Fundam. appl. Nematol., 1994, 17 (2), 97-101

Forum article

THE FUTURE OF NEMATODE SYSTEMATICS

Virginia R. FERRIS

Department of Entomology, Purdue University, West Lafayette, IN 47907-1158, USA.

Accepted for publication 26 August 1993.

Key-words : Nematode, systematics, classification, taxonomy, phylogeny, diagnostics.

In recent years, whenever plant nematologists gather for national and international meetings, a frequently heard lament is that universities, research organizations, and experimental stations are not replacing nematologists who retire with persons of similar expertise. The particular expertise referred to often turns out to be skill in traditional taxonomy/systematics of soil and fresh water nematodes. The view is widely shared that soon, in the developed countries at least, no nematologists will remain who are qualified to attend to the taxonomic needs of the discipline. Those needs include the study of phylogenetic relationships, classification, descriptions of new species, and routine diagnosis of species and subspecific categories of nematodes. The facts seem to confirm the current dismal status of nematode systematics at many institutions. Those who make hiring decisions have apparently concluded that nematode systematics is expendable. This turn of events has left many nematologists alarmed and puzzled. They point out that nematodes themselves have not diminished in importance to agriculture or in soil ecosystems, and indeed, the problems that require systematic expertise in nematology are increasing rather than diminishing. Why then this paradox? What is the future of nematode taxonomy/systematics?

Systematics in the past

Although serious study of nematodes reaches back for more than a century, a period of explosive growth in the study of soil nematodes began in many countries during the 1940s when many nematologists were trained throughout the world. Most of the leading nematologists of this period were skilled in taxonomy and a major activity for several decades was the description of new species from crop areas, the preparation of taxonomic monographs, and the building of impressive specimen collections for reference and for training students. It is not an exaggeration to say that taxonomy and systematics unified the discipline. Nematologists who could identify nematodes were in brisk demand to diagnose specimens and to organize short courses and workshops to train others, mainly in nematode identification. Graduate students proliferated as the pionners of the era passed on their expertise and value systems to the next generation who eventually replaced them. Some of these persons trained in the 1950s are still active, and most of the practicing plant nematologists today are direct descendants of the pioneers.

During the past three decades many important changes occurred in biological science generally and in the fields of taxonomy, systematics, and diagnostics in particular. A new term « biodiversity » was even created to focus attention on the variety of life forms and their differences, particularly in endangered ecosystems but also in agriculture (Ferris *et al.*, 1991). A revolution of available technologies for investigating diversity in organisms occurred, beginning in the 1960s, first with the development of scanning electron microscopy (SEM), followed by isoenzyme techniques and serology, and eventually exploding into nucleic acid data. The discovery and use in the 1980s of the polymerase chain reaction (PCR) gave added impetus to these developments.

During the same period, the techniques for using taxonomic data to make systematic inferences about relationships among organisms underwent a revolution in most of biology. In the USA, especially, biologists argued the relative merits of numerical taxonomy (Sokal & Sneath, 1963) and phylogenetic systematics (Hennig, 1950, 1966). For a detailed account of the controversies surrounding this debate see Hull (1988). The methodologies of systematic inference dramatically changed, aided by developments in computer technology, and hypotheses of relationships were derived and stated in testable form (Ferris & Ferris, 1987). At the outset in most disciplines, only classical morphological data were available for phylogenetic analysis with the new methods, but cytological, biochemical and molecular data accumulated at a rapid rate, changing the approaches to systematic research across biology generally. The theory of plate tectonics was accepted, with profound effects on the study of biogeography (Ferris, 1980).

The promises of the new technologies were understandably embraced by funding agencies and eventually by administrators of research organizations who wished to remain competitive. Although nematode taxonomy has traditionally been dominated by persons trained to use classical morphological data, some nematologists moved quickly to explore new technologies for obtain-

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ing taxonomic data. One area in which nematology did not lag was in the use of SEM, which made possible entire suites of characters previously unavailable for use (Baldwin & Powers, 1987). A few nematologists explored the use of cytogenetics and biochemical data, including isoenzymes and other proteins and later nucleic acid data. But these activities were restricted to a few laboratories, and in the main, such approaches were not generally emphasized in the training of nematology students. Indeed, the paradigm for the well-trained nematologist in the 1980s did not change much from that developed in the 1950s by the influential dynamic leaders who were responding to the needs of their day.

It is widely believed in the world of business and commerce that internal people are often unable to change their organizations even when change is needed. This occurs because radical change might adversely affect the self image, internal convictions, and support groups of those in charge. People who are comfortable in what they are doing resist change. Have we, as practicing nematologists, been too comfortable in our old patterns; and are we, therefore, responsible, at least in part, for the current dire status of taxonomy/systematics in our profession?

Classical *versus* molecular data?

Hyman and Powers (1991) conclude their excellent review of the state of the integration of molecular and classical data for nematode systematics by saying that they anticipate a significant escalation in interest in biochemical data among nematode taxonomist/systematists. The prospect of the use of biochemical data for routine taxonomic and diagnostic purposes, as well as for determining systematic relationships among species and higher taxa in nematodes, has resulted in widely differing views. Some nematode systematists prefer to continue using classical data, augmented perhaps by SEM, but are skeptical regarding the usefulness of biochemical data (Jairajpuri, 1988). Others, who have mastered the new, often expensive, biochemical technologies may appear to disdain the work of the first group. It does not help that the two groups are often separated by an age chasm or by available financial resources, or access to necessary biochemical expertise. Nematologists seem divided as well on the important issue of whether and how their data can be used for phylogenetic inference (Jairajpuri, 1988).

As a nematologist/systematist trained in classical methods, who is currently involved in the production and use of biochemical data for phylogenetic analyses, it is my opinion that most carefully collected data can contribute to our understanding, depending on the problem at hand. A tendency exists for each of us to consider our own favorite data as the final answer, however they may conflict with the data of others. I now believe that all data should be viewed with healthy skepticism when inconsistencies arise. Because living organisms are the product of evolution, the inconsistencies will disappear when relationships are eventually understood and traits can be mapped on a correct phylogenetic tree. Proper understanding of such relationships is the goal of systematics in the broad sense and is the basis for sound classification and diagnostic procedures. It can best be achieved by reciprocal illumination based on a wide spectrum of data (Mindell, 1991). History shows that no single approach is without problems and challenges, as may be illustrated as follows by data for two well-studied species of plant parasitic cyst nematodes.

Heterodera schachtii and *H. glycines* - One species or two?

Evidence from morphological and behavioral data

Heterodera glycines, soybean cyst nematode (SCN), was first thought to be a race of *H. schachtii,* sugar beet cyst nematode (SBN), but was eventually described as a separate species. Although SBN and SCN are morphologically similar, a recent compendium for identification of agriculturally important cyst nematodes lists differences in J2 stylet length, J2 tail length and shape of J2 stylet knobs as diagnostic characters to separate them (Baldwin & Mundo-Ocampo, 1991). Inasmuch as they occupy exclusive geographic areas (Miller, 1983), have some morphological differences, and are usually found on different hosts, most nematologists are comfortable with their separate species status (Luc *et al.,* 1988).

Following experiments in which he obtained fertile laboratory hybrids from SBN and SCN, Miller (1983) declared *H. glycines* to be a subspecies of *H. schachtii.* This action was not received with much enthusiasm. One reason perhaps is that in addition to these fertile interspecific hybrids between SCN and SBN, Miller (1983) also reported fertile intergeneric hybrids between *Globodera* and *Heterodera* species. If nematologists were forced to choose between strict adherence to a biological species concept (Mayr, 1969) making it necessary to combine two recognizable species of separate genera into one species, or keeping the species and genera separate, one assumes they would opt for common sense, and keep the species and genera separate. I have written on this topic on several occasions, but the explanation for the phenomenon of interfertility of distinct species is that in organisms reproductive compatibility may be viewed as an ancestral attribute which is retained or altered in a mosaic pattern during evolution. When evolving members of a lineage become separated, novel traits are acquired independently, sometimes without formation of reproductive barriers (Ferris, 1983).

Evidence from molecular data : proteins and DNA

Further data suggesting that SBN and SCN are distinct species have come from two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) protein pat-

terns. Bakker and Bouwman-Smits (1988) reported that the species have distinctive 2-D PAGE protein patterns that differ in 59 % of their protein/polypeptides, and concluded that these nematode species have accumulated protein differences during millions of years without marked changes in morphology. DNA data have also suggested good species separation. Based on restriction fragment length polymorphisms (RFLPs), Radice *et al.* (1988) reported a 14 % difference in mitochondrial DNA.

With the goal of finding further evidence of the separation of SBN and SCN, we looked at DNA sequence variation in the two internal transcribed spacers of ribosomal DNA (rDNA) of these and other species (Ferris *et al.*, 1993*a*). It is a known fact that rRNA gene areas are conserved among organisms and therefore poor hunting ground for variation in closely related species, but the rDNA spacer regions are thought to be highly variable (Hillis & Davis, 1986; Hillis & Dixon, 1991; Hyman & Powers, 1991). To our surprise the rDNA spacer sequence data for three members of the *schachtii* group, SBN, SCN, and *H. trifolii* (clover cyst nematode) were nearly identical. Intraspecific sequence differences among geographic isolates of SCN exceeded the differences between SCN and SBN in some comparisons. In contrast, the number of sequence differences between any of the *schachtii* group of species and *H. avenae* greatly exceeded our expectations.

Further surprises were in store when we studied our sequence data for the 5.8S rRNA gene. The 5.8S gene sequence proved to be highly conserved (96 % similarity) in three genera of cyst nematode species. When we compared these data with 5.8S rDNA data in the literature for other kinds of organisms, we also found remarkable similarity (75 %) with *Xenopus* (a horned toad) and with sea urchin (an Echinoderm). However, we found only 61 % similarity between our cyst nematode sequence data and published 5.8S rDNA sequence data for *Caenorhabditis elegans* (Ellis *et al.*, 1986; Ferris *et al.* 1993*b*).

Mosaic evolution

Data such as those summarized above, illustrate that all features of organisms do not evolve in unison, and that many kinds of data are essential for accurate assessment of identities and relationships. It does appear that nematode morphology, at least insofar as it is accessible with light microscopy, is a conserved feature. However, DNA sequence conservation, particularly within closely related species groups, may also exist, even in DNA regions that are assumed to be evolving rapidly, such as the rDNA spacers we investigated. Furthermore, homoplasy is a potential problem with any kind of data. Clearly, a variety of data should be considered when inferring relationships.

Phylogenies based on molecular data?

Many systematists in all groups of organisms now think that phylogenies established on the basis of phylogenetic analysis of nucleotide sequence data may prove to be most useful as a basis for establishing trees or cladograms of relationships among taxa. Such trees or cladograms can then be used as a basis for determining how other features of organisms, such as patterns of behavior or morphological changes, evolved among the taxa (Hillis & Moritz, 1990; Patterson, 1987). Phylogenies based on nucleotide sequence data are beginning to appear for animal parasitic nematodes (Nadler, 1992) and are inevitable also for plant parasitic nematodes (Ferris & Ferris, 1987; Hyman & Powers, 1991). Our experience with the rDNA spacers indicates that considerable investigation may be necessary to determine appropriate DNA or RNA regions on which to rely for a particular group at a given taxonomic level, and that one should not jump to conclusions, particularly if the results do not make sense on the basis of other reliable data. Furthermore, care must be taken in the analysis of the data. The kind of cluster analysis based on total similarity that is often used with molecular data, would place the cyst nematodes closer to *Xenopus* than to *C. elegans* if used with our 5.8S ribsosomal gene data. This would be nonsense if interpreted in terms of species relationships.

The future : nematode systematics at a crossroads

It seems clear to me that the cure for the current malaise in systematic nematology is for established nematologists to make certain that students who wish to pursue taxonomy/systematics are trained not only in the techniques of classical nematology, including respect for and use of collections and type specimens; but also in the acquisition and use of new kinds of data, particularly molecular data, with new kinds of algorithms for analysis. In most cases, such a program will require a reaching out to new disciplines and interaction with systematists of other groups of organisms to keep abreast of new developments within systematics. It may even be desirable to establish working groups of individuals with different kinds of expertise. Such an approach will assure the kind of future funding for nematode systematics that will convince administrators that nematode systematists are doing exciting science and are employable. It will also assure that the best of the classical approaches are continued.

Two areas of taxonomic research that are poised for increased future funding are nematode diagnostics and nematode biodiversity. Spectacular changes have already begun to occur in nematode diagnostics, and these changes mandate radical change in the training of nematologists. Traditionally, nematode diagnostics has relied on imprecise microscopic characters and estimates of the degree of virulence to host differentials. For several

of the notorious plant nematode pests, a switch has already begun to the use of molecular characters for species diagnosis. Isoenzymes are now in wide use for diagnosing economically important species of root knot nematodes (Esbenshade & Triantaphyllou, 1985), and a PCR method for discriminating five root knot nematode species has been developed (Powers & Harris, 1993). DNA diagnostic probes have been found for the potato cyst nematodes, *Globodera rostochiensis* and *G. pallida* (Burrows & Perry, 1988; Stratford *et al.*, 1992). In addition, analyses based on monoclonal antibodies have been developed to distinguish the two potato cyst nematodes (Schots *et al.*, 1992; Bakker *et al.*, 1993; Robinson *et al.*, 1993). The research underlying such new methods must be carried out by highly trained scientists (hopefully nematologists), but once in place the diagnosis can be performed quickly by a technician with limited training and knowledge about nematodes. Nematologists are at a crossroads, with a clear choice as to whether they will train their own students to take the leadership in this research or whether they will watch from the sidelines as new diagnostic methods are developed by persons in other (related) disciplines.

The entire area of biodiversity encompasses exciting new opportunities for investigation of intra-specific variation important to agriculture, and also opportunities for characterization of nematode occurrence, function, and relationships in multidisciplinary studies of soil and water ecosystems in temperate and tropical areas (Yoon, 1993). It is likely that well funded research opportunities in both activities will expand, and both demand expertise in new molecular methods of analysis along with training in classical approaches.

Because of the ubiquity and importance of nematodes in agriculture and in most other ecosystems, nematodes will not be ignored. If nematologists themselves do not claim the territory, it will be taken over by others, probably molecular biologists with limited perspective regarding the rich array of classical characters and special techniques that can and should be used for nematode systematics along with the newer approaches. It is imperative to nematology as a discipline that nematologists themselves embrace the new technologies and ensure that the next generation of nematologists is well trained to carry on and evolve with the rest of biological science, while retaining the essential elements of classical systematics.

Acknowledgments

Drs J. M. Ferris and J. Faghihi contributed to the author's research, which was supported in part by National Science Foundation Grant BSR 8706759 and the U.S. Dept. of Agriculture Competitive Grant 89-37231-4492.

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