosphere bacteria have an antagonistic activity against various species of plant-parasitic nematodes. Oostendorp and Sikora (1990) isolated a *Pseudomonas fluorescens* strain with antagonistic activity against the sugar beet cyst nematode *Heterodera schachtii*. Sikora (1992) reported on the antagonistic behaviour of a *Bacillus subtilis* isolate to *Meloidogyne incognita* on a number of crops. Racke and Sikora (1992) isolated two strains of

Table 1. *Effects of a leaf inoculation with* Bacillus sphaericus *(B43) and* Agrobacterium radiobacter *(G12) on the penetration of* Globodera pallida *into potato roots.*

Leaf treatment	Penetrated juveniles / root
Control H_2O + 0.1% methylcellulose	78 *ab*
Control Ringer + 0.1% methylcellulose	47 *b*
B43 + 0.1% methylcellulose	80 *a*
G12 + 0.1% methylcellulose	81 *a*

Mean values with different letters are significantly different (Duncan's Multiple Range Test; $P \leq 0{,}05$; n = 6).

rhizobacteria with antagonistic effects against the potato cyst nematode *G. pallida*. Little is known on the mode-of-action of such bacterial/nematode relationship (Sikora & Hoffmann-Hergarten, 1993). In the present study, two bacteria, *B. sphaericus* B43 and *A. radiobacter* G12 were tested for their ability to induce systemic resistance against *G. pallida*. In repeated tests, both bacteria caused large reductions in nematode penetration that were clearly systemically induced.

Systemic induced resistance has been reported in several host-pathogen systems (Kuc, 1990) and is defined as the process of active resistance dependent on physical or chemical barriers of the host plant, activated by biotic or abiotic inducing agents (Kloepper *et al.*, 1992). In earlier studies systemic resistance was usually induced against leaf pathogens. However, induced systemic resistance against root pathogens by root and stem induction has been reported by Gessler and Kuc (1982). Knowledge concerning induced resistance toward plant parasitic nematodes is scarce. Kiyohara (1986) obtained resistance against the pine wilt nematode *Bursaphelenchus xylophilus* after preimmunisation with an avirulent strain of the same nematode. Decker and Dowe (1989) reported induced tolerance in tomatoes against *Globodera rostochiensis* after infestation with *Heterodera schachtii*, which they speculated to be due to changes in plant physiology. Whether these results are based on real induced resistance or on competition or occupation of the same niche for survival is questionable. Glazer and Orion (1985) observed a 70-80% reduction in female development of *Meloidogyne javanica* after soil treatment with hydroxyurea. They attributed the effect to induced resistance. In the present study, bacteria inoculation onto leaf surfaces did not affect nematode penetration into roots. Therefore, it was not possible to demonstrate the presence on the leaf surface of a basipetal systemic mechanism affecting the root system. This could be explained by the origin of the bacteria strains used, as they were isolated from rhizosphere soil and, therefore, not adapted to leaf surface conditions.

In the present study, systemic resistance toward *G. pallida* was elicited by living as well as heat-killed cells of *B. sphaericus*. In addition, culture filtrates induced the same reaction against the nematodes, but to an even stronger degree. The resistance inducing activity of bacterial metabolites to diseases has been described in literature. Schönbeck *et al.* (1980) isolated a *Bacillus subtilis* strain whose metabolites are able to induce systemic resistance against powdery mildew on barley. Culture filtrates of several fungi and bacteria protected beans against bean rust *Uromyces phaseoli*. In our study, the weaker inducing activity of viable and dead cells could possibly be related to the lower content of active metabolites in cells than in culture filtrates.

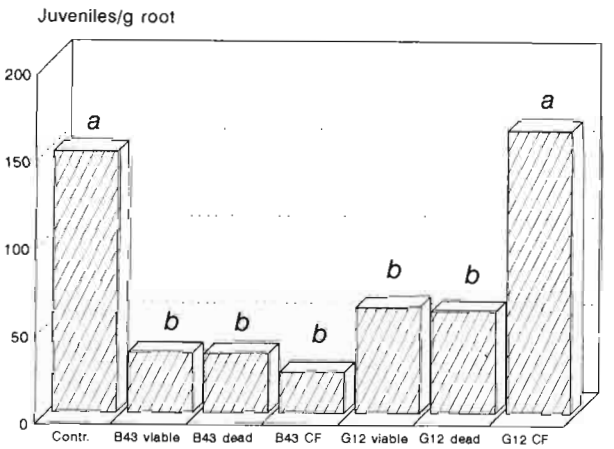

Fig. 4. *The number of juveniles of* Globodera pallida *which had invaded the bacteria-free side of a split-root system. The other side was inoculated with either viable bacteria, heat-killed bacteria or culture filtrates (CF) of* Bacillus sphaericus *(B4) and* Agrobacterium radiobacter *(G12). (Mean values with different letters are significantly different; Duncan's Multiple Range Test;* $P \leq 0.01$; $n = 6$).

Viable and heat-killed cells of *A. radiobacter* also led to a significant systemic reduction of nematode pene-

tration, whereas the culture filtrate of the bacteria showed no effect. Obviously, the mode of action of *A. radiobacter* differs from that of *B. sphaericus* and *A. radiobacter* metabolites are unable to elicit resistance. *A. radiobacter* is known to express exopolysaccarides (EPS) and lipopolysaccharides (LPS) on its surface (Sutherland, 1985). These surface structures of bacterial cell walls adhere specifically and strongly to cell wall structures of the plant (Costerton *et al.*, 1987) and are important virulence factors of plant pathogenic bacteria (Mansfield & Brown, 1986). They are also considered to affect physiological reactions in plants. EPS and LPS were found in living and in dead cells of the present G12 isolate, but not in culture filtrates (unpubl.). This could explain the activity of cells and the lack of activity of the bacterial metabolites. It is quite clear that the two bacteria strains examined in the present study have similar activity but different modes of action.

Before elucidating the exact mode of action of the induced resistance demonstrated here, a number of questions still need to be answered. Because the potato cyst nematode hatches only in the presence of a hatching factor produced by the host plant, the systemic induced effects may be due to alteration in exudates or the respective amounts of these factors in the exudates (Perry & Clarke, 1981). Secondly, specific exudates on the root surface are used by the cyst nematodes for host recognition (Zuckermann & Jansson, 1984). These components of the root exudates could be altered by the rhizobacteria inducing resistance. Thirdly, secreta in the root could be produced that repel the nematode from the root due to adverse environmental conditions as in resistant green manures. Fourthly, death of the juveniles may occur after penetration, caused by activity related to giant cell formation. These possibilities remain to be investigated. In conclusion, the results obtained here demonstrate for the first time that rhizobacteria have the ability to induce systemic resistance against cyst nematodes within the root system.

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New pathotypes of the beet cyst nematode (*Heterodera schachtii*) differentiated on alien genes for resistance in beet (*Beta vulgaris*)

Joachim MÜLLER

Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Nematologie und Wirbeltierkunde, Toppheideweg 88, 48161 Münster, Germany.

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Summary – Two populations (Schach 0 and Schach 1) of the beet cyst nematode, *Heterodera schachtii*, were used to select for new virulences to resistance genes transferred from *Beta* section *Procumbentes* into *B. vulgaris* ($Hs1^{web-7}$ and additional resistance information on chromosome 7). Both nematode populations were able to produce a few cysts which were multiplied for six successive generations on resistant plants. The two resulting populations, selected from either Schach 0 or Schach 1, were highly virulent on four monosomic additions with chromosome $^{web-7}$ or $^{pro-7}$. However, the population selected from Schach 0 was unable to break the resistance of translocation lines carrying resistance gene Hs1 from chromosome $^{pro-1}$, $^{pro-7}$ or $^{web-7}$, whereas the population selected from Schach 1 was still virulent on these translocation lines. A resistance gene $Hs2^{web-7}$ is postulated to exist on chromosome 7, having an epistatic effect on $Hs1^{web-7}$. The two new virulent pathotypes are named Schach 2 and Schach 1,2. © Orstom/Elsevier, Paris

Résumé – ***Nouveaux pathotypes du nématode à kyste de la betterave* (Heterodera schachtii) *différenciés sur des gènes de résistance étrangers introduits dans la betterave* (Beta vulgaris)** – Deux populations (Schach 0 et Schach 1) du nématode à kyste de la betterave, *Heterodera schachtii*, sont utilisées pour sélectionner de nouvelles virulences contre des gènes de résistance transférés de *Beta* section *Procumbentes* à *B. vulgaris* ($Hs1^{web-7}$ et d'autres caractères de résistance localisés sur le chromosome 7). Les deux populations du nématode ont produit quelques kystes sur des plantes résistantes et se sont multipliées sur ces mêmes plantes pendant six générations successives. Les deux populations en résultant, sélectionnées à partir de Schach 0 ou de Schach 1, montrent une très grande virulence à l'égard de quatre lignées d'addition monosomique avec le chromosome $^{web-7}$ ou $^{pro-7}$. Cependant, la population sélectionnée à partir de Schach 0, ne surmonte pas la résistance des lignées de translocation portant le gène de résistance Hs1 du chromosome $^{pro-1}$, $^{pro-7}$ ou $^{web-7}$, alors que la population issue de Schach 1 conserve sa virulence à l'égard de Hs1. Il est conclu qu'un gène de résistance $Hs2^{web-7}$, ayant un effet épistatique sur $Hs1^{web-7}$, existe sur le chromosome 7. Les deux nouveaux pathotypes virulents sont nommés Schach 2 et Schach 1,2. © Orstom/Elsevier, Paris

Keywords : *Beta procumbens*, *Beta vulgaris*, *Beta webbiana*, *Heterodera schachtii*, resistance, pathotype, virulence.

The beet cyst nematode (*Heterodera schachtii*) is a major pest in sugar beet production. As the use of nematicides is more and more restricted, alternative control methods are urgently needed. Therefore, attempts have been made in several countries to introduce monogenic resistance genes from three wild beet species of the *Beta* section *Procumbentes* into the sugar beet *B. vulgaris*. These resistance genes from *B. procumbens* Chr. Sm., *B. webbiana* Moq., and *B. patellaris* Moq. are inherited in a dominant way, but they are not easy to transfer into *B. vulgaris*. The research programmes have encountered crossing barriers, an extremely low frequency of introgression of alien genes into the genome of sugar beet, and a reduced sexual transmission of the introgressed genes. Savitsky (1975, 1978) was the first to produce monosomic additions (sugar beet with an added alien chromosome from wild beet, 2n = 19) and she later selected diploid plants (translocations, 2n = 18). She supposed that a single dominant gene was responsible for full nematode resistance. Morphological studies and isozyme analyses later proved the existence of six different chromosomes in the wild beet species carrying major resistance genes: chromosome 1 of all three wild beet species ($^{pro-1}$, $^{web-1}$, $^{pat-1}$) and chromosome 7 of *B. procumbens* ($^{pro-7}$) and *B. webbiana* ($^{web-7}$) (Löptien, 1984; Lange *et al.*, 1988; Reamon-Ramos & Wricke, 1992; Reamon-Büttner, 1994). Another monosomic addition with incomplete resistance had been described earlier (Jung *et al.*, 1986; Jung & Wricke, 1987). Here, resistance was found to be located on the added chromosome 8 of *B. webbiana* (Reamon-Ramos & Wricke, 1992; Reamon-Büttner, 1994).

Morphological studies and isozyme analyses do not give information on the quality of the resistance genes. Nematode-based investigations have been essential in identifying the specificity of different

genes. Using a virulent *H. schachtii* population selected by Müller (1992), it was possible to demonstrate the existence of different resistance genes on chromosomes $^{pro-1}$ and $^{pro-7}$, giving a specific response to the nematode populations. It was proposed to use the symbol Hs for resistance genes controlling *H. schachtii*. The different reactions to pathotypes are specified by different numbers, whereas the origin of the alien genes is indicated by an additional superscript. The two genes differentiated by the virulent nematode pathotype were named $Hs1^{pro-1}$ and $Hs2^{pro-7}$ (Lange *et al.*, 1993). Further investigations proved the existence of the same genes in *B. procumbens* and in *B. webbiana* and also of a different response in resistance between monosomic additions and translocation lines. It was concluded that resistance gene Hs1 occurs on $^{pro-1}$ as well as on $^{pro-7}$, with chromosome 7 carrying additional resistance information (Klinke, 1995; Müller & Klinke, 1996; Klinke *et al.*, 1996).

Molecular genetic technologies are also being used to isolate the alien genes for beet cyst nematode resistance and to transfer such genes to cultivated beet (Jung *et al.*, 1990, 1992; Salentijn *et al.*, 1992). After successful identification and isolation of resistance gene $Hs1^{pro-1}$ of *B. procumbens*, the gene was transferred into *B. vulgaris* and proved to be effective against *H. schachtii* (Cai *et al.*, 1997). Resistance to *H. schachtii* transferred by molecular genetic technologies is not yet available to farmers. The classical breeding techniques, however, have already resulted in marketable resistant sugar-beet hybrids and the first resistant variety was registered in France in 1996 (Mahfoud *et al.*, 1996).

Breeders used to consider *H. schachtii* to be a constant, homogeneous factor in the host-parasite interaction. From an evolutionary point of view, however, this seems unlikely. On selected resistant monosomic additions carrying chromosome $^{pro-1}$ of *B. procumbens*, a low number of cysts is usually formed. This was interpreted as an indication for the occurrence of virulence genes at low frequency in natural *H. schachtii* populations. Cysts from resistant plants were collected and tested again. After six nematode generations on plants carrying resistance of chromosome $^{pro-1}$, a virulent population was selected (Müller, 1992). This population is called a pathotype if the plant-nematode interaction is based on a gene for gene relationship (Trudgill, 1986, 1991). Further investigations showed that the virulent population was able to break resistance from chromosome $^{pro-1}$, but not from chromosome $^{pro-7}$ of monosomic additions. It was therefore considered to be a pathotype and named Schach 1 (Müller & Klinke, 1996).

The present study was undertaken to determine if more virulences exist in *H. schachtii* and if they can be used for a further differentiation of resistances derived from the *Beta* section *Procumbentes*.

Materials and methods

Nematode populations

A collection of 146 *H. schachtii* populations, obtained from different geographical origins in Germany and other European countries, is maintained at the Nematology Institute in Münster. All the populations were started from soil samples of 2-3 kg, taken from individual fields known to be infested with *H. schachtii*. Avirulent populations are maintained on rape (*Brassica napus* var. *napus*) cv. Velox. Populations virulent to resistance gene $Hs1^{pro-1}$ are multiplied on a diploid homozygous resistant translocation line with gene $Hs1^{pro-1}$. This translocation line originates from B 883 (Heijbroek *et al.*, 1988), it was called Pro1 by Müller (1992), and was obtained from the breeding company as KWS-NR1. Nematode population N° 129 was used for the experiments reported here. The avirulent population is called Schach 0, the virulent one is called Schach 1 (Müller & Klinke, 1996). Details on maintaining the populations have been described previously (Müller, 1992).

Plant materials

Resistance to *H. schachtii* in the plant material listed in Table 1 came from two wild *Beta* species of the section *Procumbentes*, *B. procumbens* Chr. Sm. and *B. webbiana* Moq. (2n = 18). Eight resistant stocks originating from interspecific breeding programmes were used. Seven stocks had been selected either from resistant monosomic additions (2n = 19) or from diploid translocation lines (2n = 18), both interbred with diploid susceptible sugar beet. Because of incomplete transmission of resistance, particularly in the addition lines, the offspring segregate to less than 50% resistant plants. The transmission rates of resistance are reported to be only 10-20% in addition lines (Lange *et al.*, 1990). The eighth stock, KWS-NR1, which contains resistance gene $Hs1^{pro-1}$, is a diploid line with homozygous resistance; its progeny is 100% resistant. One plant of the monosomic addition 14026, which contains the complete chromosome $^{web-7}$, was tested twice for its resistance to Schach 0 and was then cloned in order to obtain sufficient plant material for selection of virulence in *H. schachtii*. Cloning of the monosomic addition was done by Dieckmann-Heimburg Saatzucht, Nienstädt. Seeds of monosomic additions and diploid translocation lines were obtained from Hannover University, Institut für Angewandte Genetik. Seeds of KWS-NR1 were produced by Kleinwanzlebener Saatzucht AG, Einbeck. In all experiments, the susceptible sugar beet cv. Désirée was used as control.

Table 1. *Genetic characteristics, names of plant material, and sources of resistance.*

	Name of beet stock	Origin of added chromosome or of resistance gene	From wild *Beta* species
Monosomic additions	6019	Chromosome 7	*B. webbiana*
(2n = 19)	6022	Chromosome 7	*B. webbiana*
	8605	Chromosome 7	*B. procumbens*
	14026	Chromosome 7	*B. webbiana*
	12572	Chromosome 7	*B. procumbens*
Diploid translocation	Web 11	Chromosome 7	*B. webbiana*
lines (2n = 18)	Pro 3	Chromosome 7	*B. procumbens*
Diploid homozygous resistant translocation line (2n = 18)	KWS-NR 1	Chromosome 1	*B. procumbens*

EXPERIMENTAL DESIGN

Tests for resistance were carried out as described by Müller (1997). Loess was used as a substrate for plant cultivation in all experiments. It was obtained from the brown coal open-cast mining near Garzweiler/ Rhineland, from soil depths of 3-5 m. Steiner solution was added for better nutrient supply. The seed was treated with thiram to prevent infection with *Phoma betae*, then germinated in loess. PVC tubes (2 × 4 × 12 cm) were put into boxes containing 120 tubes each (in ten rows of twelve tubes). Single seedlings were transplanted into the PVC tubes filled with loess. The different beet stocks were planted in lines of twelve plants and these were randomised over all the boxes. The total number of plants per beet stock is indicated in Figs 2 and 3. Fourteen days later, 1000 juveniles of *H. schachtii* were inoculated per plant. The plants were cultivated in a greenhouse at *ca* 20°C, with 14 h/day artificial light between October and March. The tests were evaluated 6 weeks after inoculation. The loess was washed through a kitchen sieve (1 mm), then through a 100 µm sieve. The cysts were collected on the 100 µm sieve, together with some larger loess particles. Both, cysts and loess particles, were transferred onto a smaller 100 µm sieve and rinsed in 20% acetic acid for 5 min. The loess particles dissolved quickly and were removed by shaking the sieve in water. The cysts were transferred to a filter paper and counted under a stereoscopic microscope at 10 × magnification.

To gather reliable information on the resistance of a beet stock with respect to the virulence of the nematode population inoculated to this stock, it was necessary to test a high number of plants due to the often low transmission rates of resistance. The test conditions must be suitable to distinguish between resistant and susceptible plants, which means that their frequency distributions should not overlap. All conclusions concerning resistance/virulence depend on the correct classification of plants as resistant or susceptible.

The segregating addition and translocation lines could not be analysed statistically. All data are therefore presented in frequency distributions with a class width of ten cysts per plant. In all resistance tests, the susceptible cv. Désirée was used to obtain information on the test conditions. In general, it was found here that a minimum of 40 cysts per plant were needed. Lower cyst numbers indicated unfavourable test conditions and the risk of misinterpretation in the segregating breeding lines. Less than 30 cysts were found on resistant plants. The frequency distributions allowed a reliable distinction between susceptible and resistant plants.

Experiments and results

SELECTION FOR VIRULENCE

Two populations were used separately in this first screening for virulence to resistance located on chromosome 7: the avirulent 'natural' Schach 0 and the virulent Schach 1, which is able to break resistance gene $Hs1^{web-7}$. In Fig. 1, the different steps for virulence selection from Schach 0 are presented. The same procedure was applied when using Schach 1 instead of Schach 0. The first step was differentiation between resistant and susceptible plants in the addition line 14026 carrying the resistance gene $Hs1^{web-7}$, but also additional resistance information on chromosome 7. Both populations were able to produce a few

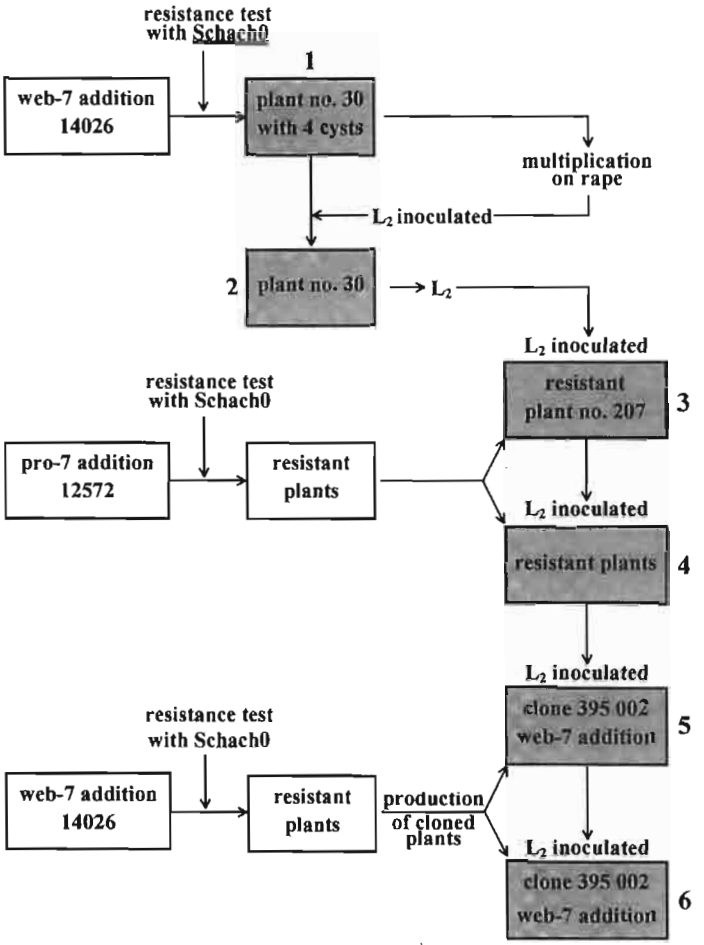

Fig. 1. *Selection for virulence to resistance located on chromosome 7 shown at the example of Schach 0. The numbers 1-6 indicate six successive generations.*

cysts on resistant plants of the monosomic addition 14026.

During the rest of the selection process, the two populations were again kept strictly separate. In the example in Fig. 1, Schach 0 produced four cysts on plant N° 30. These were multiplied on rape, the juveniles hatched and they were inoculated to the same plant N° 30. The resulting cysts constitute the second generation on resistant plant material. The third and the fourth generations were multiplied on addition line 12572, which carries chromosome $^{pro-7}$ of *B. procumbens*. This addition line was used as no more seeds of 14026 were available. To establish nematode generations five and six, a resistant plant of 14026 was cloned in cell cultures and the clone N° 395002, regenerated to normal plants, was inoculated with juveniles of the fourth generation. This clone was also used for the data presented in Table 2. After six generations, virulent populations had been selected. They were provisionally named Schach x (from Schach 0) and Schach y (from Schach 1).

The multiplication potential of these virulent populations is presented in Table 2. Resistant plants of clone 395002, grown in loess in 600 ml plastic pots, were inoculated with 3000 juveniles of Schach 0, Schach 1 and the two new virulent populations. Cysts were extracted 7 weeks after inoculation. Both populations, Schach x and Schach y, multiplied equally well and showed no difference in virulence. Only a few cysts developed from juveniles of Schach 0 or Schach 1.

Translocation lines Web 11 and Pro 3

Translocation lines Web 11 and Pro 3, carrying resistance genes $Hs1^{web-7}$ and $Hs1^{pro-7}$, respectively, reacted identically to the four nematode populations, as demonstrated by the frequency distributions in Fig. 2. The two beet stocks included both resistant and susceptible plants when inoculated with Schach 0. Schach 1 and Schach y break the resistance, in contrast to Schach x. The avirulence of Schach x was not expected as this nematode population was selected for virulence to chromosome 7, which carries resistance gene Hs1. More beet stocks with different sources of resistance were tested to explain this phenomenon.

Translocation line KWS-NR1

This diploid beet is homozygous for its resistance gene $Hs1^{pro-1}$, and consequently all plants were resistant when inoculated with Schach 0 (Fig. 3). Schach 1 overcomes the resistance, as anticipated, but Schach x is obviously avirulent. The numbers of cysts per plant produced by Schach y are not as high as expected but they do not indicate resistance in KWS-NR 1.

Chromosome-7 addition lines

Schach x and Schach y had been selected on a $^{web-7}$ addition (14026) and proved to be highly virulent (Table 2). To find an explanation for the unexpected results presented in Figs 2 and 3, the virulences of the two populations were checked on different beet stocks carrying the added chromosome 7. The additions 6019, 6022, and 8605, inoculated with the four nematode populations, segregated identically and the results are therefore combined in one frequency distribution (Fig. 3). The transmission rate of these beet stocks is low and consequently the majority of plants is susceptible, independent of the inoculated population. The distributions show, however, that these addition lines include plants resistant to Schach 0 and Schach 1, whereas all plants are considered to be susceptible to Schach x and Schach y. This confirms the results presented in Table 2.

Table 2. *Multiplication of four populations on cloned plants carrying chromosome 7 of* Beta webbiana *(monosomic addition line 14026).*

Population	Number of plants (n)	Mean of cysts/plant (± SD)
Schach 0	17	2.4 ± 2.1
Schach 1	17	1.9 ± 2.0
Schach x	18	335 ± 143
Schach y	18	321 ± 161

Discussion

Lange *et al.* (1993) postulated the existence of a resistance gene $Hs2^{pro-7}$ on chromosome 7 of the monosomic addition AN 101 as this beet stock proved to be resistant to the pathotype selected by Müller (1992). Later Klinke *et al.* (1996) demonstrated that only monosomic additions with the complete chromosome 7 give a resistant response to the pathotype but the diploid translocation lines Web 11 and Pro 3 are susceptible. They postulated the existence of more than one resistance gene on chromosome 7. As the resistance in Web 11 and Pro 3 is overcome by the pathotype - later called Schach 1 by Müller and Klinke (1996) -, it was concluded that chromosomes 1 and 7 carry the same or very similar resistance genes. Moreover genes $Hs1^{pro-7}$ and $Hs1^{web-7}$ were considered to be identical. This supports the assumption of a close relationship between *B. procumbens* and *B. webbiana* (Wagner *et al.*, 1989; Reamon-Ramos & Wricke, 1992). The results presented in Fig. 2 agree with this hypothesis.

However, the reactions of Web 11 and Pro 3 to infestation with the pathotype Schach x are inconsistent with the concept of a resistance gene $Hs1^{web-7}$ on the monosomic addition 14026. Schach x was selected on 14026 and it was able to break all resistance information of the added chromosome 7. At this point, the identity of $Hs1^{web-7}$ in Web 11 and in 14026 seemed doubtful and more information was needed. The results of further experiments (presented in Fig. 3) confirm those in Fig. 2: if chromosome 1 and chromosome 7 carry identical resistance genes and if, in addition, these genes from *B. procumbens* and from *B. webbiana* are homologous, then the translocation line KWS-NR 1 should give the same reaction as Web 11 and Pro 3 did. In fact, the population Schach x did not break resistance of KWS-NR 1 (Fig. 3).

As there was still no explanation for the unexpected results, the four nematode populations were tested on three other chromosome-7 additions. The combined data are presented in Fig. 3. The beet stocks segregated into resistant and susceptible plants when inoculated with Schach 0 and Schach 1, whereas Schach x and Schach y were able to break all resistance information. This fits with the virulence observed on addition 14026.

Another surprising fact was already evident during the process of selection for virulence. It was expected that, as the selection process started with pathotype Schach 1, an increase of virulence would occur. With Schach 0, this process was supposed to take much more time, if it occurred at all. The probability of

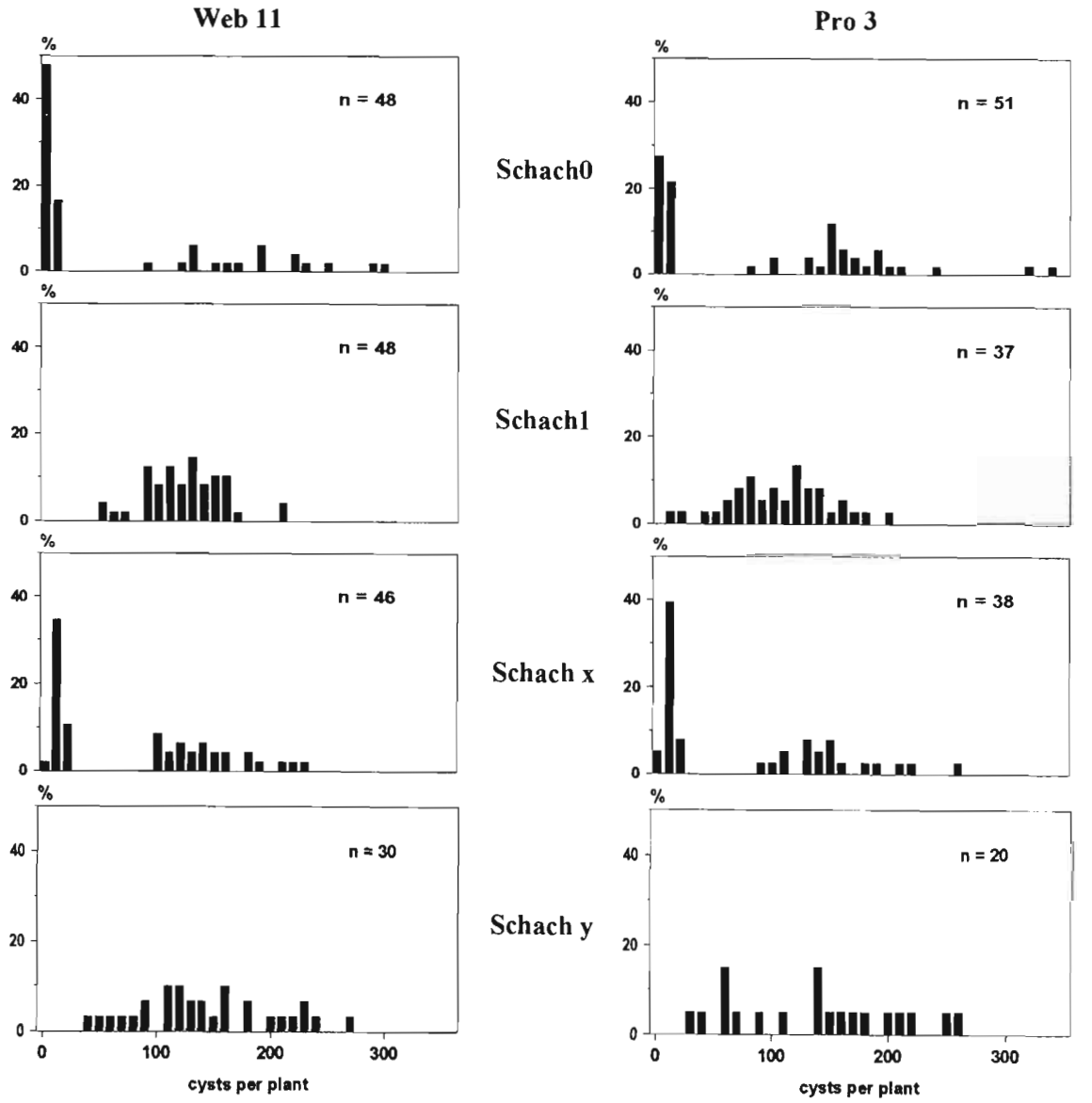

Fig. 2. Frequency distributions of cysts per plant for the translocation lines Web 11 and Pro 3 (both carrying Hs1 of chromosome 7) inoculated with four selected populations.

overcoming two independent resistance genes is much higher if one virulence is already present in the nematode. However, there was no difference in selection for virulence in the two populations as multiplication rates were almost the same (Table 2).

These contradictory observations can be explained by epistasis between the resistance genes. If resistance gene $Hs1^{web-7}$ is hypostatic in the presence of the other resistance gene, it will have no effect in the process of virulence selection. This would explain the rapid loss of resistance of 14026 to pathotype Schach 0 and it would also explain the lack of virulence of Schach x to Web 11, Pro 3 and KWS-NR 1. This epistatic gene is named $Hs2^{web-7}$. It is a dominantly inherited factor and it is probably identical with $Hs2^{web-7}$ postulated by Lange *et al.* (1993). Hs2 does not seem to be located in the vicinity of Hs1 on chromosome 7. All translocation lines carrying resistance

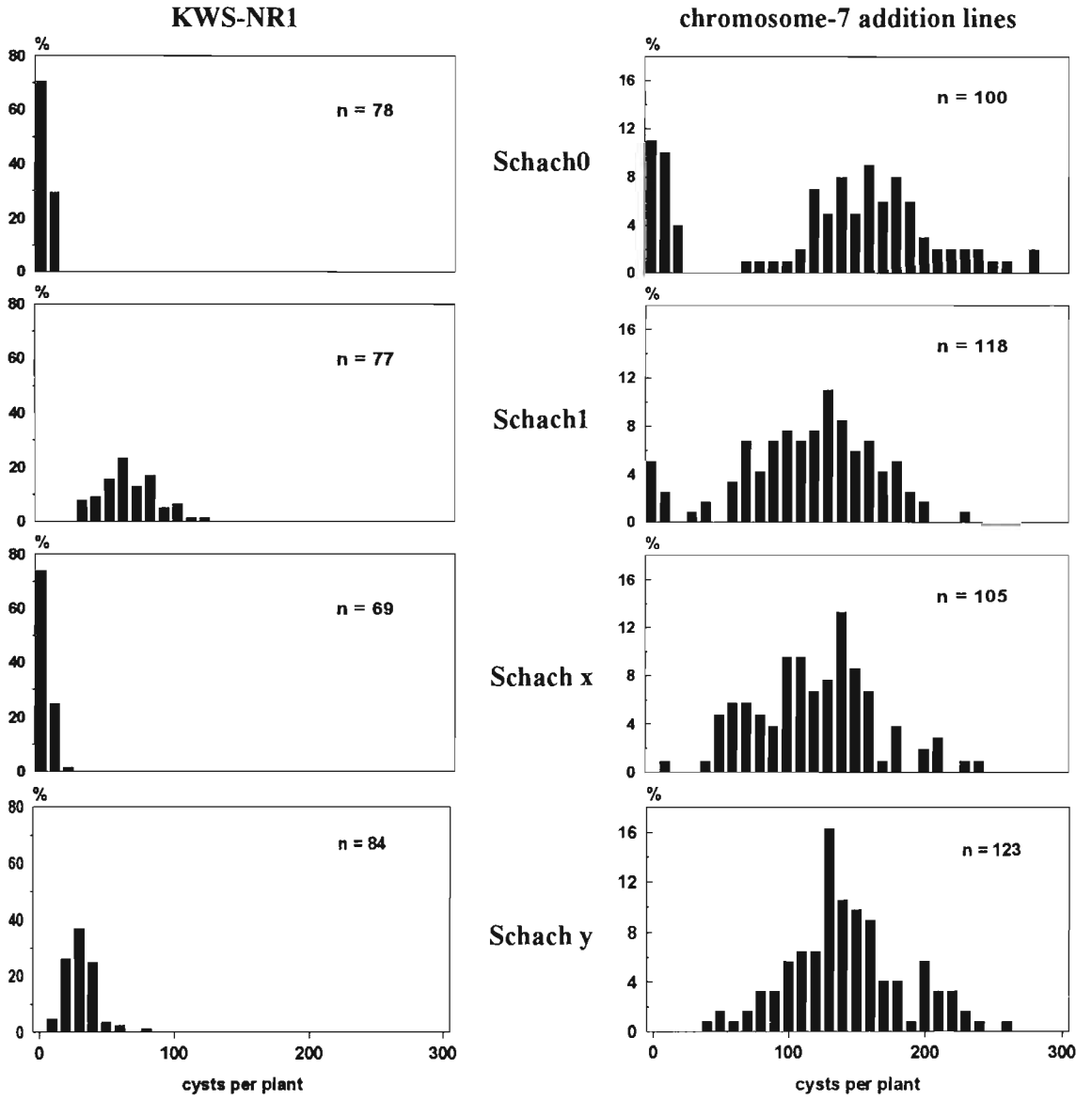

Fig. 3. *Frequency distributions of cysts per plant for the homozygous resistant translocation line KWS-NR1 and for chromosome-7 addition lines inoculated with four selected populations.*

of chromosome 7 proved to be susceptible to Schach 1, which indicates a low probability of these two genes being translocated together. According to the two resistance genes, the two new virulent *H. schachtii* populations, named previously Schach x and Schach y, are named Schach 2 and Schach 1,2 respectively. Schach 2 is virulent only to resistance gene Hs2. Schach 1,2 is able to overcome resistance of both genes, Hs1 and Hs2, even if they occur separately. Thus, we now have to distinguish between four pathotypes.

Until now, the virulent pathotypes have been selected only in the greenhouse, but we must expect to find them in field conditions as well if resistant sugar beets are grown intensively. It is important to realize that the virulence of Schach 1, which was already present when starting the selection process on 14026, was not lost after six generations without the

selection pressure of an active resistance gene Hs1 (Figs 2, 3). Therefore, it is doubtful whether alternating cultivars with different resistance genes would solve the problem of resistance-breaking pathotypes in practice. Combining different and independent resistance genes in the same cultivar is more promising, but also much more difficult to achieve.

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Morphometric studies on *Coomansus* Jairajpuri & Khan, 1977 (Nematoda: Mononchida) and descriptions of two new species from the Subantarctic region

Domingo JIMÉNEZ GUIRADO*, Wim M. WOUTS** and Antonio BELLO***

** Departamento de Biología Animal, Facultad de Ciencias, Avda San Alberto Magno s/n, Universidad de Córdoba, 14004 Córdoba, Spain,*

*** Manaaki Whenua - Landcare Research, Private Bag 92170, Auckland, New Zealand,*

*and *** Centro de Ciencias Medioambientales, C.S.I.C., Serrano 115 dplo., 28006 Madrid, Spain.*

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Summary – Four known and two new species of the genus *Coomansus* Jairajpuri & Khan, 1977, inhabiting different areas of New Zealand, the Antarctic Peninsula, and Subantarctic America, are studied. A morphometric analysis is carried out to differentiate between *C. gerlachei* (de Man, 1904), *C. mesadenus* (Clark, 1960), *C. composticola* (Clark, 1960), *C. intestinus* (Vinciguerra & La Rosa, 1990), *C. meridionalis* sp. n., and *C. magellanicus* sp. n. *C. meridionalis* sp. n. is characterized mainly by body length (2.10-2.65 mm), buccal cavity size (39-45 × 18-22 µm), apex of the dorsal tooth at 84-95 % of the total buccal cavity measured from the base, spicule length (94-110 µm), very slender lateral guiding pieces, conoid and ventrally curved tail with rounded terminus, and absence of spinneret. *C. magellanicus* sp. n. can be separated from the other species of the genus by the following combination of characters: body length (1.7-2.1 mm), buccal cavity size (32-36 × 18-22 µm), apex of the dorsal tooth at 76-80 % of the total buccal cavity from base, spicule length (97-98 µm), not very slender lateral guiding pieces, conoid and ventrally arcuate tail with broadly rounded terminus, and absence of caudal glands. © Orstom/Elsevier, Paris

Résumé – ***Étude morphométrique sur le genre* Coomansus *Jairajpuri & Khan, 1977 (Nematoda : Mononchida) et description de deux nouvelles espèces provenant de l'aire subantarctique*** – Quatre espèces déjà connues et deux nouvelles appartenant au genre *Coomansus* Jairajpuri & Khan, 1977, provenant de différentes régions de la Nouvelle Zélande, de la Péninsule Antarctique et de l'Amérique subantarctique, sont étudiées. Une analyse morphométrique a été réalisée en vue de différencier *C. gerlachei* (de Man, 1904), *C. mesadenus* (Clark, 1960), *C. composticola* (Clark, 1960), *C. intestinus* (Vinciguerra & La Rosa, 1990), *C. meridionalis* sp. n. and *C. magellanicus* sp. n. *C. meridionalis* sp. n. est principalement caractérisé par la longueur du corps (2,10-2,65 mm), la taille de la cavité buccale (39-45 × 18-22 µm), l'apex de la dent dorsale situé à 84-95 % de la longueur totale de la cavité buccale mesurée à partir de sa base, la longueur des spicules (94-110 µm), les pièces-guides latérales très minces, une queue conoïde, courbée ventralement, à extrémité arrondie, et l'absence de filière. *C. magellanicus* sp. n. peut être distingué des autres espèces du genre par la combinaison de caractères suivante: longueur du corps (1,7-2,1 mm), taille de la cavité buccale (32-36 × 18-22 µm), apex de la dent dorsale situé à 76-80 % de la longueur totale de la cavité buccale mesurée à partir de sa base, longueur des spicules (97-98 µm), pièces-guides latérales pas très minces, queue conoïde, arquée, à extrémité largement arrondie, absence de glandes caudales. © Orstom/Elsevier, Paris

Keywords: *Coomansus meridionalis* sp. n., *Coomansus magellanicus* sp. n., Mononchida, Nematoda, Subantarctica, taxonomy.

Five species of the genus *Coomansus* Jairajpuri & Khan, 1977 are known from the Antarctic and Subantarctic Territories and New Zealand Islands: *C. gerlachei* (de Man, 1904) Jairajpuri & Khan, 1977, *C. campbelli* (Allgén, 1929) Jairajpuri & Khan, 1977, *C. composticola* (Clark, 1960) Jairajpuri & Khan, 1977, *C. mesadenus* (Clark, 1960) Jairajpuri & Khan, 1977, and *C. intestinus* (Vinciguerra & La Rosa, 1990) Andrássy, 1993. These species were studied by Loof and Winiszewska-Slipinska (1993) and Andrássy (1993) who gave dichotomous keys for all large *Coomansus* species with the dorsal tooth located in the anterior half of the buccal cavity. Populations of some of these species were recorded from widely separated geographic areas, *e.g. C. campbelli,* a species described from Campbell Island, was reported by Mulvey and Jensen (1967) from Nigeria and by Coetzee (1968*b*) from South Africa. Mulvey and Jensen (1967) point out that their specimen differs from those collected near the type locality and described by Clark (1963). In our opinion, this specimen represents a different *Coomansus* species. However, Coetzee (1968*a*) later described her material as *Mononchus jugalis*, a species since transferred to the genus *Clarkus* (Jairajpuri, 1970; Jairajpuri & Khan, 1977) and synonymized

with *C. sheri* (Mulvey, 1967) by Andrássy (1983), a decision supported by de Bruin and Heyns (1992).

The present paper studies the rest of the abovementioned species, with a review of specimens previously described by Clark (1960, 1963), Chaves (1990), and Vinciguerra and La Rosa (1990) and with detailed descriptions and illustrations of more recent collections. *C. meridionalis* sp. n. and *C. magellanicus* sp. n. are described from subantarctic localities of New Zealand (Campbell Island) and Chile, respectively.

Materials and methods

For this study, the following type material was examined from The National Nematode Collection of New Zealand (Wouts, 1973): *C. mesadenus*, male and female paratypes, slides n° 410, 412, 414-418, 421-424, 430 and 431; *C. composticola*, allotype and male and female paratypes, slides n° 444, 447, 448, 450-454, 458, 465 and 466.

Other specimens, originating from Campbell Island, labelled as *M. mesadenus* by Dr Clark and as *Clarkus* in the collection of Dr Wouts were added, along with specimens of *C. gerlachei* loaned by Dr Chaves, the female paratype of *C. intestinus* loaned by Dr Vinciguerra and material collected from southern Chile by Dr Bello.

The most recently collected specimens were extracted by the Flegg (1967) technique, killed and fixed by heat in F.G. 4:1, processed in hot lactophenol, and mounted in pure glycerin.

All previously described specimens used in the present work were remeasured. The univariate analysis of variance (ANOVA) was used to test significant differences in selected characters between groups of individuals. The grouping was checked using principal component analysis (PCA), an objective ordination method which emphasizes the major pattern of the variation in order to better search for species separation (for univariate and multivariate methods see *i. al.* Sneath & Sokal, 1973; Afifi & Clark, 1990; Sokal & Rohlf, 1994).

Morphometric studies

The primary purpose of this study was to determine characters for the separation of the 53 female and 24 male specimens of the analysis. Table 1 shows the measurements of four females and four males of *C. gerlachei* described by Chaves (1990) from the Antarctic Peninsula, some male and female paratypes of *C. mesadenus* described by Clark (1960) from different localities on New Zealand Islands, specimens of the same species described by Clark (1963) from Campbell Island, and one female paratype of *C. intestinus* from Tierra del Fuego (South America). The measurements of the allotype and some paratypes of *C. composticola* are given in Table 2. Table 3 contains measurements of specimens from Campbell Island, supplied by Dr Wouts, described below as *C. meridionalis* sp. n., and of specimens collected from southern Chile by Dr Bello, named here *C. magellanicus* sp. n. The tables include all characters or variables used in this study.

A preliminary study of the data revealed significant ($P < 0.01$) linear correlations between some measurements. Thus, in females, the body length was correlated with labial diameter, buccal cavity length, buccal cavity diameter, nerve ring and excretory pore distances to anterior body end, and pharynx length. Nevertheless, there were no significant correlations with respect to height of the lip region, length of the amphid aperture, and tail length. Likewise, in males, significant correlations were found between body length and buccal cavity length, nerve ring distance to anterior body end, pharynx length, and tail length. This correlation was low with respect to the height of the lip region and length of the amphid aperture and gubernaculum length. As there were few highly significant ($P < 0.01$) correlations, a total of twenty variables for females and nineteen for males were used in the analysis.

Each single variable was analysed using one-way ANOVA, to establish which ones gave significant differences ($P \leq 0.01$) among the species (Table 4). In females, significant differences were found for twenty variables, but only for fifteen in males. Because of this, characters with less significant differences (*i.e.*, b, c and T indices and variables related to amphid and gubernaculum) were removed from the analysis.

A principal components analysis was carried out on log transformed data. In females (Fig. 1), the first two principal components account for 44.2 and 25.1 % of the total variation, respectively. The first principal component (axis I) is dominated by high positive weights for body length, labial diameter, buccal cavity length and diameter, nerve ring and excretory pore to anterior body end distances, and pharynx and vagina length. It provides an ordination of the individuals that is much in agreement with the linear measurement variables. Note that index variables are not essential elements on this axis. Consequently, this component can be interpreted as a general size axis that separates the individuals (and species) by their sizes. On the contrary, the second principal component (axis II) arranges the individuals more on the basis of index variables, a, b, c' and apex indices and tail length, which have a negative weight. This component represents shape and indicates how shapes separate individuals. Both axes provide separate ordinations of the individuals in groups that identify the species considered. Thus, *C. composticola* (below) and

Table 1. *Morphometric data of remeasured specimens of* Coomansus gerlachei, C. mesadenus *and* C. intestinus *(all measurements in µm, except L in mm)*

	C. gerlachei		*C. mesadenus*				*C. intestinus*
	Antarctic Peninsula		New Zealand Islands		Campbell Island		Tierra del Fuego
	Females	Males	Female (paratypes)	Males (paratypes)	Females	Males	Female (paratype)
n	4	4	7	11	11	1	1
L	3.51 ± 0.43 (2.85-4.07)	3.40 ± 0.18 (3.24-3.67)	3.35 ± 0.28 (3.01-3.75)	3.18 ± 0.26 (2.68-3.66)	3.35 ± 0.30 (2.93-4.05)	3.51	1.76
a	36.6 ± 5.8 (28.0-43.9)	38.0 ± 0.7 (37.2-38.8)	37.0 ± 3.7 (34.0-44.5)	35.0 ± 3.7 (28.1-41.3)	38.8 ± 3.0 (34.9-45.8)	38.2	32.5
b	5.2 ± 0.2 (4.9-5.6)	4.8 ± 0.1 (4.7-5.1)	4.5 ± 0.2 (4.2-4.9)	4.3 ± 0.2 (4.0-4.7)	4.6 ± 0.2 (4.3-4.9)	4.7	4.3
c	17.2 ± 1.6 (15.2-19.2)	20.9 ± 0.7 (20.2-22.0)	13.5 ± 1.3 (11.8-15.8)	19.4 ± 1.8 (16.7-23.5)	15.2 ± 0.9 (13.1-16.7)	22.6	12.7
c'	3.8 ± 0.2 (3.5-4.0)	2.0 ± 0.1 (1.8-2.1)	4.8 ± 0.6 (3.6-5.6)	2.5 ± 0.2 (1.9-2.8)	4.4 ± 0.3 (3.8-4.9)	2.2	4.1
V/T	52.7 ± 1.7 (51.5-55.6)	52.7 ± 1.7 (50.6-55.3)	54.3 ± 1.3 (52.5-56)	53.5 ± 4.5 (41.1-59.5)	54.5 ± 1.3 (52.8-57.1)	56.9	58.6
G_1	12.6 ± 2.3 (10.9-16.5)	-	12.9 ± 1.5 (10.4-15.4)	-	12.7 ± 0.7 (11.8-13.8)	-	16.2
G_2	12.9 ± 3.4 (10.9-18.8)	-	12.3 ± 1.3 (10.7-14.7)	-	12.7 ± 0.6 (11.7-13.7)	-	14.1
Max. body diam.	96.7 ± 8.8 (81.5-103.5)	89.4 ± 3.2 (86.5-94.5)	91.3 ± 11.1 (74.5-110.0)	91.7 ± 10.5 (76.0-115.0)	86.7 ± 9.5 (67.0-103.5)	92.0	54.0
Cuticle at head	3.2 ± 0.5 (2.5-4.0)	2.8 ± 0.5 (2.5-3.5)	5.7 ± 1.0 (4.0-7.5)	5.0 ± 0.8 (3.5-6.5)	4.5 ± 0.4 (3.5-5.0)	4.0	2.0
– at midbody	3.9 ± 0.7 (2.5-4.5)	4.4 ± 1.2 (3.0-6.0)	6.3 ± 1.3 (4.5-8.0)	6.0 ± 0.6 (5.5-7.5)	5.1 ± 0.7 (4.0-6.0)	6.5	3.0
– on tail	5.0 ± 0.2 (5.0-5.5)	4.2 ± 1.1 (3.0-6.0)	8.8 ± 0.5 (8.0-10)	6.5 ± 1.2 (5.0-8.5)	8.0 ± 1.7 (5.0-10.0)	8.5	3.5
Lat. chord	36.7 ± 3.5 (31.5-41.5)	31.4 ± 5.0 (23.5-36.0)	16.1 ± 3.4 (12.0-21.5)	16.2 ± 3.9 (11.5-23.0)	18.5 ± 2.5 (11.0-20.5)	14.0	16.0
Head diam.	43.5 ± 3.1 (39.5-48.0)	43.7 ± 0.6 (43.0-44.5)	43.1 ± 3.4 (39.0-48.0)	42.3 ± 1.6 (39.5-44.5)	44.1 ± 1.5 (41.0-46.5)	45.0	35.5
– height	14.7 ± 2.3 (13.0-18.5)	15.0 ± 0.7 (14.0-16.0)	14.9 ± 2.3 (12.0-19.0)	15.6 ± 1.4 (14.0-19.0)	15.5 ± 1.1 (12.5-17.0)	14.5	13.0
Amphid	5.2 ± 0.2 (5.0-5.5)	6.0 ± 0.5 (5.5-6.5)	6.1 ± 0.4 (6.0-7.0)	6.2 ± 0.5 (5.5-7.0)	5.2 ± 0.6 (4.5-6.5)	5.5	5.0
Bucc. cav. length	49.7 ± 1.2 (47.5-51.0)	49.5 ± 0.6 (49.0-50.5)	49.5 ± 3.7 (44.0-54.0)	49.2 ± 1.5 (47.5-52.5)	51.1 ± 1.8 (48.5-55.0)	53.0	38.0
– diameter	27.4 ± 1.2 (25.5-29.0)	25.2 ± 1.5 (22.5-27.5)	26.4 ± 2.1 (23.0-29.5)	25.1 ± 1.2 (23.5-27.0)	25.5 ± 1.5 (23.0-28.0)	23.0	21.0
Dors. tooth apex	85.4 ± 1.1 (84.6-87.3)	85.6 ± 2.1 (82.1-87.6)	87.6 ± 2.0 (85.0-91.5)	88.3 ± 2.4 (84.6-93.4)	90.7 ± 1.5 (88.5-93.0)	92.0	86.0
Nerv. ring-ant. end	201.4 ± 22.0 (166-220)	225.0 ± 4.3 (219-231)	215.4 ± 8.2 (197-221.5)	215.6 ± 20.4 (165-241.5)	214.9 ± 19.3 (179.5-255.5)	215.5	135.0
Excr. pore-ant. end	224.7 ± 22.9 (188-248.5)	248.5 ± 4.5 (242-254.5)	250.1 ± 16.0 (225.5-272.0)	243.8 ± 22.2 (183-277)	242.4 ± 20.5 (197-282)	246.0	172.5
Pharynx length	626.9 ± 60.8 (524-683)	654.7 ± 21.4 (630.5-685)	682.0 ± 29.5 (623.5-713.5)	688 ± 32.7 (611-743)	683.2 ± 43.1 (631-783.5)	692.5	374.5

End of Table 1 next page

Table 1. (End).

	C. gerlachei		*C. mesadenus*				*C. intestinus*
	Antarctic Peninsula		New Zealand Islands		Campbell Island		Tierra del Fuego
	Females	Males	Female (paratypes)	Males (paratypes)	Females	Males	Female (paratype)
Vagina length	59.1 ± 8.5 (48.5-71.5)	-	37.6 ± 1.3 (36.0-39.0)	-	45.1 ± 4.2 (40.0-52.0)	-	25.0
Tail	204.2 ± 13.0 (188-221)	162.7 ± 10.7 (147-177)	248.4 ± 10.1 (236.5-267.5)	164.1 ± 11.8 (148.5-184.5)	220.1 ± 16.9 (197.5-258)	155.0	138.0
Spicules	-	155.5 ± 6.2 (147-164.5)	-	134.5 ± 5.8 (119.5-142)	-	129.5	-
Gubernaculum	-	39.5 ± 1.0 (38.0-40.5)	-	37.3 ± 4.0 (27.5-41.5)		39.0	-
Lat. guid. pieces	-	33.5 ± 0.5 (33.0-34.5)	-	-	-	-	-
Supplements	-	10.0 ± 0.7 (9-11)	-	10.0 ± 0.7 (9-11)	-	11	-
Sperm	-	16.2 ± 1.3 (14.5-17.5)	-	8.2 ± 1.2 (7.0-10.0)	-	8.5	-
Egg length	172*	-	117, 127.5**	-	-	-	-
– diameter	66.5*	-	66, 68**	-	-	-	-

* n = 1; ** n = 2.

C. magellanicus sp. n. (above) are on the left of the graph, *C. meridionalis* sp. n. is in the center, and the specimens of *C. mesadenus* and *C. gerlachei*, two species that do not separate clearly in this analysis, are on the right of the graph.

In males (Fig. 2), the first two principal components explain 55.4 % and 13.0 % of the variance, respectively. The first principal component arranges more species by size alone than in the females, which means that the second and next components only make minor contributions to ordination. The main elements of the first axis are body length, labial diameter, buccal cavity length and diameter, nerve ring distance to the anterior body end, and pharynx, tail and spicules length. Besides spicule length, tail length is the most important character for separating the individuals, unlike in females. In the corresponding graph, showing the first two axes only, the individuals and species are plotted as for the females. From the two graphs, the separation as new species of *C. meridionalis* sp. n. and *C. magellanicus* sp. n. appears to be justified, but this is not so for the specimens of *C. mesadenus* and *C. gerlachei*. This result is confirmed by ANOVA results (Table 4) which, in the multiple range test, show high significant differences between both males and females of these two species in only few of the characters considered, *i.e.*, b and c' indices, lateral chord, and apex in addition to vagina and spicule length.

Coomansus gerlachei (de Man, 1904) Jairajpuri & Khan, 1977

(Figs 3, 4)

Four male and four female specimens of this species were available for study. Measurements are given in Table 1. They agree well with the description by Chaves (1990), except that two intestinal constrictions are clearly visible in both sexes.

Steiner (1916) reported this species from the Comores Islands, but this record is doubtful because it is based on juvenile specimens only. Mulvey (1978) reported a single male specimen from Canada. This seems to be an exceptional finding since, otherwise, the species is known only from Antarctica.

Coomansus mesadenus (Clark, 1960) Jairajpuri & Khan, 1977

(Figs 5, 6)

Measurements of *C. mesadenus* male and female paratypes from New Zealand Islands and specimens from Campbell Island are given in Table 1. They agree with those by Clark (1960), who considers this species, along with *C. gerlachei*, *C. major* (Cobb, 1893) Loof & Winiszewska-Slipinska, 1993, and *C. composti-*

Table 2. *Morphometric data of remeasured specimens of* Coomansus composticola *from New Zealand Islands (all measurements in µm, except L in mm)*

	Male (allotype)	Male (paratypes)	Female (paratypes)		Male (allotype)	Male (paratypes)	Female (paratypes)
n	1	1	14	Amphid	-	4.5	4.2 ± 0.3 (4.0-4.5)
L	3.37	3.53	2.28 ± 0.21 (1.78-2.68)	Bucc. cav. length	50.0	48.0	35.1 ± 1.6 (31.5-38.0)
a	43.0	4.8 39.1	34.9 ± 2.3 (29.8-38.2)	– . – diameter	22.5	21.0	18.0 ± 0.9 (15.5-19.0)
b	4.6		4.6 ± 0.2 (4.2-4.8)	Dors. tooth apex	90.5	88.0	90.3 ± 2.2 (85.0-93.5)
c	20.4	19.1	8.3 ± 0.4 (7.5-8.9)	Nerv. ring-ant. end	213.5	227.5	165.9 ± 10.1 (143-181)
c'	2.5	2.8	7.1 ± 0.4 (6.4-8.1)	Excr. pore-ant. end	234.5	250.5	184.8 ± 9.2 (169-203)
V/T	51.2	58.9	51.1 ± 1.4 (48.9-55.4)	Pharynx length	688.5	694.5	466.2 ± 30.2 (399-516)
G_1	-	-	9.8 ± 1.4 (8.4-12.7)	Vagina length	-	-	28.8 ± 2.7 (24.0-35.0)
G_2	-	-	9.6 ± 1.7 (7.4-12.9)	Tail	165.5	184.5	273.6 ± 17.3 (236.5-302)
Max. body diam.	78.5	90.5	65.5 ± 6.2 (56.0-78.0)	Spicules	127.0	122.0	-
Cuticle at head	4.0	3.5	2.8 ± 0.6 (2.0-4.0)	Gubernaculum	31.5	31.0	
– at midbody	4.5	4.0	3.8 ± 0.7 (2.5-5.5)	Lat. guid. pieces	29.5	30.0	
– on tail	4.5	4.5	5.9 ± 0.9 (4.0-7.5)	Suppl.	13	13	
Lat. chord	14.0	27.5	15.8 ± 3.3 (12.0-23.5)	Sperm	10.0	9.5	
Head diam.	37.5	34.5	28.9 ± 1.5 (26.5-32.0)	Egg length			124.4 ± 6.5* (112.5-131.5)
– height	13.5	11.5	11.0 ± 1.1 (9.0-13.0)	Egg diameter			57.1 ± 3.3* (51.5-61.5)

* n = 6

cola, to constitute a southern group of closely related species. Loof and Winiszewska-Slipinska (1993) separate *C. mesadenus* from *C. gerlachei* by the length of the spicules (about 70 µm in *C. mesadenus vs* 160 µm in *C. gerlachei*). Our study showed that spicule length is 120-142 µm in *C. mesadenus* and that there are slender lateral guiding pieces in the male, which were overlooked by Clark (1960). Moreover, *C. mesadenus* has reduced caudal glands and a ventrally subterminal caudal pore in the male. It can be distinguished from *C. gerlachei* by the longer tail, shorter spicules and egg and sperm size. If *C. mesadenus* and *C. gerlachei* were the same species it would occur in distant geographic areas.

Coomansus intestinus (Vinciguerra & La Rosa, 1990) Andrássy, 1993

(Fig. 6)

Measurements of the single female paratype studied are given in Table 1. These measurements are smaller

Table 3. *Morphometric data of* Coomansus meridionalis *sp. n. from Campbell Island and* C. magellanicus *sp. n. from Punta Arenas (all measurements in µm, except L in mm)*

	C. meridionalis sp. n.			*C. magellanicus* sp. n.		
	Female (holotype)	Female (paratypes)	Male (paratypes)	Female (holotype)	Female (paratypes)	Male (paratypes)
n	1	7	4	1	7	2
L	2.39	2.41 ± 0.17 (2.16-2.65)	2.39 ± 0.21 (2.09-2.67)	1.99	1.85 ± 0.78 (1.77-2.02)	1.84, 1.97
a	30.6	32.3 ± 2.8 (28.3-37.5)	31.7 ± 1.9 (29.6-34.8)	28.9	28.4 ± 1.4 (26.0-30.0)	29.6, 30.0
b	4.5	4.5 ± 0.2 (4.2-4.8)	4.6 ± 0.4 (4.3-5.2)	5.1	5.0 ± 0.2 (4.7-5.3)	5.0, 5.0
c	15.5	17.9 ± 1.6 (15.8-20.5)	23.4 ± 0.6 (22.8-24.4)	13.0	13.9 ± 0.8 (12.6-14.9)	21.4, 18.0
c'	3.5	3.2 ± 0.3 (2.8-3.5)	1.9 ± 0.1 (1.8-1.9)	3.7	3.2 ± 0.3 (2.6-3.5)	1.5, 1.9
V/T	56.6	55.1 ± 0.9 (53.9-57.0)	56.8 ± 3.7 (53.8-62.8)	52.6	53.3 ± 2.0 (51.3-56.6)	53.1, 74.1
G_1	13.3	12.3 ± 1.2 (10.0-13.8)	-	11.6	12.1 ± 0.7 (10.8-13.2)	-
G_2	12.0	12.0 ± 1.0 (11.0-14.2)	-	9.4	11.8 ± 1.3 (10.3-13.7)	-
Max. body diam	78.5	75.0 ± 8.9 (63.5-94.0)	76.2 ± 10.8 (60.0-90.0)	69.0	65.1 ± 2.9 (61.5-69.5)	62.0, 65.5
Cuticle at head	3.5	3.4 ± 0.4 (2.5-4.0)	3.4 ± 0.6 (2.5-4.0)	4.0	4.0 ± 0.6 (3.0-5.0)	5.0, 4.5
– at midbody	4.0	3.9 ± 0.9 (2.0-5.0)	4.1 ± 0.6 (3.0-5.0)	4.5	5.7 ± 0.6 (5.0-6.5)	6.0, 5.5
– on tail	6.0	6.3 ± 1.1 (5.0-8.0)	4.9 ± 0.6 (4.0-6.0)	6.5	6.8 ± 1.3 (4.5-8.5)	6.5, 6.0
Lat. chord	18.0	17.5 ± 2.8 (13.0-20.5)	19.6 ± 3.6 (15.5-25.0)	22.5	20.6 ± 2.7 (17.5-24.0)	18.5, 21.0
Head diam.	31.0	32.5 ± 1.2 (30.0-34.0)	32.2 ± 2.9 (28.0-35.0)	31.5	31.5 ± 1.4 (30.0-34.0)	32.0, 32.0
– height	12.0	12.5 ± 0.7 (11.0-13.0)	11.6 ± 1.2 (9.5-13.0)	14.0	13.7 ± 0.8 (12.5-14.5)	15.0, 13.5
Amphid	4.0	4.6 ± 0.1 (4.5-5.0)	4.9 ± 0.1 (4.5-5.0)	5.5	6.1 ± 0.4 (5.5-6.5)	6.0, 6.5
Bucc. cav. – length	41.0	41.6 ± 2.0 (39.0-45.0)	42.0 ± 1.9 (40.5-45.0)	34.0	34.4 ± 1.1 (33.0-36.0)	32.5, 33.0
– diameter	19.0	19.4 ± 0.8 (18.5-20.5)	20.0 ± 1.1 (18.5-21.5)	22.0	19.4 ± 0.9 (18.0-20.5)	20.0, 22.0
Dors. tooth apex	86.7	87.8 ± 3.7 (83.5-95.0)	87.8 ± 0.3 (87.4-88.3)	74.0	77.7 ± 1.5 (76.0-79.6)	77.0, 78.0
Nerv. ring-ant. end	157.0	175.8 ± 15.3 (153-196.5)	163.0 ± 10.0 (147.5-174.5)	123.5	113.1 ± 5.6 (101.5-121)	114.0, 115.0
Excr. pore-ant. end	182.0	194.7 ± 12.6 (177.5-211.5)	185.1 ± 12.9 (167-198.5)	145.0	140.3 ± 5.7 (128.5-148.0)	130.5, 131.0
Pharynx length	490.0	498.3 ± 36.4 (434.5-541)	482.6 ± 24.8 (448.5-507.5)	353.0	334.4 ± 8.7 (322.0-346.0)	331.5, 354.0
Vagina length	27.0	34.6 ± 4.2 (27.5-39.5)	-	34.5	29.1 ± 1.7 (25.5-31.5)	-

End of Table 3 next page

Table 3. *(End).*

	C. meridionalis sp. n.			*C. magellanicus* sp. n.		
	Female (holotype)	Female (paratypes)	Male (paratypes)	Female (holotype)	Female (paratypes)	Male (paratypes)
Tail	154.0	135.1 ± 6.8 (126.5-144)	102.4 ± 9.4 (91.5-117.5)	154.0	132.8 ± 8.2 (118.5-144.5)	86.0, 109.5
Spicules	-	-	104.0 ± 6.6 (93.5-110.5)	-	-	98.0, 97.0
Gubernaculum	-	-	28.4 ± 4.9 (20.0-33.0)	-	-	19.5, 23.0
Lat. guid. pieces	-	-	25.3 ± 1.0 (23.5-26.0)	-	-	21.0, 23.0
Supplements	-	-	12.2 ± 0.4 (12-13)	-	-	11, 12
Sperm			10.7 ± 0.4 (10.0-11.5)			9.5, 8.5

than those by Vinciguerra and La Rosa (1990) for body, pharynx and tail length. In the paratype, the uterus lacks a coiled region but a poorly developed swollen part is present. This species is a member of the aforementioned group of species and was reported from South America (Tierra del Fuego) by its original authors and from near Antarctica (Deception Island) by Andrássy (1993).

Coomansus composticola (Clark, 1960) Jairajpuri & Khan, 1977

The allotype and several male and female paratypes were examined. Measurements are provided in Table 2 and are in agreement with those by Clark (1960). Although no mention of lateral guiding pieces was made by Clark, they are present and appear very slender as in *C. gerlachei* and *C. mesadenus* (Fig. 5).

Coomansus meridionalis * sp. n.

(Figs 7, 8)

MEASUREMENTS

See Table 3.

DESCRIPTION

Female: Large, about 75 µm wide at midbody and 2.5 mm long. Body cylindrical, with truncate head, tapering slightly towards anterior end and more gradually towards posterior extremity. Habitus more or less ventrally curved when fixed, frequently G- to J-shaped, and strongly curved in the caudal region. Cuticle smooth or with obscure transverse striations. Lateral chord occupying 23 ± 3 (18-30) % of midbody diameter. Lip region separated from the adjacent body by a depression, 2.6 ± 0.16 (2.5-3) times as wide as high. Lips separated and rounded. Labial and cephalic papillae prominent, jutting out from the head contour. Amphid cup-shaped, located at the level of the cephalic depression; its aperture extending 14 ± 1 (13-15) % of lip region diameter. Buccal cavity barrel to funnel-shaped and not flattened at base, with thick walls, 2.1 ± 0.1 (1.9-2.3) times as long as wide. A dorsal tooth, forward directed, situated at the anterior region; its apex located at 87.6 ± 3.47 (83.7-95) % of the buccal cavity length from the base. Large ventrosublateral foramina visible in the basal plates. Pharynx cylindrical, muscular, surrounding the basal part of the stoma. Nerve ring located at 32.2 ± 1.9 (30-35) % of the total neck region measured from the anterior end. Excretory pore small but easily visible, situated behind the nerve ring. Pharyngo-intestinal junction not tuberculate, with conical organ short and rounded. Intestinal cells polygonal, granular, eight to ten in transverse section. Bacillary layer well developed, especially visible at the anterior and posterior regions. Genital system didelphic-amphidelphic. Ovary short, not reaching to the oviduct-uterus junction. Oocytes few in number. Oviduct consisting of a narrow distal part and a well developed proximal *pars dilatata*. A small sphincter present at the oviduct-uterus junction with an inner, poorly sclerotized part surrounded by a muscular part. Uterus consisting of a narrow coiled distal part and a large swollen proximal part filled with abundant sperm. Vagina cylindrical, extending over 45 ± 6 (34-53) % of the corresponding body diameter. Two small to medium sized sclero-

* The specific epithet is derived from Latin word *meridianus*, referring to a southern geographical location.

Table 4. *One-way ANOVA of the differences in character values, for females and males, concerning all the species of* Coomansus *studied, except* C. intestinus. *Snedecor's F values are for significant differences (P ≤ 0.01). a* = C. mesadenus, *b* = C. meridionalis, *c* = C. gerlachei, *d* = C. composticola, *e* = C. magellanicus

Character	Females		Males	
	F value	Significant differences between species	F value	Significant differences between species
1. L	70.4	a-b/a-d/a-e/b-c/b-e/c-d/c-e/d-e	26.5	a-b/a-e/b-c/b-d/b-e/c-e/d-e
2. a	14.0	a-b/a-d/a-e/b-c/b-e/c-e/d-e	5.0	a-d/a-e/b-c/b-d/c-e/d-e
3. b	18.5	a-c/a-e/b-c/b-e/c-d/d-e	4.2	a-c/a-e
4. c	90.5	a-b/a-c/a-d/b-d/b-e/c-d/c-e/d-e	–	–
5. c'	160.9	a-b/a-c/a-d/a-e/b-c/b-d/c-d/d-e	9.7	a-b/a-c/a-e/b-d/c-d/d-e
6. V/T	13.4	a-d/b-c/b-d/b-e/d-e	–	–
7. G_1	10.4	a-d/b-d/c-d/d-e	–	–
8. G_2	7.1	a-d/b-d/c-d/d-e	–	–
9. Lateral chord	36.0	a-c/a-e/b-c/b-e/c-d/c-e/d-e	6.2	a-c/b-c/c-d/c-e
10. Lip region diam.	128.7	a-b/a-d/a-e/b-c/b-d/c-d/c-e/d-e	30.9	a-b/a-d/a-e/b-c/b-d/c-d/c-e/d-e
11. Lip region height	18.9	a-b/a-d/a-e/b-c/b-d/c-d/d-e	8.7	a-b/a-d/b-c/b-e/c-d
12. Amphid	3.8	a-b/a-d/c-d/d-e	–	–
13. Bucc. cav. length	140.0	a-b/a-d/a-e/b-c/b-d/b-e/c-d/c-e	70.5	a-b/a-e/b-c/b-d/b-e/c-e/d-e
14. Bucc. cav. diameter	89.9	a-b/a-d/a-e/b-c/b-d/c-d/c-e/d-e	13.7	a-b/a-d/a-e/b-c/c-d/c-e
15. Dorsal tooth apex	41.5	a-c/a-e/b-d/b-e/c-d/c-e/d-e	12.3	a-c/a-e/b-e/c-e/d-e
16. N. r.-ant. end	70.8	a-b/a-d/a-e/b-c/b-e/c-d/c-e/d-e	34.2	a-b/a-e/b-c/b-d/b-e/c-e/d-e
17. Excr. p.-ant. end	72.2	a-b/a-c/a-d/a-e/b-c/b-e/c-d/c-e/d-e	33.0	a-b/a-e/b-c/b-d/b-e/c-e/d-e
18. Pharynx length	149.6	a-b/a-c/a-d/a-e/b-c/b-e/c-d/c-e/d-e	124.7	a-b/a-e/b-c/b-d/b-e/c-e/d-e
19. Vagina length	42.8	a-b/a-c/a-d/a-e/b-c/b-d/c-d/c-e	–	–
20. Tail	129.9	a-b/a-c/a-d/a-e/b-c/b-d/c-d/c-e/d-e	36.2	a-b/a-e/b-c/b-d/c-e/d-e
21. Spicules	–	–	47.9	a-b/a-c/a-e/b-c/b-d/c-d/c-e/d-e
22. Supplement number	–	–	12.6	a-b/a-d/a-e/b-c/c-d/c-e

tized pieces in the vagina-vulva junction. Vulva apparently a short transverse slit. Zero to three prevulval and zero to five postvulval papillae, irregularly spaced, prevulval papillae mostly absent and sometimes no vulval papillae at all. Tail conoid, ventrally curved and gradually tapering to a rounded terminus. Caudal glands indistinct and spinneret and its opening absent. Caudal papillae or pore weakly visible, apparently four on each side of the tail.

Male: General morphology similar to female. About 75 µm wide at mid-body and 2.5 mm long, posterior region more ventrally curved. Lateral chord extending over 26 ± 2 (22-28) % of midbody diameter. Lip region 2.8 ± 0.08 (2.7-2.9) times as wide as high. Amphid occupying 16 ± 2 (14-17) % of the lip region diameter. Buccal cavity 2.1 ± 0.1 (1.9-2.3) times as long as wide. Nerve ring located at 31.3 ± 1.5 (30-33) % of the total neck region measured from the anterior end. Genital system diorchid. Testes opposed with elongate, spindle-shaped sperm. *Vas deferens* and *ductus ejaculatorius* separated by a constriction with a surrounding group of eight to ten muscular bands. Ventral body contour slightly contracted in region of the anterior supplement. Ejaculatory glands in tandem, sometimes indistinct; rectal glands visible. Twelve to thirteen ventromedian supplements present, more or less regularly spaced, the anterior and posterior ones poorly developed, the rest prominent, mamilliform, and echinulate. Spicules rather thick, ventrally curved, 1.9 ± 0.2 (1.6-2.2) times as long as anal body diameter, measured along axis. Gubernaculum well developed, extending laterally to spicules, with lateral guiding pieces extraordinarily slender with fine bifurcated tip. Tail similar to female but relatively shorter and with more rounded terminus. Caudal glands reduced, subventral duct and opening present. Three subdorsal and three subventral caudal papillae on each side of the tail.

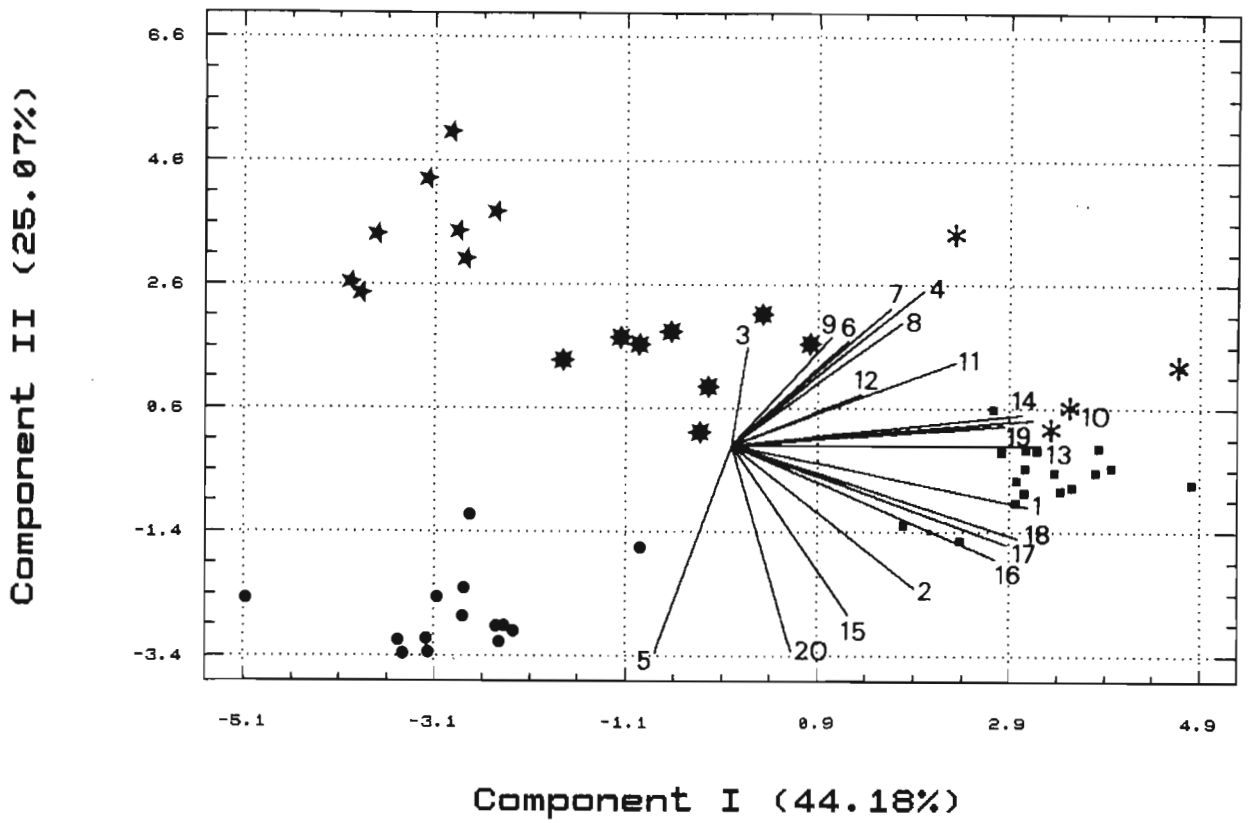

Fig. 1. *Biplot of* Coomansus *females and corresponding characters from the results of the PCA, along the two first axes.* C. magellanicus (★); C. meridionalis (✱); C. composticola (●); C. gerlachei (✲); C. mesadenus (■). *Character numbers as in Table 4.*

TYPE HABITAT AND LOCALITY

Holotype from soil under *Myrsine divaricata* on the east side of Mt. Dumas, Campbell Island, New Zealand. *Paratypes* from the same locality and the following sites from the same island: Tucker Valley, associated with *Dracophyllum scoparium* and *D. longifolium*, and northern slopes of Mt. Honey, around *Poa* sp.

TYPE MATERIAL

Holotype female on slide n° 174, labelled *Clarkus*, from Dr Wouts collection. *Paratypes*, male and female, on slides n° 114, 152, 173 and 174 from the same collection and labelled *Mononchus mesadenus*, Campbell Island S.2. and S.6., from Dr Clark collection. All material deposited in The National Nematode Collection of New Zealand.

DIAGNOSIS AND RELATIONSHIPS

C. meridionalis sp. n. is characterized by its large size (body length 2.10-2.65 mm), lip region set off by a depression, buccal cavity 39-45 × 18-22 µm or 1.9-2.3 times as long as wide, apex of the dorsal tooth at 84-95 % of the total buccal cavity length from base, V = 54-57, ventral body contour slightly contracted at the beginning of the supplement series, ejaculatory glands in tandem, spicules 94-110 µm, very slender and with finely bifurcate lateral guiding pieces, twelve to thirteen mostly echinulate and mammilla-shaped ventromedian supplements, conoid and ventrally curved tail with rounded terminus, and caudal glands and spinneret absent in the female and reduced in the male.

C. meridionalis sp. n. is closely related to *C. gerlachei*, *C. mesadenus* and *C. intestinus*. It can be separated from *C. gerlachei* by the shorter body, smaller size of the buccal cavity, intestinal constrictions absent, shorter spicules, greater number of supplements, more slender guiding pieces, and shorter tail with a more rounded terminus in both sexes. It is similar in several respects to *C. mesadenus*, but it can be distinguished by the shorter body, smaller buccal cavity, slightly greater number of supplements, and shorter and more conical shaped tail. Its measurements are quite similar

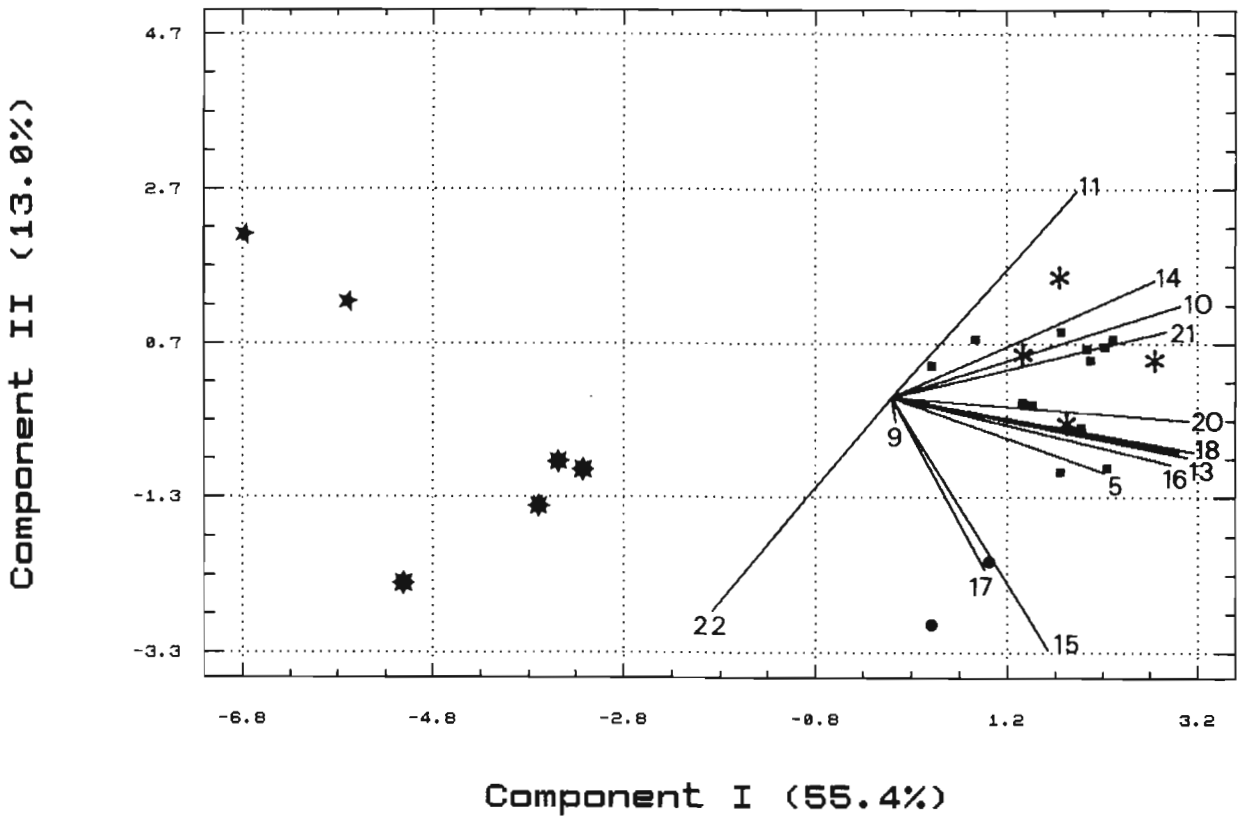

Fig. 2. *Biplot of* Coomansus *males and corresponding characters from the results of the PCA, along the two first axes.* C. magellanicus (★); C. meridionalis (✳); C. composticola (●); C. gerlachei (✲); C. mesadenus (■). *Character numbers as in Table 4.*

to those of *C. intestinus,* but it can be separated from this species by a uterus with a swollen part larger than the coiled region, lack of intestinal constrictions, and more rounded tail terminus.

Coomansus magellanicus * sp. n.

(Figs 9, 10)

MEASUREMENTS

See Table 3.

DESCRIPTION

Female: Body of medium to large size, about 2 mm long and 65 µm wide at mid-body. Body cylindrical, truncate anteriorly and tapering clearly toward posterior end. Habitus frequently G-shaped when fixed and relaxed and clearly curved in the caudal region. Cuticle smooth or with obscure fine transverse striations. Lateral chord occupying 32 ± 3 (28-38) % of midbody diameter. Lip region set off by a depression, 2.3 ± 0.13 (2.1-2.4) times as wide as high. Lips moderately separated and rounded. Labial and cephalic papillae prominent and jutting out from the head contour. Amphids cup-shaped, situated at the level of the labial constriction; their opening occupying 20 ± 1 (18-21) % of the head diameter. Buccal cavity subrectangular, flattened at base, with thick walls, 1.75 ± 0.12 (1.5-2) times as long as wide. Dorsal tooth large, forward directed, its apex located at 77.7 ± 1.5 (76-79.6) % of the stoma length measured from base. Ventrosublateral foramina visible in the basal plates. Pharynx cylindrical, muscular and surrounding the basal part of the buccal cavity. Nerve ring located at 30.8 ± 1.7 (26.8-32.5) % of the neck region measured from the anterior end. Excretory pore small and sometimes poorly visible. Pharyngo-intestinal junction not tuberculate and conical organ generally rounded. Intestine with six to eight granular polygonal cells in transverse section. Bacillary layer present, especially visible at the anterior and posterior regions. Genital system didelphic-amphidelphic.

* The specific name is the Latin gentilic adjective of Magellan, the Portuguese explorer of the geographic area where this species was found.

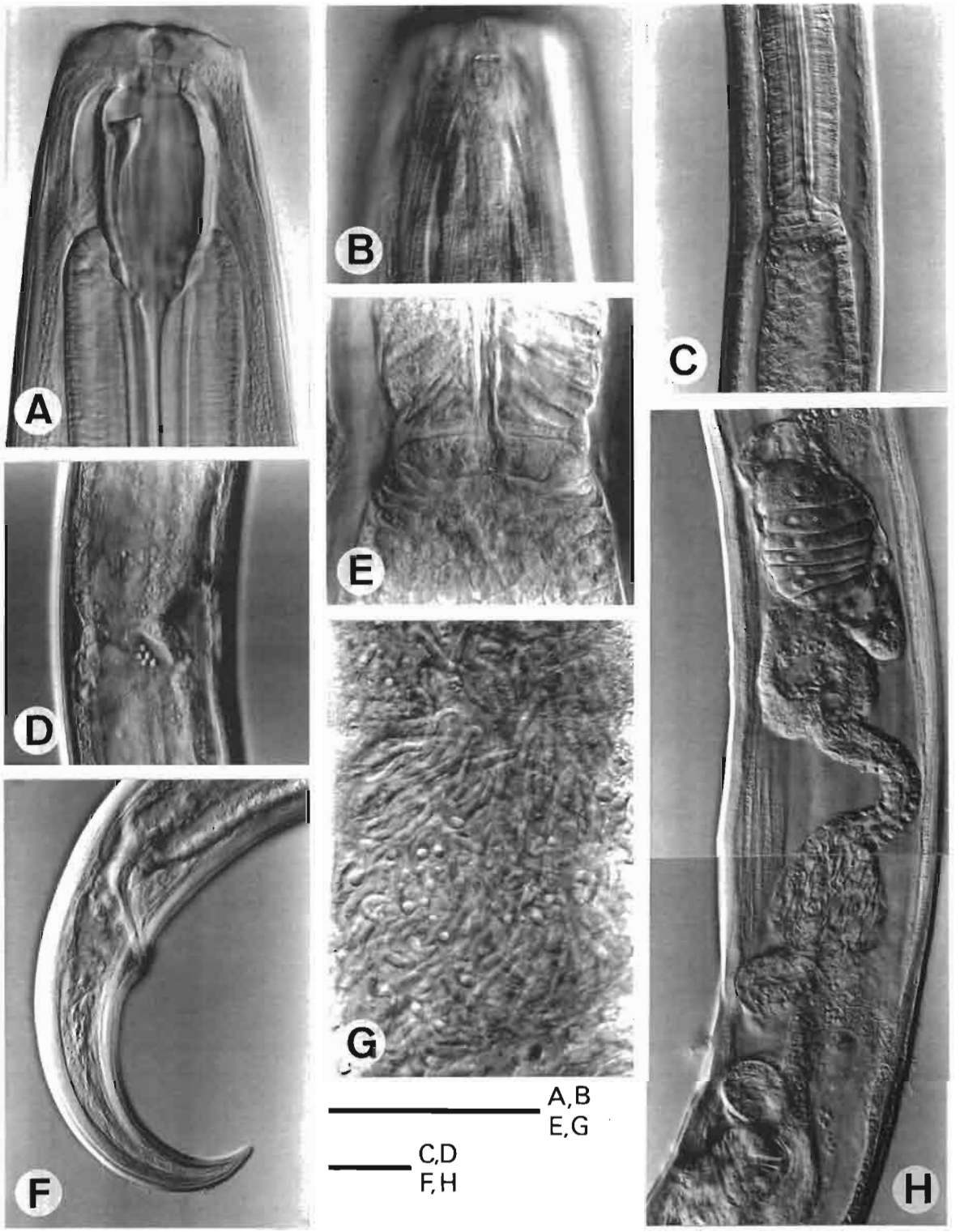

Fig. 3. Coomansus gerlachei *(specimens from Antarctic Peninsula). A, B: Head; C, E: Pharyngo-intestinal junction; D: Intestinal constriction; F: Female tail; G: Sperm; H: Female genital system. (Scale bars = 50 μm).*

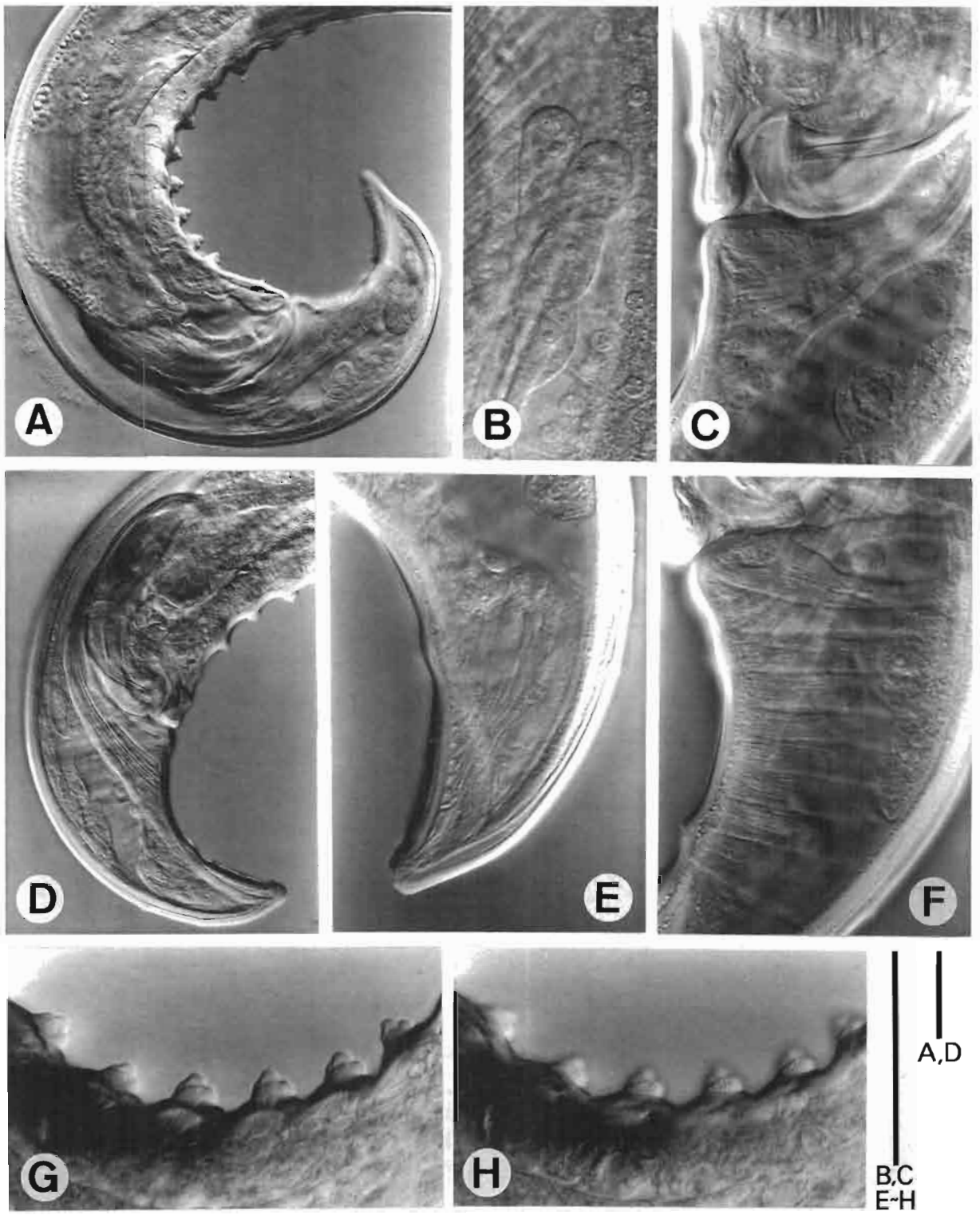

Fig. 4. Coomansus gerlachei *(specimens from Antarctic Peninsula). A, D, E, F: Male posterior region; B: Ejaculatory glands; C: Lateral guiding pieces; G, H: Supplements. (Scale bars = 50 μm).*

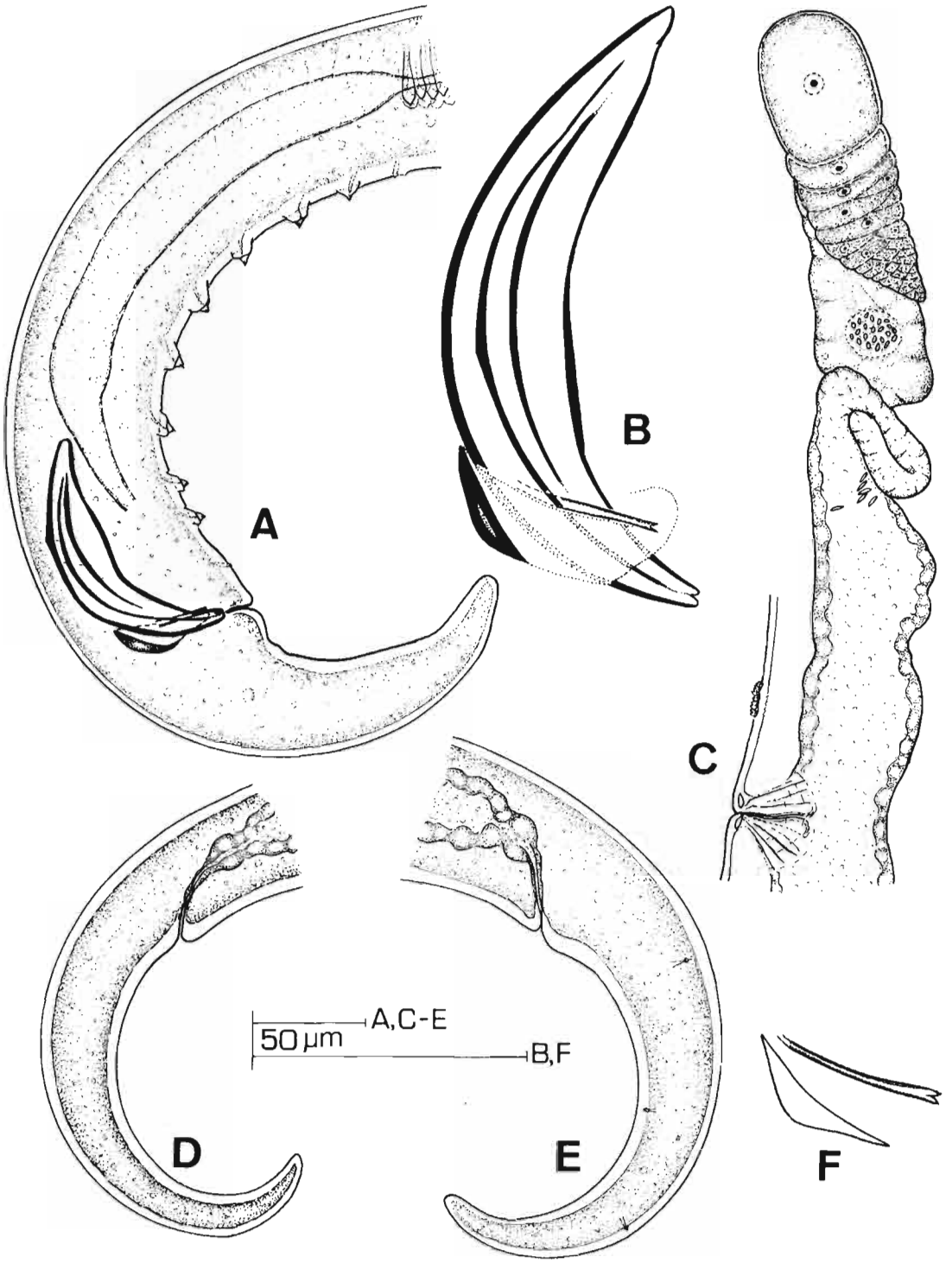

Fig. 5. Coomansus mesadenus. *A: Male posterior region; B: Spicules; C: Female genital branch; D, E: Female tail* — C. composticola. *F: Gubernaculum and lateral guiding pieces.*

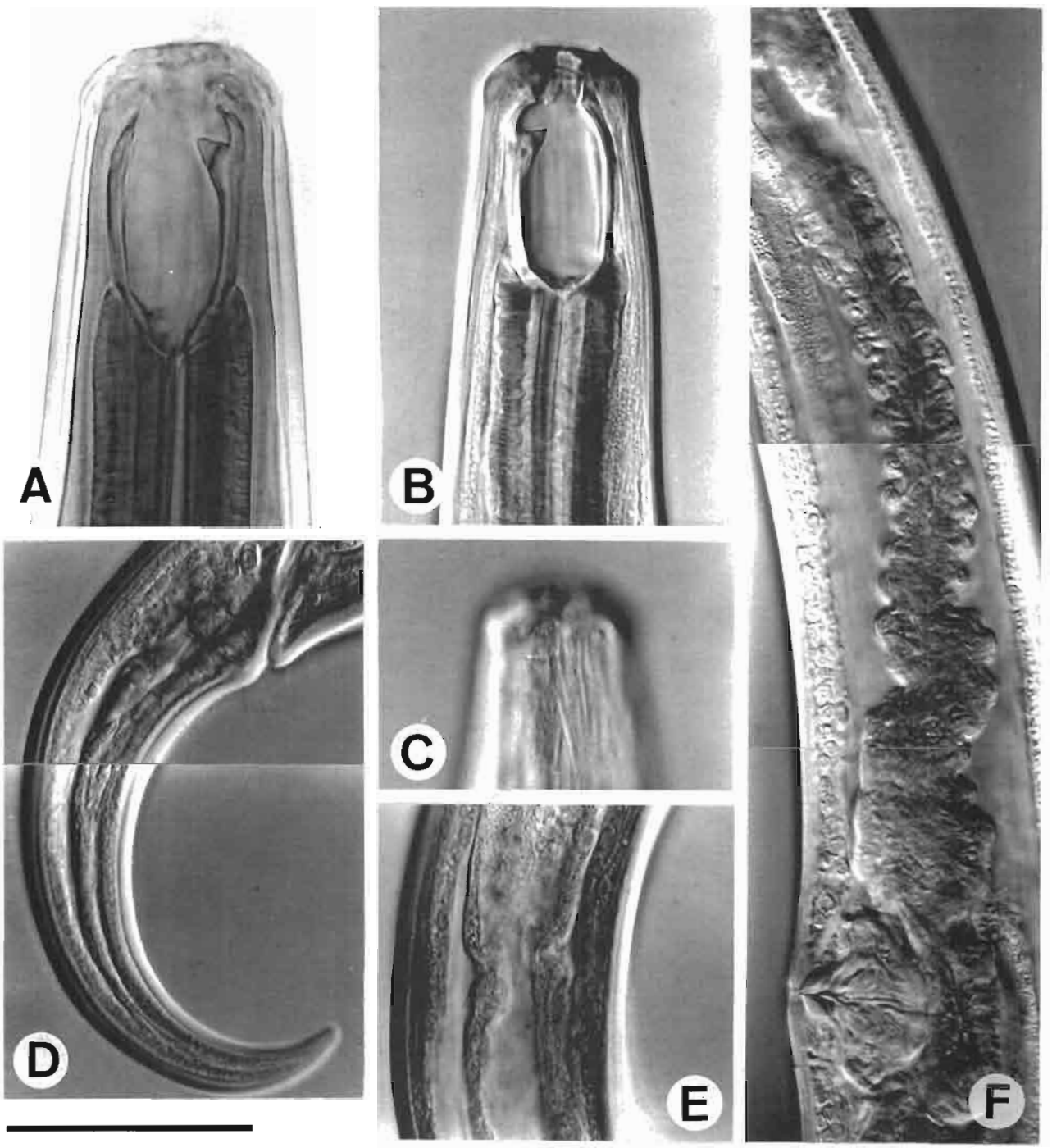

Fig. 6. Coomansus mesadenus. *A: Head—* C. intestinus *(specimen from Tierra del Fuego); B, C: Head; D: Female tail; E: Intestinal constriction; F: Female genital branch. (Scale bar = 50 μm).*

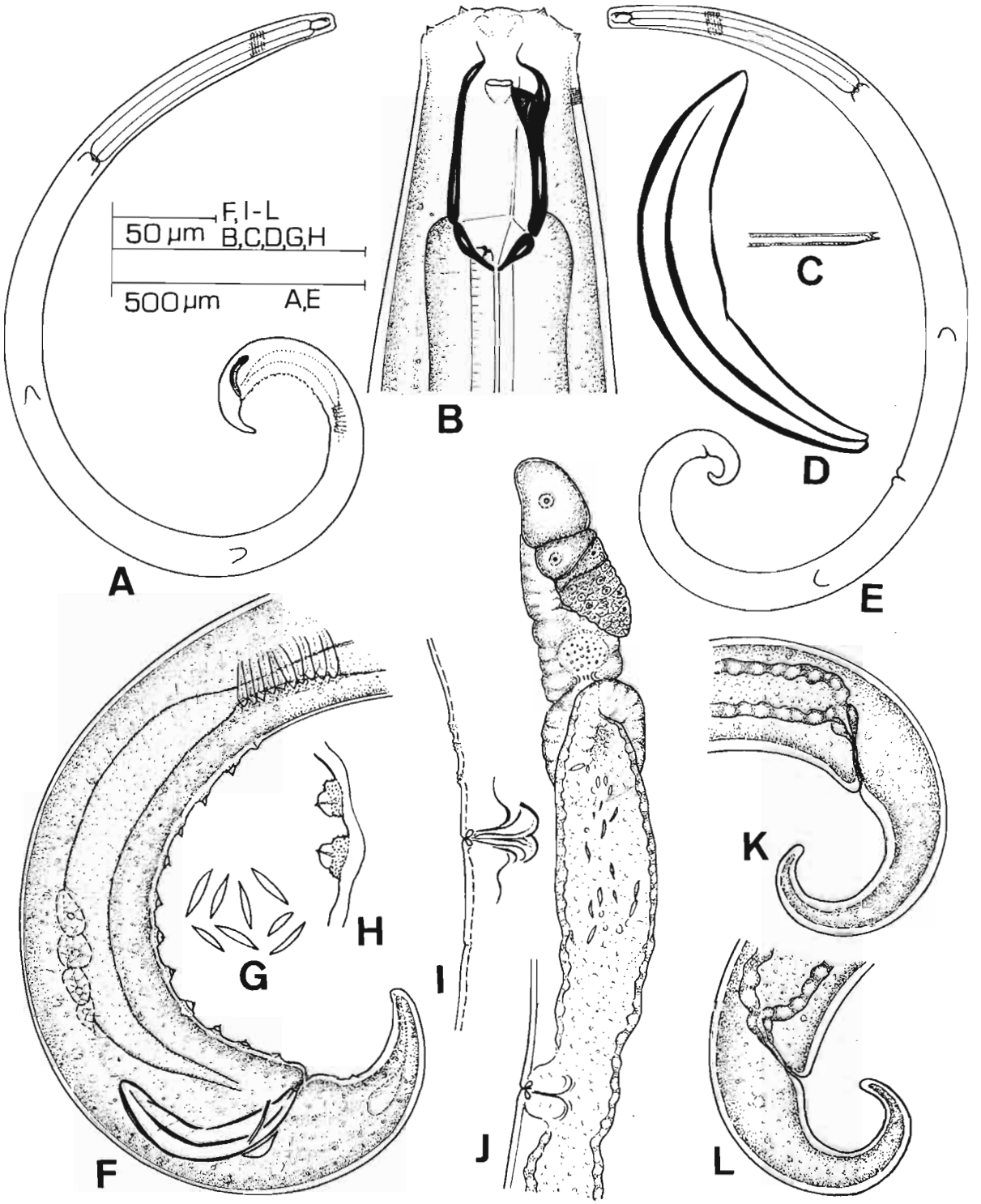

Fig. 7. Coomansus meridionalis *sp. n. A, E: Habitus; B: Head; C: Lateral guiding pieces; D: Spicules; F: Male posterior region; G: Sperm; H: Supplements; I: Vulval region; J: Female genital branch; K, L: Female tail.*

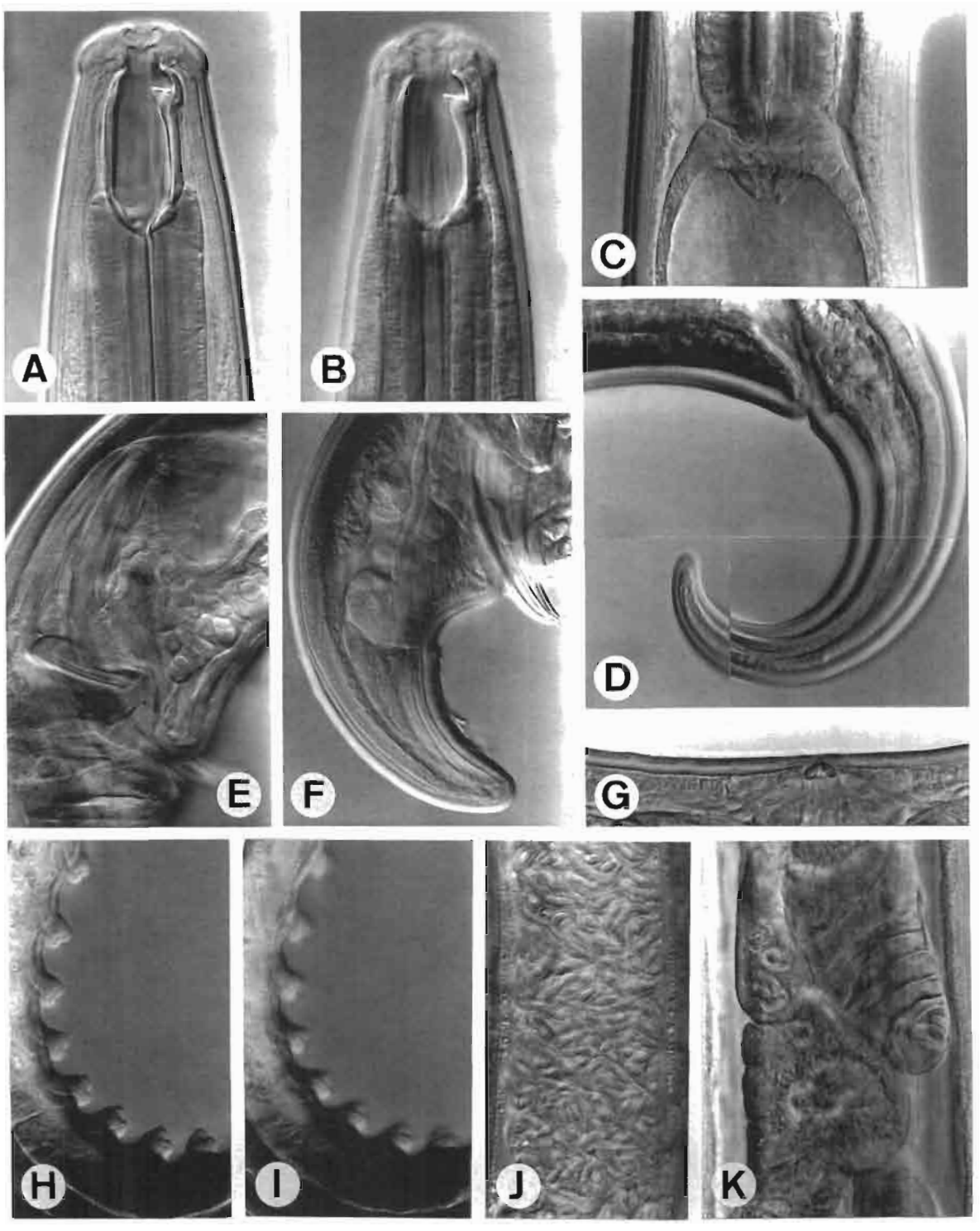

Fig. 8. Coomansus meridionalis *sp. n. A, B: Head; C: Pharynx-intestine junction; D: Female tail; E: Lateral guiding pieces; F: Male tail; G: Vulval region; H, I: Supplements; J: Sperm; K: Ovary and oviduct region. (Scale bar = 50 µm).*

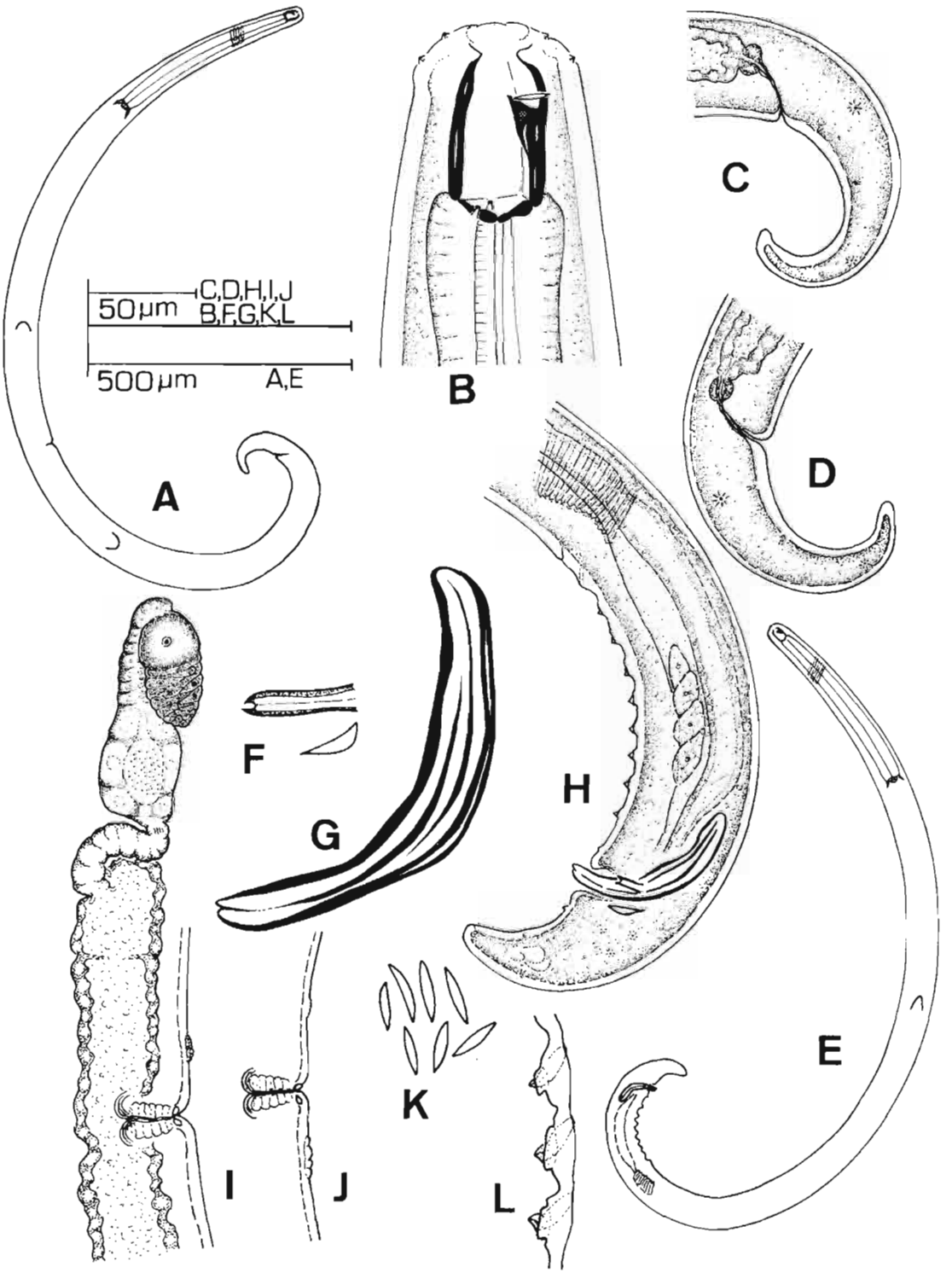

Fig. 9. Coomansus magellanicus *sp. n. A, E: Habitus; B: Head; C, D: Female tail; F: Gubernaculum and lateral guiding pieces; G: Spicules; H: Male posterior region; I: Female genital branch; J: Vulval region; K: Sperm; L: Supplements.*

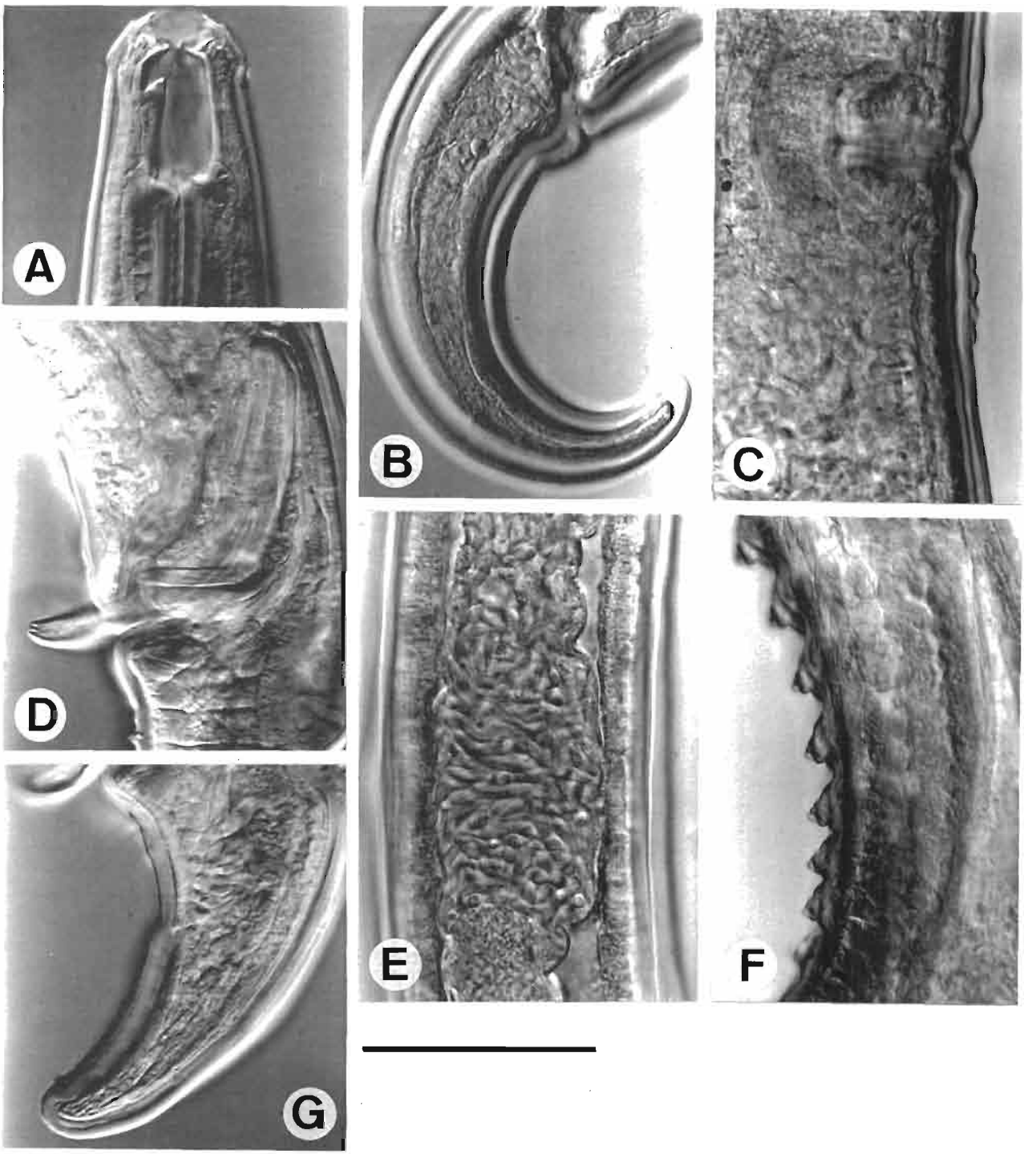

Fig. 10. Coomansus magellanicus *sp. n. A: Head; B: Female tail; C: Vulval region; D: Spicules and lateral guiding pieces; E: Sperm; F: Supplements; G: Male tail. (Scale bar = 50 μm).*

Ovary short, not reaching to the oviduct-uterus junction. Oocytes few in number. Oviduct consisting of a narrow region and a well developed *pars dilatata*. Sphincter present at the oviduct-uterus junction with an inner, poorly sclerotized part surrounded by a muscular part. Uterus consisting of a moderately developed, coiled distal part and a large swollen proximal region filled with sperm. Vagina cylindrical, extending inwards over 46 ± 3 (41-51) % of the corresponding body diameter. Small to medium sized sclerotized pieces in the vagina-vulva junction. Vulva a short transverse slit. Zero to two prevulval and zero to two postvulval papillae. Tail conoid, ventrally curved and with rounded terminus. Caudal glands indistinct, duct and opening absent. Four caudal papillae on each side of tail.

Male: General morphology similar to female but with posterior region more ventrally curved. Lateral chord extending over 30-32 % of the midbody diameter. Lip region 2.1-2.4 times as wide as high. Amphid occupying 18-20 % of the lip region diameter. Buccal cavity 1.5-1.7 times as long as wide. Nerve ring located at 29.5-31 % of the total neck region measured from the anterior end. Genital system diorchid. Testes opposed, with spindle-shaped to cylindrical spermatozoa. *Vas deferens* and *ductus ejaculatorius* separated by a well developed constriction; associated muscles consisting of about sixteen bands located just in front of the supplement series. Ventral body contour slightly contracted in this region of the body. Ejaculatory glands in tandem, often obscure; rectal glands poorly visible. Eleven to twelve ventromedian supplements more or less regularly spaced, the two anterior and one posterior ones poorly developed, the rest prominent, mamilliform, and slightly echinulate. Spicules rather thick, ventrally curved, about 1.75 times as long as anal body diameter, measured along axis. Gubernaculum moderately developed and elongate; lateral guiding pieces not especially slender, with bifurcated extremity. Tail as in female but shorter and with more rounded terminus. Caudal glands apparently reduced and duct opening subterminal. Three subdorsal and three subventral caudal papillae on each site of the tail.

TYPE HABITAT AND LOCALITY

Soil with unidentified grasses in subantarctic steppe, Punta Arenas, Chile.

TYPE MATERIAL

Holotype female in the collection of the Departamento de Biología Animal, Universidad de Córdoba, Spain. *Paratypes*: one male and one female both in the same collection, and one male and six females in that of the Centro de Ciencias Medioambientales, C.S.I.C., Madrid, Spain.

DIAGNOSIS AND RELATIONSHIPS

C. magellanicus sp. n. is characterized by its medium to large size (body length 1.7-2.1 mm), lip region set off by a depression, buccal cavity 32-36 × 18-22 µm or 1.5-2 times as long as wide, dorsal tooth in the corresponding vertical plate with the apex at 76-80 % of the total buccal cavity length from base, V = 51-57, muscles between *vas deferens* and *ductus ejaculatorius* forming approximately sixteen circular bands in front of the supplement series, ventral body contour slightly contracted at beginning of supplement series, ejaculatory glands in tandem, spicules 97-98 µm long, lateral guiding pieces not especially slender, eleven to twelve ventromedian supplements mostly conical, mamilla-shaped and slightly echinulate, conoid, ventrally curved tail with rounded terminus, and caudal glands absent in the female and reduced in the male.

C. magellanicus sp. n. can be distinguished from all above-mentioned species by its buccal cavity shape and dorsal tooth location. It is most similar to *C. campbelli* after the description by Clark (1963) and *C. major*. It differs from *C. campbelli* by a shorter body (*vs* 2-3.5 mm), smaller buccal cavity (*vs* 47-58 × 20-37 µm), apex of the dorsal tooth more anteriorly located in the buccal cavity (*vs* 69-72 %), lateral guiding pieces not especially slender and straight, and different geographic distribution (*vs* Campbell, Auckland and Antipodes Islands). It can be differentiated from *C. major* by shorter body (*vs* 3.4 mm), smaller buccal cavity (*vs* 44 × 26 µm), apex of the dorsal tooth more anteriorly located in the buccal cavity (*vs* 75 %), shorter tail (*vs* 170 µm), shorter spicule (*vs* 156 µm) absence of spinneret (*vs* presence), and different geographic distribution (*vs* Australia and Tasmania).

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Howardula neocosmis sp.n. parasitizing North American *Drosophila* (Diptera: Drosophilidae) with a listing of the species of *Howardula* Cobb, 1921 (Tylenchida: Allantonematidae)

George O. POINAR, JR.*, John JAENIKE** and DeWayne D. SHOEMAKER**

**Department of Entomology, Oregon State University, Corvallis, OR 97331, USA, and **Department of Biology, University of Rochester, Rochester, NY 14627, USA.*

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Summary – *Howardula neocosmis* sp. n. (Tylenchida: Allantonematidae) is described as a parasite of *Drosophila acutilabella* Stalker (Diptera: Drosophilidae) from Florida, USA and *D. suboccidentalis* Spencer from British Columbia, Canada. These two strains represent the first described *Howardula* from North American drosophilids. Notes on the biology of the parasite and a list to the species of *Howardula* Cobb are presented. © Orstom/Elsevier, Paris

Résumé – **Howardula neocosmis *sp. n. parasite de drosophiles nord-américaines (Diptera : Drosophilidae) et liste des espèces du genre* Howardula *(Tylenchida : Allantonematidae)*** – Description est donnée d'*Howardula neocosmis* sp. n. (Tylenchida : Allantonematidae) parasite de *Drosophila acutilabella* Stalker (Diptera : Drosophilidae) provenant de Floride, USA et de *D. suboccidentalis* Spencer provenant de Colombie Britannique, Canada. Ces deux souches représentent le premier *Howardula* décrit sur des drosophiles nord-américaines. Des notes sur la biologie de ce parasite et une liste des espèces du genre *Howardula* Cobb sont présentées. © Orstom/Elsevier, Paris

Keywords: Allantonematidae, *Drosophila*, *Howardula neocosmis* sp. n., insect nematode, parasitism.

Allantonematid nematode parasites of Drosophilidae were first reported by Gershenson in 1939 (see Poinar, 1975, for citations of nematodes from drosophilids). The first descriptions of members of this family attacking lesser fruit flies involved species of the genera *Howardula* Cobb, 1921 and *Parasitylenchus* Micoletzky, 1922 in England (Welch, 1959). In 1985, Montague and Jaenike discussed the presence of allantonematids parasitizing drosophilids in North America and subsequently Poinar *et al.* (1997) described *P. nearcticus* attacking *Drosophila recens* in New York state.

The present study describes the first species of *Howardula* parasitizing drosophilids in North America. From these studies and the report of drosophilid parasitism by allantonematids in Japan (Kimura & Toda, 1989), it is clear that allantonematid parasitism of drosophilids is widespread, demonstrating that these two nematode genera have formed species groups orientated towards infecting drosophilids as well as other acalyptrate Diptera.

Materials and methods

Adult *Drosophila acutilabella* Stalker and *D. suboccidentalis* Spencer were collected in Manasota Key, FL, USA and Peachland, BC, Canada, respectively, in 1997. Newly emerged parasitized flies were placed in culture vials containing instant *Drosophila* Medium (Carolina Biological Supply) plus a piece of commercial mushroom (*Agaricus bisporus*). They were transferred every 4 days to fresh food until they were 3 weeks old. At this age, two parasitized male flies were placed in a culture vial and two female flies from an uninfected culture were added to the same culture after 2 days. All cultures were maintained at 22 °C.

Living parasitized adult flies were dissected at different times to obtain nematodes at various stages of development. All dissections were made in Ringer's solution and the nematodes were heat killed (60 °C), fixed in TAF and processed to glycerin. All measurements were made on slide mounted fixed material, however certain morphological details, *i.e.*, stylet structure, pharyngeal gland morphology, excretory pore position, location of anus and vulva were best determined by observing living individuals.

Howardula neocosmis sp. n.

(Fig. 1)

MEASUREMENTS

Free-living vermiform female (n = 10): L = 416 (384-447) µm; greatest diameter = 16 (13-18) µm; head to middle of nerve ring = 69 (64-74) µm ; head to excretory pore = 80 (75-85) µm; stylet length = 11 (10-12) µm; tail length = 55 (52-56) µm; distance vulva to anus = 23 (22-26) µm ; head to dorsal gland orifice = 25 (22-26) µm; distance from dorsal gland

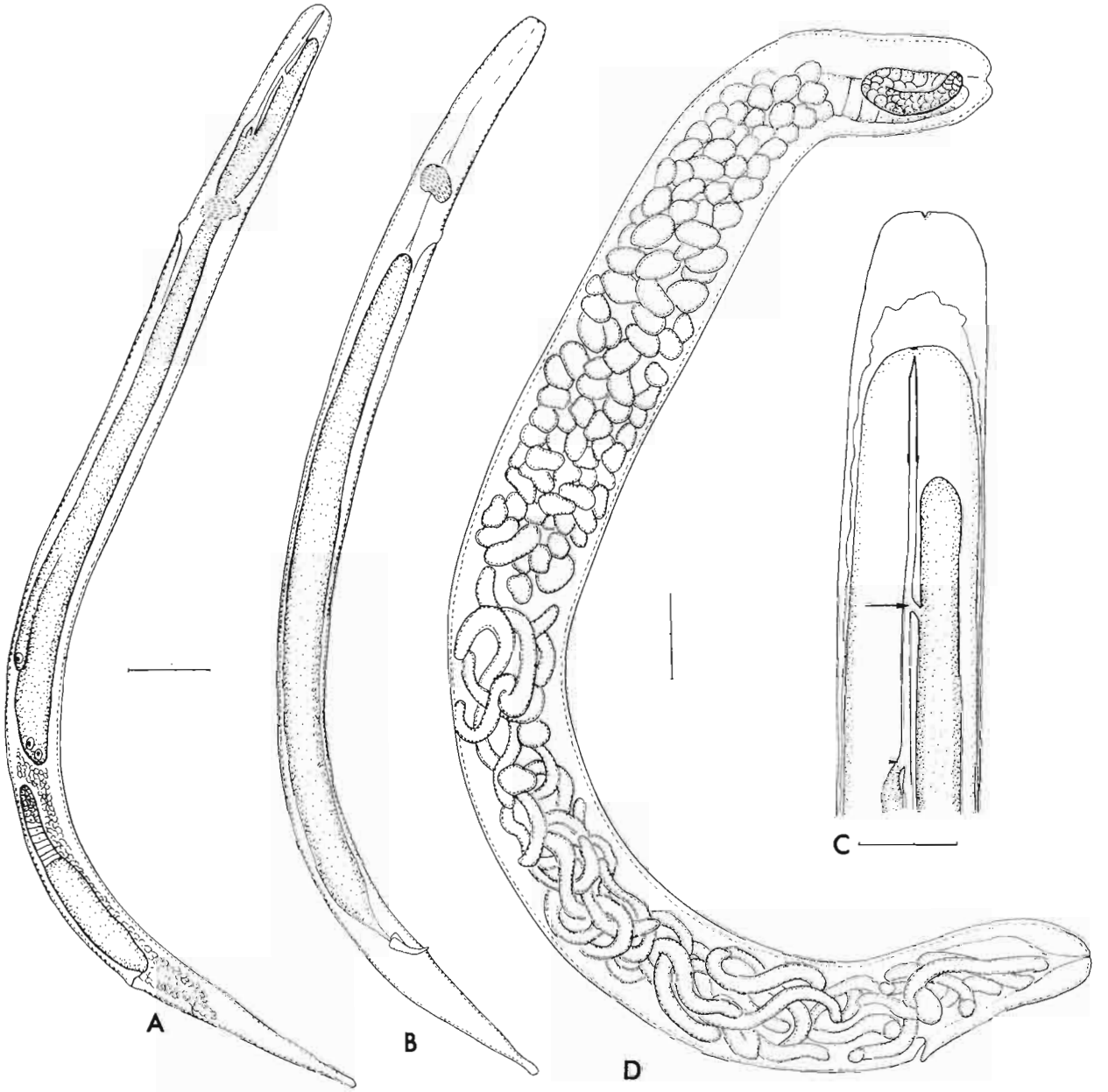

Fig. 1. Howardula neocosmis *sp.n. A: Free-living female; B: Free-living male; C: Head of free-living female with two surrounding cuticles (arrow points to opening of dorsal pharyngeal gland; arrowhead subventral gland opening); D: Parasitic female containing eggs and juveniles (Scale bars: A, B = 25 µm; C = 10 µm; D = 80 µm).*

orifice to ventral gland orifice = 15 (13-19) µm; head to tip of dorsal gland = 11 (9-15) µm ; V = 80 (78-82).

Parasitic female (n= 10): L = 1129 (960-2560) µm; greatest diameter = 125 (100-170) µm; vulva to tail tip = 152 (95-184) µm; stylet length = 9 (8-10) µm; eggs = 32 (20-43) × 20 (16-22) µm; V = 90 (87-93).

Free-living male (n = 10): L = 403 (360-435) µm; greatest diameter = 16 (14-18) µm; head to middle of nerve ring = 70 (56-75) µm; head to excretory pore =

84 (75-88) µm; tail length = 48 (44-57) µm; spicule length = 12 (11-15) µm; greatest width of spicule = 2.0 (1.9-3.0) µm.

DESCRIPTION

Free-living females: Slender body, originally enclosed in a thick, enclosing cuticle and later in the same outer thick cuticle but with an inner membranous cuticle as well. Head truncate, lip region not off-set. Stylet straight, with tip sloping toward ventral surface; stylet base slightly swollen, delineating stylet from remainder of chitinized pharyngeal tube. Distance from stylet base to dorsal gland orifice slightly greater than stylet length; distance from dorsal gland orifice to ventral gland orifice slightly longer than distance from stylet base to dorsal gland orifice; subventral glands extending posteriorally to tip of ovary. Dorsal gland extending only part of that distance; contents of the subventral glands appearing creamy white while that of the dorsal gland granular, indicating different components within. Excretory pore located posterior to the nerve ring and just posterior to the hemizonid. Lateral fields 3-4 µm wide with five striae in widest region. Vulva located on small protuberance. Vagina narrow, leading to a small uterus with no or very slight post vulvar sac; oviduct and ovary small, tip of ovary often reaching to the tip of the subventral pharyngeal glands. Tail slender, ending in a slightly swollen rounded tip.

Parasitic females: Varying in size depending on number per host (the more per host, the smaller the parasites). Cuticle thin except in tail and head region where often thick and wrinkled. Original stylet minus basal thickening portion present although this structure often obscured and shoved aside by the developing gonad (this explaining the size discrepancy between the stylets in the free-living and parasitic females). Lateral fields not discernible. Anus terminal. Vulva present although often obscured. After egg hatching, juveniles undergoing two molts in uterus. Ovoviviparous.

Males: Lacking a stylet and associated pharyngeal glands. Hemizonid prominent as swelling just above excretory pore. Lateral fields, 3-4 µm in width, present but striae faint. Spicules broad at base, with terminal fourth bent outward; bursa and gubernaculum absent. Tail elongate with faint rounded swelling at tip.

Remarks

The preceding account is based on the examination of living specimens which show morphological features much clearer than fixed material. The British Columbia strain of *H. neocosmis* sp. n. did not appear to possess any qualitative characters that separated it from the Florida strain. However, some of the measurements of the females of the British Columbia strain ranged higher than those of the Florida strain and are given below. There were no quantitative differences between the males of the two strains. In the British Columbia strain, those measurements that differed from the Florida strain in the free-living females were the length (458 [384-517] µm), the distance from the head to the middle of the nerve ring (84 [72-93] µm), the distance from the head to the excretory pore (93 [85-99] µm), the stylet length (12 [11-13] µm), and the distance from the dorsal gland orifice to the ventral gland orifice (18 [16-20] µm). The length of the parasitic females of the British Columbia strain ranged from 1920 to 3200 µm. Since at this time, clear characters separating these two populations could not be found, they will be referred to as the Florida and British Columbia strains of *H. neocosmis* sp. n.

TYPE HOST AND LOCALITY

Found in the hemocoel of *Drosophila acutilabella* Stalker collected from Manasota Key, FL, USA (Florida strain). Also found in *D. suboccidentalis* Spencer from Peachland, BC, Canada (British Columbia strain).

TYPE MATERIAL

Holotype: free-living female of the Florida strain (UCDNC 3651) - *Allotype*: male of the Florida strain (UCDNC 3652); deposited in the Nematology Collection, Department of Nematology, University of California, Davis. *Paratypes* in the collection of the Muséum National d'Histoire Naturelle, Paris, France, and in the author's collection.

DIAGNOSIS AND RELATIONSHIPS

The absence of a bursa and gubernaculum in *H. neocosmis* sp.n. separates this species from all of the other members of the genus with the exception of a group of three species which parasitize acalyptrate Muscomorpha, namely *H. marginatis* in Sphaeroceridae, *H. albopunctata* in Sepsidae and *H. aoronymphium* in Drosophilidae. The oviparous nature of the former and the long stylet of *H. albopunctatis* distinguish them from *H. neocosmis*. It is obvious that the new species is closest to the European drosophilid parasite *H. aoronymphium*. However, the following characters separate the two species: size of the spicules (11-15 in *H. neocosmis vs* 19-21 µm in *H. aoronymphium*), the distance from the head to the excretory pore in the male (80-88 in *H. neocosmis* sp.n. *vs* 98 µm in *H. aoronymphium*), the presence of basal thickenings on the stylet in *H. neocosmis* and their absence in *H. aoronymphium*, the presence of a stylet, anus and vulva in the parasitic female of *H. neocosmis* and their absence in *H. aoronymphium,* and the length of the stylet in the infective female (10-12 in

Table 1. *List of species of the genus* Howardula *Cobb, 1921 with indication of their host(s).*

Species	Hosts
Type species	
H. benigna Cobb, 1921	Coleoptera: Chrysomelidae
Valid species	
H. acarinorum Wachek,1955	Acarina
H. acris Remillet & Van Waerebeke, 1976	Coleoptera: Hydrophilidae
H. albopunctata Yatham & Rao, 1980	Diptera: Sepsidae
H. aoronymphium Welch,1959	Diptera: Drosophilidae
H. apioni Poinar, Laumond & Bonifassi, 1980	Coleoptera: Curculionidae
H. belgaumensis Raj & Reddy, 1989	Coleoptera: Chrysomelidae
H. colaspidi Elsey,1979	Coleoptera: Chrysomelidae
H. dominicki Elsey,1977	Coleoptera: Chrysomelidae
H. husseyi Richardson, Hesling & Riding, 1977	Diptera: Phoridae
H. madecassa Remillet & Van Waerebeke, 1975	Coleoptera: Nitidulidae
H. marginatis Reddy & Rao,1981	Diptera: Sphaeroceridae
H. mutilatus Devi, Rao & Reddy, 1991	Coleoptera: Mitidulidae
H. neocosmis sp. n.	Diptera: Drosophilidae
H. oscinellae Goodey, 1930	Diptera: Chloropidae
H. phyllotretae Oldham, 1933	Coleoptera: Chrysomelidae
H. saginata Rajashekar, Rao, Reddy & Reddy, 1995	Coleoptera: Chrysomelidae
H. truncata Remillet & Van Waerebeke,1975	Coleoptera: Nitidulidae
Species inquirendae	
H. claviger Warren, 1941	Acarina
H. cuneifer Warren, 1941	Acarina
H. hirsuta Warren, 1941	Acarina
H. terribilis Warren, 1941	Acarina
Species dubiae	
H. prima Rubtsov & Tshumakova, 1981	Siphonaptera
H. stenoloba Rubtsov & Tshumakova, 1981	Siphonaptera

H. neocosmis vs 14-15 [rarely 12] µm in *H. aoronymphium*.).

BIOLOGICAL OBSERVATONS

The life cycle of *H. neocosmis* sp.n. appears to be similar to those of other members of the genus. The fertilized free-living female enters a young host larva by penetrating the cuticle with the aid of its stylet and salivary glands. This was easily demonstrated when young fly larvae were placed in Petri plates with infective stage female nematodes. Up to ten nematodes were observed in a single fly larva and it appears that when penetration occurs in very young hosts, the latter may succumb to septicemia from bacteria entering the penetration wounds. By the time the infected host has emerged as an adult, the female nematodes have swollen into sausage-shaped parasites and are producing numerous eggs. These eggs hatch inside the uterus of the female and the juveniles develop and molt twice while still in this location (no evidence of a molt occurring within the egg was noted, although in one case, a molt occurred at the time the juvenile emerged from the egg). It appears that when the juveniles leave the uterus of the female and enter the host hemocoel, they have molted twice and are in the third stage. Thus the two molts seen in the free-living nematodes would account for the normal four molts in the nematodes life cycle. Exit from the host occurs from the genital and digestive tracts. Once in the environment, the nematodes molt twice to the adult stage, mate and the females search for a new host.

Species in the genus *Howardula*

There are at least 24 described species in the genus *Howardula* Cobb, 1921 (see Table 1). Four of these (*H. claviger*, *H. cuneifer*, *H. hirsuta*, and *H. terribilis*) were described by Warren (1941) only on the basis of histological sections made through the bodies of parasitized mites and are considered *species inquirendae* (Siddiqi, 1986). The two species described from fleas, *H. prima* and *H. stenoloba* by Rubtsov and Tshumakova (*in* Rubtsov, 1981) are based only on the structure of the parasitic female and we agree with Deunff (1984) that there is no evidence that these parasites belong to the genus *Howardula*. Therefore, they should be considered as *species dubiae*. The species *H. aptini* from Sharga (1932) and other *Howardula* spp. attacking thrips have been transferred by Siddiqi (1986) to the genus *Thripinema* . Thus, at this time, the host range of *Howardula* includes Coleoptera, Diptera and Acarina. Most diagnostic characters of members of this genus reside in the free-living males and females. These include the presence of a bursa and gubernaculum and size of the spicules in the male, the stylet length in the female and the overall body lengths as well as the distances from the head to the nerve ring and excretory pore in both sexes.

Discussion

As a group, the Allantonematids have a curious host range. While the great majority of described species parasitize holometabolous insects of the orders Coleoptera and Diptera, others attack mites, thrips and Hemiptera (Poinar, 1975). Fossil records of Allantonematids date back to the Tertiary, some 20-40 million years ago (Poinar, 1984, 1993; Poinar & Brodzinsky, 1986) and involve both dipteran (Drosophilidae) and coleopteran (Staphylinidae) hosts. It has been speculated that the Allantonematidae arose in the Carboniferous (Poinar, 1983), quite possibly with acarines or Hemiptera serving as the original hosts. It is not known when the two genera (*Howardula* and *Parasitylenchus*) initiated parasitic relationships with the Drosophilidae (as well as with other acalyptrate families of Diptera), but it may have occurred in the late Cretaceous or early Tertiary. The report by Gershenson (1939) listing a *Chondronema* Christie & Chitwood, 1931, infection of Drosophilidae is undoubtedly an error since this genus of parasites is only known to occur in members of the Passalidae (Coleoptera) and has now been transferred out of the Allantonematidae (Sidiqqi, 1986).

It is interesting that two allantonematid genera have adapted to the same family of hosts. While Welch (1959) reported that *P. diplogenus* and *H. aoronymphium* both occurred in separate host species in England, Poinar *et al.* (1997) noted that in New York state, *D. recens* was parasitized (even the same individuals) by both *Parasitylenchus* and *Howardula*.

Of the five reported Diptera parasitized by *Howardula* spp., all belong to the Brachycera and four are acalyptrate Muscomorpha. With the exception of *H. oscinellae* parasitizing a member of the Chloropidae, all of the *Howardula* parasitizing flies form a morphological group characterized by the loss of the gubernaculum.

Acknowledgments

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The genus *Boreolaimus* gen. n. and its six species (Dorylaimida: Qudsianematidae), nematodes from the European Arctic

István ANDRÁSSY

Eötvös Loránd Tudományegyetem, Állatrendszertani és Ökológiai Tanszék, 1088 Budapest, Puskin-u. 3, Hungary.

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Summary – A new genus, *Boreolaimus* gen. n., is proposed for five new and a known species described in this paper. *Boreolaimus* is placed in the family Qudsianematidae; it differs from other genera of the family by the wide buccal lining around spear, presence of four oesophageal nuclei instead of five and "empty" posterior part of tail. The total lack of males and spermatozoa in uterus of mature females seems to be also a characteristic feature for this genus. The genus *Boreolaimus* is also characterized by the occurrence and distribution of its member species : they all inhabit interstitial biotopes and are, as far as known, only found in the northern regions of the Globe. The members of *Boreolaimus* are: one known species, *B. enckelli* (Andrássy, 1967) comb. n., and five new species, *B. arcticus, B. borealis* (type species), *B. lapponicus, B. norvegicus,* and *B. septemtrionalis*. Although these species are closely related, they can be differentiated from each other by differences in shape and slenderness of body, shape and length of tail, presence or absence of sclerotization in vulval lips, cuticular structure of tail, and extent of "empty" region in percentage of tail length. © Orstom/Elsevier, Paris

Résumé – ***Le genre* Boreolaimus *gen. n. et ses six espèces (Qudsianematidae), nématodes provenant d'Europe arctique*** – Le nouveau genre *Boreolaimus* gen. n. est proposé pour contenir cinq espèces nouvelles et une déjà connue, toutes décrites dans cet article. *Boreolaimus* peut se ranger dans la famille des Qudsianematidae où il diffère des autres genres par la spacieuse paroi buccale entourant le stylet, la présence de quatre, au lieu de cinq, noyaux oesophagiens et une portion postérieure de la queue "vide". L'absence de mâles et celle de spermatozoïdes dans l'utérus des femelles matures paraissent être également une caractéristique du genre. Les membres du genre *Boreolaimus* sont : une espèce déjà connue, *B. enckelli* (Andrássy, 1967) n. comb., *B. arcticus, B. borealis* (espèce type), *B. lapponicus, B. norvegicus* et *B. septemtrionalis*. Bien que ces espèces soient proches l'une de l'autre, elles peuvent être séparées par des différences dans la forme et le caractère plus ou moins élancé du corps, la forme et la longueur de la queue, la présence ou l'absence d'une sclérotisation associée aux lèvres vulvaires, la structure de la cuticule caudale et la longueur de la portion "vide" de la queue, calculée en pourcentage de la longueur de celle-ci. La présence et la répartition des espèces sont également typiques pour le genre : toutes proviennent de biotopes interstitiaux et n'ont été rencontrées, à notre connaissance, que dans les régions les plus nordiques de notre globe. © Orstom/Elsevier, Paris

Keywords: Arctic, *Boreolaimus*, interstitial nematodes, morphology, reproduction, Scandinavia, taxonomy.

Dr P.H. Enckell (Zoological Institute of the University, Lund, Sweden) carried out for many years very extensive and systematic collections during the sixties in Scandinavia, with the aim of discovering the interstitial fauna of that part of Europe. He thoroughly covered all four Scandinavian countries – Denmark, Norway, Sweden, Finland – and sampled almost the entire region. A very large number of groundwater samples were collected in areas adjoining river banks and lake shores. Among several other animals, the samples contained nematodes in great number. Dr Enckell was so kind as to offer the latter to the present author for study.

Nematodes were extracted from the original samples in Lund, killed and fixed with 4-5 % formaldehyde solution, then sent to Budapest in small vials. They were transferred to glycerine and mounted in pure glycerine for microscopic examinations. Although nearly 30 years have passed since, the animals on permanent slides are still well preserved.

I have already published an article (Andrássy, 1967) about the nematodes from Enckell's collection. A report was given on the nematode fauna of the interstitial zones of two lakes (Vätter- and Torneträsk Lake) in Sweden. Also, a new species was described and named in honour of the collector, *Eudorylaimus enckelli* Andrássy, 1967. When enumerating the morphological characters of this species, I mentioned an unusual feature by which it differed from every other representative of the genus: the posterior half of the tail was completely "empty", showing no traces of body tissues or filler cuticle.

Going through more nematode material of the Scandinavian collection, I recently found that a number of specimens originating from the northernmost region (Fig. 1) strongly resembled *E. enckelli*, yet were at the same time distinctly different from that

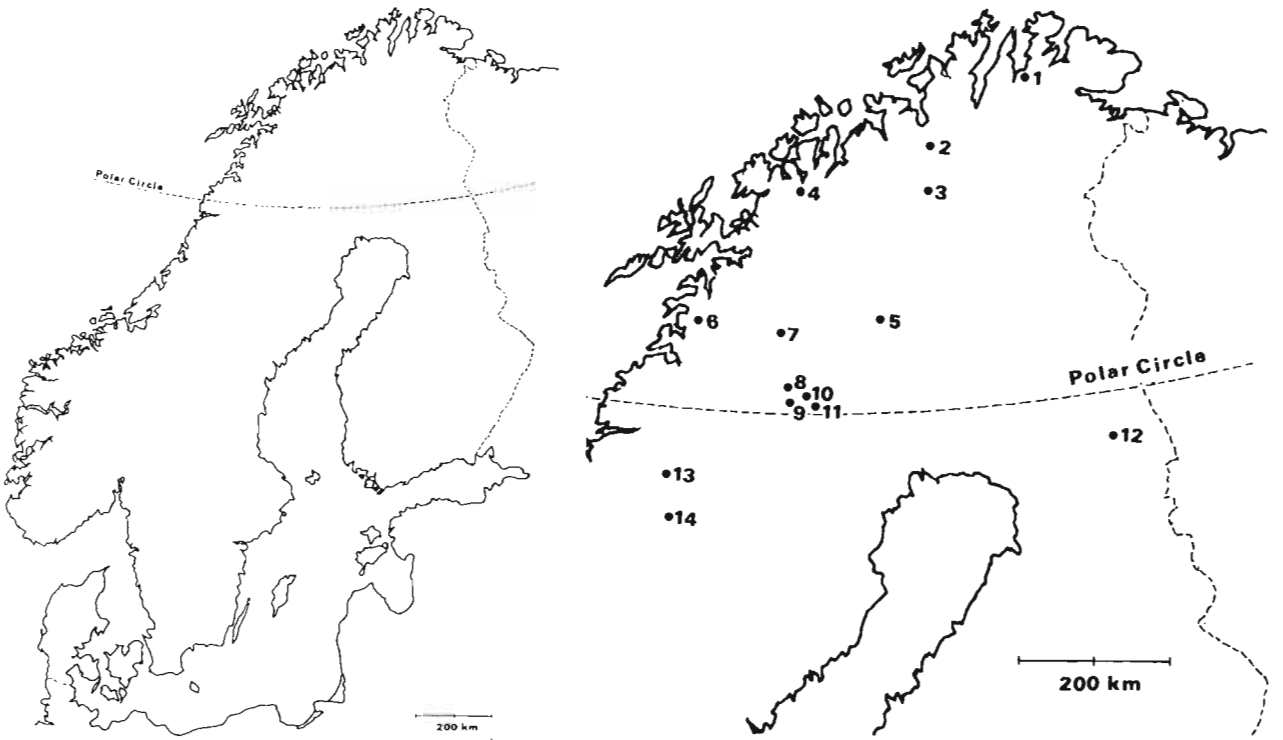

Fig. 1. Map of Scandinavia with localities of Boreolaimus *species. 1: Lake Kuvatn, Prov. Finnmark, Norway; 2: Lake Övre Trangvatn, Prov. Finnmark, Norway; 3: Lake Mieron, Prov. Finnmark, Norway; 4: Lake Sagelvvatn, Prov. Troms, Norway; 5: River Torneälv, Prov. Lappland, Sweden; 6: Bonnassjöen, Prov. Nordland, Norway; 7: Lake Satisjaure, Prov. Lappland, Sweden; 8: Lake Peuraure, Prov. Lappland, Sweden; 9: Lake Karats, Prov. Lappland, Sweden; 10: River Pärlälven, Prov. Lappland, Sweden; 11: Lake Juonajaure, Prov. Lappland, Sweden; 12: Lake Ylikitka, Prov. Lappi, Finland; 13: Lake Laisan, Prov. Lappland, Sweden; 14: River Vojm n, Prov. Lappland, Sweden.*

species. They shared, among others, the "empty" posterior part in the tail already observed in *E. enckelli*. As a result of further examinations, it became evident that these empty-tailed nematodes are part of a special group differing from all other types of *Eudorylaimus* species, and even from representatives of related genera. This group of species is here proposed as a separate genus, *Boreolaimus* gen. n.

It is hardly questionable that this new genus is a natural unit of closely related sister-species which, however, can well be differentiated from each other. *Boreolaimus* gen. n. can very well be outlined or characterized not only morphologically but also in mode of life and distribution.

Boreolaimus * gen. n.

Qudsianematidae. Body moderately slender to slender, 1.5 to 2.4 mm long. Cuticle thin, smooth, exceptionally with superficial structure. Head offset by depression, lips separate, angular. Stoma lining around spear unusually wide and reaching well behind the spear. Odontostyle straight and cylindrical, longer than cephalic diameter; its aperture occupying about one-third of its length. Guiding apparatus a long tube with widened anterior ring. Oesophagus muscular, expanding before or near middle in more steps. Oesophageal nuclei distinct, with the exception of the anterior subventral nucleus. Intestine generally folded, with enlarged cells at junction with prerectum. Prerectum with short caudal sack. Anal musculature very strong. Vulva transverse, located near middle of body. Female genital system amphidelphic; reproduction by automixis. Males absent. Tail conoid, straight to ventrally arcuate with rounded tip, as long as two to five anal diameters. Distal part of tail "empty" with very thin cuticle.

TYPE SPECIES

Boreolaimus borealis sp. n.

* Boreo-" is from the Greek word βορειοσ = northern, of the north.

OTHER SPECIES

B. arcticus sp. n.
B. enckelli (Andrássy, 1967) comb. n.
= *Eudorylaimus enckelli* Andrássy, 1967
B. lapponicus sp. n.
B. norvegicus sp. n.
B. septemtrionalis sp. n.

In general habitus and organization, the new genus corresponds to the criteria of the family Qudsianematidae and of its type subfamily (*sensu* Andrássy, 1992). It resembles the genus *Eudorylaimus* Andrássy, 1959 in some morphological features, it differs, however, not only from that and the other genera of the family but also from all known members of the suborder Dorylaimina. The uncommonly wide lining of stoma, tubular guiding sheath, (seemingly?) lacking anterior subventral nucleus in oesophagus, "empty" posterior region of tail are the main morphological characteristics of the *Boreolaimus* species. In addition, they are notable for the complete absence of males. Moreover, their occurrence also is distinctive: all of them inhabit groundwater biotopes and are distributed, as far as known, in the Arctic region of Europe.

MAJOR MORPHOLOGICAL AND BIOLOGICAL FEATURES AND THEIR TAXONOMIC VALUE

In order to present the most important morphological characters of *Boreolaimus* and to point at their constancy or variability, let us inspect the new genus more in detail.

Body shape: The members of the genus are of middle length, between 1.5 and 2.4 mm. The body length may fluctuate only a little within one species; there are smaller species, under 2 mm, species of medium length, about 2 mm, and bigger ones, above 2 mm. The figure is moderately slender to slender, the value of "a" varies between 23 and 62. Two of the species are comparatively stout (a < 30), two moderately thin (a = 30 to 40) and two definitely slender (a = 40 to 60). In fixed state the body remains more or less straight or becomes slightly bent ventrad.

Cuticle: The cuticle is definitely thin, 1 to a maximum of 2 µm over the entire body, thicker, up to 3-5 µm, only on the anterior part of tail. Optically, the cuticle consists of two layers: a thinner, more refractive outer layer and a thicker inner layer. The cuticle is smooth, at most the inner surface may show some fine annulation in the neck region (Figs 7A; 9A). Only one species shows a superficial structure of cuticle: in the anal area a very fine transverse striation and indistinct punctation can be seen (Fig. 4C). In all species, the cuticle at the level of the mouth spear is conspicuously thinner than the spear itself.

Cephalic region: The head is well separated from the adjacent region of the body by a depression. Its appearance is very similar in every species. The six lips are distinct and more or less angular, and bear rounded or somewhat conoid papillae. The diameter of the lip region varies only slightly: it measures 16 to 19 µm. The amphids open just behind the lips; they are cup-shaped and at least half as wide as the corresponding body diameter.

Buccal region: One of the principal distinguishing features of *Boreolaimus* can be seen in the mouth cavity: its lining or contour is wider (more spacious) than in other genera of Qudsianematidae. It begins with a wide atrium, then turns into a jug-shaped medial part and ends well behind the basis of the spear. The shape of the dorylaimid odontostyle is constant and there is also little variation in length: 22 to 28 µm in the genus. In each species, the variation of the stylet length does not exceed 2 µm. The spear is always longer than the diameter of head (1.2 to 1.5 times). The aperture occupies one-third of spear length or so. The structure of the guiding sheath around the spear is similar in every species: it is not a simple ring but a comparatively long tube with somewhat divergent proximal walls and widened anterior end (Figs 3A; 7A; etc.).

Oesophagus: The oesophagus consists of a slender but muscular anterior part and an extended, well muscular posterior portion, the cylindrus (see Andrássy, 1995). The length of the oesophagus is always measured from the anterior margin of the lip region to the posterior margin of the cylindrus. The length of the cylindrus cannot be defined exactly, owing to the gradual or stepwise widening of the oesophagus, but the cylindrus occupies at least half the length of the distance between anterior body end and cardia. Its posterior region is not regularly cylindrical: it starts with an anterior widening and shows a conspicuous expansion in its posterior third/fourth (*e.g.*, Figs 9B; 10B). In addition, a slight medial swelling is also present. The oesophageal gland nuclei are refractive and well visible, except for the anterior subventral nucleus (AS_1), which is completely invisible (and there is no gland opening at the place where there would be one if the nucleus was present). Whether the nucleus is truly absent or so minute as to be indistinguishable from the oesophageal tissue, cannot be decided with certainty. Whatever the case may be, this nucleus could not be observed or localized with certainty in more than one hundred specimens studied for this purpose (in some specimens of a single species, a minute, more or less nucleus-like granule could be seen at 28-30 % of the distance between dorsal nucleus and posterior end of cylindrus, well anterior to the AS_2. Was it an AS_1 indeed?) *. The

* In dorylaimid nematodes, the AS_1 nucleus is usually smaller than its partner (AS_2), but this small nucleus is generally visible.

dorsal nucleus (D) is essentially larger than the other nuclei, round-oval (*e.g.*, Fig. 8D), generally lying parallel with the body axis and rarely at a right angle to it. It is located in the proximal part of the anterior widened region of the cylindrus at a fairly constant position, near 60 % (57 to 62 %) of the distance between head and hind end of oesophagus. The visible anterior subventral nucleus (AS_2) and the two posterior subventral nuclei (PS_1 and PS_2) are similar in shape, *i.e.*, mostly round, exceptionally ovoid (Fig. 10B); in some cases AS_2 is a little bigger than each PS (Fig. 12B). Their position can be described as constant. AS_2 is located at about 40 % (36 to 44 %) of the distance between dorsal nucleus and posterior end of cylindrus, PS_1 and PS_2 are located at about 65 % (61-70 %) and 67 % (63-71 %) of the same distance, respectively. PS_1 and PS_2 are located at the beginning of the posterior expansion of the cylindrus. In one of the species, an unusual phenomenon was observed: at the level of the dorsal nucleus or a little before it, one or two 'accessory' nuclei were present (Figs 7B; 8E). They were elongate, transversely directed, and well visible in half the specimens. What are they actually and what are they used for? This remains an unanswered question for the moment.

Since the study of Loof and Coomans (1970), it has been generally accepted that the location of oesophageal gland nuclei in dorylaimid nematodes is useful in the characterization of families, genera, and even species. Of the five nuclei, the dorsal nucleus is the biggest and the most anterior. The other four nuclei are arranged in two pairs, one located in the middle, and the other one at the posterior third/fourth of the cylindrus. Their position may be measured in diverse ways: either in absolute distances in µm from anterior or posterior end of cylindrus, or in percentage of the cylindrus length or of the entire length of the oesophagus. Percentage values for nucleus positions are easier to visualize and compare at a glance than absolute measurements, but they also may cause some difficulties for the observer. Thus, percentages of oesophagus length can be relatively imprecise, *e.g.*, in terms of distance between closely spaced nuclei, while percentages of cylindrus length are often subjective as the anterior end of the cylindrus is often difficult to determine precisely (it begins by a gradual widening and not at a definite point). To avoid these problems, a new method is suggested here which is essentially a combination of old ones: the position of the dorsal nucleus is determined as a percentage of the total length of the oesophagus (from head to posterior end of cylindrus). This nucleus is big enough to be observed even under lower magnification and its location is easily specified in relation to the entire oesophageal length. However, the other four nuclei should be localized as a percentage of the distance from the dorsal nucleus (from the centre of it) to the posterior end of the cylindrus. These data give a good picture of the position of the posterior four nuclei, whether to compare them with the dorsal nucleus, or with one another. In the following descriptions of the *Boreolaimus* species, this method is applied (Fig. 2); in parentheses, the 'old' values according to Loof and Coomans (1970) are also added. Unfortunately, the openings of the oesophageal glands were in most cases so indistinct that their positions could not be measured.

Intestine: The cardia is muscular, tongue-shaped, medium long. The intestine consists of one layer of large cells; it has a wide lumen and is often folded. Compact contents can frequently be observed in the digestive tract; they may be colourless, yellowish, or greenish, occasionally quite dark. Some enlarged cells are visible in the intestine just before the intestine-prerectum junction, which is offset (*e.g.*, Figs 4A; 6C; 8C). The prerectum also possesses a wide lumen, as well as a short but distinct dorso-caudal sack due to the ventrally displaced origin of the rectum. The prerectum is 1.5 to 4.5 times, the sack 1 to 1.5 times as long as anal body diameter, but their lengths may vary within the same species. The rectum is practically straight.

Female genital system: Amphidelphic nematodes with equally developed gonads. The vulva is transverse (Fig. 12D), with sclerotized (Figs 5D; 10D) or non-sclerotized (Figs. 3C; 7C; 9D) inner lips; presence or absence of sclerotization is constant for a species and constitutes a good taxonomic character. The female genital aperture can be found near mid-body. The arrangement of the gonads can vary within one species: any of them may be seen either on the right or the left side of the intestine, but they are generally on opposite sides of the intestine in the same animal. Both ovaries are of similar length, reflexed, often reaching to the vulva; they consist of a moderate number of cells. No more than one or two mature eggs occur at the same time; they are longer than the body diameter. The oviduct is a slender and transversely striated tube lying on the ventral side; in young females, it is subterminally connected with the ovary (Figs 5E; 7E; 10E), in mature females the distance between the proximal end of ovary and the connection with oviduct is considerably longer (Figs 3D; 12E). At the junction of oviduct with uterus, a small chamber is present which may contain a few sperm-like elements, or fine granules only. No spermatozoa were ever seen in the uterus, even in gravid females. This means that males are actually lacking - a very remarkable feature of the genus *Boreolaimus* gen. n.!

Reproduction: Reproduction most probably occurs by self-fertilization (automixis). In younger females, a

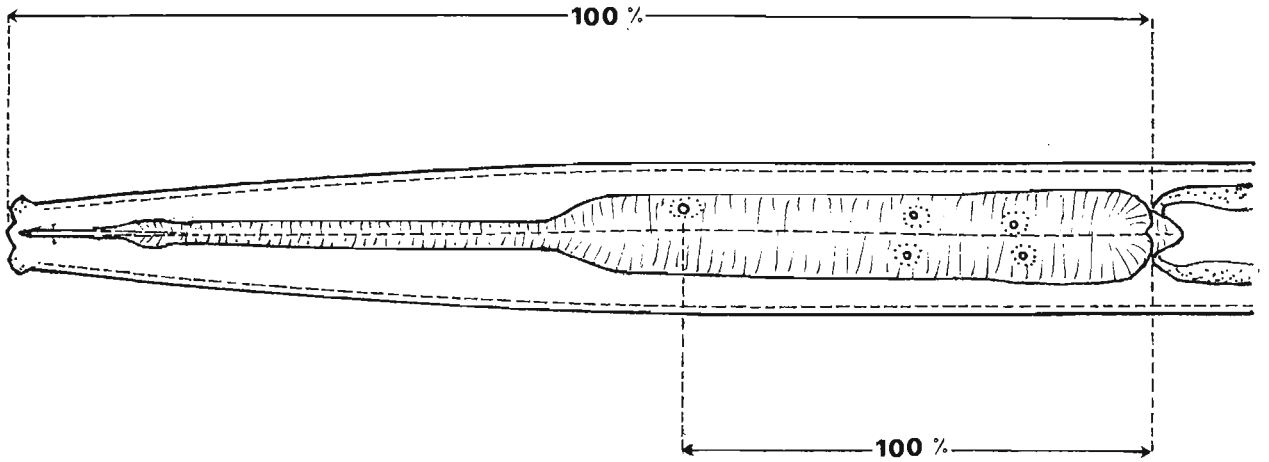

Fig. 2. *How to determine the location of the pharyngeal nuclei: The position of the dorsal nucleus is expressed as a percentage of the distance between head and posterior end of oesophagus; the positions of the subventral nuclei are given as the percentage of the distance between dorsal nucleus and posterior end of cylindrus. In the example shown here, D (dorsal nucleus) = 59 %; AS_1 (anterior subventral nucleus, first) = 48 %; AS_2 (anterior subventral nucleus, second) = 50 %; PS_1 (posterior subventral nucleus, first) = 70 %; PS_2 (posterior subventral nucleus, second) = 72 %.)*

few refractive cells or nuclei may be observed in the germinal zone of both ovaries, close to the ovary-oviduct junction. These are presumably spermatozoa (Figs 5E; 7E). Similar sperm-like elements, few in number as well, can sometimes be seen in the proximal widened end of the oviduct. Therefore, it is suggested that the ovaries - more exactly: ovotestes – first produce a limited number of male genital cells (spermatocytes), then change their function to produce oocytes (proterandry). Whether reproductively functional male specimens still occur from time to time could not be ascertained. No male specimens were ever found in the very rich Scandinavian material collected during various seasons. Thus, we may suppose that the representatives of the genus *Boreolaimus* are all monosexual, females in appearance, but in fact hermaphrodites. In theory, monosexual (autotokous) reproduction results in loss of genetic variability and, as a consequence, diminishes the ability for adaptation, which makes it seem to be less viable than bisexual (amphimictic) reproduction. When, however, the environment is constant enough - as is the case in our *Boreolaimus* species: they occur in a definite type of habitats, and in addition, in the same climatic zone of the Earth - hermaphroditic reproduction may be successful and sufficient for preserving the race. The great constancy in morphological characters within the whole genus *Boreolaimus* may thus be interpreted as an outcome of both mode of life and type of reproduction.

That male individuals are infrequent or very scarce is not unusual in nematodes, but a perfect and consistent absence of males is an extremely rare phenomenon in free-living forms. We know of only a few genera, that are rich in species and fairly frequent in certain biotopes, where males have never been found (*e.g., Bunonema, Drepanodorylaimus* *).

The material described here was interesting in another respect. When investigating the fine structure of the female genital system in Dorylaimida, Geraert *et al.* (1980) wrote: "As we never found oocytes in the oviduct, oocytes must pass quickly through each of these cells although under the light microscope a lumen is never seen". In two specimens of *Boreolaimus*, I could observe how a half-mature egg (oocyte) goes through the oviduct by actually flowing through this very tight tube, which does have a lumen. The oocytes are practically liquid in content and covered by a very thin and elastic membrane. When the most mature oocyte is ready to start from the ovary towards the uterus, it simply flows through the oviduct (Fig. 7F). Once in the uterus, it regains its concrete shape, develops a thick shell, and becomes a true egg. Whether fertilization happens in the ovary, or elsewhere on the way towards the genital opening – most probably in the receptaculum-like chamber of the oviduct – could not be ascertained in fixed material.

* I have an exceptionally rich collection of *Drepanodorylaimus* species as well as of other relative genera of the dorylaimid subfamily Afrodorylaiminae; whereas they were collected in various parts of the Earth, male specimens do not exist among any of them.

Tail: In addition to the shape of buccal cavity and guiding sheath, and to the probable lack of anterior subventral nucleus in the oesophagus, the unique structure of the tail is another important feature of *Boreolaimus*. The tail is conical, narrowing quickly at first then more slowly, straight (Figs 4A, C; 6A) or ventrally arcuate (Figs 8A; 9F, G; 13A, B), and always with a regularly rounded tip. Its length varies between two and five anal body diameters within the genus, but it is very typical and constant for each species. There are species with short tail (c' = 1.8-2.6), medium tail (c' = 3.1-3.5), and long tail (c' = 4.3-5.2). The posterior region of the tail always seems to be empty. The body tissue which fills the anterior half rapidly ends with a sharp contour, which leaves the posterior part free. The comparatively thick cuticle of the anterior tail portion suddenly becomes very thin at the level where the tail tissues end or a little before (Figs 6D; 9H; 11D; 13C). It may be that the 'empty' portion of tail is filled up with air or some crystal-clear liquor; certainly, no solid elements were seen in it. In some other dorylaimids, the posterior part of tail is sometimes also apparently empty; however, this portion may contain traces of tissues or be packed with the thickened – but transparent – cuticle. Still, if there is some actual void in the tail, it is limited in extension and called 'core'. In *Boreolaimus* species, the posterior part of tail is covered by a very thin layer of cuticle and the whole interior is apparently empty. It may be supposed that this emptiness at the hind end of body plays some role in the life of our animals - but what is that role? The length of the 'empty' section in relation to the entire length of tail is fairly constant within the same species. In the genus, it varies from 21 to 54 %. In some species it measures 1/4 (Fig. 13A, B), in other species 1/3 (Fig. 11A, B) or 1/2 (Fig. 4A-C) of tail length. It should be noted that two species of *Boreolaimus* are characterized in having distinct 'bubbles' or 'saccate bodies' in the subventral cuticle on the anterior half of the tail; these structures are constant (Figs 4A-C; 11A-C). It is furthermore characteristic for the tail that the anal musculature is stronger and wider than usual in dorylaims.

Habitat and distribution: As far as known, the mode of life and the distribution of representatives of the genus *Boreolaimus* seem to be unique. All six members of the group have come to light from one series of collections in Scandinavia. Although the species of this genus are terricolous, they do not inhabit soil types covered by plants and interwoven with roots but they live in groundwater along river banks or lake shores. In these interstitial habitats, they have been found associated with other nematodes which are generally known to occur in such biotopes. Thus, the samples studied also contained members of the following genera: *Theristus, Plectus, Hemicycliophora, Prismatolaimus, Ironus, Tobrilus, Neotobrilus, Trischistoma, Tripyla, Mononchus, Prionchulus, Mylonchulus, Eudorylaimus,* and *Discolaimoides*. However, it should be emphasized that, while these other genera of Nematoda may occur in various other habitats as well, the species of *Boreolaimus* only inhabit groundwater biotopes – as far as is known at present.

As for their distribution, all six species were discovered in Scandinavia, close to or beyond the Arctic Circle. Whether our animals also occur in other regions of the Arctic remains unknown. The nematode communities of interstitial biotopes in North Russia, North Canada, Alaska, or Greenland have not been investigated yet; the fauna of other biotopes of these territories are also hardly known.

It is of interest that no species of the genus *Boreolaimus* has been discovered in Spitzbergen, the northernmost isles of the European mainland. Although a rich nematode collection from those islands has been studied by Loof (1971), nematodes similar to *Boreolaimus* were not mentioned in this paper. One species, *Eudorylaimus maksymovi* Altherr, 1963, seems to be a little *Boreolaimus*-like as described by Loof (1971). Dr Loof kindly sent me two female individuals for comparison with my Arctic animals. However, the specimens sent by Dr Loof are typical members of *Eudorylaimus* in every respect (head practically not offset, spear weak, cuticle as thick as spear, guiding ring as usual in dorylaims, buccal cavity moderately large, intestine not folded, vulva roundish, posterior part of tail not 'empty', tail tissue, if very thin, reaching to tail tip, cuticle not thinned on hind end, etc.). However, Loof did not examine nematodes from groundwater habitats in Spitzbergen. On the other hand, it is also worth mentioning that no other investigators of interstitial biotopes – particularly Altherr – has ever described nematodes similar to *Boreolaimus*. Altherr (1972) did study groundwater samples from Sweden but his collection originated predominantly from southern regions of the country. Summa summarum: groundwater and far north, both these criteria are necessary to meet *Boreolaimus* - at least to our present knowledge.

THE SPECIES OF THE GENUS *BOREOLAIMUS*

The genus *Boreolaimus* consists of six species. One of them, *B. enckelli*, is known, the other five are new to science. The members of the genus share a great number of common morphological features (generic characters), that is, they are strongly similar to one another, but they are easily distinguished by specific morphological features.

It should be noted that other *Boreolaimus* species may be present in the European Arctic. In the material examined, I found individuals – not one or two but several – which undoubtedly belonged to *Boreo-*

laimus, but could not be identified with any of the six described species. They were in poor condition or in small numbers and could not be described as new species. Therefore, they are not included in this paper.

Boreolaimus borealis * **sp. n.**
(Figs 3, 4)

MEASUREMENTS

Holotype (female): L = 2.17 mm; a = 39; b = 3.8; c = 31; V = 55; c' = 2.0.

Females (n = 10): L = 2.00-2.38 mm; a = 39-45; b = 3.8-4.3; c = 31-34; V = 53-55; c' = 1.8-2.2.

DESCRIPTION

Females: Body long and slender, 49-54 µm wide at mid-region. Cuticle 1.8-2.0 µm thick, at spear level only about 2/3 as thick as this, thicker (4-5 µm) on anterior region of tail; smooth excepted on the anal region where fine transverse striation or punctation (the striae may show anastomoses) present. Labial region well offset by depression, 17-18 µm wide; lips angular with finely rounded papillae. Body at posterior end of oesophagus 2.6-3.0 times as wide as head. Amphids caliciform, broader than half the corresponding body diameter. Buccal cavity wide, especially at level of guide ring where it is wider than half the corresponding body diameter; reaching far behind posterior end of spear. Odontostyle straight, well developed, 24-26 µm long and about 3 µm thick, 1.3-1.5 times as long as labial diameter, 1/20-1/22 of entire length of oesophagus. Aperture shorter than 1/3 spear length. Guiding sheath long, tubular, its anterior ring-like expansion located behind spear aperture. Protractor and retractor muscles of spear well developed. Oesophagus muscular in its entire length, 500-565 µm long, widening gradually or in several steps at about 40-45 % of its length. Cylindrus somewhat expanded on both ends. Dorsal nucleus 4-5 µm, rounded-oval to oval, directed parallel with body axis, distinctly bigger than other nuclei; three sub-ventral nuclei round, conspicuous. Two posterior nuclei generally lying at the same level; closer to AS_2 than to posterior end of cylindrus. Locations of oesophageal gland nuclei given in Table 1; location following Loof and Coomans (1970): D: 57-60 %, AS_2: 75-78 %, PS_1: 85-87 %, PS_2: 85-88 %. Distance between posterior end of oesophagus and vulva longer (1.1-1.3 times) than oesophagus. Cardia wide, tongue-shaped. Intestine with wide lumen, folded, with enlarged cells just before prerectum. Prerectum 1.3-2.2 anal diameters long, with a short dorsal caudal sack; rectum straight, 1.1-1.2 anal diameters long. Vulva transverse, with non-sclerotized inner lips. Vagina 27-31 µm long, as long as or longer than half the corresponding body diameter. Female genital organ 20-

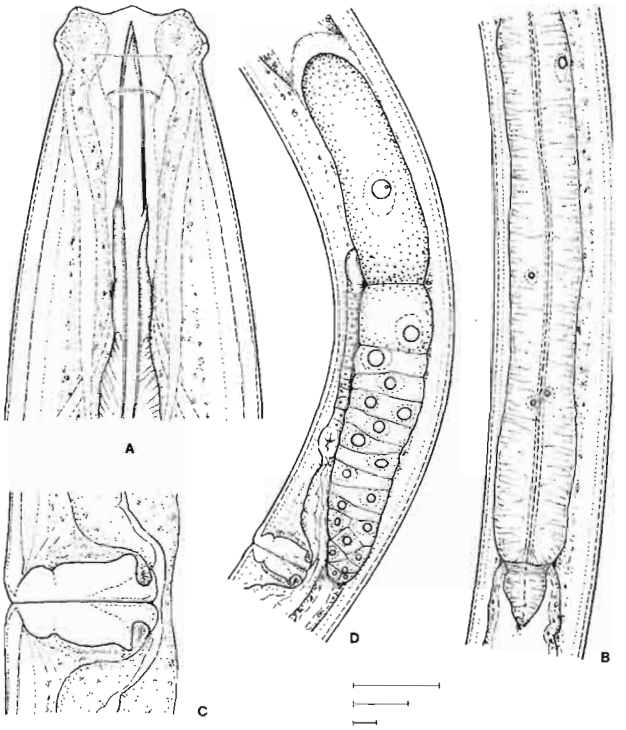

Fig. 3. Boreolaimus borealis *sp. n. A: Anterior end; B: Cylindrus with nuclei; C: Vaginal region; D: Anterior female gonad. (Scale bars = 10 µm each; upper: A, middle: C, lower: B, D).*

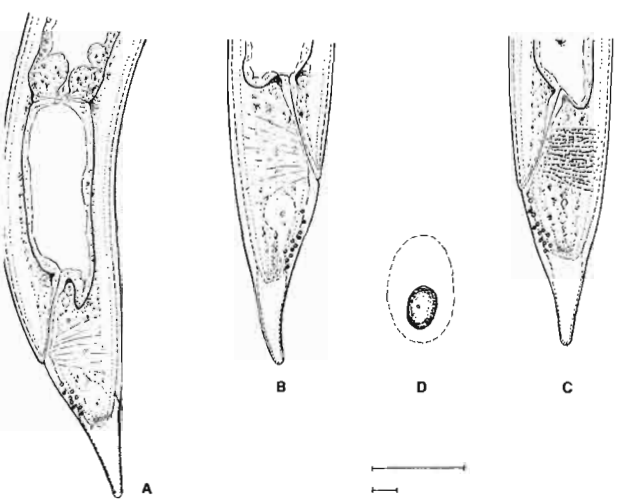

Fig. 4. Boreolaimus borealis *sp. n. A-C: Tails; D: Dorsal oesophageal nucleus. (Scale bars = 10 µm each; upper: D, lower: A-C).*

* The specific name *borealis* (Greek/Latin) means: northern, north-inhabiting.

23 % of body length in mature females; each branch four to five times as long as body diameter. Anterior gonad mostly on the left, posterior gonad on the right side of intestine. Ovaries reflexed to vulva. Oviduct with a proximal receptaculum-like chamber; junction oviduct uterus with a sphincter. Uterus never containing sperm. Mature eggs not observed. Distance between vulva and anus 13 to 15 times as long as tail. Tail short, 64-75 µm, 1.8-2.2 anal diameters long, or 3.0-3.2 % of body length; conoid, straight or with posterior part slightly bent ventrally, with rounded tip. Three features characteristic for the tail: *i*) on its anterior region cuticle finely but distinctly striated, and with a very fine punctation as well; transverse striae best visible on the dorso-lateral side of the tail and sometimes with anastomoses; *ii*) on the anterior region, but at some distance from the anal opening, small but distinct subventral 'saccate bodies' ('bubbles') sometimes present; *iii*) distal 'empty' region measuring 30-39 µm, *i.e.*, about half length of tail (47-54 %).

Males: not found.

TYPE MATERIAL

Holotype: Female, slide No. 7/Sk-B. Paratypes: fourteen females and two juveniles. All in the nematode collection of the Eötvös Loránd University, Budapest, Hungary.

LOCALITY AND HABITAT

Lake Övre Trangvatn in the Alta-elv river system, Prov. Finnmark, the northernmost region of Norway, well north of the Arctic Circle, groundwater, August 1965; collected by P.H. Enckell (Fig. 1, loc. 2).

DIAGNOSIS AND RELATIONSHIPS

The main characters are: longest species in the genus, body slender, spear long, vulva not sclerotized, tail short with striated-dotted cuticle and subventral bubbles.

Boreolaimus borealis sp. n. belongs to the short-tailed representatives of the genus, resembling in this respect *B. enckelli* (Andrássy, 1967) and *B. lapponicus* sp. n. It clearly differs from *B. enckelli* in having a body longer (2.0-2.4 *vs* 1.5-1.6 mm) and more slender (a = 39-48 *vs* 27-29), unsclerotized vulval lips, and finely ornate cuticle on tail; from *B. lapponicus* in having cuticular ornamentation on/in the tail and a longer percentage of 'empty' portion in relation to tail length. In the ornamentation of the tail, *B. borealis* is unique within the genus.

***Boreolaimus enckelli* (Andrássy, 1967) comb. n.**
= *Eudorylaimus enckelli* Andrássy, 1967
(Figs 5, 6)

MEASUREMENTS

Holotype (female)*: L = 1.56 mm; a = 29; b = 3.1; c = 21; V = 51 %; c' = 2.4.

Females (n = 6): L = 1.50-1.61 mm; a = 27-29; b = 3.2-3.5; c = 22-26; V = 52-56; c' = 2.3-2.6.

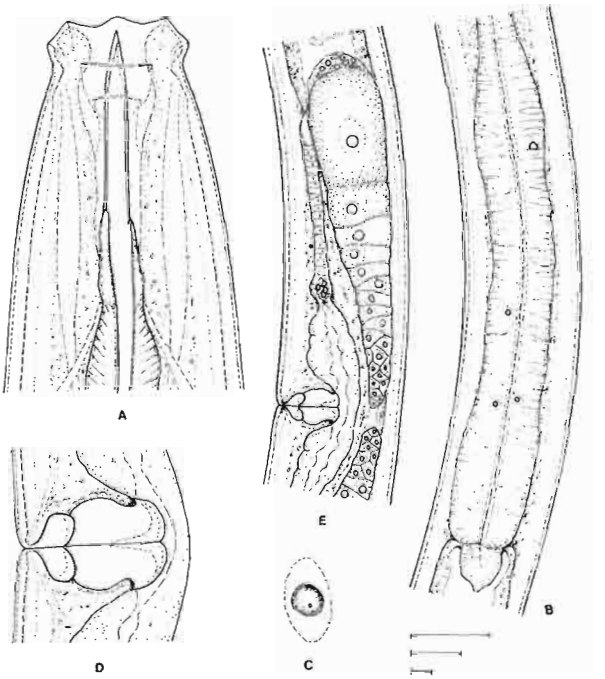

Fig. 5. Boreolaimus enckelli *(Andrássy, 1967) comb. n. A: Anterior end; B: Cylindrus with nuclei; C: Dorsal pharyngeal nucleus; D: Vulva and vagina; E: Anterior female gonad; germinal end with spermatozoa. (Scale bars = 10 µm each; upper: A, C, middle: D, lower: B, E).*

DESCRIPTION

Females: Body shorter and stouter than in other species of the genus, 52-56 µm wide at middle. Cuticle thin and smooth, 1.7-1.8 µm, somewhat thicker (2.5 µm) on anterior part of tail; half as thick as spear itself at level of spear. Cephalic region offset by depression, 17-19 µm wide, with well separated lips; papillae conoid. Body at posterior end of oesophagus about 2.5 times as wide as head. Amphids caliciform, half as wide as corresponding body diameter. Buccal

* In the original paper (1967), the measurements of the young female – still surrounded by the last larval cuticle – were mistakenly given instead of those of the mature female; the measurements of the holotype are to be found here.

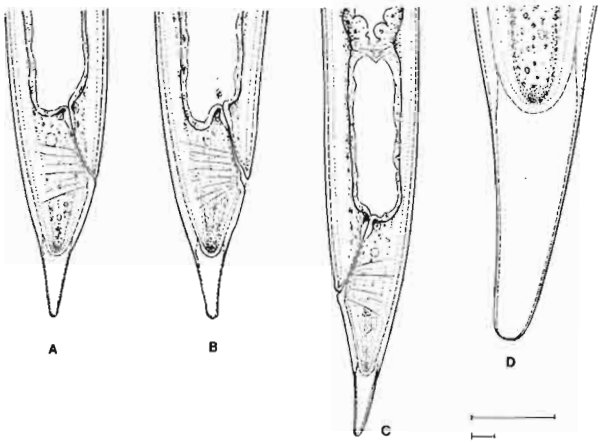

Fig. 6. Boreolaimus enckelli *(Andrássy, 1967) comb. n. A-C: Tail forms; D: Posterior half of tail with 'empty' region. (Scale bars = 10 µm each; upper: D, lower: A-C).*

cavity wide and long. Odontostyle 23-24 µm long and about 3 µm thick, 1.3-1.4 times as long as cephalic diameter, or 1/20-1/22 of oesophageal length. Aperture somewhat shorter than 1/3 spear length. Oesophagus 463-498 µm long, 29-32 % of entire length of body, gradually widening at middle. Cylindrus typical for the genus: somewhat widened on both ends. Distance between posterior end of oesophagus and vulva considerably shorter (0.6-0.7 times) than oesophagus. Dorsal nucleus oval, 4 µm, longitudinally directed; subventral nuclei round, distinctly smaller than dorsal nucleus. AS_2 much closer to the two PS than to D. Average distance between dorsal nucleus and posterior nuclei somewhat shorter than in other species of the genus. Locations of oesophageal gland nuclei given in Table 1; location following Loof and Coomans (1970): D: 60-62 %, AS_2: 76-78 %, PS_1: 84-86 %, PS_2: 85-87 %. Cardia short but fairly broad. Intestine with wide lumen and folds. Prerectum offset by constriction, 2.0-2.5 times as long as anal body diameter, with a caudal sack. Rectum 1.2-1.5 anal diameters long, straight. Vulva transverse with strongly sclerotized drop-shaped inner lips. Vagina strong, 25-28 µm, nearly half as long as body diameter. Female genital organ 22-24 % of body length; each gonad 2.8-3.5 body diameters long. Anterior gonad on the left, posterior gonad on the right side of intestine. Ovaries long, mostly reaching to vulva. One or two eggs in uterus: 80-83 × 49-51 µm, 1.4-1.5 times as long as body diameter. Distance between vulva and anus 9-10 times as long as tail. Tail 60-74 µm, 2.3-2.6 anal diameters long, or 4-5 % of body length, conoid, straight or slightly bent; tip of tail regularly rounded. 'Empty' portion 23-30 µm long, 40-50 % of tail length. Anal muscles strongly developed.

Male: not found.

LOCALITY AND HABITAT

This species was first found in groundwater near River Torne-älv, Prov. Lappland, Sweden, north of the Arctic Circle, July 1962 (Fig. 1, loc. 5). Recently it was observed in samples from Lake Satisjaure at Vietasjokk, Lule-älv water system, Prov. Lappland, Sweden, north of the Arctic Circle, in groundwater, 440 m above sea-level, July 1963 (six females and two juveniles; Fig. 1, loc. 7).

REMARKS

The main characters are: smallest species of the genus, body comparatively stout, distance between posterior oesophagus end and vulva shorter than in the other species, vulval lips sclerotized, tail short with comparatively long 'empty' portion.

I described this species as *Eudorylaimus enckelli* Andrássy, 1967 but I noted then that it differed from every member of the genus *Eudorylaimus* in the structure of its tail. There is no question that *enckelli* is a true *Boreolaimus*. In having a short tail, this species resembles *B. borealis* sp. n. and *B. lapponicus* sp. n., but it can be very easily distinguished from them by the well sclerotized vulval lips (see the descriptions of *B. borealis* and *B. lapponicus* for other differences.) In sclerotization of vulva, *B. enckelli* is similar to *B. septentrionalis* sp. n., but the tail of the present species is much shorter (60-74 *vs* 160-168 µm; c' = 2.3-2.6 *vs* 4.8-5.2) and there are no 'saccate bodies' in the cuticle.

It shall be noted that in a sample collected in Sweden, I found three females closely resembling *B. enckelli.* However, they were longer (1.7-2.0 mm), more slender (a = 33-38), the dorsal nucleus was directed transversely in the cylindrus, and the egg was longer, *i.e.*, two body diameters. Whether this form was a variant of *B. enckelli* or a separate species could not be determined because of the small number of specimens.

Boreolaimus lapponicus * **sp. n.**

(Figs 7, 8)

MEASUREMENTS

Holotype (female): L = 2.06 mm; a = 42; b = 4.0; c = 30; V = 52; c' = 2.1.

Females (n = 24): L = 1.73-2.17 mm; a = 35-42; b = 3.6-4.1; c = 26-30; V = 52-58; c' = 2.0-2.3.

* The specific name of this nematode refers to Lappland, the region of both Finland and Sweden where our species was collected.

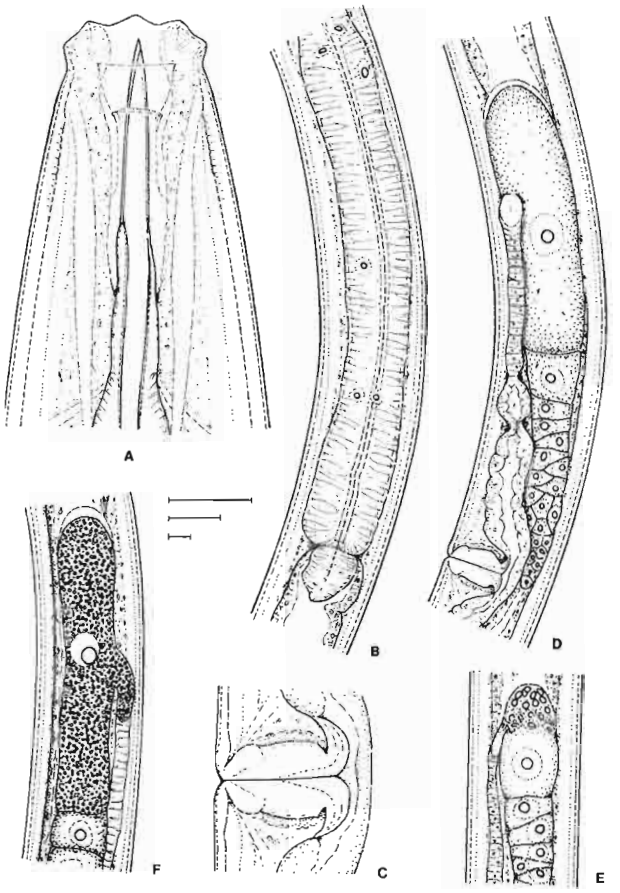

Fig. 7. Boreolaimus lapponicus *sp. n. A: Anterior end; B: Cylindrus with nuclei; C: Vulva and vagina; D: Anterior female gonad; E: Ovary-oviduct junction; germinal end of ovary (= ovotestis) showing half-mature and mature spermatozoa; F: An oocyte just pouring into the oviduct. (Scale bars = 10 µm each; upper: A, middle: C, lower: B, D-F).*

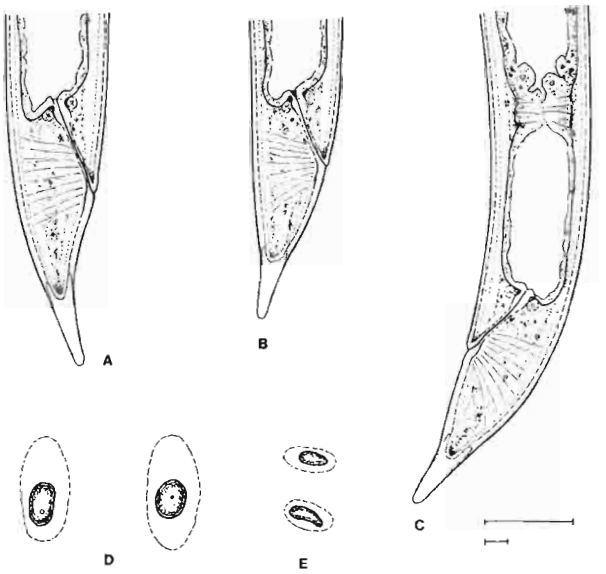

Fig. 8. Boreolaimus lapponicus *sp. n. A-C: Tail shapes; D: Forms of dorsal nuclei; E: "Accessory" nuclei in the vicinity of the dorsal nucleus. (Scale bars = 10 µm each, upper: D-E, lower: A-C).*

DESCRIPTION

Females: The longest species of the genus. Body fairly slender, 50-57 µm in mid-region. Cuticle thin, 1.5-1.7 µm, thicker (3-4 µm) on anterior part of tail, only half as thick as the spear at level of spear; smooth or very finely annulated on inner surface of neck region. Labial region offset, 17-19 µm wide, lips separate with conoid-rounded papillae. Body at posterior end of oesophagus 2.6-2.8 times as wide as head. Amphids dorylaimid, half as wide as corresponding body or a little wider. Buccal cavity very spacious and long. Odontostyle straight, 23-25 µm long and 3 µm wide, 1.2-1.4 times as long as cephalic diameter, 1/22-1/24 of oesophageal length. Aperture nearly 1/3 of spear. Guiding apparatus tubular with widened anterior end and diverging posterior walls. Oesophagus 500-575 µm long, muscular in its entire length, gradually expanding near middle; cylindrus 50-52 % of oesophagus length. Dorsal nucleus 3.5-5.5 µm, longitudinally directed. Anterior subventral nucleus (AS_2) round or oval, at 83-87 µm from dorsal nucleus. Posterior subventral nuclei (PS_1 and PS_2) globular, close to one another, 134-152 and 137-153 µm behind the dorsal nucleus, respectively. In about half of the specimens, one or two quite distinct oval, or slightly irregular 'accessory' nuclei present at level of the dorsal nucleus or somewhat (2-16 µm) anterior to it, larger than the subventral nuclei but smaller than the dorsal nucleus. In some specimens, a minute refractive particle also present at about one body diameter before the AS_2, possibly representing AS_1. Locations of oesophageal gland nuclei given in Table 1; location following Loof and Coomans (1970): D: 58-60 %, AS_2: 74-77 %, PS_1: 85-88 %, PS_2: 86-88 %. Cardia broad, tongue-shaped. Intestine often folded, with wide lumen. Prerectum offset, its lumen also wide, short, as long as 1.2-2.2 anal diameters, rectum 0.9-1.3 anal diameters long. Intestine with enlarged cells anterior to junction with prerectum. Posterior dorsal end of prerectum sack-like. Vulva transverse, inner lips not sclerotized; vagina 26-30 µm, half as long as corresponding body diameter or a little shorter. Female genital apparatus 20-24 % of body length. Each gonad 4.0-4.4 times as long as body diameter. Ovaries short in young females, longer in old females, reaching to

vulva. Oviduct ventral, with small receptaculum-like proximal chamber but not containing sperm. One or two eggs present at the same time, 102-107 × 47-49 µm, 1.8-2.0 body diameters long. No spermatozoa observed in the uterus. In young females eight to fifteen refractive cells observed at the distal ends of the ovaries, most probably spermatozoa produced by the proterandric germinative glands. Vulva-anus distance ten to fourteen times as long as tail. Tail 65-76 µm, 2.0-2.2 times as long as anal body diameter, or 3.4-3.8 % of total length of body; conical, straight or slightly bent, with regularly rounded tip. 'Empty' section 27-30 µm long, 37-44 % of tail length. Cuticle thinning suddenly a little before the beginning of this empty part. Anal muscles very strong.

Males: not found.

TYPE MATERIAL

Holotype: Female, slide No. 41/Sk-B. *Paratypes*: 40 females and three juveniles. All deposited in the nematode collection of the Eötvös Loránd University. Further paratype specimens: Three females sent to the collection of the Landbouwhogeschool, Wageningen, The Netherlands, and three females and three juveniles to the collection of the Muséum National d'Histoire Naturelle, Paris, France.

LOCALITIES AND HABITAT

Type locality: Lake Ylikitka at Hyväniemi, Oulankajoki water system, Prov. Lappi, Finland, close to the Arctic Circle, groundwater at 2 m from the lake, July 1960, collected by P.H. Enckell (47 females and 6 juveniles, Fig. 1, loc. 12).

Other localities: Lake Peuraure, Lule-älv water system, Prov. Lappland, Sweden, near the Arctic Circle, 440 m above sea-level, groundwater, July 1964 (three females, three juveniles; Fig. 1, loc. 8). Lake Karats, Lule-älv water system, Prov. Lappland, Sweden, on the Arctic Circle, 410 m above sea-level, ground water, July 1964 (one female, one juvenile; Fig. 1, loc. 9). River Pärlälven at Pärlan, Lule-älv river system, Prov. Lappland, Sweden, quite close to the Arctic Circle, ground water, July 1964 (one female; Fig. 1, loc. 10). Lake Juonajaure, Lule-älv river system, Prov. Lappland, Sweden, on the Arctic Circle, July 1964 (two females; Fig. 1, loc. 11). Lake Mieron, Alta-elv water system, Prov. Finnmark, Norway, well north of the Arctic Circle, groundwater, August 1965 (three females, two juveniles; Fig. 1, loc. 3).

DIAGNOSIS AND RELATIONSHIPS

The main characters are: comparatively large species with slender body, unsclerotized vulval lips, short tail and plain caudal cuticle.

Boreolaimus lapponicus sp. n. belongs to the short-tailed species of the genus. It is close to *B. borealis* sp. n. and *B. enckelli* (Andrássy, 1967). It can be distinguished from *B. borealis*: the cuticle of the tail is simple (striated and with 'saccate bodies' in *B. borealis*), the 'empty' part in proportion to tail length is shorter; from *B. enckelli:* the vulval lips are not sclerotized, the body is longer (1.7-2.2 *vs* 1.5-1.6 mm). If the presence of 'accessory' nuclei in front of the cylindrus were a constant feature, it would be an additional distinguishing feature for *B. lapponicus.*

Boreolaimus arcticus * **sp. n.**

(Fig. 9A-H)

MEASUREMENTS

Holotype (female): L = 1.64 mm, a = 33; b = 3.7; c = 18; V = 51; c' = 3.5.

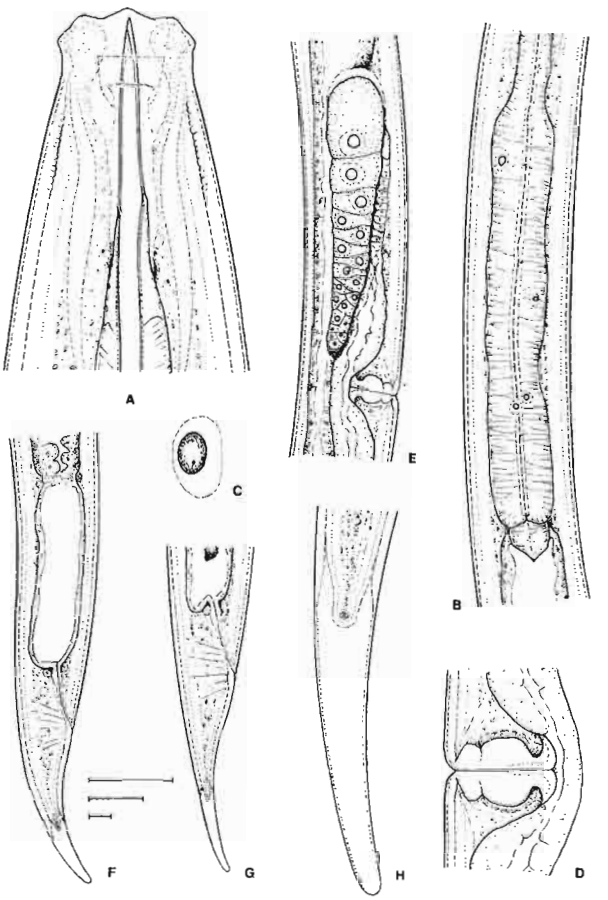

Fig. 9. Boreolaimus arcticus *sp. n. A: Anterior end; B: Cylindrus with nuclei; C: Dorsal nucleus; D: Vulva and vagina; E: Anterior female gonad; F-G: Tails; H: Posterior 'empty' half of tail. (Scale bars = 10 µm each; upper: A, C, H, middle: D, lower: B, E, F-G).*

* The specific name *arcticus* (Latin) means: arctic, originating from the Arctic.

Females (n = 18): L = 1.50-1.79 mm; a = 28-36; b = 3.6-3.9; c = 15-19; V = 44-51; c' = 3.1-3.5.

DESCRIPTION

Females: Body moderately long and slender, 42-56 µm in mid-region. Cuticle thin, 1.5-1.7 µm, hardly thicker (2 µm) on anterior region of tail, about 2/3 as thick as spear at level of spear; smooth, only subcuticle showing fine striation at neck region. Cephalic region offset by depression, 16-17 µm wide, lips separate with rounded papillae. Body at proximal end of pharynx 2.5-2.8 times as wide as head. Amphids caliciform, half as wide as body at the same level. Lining of buccal cavity wide and long as in other representatives of the genus. Odontostyle 22-24 µm long and about 2.5 µm thick, 1.2-1.3 times as long as labial diameter. Aperture shorter than 1/3 spear length. Guiding ring tubular, widened at both ends. Oesophagus 397-460 µm long, expanded over 50-55 % of its length, more rapidly than in other species. Nuclei distinct. Dorsal nucleus 4-5 µm, oval, longitudinally directed. Other nuclei round and equal in shape. Anterior subventral nucleus at 63-66 µm from dorsal nucleus, posterior nuclei at 106-112 µm and 107-114 µm from dorsal nucleus. Locations of oesophageal gland nuclei given in Table 1; location following Loof and Coomans (1970): D: 60-62 %, AS_2: 75-77 %, PS_1: 86-87 %, PS_2: 87-88 %. Cardia short, tongue-shaped. Distance between posterior end of oesophagus and vulva 0.7-0.9 times shorter than oesophagus. Intestinal lumen wide, often with greenish-blue contents; walls of intestine widened inwards just before prerectum. Prerectum 2.1-3.2 times as long as anal body diameter, with a short caudal sack; rectum 1.0-1.2 times as long as anal body diameter. Vulva transverse, not sclerotized; vagina roundish, 20-21 µm long, about 40 % of corresponding body diameter. Female genital apparatus as long as 14-20 % of body length. Anterior gonad generally to the left, posterior to the right from intestine; each 2.4-4.0 times as long as body diameter. Ovaries reflexed almost to vulva. Eggs not observed. No spermatozoa in uterus observed. Distance vulva-anus 7-9 times as long as tail. Tail long, 80-110 µm, 3.1 3.5 anal diameters, 5.5-7.0 % of body length; conoid, mostly slightly bent ventrally, rounded on tip. 'Empty' portion 26-40 µm long or 28-38 % of tail length. Cuticle narrowing suddenly before the beginning of the 'empty part'.

Males: not found.

TYPE MATERIAL

Holotype: Female, slide No. 31/Sk-B. Paratypes: 22 females and sixteen juveniles. All specimens deposited in the nematode collection of the Eötvös Loránd University, Budapest, Hungary.

LOCALITIES AND HABITAT

Type locality: A short unnamed river at Bonnassjöen, Prov. Nordland, Norway, above the Arctic Circle, groundwater, August 1965; coll. P.H. Enckell (Fig. 1, loc. 6).

Other locality: Lake Kuvatn, Prov. Finnmark, Norway, far north of the Arctic Circle, groundwater, July 1965 (three females, three juveniles, Fig. 1, loc. 1).

DIAGNOSIS AND RELATIONSHIPS

The main characters are: body moderately long and slender, vulva not sclerotized, vagina comparatively short, lying more anterior than usual, tail long, empty portion about 1/3 of tail length.

By its comparatively long tail (80-110 µm), *Boreolaimus arcticus* sp. n. clearly differs from the three short-tailed species (60-77 µm: *B. borealis*, *B. enckelli*, *B. lapponicus*), as well as from the longer-tailed one (160-168 µm: *B. septemtrionalis*). In the absolute length of tail (in µm) it is close to *B. norvegicus* sp. n., but its body is much plumper (a = 28-36 *vs* 50-62), the tail comparatively shorter (c' = 3.1-3.5 *vs* 4.3-5.0), and the "empty" section longer (1/3 *vs* 1/4 of tail length).

Boreolaimus septemtrionalis * sp. n.

(Figs 10A-E; 11A-D)

MEASUREMENTS

Holotype (female): L = 1.92 mm; a = 30; b = 3.8; c = 12; V = 50; c' = 5.0.

Females (n = 4): L = 1.62-1.95 mm; a = 23-30; b = 3.7-3.8; c = 10-12; V = 47- 50; c' = 4.8-5.2.

DESCRIPTION

Females: Body moderately long and slender, 63-71 µm at middle. Cuticle smooth, 2 µm over most of body, but thicker (2.5-3.5 µm) on anterior region of tail and about 2/3 as thick as the spear at level of spear. Cephalic region offset by depression, 18-19 µm wide, lips separate with rounded papillae. Body at posterior end of oesophagus 3.4-3.5 times as wide as head. Amphids dorylaimid, half as wide as body at the same level. Mouth cavity large, in its widest part wider than half a corresponding body diameter. Odontostyle 27-28 µm long and 3.5 µm thick, 1.3-1.5 times as long as labial diameter, 1/17-1/19 of oesophagus length; straight. Aperture occupying a little less than 1/3 spear length. Guiding ring a long tube. Oesophagus 440-506 µm long, expanded in several steps. Cylindrus somewhat widened at both ends. Oesophageal gland nuclei well visible. Dorsal nucleus almost spherical with large halo, other nuclei not globular as in

* Named after its northern occurrence; "*septemtriones*" means 'from the North' in Latin language.

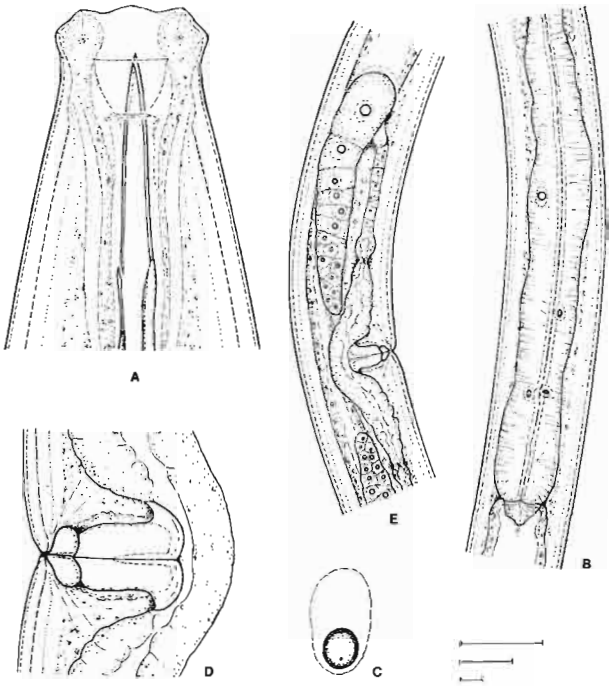

Fig. 10. Boreolaimus septemtrionalis *sp. n. A: Anterior end; B: Cylindrus with nuclei; C: Dorsal nucleus; D: Vulva and vagina; E: Anterior female gonad. (Scale bars = 10 µm each; upper: A, C, middle: D, lower: B, E.)*

the other species of the genus but plum-stone-shaped, longitudinal or transverse in direction. Anterior subventral nucleus at 65-67 µm from dorsal nucleus; posterior subventral nuclei at 117-119/118-122 µm from dorsal nucleus; average distance between AS_2 and D comparatively shorter than in other species. Locations of oesophageal gland nuclei given in Table 1; location following Loof and Coomans (1970): D : 58-59 %, AS_2: 73-75 %, PS_1: 85-86 %, PS2: 85-87 %. Cardia and intestine typical of *Boreolaimus*. Prerectum 2.5-3.2 times as long as anal body diameter, with a short caudal sack; rectum 1.1-1.4 times as long as anal body diameter. Distance between posterior end of oesophagus and vulva 0.8-0.9 times as long as oesophagus. Vulva transverse, inner lips conspicuously sclerotized. Vagina 26-30 µm, about half of the corresponding body width. Female genital apparatus 20-21 % of body length. Each gonad 2.5-3.5 body diameter long. Ovaries not reaching to vulva. One egg: 82 × 55 µm, 1.2 times as long as body diameter. No sperm in uterus. Distance vulva-anus 4.2-5.1 times as long as tail. Tail unusually long, 160-168 µm, 4.8-5.2 times anal diameter, 8-10 % of body length; conoid, arcually bent ventrad, with finely rounded tip. Cuticle with ovoid subventral 'saccate bodies' on the postanal

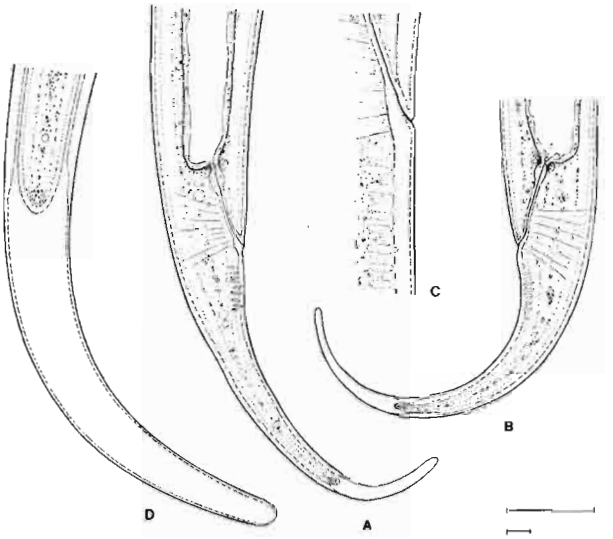

Fig. 11. Boreolaimus septemtrionalis *sp. n. A-B: Tails; C: Oval bodies in the anterior subventral region of tail; D: "Empty" portion of tail. (Scale bars = 10 µm each; upper: C-D, lower: A-B).*

region, 7-10 on each side. 'Empty' portion 26-34 % of tail length.

Male: not found.

TYPE MATERIAL

Holotype: Female, slide No. 72/Sk-B. Paratypes: one female and three juveniles. Every specimen deposited in the Nematode Collection of the Eötvös Loránd University, Budapest, Hungary.

LOCALITIES AND HABITAT

Type locality: Lake Laisan, Uma-älv water system, Prov. Lappland, Sweden, 450 m above sea-level, groundwater, July 1963; coll. P.H. Enckell (Fig. 1, loc. 13).

Other locality: Vojmån River at Henriksfjäll, Ångerman-älven water system, Prov. Lappland, Sweden, 500 m above sea-level, groundwater, August 1966 (two females and two juveniles, Fig. 1, loc. 14).

DIAGNOSIS AND RELATIONSHIPS

The main characters are: medium shape, the longest odontostyle in the genus, sclerotized vulval lips, comparatively small egg, long tail, bubbles in tail cuticle, short empty portion in body end.

Boreolaimus septemtrionalis sp. n. can easily be distinguished from every member of the genus in having an unusually long and ventrally arcuate tail (160-168 *vs* 60-110 µm in the other species). The vulval lips are well sclerotized, as in *B. enckelli*, but the long, ventrally bent tail with 'saccate bodies' and the comparatively short 'empty' part distinguish our species from *B. enckelli*.

***Boreolaimus norvegicus* * sp. n.**
(Figs 12A-E; 13A-C)

MEASUREMENTS

Holotype (female): L = 1.75 mm; a = 53; b = 3.8; c = 17; V = 46; c' = 4.7.

Females (n = 6): L = 1.74-2.04 mm; a = 50-62; b = 3.8-4.5; c = 16-21; V = 44- 48; c' = 4.3-5.0.

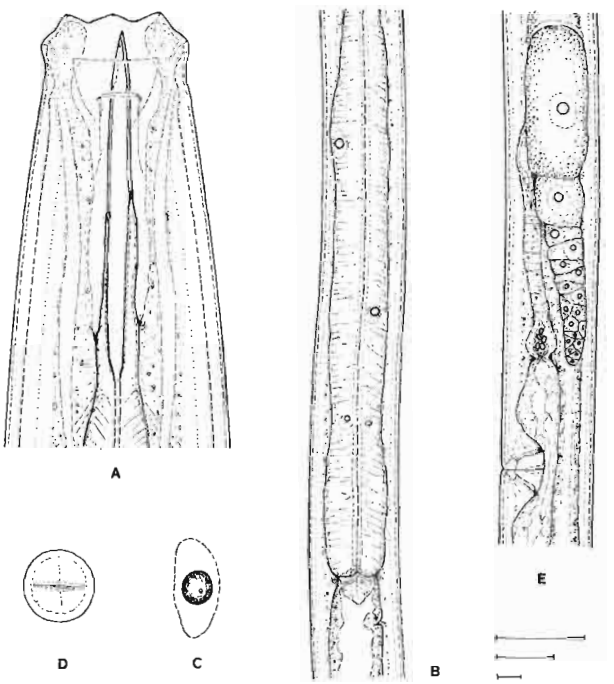

Fig. 12. Boreolaimus norvegicus *sp. n. A: Anterior region; B: Cylindrus with nuclei; C: Dorsal nucleus; D: Vulva in frontal view; E: Anterior female gonad. (Scale bars = 10 µm each; upper: A, C, middle: D, lower: B, E).*

DESCRIPTION

Females: Body extremely slender, 33-38 µm wide at mid-region. Cuticle thin, 1-1.5 µm thick on most of the body, 2 µm thick on anterior half of tail, half as thick as spear at level of spear; smooth. Cephalic region offset by depression, 16-17 µm wide; lips separate. Anterior labial papillae conoid, lateral papillae rounded. Body at proximal end of oesophagus 2.2-2.3 times as wide as head. Amphids caliciform, broader than half the body diameter at the same level. Buccal cavity spacious and very long. Odontostyle 22-23 µm, 1.3-1.4 labial diameter long, 18-21 % of oesophageal length, straight. Aperture about as long

* Found only in Norway, hence its specific name *norvegicus*.

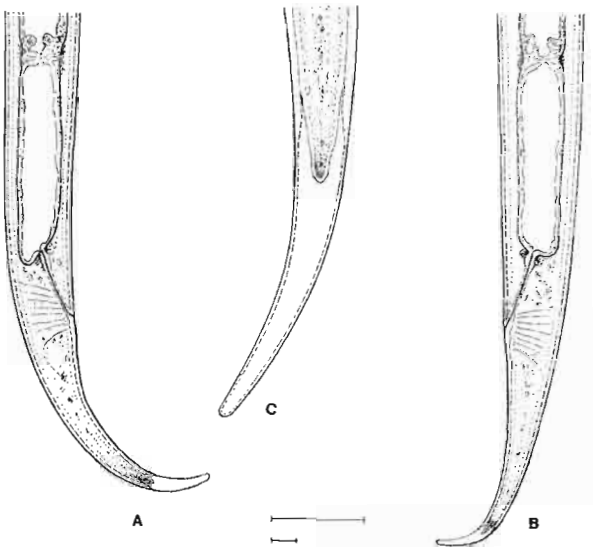

Fig. 13. Boreolaimus norvegicus *sp. n. A-B: Tails; C: Posterior 'empty' portion of tail. (Scale bars = 10 µm each; upper: C, lower: A-B).*

as 1/3 spear. Guiding ring tubular, anteriorly expanded. Oesophagus 394-495 µm long, widened at 44-48 % of its length. Cylindrus somewhat widened at both ends. Nuclei distinct. Dorsal nucleus rounded-oval, longitudinally directed, 4 µm. Other nuclei round; AS_2 hardly smaller than D. Distance between dorsal nucleus and anterior nuclei 116-131 µm and 119-131 µm. Locations of oesophageal gland nuclei given in Table 1; location following Loof and Coomans (1970): D: 59-61 %, AS_2: 74-77 %, PS_1: 84-87 %, PS_2: 85-87 %. Cardia short, tongue-like. Intestine often with greenish contents. Prerectum 3.0-4.6 times as long as anal body diameter; rectum 1.0-1.5 times as long as anal body diameter. Distance between posterior end of oesophagus and vulva 0.8-1 times as long as oesophagus itself. Vulva transverse, inner lips not sclerotized; vagina 17-19 µm, half the corresponding body diameter or a little longer, strongly widened proximally. Length of female genital organ equal to 13-19 % of body length. Each gonad 3.5-5.2 body diameters long. Ovaries not reaching to vulva. Anterior gonad mostly on the left, posterior on the right side of intestine. Mature egg not seen. No spermatozoa in uterus. Distance between vulva and anus 8-10 times as long as tail. Tail 95-110 µm, 4.3-5.0 anal diameters long, 5-6 % of body length; conoid, almost straight, with rounded tip. 'Empty' portion 20-26 µm or 21-24 % of tail length; shorter (12-17 %) in juvenile animals. Anal muscles strongly developed.

Table 1. *Position (%) of oesophageal gland nuclei in species of the genus* Boreolaimus *n. gen.*

	B. borealis sp. n.	*B. enckelli* n. comb.	*B. lapponicus* sp. n.	*B. arcticus* sp. n.	*B. septemtrionalis* sp. n.	*B. norvegicus* sp. n.
D	57-60	60-62	58-60	60-62	58-59	59-61
AS_1	–	–	–	–	–	–
AS_2	41-44	40-42	39-42	38-40	36-38	36-40
PS_1	65-68	62-64	64-70	64-66	64-65	61-67
PS_2	66-69	63-66	66-71	66-67	65-66	63-67

TYPE MATERIAL

Holotype: Female, slide No. 42/Sk-B. Paratypes: five females and nine juveniles. All specimens are deposited in the nematode collection of the Eötvös Loránd University, Budapest, Hungary.

TYPE LOCALITY AND HABITAT

Lake Sagelvvatn 50 km south of Tromsö, Sagelva river system, Prov. Troms, Norway, north of the Arctic Circle, groundwater, August 1965; coll. P.H. Enckell (Fig. 1, loc. 4).

DIAGNOSIS AND RELATIONSHIPS

The main characters are: body very slender and of middle length, vulva not sclerotized, vagina proximally expanded, prerectum long, tail long with empty portion measuring 1/4 of tail length.

Boreolaimus norvegicus sp. n. is the most slender species of the genus, which makes it easily recognizable. Among the species with unsclerotized vulva, *B. norvegicus* resembles *B. arcticus* sp. n., as both have medium-long tails; it can be distinguished from *B. arcticus* by slender body, comparatively longer tail (c' = 4.3-5.0 *vs* 3.1-3.5), and shorter "empty" part of tail (1/4 *vs* 1/3 of tail length).

Key to the species of Boreolaimus

1 – Vulval lips conspicuously sclerotized 2
– Vulval lips not sclerotized 3

2 – Tail 160-168 µm ventrally arcuate, with "saccate bodies" in its cuticle; c' about 5; L = 1.6-1.9 mm; a = 23-30; b = 3.7-3.8; c = 10-12; V = 47-50; Sweden..... *septemtrionalis* sp. n.
– Tail 60-74 µm, practically straight, with plain cuticle; c' = 2-2.5, L = 1.5-1.6 mm; a = 27-29; b = 3.2-3.5; c = 22-26; V = 52-56; Sweden .... *enckelli* (Andrássy, 1967) n. comb.

3 – Body very slender (a = 50-62); c'= 4-5 anal diameters; L = 1.7-2.0 mm; a = 50-62; b = 3.8-4.5; c = 16-21; V = 44-48; Norway..... .....*norvegicus* sp. n.
– Body not so slender (a ≤ 45), c' = 1.8-3.5 4

4 – Tail 80-110 µm; c' = 3-3.5; L = 1.5-1.8 mm; a = 28-36, b = 3.6-3.9; c = 15-19; V = 44-51; Norway..... *arcticus* sp. n.
– Tail 64-77 µm; c' = 1.8-2.3..... 5

5 – Anal region with fine cuticular striation/punctation; ventral cuticle of tail with blisters; L = 2.0-2.4 mm; a = 39-45; b = 3.8-4.3; c = 31-34; V = 53-55; Norway..... *borealis* sp. n.
– Anal region not striated; ventral cuticle of tail without blisters; L = 1.7-2.2 mm; a = 35-42; b = 3.6-4.1; c = 26-30; V = 52-58; Norway, Sweden, Finland ..... *lapponicus* sp. n.

Acknowledgements

I would like to express my special gratitude to Dr P.H. Enckell (Lund, Sweden) for giving me a free rein in his rich and valuable nematode material. Thanks are also due to Dr P.A.A. Loof (Wageningen, The Netherlands) for loaning some nematodes from his Spitzbergan material. Last, but not least, I am grateful to Dr P. De Ley (Gent, Belgium) for his friendly assistance in putting the language of the manuscript into a better English.

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Structure of the female reproductive system of *Xiphinema americanum* (Nematoda: Longidoridae)

August COOMANS and Myriam CLAEYS

Universiteit Gent, Instituut voor Dierkunde, K.L. Ledeganckstraat 35, 9000 Gent, Belgium.

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Summary – The female reproductive system of *Xiphinema americanum* differs from that of other *Xiphinema* species in several respects. The ovary has branched oocytes surrounded by epithelial wall cells in which symbiotic bacteria are embedded. The oviduct has a long *pars dilatata* which is not clearly separated from the slender part; its wall is extensively folded and extendible. The sphincter between oviduct and uterus has a sinuous lumen and 'internal musculature'. The uterus is extremely short and has a folded wall; the two uteri constitute together an extendible ovejector. Shortening of the uterus is considered to be an evolutionary trend in the *X. americanum*-group. © Orstom/Elsevier, Paris

Résumé – ***Structure du système reproducteur femelle chez* Xiphinema americanum *(Nematoda: Longidoridae)*** - Le système reproducteur femelle de *Xiphinema americanum* diffère en plusieurs points de celui des autres espèces de *Xiphinema*. L'ovaire présente des oocytes ramifiés entourés d'une paroi de cellules épithéliales dans lesquelles sont incluses des bactéries symbiotiques. L'oviducte présente une longue *pars dilatata* non clairement distincte de la partie étroite et dont la paroi, très repliée, est extensible. Le sphincter situé entre l'oviducte et l'utérus présente une lumière sinueuse et une "musculature interne". L'utérus est très court et sa paroi est également repliée; l'un et l'autre utérus forment ensemble un ovéjecteur extensible. Le raccourcissement de l'utérus est considéré comme une tendance évolutive dans le groupe *X. americanum*. © Orstom/Elsevier, Paris

Keywords: bacteria, nematode, reproductive system, symbionts, ultrastructure, *Xiphinema americanum*.

The general morphology of the female reproductive system is illustrated in detail in descriptions of most *Xiphinema* species, but not in descriptions of the species in the *X. americanum*-group.

Several ultrastructural studies on the female reproductive system in *Xiphinema* have been published (Bleve-Zacheo *et al.*, 1976; Van de Velde *et al.*, 1990*a*, *b*; Coomans *et al.*, 1992), but, apart from a few details on the structure of the sphincter and the wall of the ovary in *X. pachtaicum* (Bleve-Zacheo *et al.*, 1976), no information is available on the ultrastructure of the female reproductive system in species belonging to the *X. americanum*-group. In this paper we report on the reproductive system of *X. americanum* Cobb, 1913.

Materials and methods

Light microscopy observations are based on unstained specimens from various places in the USA, including topotype specimens, mounted on permanent Cobb slides.

For TEM, soil samples were collected in the USA by Drs R.T. Robbins and J. Halbrendt and shipped to Ghent. In the laboratory, the nematodes were extracted from the soil by the centrifugal-flotation method, using a non-toxic silica gel (Ludox AS, Du Pont de Nemours). Adult females were placed in an ice bath for a few minutes to relax. Then they were killed and fixed in ice-cooled fixative composed of 1% paraformaldehyde, 1.75% glutaraldehyde, and 1.5% acrolein in 0.1 M sodium cacodylate buffer pH 7.2. After 30 min, the nematodes were transferred to the fixative minus acrolein and were cut in pieces roughly 200-300 µm long, each piece containing one branch of the genital system. Cutting the specimens facilitates penetration of the fixative. Then, the pieces were incubated overnight in complete formula fixative at 4°C. After approximately 15 h of fixation, they were rinsed in 0.1 M sodium cacodylate buffer for 8 h. Postfixation was in 2% osmium tetroxide in 0.2 M sodium cacodylate buffer for 36 h and was followed by an *en bloc* staining of 1 h in 2% uranyl acetate. The specimens were dehydrated in a graded ethanol series and embedded in Spurr's resin.

Ultrathin sections were made on a Reichert ultracut S ultramicrotome and picked up on formvar-coated copper slot grids. The sections were post-stained in a LKB ultrostainer, for 30 min in uranyl acetate at 40°C and 5 min in lead stain at 20°C. The sections were examined with a Siemens Elmiscop 1A and a Jeol JEM 1010, both operating at 80 kV.

Results

LIGHT MICROSCOPY (Fig. 1)

The female reproductive system of *X. americanum* consists of two equally developed branches, lying either on the same side of the intestine (left or right) or on different sides. As in other species of the group,

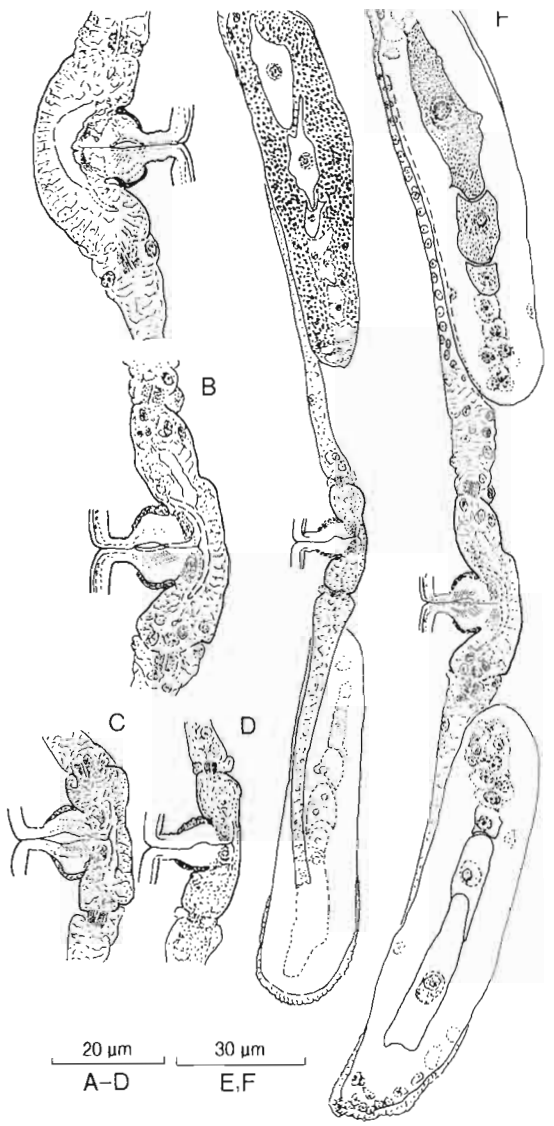

Fig. 1. Xiphinema americanum. *General morphology of female reproductive system. A-D: Variation in ovejector; E-F: Whole system (A, B, F: specimens from Wisconsin; C: specimen from Arlington National Cemetery, type locality; D, E: specimen from Rhode Island).*

symbiotic bacteria are present around the germ cells. The youngest germ cells (oogonia?) are clustered in the apical region of the ovary; they are followed by a single row of oocytes. The oviduct consists of a slender part, with 18-22 (n=4) nuclei, and a more expanded part (*pars dilatata oviductus*), with 16-20 (n=2) nuclei. However, the boundary between the slender and expanded parts is difficult to establish under the light microscope. An obscure sphincter separates the oviduct from the very short uterus. The latter, combined with the uterus of the other branch forms a short ovejector, 30.1 (24-43) µm long (n=10). The ovejector is very extendible since it can harbour an egg of roughly five times its resting length (egg size: 150-170 × 24-31 µm, n=5). The vagina has a wider proximal part with obliquely striated cuticle which is surrounded by the vaginal sphincter, and a narrower distal part to which the vulval dilator muscles attach. The vulva is a transverse slit.

TRANSMISSION ELECTRON MICROSCOPY

Ovary

The ovary of *X. americanum* consists of epithelial wall cells and germ cells as in other *Xiphinema* species, but, in addition, the ovary contains symbiotic bacteria. The outer membrane of the ovary is covered by a *basal lamina,* 60-80 nm thick. The central part of the ovary is occupied by a small number of irregularly-shaped oocytes. Narrow branched extensions of the oocytes lie between the bacteria (Fig. 2A). The oocytes have an electron-dense cytoplasm due to abundant ribosomes. The cytoplasm of the oocytes also includes clusters of electron-transparent mitochondria (Fig. 3), and, in some regions, elaborate configurations of rough endoplasmic reticulum (Fig. 2B). Some cisternae are always found close to the cell surface. The large central nucleus (about 10 µm in diameter) has a moderately electron-dense nucleoplasm, denser scattered chromatin, and a large (3-7 µm) electron-dense round to oval nucleolus. Nucleoplasm-like material is present in the perinuclear region as a narrow (Fig. 3) or wider zone. The outer nuclear membrane is coated with ribosomes. Adjacent oocytes may overlap one another over a short distance (Fig. 3).

Near its apex, the ovary is mainly filled with bacteria embedded in the epithelial wall cells. The nuclei of the epithelial cells are irregular in shape and small (5-7 × 3-5 µm), scattered, and few in number throughout the ovary itself. These nuclei contain a rather high amount of electron-dense chromatin, partly clumped against the inner nuclear membrane and partly in patches throughout the nucleoplasm. The cytoplasm of the epithelial cells is difficult to observe because of its fragmentation between the enclosed bacteria. It is less electron-dense than the

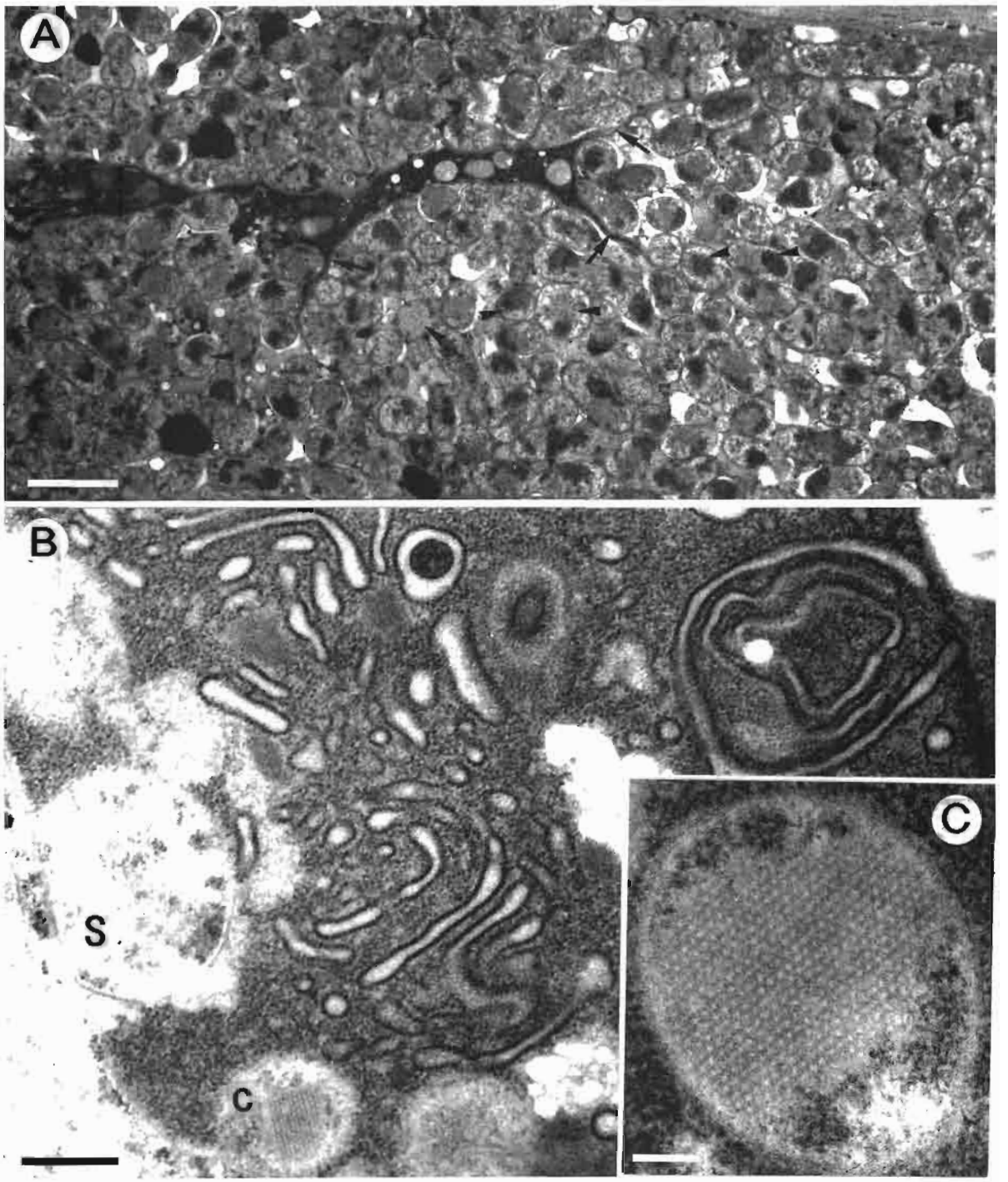

Fig. 2. Xiphinema americanum. *A: L.S. through the ovary, showing branched oocyte (arrow) and symbionts (arrowheads); B: C.S. through an oocyte, showing RER, and enclosed symbionts; C: Larger magnification of the paracrystalline S-layer of a symbiont (Scale bar: A = 2 µm; B = 0.3 µm; C = 0.1 µm. Abbreviations: c: paracrystalline S-layer, C.S.: cross section, i: intestine, L.S.: longitudinal section, m: mitochondrium, n: nucleolus, N: nucleus, o: oocyte, ov: ovary, pdo:* pars dilatata oviductus, *S: symbiont, sg: secretory granule).*

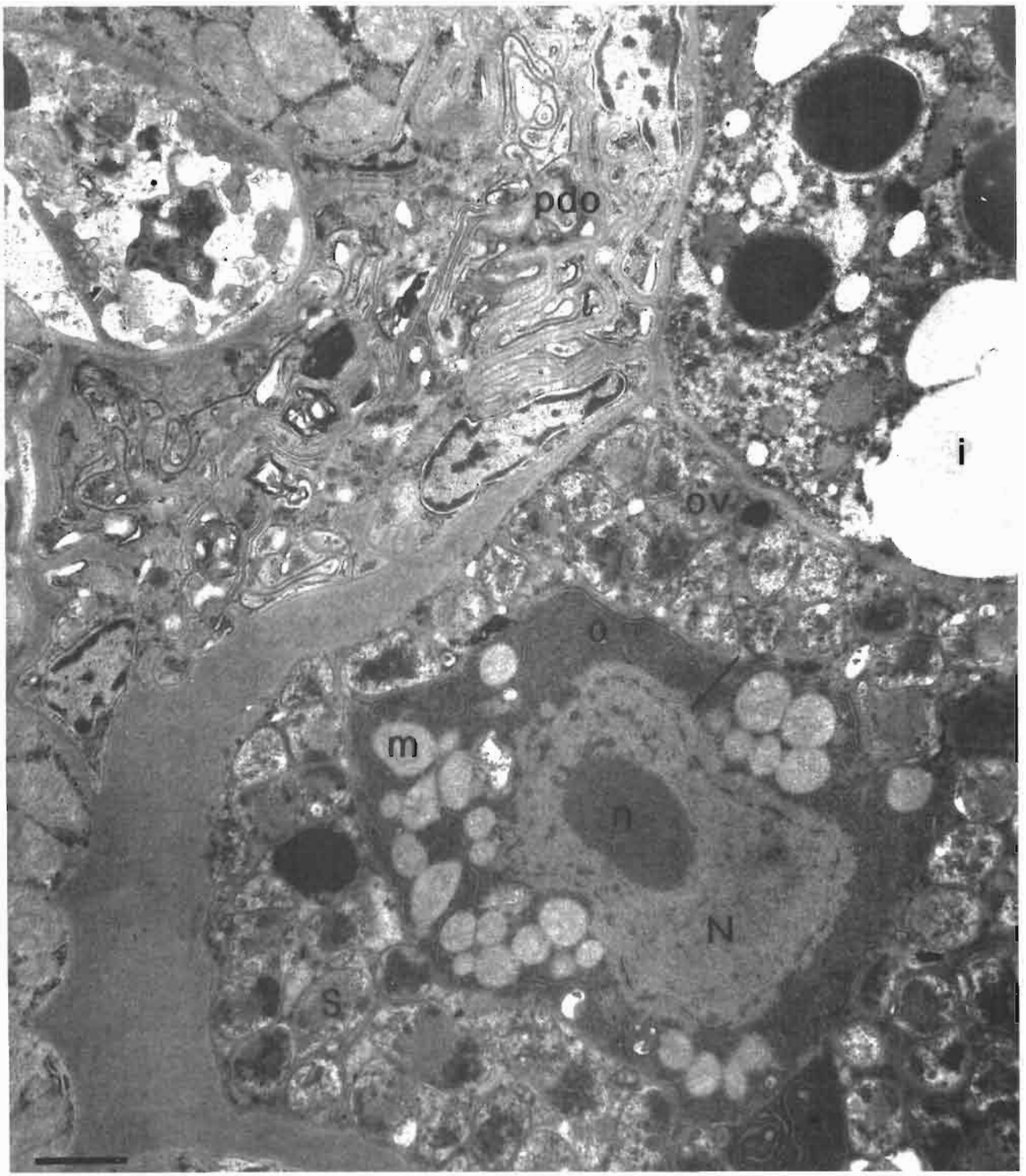

Fig. 3. Xiphinema americanum. *C.S. through ovarium and* pars dilatata oviductus *(Arrow: perinuclear nucleoplasm-like material; arrowheads: overlapping oocytes. Scale bar = 1 µm. See Fig. 2 for abbreviations).*

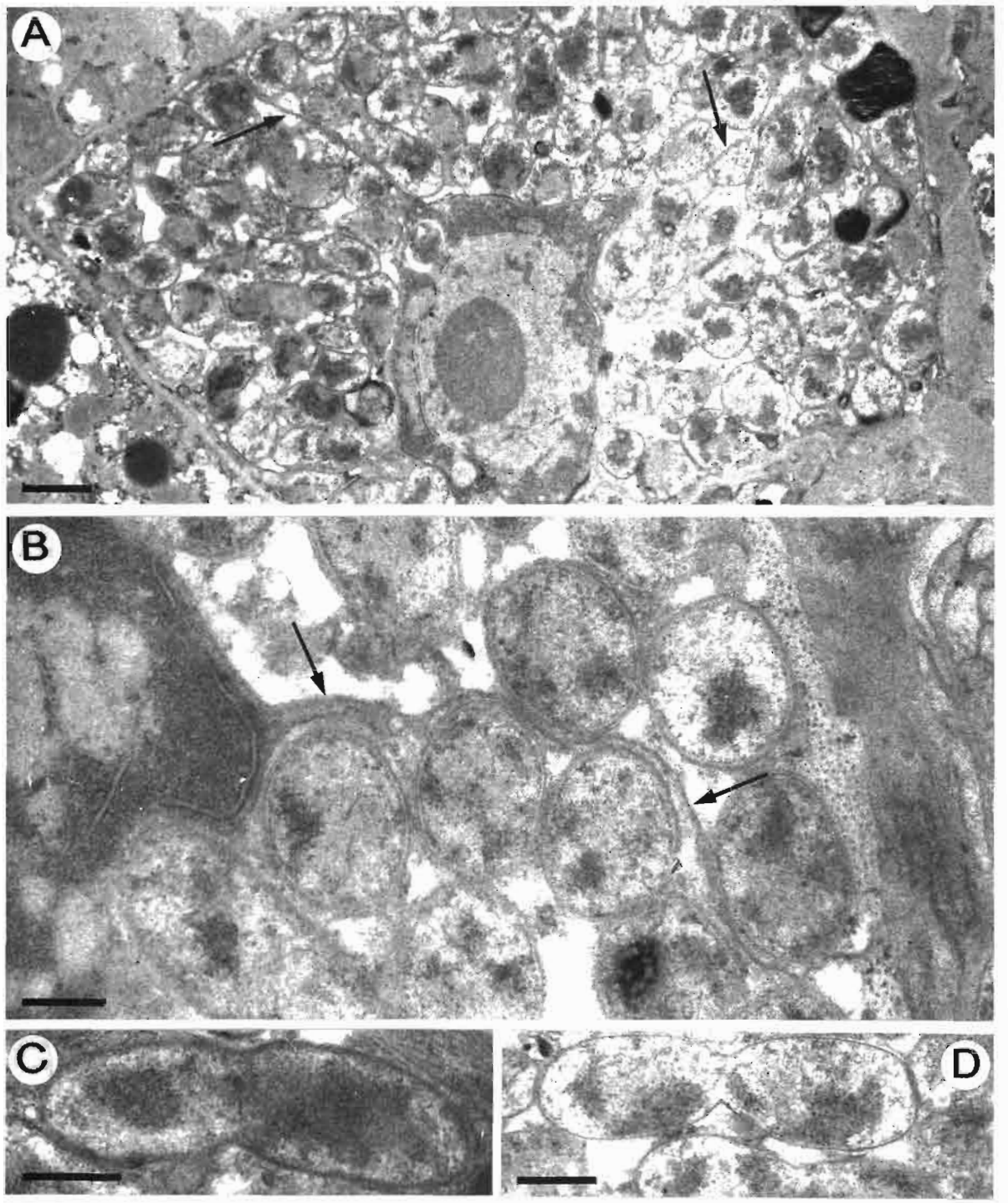

Fig. 4. Xiphinema americanum. *A: C.S. through the ovary, with central oocyte surrounded by epithelial wall cells and symbionts; B: Membranes of adjacent myoepithelial cells, separating symbionts and connecting the outer wall of the ovary with the central oocyte; C, D: Dividing symbionts (Arrows in A and B point to plasmamembranes of adjacent epithelial and myoepithelial cells. Scale bars: A = 1 μm; B = 0.3 μm; C, D = 0.5 μm. See Fig. 2 for abbreviations).*

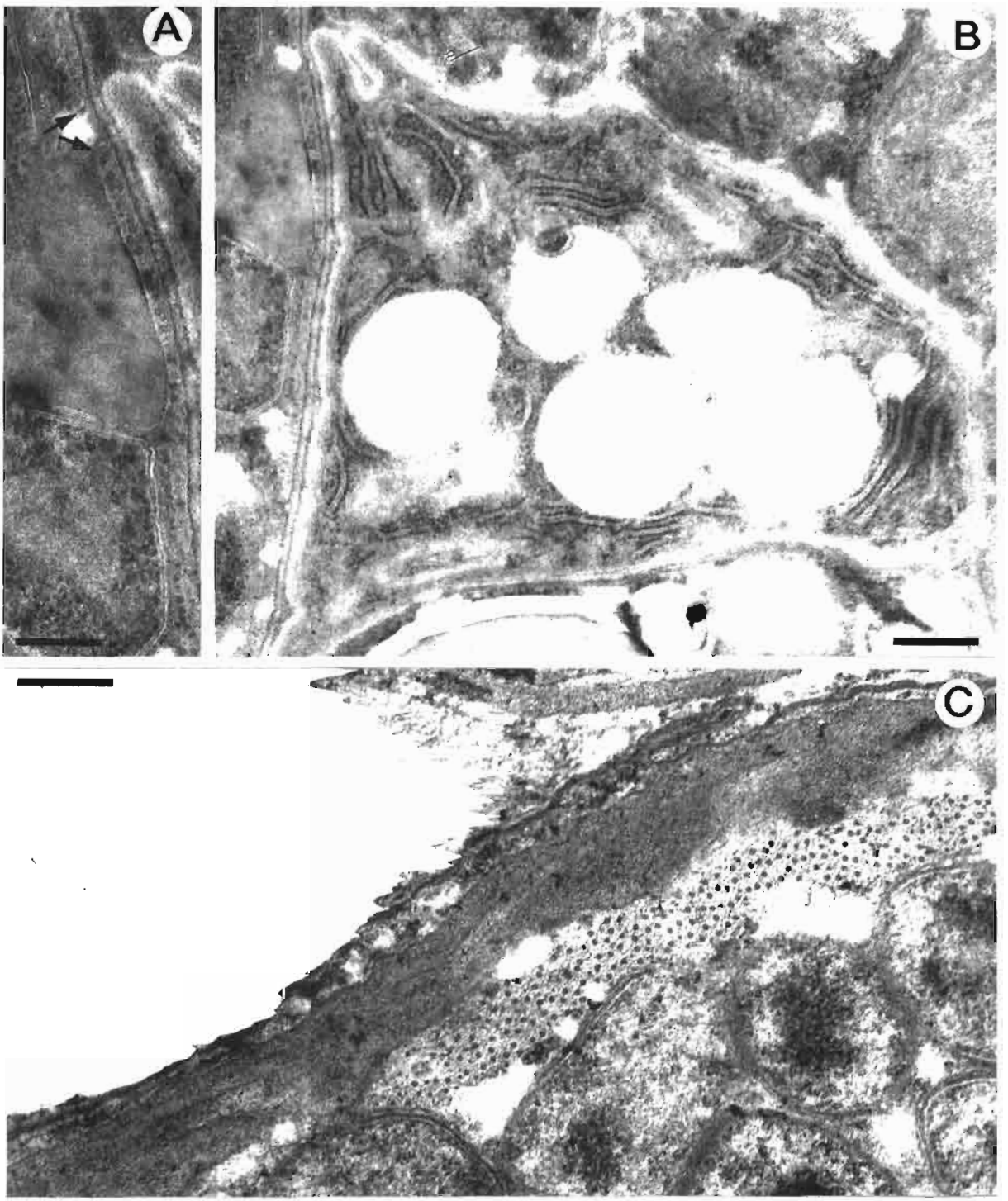

Fig. 5. Xiphinema americanum. *A: C.S. through ovarial wall about halfway in the ovary (arrows point to myofilaments); B: C.S. through the slender part of the oviduct; C: C.S. through ovarial wall close to ovarial sac (Scale bar: A = 0.2 µm; B, C = 0.3 µm. See Fig. 2 for abbreviations).*

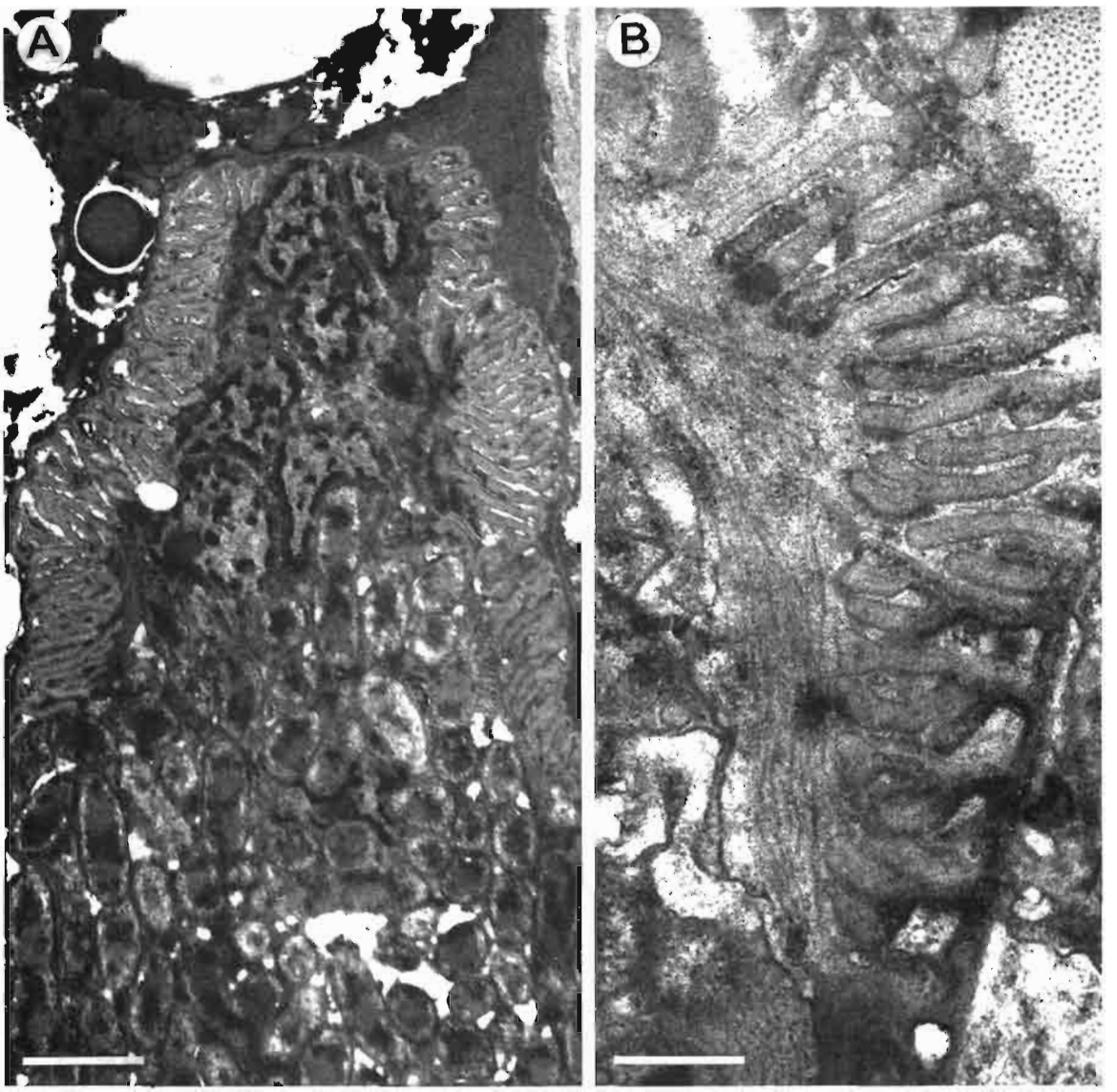

Fig. 6. Xiphinema americanum. *A: L.S. through ovarial sac; B: C.S. through wall of ovarial sac (Scale bars: A = 2 µm; B = 0.5 µm. See Fig. 2 for abbreviations).*

cytoplasm of the germ cell and contains scattered small granular inclusions.

The symbionts have the features of Gramm negative bacteria; they fill all but the central part of the ovary. Fine tissue strands and membranes running between the germ cells and the ovarial surface subdivide the bacterial mass in several sectors. These connections most likely represent the plasma membranes of adjacent epithelial cells which centrally join the plasma membrane of the germ cells (Fig. 4A, B). The bacteria are closely packed and embedded inside the epithelial cells, but occasionally a few bacteria can be observed in close contact with or even encapsulated inside an oocyte (Fig. 2B). An exceptional tangential section (Fig. 2C) shows the S-layer of a bacterium, arranged as a 2D hexagonal close packing of spheres. Size and shape of the bacteria varies according to the section; some can be observed dividing (Fig. 4C, D).

Occasional myofilaments occur at the periphery of the ovarium in a single or double layer (Fig. 5A), from about halfway its length, becoming more numerous and more regularly distributed towards the ovarial sac (Figs 4B; 5C), where the epithelial wall cells become myoepithelial cells. The myofilaments are mostly lon-

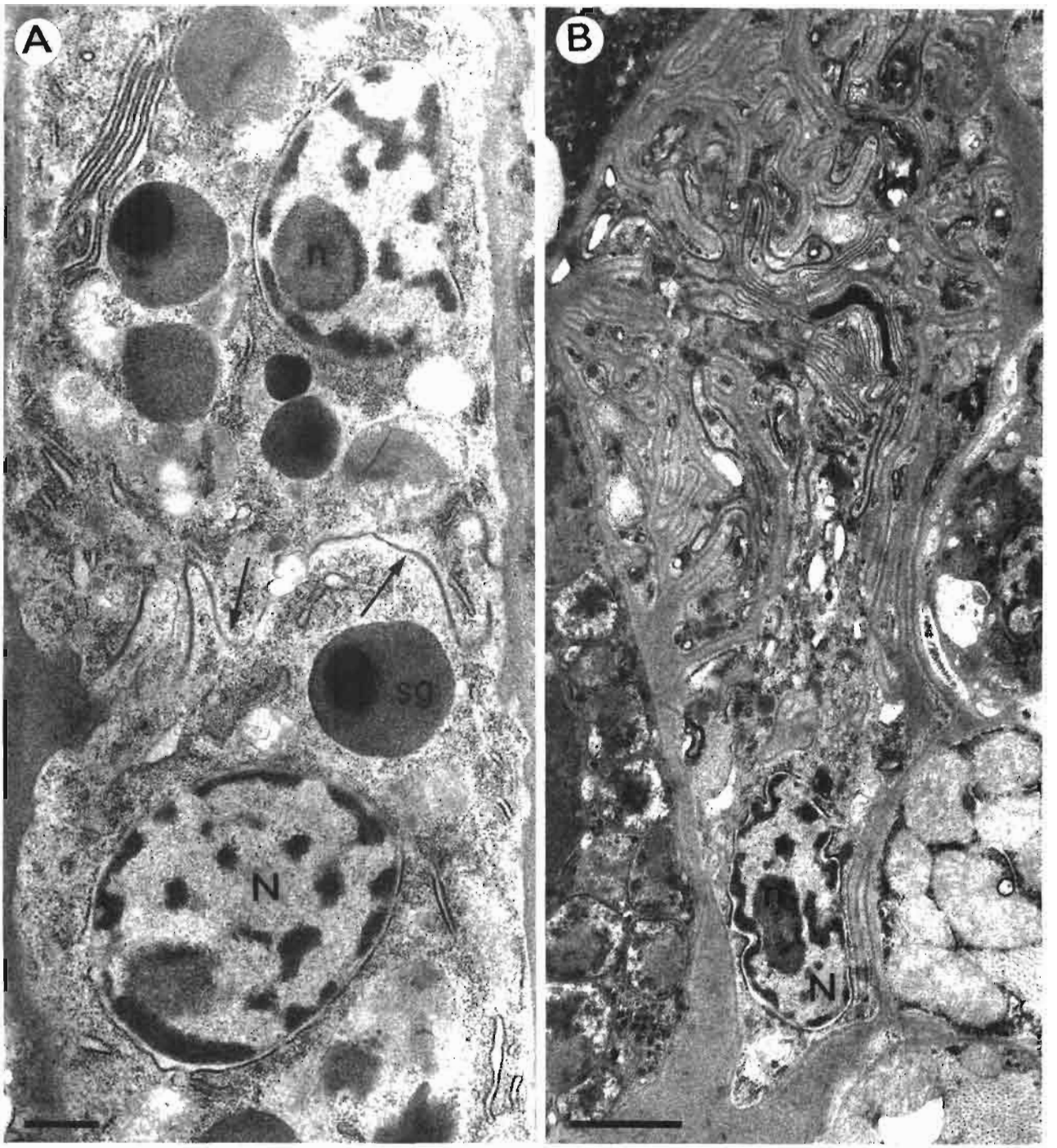

Fig. 7. Xiphinema americanum. *A: L.S. through slender part of oviduct, showing two adjacent cells; B: C.S. of pars dilatata oviductus (Arrows indicate irregular plasmamembranes. Scale bars: A =0.5 µm; B = 1 µm. See Fig. 2 for abbreviations).*

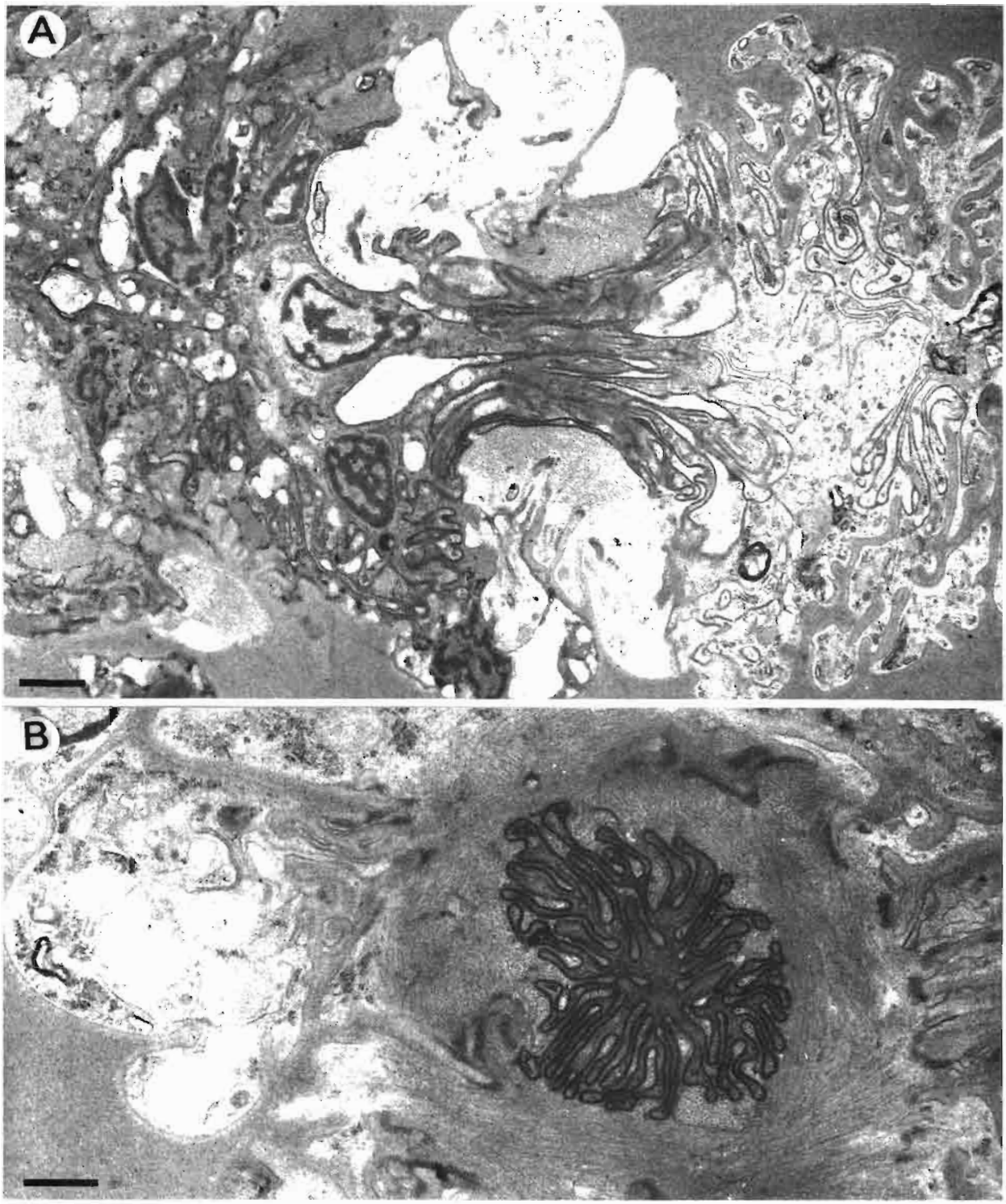

Fig. 8. *X*iphinema americanum. *Sphincter between oviduct and uterus. A: L.S; B: C.S (Scale bars: A = 1 µm; B = 0.5 µm. See Fig. 2 for abbreviations).*

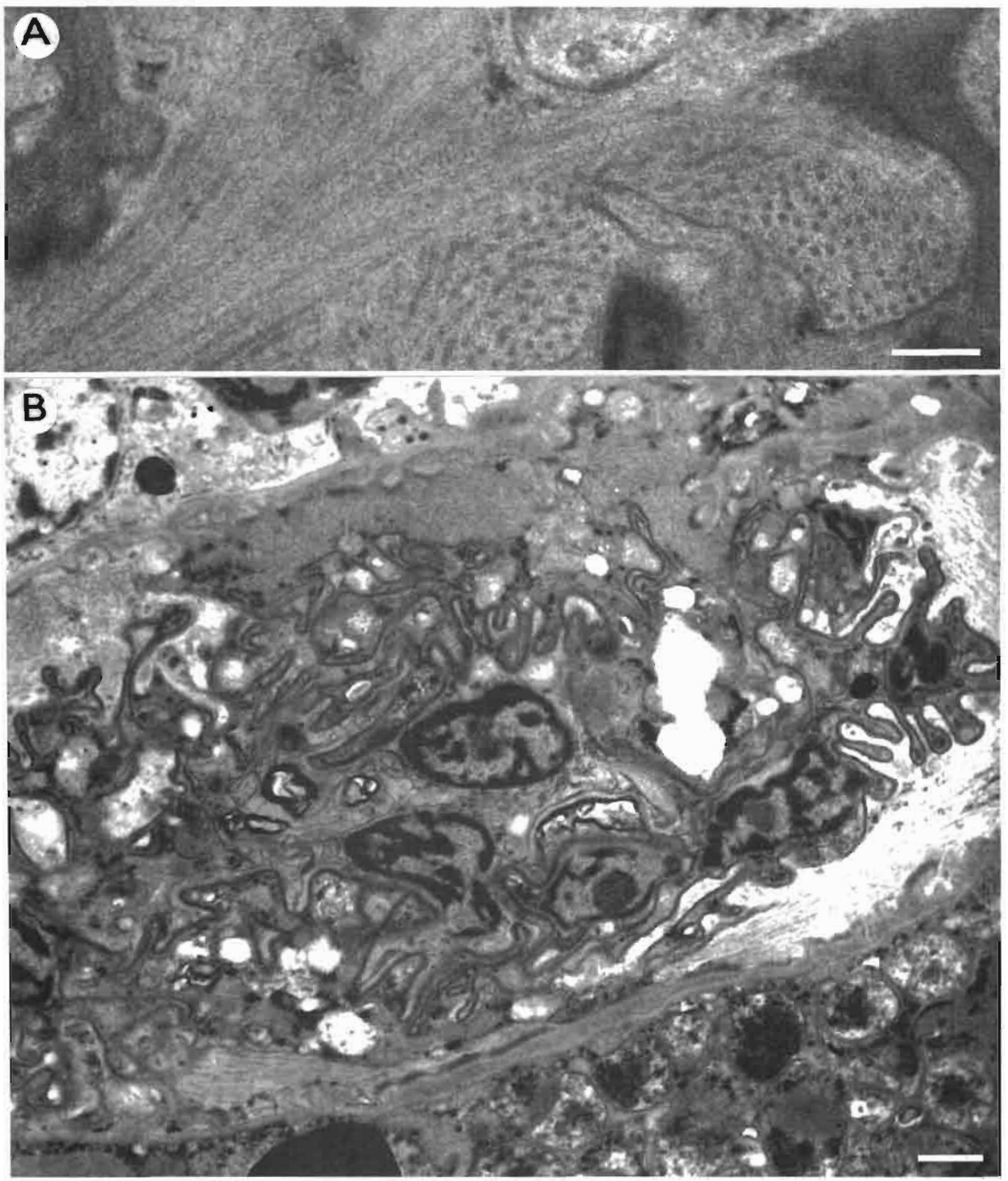

Fig. 9. Xiphinema americanum. *A: Myofilaments of sphincter; B: C.S. through uterus (Scale bars: A = 0.2 µm; B = 0.5 µm. See Fig. 2 for abbreviations).*

gitudinally orientated in the ovarial wall, but become variously arranged in the ovarial sac.

Ovarial sac

The ovarial sac is characterised by a highly folded cell membrane of the epithelial wall cells, covered by a 125-145 nm thick basal lamina. Contrary to the ovarial wall, the nuclei are closely packed (Fig. 6A). The myofilaments are more numerous, mostly located underneath the folds and variously directed (Fig. 6B). Clusters of symbionts are embedded inside the myoepithelial cells, though less numerous than in the ovary proper. This makes it easier to observe the epithelial plasma membranes between them (Fig. 6B). When present, the growing oocyte occupies a central position as in the ovary.

Oviduct

The narrow part of the oviduct is maximally 9.5 µm wide and composed of a string of cells without any apparent lumen between them. A 70-90 nm thick basal lamina covers the outer surface. The adjacent cell membranes are in close contact with each other and have an irregular course (Fig. 7A). The cytoplasm contains well developed RER cisternae and globules of variable size and variable density (Figs 5B; 7A). The nuclei are roundish (5.5-6.5 × 4-5.5 µm), with clear nucleoplasm and electron-dense chromatin. The latter is mainly clumped against the inner nuclear membrane, but also occurs in irregular patches in the nucleoplasm. The nucleolus is about 2 µm large and eccentric in position (Fig. 7A).

The *pars dilatata oviductus* is not clearly separated from the slender part, but its surface forms highly folded extensions into the pseudocoelom. The cell membranes of neighbouring cells are highly intertwined, forming intricate patterns. Cytoplasm includes rough as well as smooth endoplasmic reticulum (Figs 3; 7B), fine electron-dense material (glycogen ?), and electron-dense irregular inclusions. The nuclei are oblong, more or less flattened, 6.5-9.5 × 2.5-4 µm, located at the periphery, and sometimes bulging out of the irregular contour. Although a central lumen is present, it is difficult to observe due to extreme folding of the whole *pars dilatata*. The latter is greatly extended when the lumen contains an egg cell.

Myofilaments are absent throughout the oviduct.

Sphincter

The sphincter between oviduct and uterus consists of *i*) a highly folded inner lining surrounding a narrow and multibranched lumen (Fig. 8B); *ii*) a muscular wall comprising inner myofilaments that are mostly longitudinally arranged and outer myofilaments that are mostly circular and partly obliquely arranged

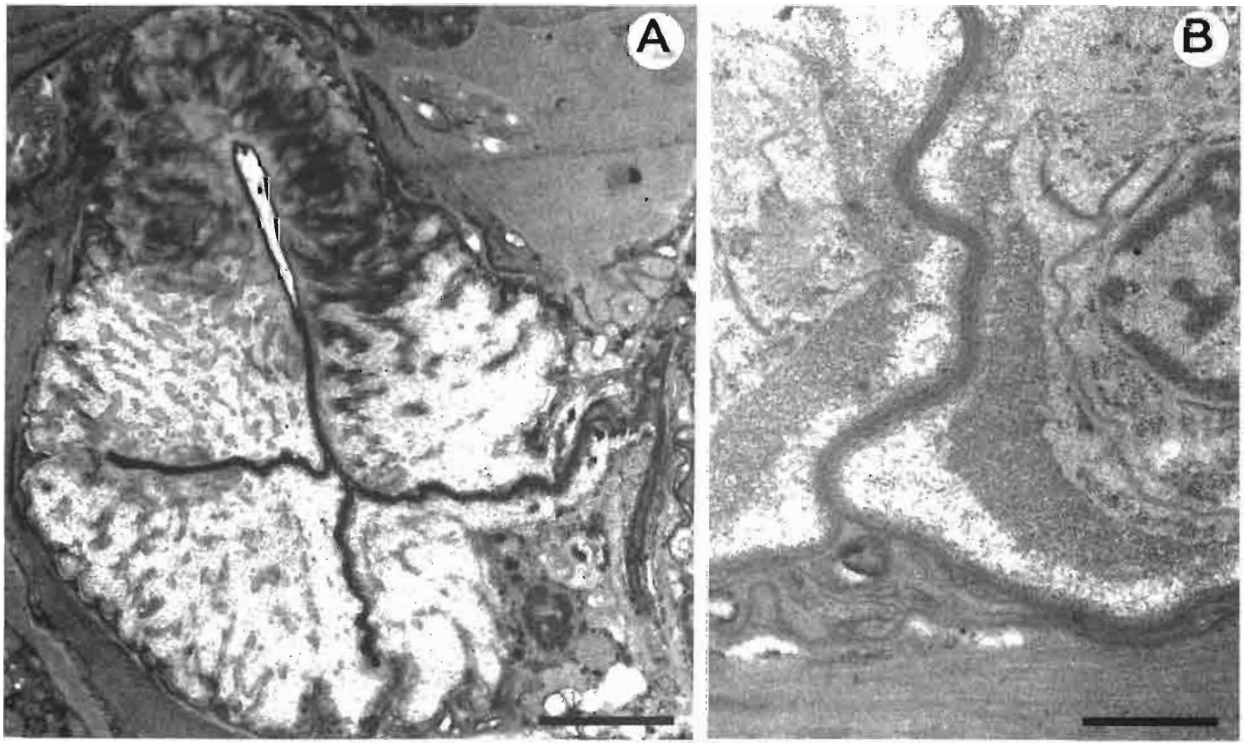

Fig. 10. Xiphinema americanum. *A: C.S. through vagina (junction with ovejector on the right); B: Detail of junction of vagina (above) with ovejector (below) (Scale bars: A = 5 µm; B = 0.5 µm. See Fig. 2 for abbreviations).*

(Fig. 9A); *iii*) several cell bodies that bulge into the surrounding pseudocoelom (Fig. 8). The plasma membrane is covered by a 100-125 nm thick basal lamina. The cytoplasm is rather electron-translucent except for the proximal part, adjacent to the uterus, where the nuclei are located (Fig. 8A). The central part (*i* + *ii*) is about 20 µm long and 10-14 µm in diameter, but the diameter value increases to 30 µm if the bulging cell bodies (*iii*) are included.

Uterus

The short uterus (or ovejector, see below) has a strongly folded inner lining and less folded outer wall, but the folding is conspicuously less developed than in the oviduct and the lumen can be more readily observed (Fig. 9B). The wall musculature is well developed and myofilaments are variously directed, but with obliquely transverse orientation predominant. The nuclei are occurring centrally as well as close to the surface and may bulge out of the contour; nuclei are oval to irregular in shape, 3.5-6 × 2-4 µm, and contain patches of electron-dense chromatin. The chromatin may be particularly abundant on the inner nuclear membrane. The uterus is covered by a 100 nm thick basal lamina.

Vagina and vulva

The vagina has a cross-shaped lumen with electron-dense lining (Fig. 10A). Its cuticular wall contains many electron-dense fibres, running between inner and outer surface and forming irregular patterns. Proximally, the longitudinal arms of the cross open into the lumen of the ovejector. At this junction, each arm is surrounded by two pairs of cells with prominent nuclei. The pair closest to the lining contains electron-dense material arranged as a strand (Fig. 10B). Distally the longitudinal arms disappear while the transverse ones become the vulva. The cuticle becomes more dense and transverse oval in shape and merges with the body cuticle at the vulva opening which is a transverse slit.

Discussion

Bleve-Zacheo *et al.* (1976) described the ultrastructure of the female reproductive system in *Xiphinema index* and *X. pachtaicum* (= *X. mediterraneum*). In the light of the present observations on *X. americanum*, it is a pity that these authors gave a single description for both species, because "no fundamental differences have been observed between the structure of the gonads in the two species...". Fortunately, the species included in the plates were clearly identified.

The structure of the ovary and gonoduct described above for *X. americanum* markedly differs from the pattern that occurs in *Xiphinema* species outside of the *X. americanum*-group (Bleve-Zacheo *et al.*, 1976; Van de Velde & Coomans, 1988; Van de Velde *et al.*, 1990*a*, *b*; Coomans *et al.*, 1992).

The differences in the ovary are the most obvious and related to the presence of symbiotic Gramm negative bacteria inside the very thick wall. Although these symbionts can be readily observed under the light microscope, their exact location inside the ovary can be assessed only with TEM. They appear to be embedded inside the epithelial wall cells and, rarely, also in the oocytes. More information about these bacteria will be published elsewhere. The small granular inclusions in the epithelial cells are probably glycogen.

The slender part of the oviduct resembles that described for other *Xiphinema* species, except that it is narrower and comprises a lesser number of cells and larger nuclei. The well developed rough endoplasmic reticulum and the presence of globules demonstrates the secretory nature of these cells. On the other hand, the *pars dilatata oviductus* differs in several respects: it is comparatively longer, not clearly separated from the slender part, and it has an extensively folded wall.

The sphincter muscle between oviduct and uterus appears to be embedded into the surrounding cell bodies. This explains why it is difficult to observe the sphincter under the light microscope. The musculature and the sinuous lumen agree with those described for *X. pachtaicum* by Bleve-Zacheo *et al.* (1976).

The uterus is extremely short and in fact both uteri together constitute the muscular ovejector. Its outer wall is more folded than in species outside of the *X. americanum*-group, which is understandable in view of the considerable expansion needed to harbour a large size egg. Personal unpublished observations on other species indicate that the shortening of the uterus is an evolutionary trend in the *X. americanum*-group.

Acknowledgements

We are indebted to Drs M. Golden, J. Hallbrendt, R.T. Robbins, and A.C. Tarjan for providing specimens of *X. americanum*.

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The genus *Xiphinema* (Nematoda: Longidoridae) in Guyane and Martinique

Antoinette SWART* and Patrick QUÉNÉHERVÉ**

** National Collection of Nematodes, Biosystematics Division, Plant Protection Research Institute, Private Bag X134, Pretoria, 0001, South Africa, and ** ORSTOM, Laboratoire de Nématologie, B.P. 8006, 97259 Fort-de-France Cedex, Martinique*

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Summary – This article presents the results of surveys conducted in Guyane and Martinique during 1994/1995. Seven species of *Xiphinema* (*X. americanum sensu lato*, *X. basiri*, *X. clavicaudatum*, *X. costaricense*, *X. filicaudatum labratum*, *X. longicaudatum*, and *X. oryzae*) are briefly described and illustrated. *X. basiri* and *X. longicaudatum* are described for the first time from South America. Three new *Xiphinema* species are described: *Xiphinema labiosum* n. sp. is characterised by peri-oral ring, small, crescent-shaped amphid aperture, lip region almost continuous with body outline, and weakly curved to almost straight habitus. *Xiphinema pseudokrugi* n. sp. is characterised by incomplete anterior reproductive branch, vulva situated almost at mid-body, slight cuticular ornamentations anterior and posterior of vulva, and a convex-conoid tail with a rounded or slightly bulging terminus. *Xiphinema seinhorsti* n. sp. is characterised by long, offset, caudal, finger-like projection on the ventral side of the tail, presence of a blind canal at the tail terminus, long odontostyle, and uterus with Z-differentiation consisting of a small number of granular bodies. © Orstom/Elsevier, Paris

Résumé – ***Le genre* Xiphinema *(Nematoda: Longidoridae) en Guyane et Martinique*** – Cet article présente les résultats de prospections conduites en 1994/1995 en Guyane et Martinique. Sept espèces de *Xiphinema* (*X. americanum sensu lato*, *X. basiri*, *X. clavicaudatum*, *X. costaricense*, *X. filicaudatum labratum*, *X. longicaudatum*, et *X. oryzae*) sont brièvement décrites et illustrées. *X. basiri* et *X. longicaudatum* sont signalés pour la première fois en Amérique du Sud. Trois nouvelles espèces de *Xiphinema* sont décrites: *Xiphinema labiosum* n. sp. est caractérisé par un anneau péri-oral, des ouvertures amphidiales petites en forme de croissant, la région labiale pratiquement continue avec le contour du corps et l'habitus de légèrement incurvé à pratiquement droit. *Xiphinema pseudokrugi* n. sp. peut être caractérisé par la branche génitale antérieure incomplète, la vulve située à mi-corps, de légères ornementations cuticulaires en avant et en arrière de la vulve et la queue de forme convexe-conoïde avec une partie terminale arrondie ou légèrement renflée. *Xiphinema seinhorsti* n. sp. est caractérisé par une projection caudale longue, en forme de doigt, séparée du reste de la queue et située sur la face ventrale de celle-ci avec présence d'un canal aveugle, un long odontostyle et l'utérus avec une différentiation Z constituée d'un petit nombre de corps granuleux. © Orstom/Elsevier, Paris

Keywords: Guyane, Martinique, nematodes, *Xiphinema*.

Seventeen species of the genus *Xiphinema* found during surveys made in 1994/1995 by the second author in Guyane and Martinique are listed below. The species marked by an asterisk have already been described in the paper by Luc and Coomans (1992) on the genus *Xiphinema* in Martinique and Guyane. As there are no major morphological differences between the populations described by Luc and Coomans (1992) and the corresponding populations of the present study, these species will not be described again in the present paper. The numbers given after the names of the various species are those of localities (see below) in Martinique and French Guyane where each species was found during the present survey.

X. americanum sensu lato: Guyane (14), Martinique (2)
X. basiri Siddiqi, 1959: Martinique (2)
* *X. brasiliense* Lordello, 1951: Guyane (6)
X. clavicaudatum Huang, Uesugi & Raski, 1987: Guyane (10)
X. costaricense Lamberti & Tarjan, 1974: Guyane (13, 17)
* *X. ensiculiferum* (Cobb, 1893) Thorne, 1937: Guyane (12)
X. filicaudatum labratum Luc & Coomans, 1992: Guyane (3, 7, 10, 16)
* *X. krugi* Lordello, 1955: Martinique (1)
X. labiosum sp. n.: Guyane (16)
X. longicaudatum Luc, 1961: Guyane (12)
* *X. macrostylum* Esser, 1966: Guyane (6)
X. oryzae Bos & Loof, 1985: Guyane (3, 7, 11)
* *X. paritaliae* Loof & Sharma, 1979: Guyane (9)
X. pseudokrugi sp. n.: Guyane (8, 10)
X. seinhorsti sp. n.: Guyana (4, 5, 7, 10)
* *X. setariae* Luc, 1958: Guyane (5, 10)
* *X. surinamense* Loof & Maas, 1972: Guyane (9, 13, 15, 16, 17)

Materials and methods

Specimens were extracted from soil using the elutriation technique (Seinhorst, 1962), killed in water by gradual application of heat, fixed in TAF, and mounted in anhydrous glycerine between coverslip slides using the slow method of Hooper and Evans (1993). Measurements and drawings were made using a Nikon Labophot-2 research microscope equipped with a drawing tube. When necessary, the body diameter was corrected for flattening according to the formula $d = \frac{1}{2}(h + v)$, as given by Geraert (1961).

The following localities were sampled:

- Martinique:

1. Rhizosphere of ferns, Rocher du Diamant
2. Rhizosphere of ornamental plants, Didier

- Guyane:

3. Rhizosphere of *Dicorynia guianensis* Amshoff, Station P6, Paracou
4. Rhizosphere of *Dicorynia guianensis* Amshoff, Botanical Track (Sample 1), Paracou
5. Rhizosphere of *Eperua grandiflora* (Aubl.) Bentham, Station P13, Paracou
6. Rhizosphere of *Eperua grandiflora* (Aubl.) Bentham, Station P6, Paracou
7. Rhizosphere of *Astrocaryum sciophilum* (Miguel) Pulle, Saut Brodel
8. Rhizosphere of Marantaceae, Saut Brodel
9. Rhizosphere of ferns, Rivière Comté
10. Rhizosphere of *Dicorynia guianensis* Amshoff, Botanical Track (Sample 2), Paracou
11. Rhizosphere of *Etlingera eliator* (Jack.) R.M. Smith, Cacao
12. Rhizosphere of *Dicorynia guianensis* Amshoff, Saül, Bois Diable
13. Rhizosphere of *Dicorynia guianensis* Amshoff, Saül, Noubel
14. Rhizosphere of *Aspidosperma* sp. Martius & Zuccarini, Saül, Mont de Boeuf Mort
15. Rhizosphere of *Dicorynia guianensis* Amshoff, Saül, Mont de Boeuf Mort
16. Rhizosphere of *Dicorynia guianensis* Amshoff, Saül, Cascade Sauveur
17. Rhizosphere of *Dicorynia guianensis* Amshoff, Saül, Mont Galboa.

In the text of the article, localities are designated by their number (1-17) in the list above.

Xiphinema americanum sensu lato
(Fig. 1)

MEASUREMENTS

Female: See Table 1.

DESCRIPTION

Female: Habitus spiral-shaped. Lip region 4-5 µm high, rounded, separated from rest of body by a shallow depression. Amphid apertures 6-8 µm wide, occupying about 66 % of lip region width, 4-5 µm from anterior end. Body pores difficult to see; distribution in neck region appearing as (n = 4): nine to twelve laterally, nine to twelve ventrally, six to nine dorsally. Vestigium seen in one specimen, about 4 µm long. Basal flanges 9-11 µm wide. Cuticle 2.5-3 µm wide at mid-body, 5-6 µm on dorsal side of tail. Body diameter at cardia 35-38 µm, at mid-body 40-48 µm, at anus 21-27 µm, and at the beginning of the hyaline part of tail, 11-14 µm. Reproductive system amphidelphic, with both branches equally developed. Each branch composed of ovary, oviduct, obscure sphincter, and short uterus (35-40 µm long). Tail conoid, dorsally convex with rounded to slightly subdigitate terminus; two caudal pores. Rectum 18-23 µm long.

Male: Not found.

Table 1. *Morphometrics of females of* Xiphinema americanum s. l. *(all measurements in µm except L in mm).*

Locality	Guyane 14	Martinique 2
n	7	1
L	1.7 ± 0.1 (1.5-1.8)	2.2
a	37.3 ± 1.9 (34.2-39.7)	54.9
b	6.2 ± 0.7 (4.8-6.9)	6.9
c	57.6 ± 5.9 (49.1-64.8)	66.6
c'	1.3 ± 0.1 (1.1-1.4)	1.3
V	52.5 ± 0.8 (52-54)	52
Tail	29.4 ± 2 (27-33)	33
Lip reg. diam.	10.4 ± 1 (9.5-11.5)	10.5
Odontostyle	88.8 ± 2.4 (86-92.5)	94.5
Odontophore	54.1 ± 1.3 (51.5-55.5)	54.5
Stylet	143 ± 3 (138-147.5)	149
Guid. ring	76.3 ± 2.7 (74.5-80)	80.5
Nerve ring	150.6 ± 10.8 (134-160)	144
Hemizonid	138.6 ± 9.7 (120-148.5)	159
h	9.5 ± 1.3 (7.5-11)	10
h%	32.3 ± 5.1 (26-38)	30

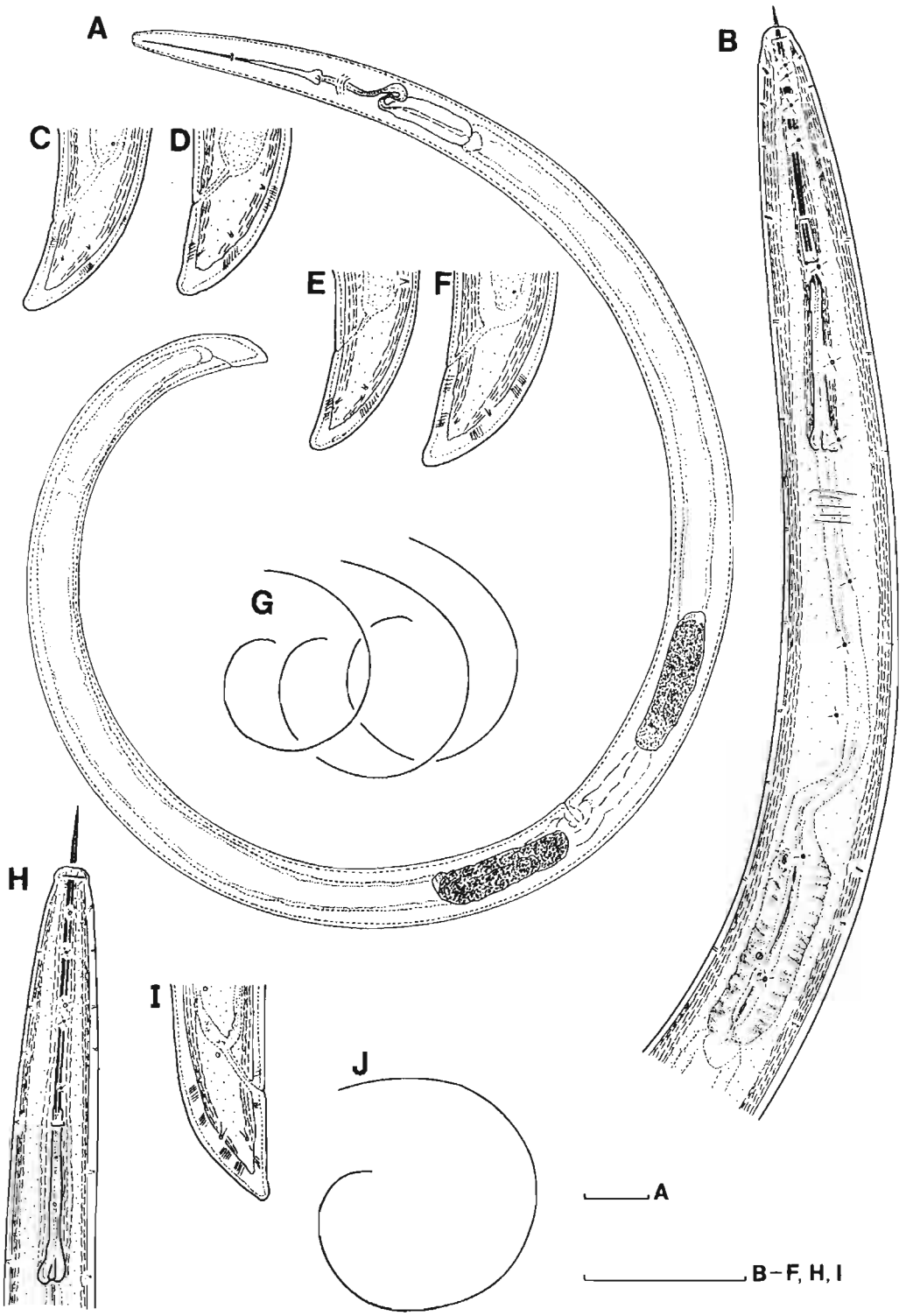

Fig. 1. *A - J:* Xiphinema americanum sensu lato, *A - G: Guyane population. A: Entire female; B: Anterior body region; C - F: Tails of females; G: Habitus; H-J, Single female from Martinique. H: Anterior body region; I: Tail; J: Habitus. (Scale bars = 50 µm.)*

REMARKS

According to the dichotomous key for the identification of species of the *X. americanum*-group (Lamberti & Carone, 1992), the specimens from Guyane belong to *X. incognitum* Lamberti & Bleve-Zacheo, 1979. To help with the identification of this difficult group, Lamberti and Ciancio (1993) subdivided it into five subgroups according to a combination of body length, position of vulva, and odontostyle length. According to this classification, the specimens from Guyane are closer to the *X. brevicollum*-subgroup (long odontostyle, relatively posterior position of vulva, and relatively short body) than to the *X. americanum*-subgroup to which *X. incognitum* belongs. Therefore, until more specimens can be obtained for measurement, we decided to regard this population as *X. americanum sensu lato.* The Guyane specimens differ from the type population of *X. incognitum* in lower a-value (34.2-39.7 *vs* 41-49) and slightly subdigitate tail terminus in some specimens (not so in the type population of *X. incognitum*). In shape of tail terminus, the Guyane specimens resemble *X. floridae* Lamberti & Bleve-Zacheo, 1979 and *X. peruvianum* Lamberti & Bleve-Zacheo, 1979. However, they differ from *X. floridae* in smaller lip region (9.5-11.5 *vs* 11.5-13.5 µm wide), lower a-value (34.2-39.7 *vs* 41-48), outline of anterior part of body (lip region separated from rest of body by shallow depression *vs* lip region separated from rest of body by an incisure), and tail shape (conoid with rounded to subdigitate terminus *vs* cuneiform tail). They differ from *X. peruvianum* in lower a-value (45-56 in *X. peruvianum*), longer odontophore (51.5-55.5 *vs* 46-52 µm), slightly lower c'-value (1.1-1.4 *vs* 1.4-1.9), and slightly different tail shape (conoid with rounded to slightly subdigitate terminus *vs* conoid elongated with subdigitate terminus).

The single female from Martinique is quite similar to the Guyane females but differs from them in body length (2.2 *vs* 1.5-1.8 mm), higher a-value (54 in female from Martinique), and clearly offset, almost button-like lip region. As it is virtually impossible to determine the species of a single female in the *X. americanum*-group, this specimen is also regarded, for the time being, as *X. americanum sensu lato.*

Xiphinema basiri Siddiqi, 1959
(Fig. 2)

MEASUREMENTS

Female: See Table 2.

DESCRIPTION

Female: Habitus hook-shaped, more curved in posterior part. Lip region 6 µm high, rounded, slightly offset. Amphid apertures 8-8.5 µm wide, occupying about 70 % of lip region width, 4.5 µm from anterior end. Body pores conspicuous; distribution in neck region: thirteen to fifteen laterally, eleven ventrally, four dorsally. Vestigium (n = 1) 3.5 µm long. Basal flanges 11-12 µm wide. Cuticle 3.5 µm wide at mid body, 9 µm on dorsal side of tail, transversely striated at both extremities of body. Reproductive system with both branches equally and fully developed. Each branch consisting of ovary 65-87 µm long, oviduct 60-85 µm long, *pars dilatata oviductus* 24-38 µm long, sphincter, *pars dilatata uteri* 30-40 µm long, and long thin convoluted uterus, 160-165 µm long with Z differentiation occupying about 8 µm of the uterus near *pars dilatata uteri.* Ovejector about 50 µm long; vagina occupying about one half of corresponding body width. Z-differentiation consisting of three to four globular inclusions. No sperm cells observed in any part of reproductive system. Prerectum distinct, occupying 11.5-11.8 % of body length, about 10.5 times corresponding body diameter. Rectum about one anal body diameter in length. Tail short, conical, distinctly digitate.

Male: Not found.

Table 2. *Morphometrics of females of* Xiphinema basiri *(all measurements in µm except L in mm).*

Locality	Martinique 2
n	2
L	3, 3.1
a	59.3, 55.5
b	7.5, 8.6
c	69, 70
c'	1.3, 1.3
V	51, 52
Tail	43, 45
Lip reg. diam.	12.5, 12.5
Odontostyle	119, 120
Odontophore	67.5, 68.5
Stylet	186.5, 188.5
Guid. ring	111.5, 107
Nerve ring	209, 175.5
Hemizonid	180,–
h	19, 21.5
h%	44, 48

REMARKS

The two females from Martinique (Loc. 2) agree morphologically and morphometrically in every respect with the descriptions of the type and other populations. This is the first report of *X. basiri* from Martinique. This species was known previously from

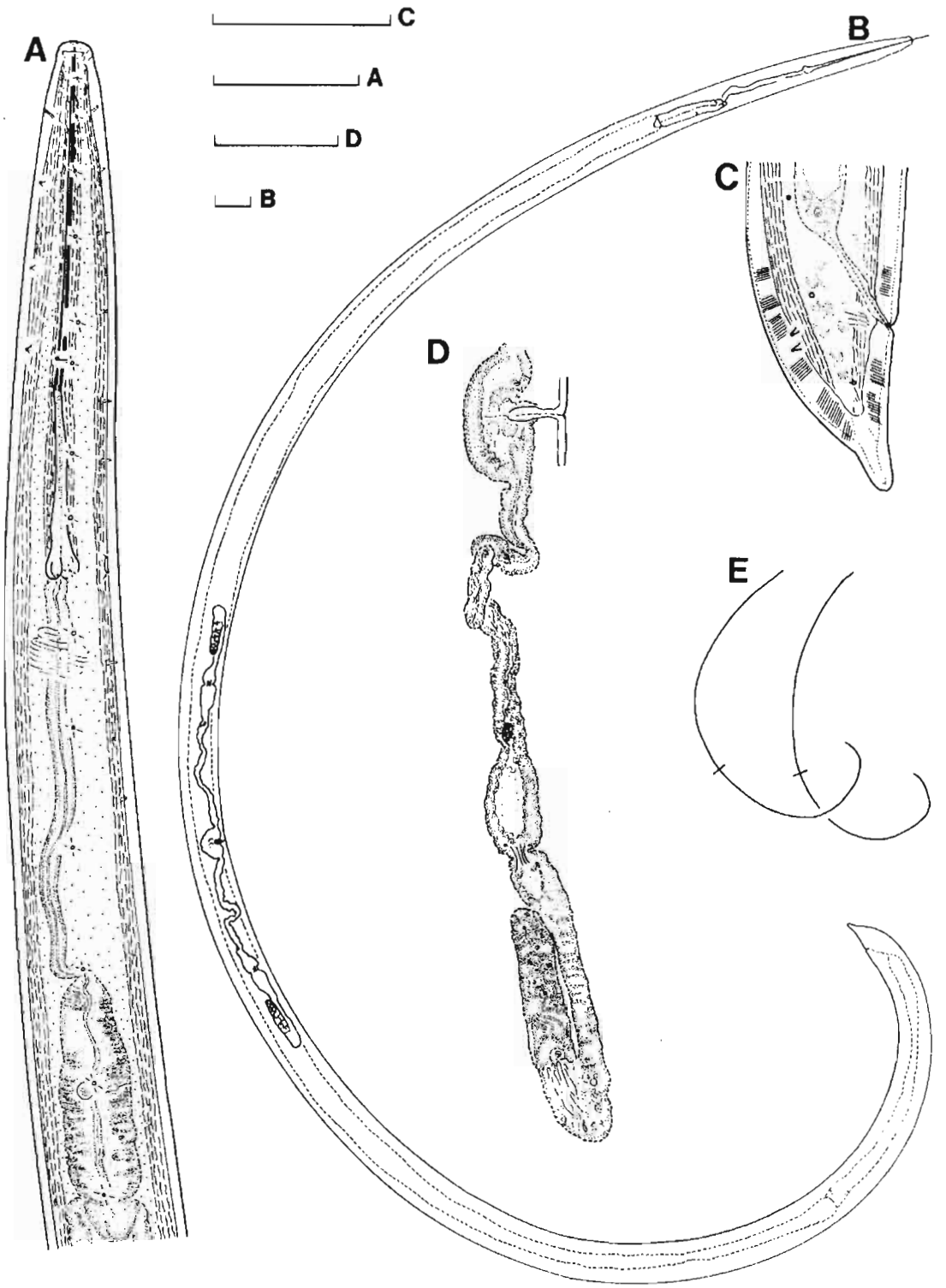

Fig. 2. Xiphinema basiri *Siddiqi, 1959. A: Anterior body region; B: Entire female; C: Female tail; D: Posterior branch of reproductive system; E: Habitus. (Scale bars = 50 µm.)*

India (Siddiqi, 1959; Bajaj & Jairajpuri, 1979; Sharma & Saxena, 1981; Javed, 1983), Sudan (Loof & Yassin, 1971; Zeidan & Coomans, 1991), Nigeria, Sri Lanka, Mexico, and Zimbabwe (Cohn & Sher, 1972).

Xiphinema clavicaudatum Huang, Uesugi & Raski, 1987
(Fig. 3)

MEASUREMENTS

Female: See Table 3.

DESCRIPTION:

Female: Habitus moderately curved to J-shaped, more curved in posterior third. Lip region rounded, about 7-9 µm high, very slightly offset from rest of body. Amphid aperture 6-8 µm wide, occupying about 45 % of body width. Body pore distribution in neck region: eleven to fourteen laterally, ten to twelve ventrally, five dorsally. Vestigium 2 µm long. Basal flanges 14-15 µm wide. Reproductive system with fully developed posterior branch and shorter, reduced anterior branch. Anterior reproductive branch consisting of

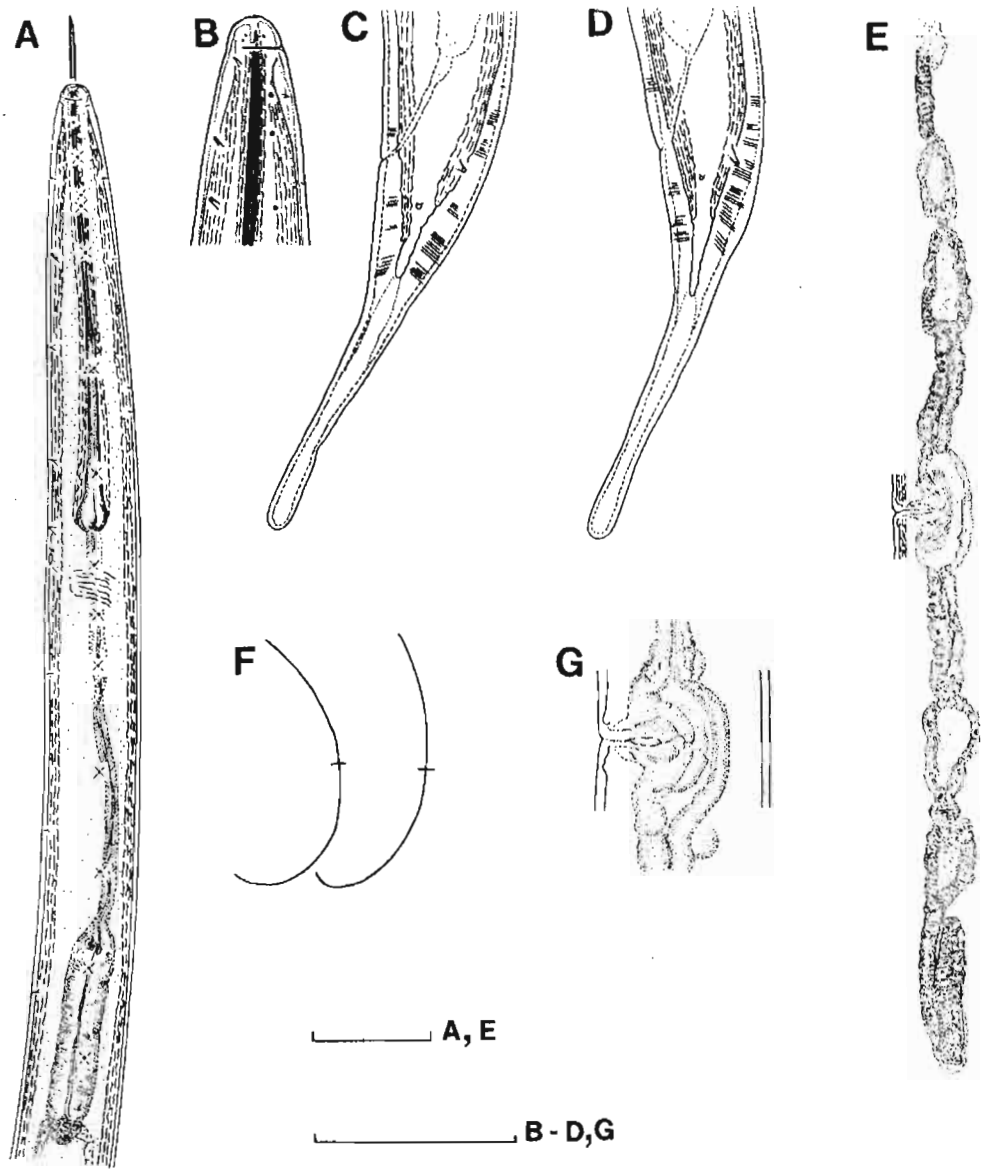

Fig. 3. Xiphinema clavicaudatum *Huang, Uesugi & Raski, 1987. A: Anterior body region; B: Lip region, lateral view; C, D: Tail region of females; E: Reproductive system; F: Habitus; G. Vulval region. (Scale bars = 50 µm.)*

Table 3. *Morphometrics of females of* Xiphinema clavicaudatum *(all measurements in µm except L in mm).*

Locality	Guyane 10
n	5
L	2.2 ± 0.1 (2.1-2.3)
a	47.5 ± 3.5 (43.1-52)
b	5 ± 0.4 (4.7-5.6)
c	22.8 ± 1 (21.7-24.2)
c'	3.6 ± 0.2 (3.3-3.8)
V	41.8 ± 1.6 (40-44)
Tail	95.9 ± 3.4 (92.5-99.5)
Lip reg. diam.	13.9 ± 1 (13-15)
Odontostyle	136.5 ± 2.1 (135-140)
Odontophore	84.6 ± 1.7 (82-86)
Stylet	221.1 ± 3.4 (217-226)
Guid. ring	127.4 ± 2.1 (125-130)
Nerve ring	246.7 ± 21.7 (210-266)
Hemizonid	195.4 ± 7.6 (188-205)
h	65.3 ± 3.6 (62-69.5)
h%	68 ± 3.4 (63.3-72)

fully developed uterus with *pars dilatata uteri*, sphincter, *pars dilatata oviductus*, short part of the slender oviduct, and a small cell mass representing the ovary. No sperm cells observed in any part of the reproductive system. Ovejector symmetrical, blind extensions apparently present on both side of the ovejector in one specimen, in others inconspicuous. No Z-differentiation or uterine spines observed. Prerectum conspicuous, 475-620 µm long, occupying about 25 % of body length. Rectum 30-35 µm long. Tail slightly curved ventrad with hyaline portion straight. Tail terminus clavate. Two pairs of caudal pores present; one pair situated subdorsally, almost at level of anal opening; second pair laterally, a short distance posteriad from first pair.

Male: Not found.

REMARKS

The population from Guyane (Loc. 10) agrees with the original description of *X. clavicaudatum* in clavate tail terminus, c-value, c'-value, pseudomonodelphic reproductive system and V-value, absence of Z-differentiation and uterine spines, and rounded lip region, almost continuous with body outline. However, this population differs from the type population in a few characters: shorter body length (2.1-2.3 *vs* 2.96-4.42 mm), shorter tail (92.5-99.5 *vs* 124-248 µm), longer hyaline terminal part (63-72 *vs* 41-53 % of tail length), slightly shorter odontostyle (135-140 *vs* 140-186 µm), and degree of reduction of the anterior reproductive branch (part of oviduct still intact, ovary reduced to a small mass of cells *vs* absence of both ovary and slender part of oviduct, *pars dilatata oviductus* reduced to a mass of cells). However, there are more similarities than differences between the Guyane specimens and the type specimens and, at least for the moment, we decided to consider the two populations as conspecific. *X. clavicaudatum* seems to be native to South America: it was described from natural vegetation in Brazil (Huang *et al.*, 1987), collected again in the Brazilian Amazonia (Germani, 1989), and it is now found in Guyane, also on natural vegetation.

Xiphinema costaricense Lamberti & Tarjan, 1974

(Fig. 4)

MEASUREMENTS

Female: See Table 4.

DESCRIPTION

Female: Habitus weakly to moderately curved ventrally. Lip region 6-7 µm high, slightly offset from rest of body. All specimens were flattened to various degrees, this possibly affecting the lip region shape and width. Amphid apertures 7-9 µm wide, at 6-7 µm from anterior end, occupying about 52 % of the lip region width. Body pores conspicuous; distribution in neck region: twelve to thirteen laterally, nine to ten ventrally, three dorsally. Vestigium small, 1.5 µm long, difficult to see. Basal flanges 17.5-20.5 µm wide. Reproductive system with anterior genital branch reduced and incomplete: ovary absent, oviduct reduced to a mass of cells (23-29 µm long), sphincter, *pars dilatata uteri* (48-55 µm long), and uterus (63-78 µm long) recognisable but reduced. Posterior genital branch complete with ovary 73-83 µm long, convoluted oviduct 40-48 µm long, *pars dilatata oviductus* 37-43 µm long, sphincter and uterus with *pars dilatata uteri* 28-33 µm long, and uterus without Z-differentiation and with tubular part 138-175 µm long. Ovejector 48-60 µm long. In a few females, an accumulation of small rounded cells, reminiscent of sperm cells,

Table 4. *Morphometrics of* Xiphinema costaricense *(all measurements in µm except L in mm).*

Locality	Guyane 13	Guyane 17	
	Female	Female	J4
n	2	8	1
L	2.6, 2.8	2.4 ± 0.1 (2.2-2.8)	2.1
a	53.6, 53.1	48.3 ± 3.5 (41.3-52.6)	45
b	4.8, 6	5.3 ± 0.6 (4.6-6.3)	2.7
c	81.1, 88.3	77.9 ± 8.8 (63.2-88.7)	58.6
c'	0.7, 0.6	0.7 ± 0.1 (0.6-0.8)	0.74
V	47, 46	45.1 ± 0.9 (44-46.4)	–
Tail	31.5, 31.5	31 ± 3.8 (27-37)	35
Lip reg. diam.	18, 18.5	15.5 ± 1.1 (14-17)	15
Odontostyle	151, 147	144.6 ± 5.4 (140-153.5)	132
Repl. od.style	–	-	149
Odontophore	86.5, 95	86.8 ± 2 (85-90)	82
Stylet	237.5, 242	231.3 ± 7.2 (225.5-242)	214
Guid. ring	148, 146	136.8 ± 4.5 (133-147)	127
Nerve ring	241, 266	252.9 ± 7.5 (247-264.5)	227
Hemizonid	226,–	214.1 ± 5.1 (207-224)	–
h	11, 10.5	9.3 ± 0.8 (7.5-10)	10.5
h%	35, 33	30.4 ± 4.7 (20-34)	30

present at the distal end of the posterior genital tract where the gonad reflexes (this may point to hermaphroditism in this species, but this needs confirmation). Tail tapering to broadly rounded terminus with main curvature on the dorsal outline. In some severely flattened specimens, the tail appearing almost hemispherical. Blind terminal canal absent. Two pairs of caudal pores clearly seen on tail, posterior to anal opening; one pair located laterally near tail terminus and one pair located almost at level of anal opening in a sublateral position.

Male: Not found.

Juvenile. General appearance as in female. Genital primordium 52.5 × 32 µm

REMARKS

The Guyane population (Loc. 13 and 17) resembles the type population both morphologically and morphometrically, except for the vulva located more posteriorly than in the type population (44-47 *vs* 36.6-37.5 %). However, it is very close to a population from Ilha Carreiro, Brazilian Amazonia, where the V-value is given as 43.1-45.8 % for eleven females (Germani, 1989).

Xiphinema filicaudatum labratum Luc & Coomans, 1992

(Fig. 5)

MEASUREMENTS

Female and *males:* See Table 5.
Juveniles: See Table 6.

DESCRIPTION

Male (n = 1): Habitus J-shaped. Lip region continuous with body outline, without peri-oral ring. Amphid opening short, crescent-shaped, 4 µm long, 7.5 µm from anterior end. Body pores conspicuous; distribution in neck region: thirteen situated laterally, seven dorsally, and about fourteen ventrally. Vestigium small, 1.5 µm long. Spicules dorylaimoid, 71 µm long, border of spicular pouch prominent. Lateral guiding pieces 16.5 µm long, each with a distinct knob at distal end. One adanal pair and five supplements present, spaced 38-48 µm, the posterior one situated 3.8 anal body diameters from anus.

REMARKS

X. filicaudatum labratum was originally described from five females found in the rhizosphere of ferns and Marantaceae near Cayenne, Guyane (Luc & Coomans, 1992). This subspecies differs from *X. filicaudatum filicaudatum* Loof & Maas, 1972 mainly in presence of a peri-oral ring, different L and V values, and absence of males. In the present survey, a single male and several females and juveniles were found in four localities in Guyane (3, 7, 10, 16).

All females agree well morphologically and morphometrically with those of the type population. Juveniles are morphologically close to the females except for shorter bodies, shorter stylets, and under-developed gonads. The genital primordiums of the J1, J2, J3, and J4 are 28, 34, 33-39, and 48-58 µm long, respectively. Noteworthy is the fact that some juveniles, particularly some J1, J2 and J3 specimens have no or slightly developed peri-oral ring. The single male has no peri-oral ring and is on average longer than the females.

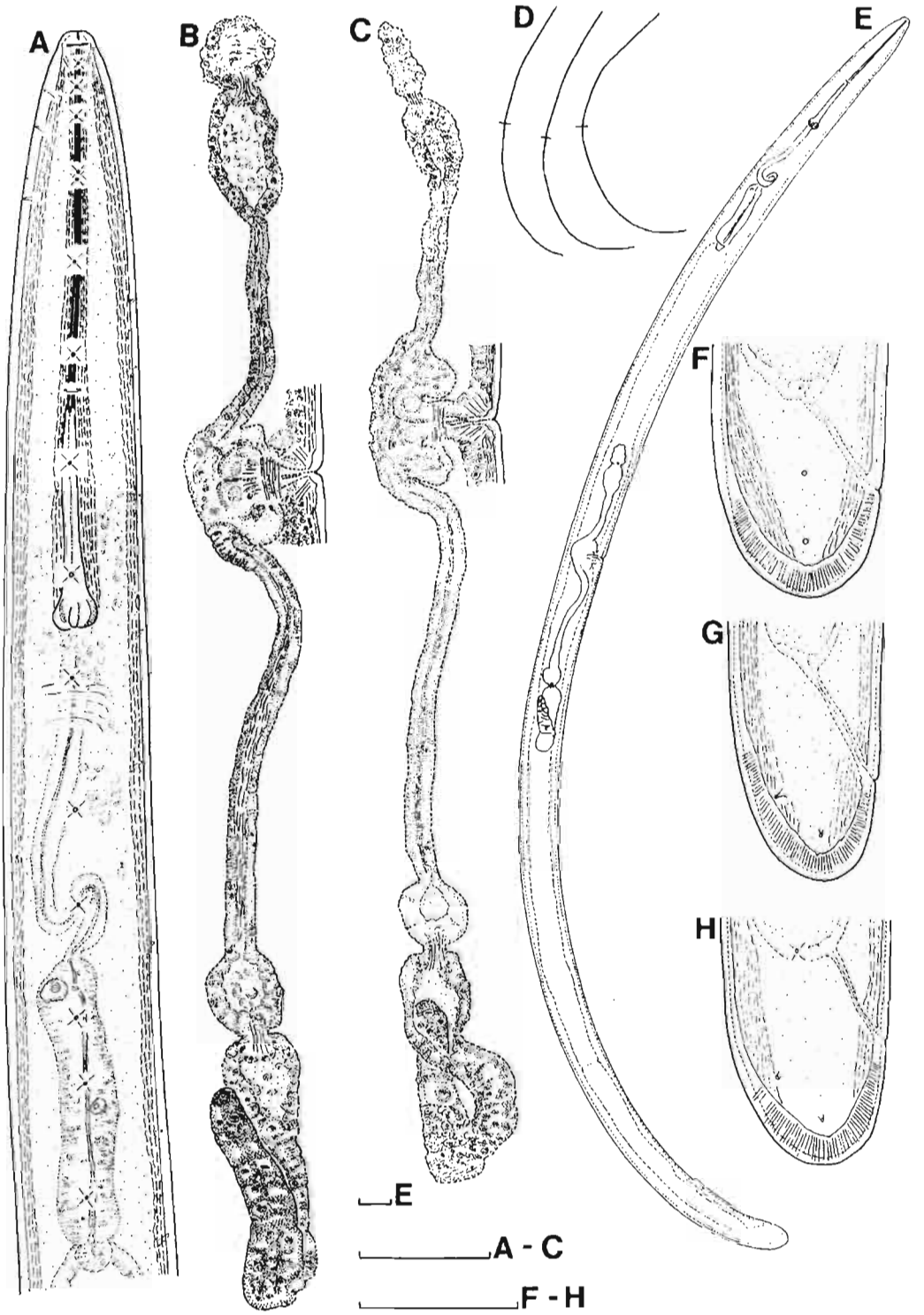

Fig. 4. Xiphinema costaricense *Lamberti & Tarjan, 1974. A: Anterior body region; B, C: Female reproductive system; D: Habitus; E: Entire female; F: Tail of severely flattened female; G: Tail of moderately flattened female; H: Tail of J4. (Scale bars = 50 µm.).*

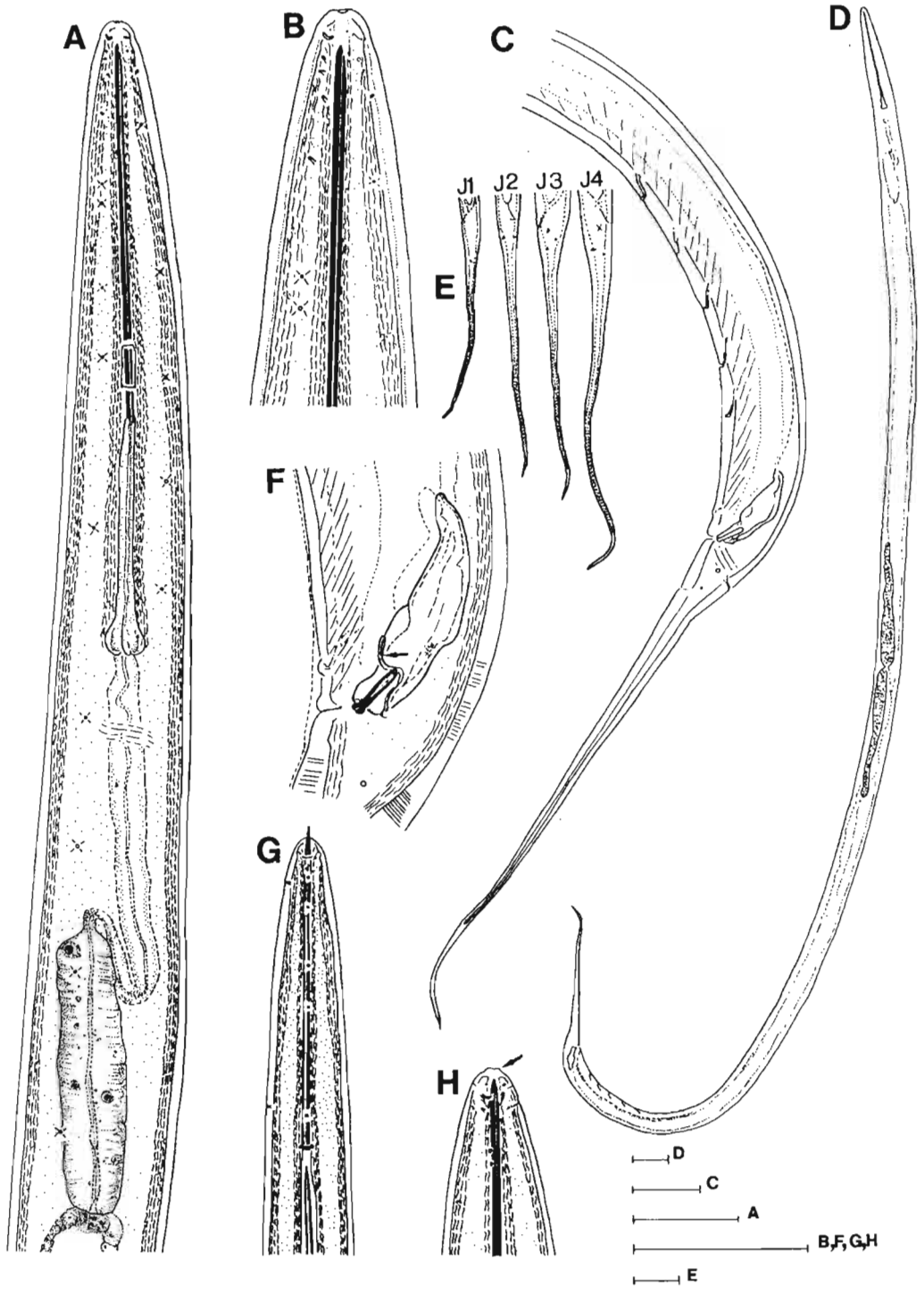

Fig. 5. Xiphinema filicaudatum labratum *Luc & Coomans, 1992. A: Anterior body region of male; B: Anterior region of male, sub-lateral view; C: Posterior region of male; D: Habitus of male; E: Tails of juvenile stages; F: Cloacal region showing border of spicular pouch (arrowhead); G: Anterior region of J1; H: Lip region of J4 showing well developed peri-oral ring (arrowhead). (Scale bars: D = 100 µm; others = 50 µm).*

Table 5. *Morphometrics of females and male of* Xiphinema filicaudatum labratum *(all measurements in μm except L in mm).*

Locality	Guyane 3	Guyane 7		Guyane 10	Guyane 16
	Female	Female	Male	Female	Female
n	1	5	1	5	2
L	3.8	3.7 ± 0.1 (3.5-3.8)	4.1	3.6 ± 0.2 (3.2-3.8)	3.5, 3.9
a	50.7	56.4 ± 3.8 (52.3-61.5)	55.7	47.4 ± 1.2 (45.8-48.9)	49.6, 49.7
b	6.2	6.4 ± 0.4 (5.9-6.8)	7.4	6.6 ± 0.5 (6.2-7.4)	6.4, 6.4
c	6.9	9.8 ± 1.2 (8.9-11.6)	9.8	8.3 ± 1.6 (6.4-10.4)	7.2, 7.9
c'	13.7	9.6 ± 1.4 (8.1-11.1)	9.7	11.9 ± 2.9 (9.3-16.2)	10.9, 14.3
V/T	43	41.9 ± 1.3 (40.9-44)	37	42.4 ± 2.3 (39.4-44.8)	40.3, 40
Tail	548	381.2 ± 36.9 (323-413)	413	444.2 ± 81.9 (348-543)	490, 500
Lip reg. diam.	16.5	15 ± 1.3 (14-17)	15	16.6 ± 0.7 (16-17.5)	16, 15.5
Odontostyle	194	189.6 ± 3.3 (186-195)	176	188.9 ± 4.1 (184.5-193.5)	179, 179.5
Odontophore	124	119.6 ± 4.3 (114.5-125)	111	118.8 ± 11.8 (101-130)	125, 125
Stylet	318	309.2 ± 6.7 (303-320)	287	307.7 ± 15.1 (288-323)	304, 304.5
Guid. ring	186	185.6 ± 1.9 (183-187.5)	172	186.3 ± 8.7 (175.5-199)	169, 174
Nerve ring	342	341.3 ± 3.4 (338-346)	316.5	338 ± 10.6 (320-348)	300, 338
Hemizonid	267	256.9 ± 16.2 (242-280)	234.5	264.1 ± 5.8 (258-270)	257, 261.5
h	22	45.5 ± 7.2 (35-55)	46	16.5 ± 3.2 (12.5-20)	41.5, 70
h%	10	12.3 ± 2.8 (9.4-16.9)	11.1	11.1 ± 3.8 (7.7-15.5)	8.5, 14

However, its small crescent-shaped amphid aperture and lip region continuous with body outline points to *X. filicaudatum labratum*. No sperm was observed in females. In body length this male is near *X. filicaudatum filicaudatum* but the peculiar guiding pieces of the male of *X. filicaudatum labratum* were not mentioned in males of *X. filicaudatum filicaudatum*. The odontostyle is also shorter (287 *vs* 308-321 μm).

*Xiphinema labiosum** n. sp.

(Figs 6, 7A-C)

MEASUREMENTS

Female: See Table 7.

* The species name *labiosum* refers to the prominent peri-oral ring.

DESCRIPTION

Female: Habitus weakly curved to nearly straight. Lip region with peri-oral ring, continuous with body outline. Peri-oral ring 7-7.5 μm wide, 2-4 μm high. Amphid aperture short, crescent-shaped, 3.5-4 μm wide, 9-10.5 μm from anterior end. Body pores inconspicuous; approximate distribution in neck region: sixteen laterally, eight dorsally, and ten ventrally. Vestigium 2-2.5 μm long. Basal flanges 18-22 μm wide. Cuticle 2.5-3.5 μm wide at mid-body, 11-13 μm at tail terminus. Reproductive system with anterior genital branch reduced and incomplete: ovary absent, oviduct reduced to a mass of cells (15-45 μm long), sphincter, *pars dilatata uteri* (65-113 μm long), and muscular uterus (50-93 μm long) reduced in length compared to that of posterior branch. Posterior geni-

Table 6. *Morphometrics of juveniles of* Xiphinema filicaudatum labratum *(all measurements in µm except L in mm).*

Locality	Guyane 3			Guyane 7				Guyane 10	Guyane 16
	J2	J3	J4	J1	J2	J3	J4	J4	J4
n	1	2	1	2	2	7	6	1	4
L	1.7	2, 2.4	2.7	1.5, 1.6	1.4, 1.8	2.3 ± 0.1 (2-2.4)	2.8 ± 0.3 (2.4-3.2)	2.9	2.8 ± 0.1 (2.7-3)
a	42.6	52.1, 42.1	46.8	51.9, 50.7	45, 48.9	49 ± 3.4 (43.4-54.2)	52.6 ± 11.4 (35.3-64.6)	52.3	51.4 ± 2 (49.5-53.8)
b	4.3	4.5, 4.9	4.5	4.5, 4.8	3.9,-	4.9 ± 0.4 (4.6-5.8)	5.1 ± 0.8 (4-5.8)	5.1	5.1 ± 0.4 (4.7-5.6)
c	5.9	7.5, 6.7	6.1	6.6, 6.2	5.3, 6.3	7.1 ± 0.8 (6.2-8.2)	7.7 ± 1.7 (7.8-9)	9.1	7.1 ± 0.2 (6.7-7.2)
c'	16	10.9, 12.6	12.8	11.2, 11.6	12.3, 12	10.6 ± 2.4 (7.4-14.3)	9.4 ± 0.8 (8.6-10.6)	8.4	11.3 ± 0.6 (10.7-11.9)
Tail	289	268, 352	448	224, 254.5	270, 285	329.5 ± 41.2 (295-388)	374 ± 65.7 (328-490)	316	398.3 ± 23 (368-418)
Lip reg. diam.	10.5	11.5, 10.5	14	9.5, 9	9.5, 9.5	11.5 ± 0.9 (10.5-13)	13.6 ± 0.4 (13-14)	16	14.5 ± 0.4 (14-15)
Odontostyle	101	124, 122	154	94.3, 99	96, 96	127.4 ± 6 (121-136)	165.6 ± 3.1 (161-168)	172	160.5 ± 2.7 (157.5-164)
Repl. od.style	125	146.5, 154	194	106, 115	118.5, 120	158 ± 5 (150.5-171)	188.8 ± 2.5 (187-193)	195	180.5 ± 2.1 (178-183)
Odontophore	67	80, 85.5	107	57.5, 55.5	67, 69	86.2 ± 3 (82-90)	106 ± 4.5 (102-113)	107	106.1 ± 4.6 (101.5-111)
Stylet	168	204, 207.5	261	151.8, 154.5	163, 165	213.5 ± 7.6 (204.5-245)	271.6 ± 6.5 (264-281)	279	266.6 ± 5.4 (259-271.5)
Guid. ring	94	115, 118	150	85.3, 88	91, 89	123.4 ± 6.1 (114.5-130)	161.3 ± 3.4 (157-164)	162	150.1 ± 6.4 (141.5-157)
Nerve ring	203	235, 229	327	188, 196	204, 196	243.1 ± 10.6 (225-252.5)	290.4 ±21.7 (260.5-312.5)	-	274.3 ± 21.2 (246-291)
Hemizonid	-	177, 190	222	141, 165	149, 152	203.8 ±12.6 (168- 223)	236.9 ± 9.4 (228.5-246)	242	224.8 ± 7.1 (217-231)
h	-	35, 37	44	27, 32	13, 35	35.6 ± 6.4 (29.5-44)	40.4 ± 10.3 (26-51.5)	15	63.8 ± 4.8 (59-70)
h%	-	13, 10.8	9.8	8, 20	4.8, 12.2	11 ± 2.2 (8.2-14)	13.4 ± 3.5 (8.6-16)	12	16.2 ± 1.6 (14.2-17.8)

tal branch complete with ovary 50-60 µm long, slender oviduct 55-75 µm long, *pars dilatata oviductus* 25-33 µm long, sphincter, *pars dilatata uteri* 55-63 µm long, and muscular uterus 137-188 µm long. Genital tract without Z-differentiation. Ovejector bean-shaped to rounded, 50-58 µm long. No sperm observed in any part of genital tract. Tail hemispherical with three pairs of caudal pores, one pair situated laterally and two pairs very near each other sublaterally. Blind terminal canal absent except in one specimen with a very faint terminal canal.

Male: Not found.

Juvenile (J4): General appearance as female except for tail, which is short, conical, and distinctly digitate with terminal peg and blind canal.

TYPE HOST AND LOCALITY

Loc. 16. Soil around the roots of *Dicorynia guianensis* Amshoff,Saül, Cascade Sauveur, Guyane (3°38' N, 53°12' W), collected by P. Quénéhervé and P. Topart in 1995.

TYPE SPECIMENS

Holotype female on slide 31784; three paratype females and one juvenile on slides 31782 and 31783 deposited in the National Collection of Nematodes at the Plant Protection Research Institute, Pretoria, South Africa.

DIAGNOSIS AND RELATIONSHIPS

X. labiosum n. sp. has the following diagnostic characters: peri-oral ring present, amphid apertures small

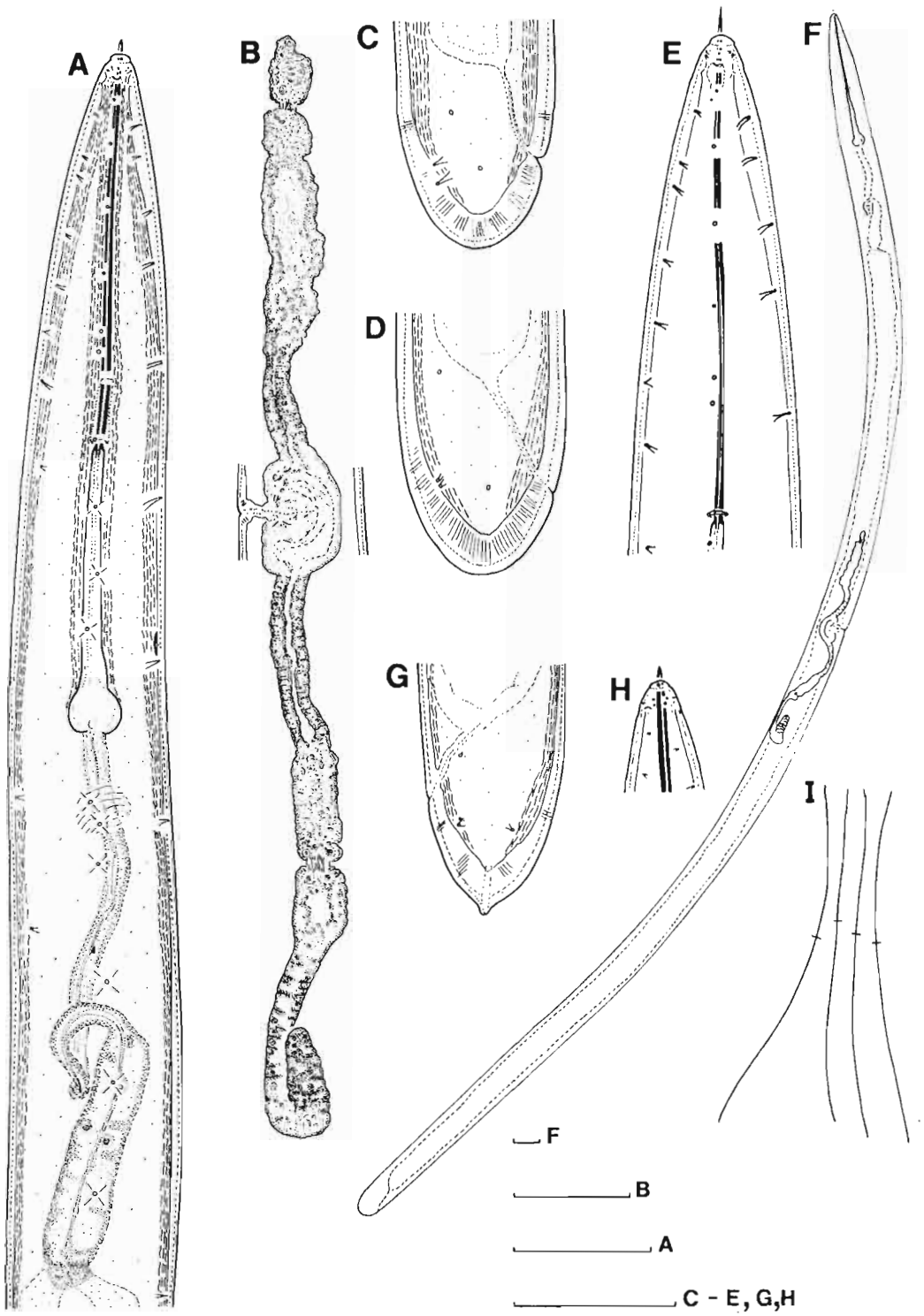

Fig. 6. Xiphinema labiosum *n. sp. A: Anterior body region; B: Reproductive system; C, D: Female tail; E: Anterior body region; F: Entire female; G: Tail of J4; H: Lip region of J4; I: Habitus. (Scale bars = 50 µm.)*

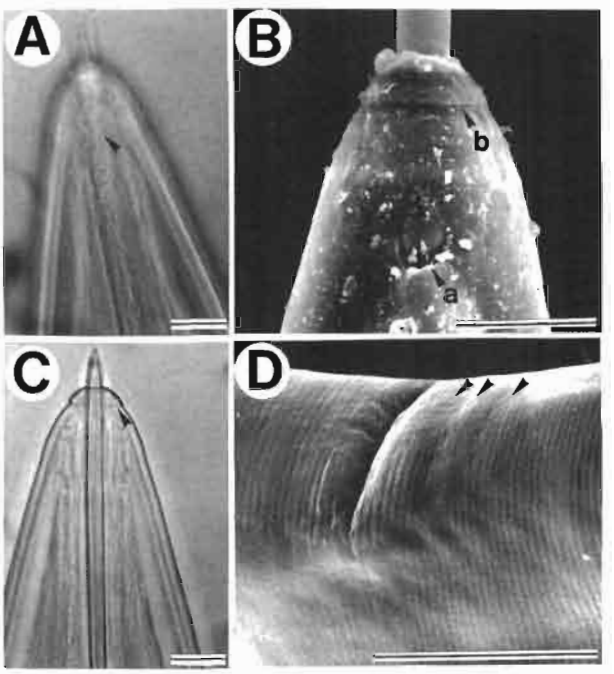

Fig. *7.* Xiphinema labiosum *n. sp. A: Lip region with light microscopy (LM), lateral view, showing small crescent-shaped amphid aperture (arrowhead); B: Lip region with scanning electron microscopy (SEM), lateral view, showing amphid aperture (arrowhead a) and peri-oral ring (arrowhead b); C: Lip region with LM, showing peri-oral ring (arrowhead) —* Xiphinema pseudokrugi *n. sp. D: Vulval region with SEM, showing slight cuticle ornamentation in the vicinity of the vulva (arrowheads). (Scale bars = 10 µm.)*

and crescent shaped, lip region almost continuous with body outline, vulva anteriorly situated, anterior branch of genital tract incomplete, and tail short, hemispherical.

X. labiosum n. sp. is morphometrically close to *X. surinamense* Loof & Maas, 1972 but can be distinguished from it by the following morphological characters: peri-oral ring (distinct *vs* absent), amphid aperture (small, crescent-shaped *vs* slit-like, occupying about one-half of corresponding body diameter), lip region shape (almost continuous with body outline *vs* separated by weak depression from rest of body), habitus (weakly curved to almost straight *vs* weakly curved to C-shaped), number of caudal papillae (three *vs* two pairs), and tail shape of J4 (short, conical, distinctly digitate *vs* hemispherical). In the presence of a peri-oral ring, small crescent-shaped amphid apertures, and lip region continuous to almost continuous with body outline, *X. labiosum* sp. n. resembles only *X filicaudatum labratum* Luc & Coomans, 1992. It is quite different from this species in tail shape (hemispherical *vs* filiform), shorter stylet (262.5-270.5 *vs* 294-314 µm), and shorter body (2.6-2.7 *vs* 3.27-3.76 mm).

Table *7. Morphometrics of* Xiphinema labiosum *n. sp. (Loc. Guyane 16) (all measurements in µm except L in mm).*

	Females		J4
	Holotype	Paratypes	
n	1	4	1
L	2.7	2.6 ± 0.1 (2.6-2.7)	2.5
a	66.1	63.1 ± 4 (56.9-66.9)	58.4
b	6.1	5.9 ± 0.1 (5.8-6.1)	5.5
c	109.2	98.8 ± 10.2 (85-109.2)	70.9
c'	0.6	0.6 ± 0.1 (0.6-0.7)	0.9
V	43	42.2 ± 1.6 (39.6-43.9)	–
Tail	25	27 ± 2.2 (25-30)	35
Lip reg. diam.	15	13.5 ± 1.8 (10.5-15)	12
Odontostyle	156.5	156 ± 2.2 (153-159)	132
Repl. od.	–	–	158
Odontophore	114	109.5 ± 3 (107-114)	101
Stylet	270.5	265.5 ± 3.1 (262.5-270.5)	233
Guid. ring	147	148.7 ± 3 (147-151.5)	132
Nerve ring	281	288.3 ± 4.7 (281-291.5)	–
Hemizonid	223	21701 ± 5 (212-223)	–
h	13	12.2 ± 0.8 (11-13)	14
h%	52	45.2 ± 4.3 (42-52)	40

X. labiosum is represented in the polytomous key of Loof and Luc (1990) by the following code: A2-B4-C7b(a); D6-E34-F3-G4-H1(2)-I12-J5a-K?-L?.

Xiphinema longicaudatum **Luc, 1961**
(Fig. 8)

MEASUREMENTS

Female: See Table 8.

Table 8. *Morphometrics of females of* Xiphinema longicaudatum *(Loc. Guyane 10) (all measurements in µm except L in mm).*

n	5
L	2.9 ± 0.1 (2.8-3)
a	65.3 ± 7.6 (59.8-74)
b	6 ± 0.4 (5.7-6.4)
c	10.2 ± 0.4 (9.7-10.7)
c'	8.2 ± 0.3 (7.9-8.5)
V	39.4 ± 0.3 (39-39.7)
Tail	284.8 ± 9.3 (275-295)
Lip reg. diam.	14.3 ± 0.5 (14-15)
Odontostyle	152.8 ± 2 (150-155.5)
Odontophore	93.7 ± 2.3 (90-96)
Stylet	246.5 ± 2.9 (243-249.5)
Guid. ring	137.2 ± 5.9 (130-146)
Nerve ring	229.5 ± 19.8 (210-263)
Hemizonid	188.7 ± 26.4 (167-218)
h	106 ± 3.9 (105-110)
h%	37.3 ± 1.7 (35-39)

DESCRIPTION:

Female: Habitus moderately ventrally curved, more curved in posterior third. Lip region rounded, slightly offset from rest of body. Amphid aperture 6.5-7 µm wide, curved, occupying 46-50 % of lip region width, situated about 6 µm from anterior end. Body pores conspicuous; distribution in neck region: ten to thirteen laterally, ten ventrally, three dorsally. Vestigium 3.5-5 µm long. Basal flanges 16-17.5 µm wide. Reproductive system with fully developed posterior branch and degenerate, much shorter anterior branch. Anterior branch with shorter tubular uterus, well-defined *pars dilatata uteri*, sphincter, oviduct and ovary represented by short mass of cells. Tail slightly ventrally curved with terminus bluntly pointed. Two caudal pores present.

Male: Not found.

REMARKS

X. longicaudatum has been described only from Africa, in Ivory Coast, Nigeria, and Cameroon (Luc, 1961; Luc & Hunt, 1978; Sakwe & Coomans, 1993, respectively). The specimens from Guyane (Loc. 10) agree well with the published descriptions of the species, except for longer tail (275-295 *vs* 166-241 µm) and shorter hyaline portion in relation to the tail length (35-39 *vs* 58-75 %). The amphid aperture is also slightly shorter compared to the lip region width (46-50 *vs* 55 % in *X. longicaudatum* from Cameroon) and the numbers of lateral and ventral pores in the neck region are different from those of specimens from Cameroon (ten to thirteen and ten *vs* 22 and seven). The only other species similar to the Guyane specimens is *X. filicaudatum filicaudatum* Loof & Maas, 1972. However, our specimens differ from that species in shape of lip region (slightly offset *vs* continuous), shorter stylet (243-249.5 *vs* 308-333 µm), shorter body (2.8-3 *vs* 4 6-4.70 mm), and shorter tail (275-295 *vs* 363-545 µm). In spite of the few minor differences with the African specimens, we consider the Guyane specimens as pertaining to *X. longicaudatum*.

Xiphinema oryzae Bos & Loof, 1985
(Fig. 9)

MEASUREMENTS

Female, male and *juvenile:* See Table 9.

DESCRIPTION

Female: Habitus weakly to moderately curved ventrally. Lip region 6-7 µm high, rounded, slightly offset from rest of body. Amphid aperture 8-9 µm wide, curved, occupying about 60 % of the lip region width, at 6-7 µm from anterior end. Body pores conspicuous; distribution in neck region: fourteen to nineteen laterally, eight to ten ventrally, three to five dorsally. Vestigium 3-4 µm long. Basal flanges 12-14 µm wide. Reproductive system with two fully developed branches, sperm usually present in uterus. Z-differentiation consisting of numerous large angular to globular structures, adjacent to the *pars dilatata uteri*. Tail filiform, curved ventrally, terminus bluntly pointed. Two caudal pores present on either side of the tail.

Male: General appearance as in female, with the following exceptions: Habitus ventrally curved into a J-shape. Spicules 58-59 µm long; gubernaculum 19 µm long. Supplements consisting of an adanal pair and two to four medioventral papillae. Three caudal pores present on either side of the tail.

Juveniles: General morphology as in females. Genital primordium in J2 and J4 23-27 and 36-53 µm long, respectively.

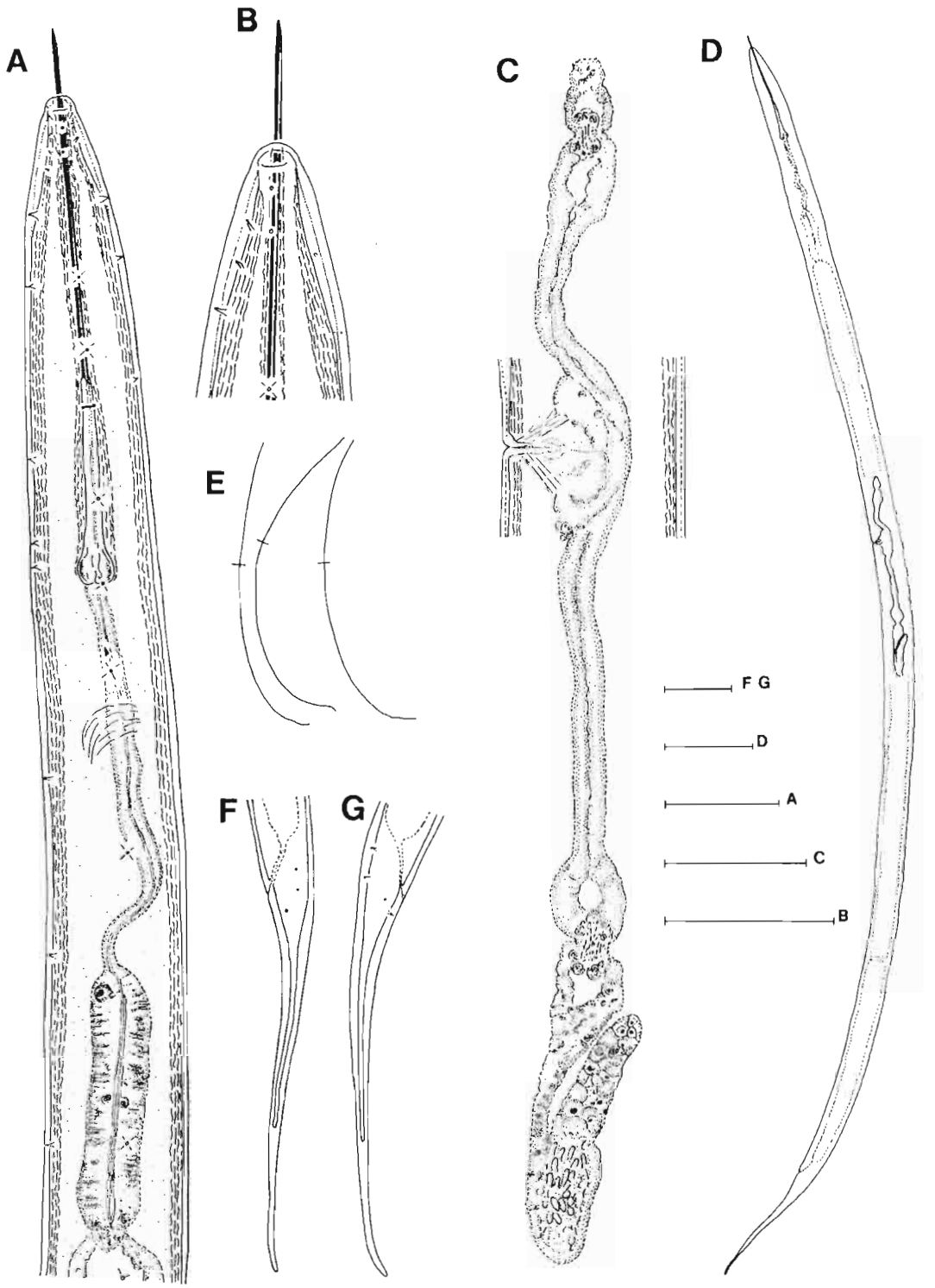

Fig. 8. Xiphinema longicaudatum *Luc, 1961. A: Anterior region; B: Anterior region, lateral view; C: Reproductive system; D: Habitus; E: Differences in habitus of three females; F, G: Tail region of two females. (Scale bars: D = 0.2 mm; others = 50 µm).*

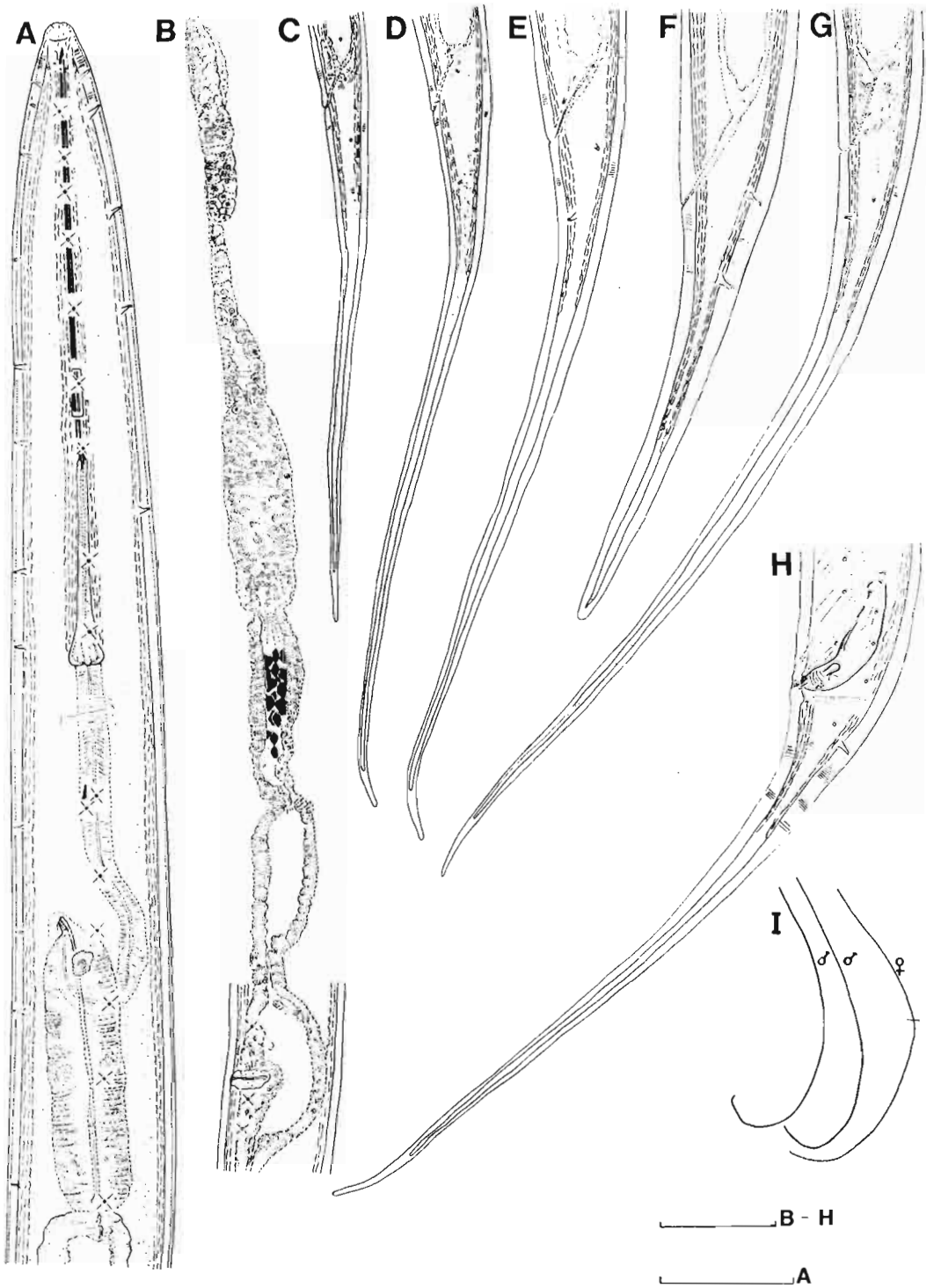

Fig. 9. Xiphinema oryzae *Bos & Loof, 1985. A: Anterior body region of male; B: Anterior genital branch; C: Tail of ♀2; D: Tail of ♀3; E: Tail of ♀4; F: Atypical female tail, presumably broken at some stage; G: Typical female tail; H: Male tail; I: Habitus. (Scale bars = 50 µm.)*

Table 9. *Morphometrics of* Xiphinema oryzae *Bos & Loof, 1985 (all measurements in µm except L in mm).*

	Guyane 3		Guyane 7			Guyane 11				
	Female	Male	Female	Male	J2	Female	Male	J2	J3	J4
n	1	1	2	1	1	1	1	2	3	5
L	–	3.4	3.7, 4.2	4	1.5	3.5	3.4	1.8, 2	2.3-2.4	2.9 ± 0.3 (2.6-3.2)
a	–	59.3	71.8, 72.4	82.4	48.2	45.9	53.3	52.4, 47.6	50.1-51.5	50.8 ± 0.7 (50.2-51.2)
b	–	8.9	7.8, 9.2	8.7	4.6	5.9	7.7	5.2, 6	5.3-6	7.3 ± 0.9 (6.6-8.5)
c	–	10.9	8.8, 10.5	11.3	12.4	8.1	10.9	7.1, 7.6	7.4-9	9.9 ± 1.8 (8.2-12.4)
c'	–	8	13.3, 12.6	9.7	6.3	10.5	7.3	13.2, 10.9	9.1-11.5	9.5 ± 0.7 (8.7-10.5)
V / T	–	–	41.8, 39.5	41	–	43	36	–	–	–
Tail	broken	312	415, 395	350	119	433	310	248, 260	253-303	299.5 ± 32.1 (261-335)
Lip reg. diam.	14.5	12.5	12.5, 14	14.5	-	13.5	13	9, 9.5	10-11.5	12
Odontostyle	166.5	154	152, 162.5	152	86.5	151.5	156	87, 88	106-111	129.1 ± 5.1 (123-135.5)
Repl. od.style	–	–	–	–	99	–	–	113, 119	128.5-135.5	150.5 ± 2.3 (147.5-153)
Odontophore	84	80	81.5, 84.5	81.5	51	75	78	52, 54	60-61	69.6 ± 1.8 (68-72)
Stylet	250.5	234	233.5, 247	233.5	137.5	226.5	234	139, 142	166-171	198.8 ± 4.8 (193-204)
Guid. ring	161	148	142, 158.5	147	82.5	138	150	78.5, 85.5	101-104	117.9 ± 5.1 (112-124.5)
Nerve ring	254	255	260,-	262	147	–	–	136.5, 146	177-198	203 ± 8.1 (193-211)
Hemizonid	222	–	223, 235	227	141	217	244	–, 140	169-179.5	198.1 ± 4.1 (193.5-201)
h	–	54	34, 42.5	37	23.5	46	36	22.5, 5	8.8-22.5	15.7 ± 8.7 (7.5-23.8)
h%	–	17	8, 10.8	10.6	19.7	10.6	11.6	9, 1.9	3.5-7.4	5.2 ± 2.9 (2.2-8.3)

REMARKS

These specimens are similar to the *X. oryzae* specimen described by Luc and Coomans (1992) from Guyane. However, the two males from the present populations are the first to be described from South America. As noted by Luc and Coomans (1992) in the specimen they described, the present females of *X. oryzae* differ from those of the type population by longer body, much longer tail, difference in Z-differentiation, and longer odontostyle. Compared to the single male in the type population, the present males have longer body (3.4-4.0 *vs* 2.79 mm), longer tail (310-350 *vs* 155 µm), and longer odontostyle (233.5-234 *vs* 180 µm). No ventromedian supplements were visible in the males of the present populations from Guyane.

*Xiphinema pseudokrugi** n. sp.

(Figs 10, 7D)

MEASUREMENTS

Female: See Table 10.

DESCRIPTION

Female: Habitus hook-shaped, curvature more pronounced in posterior one-third. Lip region 4.5-5 µm high, rounded, offset from rest of body by a weak depression. Body tapering anteriad; diameter at base of pharynx about four times width of lip region. Amphid aperture 6 µm wide, occupying about 60 % of lip region width. Cuticle 1.5-2.5 µm wide in mid-

* The species name *pseudokrugi* refers to the resemblance to *X. krugi*.

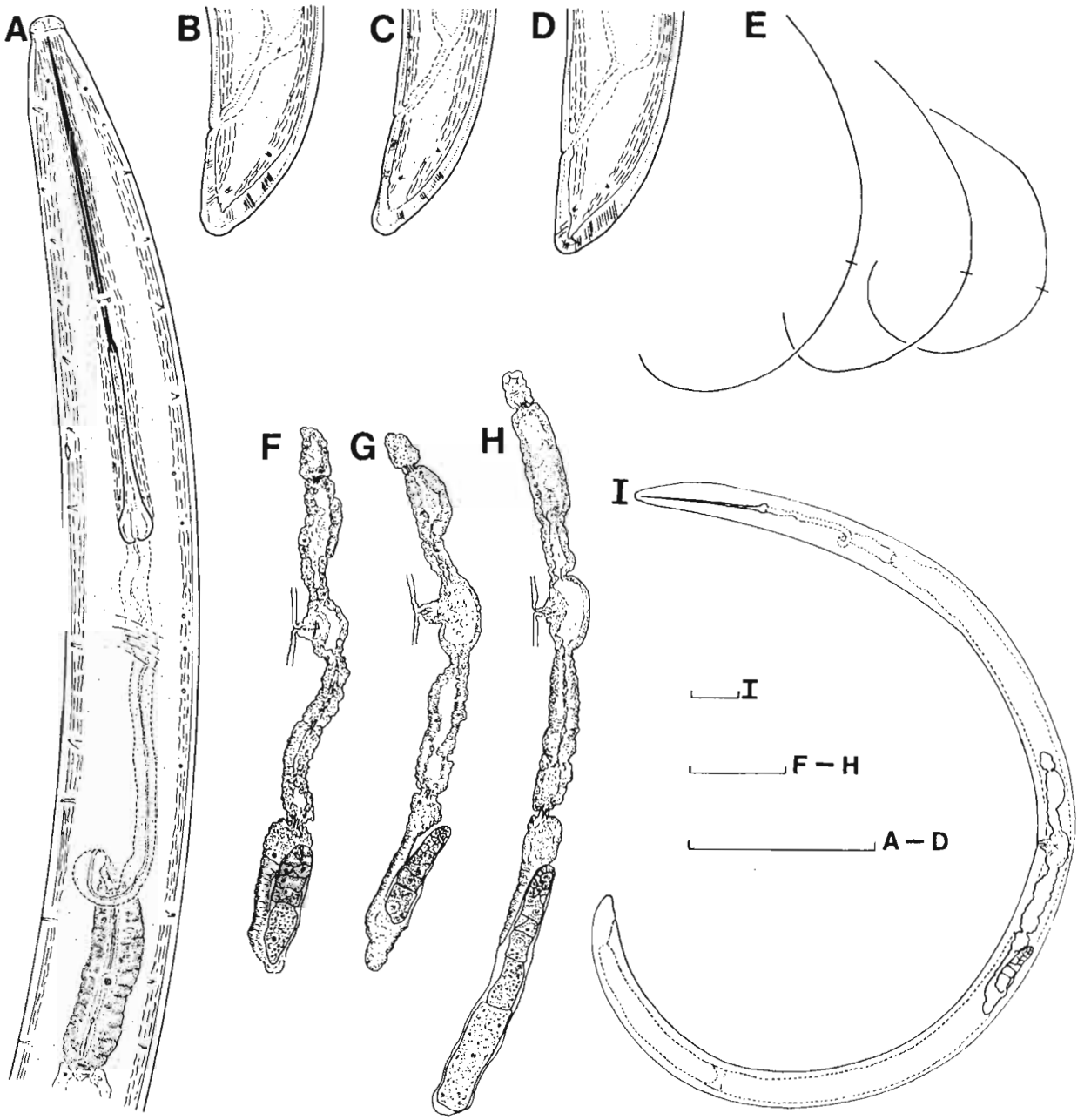

Fig. 10. Xiphinema pseudokrugi *n. sp. A: Anterior body region of holotype; B - D: Tail shape of three different females; E: Habitus; F - H: Reproductive systems of three different females; I: Entire holotype female. (Scale bars = 50 µm.)*

body, 3-4 µm near lip region, 4.5-6 µm on dorsal side of tail. Cuticle on tail and near lip region with faint radial striae. Body pores small, difficult to see; approximate distribution in neck region: fifteen laterally, thirteen ventrally, and three dorsally. Basal flanges 8.5-11 µm long. Pharynx 274-336 µm long, basal bulb 55.5-60 × 15-17 µm. Pharyngeal glands and opening obscure: DO located at about 10 % of basal bulb length, DN at about 12 %, SN_1 and SN_2 at about 50 %, and SO at 70 %. Reproductive system with fully developed posterior branch and reduced, much shorter anterior branch with ovary absent, ovi-

Table 10. *Morphometrics of females of* Xiphinema pseudokrugi *n. sp. (all measurements in µm except L in mm).*

	Guyane 10		Guyane 8
	Holotype	Paratype	
n	1	1	5
L	1.4	1.4	1.5 ± 0.1 (1.4-1.6)
a	33.8	33.7	46.7 ± 3.7 (42.1-51.8)
b	5	4.5	4.7 ± 0.3 (4.2-5)
c	52.8	50.2	47.1 ± 1.9 (44.5-49.3)
c'	1.1	1.1	1.5 ± 0.1 (1.4-1.7)
V	47.1	48.2	49 ± 0.9 (47.6-50.1)
Tail	26.5	27.5	30.5 ± 2.9 (26.5-33.5)
Lip reg. diam.	10	10	10 ± 0.3 (9.5-10.5)
Odontostyle	86	88.5	100.1 ± 3.3 (95-102.5)
Odontophore	55	53	55 ± 1.6 (53.5-57.5)
Stylet	141	141.5	155.1 ± 4.5 (148.5-159)
Guid. ring	78	82.5	86.2 ± 2.2 (83-89)
Nerve ring	145.5	161	166 ± 9.6 (155-178)
Hemizonid	140	142.5	148.4 ± 5.7 (138.5-153)
h	6	5.5	5.6 ± 0.9 (4.5-7)
h%	23	20	18.1 ± 3.7 (13.8-23)

duct reduced to a small mass of cells, and sphincter and uterus recognisable but reduced. *Pars dilatata uteri* larger in the anterior than in the posterior branch, with large cells in one specimen (Fig. 10H). Dimensions of the reproductive system as follows: anterior branch: tubular uterus 22-34 µm, *pars dilatata uteri* 34-62 µm, oviduct reduced to a mass of cells 22-28 µm, ovejector 28-39 µm; posterior branch: tubular uterus 45-67 µm, *pars dilatata uteri* 22-25 µm, *pars dilatata oviductus* 21-28 µm, oviduct 45-53 µm, ovary 70-146 µm. Vagina reaching halfway across body. Slight cuticular ornamentation occurring anterior and posterior of the vulva. In most specimens, prerectum obscure, constituting 14 % of body length. Tail convex-conoid with main curvature on dorsal outline, the ventral outline almost straight. Tail terminus varying from almost smoothly rounded to distinctly bulging. No terminal canal observed but outermost cuticular layer appearing disjointed at the tail extremity in most specimens. Two pairs of caudal pores observed; one pair near terminus in lateral position, one pair closer to anus, subdorsal in position.

Male: Not found.

Type host and localities

Loc.8: Rhizosphere of Marantaceae, Saut Brodel, Guyane (4°15' N, 52°40' W). Loc. 10: rhizosphere of *Dicorynia guianensis* Amshoff; Paracou, Guyane (5°20' N, 52°50' W). Collected by P. Quénéhervé and P. Topart in 1995.

Type specimens

Holotype female on slide 31795, six paratype females on slides 31794 and 31795 deposited in the National Collection of Nematodes, Plant Protection Research Institute, Pretoria, South Africa.

Diagnosis and relationships

X. pseudokrugi n. sp. is characterised by incomplete anterior genital branch with oviduct reduced to a single mass of cells and sphincter and uterus recognisable but reduced; Vulva situated almost at mid-body, slight cuticular ornamentation anterior and posterior of vulva, tail convex-conoid with smoothly rounded to bulging terminus.

X. pseudokrugi n. sp. is most closely related to *X. krugi* Lordello, 1955. However, it differs from that species in position of vulva (V = 47.1-50.1 *vs* 28.6-36), slight cuticular ornamentation around vulva (absent in *X. krugi*), somewhat shorter odontostyle (86-103 *vs* 94-129 µm), shorter odontophore (53-57.5 *vs* 60-84 µm), shorter stylet (141-159 *vs* 154-207 µm), and shorter body (1.38-1.58 *vs* 1.55-2.59 mm). The morphometrics of *X. krugi* are based on the description of Luc and Hunt (1978). *X. pseudokrugi* n. sp. is also morphologically near to *X. paulistanum* Carvallo, 1965 but can be distinguished mainly by the following characters: position of vulva (V = 47.1-50.1 *vs* 41.5), tail shape (convex-conoid with terminus from smoothly rounded to distinctly bulging *vs* conical with short, thick terminal bulge), shorter odontostyle (122 µm in *X. paulistanum*), shorter odontophore (60 µm in *X. paulistanum*), and shorter body (1.76 mm in *X. paulistanum*). By stylet length, reduced anterior branch of reproductive system, and tail morphology, *X. pseudokrugi* n. sp. also resembles *X. llanosum* Siddiqi & Lenné, 1990. *X. pseudokrugi* n. sp. differs from *X. llanosum* in position of vulva (V = 42-47 in *X. llanosum*), degree of reduction of anterior reproductive branch (uterus and sphincter recognisable but reduced *vs* uterus and sphincter normal, as long as posterior branch), shorter body (2.3-2.7 mm in *X. llanosum*), lower c-value (44.5-52.8 *vs* 86-114), and higher c'-value (1.1-1.7 *vs* 1-1.2).

X. pseudokrugi n. sp. belongs to Group 2 in the polytomous key of Loof and Luc (1990). Its code is: A2-B4-C6b-D45-E5(6)-F12-G12, H2-I3-J?-k?-L-?. It is close to *X. krugi*, but differs in E.

REMARKS

This is the first report of a *Xiphinema* sp. from South America with vulval ornamentation. The other five species with similar ornamentation are all described from South Africa (Swart, 1994) and are characterised by conical to long tails (c' = 2-7.8 in these species).

*Xiphinema seinhorsti** n. sp.

(Fig. 11)

MEASUREMENTS

Female and *juveniles:* See Table 11.

DESCRIPTION

Female: Habitus from weakly curved ventrad to J-shaped. Cuticle with two well-defined layers, thickened and striated towards both extremities. Cuticle thickness: 4.5-6 µm in neck region just posterior of lip region, 3.5-5 µm at mid-body, 11-13 µm on dorsal side of tail. Lateral chord 14-18 µm wide at mid-body. Body pores numerous and conspicuous; distribution in neck region: seventeen to 24 situated laterally, four to five situated dorsally, ten to fourteen ventrally. Lip region high, rounded when viewed dorso-ventrally; broadly rounded and set off from rest of body by a shallow depression when viewed laterally. Amphidial fovea stirrup-shaped; aperture occupying about 66 % of lip region width and situated at the level of the shallow depression. Odontostyle and odontophore well-developed; flanges well-defined, 12-15 µm wide. Vestigium 1.5-4 µm long, situated in anterior region of slender part of pharynx. Hemizonid well-defined, from slightly anterior to just posterior of flanges. Nerve ring surrounding anterior part of slender pharynx at a short distance behind the flanges. Basal bulb 97-126 × 23-30 µm. Ventrosublateral pharyngeal gland nuclei located at about mid-bulb, both on the same level or one slightly posterior to the other. Position of gland nuclei and gland outlets (n = 8): DO = 4-6 %, DN = 4-10 %, SO = ±48 %, SN_1 and SN_2 = 45-57 %. Ventrosublateral gland outlets obscure in most specimens. Cardia conical, sometimes flattened, surrounded by intestine. Prerectum difficult to measure in most specimens, 215-334 µm long (6-9 % of body length) in six specimens. Rectum well-defined, 30-50 µm or 0.8-1.2 times anal body diameters long. Tail short, convex-conoid with greater curvature dorsally, with exceptionally long finger-like projection or peg, clearly offset from rest of tail and situated to ventral side of tail. Blind canal present at tail terminus (some specimens with shorter pegs, perhaps due to injury). Two pairs of caudal pores present: one pair situated in a subventral position, a short distance posterior of anus; second pair in subdorsal or sublateral position, slightly posterior of subventral pair (this pair absent or obscure in some specimens). Female reproductive system amphidelphic with the two genital branches equally developed. Each branch consisting of reflexed ovary (50-92 µm), slender part of oviduct (55-95 µm), *pars dilatata oviductus* (22-36 µm), sphincter, large *pars dilatata uteri* (54-69 µm), long, tubular, sometimes convoluted uterus (114-182 µm) containing a weakly developed Z-differentiation, well demarcated ovejector (60-80 µm), and vagina (29-34 µm) stretching about halfway across the body width. Z-differentiation consisting of three to four small granular bodies (2-5 × 1-3 µm), situated 25-49 µm from the *pars dilatata uteri*. In some specimens, the Z-differentiation is obscure and appears to consist of a few small granules. As the uterine wall enclosing the Z-differentiation is not particularly muscular or thickened, the differentiation represents a pseudo Z-organ (according to Loof & Luc, 1990). Vulva a transverse slit. Dimensions of one intra-uterine egg: 95 × 22 µm; shell 1-3.5 µm thick. No sperm found within reproductive system.

Male: Not found.

Juveniles: One third stage (J3) and one fourth stage (J4) juvenile found. The J3 has a fully developed replacement odontostyle, surrounded by a protective sheath. The developing gonad is a flattened structure (56 × 7.7 µm) filled with several large cells with prominent nuclei. Also, the habitus is almost straight whereas the J4 has C-shaped body, which suggests that the J3 is moulting (see Samsoen & Barbez, 1982). The developing gonad of the J4 is a compact, roundish mass of cells (44 × 27 µm) and the length of the replacement odontostyle corresponds well with that of the odontostyle of the adults. In both juveniles, the tail differs from that of adult females by being longer, elongate, and without an offset peg. The rest of morphology resembles that of adult females, except for a shorter body.

TYPE HOST AND LOCALITIES

Loc. 10: Soil around roots of *Dicorynia guianense* Amshoff, Paracou, Guyane (5°20' N, 52°50' W). Collected by P. Quénehérvé in 1994.

Other localities: Loc. 4, 5, and 7 (see above).

TYPE SPECIMENS

Holotype and three paratype females on slide 30844 and six paratype females and one juvenile on slides 30845 and 30846 deposited in the National Collection of Nematodes at the Plant Protection Research Institute,

* The species name commemorates the late Dr J.W. Seinhorst.

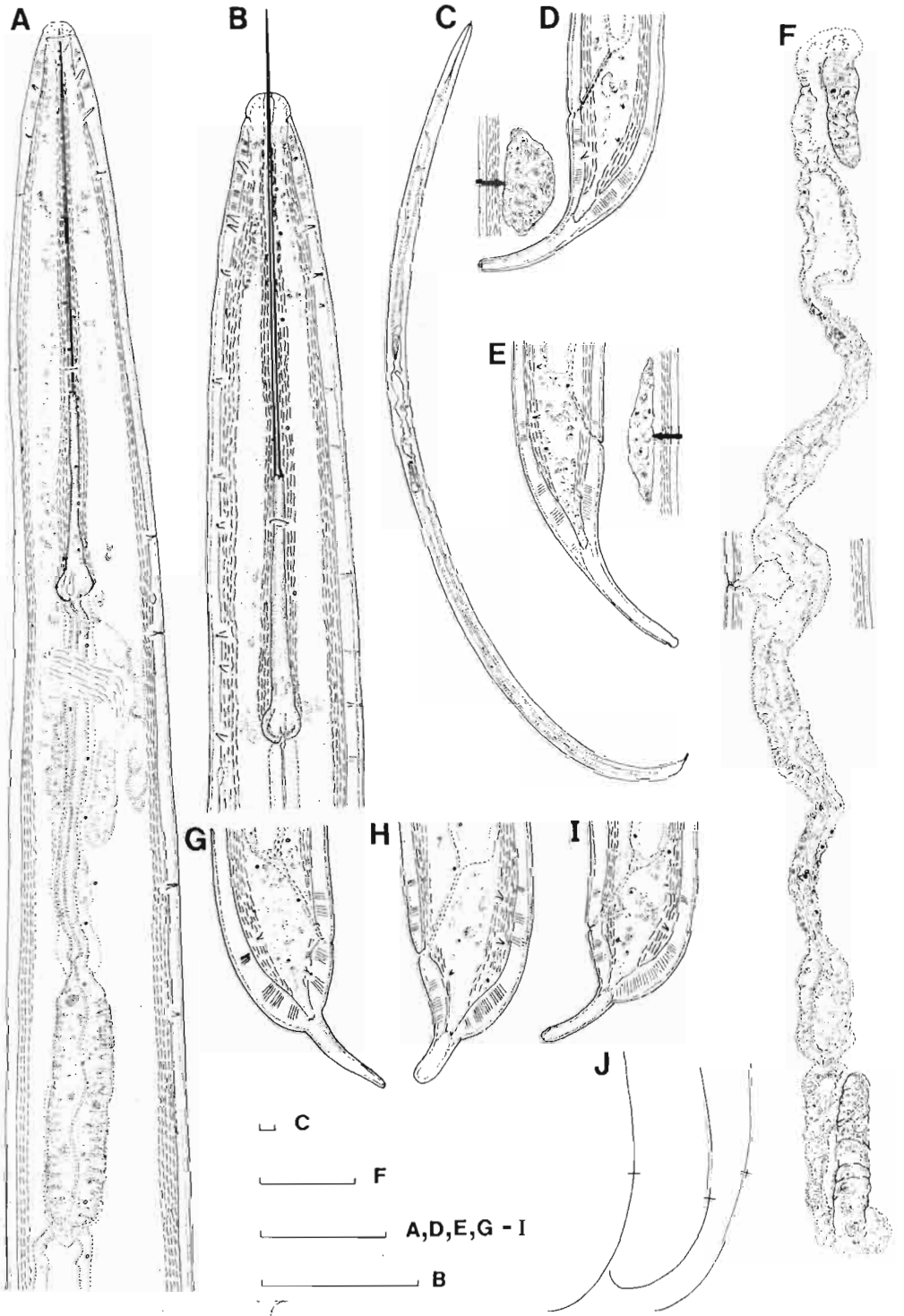

Fig. 11. Xiphinema seinhorsti *n. sp. A: Anterior body region, lateral view; B: Anterior region, dorso-ventral view; C: Entire female; D: ♂4, tail region and developing gonad (arrowhead); E: ♂3, tail region and developing gonad (arrowhead); F: Female reproductive system; G - I: Female tail regions; J: Habitus. (Scale bars = 50 µm.)*

Table 11. *Morphometrics of* Xiphinema seinhorsti *n. sp. (all measurements in µm except L in mm).*

	Guyane 4				Guyane 5	Guyane 7	Guyane 10
	Holotype female	Paratype female	J3	J4	Female	Female	Female
n	1	12			8	12	3
L	3.3	3.5 ± 0.3 (3.1-4.3)	2.9	2.8	3.5 ± 0.3 (3.1-4)	3.3 ± 0.2 (2.9-3.6)	3.1-3.3
a	51.4	68.1 ± 6.9 (57.5-82.1)	67	64.2	63.8 ± 9.5 (49-73.2)	67.5 ± 3.8 (62.6-74)	56-57.9
b	7.7	7.7 ± 0.9 (6.2-9.2)	9.9	6.4	7.9 ± 1 (7.1-10.4)	7.3 ± 0.4 (6.9-8.3)	6.7-7.5
c	57.2	63.7 ± 8.7 (46.6-76.4)	29.3	30.8	64.2 ± 10.1 (50.8-78.5)	67.9 ± 6.8 (58.3-77.3)	63.3-67.1
c'	1.4	1.4 ± 0.2 (1.2-1.6)	2.9	2.5	1.4 ± 0.2 (1.2-1.6)	1.2 ± 0.1 (1-1.4)	1.2
V / T	39.9	39.4 ± 1.9 (36.4-43.6)	-	-	40 ± 1 (39-41.5)	41.4 ± 1.1 (39.5-42.8)	36.9-41.5
Tail	58.5	56.2 ± 5.8 (49-67)	100	89.5	54.8 ± 6.3 (48-62)	47.9 ± 2.9 (42-51)	49-49.5
Lip reg. diam.	12.5	13.7 ± 0.7 (12.5-15)	12	12.5	13.5 ± 0.6 (13-14.5)	13 ± 0.7 (12-14)	13-15
Odontostyle	175.5	156.7 ± 7 (147.5-175.5)	137	135	150.2 ± 4.1 (145-156.5)	144.9 ± 5.9 (138 0-157.5)	150-155.5
Repl. od.style	-	-	147	161			
Odontophore	85	85.2 ± 2.7 (81.5-90)	78.5	76	83.3 ± 2.3 (80.5-87.5)	79.2 ± 1.5 (76.5-81.5)	84-88
Stylet	260.5	241.9 ± 8.2 (230-260.5)	215.5	211	233.5 ± 5 (228.5-243.5)	224.1 ± 6 (217-236.5)	234-240.5
Guid. ring	175	148.9 ± 10.1 (136.5-175)	96	124	144.5 ± 4.3 (137.5-149.5)	139.2 ± 9 (121-149)	146.5-161
Nerve ring	264	259.4 ± 10.8 (236.5-276)	152	225	255.4 ± 7.8 (244-265)	244.1 ± 21.1 (204-273)	238-241
Hemizonid	231	230.3 ± 8.3 (214-243)	143	203	223.8 ± 6.6 (217.5-233)	207.9 ± 14.5 (173-221)	200-219.5
h	29	32 ± 5 (22-40)	54.5	44	32.1 ± 5.3 (25-40)	24.6 ± 3.1 (20.5-29)	30-32
h%	50	56.9 ± 7.3 (44.4-61.8)	54.5	49	58.4 ± 4.3 (50-64.5)	51.4 ± 5.6 (40.2-56.9)	61.2-65.3

Pretoria, South Africa. Three paratype females and one juvenile deposited in the collection of the Muséum National d'Histoire Naturelle, Laboratoire de Biologie Parasitaire, Protistologie, Helminthologie, Paris, France.

DIAGNOSIS AND RELATIONSHIPS

X. seinhorsti n. sp. is characterised by a long caudal, offset, fingerlike projection located on the ventral side of tail, presence of a blind canal at the tail terminus, long odontostyle, long odontophore, and pseudo-Z-organ consisting of a small number of small granular bodies in the uterus.

X. seinhorsti n. sp. is closely related to four *Xiphinema* species: *X. imambaksi* Loof & Maas, 1972, *X. mammatum* Siddiqi, 1979, *X. stockeri* Kruger & Heyns, 1985, and *X. manubriatum* Luc, 1975. The new species is closest to *X. imambaksi*, especially in the appearance of the peg, but it can be distinguished by the following characters: longer body (2.9-4.302 *vs* 2.5-3 2 mm), longer odontostyle (138-175.5 *vs* 119-136 µm), longer odontophore (76.5-90 *vs* 64-70 µm), longer peg (22-40 *vs* 18-20 µm), smaller number of caudal pores (two *vs* three pairs), Z-differentiation different (few small granular bodies *vs* distinct sclerotized apophysis), and vulva slightly more anterior (V = 36.4-43.6 *vs* 40-45). *X. seinhorsti* n. sp. differs from the female of *X. mammatum* mainly in longer odontostyle (110 µm in *X. mammatum*), longer odontophore (73 µm in *X. mammatum*), different kind of Z-differentiation (stellate uterine spines in *X. mammatum*),

longer peg, located on ventral side of tail (13 µm long peg, situated on central axis in *X. mammatum*), and J4 with longer tail (89.5-100 *vs* 49 µm). *X. seinhorsti* n. sp. can be distinguished from *X. stockeri* by longer odontostyle (98.3-105 µm in *X. stockeri*), longer odontophore (65-75 µm in *X. stockeri*), difference in position and length of peg (on central axis of body, 11-12.5 µm long in *X. stockeri*), longer tail (40-50 µm in *X. stockeri*), and difference in Z-differentiation (15-28 irregular granular bodies in *X. stockeri*). *X. seinhorsti* n. sp. differs from *X. manubriatum* mainly in longer body (1.77-2.23 mm in *X. manubriatum*), vulva more anterior (51.3-56.3 % in *X. manubriatum*), different kind of Z-differentiation (Z-organ present in *X. manubriatum*), and longer odontostyle and odontophore (132-144 and 61-70 µm, respectively, in *X. manubriatum*).

In the polytomous key of Loof and Luc (1990), *X. seinhorsti* n. sp. is represented by the following code: A4-B2-C5A-D(4)5-E34-F34-G34-H2-I23-J2-K?-L1. It is close to *X. imambaksi* but differs in c-value.

REMARKS

One Guyane specimen (Loc. 7) has smaller body (2.9 mm), odontostyle (138 µm), and odontophore (76.5 µm). Its lip region, tail, and reproductive system morphology agrees well with the description of the new species, which warrants its inclusion in this species.

Acknowledgements

Thanks are due to Prof. A. Coomans (Rijksuniversiteit Gent, Belgium) and Dr J. Heyns (Rand Afrikaans University, South Africa) for useful discussions, Mr P. Topart (ORSTOM) and Mr S. Marie-Luce (INRA) for technical assistance, Mr H. Van Tonder (Plant Protection Research Institute, Pretoria, South Africa) for the SEM work, and Mr S.P. Swart for typing the manuscript.

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Longidorus seinhorsti sp.n. (Nematoda: Dorylaimoidea) from The Netherlands

Vlada PENEVA*, Pieter A.A. LOOF** and Derek J.F. BROWN***

**Central Laboratory of General Ecology, 2 Gagarin Street, 1113 Sofia, Bulgaria,*
***Department of Nematology, Wageningen Agricultural University, The Netherlands, and*
****Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK.*

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Summary – A description is provided of *Longidorus seinhorsti* sp.n., a bisexual species associated with grass growing in coarse alluvial sand in The Netherlands. The species is characterised by its medium body length (4.6-6.2 mm), slightly expanded and anteriorly flattened head region, symmetrically bilobed amphidial pouches, odontostyle 110-128 µm long, and elongate, conoid tail (36-46 µm). Males have short spicules (50-57 µm) and a row of fourteen to sixteen supplements. © Orstom/Elsevier, Paris

Résumé – Longidorus seinhorsti *sp. n. (Nematoda: Dorylaimoidea) provenant des Pays-Bas* – Description est donnée de *Longidorus seinhorsti* sp. n., espèce bisexuée associée aux graminées poussant sur un sol alluvial grossier des Pays Bas. Cette espèce est caractérisée par: corps de longueur moyenne (4,6-6,2 mm), région antérieure en relief et légèrement aplatie frontalement, poches amphidiennes à bilobation symétrique, odontostyle long de 100-128 µm, queue allongée-conoïde (36-46 µm). Les mâles présentent des spicules courts (50-57 µm) et une rangée de quatorze à seize suppléments. © Orstom/Elsevier, Paris

Keywords: Grass, *Longidorus*, nematode, The Netherlands.

As part of a study of morphological variability present in *Longidorus vineacola* Sturhan & Weischer, 1964 (see Brown *et al.*, 1997) the late Dr J.W. Seinhorst provided one of us (DJFB) with specimens from several populations of *Longidorus* occurring in The Netherlands, which had been tentatively identified as representing *L. vineacola*. Amongst this material were several slides containing specimens from a population from Vortum-Mullem, which Dr Seinhorst had identified as being similar to, but not identical with, *L. vineacola*. Dr Seinhorst also provided some preliminary drawings and measurements (Fig. 1) of these nematodes which suggested that the specimens were not *L. vineacola* but represented an undescribed species. Also, on one slide he indicated a female specimen as the potential holotype. Brown *et al.* (1997) referred to this species as *L. pseudoelongatus* Altherr, 1976 but subsequently, whilst preparing a redescription of that species (unpubl.) one of us (P.A.A.L.) confirmed that the specimens from Vortum-Mullem represented an undescribed species. Here we provide a description of this species.

Specimens were provided mounted in glycerine on permanent aluminium double slides by the late Dr J.W. Seinhorst.

Corresponding author: D.J.F. Brown.

*Longidorus seinhorsti** sp.n.

(Figs 1, 2)

MEASUREMENTS

See Table 1.

DESCRIPTION

Female. Body very slender, C- or G-shaped when heat relaxed. Head region slightly expanded, anteriorly flat, rounded laterally, set-off by a shallow depression. Labial papillae prominent, nerve canals very thick. Cuticle with two layers of equal thickness and with fine transverse striae; cuticle along the body about 2.5 µm thick, 3 µm at postlabial region and 6 µm on tail. Hypodermal chord 16 (14-18) µm wide (n=10). Numerous lateral body pores, five to seven in the odontostyle region, six dorsal and eight ventral cervical pores. Amphidial pouches large, extending more than half the distance between anterior end and guide ring, symmetrically bilobed. Nerve ring single. Muscular bulb plump, oesophago-intestinal valve elongate, bluntly conoid. Location of oesophageal gland nuclei typical for genus. Nuclei of dorsal and subventral glands situated at 35 (29-39) (n=7) and 52 (48-56) % (n=11); opening of the dorsal gland (DGO) at 11-13% (n=4) and opening of the subventral glands (SVO) 86-92% (n=4) of the distance from

* Named in memoriam of the late Dr J.W. Seinhorst.

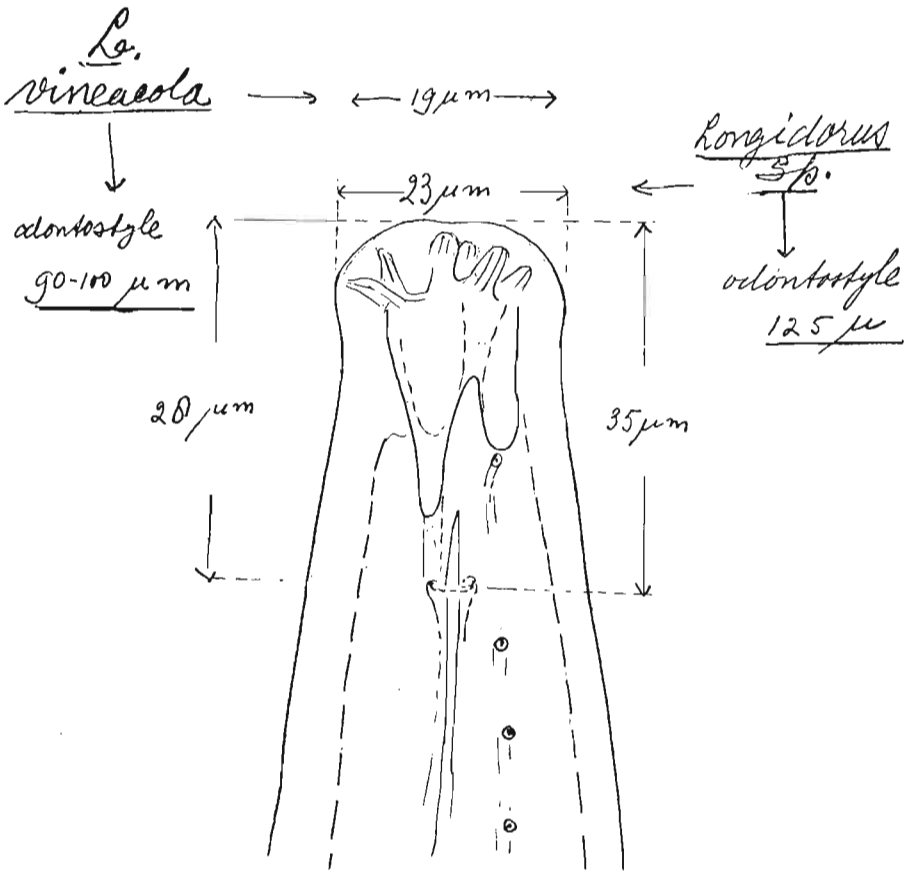

Fig. 1. *The original figure drawn by the late Dr J.W. Seinhorst showing a stylised representation of the anterior end of a* Longidorus *nematode in which the differences between* Longidorus vineacola *and* L. seinhorsti *sp. n. are displayed.*

anterior end of oesophageal bulb, respectively. Dorsal gland nuclei 1.5 µm diam. and subventral glands nuclei 3 µm diam. Vulva a transverse slit, vagina extending to *ca* half a corresponding body diameter, or slightly less; *pars distalis vaginae* and moderately thick walled *pars proximalis vaginae* 13-15 µm and 8-11 µm long, respectively. Uteri 180-289 µm long, thick walled, with conspicuous lumen, filled with sperm cells in fertile females; well developed sphincter between uterus and *pars dilatata oviductus*. *Pars dilatata oviductus* 75-134 µm long, containing sperm cells in fertile females, slender part of oviduct 92-154 µm long; caecum 43-70 µm long and ovarium 87-193 µm long. Prerectum 526 (396-633) µm long (n=5), rectum about 0.8 (0.7-0.9) of body diameter at anus. Tail elongate conoid, slightly ventrally curved, terminus broadly rounded; two pairs of lateral pores present.

Male: Body C-shaped, more strongly curved at posterior end. Two pairs of adanal supplements followed by a row of twelve to fourteen. Postcloacal papilla well developed. Tail bluntly conoid with a rounded terminus.

Juveniles: Only four specimens available. On the basis of lengths of body, odontostyle, replacement odontostyle and developing gonad, and the position of the replacement odontostyle in relation to the odontophore, they can be assigned to three stages (J2, J3, and J4). In J2 the head region is less expanded than in the subsequent stages and adults.

Diagnosis and relationships

Longidorus seinhorsti sp.n. is a bisexual species characterised by its medium body length (4.6-6.2 mm), slightly expanded and anteriorly flattened head region, symmetrically bilobed amphidial pouches, odontostyle 113-128 µm long and elongate conoid tail (41-46 µm). Male with short spicules (50-57 µm) and two pairs of adanal supplements followed by a row of twelve to fourteen supplements.

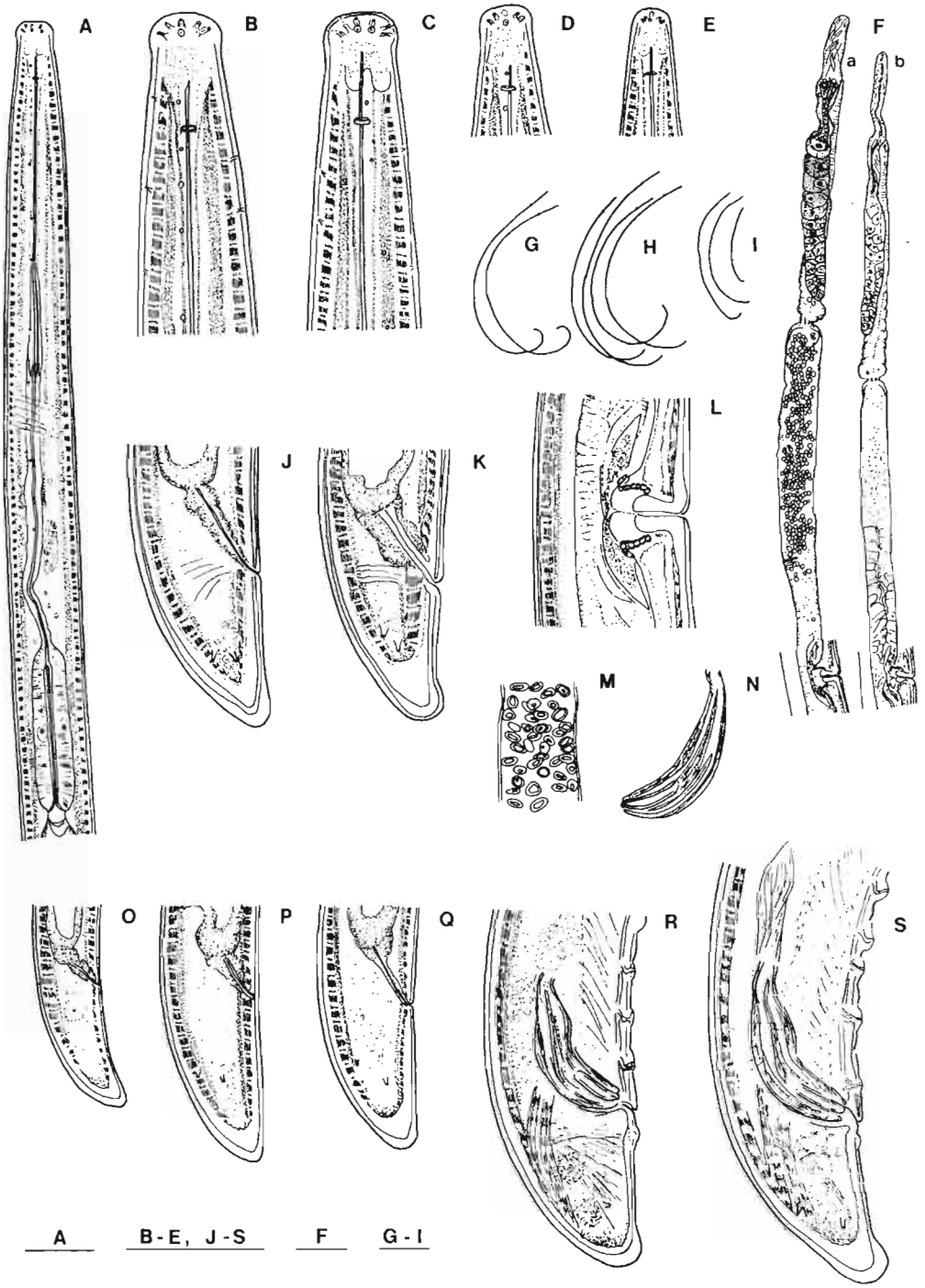

Fig. 2. Longidorus seinhorsti *sp.n. A: Female oesophageal region; B: Female head region; C: Male head region; D: Third stage juvenile head region; E: Second stage juvenile head end; F: Anterior genital branches of, a) fertile, and b) virgin females; G-I: Habitus of males, females and juveniles fourth, third and second stage, respectively; J, K: Female tail ends; L: Vaginal region; M: Sperm cells; N: Spicule; O-Q: Juvenile tail ends, second, third and fourth stage, respectively; R, S: Male tail ends (Scale bars: A, B - E, J - S = 40 µm; F = 20 µm; G -I = 1 mm).*

Table 1. *Measurements of* Longidorus seinhorsti *sp.n. (All measurements in µm, except L in mm).*

	Holotype	Females	Males	J2	J3	J4
n		13	4	1	2	1
L	5.1	5.5±0.35 (4.6-6.2)	5.3-5.6	1.7	2.6,3.0	3.4
a	118	122±7.8 (107-133)	108-138	69	89,91	113
b	11.7	13.1±0.9 (11.7-15.3)	11.3,12.3*	5.7	7.1,8.5	8.9
c	113	129±7.8 (113-140)	121-146	50	58,78	85
c'	1.3	1.3±0.11 (1.0-1.4)	1.1-1.3	1.8	1.6,1.8	1.4
V/T	53	49±2.0 (47-53)	32, 33*	-	-	-
Odontostyle	125	121±4.7 (113-128)	110-124	76	93, -	105
Odontophore	61	65±3.8 (55-69)	59-70	42	54, -	54
Repl. od. style	-	-	-	83	101,103	120
Bulb length	88	91±5.5 (84-102)	79-92**	62	66,73	77
Bulb diam.	21	22 (21-29)	19-22**	13	17,18	18
Tail	45	42±3.1 (41-46)	42-47	33	44,45	40
h	17	15 (9-19)	6-12**	8	10,12	10
Ant. end to guide ring	32	32±0.8 (30-33)	31-32	18	23,24	25
Neck length	435	418±27.5 (375-457)	443-476**	291	354, 364	384
Ant. end to nerve ring	192	200±9.1 (192-218)	203-208**	133	170, 168	174
Diam. at lip region	21	21 (21-22)	21-22**	11	14,15	15
Diam. at mid-body	43	45±3.1 (40-49)	39-49	24	29,33	30
Diam. at anus	36	33±1.4 (31-36)	33-38	18	25,28	28
G1/T1	8	9 (7-11)	8-11**	-	-	-
G2/T2	7	9 (6-12)	7-9**	-	-	-
Spicules	-	-	50-57	-	-	-

* n = 2; ** n = 3.

The code for identifying the new species when using the identification key of Chen *et al.* (1997) is: A-45, B-4, C-3, D-2, E-2, F-23, G-23, H-4, I-2.

Based on the codes provided in the identification key, the new species is similar to *Longidorus dunensis* Brinkman, Loof & Barbez, 1987, which is a parthenogenetic species having a shorter odontostyle (95-106 µm) and odontophore (54-63 µm), a narrower lip region (14-16 µm), DN is situated more anteriorly (20-29%), and SVN more posteriorly (51-60%) than in the new species. The new species is also similar to: *L. paraelongatus* Altherr, 1974, which was described from a single female, and which has a longer body (7.35 mm) and odontostyle (140 µm), a slightly narrower labial region (calculated from Fig. 10a of Altherr, 1974, who did not provide information in the text), amphids not bilobed, and tail broader at the tip; *L. cylindricaudatus* Kozlowska & Seinhorst, 1979 which differs by having a longer odontostyle (128-140 µm), a narrower lip region (12 µm), a more posterior guide ring (33-38 µm), different tail shape, and males extremely rare; *L. cohni* Heyns, 1969 in which the lip region is narrower (16-18 µm), the body is longer (7.8-8.8 mm) and more slender (a=165-225), and the location of the oesophageal gland nuclei is abnormal; *L. closelongatus* Stoyanov, 1964 which has a narrower (17 µm) and more expanded lip region, males are rare, and the location of the oesophageal gland nuclei is abnormal; *L. arenosus* Kankina & Ivanova, 1986 has a longer body (7.13-9.16 mm), a narrower (18 µm, measured from the illustration) and more expanded, strongly off-set lip region, and a more anterior guide ring (22-27 µm).

TYPE HOST AND LOCALITY

Rhizosphere of grass growing in coarse alluvial sand, 1000 m from the southern bank of the river Maas, Vortum-Mullem, The Netherlands (51°38'N, 05°59'E).

TYPE MATERIAL

Holotype (slide number WT 3251), three paratype females, two paratype males and four juveniles deposited in the collection of the Agricultural University, Wageningen, The Netherlands (slide numbers WT 5231 to WT 5235) . The holotype female designated by Dr Seinhorst was in poor condition which required us to designate a different specimen as the holotype. Three paratype females and one paratype male deposited in the nematode collection of the International Institute of Parasitology, St. Albans, UK; four paratype females deposited in the collection at the Muséum National d'Histoire Naturelle, Paris, France; two paratype females and one paratype male deposited in the USDA Nematode Collection, Beltsville, MD, USA.

REMARK

A relatively long caecum is present in all females (Fig. 2 Fa, b). In fertilised females, this structure becomes thicker, presumably as a result of physiological changes induced in the spindle-shaped epithelial cells (Coomans, 1965) as a pre-requisite for receiving fertilised eggs from the ovary. In one specimen an oocyte was observed partly entered into the caecum and with two sperm cells associated with invaginations of the oocyte. Thus, the caecum appears to be the place in the reproductive tract where fertilisation of oocytes occurs.

Acknowledgements

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Meloidogyne javanica-Rhizoctonia solani disease complex of peanut

Salah M. ABDEL-MOMEN and James L. STARR

Department of Plant Pathology and Microbiology, Texas A & M University, College Station, TX 77843-2132, USA.

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Summary – The interaction of *Meloidogyne javanica* and *Rhizoctonia solani* was studied on peanut in greenhouse and field microplot experiments. The effects of *R. solani* on reproduction of *M. javanica* was variable, with nematode *Pi* having a greater effect on nematode reproduction than did the presence of *R. solani*. In microplot tests with a factorial design with four nematode *Pi* and two levels (1995) or three (1996) levels of *R. solani*, peanut pod rot and root colonization by *R. solani* were increased by the presence of *M. javanica* ($P \leq 0.05$), and the total amounts of pod rot and root colonization were positively related ($r^2 > 0.85$) to *Pi* of *M. javanica*. Pod yield was more suppressed by both pathogens than by either pathogen alone, and was negatively related ($r^2 > 0.86$) to *Pi* of *M. javanica* in both microplot experiments. The data confirm the interaction of *M. javanica* and *R. solani* on peanut. © Orstom/Elsevier, Paris

Résumé – ***Le complexe pathogène de l'arachide* Meloidogyne javanica - Rhizoctonia solani** – L'interaction entre *Meloidogyne javanica* et *Rhizoctonia solani* a été étudiée sur arachide en serre et en microparcelles au champ. L'action de *R. solani* sur la reproduction de *M. javanica* est variable, la *Pi* du nématode ayant une plus grande influence sur sa reproduction que la présence de *R. solani*. Lors d'expériences en microparcelles ayant une disposition factorielle et comportant quatre niveaux de *Pi* du nématode et deux (1995) ou trois (1996) niveaux de *R. solani*, la pourriture des gousses d'arachide et la colonisation des racines par *R. solani* sont accrues en présence de *M. javanica* ($P \leq 0,05$) ; les valeurs totales de la pourriture des gousses et de la colonisation des racines sont corrélées positivement ($r^2 > 0,85$) à la *Pi* de *M. javanica*. La récolte en gousses est plus affectée en présence des deux agents pathogènes que si un seul d'entre eux est présent, et cette récolte est corrélée négativement ($r^2 > 0,86$) aux *Pi* de *M. javanica* dans l'une et l'autre expériences en microparcelle. Ces données confirment donc l'interaction entre *M. javanica* et *R. solani* sur l'arachide. © Orstom/Elsevier, Paris

Keywords: *Arachis hypogaea*, disease complex, *Meloidogyne javanica*, peanut, *Rhizoctonia solani*, root-knot nematode.

Nematode-fungal disease complexes, especially those involving *Meloidogyne* spp., are common on many crops (Golden & Van Gundy, 1975; Diomandé *et al.*, 1981; Abawi & Barker, 1984; Starr *et al.*, 1989). The association of nematodes and fungi on plants may cause synergistic, additive, or antagonistic effects with respect to disease development and yield suppression. Synergistic associations are generally attributed to the enhancement of fungal infections due to the physiological effects on the plant of nematode parasitism (Golden & Van Gundy, 1975; Starr & Aist, 1977). In the synergistic association of *Meloidogyne incognita* and *Rhizoctonia solani* on okra and tomato, roots of both crops were colonized to a greater extent by *R. solani* in the presence of *M. incognita* compared to colonization of plants exposed to *R. solani* alone (Golden & Van Gundy, 1975). Siddiqui and Husain (1992) reported a similar effect of *M. incognita* on the colonization of chickpea roots by *Macrophomina phaseolina*. Infection of cotton by *M. incognita* increased the susceptibility of wilt-susceptible and wilt-resistant cotton genotypes to *F. oxysporum* f. sp. *vasinfectum* (Starr *et al.*, 1989; Jeffers & Roberts, 1993).

Not all associations of nematodes with soilborne fungal pathogens result in synergistic effects. Starr *et al.* (1996) reported that the effects of *Sclerotium rolfsii* and *M. arenaria* on yield of peanut and incidence of southern blight were additive over a range of different inoculum levels of both pathogens. Jorgenson (1970) reported antagonistic effects for the association of *Heterodera schachtii* and *F. oxysporum* with respect to growth of sugar beets.

Meloidogyne javanica (Tomaszewski *et al.*, 1994) and *R. solani* (El-Wakil *et al.*, 1984) are important pathogens of peanut (*Arachis hypogaea*) in Egypt and have been reported to be involved in disease complexes either together or with other organisms on several crops (Batten & Powell, 1971; Golden & Van Gundy, 1975; Sankarialingman & McGawley, 1994; Walker, 1994), but their association on peanut has not been investigated previously. The objective of this study was to quantify the effect of the association of these two pathogens on peanut.

Materials and methods

Rhizoctonia solani was isolated from peanut roots exhibiting symptoms of root rot and root galling and Koch's postulates fulfilled (data not shown). The isolate of *R. solani* used for all experiments was identified

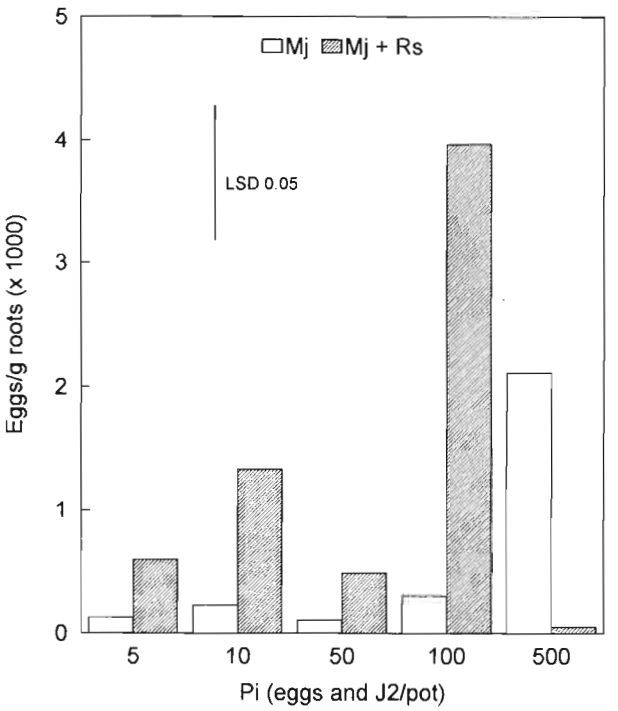

Fig. 1. *Effect of root infection by* Rhizoctonia solani *on reproduction of* Meloidogyne javanica *on peanut in a greenhouse experiment.*

as belonging to anastomosis group (AG)4 by comparison with isolates of known AG. *Meloidogyne javanica* was obtained from potato in Texas, and was parasitic on peanut (Abdel-Momen & Starr, 1997).

Meloidogyne javanica was reared on tomato (*Lycopersicon esculentum*) cv. Rutgers and eggs for inoculum were extracted from infected roots with 0.5 % NaOCl (Hussey & Barker, 1973). *Rhizoctonia solani* was maintained on potato dextrose agar. Inoculum of *R. solani* was prepared by growing the fungus on autoclaved oat grains for 15 days at 27 °C. Colonized oat grains were air-dried and then ground in a blender for 15 s prior to use as inoculum.

The effect of *R. solani* on reproduction of *M. javanica* was determined in a greenhouse experiment using a two × six factorial design. Two inoculum densities of *R. solani* (0 and 0.1 g/pot) and six initial population densities (*Pi*) of *M. javanica* (0, 5, 10, 100, and 500 eggs/pot) were tested. Thirty-cm-diam. pots were filled with a loamy sand soil, infested with each pathogen alone or combined. Seeds of peanut cv. Florunner were germinated in moist paper towels and one seedling was transplanted to each pot. To estimate nematode reproduction, as number of eggs per gram roots, eggs were extracted from infected roots with 0.5 % NaOCl (Hussey & Barker, 1973) at 8 weeks after planting.

The combined effects of *M. javanica* and *R. solani* on nematode reproduction, root colonization and pod rot by *R. solani*, and peanut yield was tested in 1995 and 1996 in field microplots. Microplots were plastic cylinders (55 cm diam × 45 cm deep) filled with and buried in loamy sand soil (85 % sand, 7 % silt, 8 % clay, pH 7.5). Microplots were fumigated before planting with metham sodium (380 L/ha) to eliminate existing pathogen populations. For these microplot experiments, nematode inoculum was prepared by chopping infected tomato roots into small fragments and mixing these fragments with the infested soil from which the roots were obtained. This mixture of infested soil and infected root fragments was then mixed with pasteurized sand (1:2 v/v). The number of second-stage juveniles (J2) and eggs in the inoculum mix was estimated from 500 cm^3 samples following elutriation (Byrd *et al.*, 1976) and centrifugation (Jenkins, 1964). Eggs were extracted with 0.5% NaOCl (Hussey & Barker, 1973) from root fragments collected during elutriation. Different amounts of this highly infested soil was mixed into the upper 25 cm of microplot soil to obtain the desired nematode *Pi* in each microplot. Ground oat grain inoculum of *R. solani* was also incorporated into the upper 25 cm of soil in the appropriate microplots. Eight seeds of Florunner peanut were planted in each microplot immediately after infesting the soil, microplots were thinned to three plants/microplot following emergence.

A factorial experimental design for microplot tests was used to determine the effects of each pathogen. In 1995, two inoculum densities of *R. solani* (0 and 0.01 g/500 cm^3) and four *Pi* of *M. javanica* (0, 5, 10, and 50 eggs and J2/500 cm^3 soil) were tested. In 1996, three inoculum densities of *R. solani* (0, 0.01, and 0.02 g/500 cm^3) and four *Pi* of *M. javanica* (0, 10, 50, and 100 eggs and J2/500 cm^3) were tested. There were five replications of each treatment each year.

Microplots were sampled twice, once at 8 weeks after planting and again at crop maturity (140 days after planting) to estimate nematode population densities. A composite soil sample of eight cores, each 2.5 cm diam. × 25 cm deep, was collected from each microplot. J2 were extracted from 500 cm^3 of soil by elutriation (Byrd *et al.*, 1976) and centrifugation (Jenkins, 1964). Eggs were extracted from root fragments collected during elutriation with 0.5 % NaOCl (Hussey & Barker, 1973). Reproductive factors (*Rf*) were calculated as midseason (*Pm*) or final (*Pf*) nematode population densities divided by *Pi*.

Peanuts were dug at 140 days after planting, air-dried, and the pods were harvested by hand. To

estimate the percentage of rotted pods, 100 pods were selected randomly from the yield of each microplot and examined for symptoms of pod rot. The percentage root colonization by *R. solani* for each treatment was determined in 1996. One hundred root segments (each 1 to 2 cm long) were taken arbitrarily from the roots of each microplot, rinsed with tap water, surface sterilized with 0.5 % NaOCl for 1.5 min, rinsed with sterilized water, placed in 10 cm diam. Petri dishes containing tannic acid-benomyl-agar medium, a selective medium for *R. solani* (Flowers, 1976), and incubated at 27 °C. Percentage of peanut roots colonized by *R. solani* was estimated based on the number of root segments from which colonies of *R. solani* developed.

Data from different experiments were subjected to analysis of variance using the general linear model procedures of SAS (SAS Institute, Cary, NC 27511, USA) to determine the effects of treatments on nematode population densities, pod rot, root colonization by *R. solani*, and pod yield. Mean separations were made using Fisher's LSD.

Results

Rhizoctonia solani did not affect the reproduction of *M. javanica* at nematode *Pi* of 5, 10, or 50 eggs/pot, but at a *Pi* of 100 eggs/pot in greenhouse tests, the final number of nematode eggs per gram roots was greater ($P \leq 0.05$) in the presence of *R. solani* compared to that in the absence of *R. solani* (Fig. 1). At a *Pi* of 500 *M. javanica* eggs/pot, the final number of eggs per gram roots was reduced ($P \leq 0.01$) by 98 % in the presence of *R. solani* compared to that produced in the absence of *R. solani*.

In microplot experiments, the midseason Rf values of *M. javanica* were not affected ($P \leq 0.05$) by the presence of *R. solani* (data not shown). At crop maturity, there was a general trend for *Rf* values to decline with increasing *Pi* (Fig. 2). The effect of *R. solani* was to increase *Rf*, but this trend was significant only in 1996 ($P \leq 0.05$).

No symptoms of pod rot were observed in plots not infested with *R. solani*. The percentage of pods with symptoms of rot caused by *R. solani* was greater ($P \leq 0.05$) in plots also infested with *M. javanica* than in plots infested only with *R. solani* (Fig. 3), and was positively related to the *M. javanica Pi* in both years ($r^2 = 0.86$ for 1995 and $r^2 = 0.89$ for 1996; $P \leq 0.01$). An increase in the concentration of inoculum of *R. solani* from 0.01 to 0.02 g/500 cm^3 did not affect the percentage of rotted pods in 1996 ($P \leq 0.05$).

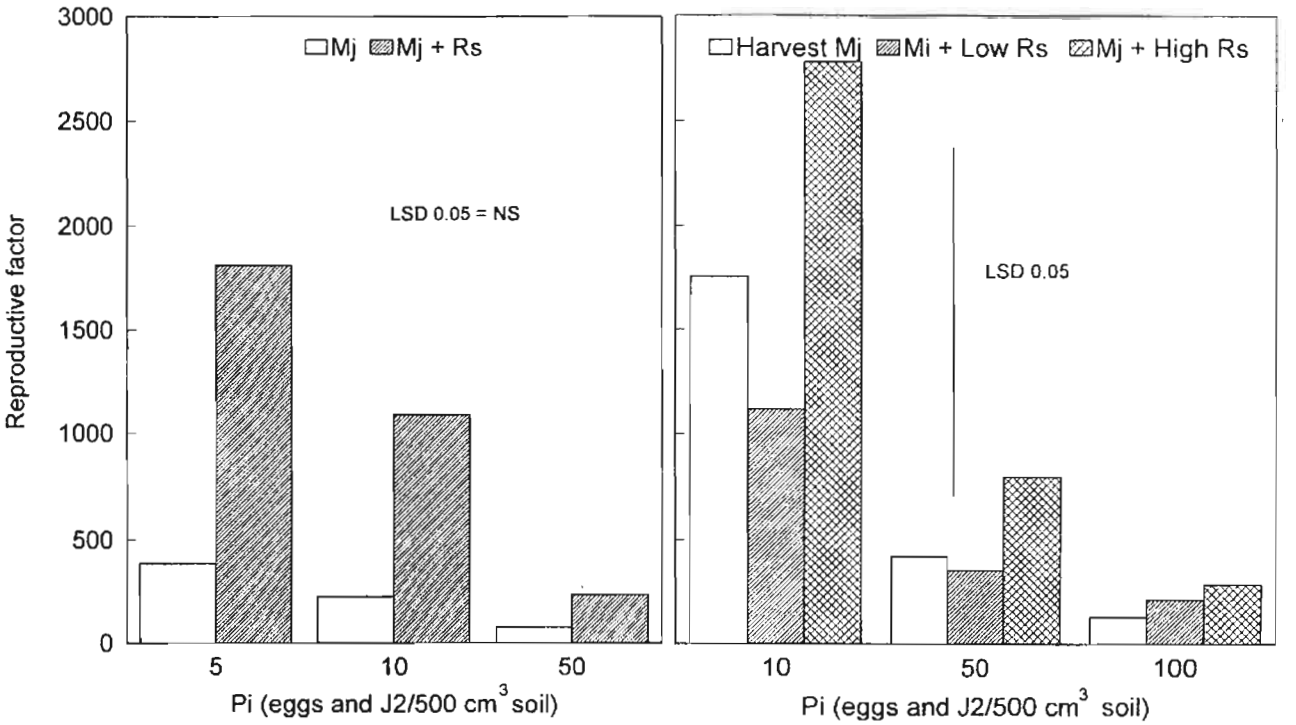

Fig. 2. *Effect of root and pod rot caused by* Rhizoctonia solani *(Rs) on the reproductive factor (Rf) of* Meloidogyne javanica *on peanut in field microplots (Left: 1995. Right: 1996. Low Rs and High Rs = 0.01 g and 0.02 g of inoculum per 500 cm^3 soil, respectively).*

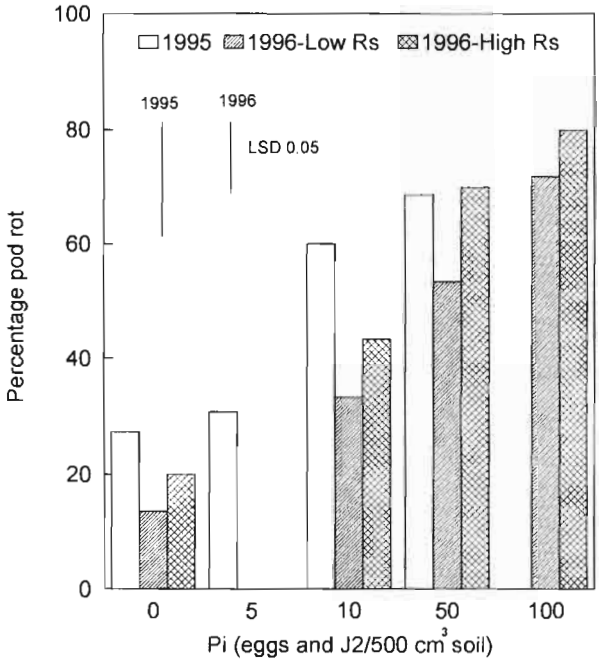

Fig. 3. *Effect of initial population densities* (Pi) *of* Meloidogyne javanica *on percentage pod rot of peanut by* Rhizoctonia solani *(Rs) in field microplots (Low Rs and High Rs = 0.01 g and 0.02 g of inoculum per 500 cm³ soil, respectively).*

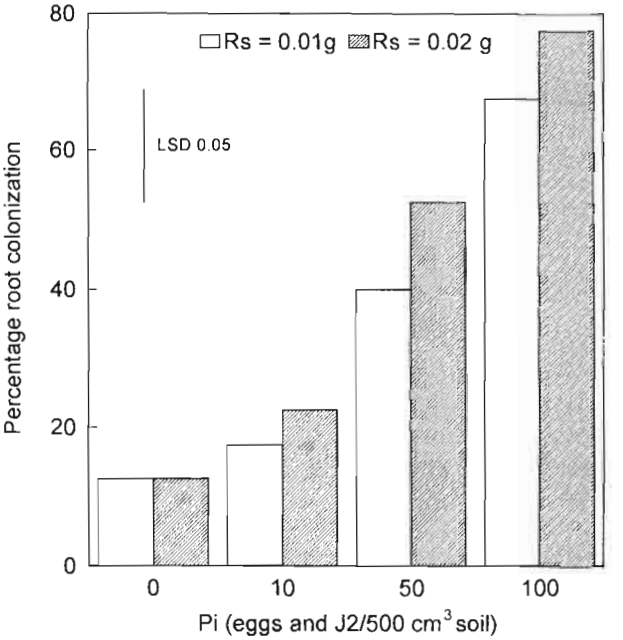

Fig. 4. *Effect of initial population densities* (Pi) *of* Meloidogyne javanica *on percentage colonization of peanut roots by* Rhizoctonia solani *(Rs) in field microplots in 1996.*

Colonization of peanut roots by *R. solani* also was positively related to *M. javanica Pi* (r^2 = 0.83; $P \leq 0.01$; Fig. 4), and visual examination of root segments indicated that the fungal growth was concentrated on and around root galls. The increase in *R. solani* inoculum from 0.01 to 0.02 g/500 cm³ soil did not affect the percentage of root colonized by *R. solani* ($P \leq 0.05$).

Both *R. solani* and *M. javanica* suppressed yield of peanut ($P \leq 0.05$) in 1995 and 1996 (Fig. 5), and the interaction of the two pathogens was significant ($P \leq 0.05$) in both years. A negative relationship between *M. javanica Pi* and pod yield was observed in both years (r^2 = 0.88 in 1995 and r^2 = 0.86 in 1996; $P \leq 0.01$). In 1996, *R. solani* alone at 0.02 g/500 cm³ soil caused greater ($P \leq 0.01$) yield suppression than that caused by 0.01 g/500 cm³ soil.

Discussion

The pathogenicity of *R. solani* on peanut was confirmed by fulfilling Koch's postulates. This is consistent with previous reports of other isolates of *R. solani* AG-4 causing limb (Barnes *et al.*, 1989) and pod rot of peanut (Baird *et al.*, 1993). Parasitism of peanut by *M. javanica* (Tomaszewski *et al.*, 1994; Abdel-Momen & Starr, 1997), which is rare on peanuts in the United States, also was confirmed.

As reported with other crops (Batten & Powell, 1971; Golden & Van Gundy, 1975; Sankarialingman & McGawley, 1994; Walker, 1994), the combined effects of *M. javanica* and *R. solani* on yield of peanut were consistently greater than the effects of either pathogen alone. The synergistic nature of this association was most evident at the lower nematode *Pi* levels. At the highest population densities of *M. javanica* and *R. solani*, the damage by each pathogen alone was sufficiently large that no synergistic effect was observed. *Meloidogyne javanica* also increased the peanut pod rot and root colonization by *R. solani*. Colonies of *R. solani* were most frequently observed to develop from the gall tissue of peanut roots in the present study. This observation was consistent with the hypothesis of Golden and Van Gundy (1975) that *R. solani* is more attracted to the gall tissue compared with nongalled root tissues.

There was no consistent trend with regard to effects of *R. solani* on the reproduction of *M. javanica*. Overall, the *Pi* had a greater influence on final nematode population densities than did the presence or absence of *R. solani*. Premature senescence of peanut due to combined infection by *M. javanica* and *R. solani*, however, would be expected to limit total reproduction by *R. solani*, as was observed in the *Fusarium*

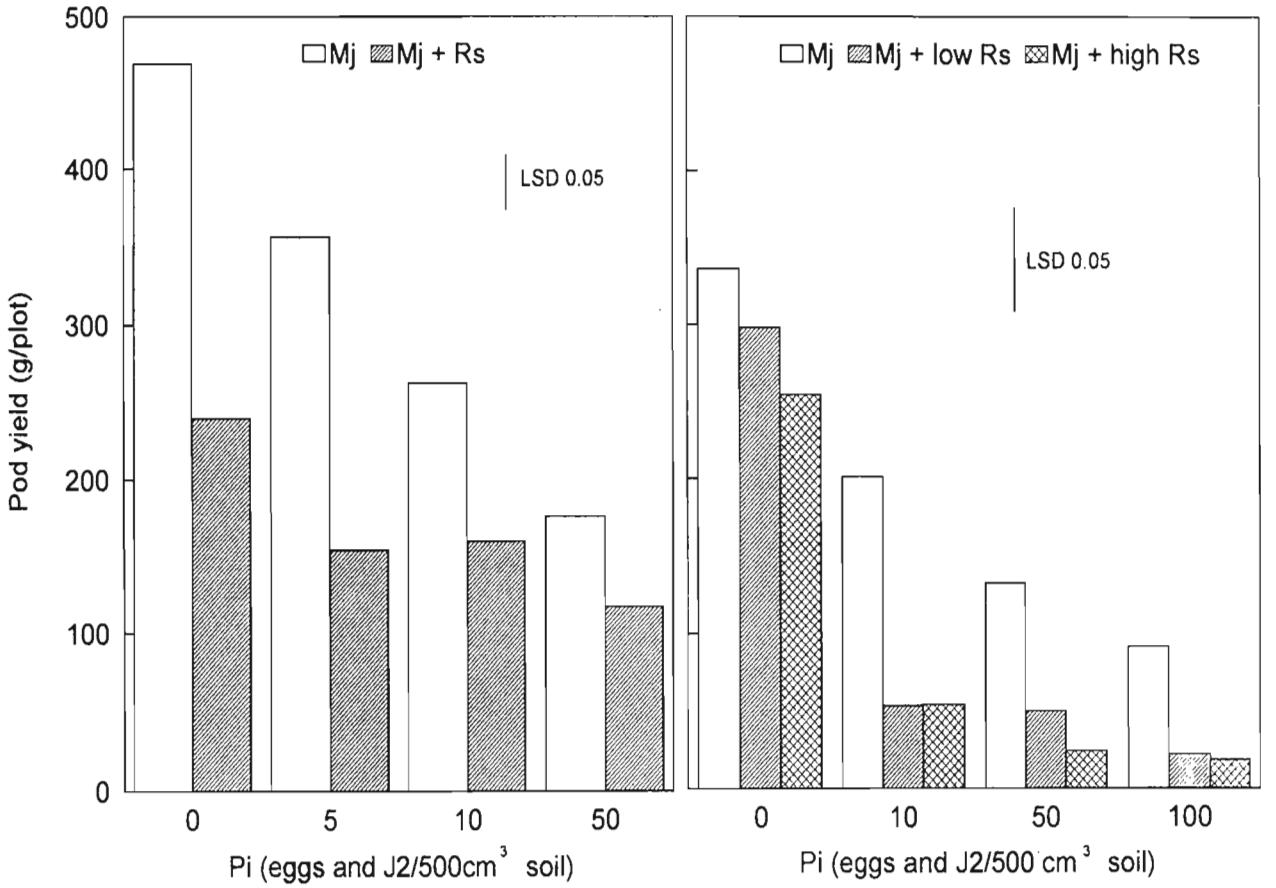

Fig. 5. *Combined effects of* Meloidogyne javanica *(Mj) and* Rhizoctonia solani *(Rs) on pod yield of peanut in field microplots (Left: 1995. Right: 1996. Low Rs and High Rs = 0.01 g and 0.02 g of inoculum per 500 cm³ soil, respectively).*

wilt/root-knot nematode complex of cotton (Starr *et al.*, 1989).

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The dynamics of the decline of the cereal cyst nematode, *Heterodera avenae*, in four soils under intensive cereal production

Brian R. KERRY and David H. CRUMP

Entomology and Nematology Department, IACR Rothamsted, Harpenden, Herts, AL5 2JQ, UK.

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Summary – Changes in the population density of the cereal cyst nematode, *Heterodera avenae*, were monitored in four soils in microplots for 10 years. For the first 4 years, half of the microplots were treated each year with drenches of formalin (38 % formaldehyde) prior to sowing with susceptible cereals. Thereafter, plots were sown with susceptible or resistant spring barley or ryegrass to manipulate the numbers of female nematodes and eggs produced each year. After the fourth application of formalin, the densities of the nematophagous fungi, *Nematophthora gynophila* and *Verticillium chlamydosporium*, were estimated under each crop in untreated soil and soil previously treated with the partial soil sterilant. Formalin significantly increased nematode populations and reduced the densities of the nematophagous fungi in all soils. By the second year of formalin application, nematode population densities in all soils had increased to levels which caused crop failure in winter wheat; thereafter, populations declined under spring barley unless applications of formalin were continued. Despite continuous cropping with susceptible cereals, populations of the nematode were often not detectable in plots after the eighth year. Changes in the densities of spores of the fungi in soil were closely related to changes in the numbers of nematodes. In soils previously treated with formalin, the fungi had increased to levels similar to those found in naturally suppressive soils within 3 years of discontinuing formalin application and population densities of the nematode had declined to the same levels as in untreated soils. © Orstom/ Elsevier, Paris

Résumé – ***Dynamique du déclin du nématode à kystes des céréales,* Heterodera avenae, *sur quatre sols mis en culture intensive de céréales*** – Les changements affectant la densité de population du nématode à kystes des céréales, *Heterodera avenae*, ont été observés pendant 10 années dans des micro-parcelles contenant quatre types de sol. Pendant les 4 premières années, le moitié des micro-parcelles ont été traitées annuellement par un arrosage à l'aide de formol (38 % d'aldhéhyde formique) précédant un semis de céréales sensibles. Les parcelles ont été ensuite ensemencées en orge de printemps, sensible ou résistant, ou en rye-grass de façon à estimer le nombre de femelles et d'oeufs produits chaque année. Après la quatrième année de traitement au formol, la densité des champignons nématophages *Nematophthora gynophila* et *Verticillium chlamydosporium* a été estimée pour chaque culture dans les sols non traités et dans ceux traités auparavant avec le produit partiellement stérilisant. Le formol augmente les populations du nématode et diminue la densité des champignons nématophages. A la deuxième année d'application de formol, la densité des nématodes s'est accrue dans tous les sols pour atteindre un niveau provoquant une diminution de récolte chez le blé d'hiver ; les populations déclinent ensuite sous orge de printemps même si les applications de formol se poursuivent. Après la huitième année, malgré une culture continue de céréales sensibles, les populations du nématode n'étaient souvent plus détectables. Les modification dans la densité des spores de champignon présentes dans le sol sont étroitement liées aux changements dans le nombre de nématodes. Dans le sols traités auparavant au formol, le niveau des champignons s'est accru pour atteindre des valeurs similaires à celles observées dans des sols naturellement intolérants (= suppressive soils) au cours de 3 années sans application de formol, et les densités de population du nématode ont diminué pour atteindre des niveaux similaires à ceux des sols non traités. © Orstom/Elsevier, Paris

Keywords: *Heterodera avenae*, nematophagous fungi, *Nematophthora gynophila*, population dynamics, suppressive soils, *Verticillium chlamydosporium*.

In several countries, soils have been identified in which the population densities of specific plant parasitic nematodes have declined under monocultures of susceptible crops because nematophagous parasites and antagonists have increased, in some cases, to densities which limit nematode multiplication (Stirling, 1991). The causal agents of this natural control have been identified and include several species of nematophagous fungi and the bacterium *Pasteuria penetrans*. These parasites increased sufficiently to prevent the multiplication of cyst and root-knot nematodes, respectively, within 3-5 years of continuous cropping. Few detailed studies have been made on the population dynamics of nematodes and their natural enemies in suppressive soils and there is a dearth of quantitative methods for estimating the numbers of nematophagous microbial parasites. In the laboratory, Jaffee *et al.* (1992, 1993) have done pioneering research on the quantitative relationships between both endoparasitic and trapping fungi and their nematode hosts, and

have provided much valuable information on the density dependence of these interactions. In Florida, the impact of *P. penetrans* on the population dynamics of *Meloidogyne arenaria* has also been studied on a range of crops (Oostendorp *et al.*, 1991; Chen *et al.*, 1997). This paper describes a 10-year study done in small plots out-of-doors in which the population dynamics of the cereal cyst nematode, *Heterodera avenae*, were investigated in four soils containing nematophagous fungi.

The decline of populations of *H. avenae* under monocultures of susceptible cereals was first reported in the UK (Collingwood, 1962) and is now a widespread phenomenon throughout northern Europe (Kerry, 1982). Kerry *et al.* (1982*a*,*b*) demonstrated that the suppression of nematode multiplication was caused by the nematophagous fungi *Nematophthora gynophila* and *Verticillium chlamydosporium* which parasitised female nematodes and their eggs in the rhizosphere. *N. gynophila* prevented the formation of nematode cysts, which are the survival stage of the nematode between crops, and *V. chlamydosporium* reduced fecundity and the numbers of healthy eggs. The partial soil sterilant formalin (38 % formaldehyde) applied as a soil drench resulted in increased nematode multiplication (Williams, 1969) and was demonstrated to reduce parasitism of cereal cyst nematodes by parasitic fungi (Kerry *et al.*, 1980, 1982*b*). Applications of formalin and the fungicide Captafol have been used in the field to estimate the level of natural control exerted by these fungi (Stein, 1993).

Natural control of cereal cyst nematodes has proved the most sustainable method of nematode management in intensive agriculture but it is slow to establish in soils and difficult to exploit. However, little is known of the dynamics of the decline phenomenon. The study described in this paper uses different crops and formalin soil drenches to investigate the relationship between the population densities of the cereal cyst nematode and its fungal parasites under intensive cereal cropping in microplots.

Materials and methods

Soil from four sites (Table 1) was used at IACR-Rothamsted to fill 96 microplots (24 for each soil) made from polypropylene cold water tanks (Osma, Hayes, UK) which were 625 × 488 × 510 mm (length x width x depth) in size and had five drainage holes (diam. 20 mm) drilled in their bases. The tanks were buried in sand and ballast on a gravel base and the area surrounded by a retaining wall. Each site had been selected for its history of intensive cereal cropping and all had soils which were thin and free-draining, lying over chalk; each soil was naturally infested with *H. avenae* and contained both *N. gynophila* and *V. chlamydosporium*. Three of the soils were calcareous silt loams and the soil from the site at Sutton Veny contained significant amounts of organic matter; all soils were alkaline (Table 1).

For the first 4 years, 94 ml of formalin (38 % formaldehyde) was applied to twelve of the plots of each soil (48 plots in total) 3-4 weeks before the cereal crop was sown (Table 2). The application of formalin was equivalent to 3000 L ha^{-1}, a rate demonstrated to cause significant increases in *H. avenae* population densities (Williams, 1969) and decreases in the density of parasitic fungi (Kerry *et al.*, 1980). The formalin was applied in 3.5 L of water as a soil drench to each plot. Before each spring barley crop, the equivalent of 376.5 kg ha^{-1} 0:20:20 fertilizer with 188.3 kg ha^{-1} Nitrochalk and 188.3 kg ha^{-1} $MgSO_4$, were applied to the seed bed; a second similar rate of Nitrochalk was applied 6 weeks later. In the second year of the experiment, winter wheat cv. Maris Huntsman was sown and received 376.5 kg ha^{-1} 13:13:20 fertilizer in the seed bed and 100 kg ha^{-1} Nitrochalk in the spring. The perennial ryegrass received fertilizer applications in the spring at rates similar to that applied to the barley crops. All cereal crops were sown

Table 1. *Characteristics of the four soils used in the microplots (all shallow soils over chalk).*

Site	pH	Soil type	Soil texture (%)			*H. avenae* (eggs g^{-1} soil)	Fungal density (spores g^{-1} soil)	
			sand 2000-63 µm	silt 63-2 µm	clay < 2 µm		Ng *	Vc**
Crux Easton	7.5	Silt Loam	6.7	73.3	20.0	37	41	39
South Tidworth	8.1	Calcareous silt	31.5	54.8	13.7	20	5	218
Sutton Veny	7.9	Calcareous humose silt	29.1	58.3	12.6	41	140	434
Devizes	8.1	Calcareous silt	37.1	53.2	9.7	50	27	621

* Ng = *Nematophthora gynophila*
** Vc = *Verticillium chlamydosporium*

Table 2. *Management of the four soils.*

Year	Crop*	Treatment	Date of formalin application
1	s. barley (S) cv. Athos	formalin**	2 March
1	w. wheat (S) cv. Maris Huntsman	"	16 September
3	s. barley (S) cv. Athos	"	13 March
4	s. barley (S) cv. Athos	"	15 February
5-8	s. barley (S) cv. Triumph; s. barley (R) cv. Tyra; Perennial ryegrass	No formalin	–
9	s. barley (S) cv. Triumph; s. barley (R) cv. Vista; Perennial ryegrass	"	–
10	s. barley cv. Klaxon	"	–

* Spring (s) barley and winter (w) wheat, susceptible (S) or resistant (R) to *H. avenae.*
** Formalin (38% formaldehyde) applied cumulatively to half the plots of each soil. Years 1-4 and 10, all plots sown with the same cereal. Years 5-9 three crops were sown each to one-third of the plots containing either untreated soil or soil previously treated with formalin.

by hand in four rows 15 cm apart at seed rates equivalent to 181.5 kg ha^{-1}; the ryegrass was broadcast at 33 kg ha^{-1}. Pesticide applications were made as required in accordance with manufacturer's instructions. The microplots were grouped in fours with one of each being filled at random with one of the four soils; all plots within each group received the same treatment (formalin or untreated) and were sown with the same crop. Although treatments were assigned at random, each soil - crop - treatment combination was represented within each block of 24 plots when three crops were sown; in the first 4 years of the experiment, when a single crop was sown, each block contained three replicates of each soil – treatment combination. At each harvest, the two central rows of each plot of cereals were cut at ground level and removed for drying at 80 °C overnight; the grain was removed and straw and grain weights at 15 % moisture content were estimated separately. The ryegrass was cut each year but yields were not recorded.

Ten soil cores (2.5 × 15 cm) were removed at random from each plot at the time of preparing a seed bed but prior to the application of formalin or, after this treatment ceased, samples were taken from the seed bed or the ryegrass sward in spring at the time the barley crop was sown. The cores were bulked, screened through a 4 mm aperture sieve, thoroughly mixed and a 50 g subsample removed to determine the moisture content. Soil was stored in sealed polythene bags at 4 °C for up to 4 weeks before they were further processed for the extraction of nematode cysts and fungal propagules. Cysts were extracted from 100 ml sub-samples of soil from each plot. The subsamples were weighed and then washed through a fluidising column (Trudgill *et al.*, 1972) as modified by Kerry (1975). The population densities of nematodes were estimated in terms of the number of healthy eggs g^{-1} air-dried soil using standard methods (Southey, 1970). At the beginning of the fourth year and thereafter, the resting spores of *N. gynophila* and *V. chlamydosporium* were extracted from a 25 g sub-sample of soil by the wet sieving – centrifugation method described by Crump and Kerry (1981) and the numbers of spores g^{-1} air-dried soil estimated. As this method was very time consuming, soil samples from only half the experiment (two replicates from each soil, crop and treatment) were processed; the same two blocks of the experiment were used for the estimation of fungal population densities each year. At the end of the experiment, estimates of the densities of chlamydospores of *V. chlamydosporium* were compared with those of the densities of colony forming units (cfu) of the fungus as assessed on dilution plates of a semi selective medium (Kerry *et al.*, 1993), a method not available when the experiment was initiated.

Results

In both treated and untreated soil, population densities of the nematode increased in the first year of spring barley cv. Athos but decreased between year 2 and 3 when winter wheat cv. Maris Huntsman was grown (Fig. 1). Population densities in untreated soil were > 100 eggs g^{-1} soil after the first year, but they declined after the second year to densities of < ten eggs g^{-1} soil by the seventh year despite the continuous cropping of susceptible barley cultivars (Fig. 1). After the first year of the experiment, nematode population densities were significantly greater ($P \leq 0.001$) in all soils treated with formalin than in untreated soils, and this effect persisted throughout the experiment. However, after year 7, population densities in all soils were < two eggs g^{-1} soil and differences between treated and untreated soils were probably of little biological significance. Nematode population densities declined in all plots after the fourth year, when applications of formalin ceased; by the eighth year, there was little difference between densities of the nematode in untreated and previously treated soil under all crops. After year 8, the nematode was unde-

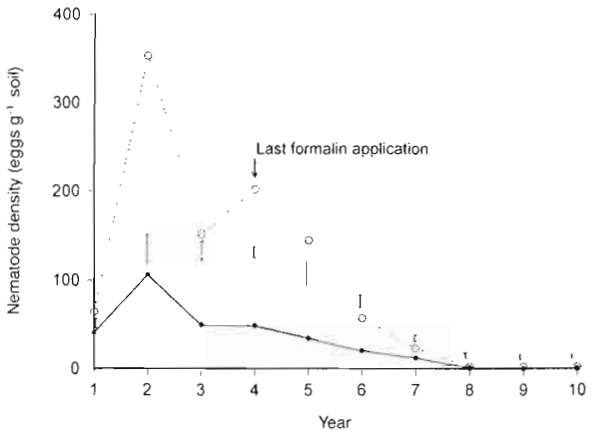

Fig. 1. *Changes in the mean density (eggs g^{-1} soil) of* Heterodera avenae *on susceptible cereals in untreated soils and those treated with formalin (○-----○ soil treated with formalin; ●——● untreated soil).*

tectable in more than 60 % of the plots, even in plots where susceptible cereals had been grown continuously, so statistical analysis was not used on data from these last two years of the experiment. The population trends were similar in all soils but nematode densities were significantly smaller in the soil from the Tidworth site and were greatest in formalin treated soils from Sutton Veny and Devizes (Table 3). At the time of the second application of formalin, and again in year 4, the differences in nematode population densities in treated and untreated soil were significantly smaller in soil from Crux Easton than from the other sites (Table 3). Rates of decline under susceptible barley were significantly slower than under resistant barley in both treated and untreated soil (Table 4); the rate of decline under grass was similar to that under resistant barley in untreated soil but similar to that under susceptible barley in soil previously treated with formalin. Populations of the nematode decreased most rapidly in untreated soils under resistant barley.

In the first 2 years, yields of neither spring barley nor winter wheat (year 2) were affected by the soil type or its treatment with formalin despite considerable differences in the densities of the nematode (Table 5). However, in the second year, when nematodes were most numerous, the yields of winter wheat were very low in all plots and the crop failed. There was a significant interaction ($P \leq 0.001$) between the effects of treatment with formalin and soil type on spring barley yields in years 3 and 4; in soil from Devizes, the application of formalin tended to decrease yields whereas in soil from Tidworth, yields were increased by the treatment. Spring barley yields between the third and seventh years tended to be

Table 3. *Effect of annual pre-planting applications of formalin on the population densities (eggs g^{-1} soil) of cereal cyst nematode in four soils.*

Soil	Year							
	1		2		3		4	
	–°	+	–	+	–	+	–	+
Crux Easton	42	33	183	252	53	140	78	196
South Tidworth	12	27	39	154	19	90	7	90
Sutton Veny	28	53	69	335	47	192	41	287
Devizes	50	49	91	274	71	200	61	261
Mean	33	41	96	254	48	156	47	208
$\pm SE_{DIFF}$	5.7		19.9		14.6		11.1	
	NS		***		***		***	
$\pm SE_{DIFF}$ interactions	11.4		39.8		29.2		22.2	
	NS		**		NS		***	

° Untreated soil (–) and soil treated with formalin (+).

Table 4. *Decline of* Heterodera avenae *population densities (eggs g^{-1} soil) under three crops in untreated soil (–) and soil treated (+) with formalin for the previous four years.*

Crop	Treatment	Year			
		5	6	7	8
Susceptible barley	–	34	20	12	1
	+	144	57	23	2
Resistant barley	–	26	3	1	0
	+	119	26	6	2
Ryegrass	–	34	8	3	1
	+	175	66	22	2
$\pm SE_{DIFF}$		29.4	10.5	3.7	0.6

Means of sixteen replicates; four soils.

significantly larger in soil from Tidworth than from the other sites and often smaller in soil from Devizes (Tables 5, 6). For the first two years following the last formalin application, yields of barley from treated soil remained significantly smaller than those from untreated soil but thereafter there was no effect of treatment (Fig. 2); there were no significant interactions between this previous treatment and soil type or cereal cultivar. Resistant barley produced greater yields than those from the susceptible cultivar in all soils only in years 7 and 8 (Table 6). After year 8 when nematode populations were small, yields of resistant and susceptible barley were similar in all plots. Grain yields were similar in all plots in the final year of the experiment and the data are not presented; yields of straw showed similar trends to those for grain and are not included.

Table 5. *Yields of spring barley cv. Athos and winter wheat cv. Maris Huntsman (year 2) in four soils infested with cereal cyst nematode and treated with formalin or left untreated.*

Soil	Year											
	1			2			3			4		
	–	+	Mean	–	+	Mean	–	+	Mean	–	+	Mean
Crux Easton	4.45	4.25	4.35	2.50	2.12	2.31	4.24	4.03	4.14	3.63	3.21	3.42
South Tidworth	4.98	4.75	4.87	2.52	1.86	2.19	3.87	5.52	4.70	4.77	5.57	5.17
Sutton Veny	4.07	4.69	4.38	2.67	2.75	2.71	4.02	4.36	4.19	4.26	4.56	4.41
Devizes	4.43	4.72	4.58	2.71	2.57	2.64	3.89	3.69	3.79	4.80	3.63	4.42
Mean	4/48	4.60		2.60	2.33		4.01	4.40		4.36	4.24	
$\pm SE_{DIFF}$	0.22		0.31	0.17		0.24	0.16		0.22	NS		0.22
	NS		NS	NS		NS	*		**			***
$\pm SE_{DIFF}$ interaction	0.44			0.34			0.32			0.31		
	NS			NS			***			***		

Means of twelve replicates.

Table 6. *Yields of susceptible (S) and resistant (R) cultivars of spring barley grown continuously in four soils infested with cereal cyst nematode.*

Soil	Year														
	5			6			7			8			9		
	S°	R°°	Mean	S	R	Mean	S	R	Mean	S	R	Mean	S	R	Mean
Crux Easton	2.47	2.99	2.73	5.41	5.41	5.41	2.57	5.00	3.79	3.71	4.63	4.17	4.62	5.03	4.82
South Tidworth	3.94	3.51	3.72	5.53	4.15	4.84	4.04	4.79	4.41	4.26	4.49	4.38	4.93	5.00	4.96
Sutton Veny	2.63	2.74	2.68	5.67	4.46	5.06	2.37	3.52	2.94	4.25	4.62	4.44	4.91	5.38	5.15
Devizes	2.51	2.81	2.66	4.38	4.21	4.30	2.89	4.59	3.74	4.42	4.90	4.66	4.60	4.59	4.59
Mean	2.89	3.01		5.25	4.56		2.97	4.47		4.16	4.66		4.77	5.00	
$\pm SE_{DIFF}$	0.25		0.35	0.34		0.47	0.30		0.42	0.20		0.29	0.26		0.37
	NS		**	*		NS	***		*	*		NS	NS		NS

° cv. Triumph except year 7 when cv. Georgie was grown.
°° cv. Tyra except year 9 when cv. Vista was grown.
Means of four replicates.

At the beginning of the fourth year, the numbers of spores of both *N. gynophila* and *V. chlamydosporium* were significantly fewer ($P \leq 0.001$) in soils treated with formalin for four years than in untreated soil (Table 7). *N. gynophila* and *V. chlamydosporium* were most abundant in untreated soils from Sutton Veny and Devizes, and least common in soils from Tidworth and Crux Easton. In general, as nematode populations declined under susceptible barley both in soils previously treated with formalin and in untreated soils, there was a tendency for spore numbers in soil to increase (Fig. 3). However, the spore densities in soil previously treated with formalin appeared to increase erratically, especially those of *N. gynophila*, although in years 7 and 9 even in these plots the densities were similar to or greater than those in untreated soil in year 5 (Fig. 3). Between years 5 and 9, spore densities of both fungi increased under susceptible barley both in soils previously treated with formalin and in untreated soils (Table 8). However, under resistant barley and ryegrass, which supported few nematodes, the spore densities declined in untreated soil and failed to increase in treated soil. Although there were significant differences ($P \leq 0.001$) between the densities of spores in the different soils, the effects of crop and treatment with formalin were similar in each and the data are not presented separately. The relationship between estimates of the density of

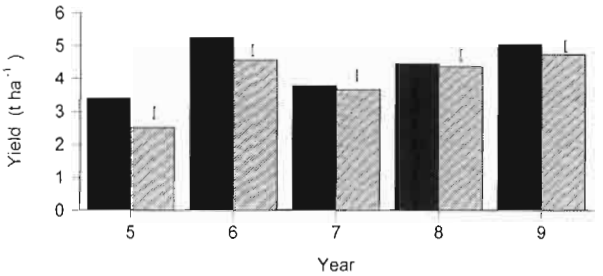

Fig. 2. *Mean yields (t ha^{-1}) of spring barley in soils previously treated with formalin (grey) and untreated soil (black).*

Table 7. *Densities (spores g^{-1} soil) of* Nematophthora gynophila *(Ng) and* Verticillium chlamydosporium *(Vc) at the beginning of the fourth year in four untreated soils (–) and those drenched each year for four years with formalin (+).*

Soil	Treatment	Fungus	
		Ng	*Vc*
Crux Easton	–	41	39
	+	2	9
South Tidworth	–	5	218
	+	3	78
Sutton Veny	–	140	434
	+	46	231
Devizes	–	27	621
	+	2	85
Mean	–	53	324
	+	13	100
SE_{DIFF}		8.7	29.7
		***	

Means of six replicates.

V. chlamydosporium based on the physical extraction of chlamydospores and that derived from the numbers of cfu developing on the selective medium was examined for the last sampling only. The effect of formalin on the density of cfu in soil was similar to that for spores and data for this comparison are not presented. However, chlamydospores formed a significantly greater proportion of the total number of cfu in soil from Devizes than in soil from Crux Easton and differences in the density of the fungus in each soil based on estimates of the numbers of spores are not consistent with those based on cfu (Table 9); soil from Devizes, which consistently contained the greatest densities of chlamydospores, often had the smallest numbers of cfu. Although there were similar numbers of cfu under the three crops, chlamydospores were most

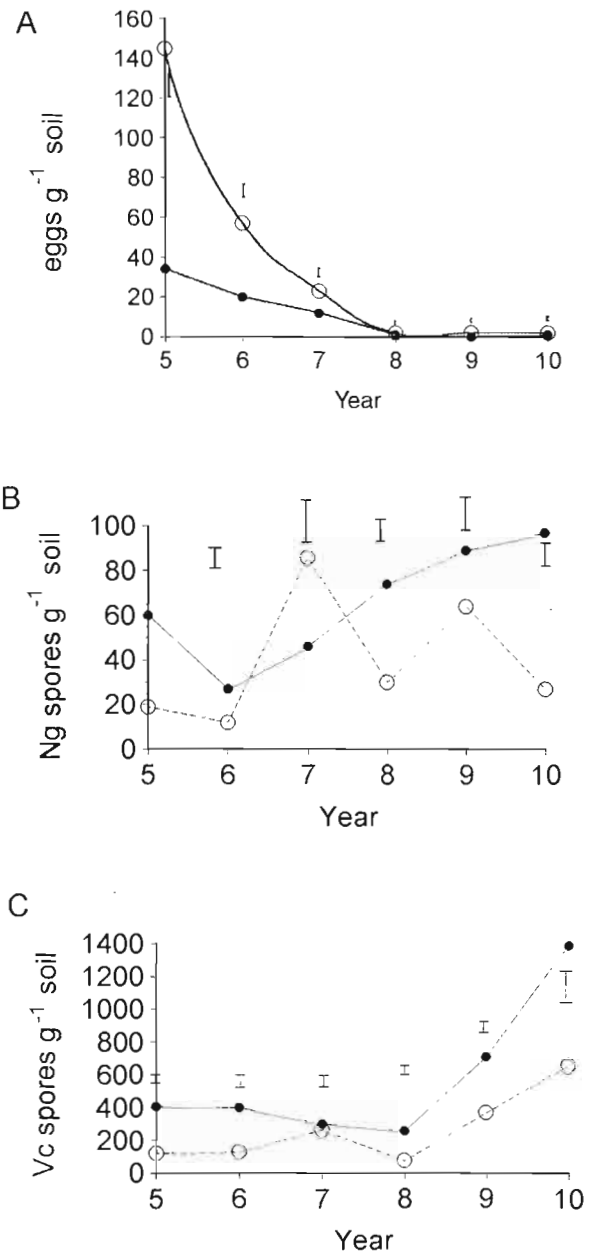

Fig. 3. *Changes in the mean numbers (eggs g^{-1} soil) of cereal cyst nematode (A) on susceptible barley and densities of resting spores of* Nematophthora gynophila *(B) and* Verticillium chlamydosporium *(C) in soil previously treated with formalin and untreated soil (Mean nematode counts from sixteen replicates; mean spore counts from eight replicates; ○-----○ soil previously treated with formalin; ●——● untreated soil).*

abundant ($P \leq 0.05$) in soil under susceptible barley (Table 9).

Table 8. *Densities (spores g^{-1} soil) of* Nematophthora gynophila *and* Verticillium chlamydosporium *after one and four years under different crops in soil previously treated with formalin and in untreated soil.*

Crop	Treatment	*Nematophthora gynophila*				*Verticillium chlamydosporium*			
		Year 5		Year 9		Year 5		Year 9	
		Count	Mean	Count	Mean	Count	Mean	Count	Mean
Barley - S	–	27		63		401		511	
			20		54		266		386
	+	12		44		131		260	
Barley - R	–	59		29		374		157	
			38		23		224		119
	+	16		16		73		81	
Ryegrass	–	54		20		204		147	
			34		15		133		103
	+	14		10		61		58	
SE_{DIFF}		7.6	5.4	13.3	9.4	66.6	47.1	52.9	37.4
		***	**	NS	***	***	*	***	***

Means of eight replicates.

Table 9. *Differences in the numbers of colony forming units (cfu) and chlamydospores (chlam.) of* Verticillium chlamydosporium *in four soils after five years of three crops.*

Soil*	Susceptible barley		Resistant barley		Ryegrass	
	cfu	chlam.	cfu	chlam.	cfu	chlam.
Crux Easton	2242*	247	1054	37	2333	119
South Tidworth	1504	979	2596	270	4879	191
Sutton Veny	5496	1208	4963	371	6500	446
Devizes	1583	1620	1488	373	1257	830
Mean	2706	1013	2525	263	3742	396
SE_{DIFF}		(crop *vs* propagule)		± 493 ($P \leq 0.05$)		
SE_{DIFF} interaction		(soil *vs* propagule)		± 427 ($P \leq 0.01$)		

Means of four replicates.

* Numbers of propagules g^{-1} soil.

Discussion

After partial soil sterilisation with formalin, populations of the cereal cyst nematode built up to very large densities in the four soils, and in individual plots populations > 600 eggs g^{-1} soil were recorded. However, there were differences between the soils in their ability to support the multiplication of the nematode, and populations in soil from the Tidworth site were significantly smaller than those in soil from elsewhere. In the second year, when nematode populations were at a peak in all soils, the winter wheat crop failed and a mean yield of only 2.47 t ha^{-1} was obtained. Wheat is more susceptible to damage than barley (Gair, 1965) but winter wheat is a relatively poor host for the nematode and post-crop population densities were smaller than those observed following barley. In the third year of the experiment, yields of spring barley were larger in formalin treated soil than in untreated soil despite the differences in the numbers of nematodes. This effect was dependent on the soil and may indicate that in soil from Tidworth and Sutton Veny formalin was affecting other factors than the nematode, such as soil nutrient availability or soil borne fungal pathogens, which were limiting crop yields. However, by the fifth year, yields of barley were on average 27 % smaller in formalin treated soil in which the nematodes were more numerous, than in untreated soil; this effect was reduced to 3 % by year 8 when populations of the nematode were similar and very small in both treated and untreated soil. The differences in mean yield observed between treated and untreated soil may also be related to the change of barley cultivar after year 4; cv. Athos may be more tolerant of nematode attack than the other cultivars used.

In untreated soil from each of the four sites, the nematode populations declined, even under continuous susceptible cereal crops and the nematode was often undetectable in many plots after the eighth crop, despite the intensive soil sampling procedure used. However, when these same soils were treated with a partial soil sterilant, the nematode was capable of multiplying to densities which severely reduced yields and these damaging populations were maintained when repeated applications of the formalin sterilant were

made. It is clear that the soils and susceptible cereal cultivars used were capable of supporting large nematode populations and that a soil factor(s) removed by the formalin was limiting nematode populations. These results confirm earlier observations in which the decline of *H. avenae* populations under intensive cereal production has been reported (Kerry, 1982). Several workers have associated this decline with the parasitism of adult female and egg stages of the nematode (Kerry, 1982; Schonhammer & Fischbeck, 1985).

Estimations of the numbers of nematodes parasitised by fungi and the impact of these natural enemies on the population dynamics require intensive, destructive sampling (Kerry *et al.*, 1982*b,c*) which was inappropriate for the small plots used in this long term study. Hence, the activities of the fungi were estimated by measuring changes in the densities of the resting spores of the two main causal agents of the decline phenomenon, *N. gynophila* and *V. chlamydosporium*. Although it had proved difficult to relate changes in densities of resting spores of these fungi with the levels of parasitism of the nematodes in a single growing season in field trials at three sites (Kerry *et al.*, 1982*c*), it was possible to detect significant differences between the numbers of spores found in suppressive soils and those in which the nematode multiplied (Crump & Kerry, 1981). In this study, in which plots were sampled only annually but more intensively than in previous field trials, it was possible to demonstrate that applications of formalin significantly reduced the numbers of spores of both *N. gynophila* and *V. chlamydosporium* in each of the four soils and that large nematode infestations were associated with small spore densities and small nematode populations with large spore densities. Also, in the absence of further formalin applications over a 4-year period, the numbers of spores of both fungal species increased in all soils where there were nematode females and eggs on susceptible barley crops, but the numbers of spores decreased under resistant barley or ryegrass on which these nematode stages were scarce or absent. Thus, it was possible to relate changes in the densities of the nematode with those of the fungi as estimated by the numbers of spores in soil. The densities of spores found in the untreated soils of year 4 and in the previously formalin treated soils by year 7 were similar to those reported from known suppressive soils (Crump & Kerry, 1981; Kerry *et al.*, 1982*a*). However, the size of soil sample (25 g) and the processing method used appeared not to be sufficiently sensitive to estimate reliably changes in the annual density of these fungi. Although general increases in the numbers of spores in soil under susceptible barley were observed as nematode populations declined, there was much variation in the estimates of fungal densities in samples taken from the same soil in the same year and in estimates between years. Also, in the case of *V. chlamydosporium*, the use of the selective medium demonstrated that the fungus was much more abundant in some soils than was indicated by counts of the chlamydospores alone. Isolates of *V. chlamydosporium* differ in their ability to produce chlamydospores *in vitro* (Kerry *et al.*, 1986) and may also differ in this ability in soil. Also, the selective medium may differ in its efficacy in estimating the abundance of the fungus in different soils (Kerry *et al.*, 1993); although there is a general positive and linear relationship between the abundance of chlamydospores in soil and the densities of propagules of the fungus, there is much variation between soils (Kerry *et al.*, 1993), which makes it difficult to interpret detailed observations on changes in fungal abundance. Interestingly, chlamydospores not only formed a greater proportion of the total numbers of propagules in some soils, such as that from Devizes compared to that from Crux Easton, but they were also significantly more abundant in soils under susceptible barley than under resistant or poor hosts for the nematode. The fungus may depend on the nematode to produce its resting structures in large numbers and ensure its long term survival in soil.

Changes in the densities of resting spores of both the obligate parasite, *N. gynophila*, and the facultative parasite, *V. chlamydosporium*, were closely associated with changes in the densities of the nematode in soil not treated with formalin. Populations of the nematode declined to densities which did not affect the yield of tolerant crops in 4 years and the two species of nematophagous fungi increased, within 3 years, to levels previously reported to be associated with the decline of the nematode (Crump & Kerry, 1981). The rates of decline of the nematode under resistant barley and ryegrass were similar to those reported elsewhere (Gair, 1965; Gair *et al.*, 1969); but it was not possible to demonstrate a positive density dependent effect of the fungi on nematode populations as has been observed by Jaffee (1992), who found that the weakly saprophytic fungus, *Hirsutella rhossiliensis* parasitised few nematodes at low densities and exerted little population control unless nematode hosts were abundant. In our studies, the estimates of fungal densities may have been too variable for the detection of density dependence; indeed, Jaffee's research was done in small microcosms to avoid such variation in sampling. Although the numbers of spores of both fungi were associated with changes in the density of *H. avenae*, it might be expected that parasitism by the obligate parasite *N. gynophila* would show greater density dependence on the host than *V. chlamydosporium*, which may grow saprophytically in soil. The latter species was the more prevalent in the soils used in this

experiment and populations of *H. avenae* declined more slowly in the years in which the nematode was abundant compared to those when the populations were smaller (< twenty eggs g^{-1} soil). This study has provided further evidence of the decline of populations of *H. avenae* under susceptible cereal crops and, for the first time, has provided information on the associated dynamics of the nematophagous fungi which cause this phenomenon. Changes in the densities of the nematophagous fungi to levels which were associated with significant reductions in *H. avenae* populations appeared to take 3-4 years, even in soils where the fungi were established. It remains to be seen whether the speed of such changes can be increased by applications of selected isolates of these fungi to provide a more easily managed strategy for biological control.

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Dynamics of damage to tomato by *Meloidogyne incognita*

Mahmoud E. EHWAETI, Mark S. PHILLIPS and David L. TRUDGILL

Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK.

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Summary - Damage due to *Meloidogyne incognita* on tomato (cv. Moneymaker) growing in large pots (3.65 kg dry soil) was related to initial population density (*Pi*). Damage was assessed after 42 and 135 days and only the highest *Pi* used (20 viable eggs/g soil) caused a statistically significant decrease in growth at both harvests. After 135 days, growth was also decreased significantly by *Pi* = 0.8 and 4.0 eggs/g soil. The decrease in growth was proportionally much greater after 135 days than after 42 days, as was reflected by the estimated values of *m* (the minimum yield), *T* (the tolerance limit) or *z* (a constant close to 1.0 which reflects the damage caused by a single nematode or where several generations are involved, all its progeny). The increased damage after 135 days was associated with a greatly increased final population density (*Pf*) of *M. incognita*. The greatest increase in population density (*Pf*/*Pi* = 273) occurred at the lowest effective initial population density used (*Pi* = 0.03 eggs/g soil), but the greatest final population (*Pf* = 90 000 nematodes/pot) occurred with *Pi* = 0.16 viable eggs/g soil. The effects on tomato growth of drought and of *M. incognita* were additive, and both decreased stomatal conductance. However, neither affected the ^{13}C discrimination in either upper or lower leaves, but the percentage of N in the leaf dry matter was greater in infested than uninfested plants. © Orstom/Elsevier, Paris

Résumé - ***Dynamique des dommages causés à la tomate par* Meloidogyne incognita** - Les dommages causés par *Meloidogyne incognita* à des tomates cv. Moneymaker poussant dans des pots de grande taille (3,65 kg de sol sec) ont été mis en relation avec la densité initiale de la population du nématode (*Pi*). Les dommages sont estimés après 42 et 135 jours de culture : seule la *Pi* la plus élevée (20 œufs viables/g de sol) cause une diminution statistiquement significative de la croissance aux deux dates de récolte. Après 135 jours, la croissance est également significativement diminuée par des *Pi* de 0,8 et 4,0 œufs/g de sol. La diminution de croissance après 135 jours était proportionnellement beaucoup plus forte qu'après 42 jours, ainsi qu'il l'était indiqué par les valeurs estimées de *m* (récolte minimum), *T* (limite de tolérance) et *z* (une constante proche de 1,0 reflétant les dommages causés par un seul nématode ou, dans le cas de plusieurs générations, par la totalité de sa descendance). Cette augmentation des dégâts après 135 jours est associée au très fort accroissement de la population finale (*Pf*) de *M. incognita*. La plus forte augmentation de la densité de population (*Pf*/*Pi* = 273) se rencontre aux densités efficaces les plus faibles de la population initiale (*Pi* = 0,03 œuf/g de sol), mais la population finale la plus élevée (*Pf* = 90 000 nématodes par pot) résulte d'une *Pi* = 0,16 œufs viables/g de sol. Les effets de la sécheresse et de *M. incognita* sur la tomate s'additionnent et l'une et l'autre diminuent la conductivité des stomates. Cependant, ni l'une ni l'autre n'affectent la discrimination du ^{13}C entre feuilles inférieures et supérieures ; toutefois, le pourcentage de N dans la matière sèche des feuilles est plus élevé chez les plantes infestées que chez les témoins. © Orstom/Elsevier, Paris

Keywords: Drought, host status, *Meloidogyne incognita,* modelling, stable isotopes, stomatal conductance, tolerance, tomato, water relations.

Several equations have been used to describe the relationship between crop yields and the initial population density of nematodes (*Pi*) with a single generation per year, such as potato cyst nematode (PCN or *Globodera* spp.). These include a log linear model suggested by Oostenbrink (1966):

$$Y = a + b\ (\log Pi) \qquad (1)$$

where Y is the expected yield for a given value of *Pi*, and the exponential model of Seinhorst (1965):

$$\{Y = Ymax\ [m + (1 - m)z^{Pi - T}] \text{ if } Pi > T \text{ and } Y = Ymax \text{ if } Pi > T\} \qquad (2)$$

with four parameters: *Ymax* - the expected yield in the absence of nematodes; T - the 'tolerance' limit, the threshold for *Pi* below which no damage occurs; z - the rate at which the increasing *Pi* decreases expected yield; and m - the ratio of minimum yield at high values of *Pi* and the maximum value when $Pi = 0$. Later Seinhorst (1986) used a modified version of equation (2) based on a number of experiments where he had observed that $z^T = 0.95$, thus giving

$$\{Y = Ymax\ [m + (1 - m)0.95^{(Pi/T - 1)}] \text{ if } Pi > T \text{ and } Y = Ymax \text{ if } Pi = T\} \qquad (3)$$

Elston *et al.* (1991) proposed an inverse linear model which has two parameters. Whilst the log-linear and inverse linear models are descriptive, that of Seinhorst (1965) includes the concept of a tolerance limit (T), and of a minimum yield (m). The value of m is expressed as a proportion of the nematode-free growth and depends on several factors, including the amount of growth achieved during the initial delay before nematode attack started. The longer the delay (or the larger the planting material), the greater the growth before attack and the greater the value of m (Seinhorst & Kozlowska, 1977; Seinhorst, 1995).

The Seinhorst equation has also been used to model damage by nematodes with several generations per growing season. Duncan and Ferris (1983) modelled the effects of the root-knot nematodes *Meloidogyne javanica* and *M. incognita* on the growth of cowpea cv. Californian Blackeye No 5 and showed that damage was greater with the *M. javanica* than with *M. incognita* for which the cowpea was a poorer host. McSorley *et al.* (1992) found that the effect of *M. arenaria* on the yield of peanut could be effectively modelled using the Seinhorst equation but observed values of T as low as 0.01 eggs/cm^3 soil, and recently Di Vito *et al.* (1997) obtained a low estimate of T (0.13 eggs/cm^3 soil) for kenaf growing in microplots and attacked by *M. incognita*. In contrast, Vrain (1982) found that damage to carrots by *M. hapla* could not be satisfactorily modelled by the Seinhorst equation.

Seinhorst (1995) simulated up to three generations of *Heterodera avenae* by applying the inoculum at sowing and/or once or twice at later dates. Except at the highest inoculum densities (more than 14 eggs/g soil), the relative weights of oat plants after 97 days growth were found to be the products of the relative weights that would have been obtained after single inoculations on the same dates. The greater sensitivity to additional damage observed with the most heavily infested plants was attributed to their development being retarded and to the effects of the 'second mechanism of damage'.

The severity of crop loss resulting from root-knot nematode attack is influenced by environmental factors. Various authors (Niblack *et al.*, 1986; Windham & Barker, 1986; Barker & Noe, 1987; Barker & Weeks, 1991; McSorley *et al.*, 1992) showed that damage by root knot nematodes is influenced by host crop and cultivar, by soil type and by the year in which the experiment was done. Understanding how the effects of the nematode and of the environment might interact depends on understanding how the crop is damaged by the nematode. Drought coupled with infection of roots by potato cyst nematodes decrease leaf water potentials (Haverkort *et al.*, 1991) and the two can interact to further decrease yield. Similarly, O'Bannon and Reynolds (1965) reported that mild water stress decreased water consumption of cotton plants infected with *M. incognita* much more than that of uninfected plants and Meon *et al.* (1978) reported that *M. javanica* infection increased the suction pressure in tomato roots, especially when soil water was restricted, without greatly affecting transpiration. Rahi *et al.* (1988) observed that both *M. incognita* and *M. javanica* increased water stress within the leaves of tobacco and greatly decreased the efficiency of water use per gram of dry matter produced and Wheeler *et al.* (1991) found that the effects on the yield of tobacco of drought and of *M. incognita* were additive. Meon *et al.* (1978) proposed that damage by *M. javanica* to tomato involved disruption of the xylem within the galls and, consequently, increased water stress in infected plants. It seems likely, therefore, that the availability of soil water may be one of the environmental factors that could influence the relationship between yield and nematode density.

A recent survey involving countries in east and west Africa, in the Caribbean and South America suggested that damage by root knot nematode was widespread and severe in crops of tomato grown by local farmers (average gall indices of 5 to 6, on a 1-10 scale; Triviño, unpubl.). It appears that, in general, it is the good hosts for root knot nematodes which are most severely damaged. The main objective of the experiment reported here was to test the hypothesis that on a good host (tomato) initially non damaging populations of *M. incognita* can multiply so greatly that they become damaging later in the growing season. The experiment also examined the effects of *M. incognita* on the water relations of tomato and the interaction with drought.

Materials and methods

A single experiment was done in large pots (23 cm diam, 5.4 dm^3) in a glasshouse with heating and vents set at 26°C during the day and 22°C at night. The effects on the growth of well-watered susceptible tomato cv. Moneymaker of six population densities of *M. incognita* were determined and compared at three of those population densities with the effects on plants which were partially subjected to drought. Plants (five replicates) were harvested 42 days and 135 days after inoculation so as to be able to compare the effects on growth early in the growing season with those that occurred later.

To produce the inoculum, 4-week-old tomato plants cv. Moneymaker were transplanted into 15 cm diam. pots filled with a peat/sand mixture and infected with chopped roots containing egg-masses of *M. incognita*. The pots were maintained at 25-26°C in a glasshouse for 2 months, after which the infected roots with egg-masses were cleaned of debris, cut in 2 to 5 cm long pieces, and then stirred for 4 min in 1% NaOCl

solution at 23°C to release the eggs (Hussey & Barker, 1973). The suspension of eggs was collected on a 38 µm mesh sieve and quickly washed several times with tap water. As some eggs are killed during extraction with NaOCl (Ehwaeti *et al.*, 1998) a hatching test was performed to determine the proportion of hatchable eggs.

The pots were inoculated with 360 ml of egg suspension during filling with 3.8 kg (3.65 kg dry weight) of sterilised, moist, mechanically mixed soil (40% peat and 60% sand). Six densities of *M. incognita* (0, 160, 800, 4 000, 20 000, 100 000 eggs/pot; equivalent to 0.0, 0.044, 0.22, 1.1, 5.5, and 27.4 eggs/g dry soil) were applied and mixed in by hand during filling to try to ensure that the eggs were distributed throughout the soil. Each pot was placed in a saucer and a 2-cm layer of polythene granules added to the surface to prevent water loss. The pots were watered to 33% moisture and 3 days later planted with a tomato seedling with two true leaves. There were ten replicates of each treatment.

A further ten replicates inoculated with 0, 4 000, or 100 000 eggs/pot received the reduced watering treatment (drought). These pots were initially watered to 22% moisture. Two pots without tomato plants were used to estimate water losses from the soil surface.

The pots were watered to a standard weight (progressively adjusted to allow for the estimated increase in plant weight) equivalent to 30 and 20% moisture for the well-watered and drought-subjected, respectively. The amounts of water added to each pot were recorded. The well-watered plants were watered when they had used more than 200 g of water, but the drought-subjected plants were not watered until they showed signs of wilting. The frequency of watering depended on water use, so that smaller plants with lower transpiration rates were watered less frequently than larger plants

The pots were randomised within ten blocks that were rotated and plants re-randomised each week to try to ensure each pot experienced a similar environment. Fertiliser was added weekly as a liquid feed (Vitafeed III; N 19%, P_2O_5 19%, K_2O 19%), a balanced feed, suitable for a wide range of plants and conditions. The plants were sprayed twice with Benlate and Calixin to control powdery mildew.

Five blocks were harvested 42 days after planting (first harvest) and the remaining five blocks after 135 days (second and final harvest). At each harvest the plant height and fresh and dry weight of the tops were determined. The roots were washed free of soil and the numbers of galls counted (first harvest), or scored on a 1-5 scale (second harvest). The nematodes per root system were determined by staining the whole root system (first harvest), or 10% of the chopped and mixed root system (second harvest), with acid fuschin in lactic acid. The roots were examined in glycerol (first harvest) under a stereomicroscope, or after blending in 150 ml water for 30 s at low and then high speed (second harvest), and the numbers of nematodes present and stages of development determined.

The effects of *M. incognita* and of drought on water relations were determined once a week between 13-16 h over 15 weeks by measuring the stomatal conductance (gs) on the abaxial and adaxial side of the upper leaflet of the youngest full-grown leaf of every plant using a diffusion porometer (MK3, Delta-T Devices Ltd, Cambridge, UK). In addition, immediately before the final harvest, two leaves were taken from each plant. One came from the lower part of the plant (the ninth leaf) and one from the upper part (the fourth leaf) counting from the top of the plant. The leaves were dried at 80°C for 48 h, ground to a fine powder and sub-samples of 1 mg were combusted, and the resulting CO_2 was analysed for the relative abundance of ^{13}C and ^{12}C, the ratio of which is thought to reflect stomatal conductance (Farquhar *et al.*, 1982). The ratios of $^{13}C/^{12}C$ of the samples were expressed as $^{13}\delta C$ (per thousand); the carbon isotopic composition was determined in 1 mg samples relative to Pee Dee Belemnite standard ($^{13}\delta C$) using a ratio mass spectrometer (Europa Scientific, UK). During the carbon analysis, the percentage of N and its isotope composition were also determined.

Results

In the hatching test, 73% of the eggs hatched and this is assumed to reflect the true level of viability. The effective inoculum densities were, therefore, 0.0, 0.03, 0.16, 0.80, 4.0, and 20.0 viable eggs/g dry soil. These are the values used hereafter.

At the first harvest, the numbers of galls and estimated numbers of nematodes per root system and per g root progressively increased with inoculum densities up to 4.0 viable eggs/g soil, but slightly decreased with 20 eggs (Table 1). Percentage invasion was low throughout; ranging from 4.9% with the lowest inoculum density to 0.7% of viable eggs with the highest inoculum density. The numbers of galls and the total numbers of nematodes were slightly lower in the drought treatments compared with the corresponding well-watered plants but the differences were not significant.

After 135 days, the greatest number of nematodes was produced by plants inoculated with 0.16 eggs/g soil and the greatest number per g root by those inoculated with 0.8 eggs (Table 1). The highest inoculum density (20 eggs/g soil) produced plants with the smallest root systems (Table 2) and the fewest nematodes at the second harvest. The reproduction rate (final population/initial population [*Pf/Pi*]) progres-

Table 1. *Number of galls or gall index and numbers of nematodes per plant or per pot 42 and 135 days after planting.*

Eggs, *Pi* Per pot	Viable/g	First harvest (42 days)			Second harvest (135 days)		
		Number of galls	Number of nematodes/plant		Gall index *	Total nematodes per pot (*Pf*)	Pf/Pi
			Females	J2			
			Well watered				
0	0.00	0	0	0	1.0	0	0
160	0.03	5	4	1	2.2	43 642	273
800	0.16	16	13	3	3.6	90 052	113
4 000	0.80	89	81	11	3.2	78 892	19.7
20 000	4.00	531	512	54	2.6	16 221	0.81
100 000	20.00	486	487	45	2.7	11 015	0.11
			Drought-subjected				
0	0.00	0	0	0	1.0	0	0
4 000	0.80	63	58	7	3.2	74 204	18.6
100 000	20.00	427	416	35	2.8	19 680	0.2
LSD 5%		167	188	36	1.0	47 939	-

Results are means of five replicates.
*On a 1-5 scale.

Table 2. *Growth of tomato plants 42 days after planting.*

Pi Viable eggs per g soil	Number of leaves	Plant height (cm)	Fresh weight				% dry matter (shoot only)	Shoot to root ratio
			Shoot (g)	Root (g)	Total (g)	Shoot (dry weight) (g)		
				Well watered				
0.00	16.2	55	90	4.4	94.4	9.4	10.4	21
0.03	15.4	55	85	4.3	89.3	9.8	11.6	20
0.16	15.8	56	89	5.0	94.0	9.7	10.9	18
0.80	16.6	57	79	3.4	82.4	9.2	11.5	23
4.00	16.0	55	77	3.8	80.3	8.8	11.5	20
20.00	14.0	42	57	4.1	63.1	7.8	13.6	14
				Drought-subjected				
0.00	14.8	48	62	2.6	64.6	7.0	11.3	24
0.80	14.6	48	58	2.4	50.4	6.4	11.1	24
20.00	14.0	34	37	2.7	39.7	4.1	11.8	13
LSD 5%	4.8	12	27	1.5	28.3	2.4	1.3	6.2

Results are means of five plants.

sively decreased from × 273 at $Pi = 0.03$ eggs/g, to × 0.11 at $Pi = 20$ eggs/g soil for well-watered plants and × 0.20 for the equivalent drought-subjected plants (Table 1). When the mean numbers of nematodes per plant at first harvest were used as the Pi, then the reproductive rate increased to × 8728 for the plants inoculated with 0.03 eggs and to × 21 for those with 20 eggs/g soil.

At the first harvest, compared with the uninfected control plants, only the highest inoculum density (20 viable eggs/g dry soil) affected plant growth; mean plant weight, shoot fresh weight, total fresh weight and the root/shoot ratio were all significantly ($P \leq 0.05$) decreased (Table 3). Leaf number was not significantly affected, but height was decreased ($P \leq 0.05$), indicating that internode length had been decreased. It was calculated that the highest inoculum density also decreased leaf size; a mean of 4.1 g of top weight per leaf for the most heavily infested plants compared with 5.6 g per leaf for the uninfected control plants. The highest inoculum density significantly increased percentage dry matter in the shoot (Table 3).

At the second harvest (Table 2), shoot fresh weight and numbers of fruit and total fresh weight were significantly ($P \leq 0.01$) decreased by the three highest inoculum densities (0.8, 4.0 and 20.0 eggs/g soil). The shoot/root ratio was also significantly ($P \leq 0.05$) decreased with 0.16, 0.80, and 4.0 eggs/g soil. However, compared with the uninoculated plants, the lowest inoculum density (0.032 eggs/g soil) significantly ($P \leq 0.05$) increased shoot and root fresh and total dry weight.

Using equation (1), the relationship between total plant weight as a percentage of the uninoculated value at the first harvest against inoculum density (log viable eggs/pot) was examined and showed (Fig. 1) a significant negative, log-linear relationship ($P \leq 0.05$) accounting for 68% of the variance.

$$Y = - 10.55(\log Pi) + 123$$

At the second harvest there was a much greater negative, linear relationship ($P \leq 0.0007$, variance accounted for 98%) between log Pi and total fresh weight as a percentage of the uninoculated (Fig. 1).

$$Y = - 38.07(\log Pi) + 199$$

It could be argued that six data points are insufficient to fit the Seinhorst equations, but nonetheless an attempt was made to estimate the parameters in equations (2) and (3) (Table 4). Parameters were estimated using maximum likelihood in the statistical package GENSTAT (Payne *et al.*, 1987). Simultaneously estimating all four parameters in equation (2) proved difficult without fixing the estimates of at least two of them. Equation (3) presented no difficulty in estimating all parameters simultaneously and described the data more effectively than equation (2) (Fig. 2). For the first harvest the model accounted for 86.2% of the variation and for the second harvest

Table 3. *Growth of tomato plants 135 days after planting.*

Pi Viable eggs per g soil	Plant height (cm)	Fresh weight				Dry weigth shoot + fruit (g)	Shoot to root ratio
		Shoot (g)	Root (g)	Fruit (g)	Total (g)		
			Well watered				
0.00	124	232	53	107	391	43	4.4
0.03	131	302	90	69	461	51	3.4
0.16	119	183	91	84	388	39	2.0
0.80	115	157	68	30	255	40	2.3
4.00	103	71	32	30	132	28	2.3
20.00	91	55	14	0	69	19	3.9
			Drought-subjected				
0.00	109	199	45	36	280	35	4.4
0.80	99	139	58	22	219	28	2.5
20.00	69	28	9	0	37	10	3.2
LSD 5%	12	27	22	45	82	6.3	2.0

Results are means of five plants.

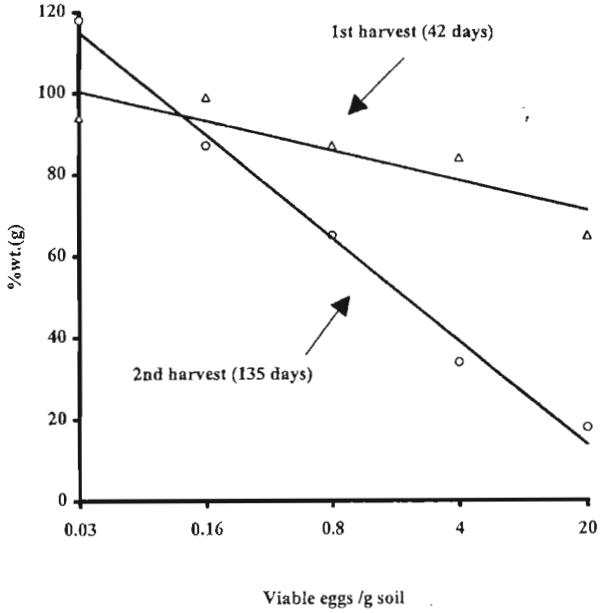

Fig. 1. Total fresh weight at the first (42 days) and second harvests (135 days) as a percentage of the uninoculated treatment regressed against viable eggs per g soil on a log. scale. Results are means of five replicates.

94.6% of the variation. Using equation (2), with the estimated values of T and of $Ymax$ fixed, the value of z was estimated to have decreased from 0.88 at the first to 0.42 at the second harvest (Table 4). With equation (3), the estimates of m and of T had decreased from 0.66 (of $Ymax$) and 0.34 eggs per g soil respectively at the first harvest to 0.22 (of $Ymax$) and 0.06 eggs per g soil at the second harvest.

Plant height, shoot fresh and dry weight and root fresh weight were less, at both harvests, for drought-subjected compared with the equivalent well watered plants (Tables 2, 3). At the first harvest, the percentage differences (-32%, -27%, and -35%) in total fresh weight were similar for the three inoculum densities (0.0, 0.8, and 20 eggs/g soil, respectively). At the second harvest, the relative decrease associated

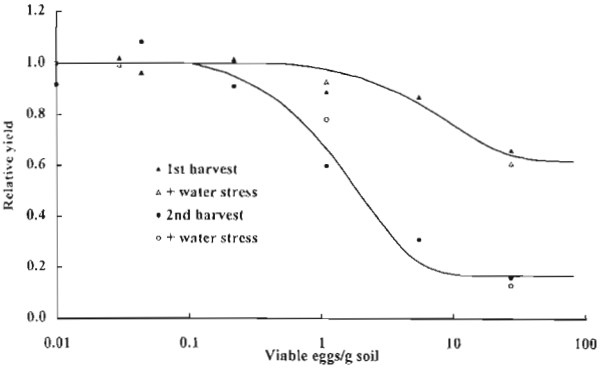

Fig. 2. Relationship between total fresh weight at the first (42 days) and second harvests (135 days) and viable eggs per g soil as described by fitting parameters to the Seinhorst yield loss model (equation 3).

with the drought treatment was less than at the first harvest for plants with 0.0 and 0.8 eggs/g soil (-29% and -14%, respectively) but greater for the plants inoculated with 20 eggs/g (-47%). However, there was no significant interaction between the nematodes and the drought treatment (Fig. 2).

Water used per plant was decreased overall *ca* 35% by drought, and increasingly by the three highest inoculum densities with the most heavily infested, well watered and drought-subjected plants having 72 and 76% decreases, respectively, compared with the corresponding uninfected plants. After 42 days, water use per g dry matter produced had been largely unaffected by drought or by the *M. incognita*, but after 135 days drought and the highest inoculum density had significantly ($P \leq 0.05$) increased water use efficiency (data not shown).

The porometer measurements before the first harvest showed that, compared with the well watered, uninoculated plants, stomatal conductance (Table 5) tended to be decreased by drought but was unaffected by *M. incognita*, except perhaps for the highest inoculum density. Between the first and second harvests, stomatal conductance was slightly decreased by the drought treatment and by 0.16, 0.8 and 4.0 eggs/g soil

Table 4. *Estimates parameters for equations (2) and (3) based on total fresh weights (* indicates parameters fixed).*

Harvest	Equation (2)					Equation (3)		
	T^*	Y_{max}^*	m	Z	Z^T	T	Y_{max}	m
First	0.3	91	0.61	0.88	0.90	0.342	96.28	0.664
Second	0.032	415.0	0.23	0.42	0.92	0.057	422.6	0.221

Table 5. *Mean stomatal conductance measured weekly.*

Pi viables eggs per g soil	Stomatal conductance (cm/s)			
	To 42 days		To 135 days	
	Watered	Drought-subjected	Watered	Drought-subjected
0.00	1.53	1.34	0.29	0.25
0.03	1.72	-	0.35	-
0.16	1.84	-	0.26	-
0.80	1.75	0.92	0.27	0.22
4.00	1.90	-	0.24	-
20.0	0.96	1.24	0.19	0.19
LSD (5%)	0.92		0.09	

Results 42 and 135 days are means of ten plants measured on three occasions, and of five plants measured on nine occasions, respectively.

of *M. incognita*. The highest inoculum density (20 eggs/g soil) significantly ($P \leq 0.05$) decreased the stomatal conductance of the well-watered plants, which was not further decreased by drought.

Neither nematodes nor drought had any effect on the ^{13}C composition of either the lower leaves or upper leaves (Table 6). All the values were close to -28 and, with one exception, none were significantly different from the well-watered, uninoculated control plants. The exception was the drought-subjected plants with 0.8 viable eggs/g soil where the value discrimination in favour of ^{12}C increased ($P \leq 0.05$) rather than decreased as might have been expected if the plants were water stressed.

The percentage of N was significantly ($P \leq 0.05$) increased in the lower leaf dry matter by both drought and by the highest level of nematode infection and in the upper leaves by the highest level of nematodes only (Table 6). The intermediate level of infection had no consistent effect on the lower leaves but consistently increased the percentage of N in the upper leaves of the drought-subjected plants.

Table 6. *13δC values for upper and lower leaves and percentage of nitrogen (%N) in leaf dry matter at 135 days.*

Pi viable eggs per g soil	$^{13}\delta C$		%N	
	Lower leaves	Upper leaves	Lower leaves	Upper leaves
		Well watered		
0.00	-28.3	-28.3	4.75	4.95
0.03	-28.1	-27.7	4.90	5.03
0.16	-28.3	-27.7	4.70	5.27
0.80	-27.9	-27.6	4.58	4.77
4.00	-28.0	-27.3	4.53	5.28
20.00	-27.9	-27.7	5.94	6.31
		Drought-subjected		
0.00	-28.1	-28.2	5.66	4.63
0.80	-29.9	-27.7	4.91	5.72
20.00	-27.9	-27.8	6.22	6.72
LSD (5%)	-0.60	-0.70	0.86	0.99

Discussion

We hypothesised that on good hosts, initially small populations of *M. incognita* which cause little or no damage at the start of the growing season, can increase so greatly that they cause substantial damage later in the growing season. Tomato cv. Moneymaker fulfilled the requirement of being a good host, as shown by the rates of the population increase of the *M. incognita* at the lower population densities, and the results obtained with it broadly supported the original hypothesis.

The general principle (Seinhorst, 1967) that the multiplication rate of sedentary nematodes is density dependent was again confirmed with the greatest rate of increase observed at low values of *Pi*. With an inoculum of 0.03 eggs/g soil there was a × 273 increase in the population density over the whole of the experiment compared with × 0.11 increase with a *Pi* of 20.0 eggs/g soil. However, as found with *M. arenaria* by McSorley *et al.* (1992), the highest *Pf* was produced by intermediate values of *Pi* and it was again demonstrated that on a good host, and in favourable conditions, small populations can become very large during the lifetime of one crop.

A much greater proportional effect on plant growth (total plant weight) of the intermediate inoculum densities at the second as compared with the first harvest was demonstrated, supporting the hypothesis that initially non-damaging population densities can increase so greatly that they become damaging during the lifetime of an annual crop. Also, the slope of the linear regression of total plant weight as a proportion of the uninoculated yield against increasing log inoculum density was much greater at the second than at the first harvest. Using the Seinhorst equation (3) to fit lines to the data showed that, for the first and second harvests, the respective estimates of T (the tolerance limit) and of m (the minimum yield) were always higher for the first harvest data then for the second harvest data. As at planting, the plants were small (< 2.0 g), their contribution to m at either harvest would have been small. Estimating z in equation (2) showed that it was also much higher for the first harvest (where it measures the damage caused by a single nematode) compared with the second harvest (where it measures the damage caused by a single nematode and all its progeny).

Equation (3) has the assumption that $z^T = 0.95$, which is largely derived for observations with *Globodera* spp. (Seinhorst, 1986) with one generation in a growing season. Applying this assumption to the data for the second harvest, where there will have been up to three generations of the *M. incognita*, still resulted in equation (3) providing a better fit to the data than equation (2). This approach has also been successfully used by Di Vito *et al.* (1985, 1997) to describe yield losses in relation to *M. incognita* on *Capsicum* and *Hibiscus*, respectively.

An important conclusion from these results is that the estimated threshold for damage (T) at the second harvest of 0.057 viable eggs per g soil is so low that it is on the border line of detection, *i.e.*, 11.4 viable eggs of J2 in 200 g soil. In a series of studies of *M. arenaria* damage to peanut in microplots McSorley *et al.* (1992) reported closer fits with the Seinhorst equation (r^2 ranged from 0.79 to 0.98) and similar low T values (as low as 1.0 egg/100 g soil), except in one trial where the value of T was higher (23 eggs/100 g soil). Their estimated value of z varied from 0.90 to 0.99, indicating that *M. arenaria* was much less damaging to peanut than was *M. incognita* to tomato.

The lowest inoculum density (0.03 eggs/g soil) had apparently increased tomato growth at the end of the experiment. A similar increase was observed with a *Pi* of 0.31 eggs per g soil by Niblack *et al.* (1986) with *M. incognita* on partially resistant soybean cultivars Bragg and Braxton in two microplot experiments. In contrast the yield of the susceptible cultivar GaSoy 17 was greatly reduced at *Pi* of 0.31 eggs.

Under ideal growing conditions, nematodes may cause only moderate damage, whereas under periods of drought, or other stress factors, they may cause considerably more damage (Wallace, 1973). Moreover, the relative host sensitivity of a plant measured under glasshouse conditions, where there is minimal stress, frequently differs from that of plants grown under field conditions. The drought stress applied in this experiment decreased the growth of the uninfested and of the *M. incognita* infested plants equally and generally reduced their stomatal conductance compared with the comparatively well watered plants. However, there was no evidence of an interaction between the stress induced by the drought and that of the *M. incognita*. Wheeler *et al.* (1991) also found that the effects of damage to tobacco by *M. incognita* and the effects of drought were additive and not interactive.

Although both the *M. incognita* and drought decreased stomatal conductance, indicating that both increased water stress, this was not reflected in the ratio of the stable isotopes 12/13 of carbon. Drought generally decreases the degree of discrimination so that a value of -21 is typical of drought stressed plants (Haverkort & Valkenburg, 1992). However all our plants had similar values close to -28. That the drought-subjected plants grew less well than the well watered plants suggests that they were water stressed for at least some of the time but this was not reflected in the carbon isotope ratio. Consequently, the stomatal conductance results are the more meaningful and they support the hypothesis that a damaging infection with *M. incognita* does increase water stress, leading to

reduced growth. The increased percentage nitrogen in the leaf dry matter of the most heavily infested plants indicates that they were not N deficient, and that some other factor, such as water stress, must have been responsible for their poor growth. However, the possibility has not been excluded of other physiological effects being involved in reducing the growth of *M. incognita* infested plants.

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