

Analysis of Developmental Stages and Morphological Characteristics in *Xiphinema* species Juveniles (Nematoda: Longidoridae)

Abstract

This study investigates the developmental stages and morphological characteristics of *Xiphinema* species during their juvenile stages (J1, J2, and J3). Through detailed analysis, this research examines the transformations and adaptive traits exhibited by these nematodes throughout their early life stages. . We conducted an examination of morphological traits and gathered essential measurements from the specimens. An observed occurrence involves the replacement of odontostyle in the pharyngeal region across all three juvenile stages. The initial juvenile stage of *Xiphinema* ranged in body length from 750.1 to 1104.8 μm , followed by the second stage spanning 1166.1 to 1705.1 μm , and the third stage ranging from 1752.8 to 2339.2 μm . The length of replacement odontostyle during these stages occurred within specific ranges: 55.1-80.1 μm in the first stage, 81.1-105.0 μm in the second stage, and 107.7-126.1 μm in the third stage. Detailed morphological observations, accompanied by microphotographs, provide insights into the structural changes characterizing each developmental phase.

Key words: Juvenile stages, Development, *Xiphinema*

1. Introduction

The genus *Xiphinema* Cobb, 1913 (family Longidoridae), commonly known as dagger nematode, includes more than 275 species that feed as an ectoparasite of plants (Xu *et al.*, 2019). It has worldwide distribution and causes damage to diverse wild and cultivated crops and transmitting plant viruses (Taylor and Brown, 1997). The genus comprises the largest number of species among the genera of the order dorylaimida and perhaps of the phylum Nematoda. The identification of juvenile and adult nematodes within the genus *Xiphinema* relies significantly on the discernible presence of a substitute odontostyle within the esophageal corpus (Bos *et al.*, 1984). Particularly, the distinctive positioning of the replacement odontostyle, where its tip coincides with the base of the odontophore, serves as a defining characteristic for distinguishing first-stage juveniles from other developmental stages (Robbins *et al.*, 1996). Throughout the molting process, the operational odontostyle is shed, making way for the forward relocation of the replacement odontostyle to occupy its functional role. Notably, a novel replacement odontostyle takes shape in all stages except the adult phase, underscoring a unique developmental sequence (Alkemade *et al.*, 1989). Consequently, the length of the operative

odontostyle in any given stage closely mirrors that of the replacement odontostyle in the antecedent stage, further emphasizing this intricate developmental pattern (Yuste *et al.*, 2016).

2. Materials and methods

2.1 Sampling, nematode extraction

The measured quantity of sub-sample (200 cc) from the composite sample was washed by Cobb's decanting and sieving technique (Cobb, 1918); the sieves of 20, 60, 200 and 325 mesh size were used to catch the target nematode populations. The catches of 60, 200 and 325 were further cleaned through modified Baermann's technique (Schindler, 1961). Here the residues passed through a double layer tissue paper supported on an aluminium wire-gauge, and the entire assembly was kept over a glass Petriplate of 10 cm diameter filled with tap water. It was left undisturbed for overnight at room temperature. Nematode suspension thus collected was labeled with details of host, date of collection, locality and collector's name and kept properly for further studies.

2.2 killing and fixing

Several fixatives have been recommended for killing and fixing the nematodes for dorylaimids. However, TAF (7.6 ml formalin, which contains 37% formaldehyde, 2 ml Tri-ethylamine, 90.4 ml Distilled water for 100 ml solution) was used for killing and fixing of nematode specimens. Mass fixation of nematode in the suspension was done with the help of hot TAF at 60°C (for simultaneously killing and fixing). The fixed nematodes were then observed under stereobinocular compound microscope and identified the target nematode genera based on morphological characters from the mixed populations (Seinhorst *et al.*, 1959).

2.3 Statistical Analysis

Morphometric data recorded for the populations of *Xiphinema* were further analyzed on Microsoft Excel for determining values of De Man's, maximum-minimum (range), mean, standard deviation (SD) etc.

3. Results

The research findings reveal interesting insights into the developmental stages of *Xiphinema* nematodes, particularly in terms of their morphological characteristics and the presence of replacement odontostyle. The occurrence of replacement odontostyle observed in the pharyngeal

region across all three juvenile stages (J1, J2, and J3). This phenomenon underscores the dynamic nature of *Xiphinema* nematode development. The specific localization of these replacement structures offers distinctive markers for each developmental stage, providing a basis for accurate identification and classification. The juveniles similar to adult female, but small in size and absence of reproductive organs. Replacement odontostyle are seen in pharyngeal region of all three juveniles (J1, J2 and J3) stages.

Description (Fig. 1, for measurement sees Tables 1)

3.1 Juvenile stage 1: In the case of the juvenile stage 1 (J1), the identification criterion hinges on the precise positioning of the replacement odontostyle. Notably, the anterior tip of the replacement odontostyle is subtly projected at the juncture between the functional odontostyle and the odontophore. This distinctive trait serves as a pivotal characteristic for categorizing nematodes within this developmental stage. Body 750.1-1104.8 μm and Replacement of odontostyle 55.1-80.1 μm long (Fig. 1, A Tables 1).

3.2 Juvenile stage 2: As the nematodes progress to the juvenile stage 2 (J2), a compelling shift in the positioning of the replacement odontostyle becomes evident. Specifically, the anterior tip of the replacement structure becomes embedded within the basal flanges. This transformation further emphasizes the intricacies of *Xiphinema* nematode development and highlights the importance of meticulous morphological observations. Body 1166.1-1705.1 μm and Replacement of odontostyle 81.1-105.0 μm long (Fig. 1, B Tables 1).

3.3 Juvenile stage 3: The journey culminates in the juvenile stage 3 (J3), where spatial arrangement of the replacement odontostyle anterior tip is observed. Unlike the preceding stages, the anterior tip of the replacement odontostyle is situated at a discernible distance from the flanges. This unique positioning offers a distinct characteristic that aids in the accurate classification of nematodes in this advanced developmental stage. Figure 1 provides a visual representation of this distinct morphological feature. Body 1752.8-2339.2 μm and Replacement of odontostyle 107.7-126.1 μm long (Fig. 1, C Tables 1).

4. Discussion:

The findings of this research shed light on the developmental stages of *Xiphinema* species, specifically focusing on the morphological characteristics associated with their juvenile phases

(Brown *et al.*, 2016). The presence of replacement odontostyle across all three juvenile stages (J1, J2, and J3) holds significant implications for understanding the adaptive strategies of *Xiphinema* species (Fadakar *et al.*, 2021). The distinct positioning of the replacement odontostyle in each stage serves as a reliable marker for characterizing the developmental phases (Loof, 1993 and loof, 1996).

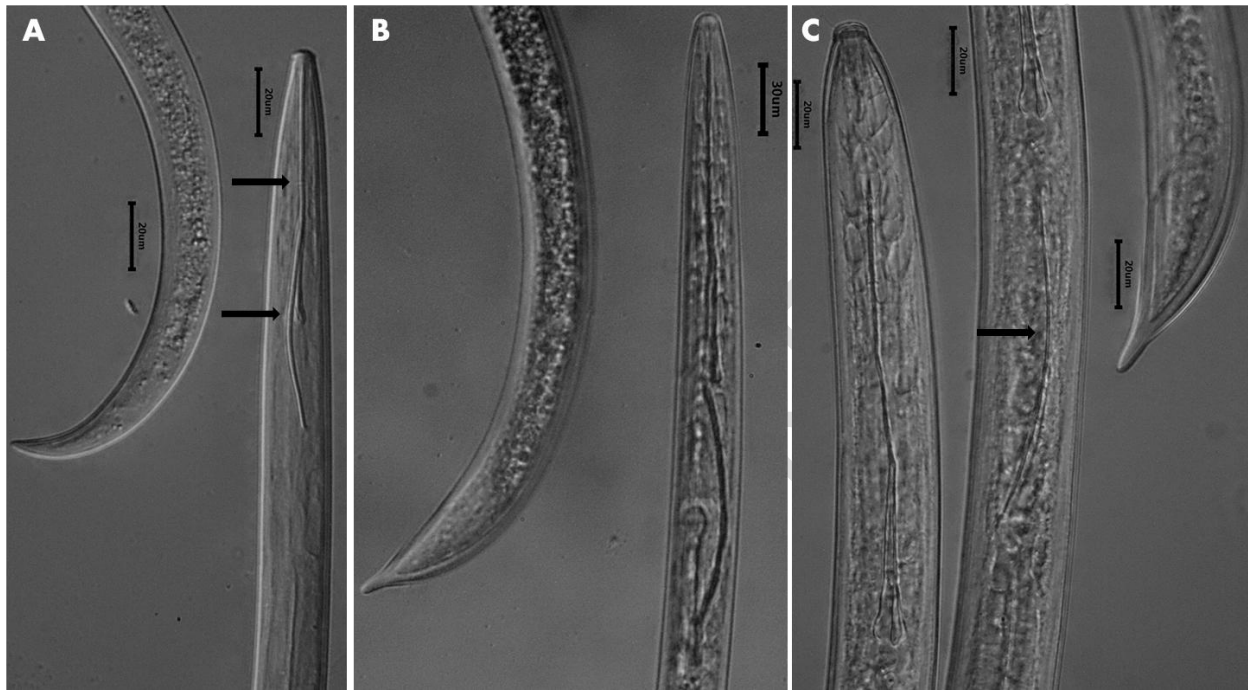


Fig.1 Light microphotographs of *Xiphinema* species (juveniles J1-J3) **A-C**, **A** First stage juvenile- anterior & posterior body region with arrows pointing position of guiding ring and flanges **B** Second stage juvenile- anterior and posterior region along with replacement of odontostyle **C** Third stage juvenile- anterior and posterior body region with arrow pointing replacement of odontostyle. (Scale bars: A, B & C = 20 μ m)

Table 1. Measurements of juveniles of *Xiphinema basiri* (mean \pm SD, with range). All measurements are in μ m

Characters	J1 (n=16)	J2(n=21)	J3 (n=17)
L	900.9 \pm 95.7 (750.1-1104.8)	1490.9 \pm 162.1 (1166.1-1705.1)	2009.39 \pm 172.1 (1752.8-2339.2)
a	49.8 \pm 5.8 (39.3-59.0)	56.6 \pm 10.1 (38.90-71.1)	52.05 \pm 4.0 (46.9-59.7)
b	4.0 \pm 0.4	5.4 \pm 0.8	5.42 \pm 0.7

	(3.1-4.7)	(4.1-7.0)	(4.7-7.1)
c	32.7 ±4.8 (23.4-43.0)	35.3±5.8 (24.2-47.1)	36.41±3.6 (30.2-42.4)
c'	2.2±0.3 (1.9±2.7)	2.4±0.5 (1.7-3.3)	2.12±3.6 (1.8-2.4)
Odontostyle length	57.6±6.9 (47.7-67.7)	75.0±3.8 (68.4-79.3)	100.26±8.7 (81.5-110.7)
Odontophore length	35.3±2.4 (32.2-39.1)	44.2±3.6 (36.1-49.0)	49.82±10.1 (32.3-60.0)
Total Stylet length	93.0±93.0 (79.9-104.6)	119.2±6.0 (104.5-127.5)	150.08±17.3 (113.8-164.6)
Head to guiding ring	41.3±3.7 (35.1-48.1)	62.8±4.1 (55.6-69.5)	86.66±12.2 (70.7-100.0)
Flanges width	6.9±0.1 (6.8-7.1)	7.8±0.6 (7.0-9.0)	8.71±0.6 (7.7-10.0)
Pharyngeal length	223.7±10.1 (206.6-241.5)	277.4±22.4 (241.2-309.1)	373.49±35.0 (304.5-426.0)
Pharyngeal bulb length	55.0±4.6 (47.7-61.3)	71.2±5.5 (61.1-79.5)	85.85±4.2 (79.9-92.3)
Pharyngeal bulb dia.	10.7±0.8 (10.0-12.1)	14.0±0.7 (13.0-15.0)	17.41±1.0 (15.2-18.5)
Replacement of odontostyle	66.9±6.0 (55.1-80.1)	90.9±7.7 (81.1-105.0)	118.06±5.9 (107.7-126.1)
Body diam. at lip region	6.7±0.7 (6.1-7.7)	8.0±0.4 (7.5-8.5)	10.35±1.1 (9.0-11.5)
Mid body width	18.2±2.0 (16.1-21.5)	26.7±2.6 (22.0-30.0)	38.73±3.5 (32.7-45.0)
Body diam. at anus	12.6±0.8 (11.1-13.8)	18.7±2.4 (15.0-22.0)	26.29±1.8 (23.1-28.3)
Hyaline tail region	4.5±0.8 (3.8-6.2)	8.9±1.5 (7.0-12.0)	16.71±2.0 (13.8-20.0)
Tail length	27.8±2.4 (25.1-33.0)	42.8±4.9 (35.0-50.0)	55.41±4.1 (49.2-63.1)

The precision with which these markers distinguish one stage from another emphasizes the importance of meticulous morphological observations in taxonomic studies and ecological research. Furthermore, this study contributes to the broader knowledge of nematode biology,

offering a foundation for more in-depth investigations into the functional significance of these morphological changes.

While this research delves into the morphological aspects of *Xiphinema* species juvenile development, there remain a questions about the underlying mechanisms driving these changes. Future studies could explore the genetic and molecular factors that regulate the development of replacement odontostyle and how these changes influence the nematodes' interactions with their surroundings.

5. Conclusion:

In conclusion, the investigation into the developmental stages of *Xiphinema* species has revealed insights into the morphology and adaptive traits exhibited by these organisms during their juvenile phases. The presence of replacement odontostyle and their distinctive positioning provides a novel perspective on the growth and development of these nematodes. The intricate changes observed across stages underscore the dynamic nature of *Xiphinema* species development, potentially influenced by environmental cues and interactions with host plants. These findings contribute to our understanding of nematode biology and their potential implications for agricultural and ecological systems. The accurate classification facilitated by the localization of replacement odontostyle provides a practical tool for researchers and taxonomists. Furthermore, the study opens avenues for further research into the functional roles of these morphological changes and their significance in nematode adaptation and evolution. Ultimately, this research advances our comprehension of the complex life history of *Xiphinema* species, offering a foundation for continued exploration into the mechanisms driving their growth, development, and interactions within their ecosystems.

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