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Aspects of Biology and Development of *Xiphinema americanum* and Related Species[1]

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Abstract: Identification of *Xiphinema americanum*-group nematodes is based on relatively subtle morphological and morphometric differences, many of which overlap. The significance and importance of these separations cannot be assessed without a basic understanding of the biological differences between species. Currently, information is accumulating on *Xiphinema* biology, development, and genetics that will help to confirm or refute the current systematics of species in this group. Recently, it was demonstrated that *Xiphinema* species pass through either three or four juvenile stages before becoming adults. This new and fundamental information divides the genus and the *X. americanum* group into subgroups based on their developmental evolution and provides new insight into the taxonomy and systematic positions of the species.

Key words: biology, development, morphology, morphometric, nematode, species, virus-vector, *Xiphinema americanum*, *Xiphinema* spp.

Xiphinema americanum, the type species of the genus, was originally described in 1913 by Cobb (7). In 1968, Lima (24) suggested that *X. americanum* was actually a complex of morphologically similar species, and he recognized seven parthenogenic species within the *X. americanum* group. Tarjan (31) examined 75 populations from around the world and concluded that only four species were valid, whereas the others were morphological variants of *X. americanum*. In 1974, Heyns (16) described species of *Xiphinema* from South Africa and indicated that although the separation of species was not satisfactory, several species proposed by Lima were probably valid. Several additional species in the *X. americanum* group were described between 1969–79. In 1979, an examination of several hundred populations from worldwide collections by Lamberti and Bleve-Zacheo (20) resulted in a major taxonomic revision. They recognized 25 valid *X. americanum*-group species with 15 of them new to science. Additional species have been described since, and the most recent key to the *X. americanum* group recognizes 38 species (21).

The *X. americanum* group is one of nine recognized groups of *Xiphinema* species (26). The species group concept is an aid to rapid initial identification by grouping nematodes according to shared key characters. The characters upon which the groups were established include the structure of the female genital tract, uterine differentiations, tail shape, and the coefficient c′. The species groups are useful for identification purposes but have no value as taxonomic units, nor are any phylogenetic relationships implied in their assembly. Although species in the *X. americanum* group are easy to differentiate from species outside this group, the taxonomy of *X. americanum*-group nematodes remains controversial.

Separation of *X. americanum*-group species is based on relatively subtle morphological and morphometrical differences, many of which overlap (20). Tarjan (31) recognized earlier that "the main problem with this group has been [the inability] to determine the existence of a clear demarcation between what the investigator feels are discrete species. Overlap of diagnostic criteria across such a delimitation tends to obscure recognition of the different species." Even after the taxonomic reappraisal of the group in 1979, Heyns (17) commented, "In spite of much careful work on

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the morphology and taxonomy of *Xiphinema,* it is becoming increasingly difficult even for the specialist to attempt species identification in this group. Uncertainty and confusion regarding the identity of many species are increasing rather than diminishing, especially with the increase in the number of uniparental species." In the same paper, he expressed his view that additional supporting evidence was needed to validate the separation of species. He went so far as to "contend that morphological characters are not only insufficient in differentiating [biological] species, but are in many instances also insufficient, contradictory as it may seem, for differentiating morphospecies, and this may be particularly true in monosexually reproducing groups, of which the *X. americanum* group is a good example." Although Heyns advocated the use of additional criteria to verify species designations, he did not specify which type of information would be most useful.

In a discussion of nematode taxonomy, Jairajpuri (19) distinguished between the concepts of "biological" species and "phenetic" or morphospecies. While conceding that only biological species exist in nature, he noted that the morphospecies concept is "more practical and will continue to dominate nematode taxonomy for a very long time to come." This is not to say that taxonomists should rely exclusively on morphology to designate species, but rarely is there enough information available on the biology of specimens to be of much use. In principle agreeing with Heyns, Jairajpuri stated that "information on all aspects [of nematode biology] would be of immense value for supporting conclusions drawn from morphology, but these should not be a prerequisite for taxonomic descriptions as it would amount to restricting taxonomic growth and freedom." According to Loof (25), "the history of taxonomy shows that the description of morphospecies precedes the development of the biological, polytypical species concept." Thus, despite the controversial status of *X. americanum*-group taxonomy, it provides the framework for research on the biology of this group. Information on biological differences (or lack thereof) between species will either support or refute the present nomenclature. The remainder of this paper focuses on selected aspects of *X. americanum*-group taxonomy, biology, and development.

Morphospecies And Development

The difficulty of identifying *X. americanum*-group nematodes is a consequence of the lack of uniquely distinguishing characters. Recently, however, Halbrendt and Brown (14) confirmed a fundamental difference in development between species, which is manifest as either three or four juvenile stages in the life cycle. The number of juvenile stages was determined unequivocally from measurements of the functional and replacement odontostyles of a representative sample of the population.

First-stage juvenile and adult nematodes are easily distinguished from all other developmental stages; therefore, the upper and lower range of stylet measurements for the species can readily be determined. First-stage juveniles are distinguished by the unique position of the replacement odontostyle (i.e., the tip of the replacement odontostyle overlapping the base of the odontophore), whereas adults are sexually mature and lack a replacement odontostyle, unlike the other stages (6). At each molt, the functional odontostyle is shed and replaced by the replacement odontostyle of the previous developmental stage. Because of this developmental phenomenon, measurements of these structures can be used to define natural groupings, which identify all the intermediate stages of development (Fig. 1).

The number of juvenile stages in the life cycle of a species has not yet been utilized as a taxonomic character. Although the addition of such a character would obviously make the present system more cumbersome, this character could be very useful, and we feel that it should be carefully con-

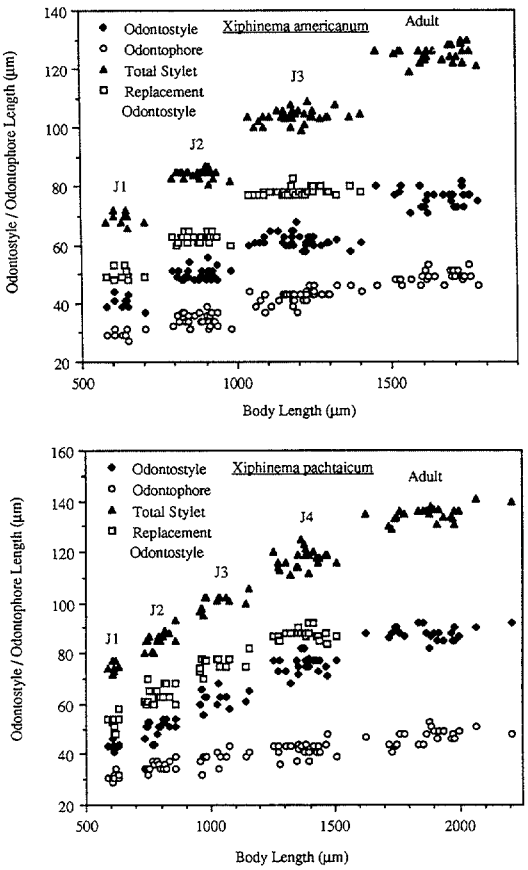

FIG. 1. Scatter diagrams showing that measurements of the odontostyle, replacement odontostyle, and body length effectively delimit the size range of each developmental stage of two species of the *Xiphinema americanum* group.

sidered. The number of juvenile stages for a species is a discrete and unambiguous character that has the potential to easily and clearly separate the *X. americanum* group into two subgroups. This dichotomy may effectively resolve some taxonomic questions where descriptions of adult nematodes alone are insufficient to distinguish the species unequivocally. Another advantage of this character is that preserved juvenile specimens would provide permanent reference material, such as is highly regarded for the type specimens.

Heretofore, juveniles have generally not been considered useful in taxonomic descriptions, and morphometric data of juveniles is lacking for most species. Table 1 lists *X. americanum*-group species whose number of juvenile stages has been determined. We propose that this number be included as a diagnostic character for distinguishing species.

Life Cycle and Biology

Detailed research on nematode biology can best be accomplished under controlled conditions in the laboratory and greenhouse. However, numerous attempts to culture *X. americanum*-group nematodes have been fraught with frustration. Although some cultural work has had limited success, *X. americanum* appears to be not as easily or reliably manipulated as many other plant-parasitic nematodes. Why these nematodes respond poorly in culture is not known, but extreme sensitivity to moisture tension, temperature fluctuation, physical handling, oxygen deprivation, or other such factors have been suggested (13,27,33). The ability to successfully culture *X. americanum* would undoubtedly add much to our knowledge on the biology of the group.

TABLE 1. *Xiphinema americanum*-group species having a known number of juvenile stages, and their geographic occurrence.

Species	Number of juvenile stages	Location	Literature reference
X. americanum	3	United States, South Africa	(14,15)
X. brevicolle	4	United States	(Robbins, unpubl.)
X. bricolense	3	Canada	(15)
X. californicum	3	Peru, United States	(1,14)
X. incognitum	4	Japan	(29)
X. madeirense	4	Madeira	(4)
X. pachtaicum	4	Bulgaria, South Africa	(14,15)
X. rivesi	3	Canada, United States	(1,14)

Some details of *X. americanum* biology have been extrapolated from field data and greenhouse experiments. Jaffee et al. (18) recorded the relative abundance of various life stages present in field populations over a 2-year period. From these data they concluded that the life strategies of *X. americanum* and *X. rivesi* were K-selected (i.e., they had a long life span and low reproductive rate) as opposed to r-selected (i.e., a short life span and high reproductive rate). These conclusions agree with life cycle studies done on other *Xiphinema* populations. The reproductive rate and longevity of *X. americanum*-group nematodes are unknown but probably are similar to *X. diversicaudatum,* which is estimated to produce an egg every 21 degree days above a threshold temperature of 5 C. In southern England, this estimate yields 68 eggs per year, and the life span is estimated to be 3 to 5 years (3,8). Under greenhouse conditions, Halbrendt and Brown showed that *X. americanum* can develop from egg to adult in 7 months (unpubl.).

In an attempt to rear *X. americanum* populations from single females, Halbrendt recovered offspring from only 48 of 420 females after one year (11.4% success). Forty-five of these breeding lines contained less than 42 nematodes each, and the remaining three contained over 100 (Halbrendt and Brown, unpubl.). With such a low reproductive rate and without knowing the optimal culture conditions, advances in our understanding the biology of *X. americanum*-group species will probably not be rapid.

Morphometric Variation

One of the most perplexing issues that continues to obscure the significance of various characters for species designation is the unknown factor of morphometric variation. How variable are the characters used for identification? Do factors such as temperature or host influence these characters? These are examples of biological questions best answered by controlled laboratory and greenhouse experiments. However, as stated in the previous section, answers to such questions will not be obtained quickly. When such experiments are performed, they will probably involve only the most common species, as some species in the *X. americanum* group are rare and have been identified as only the type specimens from a single population.

Because morphometric studies of various nematode species have indicated that stylet length is consistently one of the least variable taxonomic characters (2,9,10,32), stylet length would seem useful for species designation. However, stylet length data from field populations of *X. americanum* have displayed a relatively broad range (11,12,22). Recent research (Halbrendt and Brown, unpubl.) showed that stylet measurements obtained from populations descending from single *X. americanum* females fell into two distinct groups. Stylet lengths of one group were at the lower range of measurements assigned to *X. americanum,* whereas stylets of the other group were at the upper range. Within each group, however, stylet measurements were the least variable morphometric character. It appeared from these data that the reported stylet variability in field populations could be accounted for by the relative proportion of "long" and "short" stylet nematodes in the sample.

Biogeography

The symposium paper by Robbins (28) reviews the distribution of *X. americanum*-group species in North America, and a recent paper by Brown and Taylor (5) contains information on their geographic occurrence in Europe and the Mediterranean region. Knowledge of *Xiphinema* biogeography is essential to determine the natural range of nematode and nematode-transmitted virus diseases and to establish phytosanitary regulations to prevent the introduction and spread of these pathogens. Biogeography can also provide insights into the evolutionary history of the group and the origins of virus–vector associations. For these reasons, accurate

identification of species and an understanding of their biological differences are essential.

Many *Xiphinema* species reportedly have only a limited geographic range, but a few have been identified from widely separated locations. For example, *X. americanum* and *X. pachtaicum* have been reported from North America, Europe, South Africa, and Asia (23,30). Such a vast distribution of a soil-borne organism with limited means of dispersal needs to be carefully considered and verified, especially if one wishes to attach evolutionary significance to the data. Agricultural practices have transported many nematodes outside of their natural range; therefore, some reports of *Xiphinema* distribution may not accurately indicate the natural geographic range of the nematodes.

Conclusion

A taxonomic revision that establishes a number of new species is most useful when it facilitates understanding the biological functions and relationships of the organisms, and if the criteria for species separation are readily distinguishable. The taxonomy of the *X. americanum* group has challenged nematologists during the 25 years since Lima (24) first reported the occurrence of several distinct morphotypes, and especially for the last 13 years. The group consists of many morphologically similar species, but the lack of uniquely distinguishing characters makes identification difficult. The significance and usefulness of these separations cannot be fully appreciated until fundamental biological information about these nematodes is obtained, such as their ability to transmit viruses, their host preferences, and morphological variation within populations under different host and environmental conditions.

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