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SYSTEMATICS AND IDENTIFICATION OF PLANT-PARASITIC NEMATODE GENERA

Michel Luc

Nématologiste ORSTOM
Muséum national d'Histoire naturelle, Laboratoire des Vers
61, rue de Buffon, 75005 Paris, France

The title of this communication requires the definition of what are systematics, identification, and genera. We feel there is no need to define what is a plant-parasitic nematode.

Several definitions have been offered for systematics. Recently, Matil, Tassy and Goujet (1987), have proposed the following: "Systematics is the study and the description of the diversity of living organisms, the search for nature and causes of their differences and resemblances, the assessment of their relationships and the setting up of a classification reflecting such relationships." This is a rather long definition, but it has the merit to give a kind of research program of which each step represents a part of the systematical work.

Identification is the process by which a taxonomical name is given to an organism. In nematodes, identification most generally refers to generic and specific ranks.

We immediately see from these definitions that systematics and identification are dependant from each other because, i) the name given to the organism to be identified refers to a classification, itself the result of systematic studies; ii) systematics must be based on properly identified specimens.

Definitions of the genus are numerous, and sometimes contradictory, which is not surprising because the concept of genus is in fact a human concept. The International Code of Zoological Nomenclature (1985) defines it as: "the group next below the family group and above subgenus." This gives data on place of the genus in the nomenclatural hierarchy but gives no precision on its content. According to Mayr (1969) the genus is: "a taxonomic category which contains a single species or a monophyletic group of species, and separated from all the taxa of same rank [i.e. other genera] by a conspicuous discontinuity." This definition is not clear enough to serve as a working definition of the concept.

Among other definitions we may cite the following one, rather ironic, from Plateaux (1981): "The genus is a group of species that a non-specialist is unable to recognize from each other." Such a statement supposes that a non-specialist is able to recognize a genus; unfortunately this is not always the case.



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It is obvious from the above that the definitions given for systematics, identification and genus, are loose, variable, and submitted to revision, and this is a problem for systematists and identifiers.

With the plant-parasitic nematodes, an additional problem is the fact that characters used for determination (otherwise named the "key characters") and characters used for systematics, i.e. for the definition of taxa of generic --and in many cases suprageneric-- levels, are often the same. This is mostly due to the fact that these small animals are transparent, and so nearly all structures, internal as well external are perceived simultaneously under light microscope. Consequently, the characters that are the most easily seen, such as number of female genital branches, ornamentation of cuticle and of lateral field, shape and position of the basal glandular part of the oesophagus, etc., have been considered as the most important. As there are few such characters, they have been used for two purposes: the construction of dichotomous identification keys, and the definition of taxa at various levels. It is obvious that this practice leads to a confusion between identification and systematics.

Such a confusion does not exist, or is present at a lesser degree, in other groups of living organisms where identification and systematics are two clearly different processes. When trying to identify a plant, the botanist does not try at first to find whether it has one or two cotyledons. Characters used for botanical determination are mainly secondary ones, such as shape of leaves, color of flower, etc. In Mammalia, the order Primata, grouping apes and man, is now only defined by a special structure and arrangement of the bones of the internal ear. Of course other characters, easier to observe, are used for the identification of members of this order.

Taxonomists must reevaluate all characters describing the plant parasitic nematodes and mainly Tylenchina¹, and try to separate key characters from taxonomic / systematic characters. Such a critical examination has been performed in various groups at suprageneric level:

- the classification of Andrassy (1976), based on the presence of one or two female genital branches is now generally rejected. This character is accepted only at generic level, or even at specific level in *Helicotylenchus*, *Radopholus*, etc.;

- the grouping of genera with inflated, more or less globose, sedentary females in the Heteroderoidea of Golden (1971) received little acceptance, and it is recognized now that genera like *Rotylenchulus*, *Nacobbus* or *Heterodera* represent the end of a similar evolutionary process in different families;

- the Neotylenchoidea Thorne, 1941 characterized by a non valvular median oesophageal bulb have recently been shown to be an artificial grouping (Fortuner & Raski, 1987).

This reappraisal of characters must also be made at generic level, and for that purpose some general principles can be proposed:

- the characters linked with the cuticle often have received a too great importance. This is clear in Belonolaimidae where numerous genera have been defined differing mostly by the number of lines (3, 4, 5) in the lateral field. Such a character constitutes a good help for specific determination; it may also be used to define "nests of species" (Fortuner, 1989) inside the genus that have no taxonomical value; but it cannot be used alone, or with other characters of the same type, for the definition of genera. For this

¹ In the present lecture, all taxonomic designations concerning the Tylenchina follow the recent reappraisal of this group by Luc *et al.* (1987) and Maggenti *et al.* (1987, 1988)

reason a number of genera in Belonolaimidae have been rejected in a recent study of that family (Fortuner & Luc, 1987). However, it is difficult to apply this rule to Criconematinae. In this subfamily most characters used to separate genera are those describing the ornamentation of the cuticle. If a criconematid were to be stripped of its cuticle, it would lose all its generic characters, and it would resemble all the other criconematid species, except for body and stylet lengths, and shape of the post vulvar part. In this and other traits (e.g. ontogeny of the female genital system, see below), the criconematids appear as quite distinct from the rest of the Tylenchina, which justifies the division of Tylenchina into two superfamilies.

- the number of female genital branches (monodelphy vs didelphy) must be carefully evaluated in various groups. Nematodes with only one anterior female genital branch belong to one of several categories:

- Criconematoidea, where all species are monodelphic, and where the female genital primordium never shows the initiation of a posterior branch;

- monodelphic Tylenchoidea where the genital primordium have two branches. In adult females, the posterior branch is reduced to a post-uterine sac. In some genera, the species show a continuous variation (morphocline) from two equal and functional branches, to a posterior branch shorter but functional, to a posterior branch reduced, differentiated but apparently not functional, and to an undifferentiated post uterine sac. If all other characters are similar, there is no reason to place these species in separate genera. This situation is found for example in *Helicotylenchus* (= *Rotylenchoides*) and *Radopholus* (= *Radopholoides*);

- however, in the majority of monodelphic Tylenchoidea, monodelphy is a good character, stable in a given group, clearly separating this group from didelphic genera. In such cases, mono- or didelphy can be used for both systematic and determination purposes.

- the aspect of oesophageal glands, either abutting the intestine or overlapping it over various distances, also must be carefully evaluated. In Criconematoidea, the oesophagus has the same characteristic structure in all groups. In Tylenchoidea, the oesophageal glands overlapping or abutting the intestine generally are accepted as a good character, at generic and even family level. However, it is not always constant in some genera (*Pratylenchoides*) or even species (*Ditylenchus*). If it is the only difference between two genera resembling each other by all other characters, the validity of the separation of these genera may be questioned. In Belonolaimidae *Telotylenchus* and *Quinisulcius*, both with overlapping glands, have been considered as junior synonyms respectively of *Tylenchorhynchus* and *Trichotylenchus*, both with abutting glands (Fortuner & Luc, 1987)

- the structure of the columned uterus, or crustaformeria, is an important systematic character, that can be used at family level in Tylenchoidea. It has been observed (Geraert, 1986) that in families with many ancestral characters (Tylenchidae, Anguinidae, Dolichodoridae), the columned uterus presents four rows of cells, sometimes with a secondary derivation to multiple rows, whereas in more derived families, the basic number of rows is reduced to three. This could be difficult to use for determination, but is important for the placement of genera in the appropriate family. It is permitted to separate *Dolichodorus sensu lato* (Dolichodoridae) from other genera bearing a superficial resemblance, but actually pertaining to Belonolaimidae.

Certainly other characters may be subjected to a similar analytical process. Such characters have in common (to the exception of the last one cited perhaps) to be easily seen by normal optical means and to be used both for taxonomy and determination.

Other characters cannot be seen by optical microscopy, and thus cannot be commonly used for routine identification, at least at the moment, but they are often very useful to assess the systematic position of taxa of various ranks. Several examples are given below:

- histological study of root tissues modifications due to the presence of sedentary nematodes may be useful to separate some genera:

- in some genera of Heteroderinae, the developing female induces the formation of a single giant cell provided with a single giant nucleus, whereas in the other genera the nurse cell system is a syncytium, with numerous smaller nuclei. The first situation is believed to be ancestral;

- the superficial layers of roots attacked by *Trophotylenchulus* (Tylenchulidae) form a capsule that eventually protects the external part of the female and the eggs, whereas no apparent superficial modification is caused by *Tylenchulus* (Cohn & Kaplan, 1983). This is the main taxonomic character which differentiates these two genera, otherwise very close in their morpho-anatomy;

- TEM could be of some help too. For example it is used to characterize the structure of the cuticle of females and cysts of Heteroderinae. In some genera the cuticle includes a D-layer, which is absent in others. This character appears to be constant in a given genus, with few exception (*Cactodera betulae* is devoid of a D-layer present in other species of the genus).

- SEM photographs of superficial features are now very commonly used. They are mostly used to reveal the morphology of the lip region in face view, i.e. the "face", of the nematodes. Results of such examinations have been used at different taxonomic levels, as shown by the examples below:

- at specific level: SEM graphs of the face solved the problem of the placement of the species *Pratylenchoides magnicauda* (Pratylenchidae) that had been earlier considered a *Tylenchorhynchus* and then an *Amplimerlinius* (Belonolaimidae). *Pratylenchoides*, the most ancestral genus in Pratylenchidae, links this family with Belonolaimidae, because some of its species, such as *P. magnicauda*, have an abutting, or nearly abutting, glandular part of oesophagus, more characteristic of Belonolaimidae. Males, with oesophagus and stylet conspicuously reduced in *Pratylenchoides*, but not in Belonolaimidae, generally allow an easy determination, but males are unknown for *P. magnicauda*. The placement on this species in one or the other family was controversial until SEM face views were taken, clearly resembling face view of other *Pratylenchoides*, and conspicuously different from face views in *Amplimerlinius* (Baldwin, Luc & Bell, 1983);

- at generic level: the family Dolichodoridae contains only three genera : *Dolichodorus*, *Neodolichodorus* and *Brachydorus*. The first two present a very long stylet, a short female tail and a conspicuous striation of the labial area whereas opposite characters were considered in *Brachydorus*. *Dolichodorus* differs from *Neodolichodorus* mainly by the female tail hemispherical spiked vs rounded, and three lines in the lateral field vs four lines. However some species of *Dolichodorus* were described with a conoid tail, some species of

Neodolichodorus have a shorter stylet and one species of *Brachydorus* was found with a longer stylet; also, the labial area of the latter genus is actually striated, as in the other two genera. It was thought at first that the characters above were not diagnostic at generic level but SEM face views revealed a very distinctive shape and position of the amphidial slits in the three genera: they are small and laterally directed in *Dolichodorus*, small and dorso-ventrally directed in *Neodolichodorus*, and large and oblique in *Brachydorus* (Luc & Fortuner, 1987; Raski & Luc, 1988). This is a good example of three genera that can be easily identified using key characters (tail shape; number of lines in the lateral field), but whose taxonomic validity was proved by systematic characters only seen with SEM;

- at subfamily level: the SEM face view is one of the most obvious common characters used to define the Heteroderinae. In the female, the oral disc is roughly squarish, elevated, and conspicuously detached from the lip sectors. The lip sectors are rounded, notably larger, and completely fused to form a roundish annulus. This structure is found in females of all genera of Heteroderinae; and it has not been observed outside this subfamily (Luc, Maggenti & Fortuner, 1988). It was also observed in the genus *Verutus* that has some characters at variance with the description of the Heteroderinae. Because of its female face organization, the genus is now confirmed as a member of the subfamily, where it represents the most ancestral known genus.

To summarize and conclude, I intend to draw the attention of the participants to this workshop on identification to the following points:

- the characters used for identification and those used for systematics are often the same in plant-parasitic nematodes, but their weight and relative importance may be not be the same for both uses;

- modern systematics studies must take into account also characters other than those describing the morpho-anatomy of the nematodes; it is often difficult to use such characters for identification;

- consequently they are some reasonable doubts that a system of identification developed from our work could alone serve as a basis for hypothetical future "new" developments of systematics of plant parasitic nematodes. However, there is no doubt that the sum of expertise represented by the participants to this workshop will afford an up-to-date, precise and high quality information, which will be useful for both systematists and identifiers.

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DISCUSSION

Loof: Dr. Luc, I am very glad that you underlined the difference between key characters and taxonomic characters. This is the kind of thing I am always hammering on in my taxonomy lectures. There seems to be an intuitive feeling, that cannot be combatted too much, that a character that is very conspicuous and easy to see is by necessity an important taxonomic

character. In fact, there is no connection, and a character that may be insignificant as to its magnitude may be very fundamental for systematics, while a character that has a profound effect upon morphology may be taxonomically very unimportant. I am glad you emphasized the fact that key characters and taxonomic characters are two very different things, and that an identification key needs not be a copy of a classification system.

Fortuner: You said that the result of the work that will be done during this workshop is not to be used directly for future classification. Does that mean that you consider that nests of species (and I will define this later) cannot be used as basic bricks for building future classification?

Luc: I wanted to emphasize the fact that the morpho-anatomical data we are going to use to describe promorphs and nests of species cannot be used alone --and I insist on the word alone-- for proposing new systems of classification, while characters linked to host-parasite relationships, SEM and TEM examination, esterase profiles, number of chromosomes, etc., cannot be considered for determination, but they could be used for systematics.