

Morphometric Evidence for Three Juvenile Stages in Some Species of *Xiphinema americanum sensu lato*¹

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Abstract: One to two hundred nematodes from each of seven *Xiphinema americanum*-group populations were measured to determine the range of stylet and body lengths for juveniles and adults. First-stage juveniles were identified by the position of the replacement odontostyle (i.e., the tip of the replacement odontostyle overlapped the base of the odontophore). Nematodes were identified as second stage if the functional odontostyle was the same length as the replacement odontostyle of the first stage. Subsequent stages were similarly identified by establishing the range of corresponding replacement and functional odontostyle lengths. In all populations examined, this procedure created natural divisions that clearly grouped nematodes by stylet and body length. Presumably these groups identified all juvenile and adult stages. Populations of *X. americanum*, *X. rivesi*, and *X. californicum* from the United States had three juvenile stages, but a population of *X. pachtaicum* from Bulgaria had four juvenile stages.

Key words: juvenile, morphometrics, nematode, virus-vector, *Xiphinema americanum*, *X. californicum*, *X. pachtaicum*, *X. rivesi*.

Presence of a replacement odontostyle in the esophageal corpus is one of the most readily distinguishable features separating juvenile from adult nematodes in the genus *Xiphinema*. Moreover, first-stage juveniles are easily distinguished from other stages by the unique position of the replacement odontostyle (i.e., the tip of the replacement odontostyle overlapping the base of the odontophore). During molting, the functional odontostyle is shed and the replacement odontostyle moves forward to take its place. A new replacement odontostyle forms in all stages except in the adult. Because of this developmental pattern, the functional odontostyle of any stage is virtually identical in length to the replacement odontostyle of the preceding stage (6). As a consequence, measurements of replacement and functional odontostyles have been reported to easily and reliably separate developmental stages of *Xiphinema*, provided only one species is present in the sample (2,5,6,11,12,18,19,

22,23). Many taxonomic descriptions do not include measurements of juveniles; however, it is widely accepted that *Xiphinema* spp., including those comprising the *Xiphinema americanum* Cobb, 1913, species group, pass through four juvenile stages (8,10,20).

Species of the *X. americanum* group in North America are important as vectors of several nepoviruses (3,21). Although population dynamics of these nematodes have been reported, the stylet measurements necessary for separating life stages were not included (9,14–16). In a recent study of *X. californicum* from Peru, Alkemada and Loof (1) could not identify four juvenile stages and suggested that only three were present, based on morphometric evidence. They emphasized the relationship between functional and replacement odontostyles in recognition of juvenile stages. Our study evaluated the use of odontostyle, total stylet, and replacement odontostyle lengths to identify juvenile stages in North American populations of the *X. americanum* group.

MATERIALS AND METHODS

Specimens from seven *X. americanum*-group populations were measured. Geographic occurrence of the populations were as follows: three populations of *X. americanum sensu stricto* (s.s.), one each

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from Pennsylvania, Arkansas, and California, two populations of *X. rivesi* from Pennsylvania, one population of *X. californicum* from California, and one population of *X. pachtaicum* from Bulgaria. Nematodes were extracted from soil by decanting and sieving (4) followed by a 2-day recovery in a Baermann funnel. Nematodes were heat relaxed in a 45 C oven for 30 minutes and fixed in warm triethanolamine-formalin (TAF) (7,9). Specimens were temporarily mounted for measurement in fixative on microscope slides with coverslips supported by cellophane tape. About 70–75 randomly selected nematodes were measured to determine the size distribution of nematodes in the sample. Additional specimens were selected when necessary to equalize the representation of all size ranges in the population. Measurements of odontophore, odontostyle, total stylet, and replacement odontostyle were made at 450 \times using an eyepiece reticle. Body length was measured with a zoom lens on a stereoscope at approximately 80 \times with the aid of video imaging software on a Macintosh II computer.

RESULTS

Range of stylet lengths denoting each group was established by means of frequency distribution or scatter diagrams. A frequency distribution graph of 200 *X. rivesi* stylet measurements produced three groups for replacement odontostyle and four groups for total stylet, corresponding to three juvenile stages and an adult stage (Fig. 1). Scatter diagrams were preferred, however, because separation of the groups was readily achieved with fewer nematodes. One hundred specimens of *X. californicum* produced four natural groupings that established size ranges for each characteristic measured (Fig. 2). These four groups corresponded to three juvenile stages and an adult stage. Similar scattergrams resulted when the same information was plotted for other populations. All North American populations produced four groups, and the Bulgarian population produced five (data not shown).

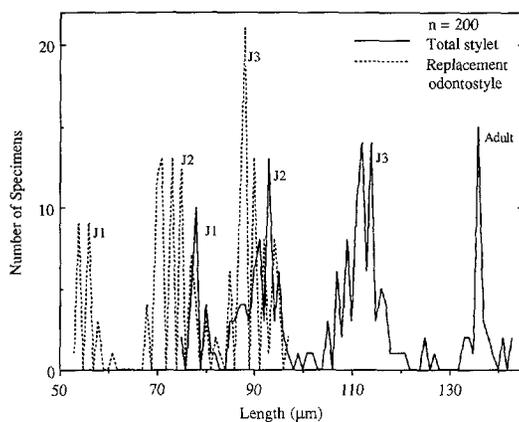


FIG. 1. Frequency distribution of total stylet and replacement odontostyle lengths for a population of *Xiphinema rivesi* from Pennsylvania.

Mean lengths for functional and replacement odontostyles of each naturally occurring group were calculated for each *X. americanum*-group population. In every case, there was good agreement between the replacement and functional odontostyle lengths of successive stages. In most instances, the mean length of the functional odontostyle was slightly shorter than the preceding replacement odontostyle length (Fig. 3). Body measurements alone did not readily separate nematodes into identifiable groups corresponding to life stages (data not shown).

DISCUSSION

Coomans and DeConinck (6) studied an unidentified *Xiphinema* sp. at various stages

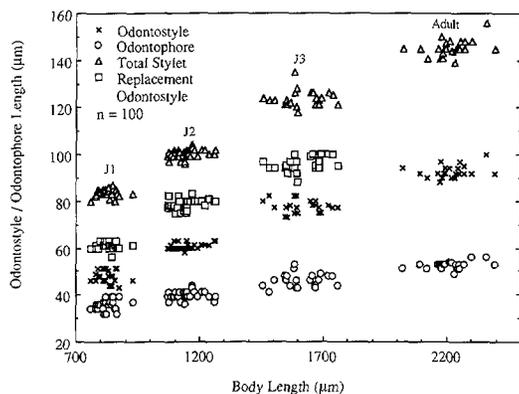


FIG. 2. Scatter diagram plotting the length of all stylet measurements vs. body length for a population of *Xiphinema californicum* from California.

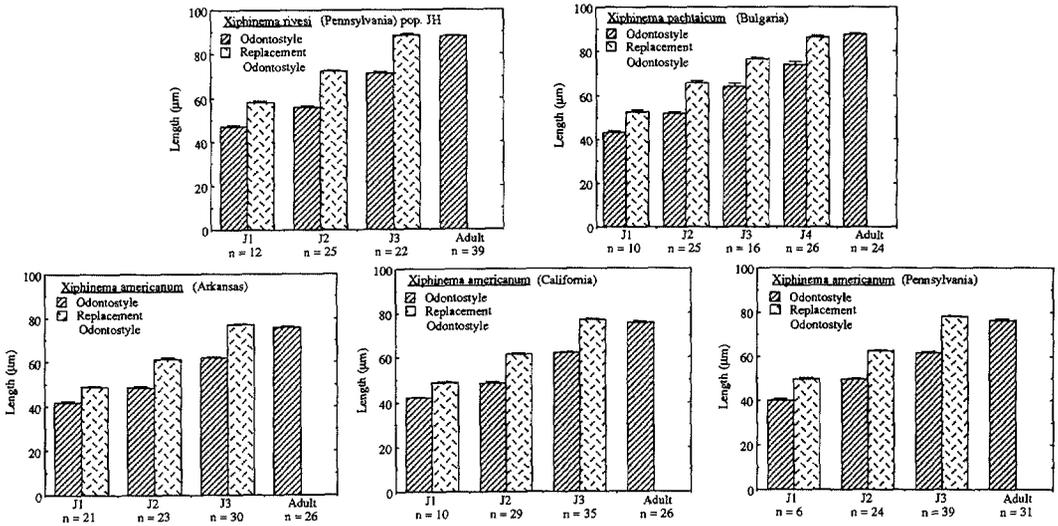


FIG. 3. Length of functional and replacement odontostyle for *Xiphinema rivesi*, *X. pachtaicum*, and three populations of *X. americanum* s. s. (mean \pm SE).

of molting to determine the sequence of events in stylet development. From these observations, they ascertained that the replacement odontostyle found in the esophageal corpus of juveniles becomes the functioning odontostyle of the subsequent stage. Furthermore, they showed that the relationship provided by the increased length of replacement and functional odontostyles through successive molts is an indicator of the number of juvenile stages in the life cycle. The advantage of stylet measurements lies in the fact that the length of the replacement odontostyle may be used to predict the odontostyle length of the succeeding stage. The population they studied possessed four juvenile stages, and measurements of replacement and functional odontostyles of sequential stages were in close agreement. They noted that the functional odontostyle was slightly shorter than the preceding replacement odontostyle, which they presumed was due to shrinkage resulting from solidification of the new odontostyle. They also described the formation of a new odontostyle tip (mucro) during the last molt, which they interpreted to be "an indication of a previous fifth moult which has been lost during evolution."

Alkemada and Loof (1) were first to sug-

gest that some species of the *X. americanum* group may only have three juvenile stages, but at least two earlier papers reported only three juvenile stages in other Longidorid nematodes. Yagita (24,25) provided convincing evidence for three juvenile stages in *Longidorus martini* (Merny), the natural vector of mulberry ringspot nepovirus, and morphometric data collected by Shishida (19) indicated that only three juvenile stages occurred in *Xiphinema chambersi* (Thorne). Alkemada and Loof reviewed the literature on *X. americanum*-group juveniles and reported that some published measurements clearly indicated four juvenile stages, whereas others were ambiguous (1). In these latter cases, measurements overlapped to such a degree that it is likely only three juvenile stages were present (*X. rivesi* (8), *X. californicum*, and *X. americanum*) (10).

Our data separated juveniles (with a replacement odontostyle) in six populations of North American *X. americanum sensu lato* (s. l.) into three readily identified groups based on length of body and stylet. The same criteria applied to a European population of *X. pachtaicum* identified four groups. Furthermore, the calculated mean values for replacement and functional odontostyles of all populations showed the

same subtle shrinkage phenomenon noted by Coomans and DeConinck (Fig. 3). The simplest interpretation of these data is to assume that these naturally occurring groups resulted from the size differences of juvenile stages. There does not appear to be a satisfactory alternative explanation that could account for four juvenile stages occurring in our North American populations.

On the basis of the uniform increase in stylet length between one group and the next, it appears unlikely that one developmental stage was missing from each of the six populations from North America. Equally unlikely is the possibility that two developmental stages were virtually identical, with the stylet measurements not changing between stages. This explanation is contrary to our understanding of stylet development. If either phenomenon were possible, however, the same could occur in other members of the Longidoridae reported to have four juvenile stages. In our population of *X. pachtaicum*, for example, perhaps five or six juvenile stages occur. The evidence to support the presence of three juvenile stages in our North American populations is the same justification by which other nematodes are reported to have four juvenile stages. Our data support the conclusion that a fundamental difference in development exists between our populations of North American *X. americanum* s. l. and *X. pachtaicum* and also many other members of the Longidoridae.

Furthermore, if species of *Xiphinema* have lost a developmental stage in the course of evolution, as proposed by Coomans and DeConinck, then it may also be possible for some species to have lost an additional stage (6). Perhaps the only unequivocal means of documenting the number of juvenile stages for a particular species is to follow development from egg-hatch to adult. In the absence of such information, the formation of natural groups based on an easily recognized characteristic such as the lengths of the functional and replacement odontostyle is the

most convenient indicator of juvenile stages.

In light of our findings and those of Alkemada and Loof, the number of juvenile stages for members of the *X. americanum* group should be carefully reexamined. The available literature documenting juvenile measurements is sparse, but some species appear to have four distinct stages, whereas others appear to have only three. The number of juvenile stages for the majority of species, however, has not been determined. Considering the controversial status of *X. americanum*-group taxonomy (10), it seems likely that additional information documenting the number of juvenile stages may be useful in a reassessment of the group. At present the *X. americanum*-group species that appear to have three juvenile stages include *X. americanum* s. s., *X. rivesi*, and *X. californicum* from the United States (this study), *X. californicum* from Peru (1), and *X. rivesi* from Ontario, Canada (1). The species with four juvenile stages include *X. pachtaicum* from Bulgaria (this study), *X. incognitum* from Japan (19), *X. guirani* from Madagascar (13), and *X. exile* from Portugal (18).

Further research is required, but it would appear from present evidence that two patterns of juvenile development are emerging. In the "Old World," the *Xiphinema americanum* group of species appear to have four juvenile stages, whereas species in the "New World" have only three such stages. This may indicate an early evolutionary separation in the *X. americanum* group, possibly coinciding with the divergence of the Americas with Africa and Europe during the early to late Cretaceous period 130 to 80 million years ago (17). In addition, the populations from the United States used in our study are vectors of tobacco and tomato ringspot nepoviruses. The species with four juvenile stages have not been reported to vector viruses. Although there would not appear to be any biological reason for this correlation, this observation may provide a convenient characteristic for distinguishing virus vec-

tor and nonvector species in the *X. americanum* group.

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