Historical Perspectives of Nematode Taxonomy

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It is especially appropriate on this twenty-fifth anniversary of the founding of the Society of Nematologists to reflect on and review the status of nematode taxonomy. It is appropriate because the science is in an exciting period facing significant changes brought on in part by new tools, methods, and concepts. Also, the explosive increase in new species and new taxa at all levels has provoked significant questions as to the validity of our classification systems.

Taxonomy is a broad subject which, according to Sneath and Sokal (29), includes taxonomy *sensu stricto* (the theoretical study of classification), systematics (study of organisms and of relationships among them), classification (ordering of organisms into groups on the basis of their relationships), and identification (assignment of unidentified organisms to the correct class once a classification has been established).

Our review will include 1) the preparation of individual specimens, 2) the description of species, and 3) the concepts that governed the definitions of taxa at specific and higher levels. Although this review mostly concerns plant-parasitic species, the principles should apply throughout the Phylum Nemata.

PREPARATION OF SPECIMENS

Optimum preservation of specimens is an essential step leading toward the description and definition of a species. Direct examination of fresh material in temporary water mounts as soon as possible after relaxation by heat is one of the best procedures for clarity, especially for internal structures. Obviously, the time constraint and need for study over longer periods limit the usefulness of this technique.

The relaxation and killing process is critical for good preservation of specimens; cold fixatives produce excessive distortion, whereas, overheating results in destruction of internal organization and obscures structures. Heating to 60 C and cooling as quickly as possible after reaching this temperature is still the preferred method for relaxing and killing nematode specimens.

Fixatives have caused even greater damage to specimens. Corrosive sublimate (bichloride of mercury with osmic acid), commonly used by N. A. Cobb, is extremely caustic and destroys most of the delicate structures of nematodes, greatly limiting the value of studies made on such specimens. For example, the genus *Nemonchus* proposed by Cobb (7) later was shown by Thorne (32) to be based on artifacts in the badly fixed specimens of its type species, *N. galeatus*. *N. galeatus* is now considered to be a synonym of *Hoplolaimus coronatus*.

Currently, the most commonly used fixative–preservative is formalin. It has been our experience that commercial grade formalin is not suitable; reagent quality is needed. It should be stored with calcium carbonate ($CaCO_3$) to neutralize its acidity. Filtration at time of use is a simple procedure to remove any particles of the carbonate (1). FAA is very useful for many species, but the acetic acid component may adversely affect specimens even after mounting. For such species, triethanolamine-formalin (TAF) (13) is preferred, but exposure should be limited to 24 hours or less or excessive clearing of specimens may occur. In sum, despite many years of use, fixatives–preservatives have many limitations, and improved materials and (or) procedures are still needed. Optimum fixation for a given species requires selection of the best procedure precisely for that species.

For mounting on slides, glycerin remains the best medium to date. Canada balsam was used earlier, another favorite of Cobb's, but it proved to be objectionable because many specimens were damaged in the preservation process and remounting was difficult. Many type specimens preserved in balsam are essentially useless for study today, whereas glycerin-mounted specimens usually survive indefinitely and can be recovered easily for repositioning, sectioning, or scanning electron microscope (SEM) preparations.

Our understanding of the morphology of nematodes has been greatly enhanced by increasing uses of electron microscopy. The application of scanning electron microscopy, especially, has been one of the most significant advances in recent years. Although SEM is limited to external structures, vastly improved insights are now possible for details invisible by light microscopy.

Transmission electron microscopy (TEM) is another valuable new tool. It is rarely used directly for purposes of classification and even less so for identification, but better knowledge of ultrastructure may solve certain taxonomic problems. TEM has been useful in the discovery of new characters in the body wall of heteroderid females and in the discovery of homology of pore phasmids of heteroderid juveniles (2,5). This knowledge ultimately will contribute to a better classification of the Heteroderidae. Another result of detailed ultrastructure studies is that once the exact arrangement of organs is known, it becomes possible to recognize this arrangement even with the light microscope. Electrophoresis, chromatography, serology, DNA characterization, and the use of monoclonal antibodies are exciting new techniques which hold promise of distinguishing specific differences not detectable by other means.

Morphology is only one among several sources of data that can be used to characterize a species. Increased knowledge of the biology of nematodes is undoubtedly among the most significant advances helping to understand speciation and relationships. Many examples can be found where differences in comparative life habits support taxonomic classifications; e.g., feeding mechanisms and host plant responses were essential supportive factors in establishing *Trophotylenchulus* as a distinct taxon separate from *Tylenchulus* (12). Another example is in the Paratylenchinae, where *Gracilacus* has been questionable as a taxon separate from *Paratylenchus*. Discovery of a new species of *Gracilacus* by Cid del Prado-Vera and Maggenti (unpublished) under the bark of redwood roots has given evidence of a life habit very different from *Paratylenchus*. This information is important in confirming the status of *Gracilacus*. Chromosome counts have been used to differentiate *Meloidogyne* species for some years now. More recently (22), this character (plus male behavior) has been the basis for separating *Radopholus citrophilus* Huettel, Dickson and Kaplan, 1984 from *R. similis* (Cobb).

As more knowledge is gained, biological differences, including life cycles, reproduction, etc., combined with morphological distinctions, give greater validity to higher classification categories from generic level upwards. However, morphology is still the major source of information presented in the descriptions of new species.

Description of Species

The way species have been described, including measurements and illustrations, has varied greatly during the history of nematode systematics. The quality of early illustrations was widely variable. For instance, Bastian (3) published simple outline drawings mostly of the anterior end, including esophagus and tail at low magnifications. These were crowded 70 or more to a page with little detail shown. In contrast, illustrations by de Man (15), Cobb (8), and others were lavish, very artistic with full length reproductions of the entire nematode, showing all the organs, often with their names as in a textbook. At the same time, the descriptions were lacking in many details that could help identify the nematode. For example, the illustrations of *Dolichodorus heterocephalus* by Cobb (8) do not give clear detail of the esophago-intestinal junction and the genital branch (columned uterus). In the drawings of *Hoplolaimus coronatus* by Cobb (9), the prominent cephalic framework is obscured by external details, and in *Tylenchus cancellatus* Cobb (11), the junction between esophagus and intestine is poorly defined and the shape and structure of the spermatheca obscured by extraneous details. Thorne and Swanger's (35) monograph of *Dorylaimus* and Thorne's Dorylaimoidea (33) were exceptional, with detailed drawings at higher magnifications and artfully spaced for maximum visibility and reference. However, most illustrations generally have not included variability, only one shape per organ being given.

Measurements for the most part were given for a single specimen. When several specimens were measured, only the mean, without the range or standard deviation, was given. The de Manian formula has been one of the most widely used for many years. Besides total length (L), it includes alpha (L/width), beta (L/distance from anterior end to end of esophagus) and gamma (L/tail). The use of these ratios has been shown to pose various statistical problems (18,24), but they continue to be given in every specific description. Cobb (1913) proposed a much more elaborate scheme of measurements and formulae for ratios, but this never found wide acceptance among taxonomists and was completely abandoned in the 1940s.

Descriptions were brief among the earliest workers. Details necessary for differentiating from other taxa were lacking. Later, verbose, rambling descriptions were preferred, including many nonrelevant characters or observations, but omitting many important details. Cobb's (6) description of *Tylenchus olaae* which begins with the words, "The colorless or yellowish transparent cuticle is a striking feature of this worm . . .," and the lengthy description of *Paratylenchus nanus* Cobb (10) are examples.

Diagnosis often was missing and in fact was not required by the Code of Nomenclature for works published before 1931 (Art. 12). Type material was not mentioned, and most types were lost or never saved. Though highly desirable, designation of type specimens is still not required by the International Code of Zoological Nomenclature.

In the more recent past, illustrations became more schematic and stylized. The organs represented were not realistic and did not correspond to the actual feature in the specimens. Very few illustrations were given. Generally, only part of the animal was shown, those selected as the differentiating characters according to the author's judgment. Sher's (27) redescription of *Helicotylenchus dihystera* is one example of this practice.

Measurements were obtained from several paratypes, but only the range, sometimes the mean, was given. De Manian ratios and other similar ratios were widely used.

Descriptions were concise, consisting of a short statement of the few identifying characters. Diagnoses were usually given, but fell short of achieving their purpose (19).

Type material was saved. Perhaps the first formal designation of type specimens was made by Chitwood (4), when he established topotypes and syntypes for some of the *Meloidogyne* species. Up to that time, it was the usual practice to give no references, descriptions, or measurements for type specimens.

The overall quality of descriptions has dramatically improved in recent years. Illustrations now tend to be more realistic and show the actual shapes observed in specimens. The quality still is quite variable. Some authors give detailed figures using knowledge gained by TEM (25,26) and SEM (indispensable for greater detail of cuticular characteristics of face view, amphid shapes, lateral field, and structures associated with the vulva, caudal alae, and (or) cloaca). Other authors rely on starkly schematic, outline drawings. More emphasis is now being given to variability. Authors often present many different representative shapes observed in their species.

Measurements now are more representative and useful. In more and more descriptions, standard deviations are given in addition to the mean and range. Unfortunately, many authors still give only the mean or, in some cases, only the range. In some recent descriptions, actual measurements are given in addition to the ratios. This practice, however, seems impractical in view of the great volume of data implicit in such reporting. To save space, some authors (or review editors) have started grouping measurements in tables, where all statistical parameters (mean, standard deviation, range, eventually coefficient of variability, etc.) can be conveniently displayed.

Descriptions are more complete, including many characteristics that may or not be diagnostic. Goodey (21) expressed the belief that new species should be described in exhaustive detail, which may be desirable but is not practical. Of much greater importance is preservation of type specimens to be available for future studies as needed. Type material is now almost universally saved and catalogued. Unfortunately, some authors persist in keeping type material in their personal collections in disregard of Recommendation 72D of the International Code of Zoological Nomenclature.

It is encouraging to note the progress and improvements evident in the quality of preserved specimens, the increasing number of specimens of a given species available for systematic studies, the broader distribution and deposition of types, and more thorough descriptions of new taxa. However, there is much room for future improvements, especially concerning more precise selection of methods of fixation and processing to maximize preservation of structures of the entire specimen. Also, availability of type specimens to other researchers is far from satisfactory. Correct and complete descriptions of populations are necessary, but in the end, it is the concepts that govern the proposal of a new name that are the decisive factors in its validity and eventual acceptance by the nematological community. Here again, these concepts have been rapidly changing, both at the specific as well as higher levels of classification.

The Species Concept

For almost 100 years, starting from the mid-1800s, nematode species descriptions were based mainly on typological concepts. These concepts considered species as completely defined in reference to their type, an "ideal" representation of the species. Individual variations were disregarded, and members of a population were held to be replicas of the "type". Because of limitations of extraction methods, species often were described from a single, and often only, available specimen. This was judged to represent the "type" even when more specimens were available for study. One notable exception was Dujardin (16), who gave ranges for several measurements on many of the species he studied.

The typological approach was followed by many authors even as late as the 1960s, when species were described very succinctly and only one value given for each measurement (34). This typical measurement often was the average of several individual measurements, but lack of range and (or) standard deviation gave no clue as to the nature and extent of deviations, which suggested that for those authors, a species should conform to a "type."

Gradually, an idea of variability began to appear in nematological taxonomy. Species were described from larger samples (5–15 specimens) from the type population. The philosophy was, and often still is, typological in the sense that any specimen–population not identical to the type may be considered as belonging to a different species.

Characteristic of this approach is the use of the "range" for measurements. Specimens out of the range for one measurement are, for some taxonomists, considered to be out of the species definition, because they do not fit the ideal "type" of the species. One result of this approach has been a rapid multiplication in the number of nominal species.

Beginning in the 1950s, some authors began to measure and describe several populations in addition to the type population when redescribing known species as well as for descriptions of new species. Raski and Golden (23) were among the first to use this approach in nematology. Initially, such populations were cited as isolated examples of the species. Because the means and the standard deviations were seldom, or never, given, it is not possible to utilize fully these descriptions for a better understanding of the extent of variability of the species.

A more elaborate concept of a composite description of species was discussed by Fortuner (18) and used by Fortuner, Maggenti, and Whittaker (20) in the redescription of *Helicotylenchus pseudorobustus*. Application of such statistical definitions is especially useful for complex species groups such as *Helicotylenchus*, which are abundant, widely distributed and quite variable in morphology.

The definition of composite descriptions is possible only in a very small percentage of the nominal plant-nematode species. Most species are known from only one, or a few, populations, each studied from few specimens. New taxa will continue to be described from a few specimens from one locality, and rightly so, when the new species extends our understanding of a genus or brings to the attention of nematologists the existence of a potentially dangerous parasite. It is hoped, however, that the statistical descriptions of species will be used more often by all nematologists.

Descriptions and definitions of species were discussed first, because species are the blocks from which classifications are built. At higher levels, many successive or concurrent classification schemes have been proposed over the years for plant nematodes. Authors at times have held very divergent views on how nematodes should be arranged because of differences in their approach to taxonomy or because of the nature of the taxonomic material they held.

Higher Level Classification

Most early attempts to classify nematodes were handicapped because of the few species known, most of which were inadequately described. Groupings were made on superficial resemblances. For example, de Man (14) placed four genera—*Tylopharynx, Tylencholaimus, Tylenchus,* and *Aphelenchus*—in one family, *Tylolaimidae*, whereas today they are in three separate orders.

Filip'ev (17) published one of the first attempts to classify the Tylenchinae (equivalent to Tylenchina today). In this article, Filip'ev placed *Dolichodorus heterocephalus* with genera having head without chitinization, because Cobb's (8) description of this species omitted this characteristic. Furthermore, he grouped *Hemicycliophora* and *Macroposthonia* with genera having degenerate stylet, because they were described from males only, which were entirely lacking a stylet. *Ditylenchus* was characterized as having "bursa caudal." In all, only 19 genera were included in the classification of Filip'ev, so his picture was far from complete.

The recent history of plant-nematode classification has been influenced by two major concepts: 1) Classification must make identification easy. Siddiqi (28) began a chapter on taxonomic methods with these words: "Taxonomy is a fundamental science which deals with the recognition of taxa. . . ." 2) A "big" genus must be broken into smaller units. By big, most nematologists mean a genus with more than 50 species. This may be compared to the situation in entomology, where much larger genera are well accepted. For example, the genus *Aphis* includes more than 1,500 species.

For other authors, the most important consideration is monophyly. Identification comes later when the problem occurs to fit taxa into their existing categories.

Recent developments have brought about renewed interest in taxonomic philosophies with the proposal of the phenetic and the cladistic schools and the redefinition of evolutionary taxonomy. Whichever school is followed, modern classifications are based on more reasoned, deliberate, and sound concepts. These have had invaluable assistance from better knowledge of nematode biology. For example, *Deladenus* was placed in Allantonematidae after the discovery of its double cycle. Remote areas and uncultivated native habitats such as arctic, antarctic, deserts, virgin tropical forests, etc., have protected relict forms, collections of which help us overcome the lack of fossils in our attempt to discover past evolution; e.g., *Antarctylus* with links to *Helicotylenchus, Acontylus* and *Senegalonema* with links to *Rotylenchulus, Basirienchus* with links to *Basiria*, etc.

As more and more species have been described from such areas and other cultivated areas in the world, our knowledge of nematode diversity has likewise increased. Evolution has become apparent in certain characters, and large gaps in our understanding of nematode relationships are being filled. For example, when *Brachydorus* was proposed on the basis of a single species, this genus was readily differentiated from *Dolichodorus*. Since then new species have been described in both genera until it has become apparent that the differences in stylet lengths and tail shapes were only the extremes in a continuous range of sizes and shapes, and the two are indeed congeneric.

The last 10 years have seen the publication of many interesting studies in systematics, resulting in several new classifications for various taxa. The principles of cladism have been applied to the family Heteroderidae (Baldwin), Leptonchoidea (Ferris), Longidoridae (Coomans), Nemata (Lorenzen). Studies of numerical taxonomy have been published. The present authors, together with Maggenti, Luc, and Geraert, have been preparing a general revision of Tylenchina, following the principles of evolutionary classification.

These modern classifications often are based on hypotheses that need to be tested further, and much remains to be done on the study of diagnostic characters and evaluation of their role as evolution markers.

Once a classification system is created, it remains for the taxonomist to offer methods to assign unknown organisms into their correct taxonomic category. There again, the methods used for identification are rapidly changing.

Identification

Dichotomous keys have long been the principal tool for nematode identification. These sequential keys employ one or two characters at a time. Paragraph keys were the first to be used in nematology and were favored, for example, by Cobb. Scientists from the USSR and Eastern Bloc countries are still using this kind of identification key. Goodey and Thorne introduced the line or bracket key, where both terms of each dichotomous alternative are presented close to each other. An advantage to this second kind of key is that it can be followed backwards. Dichotomous keys have worked well and are still usable to identify species in small genera with well-defined specific criteria. However, they cannot easily handle variability of key characters, and they are difficult to update.

Tabular keys are more accurate than dichotomous keys because comparisons utilize all available characters simultaneously. They are also easy to update and useful in accounting for intraspecific variability. Generic compendiums have long been used in nematology, one of the first of which was presented by Tarjan (31). The first true tabular key seems to be the one Stegarescu (30) proposed for *Longidorus*. The only drawback for this kind of key is that it soon becomes very difficult to use when it has to accommodate more than a dozen characters and more than two or three score species. It then becomes necessary to use a computer to do the necessary lengthy comparisons.

Conclusion

Nematode taxonomy has long developed without taking into consideration some of the theoretical questions raised by its uses and methods. The last 25 years have seen the introduction of new taxonomic philosophies, new statistical procedures, new research tools, etc., many of which are currently accepted and utilized. It remains to use them to solve practical taxonomic questions and propose general nematology classification and identification schemes that are accurate and easy to grasp.

Literature Cited

1. Baker, J. R. 1945. Cytological technique. London: Methuen.
2. Baldwin, J. G. 1983. Fine structure of body wall cuticle of females of *Meloidogyne charis, Atalodera lonicerae*, and *Sarisodera hydrophila* (Heteroderidae). Journal of Nematology 15:370–381.
3. Bastian, H. C. 1865. Monograph on the Anguillulidae, or free nematoids, marine, land, and fresh-water; with descriptions of 100 new species. Transactions of the Linnean Society, London 25:73–184.
4. Chitwood, B. G. 1949. Root-knot nematodes—Part 1. A revision of the genus *Meloidogyne* Goeldi, 1887. Proceedings of the Helminthological Society of Washington 16:90–104.
5. Cliff, G. M., and J. G. Baldwin. 1985. Fine structure of body wall cuticle of females of eight genera of Heteroderidae. Journal of Nematology 17:286–296.
6. Cobb, N. A. 1906. Free living nematodes inhabiting the soil about the roots of cane, and their relation to root diseases. Pp. 163–195 *in* Fungus maladies of the sugar cane, with notes on associated insects and nematodes. Bulletin 5, second ed., Hawaiian Sugar Planters' Association Experiment Station, Division of Pathology and Physiology.
7. Cobb, N. A. 1913. New nematode genera found inhabiting freshwater and non-brackish soils. Journal of the Washington Academy of Science 3:432–444.
8. Cobb, N. A. 1914. The North American free-living freshwater nematodes. Contributions to a science of nematology, 2. Transactions of the American Microscopical Society 33:69–134.
9. Cobb, N. A. 1923. An amendation of *Hoplolaimus* Daday, 1905, nec auctores. Journal of the Washington Academy of Science 13:211–214.

10. Cobb, N. A. 1923. Notes on *Paratylenchus*, a genus of nemas. Journal of the Washington Academy of Science 13:254–257.

11. Cobb, N. A. 1925. Biological relationships of the mathematical series 1, 2, 4, etc., with a description of a new nema, *Tylenchus cancellatus*. Contributions to a science of nematology XV. Journal of the Washington Academy of Science 15:235–283.

12. Cohn, E., and D. T. Kaplan. 1983. Parasitic habits of *Trophotylenchulus floridensis* (Tylenchulidae) and its taxonomic relationship to *Tylenchulus semipenetrans* and allied species. Journal of Nematology 15:514–523.

13. Courtney, W. D., D. Polley, and V. L. Miller. 1955. TAF, an improved fixative in nematode technique. Plant Disease Reporter 39:570–571.

14. De Man, J. G. 1876. Onderzoekingen over vrij in de aarde levende nematoden. Tijdschr. ned. dierk. Ver. 2:78–196.

15. De Man, J. G. 1884. Die frei in der reinen Erde und im süssen Wasser lebenden Nematoden der niederländischen Fauna. Eine systematische-faunistische Monographie. Leiden.

16. Dujardin, F. 1845. Histoire naturelle des helminthes ou vers intestinaux. Paris.

17. Filip'ev, I. N. 1936. On the classification of the Tylenchinae. Proceedings of the Helminthological Society of Washington 3:80–82.

18. Fortuner, R. 1985. Statistics in taxonomic descriptions. Nematologica 30:187–192.

19. Fortuner, R. 1985. A proposal for better diagnoses. Revue de Nématologie 8:175–177.

20. Fortuner, R., A. R. Maggenti, and L. M. Whittaker. 1984. Morphometrical variability in *Helicotylenchus* Steiner, 1945. 4: Study of field populations of *H. pseudorobustus* and related species. Revue de Nématologie 7:121–135.

21. Goodey, J. B. 1959. Data to be considered, observed and, where possible, reported upon when presenting descriptions of new species. Nematologica 4:211–216.

22. Huettel, R. N., D. W. Dickson, and D. T. Kaplan. 1984. *Radopholus citrophilus* sp. n. (Nematoda), a sibling species of *Radopholus similis*. Proceedings of the Helminthological Society of Washington 51:32–35.

23. Raski, D. J., and A. M. Golden. 1966. Studies on the genus *Criconemoides* Taylor, 1936 with descriptions of eleven new species and *Bakernema variabile* n.sp. (Criconematidae: Nematode). Nematologica 11:501–565.

24. Roggen, D. R., and R. Asselberg. 1971. The use of ratios in nematology. Nematologica 17:187–189.

25. Shepherd, A. M., and S. A. Clark. 1976. Structure of the anterior alimentary tract of the passively feeding nematode *Hexatylus viviparus* (Neotylenchidae: Tylenchida). Nematologica 22:332–342.

26. Shepherd, A. M., S. A. Clark, and D. J. Hooper. 1983. *Hexatylus viviparus* (Nematoda, Tylenchida, Hexatylina): head skeleton morphology and a comparison of head symmetry with that of *Ditylenchus dipsaci* (Tylenchina). Revue de Nématologie 6:275–283.

27. Sher, S. A. 1966. Revision of the Hoplolaiminae (Nematoda) 6. *Helicotylenchus* Steiner, 1945. Nematologica 12:1–56.

28. Siddiqi, M. R. 1986. *Tylenchida* parasites of plants and insects. Commonwealth Institute of Parasitology U.K.

29. Sneath, P. H. A., and R. R. Sokal. 1973. Numerical taxonomy. San Francisco: W. H. Freeman and Company.

30. Stegarescu, O. 1966. Application of the polytomous principle to determination of nematodes of the genus *Xiphinema* Cobb. Dans: Politomićeskij princip oprendelenija životnyh i rastenij, 55–58.

31. Tarjan, A. C. 1964. A compendium of the genus *Tylenchorhynchus* (Tylenchidae: Nematoda). Proceedings of the Helminthological Society of Washington 31:270–280.

32. Thorne, G. 1935. Notes on free-living and plant-parasitic nematodes, 2. Proceedings of the Helminthological Society of Washington 2:96–98.

33. Thorne, G. 1939. A monograph of the nematodes of the superfamily Dorylaimoidea. Capita Zoologica 8:1–261.

34. Thorne, G., and R. B. Malek. 1968. Nematodes of the Northern Great Plains. Part 1. *Tylenchida*. (Nemata: Secernentea). Bulletin 31, South Dakota Agricultural Experiment Station, Brookings, S.D.

35. Thorne, G., and H. H. Swanger. 1936. A monograph of the nematode genera *Dorylaimus* Dujardin, *Aporcelaimus* n.g., *Dorylaimoides* n.g. and *Pungentus* n.g. Capita Zoologica 6:1–223.