PART II

STUDY OF INTRASPECIFIC VARIATIONS
The characters that are used in nematode taxonomy are known to vary among and within the populations of a single species. These variations may be host induced or due to differences in the environmental conditions or geographical distribution etc. Goodey (1952) demonstrated host induced variations in body length and dorsal or ventral positions of the oesophageal bulb in *Ditylenchus destructor*. Rhode & Jenkins (1957) found out that body length and body width of *Trichodorus christei* vary inversely with the temperature of soil. Taylor & Jenkins (1957) who worked with *Pratylenchus* spp. and Coomans (1962), with *Rotylenchus goodeyi* found that the deviations from mean were lowest for vulva position and greatest for tail shape. Sturhan (1963) found that body width, oesophageal and tail lengths of *Xiphinema* and *Longidorus* species exhibit negative allometric growth with body length. Tarjan (1964) noticed differences between the north-western and south-eastern populations of *X. bakeri* in body length, \( a \), \( b \), posterior gonad length and \( c' \) ratios. Gysels (1964) observed that the temperature influences the allometric growth of *Panagrellus silusiae*. Fisher (1965) demonstrated variations due to host plant and temperature in the body length and stylet length of *P. nanus*. The host species and host variety, host physiology and the geographical origin of a population was found to influence the variations in morphometric and allometric characters in *T. christei* and the stylet length and value of \( V \) were the least variable characters (Bird & Mai, 1967). The variations among different populations of *X. americanum* from
different parts of the world were possibly due to geographical latitude, temperature, etc. (Tarjan, 1969). Loof & Maas (1972) observed intraspecific variations among the populations of *Xiphinema* species found in Surinam and concluded that body dimensions alone are unsatisfactory for distinguishing species and qualitative characters should be given at least equal weight. Heyns (1974a & b) studied intraspecific variations in *X. brevicolle* and *X. elongatum* which can be correlated with the geographical distribution of the populations.

In the present study, intraspecific variations in *Xiphinema basiri* and *X. insigne* have been studied. The variations in these species were studied from two different angles. In *X. basiri* only a single local population (from the garden of Botany Department, Aligarh Muslim University campus) was studied in detail for determining the morphometric and allometric variations in the adults and juveniles. In *X. insigne* which is a very widely distributed species in India several populations were obtained from different parts of the country and variability within these populations was found out. For both species variations within or/and between population(s) were found. These studies are likely to have direct bearing on the taxonomy of this genus and also related groups.
MORPHOMETRIC AND ALLOMETRIC VARIATIONS IN XIPHINEMA BASIRI

Morphometric and allometric variations were observed in almost every character in the population of Xiphinema basiri that was studied in the present work. These variations are basically different for each character.

MORPHOMETRIC VARIATIONS

Body length: Body length in adults ranges from 2.55-3.61 mm with S.D. ± 0.3 mm. The coefficient of variability of this character is 0.09 in adults showing that it is quite variable.

In juveniles, the body length measures 1.21 ± 0.14, 1.65 ± 0.2, 2.35 ± 0.2 mm in L₂, L₃, and L₄ respectively. The minimum variation was observed in L₄ where C.V. = 0.10. The value of C.V. in L₂ and L₃ = 0.12. This high value of C.V. shows that the total body length is also quite variable in the juveniles.

Body width: In the adults the body width ranges from 43-57 um with S.D. ± 5.0 and C.V. = 0.10.

Among the juveniles the body width measures 26 ± 3.0, 31 ± 3.0 and 39 ± 4.0 in L₂, L₃ and L₄ respectively and have C.V. 0.12, 0.09 and 0.10.

This high value of C.V. in adults and the juveniles clearly shows that body width is a greatly variable character.
Almost same value of C.V. (0.09-0.12) also shows that this character is variable to same extent in each stage of the life cycle.

**Odontostyle lengths:**

**Functional odontostyle length:** The functional odontostyle length is least variable of all the morphometric characters that were studied. In adults of *X. basiri* the variability is only 3% of its length and measures 119 ± 3.4 um.

Among juveniles, the C.V. of functional odontostyle length is 0.04, 0.03 and 0.03 in L<sub>2</sub>, L<sub>3</sub> and L<sub>4</sub> respectively and is least variable of all the other characters measured. The odontostyle length measures 62 ± 2.4 in L<sub>2</sub>, 80 ± 2.9 in L<sub>3</sub> and 99 ± 3.4 in L<sub>4</sub>.

The C.V. 0.03-0.04 clearly shows that odontostyle length is variable to almost same extent in each stage of the life cycle.

**Replacement odontostyle length:** This varies 3.2%-5.2% of its length in juveniles. In L<sub>2</sub>, L<sub>3</sub> and L<sub>4</sub>, it measures 80 ± 4.2, 98 ± 3.1 and 120 ± 5.3 respectively with C.V. = 0.05 in L<sub>2</sub>, 0.03 in L<sub>3</sub> and 0.04 in L<sub>4</sub>. This shows that the replacement odontostyle length is also a less variable character.

**Odontophore length:** This character is quite constant having C.V. 0.04 in adults, 0.05 in L<sub>2</sub>, 0.05 in L<sub>3</sub>, 0.06 in L<sub>4</sub>. This shows that odontophore length varies to a limited extent in each stage of the life cycle.
Guiding ring: The position of guiding ring from the anterior end is also a constant character with C.V. 0.06 in adults (distance 101 ± 6.9). Among the juveniles 54 ± 3.52, 90 ± 4.10 in L₂ and L₄ with C.V. 0.06 and 0.05 respectively.

Oesophageal length: A few specimens have abnormal oesophageal lengths measuring 518 um against the 402 um which is the average length in normal adults. In adults this character is variable with C.V. = 0.07.

The variations of oesophageal length are similar for each of the juvenile stages, the C.V. being equal to 0.07, 0.08, 0.08 in L₂, L₃ and L₄ respectively and measuring 249 ± 16.1, 305 ± 24.2 and 375 ± 28.4 respectively.

Basal oesophageal bulb: It is comparatively less variable in adults (C.V. = 0.06), and measures 101 ± 6.1, although its length is affected by the position of the odontostyle. In juveniles, the L₃ stage exhibits more variations than in any other juvenile stage with C.V. = 0.12 (length = 73 ± 9.2) as compared to C.V. = 0.06 and 0.07 in L₂ and L₄ respectively.

Gonad length: The length of anterior and posterior gonads are quite variable having C.V. = 0.13 and 0.17 respectively. These variations are due to unequal coiling of the muscular part of the uterus.

Vaginal length: The length of vagina varies 3.6% of its length and measures 27 ± 2.4 um.
**Anal body-width:** The C.V. of anal body-width is 0.08, 0.13, 0.09, 0.09 and measures 17 ± 1.4, 21 ± 3.7, 26 ± 2.5 and 27 ± 2.4 in L₂, L₃ and L₄ and the adults respectively. This indicates that the anal body-width shows more variations in L₃ than in any other juvenile stage or the adults.

**Tail length:** It varies 7.1% of its length and measures 42 ± 3.0. In juveniles the maximum variations were observed in L₂ where C.V. = 0.10 against 0.06 and 0.07 in L₃ and L₄.

**ALLOMETRIC VARIATIONS**

**Body length/body width:** The ratio a varies from 54-75 with S.D. = 5.3 and C.V. = 0.08 in adults. Although the body width has a tendency to increase with the increase in the body length but it does not increase in the same proportion. Further, C.V. of a = 0.08 which is only a little less than C.V. of body width.

In the juveniles, the ratio a is quite variable in each stage. The maximum variations were observed in L₄ where C.V. = 0.10 and minimum in L₃ where it is only 0.07. The value of a in L₂, L₃ and L₄ was found to be 48 ± 4.0, 54 ± 4.0 and 61 ± 6.3 respectively.

**Body length/oesophageal length:** This ratio was found to be 7.6 ± 0.57 with C.V. = 0.07. Since C.V. of b is 0.075 while that of total oesophageal length is 0.07 and of the oesophageal bulb is 0.06, it is clear that ratio b is more variable than the oesophageal length or the length of oesophageal bulb in adults.
In the juveniles too, the value of $b$ is quite different for developmental stages showing that oesophageal length does not increase in the same proportion as does the total body length. The value of $c$ in $L_2$, $L_3$ and $L_4$ was found to be $4.8 \pm 0.7$, $5.4 \pm 0.7$ and $6.3 \pm 0.5$ respectively.

**Body length/tail length:** It is clear from Fig. 16 that increase in the body length is independent of tail length in adults. The $c$ value is correlated with total body length showing that this ratio is not a good taxonomic character as was reported by Clark (1962). The C.V. of $c = 0.10$ (mean $= 75.2 \pm 7.3$) while that of tail length itself is only 0.07 showing that the tail length is more constant than ratio $c$ in adults.

In juveniles, the $c$ value is more variable than the tail length with maximum C.V. = 0.11 in $L_2$ and 0.09 in $L_3$ and $L_4$. The $c$ value in $L_2$, $L_3$ and $L_4$ was found to be $26.1 \pm 3.0$, $32.9 \pm 3.0$ and $49.0 \pm 4.6$ respectively. As in adults, $c$ has a tendency to increase with the total body length.

**Body length/functional odontostyle length:** There is no relation between the body length and the length of the functional odontostyle. The odontostyle length is independent of body length in adults as well as in juveniles (Fig. 15).

**Body length/replacement odontostyle length:** There exists no correlation between these two structures, both being quite
independent of each other in the juveniles.

**Oesophageal length/functional odontostyle length:** The functional odontostyle length is independent of oesophageal length which is quite clear from the Fig. 17. This also shows that the functional odontostyle is consistent in length in adults and juveniles.

**Oesophageal length/replacement odontostyle length:** The growth of replacement odontostyle is correlated with the growth of oesophagus (Fig. 17). Also coefficient of rank correlation between these two characters was found to be significant at 5% level of probability in each juvenile stage.

**Body length/vulva position:** The vulva position was found to be correlated with total body length (Fig. 18). This character is least variable of all the morphometric and allometric characters that were measured having C.V. = 0.024.

**Anterior gonad length/posterior gonad length:** Anterior gonad length was found to be quite independent of posterior gonad length. This may be due to unequal coiling of uteri of two gonads.

**DISCUSSION**

Various morphometric and allometric characters vary to different degrees in a population of *Xiphinema basiri*. The length of the odontostyle and V are the least variable characters in this species and so they can safely be used to differentiate this
species from other closely related species. These results are similar to those of Bird & Mai (1967) for Trichodorus christei, Tarjan (1969) for Xiphinema americanum, Wu (1960) for Ditylenchus destructor and Azmi & Jairajpuri (in press) for Helicotylenchus indicus. The position of fixed guiding ring from the anterior end, odontophore length, length of the oesophageal bulb and tail vary to moderate degrees. All other characters are very much variable and should be used with caution. The body length, lengths of the oesophagus and tail exhibit negative allometric growth with the body length which agrees with the observations of Sturhan (1963). All the morphometric and allometric characters vary to almost the same extent in the juvenile stages as well as in the adults. This shows that the variability of a character is determined from the very first juvenile stage in the life.

Xiphinema basiri comes close to X. coxi Tarjan, 1964 and X. ifacolum Luc, 1961. Cohn & Sher (1972) synonymised X. ifacolum with X. basiri owing to the overlapping values of L, a, b, c, V, length of odontostyle and tail shape and in the presence of 'Z' organ. Luc & Dalmasso (1975) rejected this synonymy on the basis of differences in the 'Z' organs (X. ifacolum has a typical 'Z' organ while X. basiri a pseudo 'Z' organ) and the structure of tail tip (in X. ifacolum the inner surface of the cuticle of the tail tip forms a thin and regular blind canal surrounded apically by a thin muff but in X. basiri the tail tip is with a large conical blind canal without any apical muff). The views of Luc & Dalmasso on this synonymy appear to be quite correct.
*X. basiri* and *X. coxi* differ only in the position of vulva (V=40-46 in *X. coxi*) but as was made clear above the position of the vulva is least variable in *X. basiri*, and consequently the two species are clearly different from each other.

Siddiqi (1959) described *Xiphinema basiri* from around the roots of *Citrus sinensis* from Aligarh, India. Yadav & Varma (1967) reported its frequent occurrence in Rajasthan in association with fruit-trees. It is also known to occur in some other countries, e.g., Sudan (Yassin, 1974); Ceylon, Mexico and Rhodesia (Cohn & Sher, 1972).

This species causes galling of the root tips of tomato (cf. Roy, 1975) and is suspected to transmit cowpea mosaic virus (cf. Caveness et al., 1975).
Loos (1949) described *Xiphinema insigne* collected from the soil around the roots of soursop, coconut and grasses from Kurenegala, Ceylon. Siddiqi (1959) described a very similar species, *X. indicum* from the soil around the roots of *Grewia asiatica* L. from Aligarh, India. Tarjan & Luc (1963) synonymised the two species after comparing the syntypes of *X. insigne* and paratypes of *X. indicum*. Cohn & Sher (1972) and Loof & Maas (1972) accepted this synonymy. Cohn (1969) reported *X. insigne* from several localities in Israel and described the males of this species for the first time. Saigusa & Yamamoto (1971) described females and a single male of *X. insigne* from Japan. However, these specimens from Japan have longer bodies and tails. Southey (1973) though regarding *X. indicum* to be a synonym preferred to retain two types under *X. insigne*, viz., *indicum*-type (e.g., specimens of Siddiqi, 1959) and long-tail type (e.g., specimens of Saigusa & Yamamoto, 1971).

*Xiphinema insigne* (syn. *X. indicum*) is widespread in India as it was found in soil from the hills, foot-hills, from the banks of a hot-water sulphur spring, from marshy places as well as from the plains. It was usually found associated with the roots of fruit trees. The specimens from the type locality (Aligarh) of *X. indicum* also include 4 males which have been
recorded for the first time from India and are described here in detail. In the following an account is given of variability in 23 populations of *X. insigne* collected from the following hosts and localities in India:


Observations were made on specimens fixed in 4% hot formalin and dehydrated to pure glycerine. The dimensions of adult females, males and juveniles of 1-23 populations are given in Table IX & IX.

**Female:**

**Adults:** The body when fixed curves ventrally to a 'C' shape. It is long, slender and tapers at both ends, with the taper towards the hind end more pronounced in populations 19-23. Cuticle in 2 layers, 2-8 um thick, 2-3 um at mid-body and 3-8 um on tail. Lateral chords are 1/4th to 1/3rd of mid-body width.

**Lip region** is almost flat and slightly offset in populations 19-23 (Fig. 8,C), but is rounded and set off in populations 1-18 (Fig. 8,B&D). Amphids are stirrup-shaped with slit-like apertures which are 6-8 um or nearly 3/5th the labial-width and located 3-5 um from the anterior end of body. Odontostyle
length ranges from 80-110 µm (avg. 102); populations 1-18 have odontostyle measuring 94-110 µm (avg. 104), 19 and 20 have 80-92 µm (avg. 89) and 21-23 have 90-95 µm (avg. 93). Odontophore length ranges from 55-64 µm (avg. 63). It shows a range from 59-64 µm (avg. 63) in populations 1-18 compared to 55-59 µm (avg. 57) in other populations. The position of the fixed guiding ring from the anterior end varies from 50-110 µm (avg. 97); it is more posteriorly located at 92-110 µm (avg. 100) in populations 1-18 as compared to 80-92 µm (avg. 84) in populations 19-23.

A mucro (vestigeal odontostyle) with anteriorly directed tip is usually present in the anterior slender part of oesophagus. However, in one specimen the mucro was found within the walls of the odontophore. Length of the oesophageal bulb ranges from 66-104 µm (avg. 83), in populations 1-18 measuring 66-92 µm (avg. 80), and in populations 19-23 87-104 µm (avg. 95). The lumen of the anterior part of bulb is thin-walled and resembles the lumen in the anterior slender part of the oesophagus, but just behind the opening of the dorsal oesophageal gland it becomes heavily cuticularized, the heavy cuticularization disappearing in front of the oesophago-intestinal junction. No distinct differences were noticed in the position of oesophageal gland nuclei and their orifices between various populations. The position of oesophageal gland nuclei and their orifices in populations 1-23 are as follows:

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<td>RS₁N</td>
<td>53-59%</td>
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<td>DN</td>
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The nerve ring surrounds the anterior slender part of oesophagus at 60-92 μm (avg. 70) from the base of odontophore. Cardia short and conoid. Prerectum is 18-25\% of total body length and 18-25 (avg. 22) anal body-widths long. It is distinguishable from the intestine in being narrow and in having a few granules in its cells. Rectum 1.2-1.5 times the anal body-width long.

Genital branches are amphidelphic; each sexual branch consisting of a reflexed ovary, slender and expanded parts of oviduct, a sphincter, and expanded and slender parts of uterus. The uteri are connected to a vagina which opens through a conspicuous vulva at 29-32\% of total body length. In populations 19-23, both the ovaries are of almost same size (Fig. 10, E), while in populations 1-18 the anterior ovary shows much variability in its size and development (Fig. 10, A-D & F-H). Anterior ovary in these populations is usually smaller (less than 1/4th the length of posterior ovary), but it is not non-functional as was thought by Luc (1961) since developing oocytes are present in the hind part of the ovary as well as in the expanded part of the oviduct of the anterior sexual branch. The frequency of oocyte development in the anterior branch is much less than in the posterior sexual branch. However, the anterior ovary in these populations is not always reduced and sometimes may be as developed as the posterior one and rarely even more developed. The above statements hold true only for the ovaries and not for the other associated structures which are always
almost equally developed in both the sexual branches. In an aberrant specimen from population 12 the posterior ovary was missing, but the oviduct and uterus were of normal lengths (Fig. 10,H). The eggs may be rounded-oval to elongate-oval. The size of eggs within the uterus also varies. The majority of the eggs that were measured fall in the range of 150-165 µm (avg. 155) x 32-40 µm (avg. 36). However, often the eggs are as small as 60 µm in length and may reach to a maximum of 200 µm but their widths show little variation from the average (Fig. 10,A & D).

The tail is also quite variable in shape and size (Fig. 9). The shape varies from conoid to elongate-conoid. It measures 66-156 µm (avg. 90); in populations 1-18 it is 66-92 µm (avg. 82), in 19 and 20 86-120 µm (avg. 106) and in 21-23, 110-156 µm (avg. 134). Its ratio with anal body-width is from 3.0-8.5; varying from 3.0-4.3 (avg. 3.4) in populations 1-18, and 4.4-8.5 (avg. 6.0) in populations 19-23. Ratio of tail length / hyaline portion of tail varies from 5.0-20.0 (avg. 7.5); in populations 1-18 it varies only from 5.0-7.5 (avg. 6.0) but in populations 19-23 it ranges from 9.5-20 (avg. 14). The number of caudal papillae vary from 3-4.

Male (Fig. 8,A,B,E-H): Four males were obtained from the type locality of Xiphinema indicum which resemble the females in all morphological characters except the followings:
Lip region rounded and offset. Cuticle 2 um thick at mid-body and 5 um on tail. Amphids stirrup-shaped with slit-like apertures. Odontostyle 101-104 um long with 58-61 um long odontophore. The fixed guiding ring is 93-98 um from the anterior end. Oesophageal bulb 4 times as long as wide. The position of oesophageal gland nuclei and their orifices similar to that of the females. The spicules are sharply ventrally curved measuring 53-58 um along their mid-axis. They are supported by finger-like lateral guiding pieces which are 16 um long. Supplements consist of an adanal pair and a series of 3-5 ventromedians which are spaced as shown in Fig. 8,E.

One of the males in addition to possessing the normal male reproductive organs was also found to have the genital primordium that occurs in the fourth stage female juveniles (Fig. 8,G) in its normal position, i.e., at 32% of total body length. Such a case of male intersexuality is reported here for the first time in Dorylaimida although female inter-sexuality has earlier been reported in dorylaims (cf. Luc, 1961 in *Xiphinema attorodorum*; Jairajpuri & A. H. Siddiqi, 1964 in *Tyleptus striatus*; Aboul-Eid & Coomans, 1966 in *Longidorus macrosoma*; Lamberti et al., 1975 in *X. ingens*).

Tail is conoid-digitate about 1.5 times the anal body-width long and is provided with 2 papillae on each side.

A single male was found in population 22. Although
females of this population have on an average longer tails this male was identical in almost all characters including tail length to the males of population 1 described above.

These males collected from India resemble the males described by Cohn (1969) from Israel differing only in having a less pointed tail and in the number of copulatory muscles. The males described by Cohn have only 11 copulatory muscles up to the 3rd ventromedian supplement while these males have 31 up to this level.

The male described by Saigusa & Yamamoto (1971) has a longer body (2.52 mm) and 3 caudal papillae on each side of tail.

A single fourth stage male juvenile was present in population 14. It had no functional odontostyle which is presumably abnormal. The replacement odontostyle was 101 um long, lying within the walls of the oesophagus with its anterior end just piercing the odontophore which was deformed.

Spicules well developed, about 1.5 times the anal body-width long. Supplements consist of an adanal pair and 2 ventromedians spaced as shown in Fig. 8,G.

Tail conoid-digitate with 2 caudal papillae on each side.

**Juveniles:** The genital primordium occurs at 29-37% of the body length, which conforms fairly well with adult females. Genital primordium in second stage juveniles is small and consists
of only 4 cells, in third stage it becomes elongate-oval and is made up of 12 cells, and in the fourth stage 24-32 cells. Other characters such as position of fixed from the anterior end, shape and size of tail, values of c and c' ratios are useful in differentiating the juveniles of a single population, or juveniles of populations 1-18, or of 19-23 when considered separately, but if all the populations are mixed together these characters prove to be of little value because of the variability and overlap between successive stages. The measurements of various characters of these two groups are given in Table X. In general, each juvenile stage of populations 1-18 has a slightly longer odontostyle, more posterior position of the fixed guiding ring, shorter tail and lower values of c' ratio than those of populations 19-23. The hyaline non-protoplasmic portion is almost completely absent in the first stage juveniles of population 23 but is distinct in the first stage juveniles of population 1. (First stage juveniles were recorded only in these populations).

Discussion: The study of 23 populations of Xiphinema insigne from India shows much variability within this species especially with respect to lip-region, odontostyle, odontophore, spear guiding ring, gonads, tail etc. The juveniles of the different populations also vary much. Because of this, these populations can be divided into two distinct groups 1) insigne-form and ii) indicum-form. The insigne-form populations (19-23)
differ from the *indicum*-form populations (1-18) mainly in the following characters:

i) Body taper towards posterior extremity is more pronounced,
ii) Flat lip region almost continuous with the body,
iii) Shorter odontostyle,
iv) More anterior fixed guiding ring,
v) Longer oesophageal bulb,
vi) Equally developed anterior and posterior ovaries,
vii) Longer tail and greater value of c',
viii) Longer tail length / hyaline portion ratio, and
ix) Absence of hyaline non-protoplasmic portion in first stage juveniles.

Depending upon one's assessment, the above differences between the two groups of populations may be given varying status. Some may regard them as representing different species, with populations 1-18 identifiable as *Xiphinema indicum* Siddiqi, 1959, while populations 19-23 could be *X. insigne*. Another course may be to accept them as subspecies which may add to the existing confusion. A third opinion may be to consider these differences as intra-specific variations of *X. insigne*, a view held by the present author.

In spite of the fact that there are some morphological differences between the two groups of this species, they are neither convincing nor clear-cut and when a large number of specimens were studied different characters were found to
overlap (e.g., shape of lip region, odontostyle and tail lengths etc.). Also when the two forms are compared with *X. insigne* (lectotype after Tarjan & Luc, 1963) and *X. indicum* Siddiqi, the author has failed to find a single reliable and consistent character by which the two species can be recognised as distinct (Table XVIII). *X. insigne* is therefore regarded as an extremely variable species and the synonymy of *X. indicum* as proposed by Tarjan & Luc (1963) is further confirmed by this study.

*Xiphinema insigne* is closely related to *X. simillimum* Loof & Yassin, 1971 and *X. orbum* Siddiqi, 1964. *X. simillimum* described from the Sudan resembles the indicum-form in nearly all its characters, the only difference being the presence of an undifferentiated anterior sexual branch in *X. simillimum* though it is quite long (110 um) and is slightly differentiated in one of the paratypes. *X. orbum* is distinct from *X. insigne* in having a more slender body (a=90), in having the different parts of anterior sexual branch greatly reduced and in having only 2 caudal papillae on each side of tail.