NEMATODE PARASITES OF EARTHWORMS

Dissertation
submitted in partial fulfilment for the degree of
MASTER OF PHILOSOPHY
IN
ZOOLGY

TALAT ANWAR

Department of Zoology
Aligarh Muslim University, Aligarh
August, 1977
This is to certify that the entire research work which has been presented in the dissertation entitled "Nematode parasites of Earthworms" by Talat Anwar was carried out under my supervision and I allow her to submit it to the Aligarh Muslim University for the award of the degree of Master of Philosophy.

(Durdana S. Jairajpuri)
Ph.D.
Supervisor
ACKNOWLEDGEMENTS

My sincere thanks are due to Dr. (Mrs.) Durdana Jairajpuri for her valuable guidance and selfless help during the progress of this work.

I am also indebted to Prof. S.M. Alam, Head, Department of Zoology for providing laboratory facilities. I am particularly indebted to Dr. M. Shamim Jairajpuri and to all research scholars of nematology lab for their constant help during this work. Special thanks are due to Miss Humaira Khatoon for constant encouragement and help. I am also thankful to the Council of Scientific and Industrial Research for financial help.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Systematic position of nematodes of earthworms and <strong>Athusia</strong> n. gen.</td>
<td>6</td>
</tr>
<tr>
<td>Material and Methods</td>
<td>8</td>
</tr>
<tr>
<td>Description of Taxa</td>
<td>11</td>
</tr>
<tr>
<td><strong>Athusia gracilis</strong> n. sp.</td>
<td>11</td>
</tr>
<tr>
<td><strong>Athusia natai</strong> n. sp.</td>
<td>15</td>
</tr>
<tr>
<td><strong>Athusia pharatomae</strong> n. comb. Timm, 1959</td>
<td>15</td>
</tr>
<tr>
<td>Discussion</td>
<td>17</td>
</tr>
<tr>
<td>Detailed morphology of <strong>Athusia gracilis</strong></td>
<td>18</td>
</tr>
<tr>
<td>The juvenile stages</td>
<td>26</td>
</tr>
<tr>
<td>Intra-specific variation in <strong>Athusia gracilis</strong></td>
<td>29</td>
</tr>
<tr>
<td>References</td>
<td>34</td>
</tr>
<tr>
<td>Plates</td>
<td>I - V</td>
</tr>
</tbody>
</table>

---
INTRODUCTION

The nematodes are one of the largest groups in the animal kingdom comprising about 500,000 species and are therefore second only to the insects as far as the number of species is concerned. Hyman (1951) has pointed out that at least 100,000 nematodes species are parasitic in vertebrates, and quite a large number are parasites of invertebrates. A vast number are plant-parasitic, whereas the free-living species exceed the parasitic ones. Most nematode species are microscopic, may be free-living or parasitic in the body fluids of their hosts such as in blood and lymph. The nematodes that live in the intestine of their host are generally larger in size. Within a host the nematodes may parasitise any organ including the eye, brain, mouth, tongue, stomach, intestine, lung, heart, liver, etc. Perhaps Cobb has correctly said "they occur in arid deserts and at the bottom of lakes and rivers, in the water of hot springs and in the polar seas where the temperature is constantly below the freezing point of fresh water. They occur at enormous depths in Alpine lakes and in the Ocean. As parasite of fishes they traverse the seas, as parasite of birds they float across continents and over high mountain ranges". 
Most nematodes are bisexual, some unisexual. They may be oviparous or viviparous. Some forms may change from oviparous to viviparous, being oviparous in early life and becoming viviparous with age. In many species males are rare and not entirely necessary for reproduction, while in others males are entirely unknown.

The life of nematodes is usually a simple story hatching from the egg as a small organism similar to the adult except for the sexual character. It goes through a series of stages marked by ecdysis and then to the adulthood.

Nematodes have great economic importance for man because of a large number of them causing diseases in him and his livestock. The hookworms, Ascarids, Filarids, Trichinella, Entamoeba etc., are very important because they are the causative agents of well known diseases. There are a large numbers of other vertebrate parasites inhabiting birds, fishes, reptiles etc., causing a great damage to these animals. It is due to this that much research work was done on vertebrate nematodes, and also recently on plant-parasitic nematodes which are of agricultural importance.

In the past years the nematologists have concentrated mainly on those group of nematodes which parasitise man, his livestock or crops. The work on nematodes of invertebrate animals started comparatively late. First, the juvenile
stages of nemithid nematodes were found in the terrestrial or freshwater insects. It was Kühn (1905) who for the first time observed larvae of Paramermis contorta, a nematode parasitising the insect midge, Chironomus. In 1923, Cobb found Astigmus degusdata parasitic in grasshoppers. Many other workers like Coodey (1941), Christie (1937), Baylis (1921) observed juveniles of nematodes attacking insects. The genera, Rhigonema Cobb, 1898; Dendicae Artigas, 1930 and Ichthyoscocephalus Artigas, 1926 belonging to the family Rhigonematidae were found in the Arthropods. Basir (1956) did excellent work on the nematode parasites of Arthropods. Most of the work done so far on invertebrates nematodes is confined to insects, snails or millipedes.

Oligochaetes were completely ignored until Pierantoni (1923) described some species of nematodes from the coelome of earthworms and recognised three families Cephalonematidae, Onychidae and Drilonematidae. Baylis and Daubney (1926) discussed Pierantoni's classification and placed all the genera under family Drilonematidae. The latter name has been justifiably emended to Drilonematidae by Chitwood and Chitwood (1935) and was placed as an appendix of Rhabditoida.

Baylis (1943) gave a comprehensive account of the taxonomy of nematodes of earthworms and descriptions of 7 new species. Timm (1959–1967) in a series of papers described the nematodes that occur in the body cavities of earthworms and added a number of new genera and species. It is interesting
to note that the majority of the nematodes from the earthworms described by the various workers as well as a new species which is being described in this work were provided by Prof. G.E. Gates, a well known world authority on Oligochaetes. Absolutely no work has been done in India on the nematodes of earthworm except for a brief report by Ali and Fakhruddin (1970) who described a species belonging to the genus *Siconemella*. Since the majority of the nematodes of earthworms have been reported from South Asia the author undertook the study of these interesting worms with a view to clarify their systematic position.

The present work gives the detailed description, intraspecific variations etc., of *Anthisia gracilla*, new genus new species from the earthworms in Aligarh. Another new species of the same genus was found in the collections sent by Prof. Gates. In addition to this a review of the classification of nematodes of earthworm has also been provided.
<table>
<thead>
<tr>
<th>Superfamily</th>
<th>Family</th>
<th>Subfamily</th>
<th>Genus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drilonematoida</td>
<td>Ungellidae</td>
<td>Ungellinae</td>
<td>Ungella (Cobb 1922)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Synicomma (Baylis)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Siconemidae Timm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ciftonia Timm 1962</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Siconemella Timm</td>
</tr>
<tr>
<td></td>
<td>Homungellidae</td>
<td></td>
<td>Homungella Timm</td>
</tr>
<tr>
<td></td>
<td>Cregroceridae</td>
<td></td>
<td>Cregrocerus Baylis</td>
</tr>
<tr>
<td></td>
<td>Onyclidae</td>
<td></td>
<td>Dionyx Pierantoni</td>
</tr>
<tr>
<td></td>
<td>Cephalonemidae</td>
<td></td>
<td>Cephalonema Pierantoni</td>
</tr>
<tr>
<td></td>
<td>Pharyngnematidae</td>
<td>Pharyngnematinae</td>
<td>Pharyngnema Timm, Scolexophiloidea (Timm)</td>
</tr>
<tr>
<td></td>
<td>Scolexophilidae</td>
<td></td>
<td>Scolexophiloidea (Timm)</td>
</tr>
</tbody>
</table>
SYSTEMATIC POSITION OF NEMATODES OF EARTHWORMS
AND
ATUSIA N. GEN.

Nematode parasites of earthworms were classified at
different times by different nematologists but no satisfactory
position of these worms could be established. Pierantoni
(1916) studied many worms from the body cavity of earthworms
and classified them into three families as Cephalonemididae,
Onyldidae and Drilonemididae without assigning them to higher
taxa. The family name Cephalonemididae and Onyldidae Pierantoni
(1916) became in valid as the genera of these families were
preoccupied.

Baylis and Dauney (1925) discussed Pierantoni's
classification and placed all the known genera in a single
family Drilonemididae Pierantoni, 1916. Chitwood and Chitwood
(1935) changed the family name from Drilonemididae to
Drilonematidae which they placed as an appendix of the super-
family Rhabditoida. In 1950, Chitwood and Chitwood created
a new superfamily Drilonematoida and included all the
nematode parasites of earthworms under this superfamily in
order Rhabditida. Two families were recognised under the
superfamily Drilonematoida differing from one other on the
presence and absence of cephalic hooks. The family Drilonematidae
with subfamilies Drilonematinae and Pharyngonematinae and the family Unigallidae. Two other families namely Scolecocephillidae and Crengocercidae were included as an appendix.

Timm (1959-1967) described a number of species of nematode of earthworms. In 1959, he emended the diagnosis of the genus Pharyngonema. The genus did not fit into the family diagnosis of Drilonematidae. Therefore he raised the subfamily Pharyngonematinae to a family rank and placed it under the superfamily Oxyuroidea.

In the present work the genus Pharyngonema has been considered genus in inquirenda and a new genus Athusia has been proposed under the family Pharyngonematidae, superfamily Oxyuroidea, Order Rhabditida.
Collection of the host:

For the present work earthworms were collected from various regions of Aligarh. The earthworms, if not dissected immediately were kept in wet soil in the laboratory. Nearly 3,000 specimens of *Phasitina posthuma* were dissected and examined for the presence of nematodes.

Live earthworms were first washed in tap-water and then placed in the petri-dishes. An incision was made along the length of the earthworm. The coelomic contents were then mixed thoroughly in a small amount of water and the petri-dishes were kept undisturbed for a few minutes allowing the heavy soil particles to settle down at the bottom. The parasites, if present, can be easily located because of the movements under the low power of the stereoscopic binocular microscope.

Isolation of parasites:

The nematodes were picked with the help of a fine needle and were kept in small amount of water in a cavity-block. The nematodes remain quite active for about an hour. The worms so recovered were first studied under the
stereoscopic binocular microscope as this often provides some valuable information about their colour, shape, movements as well as some morphological details.

**Killing and fixation:**

When the parasites became fully relaxed the excess amount of water was removed from the cavity-block with the help of a dropper and hot 70% alcohol was added for the killing and fixation of the nematode.

**Dehydration and clearing:**

The nematodes fixed as above were transferred to cavity-blocks containing glycerine-alcohol (5 parts glycerine and 95 parts 70% alcohol). The cavity blocks were then kept in a desiccator at room temperature for gradual dehydration. After about 3-4 weeks the nematodes are ready for preparing permanent glycerine mounts. The nematodes were mounted either on glass or aluminium slides. When the latter was used the specimen were placed between two coverslips in a drop of pure glycerine, around the specimen pieces of glass-wool or plastic wire of suitable thickness were arranged to prevent the flattening or crushing. Some time specimens were also cleared and mounted in lactophenol.

**Cross sections of body:**

The on face view and cross sections of body at different levels were also studied. A worm which was already cleared in glycerine was transferred to a drop of melted glycerine.
jelly and then with the help of very sharp knife a cut was made as close to the anterior end as possible under the binocular microscope. The cut end was so manipulated as to have the anterior extremity facing upwards. This was then transferred to a drop of glycerine on an aluminium slide with the help of sharp needle and was covered with a coverslip. The cross sections of different regions of the body were similarly prepared.

Measurement and drawings:

The measurements of the specimens were taken with the help of an ocular micrometer. De Man's (1884) formula for representing the dimensions of the nematodes was used.

The diagrams were drawn with the help of a camera lucida.
DESCRIPTION OF TAXA

GENUS ATHUSIA N. GEN.

Pierantoni (1923) described rather poorly a nematode from an Oligochaet Necrasocolax mekongensis. He proposed a new genus new species, Pharmannema mekongensis for these worms. Timm (1959) collected some nematodes from Pharetima posthuma and identified them as a new species of Pharmannema. According, he emended the generic diagnosis so as to supplement the insufficient and the meagre account of the genus as given by Pierantoni.

A large number of the worms were collected from the Coelomic cavity of Pharetima posthuma in Aligarh. Study of these nematodes revealed that they are closely related to Pharmannema as conceived by Timm. The inadequate description and extremely poor illustrations of the genus Pharmannema by Pierantoni leave much to be desired as was also mentioned by Timm. In the opinion of the present author the identity of Pharmannema is questionable and it appears quite justified to regard it as a genus inquirenda. A new genus, Athusia is therefore established for the species obtained in India as the one described by Timm. A closely related but different species
was present in the collection of nematodes sent to Dr. M. Shamim Jairajpuri by Prof. G.E. Gates. It has also been described.
ATHUSIA N. GEN.

Diagnosis: Small and stout worms. Mouth bordered with or without lips. Buccal cavity small, present or absent. Four large submedian labial papillae in outer circle and four small submedian papillae in the inner circle present.


Tail conoid. Phasmids small.

Type species: Athusia gracilis n. sp.


Athusia gatasi n. sp.

ATHUSIA GRACILIS N. SP.

(Fig. )

Cuticle finely striated, slightly brownish in appearance. Anterior end of body bluntly rounded provided with 2 very small hooks. Mouth surrounded by 3 small lips, one dorsal and 2 ventrolateral in position. The amphids are large and circular. Cephalic papillae 8 in number, 4 larger submedian in outer circle and 4 smaller submedian in inner circle.
Esophagus is highly muscular, oval and lacking a valve. Intestine is a straight tube. The intestinal cells are packed with large globules. The nerve ring surrounds the intestine just behind esophagus. Excretory pore near the distal end of the anterior ovary.

Female reproductive system amphidelphic. Ovaries are either outstretched or reflexed, the anterior ovary is longer than the posterior ovary which is generally reflexed. Vulva slit-like, a little behind the middle of body. Vagina a muscular tube. Anterior uterine branch is also longer than the posterior branch and also contains more eggs than the latter. The females are of two types depending upon the stage of development in the egg. In oviparous females the eggs are small, double-walled, and pear-shaped, 5-11 in number. In ooviviparous females the eggs are elongated, single-walled and brownish in colour, maximum number being not more than 5.

The cuticle of the anal region is thick, tail muscles spread out in a fan-like fashion. The tail abruptly narrows to a fine spike with a finely rounded tip. Phasmids are situated just posterior to the anus and are pore-like.
ATHUSIA GATESI N. SP.

Description:

(The description based on one intact and one damaged female which were sent by Prof. G.E. Gates).

Body stout, tapers slowly to a rounded anterior end and a tapering posterior end. Cuticle apparently smooth, most probably the striaation are so fine that they are easily observed. Mouth is provided with 4 sclerotized teeth. Amphids are large and papilla-like. The mouth leads to a buccal cavity which is slightly cuticularized and surrounded by esophageal tissues. Valves are present just below the cup-like buccal capsule. Esophagus is highly muscular, posteriorly it forms bulges which project into the lumen of the intestine which is at first wide but gradually narrows. The intestine becomes abruptly narrow to form rectum. Tail is conoid with an acute terminus. Phasmids are tiny, pore-like, situated just below the anus.

Vulva located behind middle of body, slit-like. Vagina small and muscular. Gonads amphidelphic. Anterior sexual branch longer than the posterior branch. 3-6 eggs in the uterus: elongated and double walled; containing developing embryo.

*Athusia phreatima* new comb.
<table>
<thead>
<tr>
<th>Characters</th>
<th><em>Athysia gracilis</em> n. sp.</th>
<th><em>Athysia gatlesi</em></th>
<th><em>Athysia pheretimae</em> after Timm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Holotype</td>
<td>Paratypes</td>
<td>Holotype</td>
</tr>
<tr>
<td>Body</td>
<td>2.42 mm</td>
<td>1.5-2.6 mm</td>
<td>3.7 mm</td>
</tr>
<tr>
<td>Body width</td>
<td>266 μm</td>
<td>120-266 μm</td>
<td>178 μm</td>
</tr>
<tr>
<td>Cuticle thickness</td>
<td>5 μm</td>
<td>4-5 μm</td>
<td>4 μm</td>
</tr>
<tr>
<td>Hooks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>2</td>
<td>2-4</td>
<td>-</td>
</tr>
<tr>
<td>Size</td>
<td>5 μm</td>
<td>4-5 μm</td>
<td>-</td>
</tr>
<tr>
<td>Buccal cavity *</td>
<td>-</td>
<td>-</td>
<td>130 μm</td>
</tr>
<tr>
<td>Esophagus *</td>
<td>128 μm</td>
<td>88-181 μm</td>
<td>240 μm</td>
</tr>
<tr>
<td>Nerve ring (Position from anterior end)</td>
<td>173 μm</td>
<td>88-180 μm</td>
<td>-</td>
</tr>
<tr>
<td>Vulva ** (Position from anterior end)</td>
<td>69%</td>
<td>54-69%</td>
<td>58%</td>
</tr>
<tr>
<td>Anterior gonad ***</td>
<td>59%</td>
<td>40-59%</td>
<td>55%</td>
</tr>
<tr>
<td>Posterior gonad ***</td>
<td>22%</td>
<td>21-28%</td>
<td>35%</td>
</tr>
<tr>
<td>Tail *</td>
<td>133 μm</td>
<td>88-173 μm</td>
<td>280 μm</td>
</tr>
<tr>
<td>Eggs</td>
<td>70x20 μm</td>
<td>70-75x20-25 μm</td>
<td>75x25 μm</td>
</tr>
</tbody>
</table>

* Length
** Position from anterior end expressed as percentage in relation to body length.
*** Total length expressed as percentage in relation to body length.
DISCUSSION

_Athusia gracilis_ differs from _Athusia pharatinum_ in the presence of a pair of hooks anterior to the lips, stoma is shorter than in _A. pharatinum_ and _A. gatac_, in the presence of three small lips which are absent in _A. pharatinum_. The esophagus and tail are shorter whereas the vulva is located more posteriorly. The anterior ovary of _Athusia gracilis_ is nearly thrice as large as in _A. pharatinum_ and the ova of the former are much smaller than in the latter species.

_Athusia gatac_ varies from _A. gracilis_ in the absence of lips. It differs from both the known species _A. gracilis_ and _A. pharatinum_ in the presence of 4 sclerotized teeth, a large cup-like buccal capsule with valvular arrangement and a conoid tail. The intestine abruptly tapers and forms a fine tube like rectum which is absent in other two species.
DETAILED MORPHOLOGY OF ATHUSIA GRACILIS

The specimens of Athusia gracilis are brownish white in colour. Upon fixation they assume a straight posture. They have short bodies which are blunt anteriorly but taper towards the posterior end. The shape of tail is greatly variable.

Cuticle:

The cuticle is smooth, without any striations. It is double layered, the outer one thicker and the inner thinner. The thickness of the two cuticle layers ranges from 4 µm to 5 µm. The cuticle is more thickened at vulva and anus and at extremities.

Lateral chords:

The lateral chords prominent appearing as bulges on the two sides of the body. These markings are more prominent in middle part of the body. They originate just behind the lip region. In cross section at the level of esophagus the lateral sides show only convex ridge like structure. In the region of anterior portion of intestine the ridge forms a depression in the cuticle thus giving rise to two ridges. In the middle part of the body the cross section shows two grooves and three ridges. The width of lateral fields is
maximum at the vulva. In the post vulval region the lateral fields begin to narrow and on reaching the tail the lateral lines run close to each other and ultimately converge.

**Amphida and phasmids:**

In *Athisia gracilis* amphids are circular and papilla like projecting above the contour of lips. They are innervated by minute nerve fibers from the cephalic sensory nerves. Phasmids are small pore-like structure, situated just below the anus.

**Hypodermis:**

The hypodermis is a rather thin syncytial layer which is thickened at four points to form the longitudinal chords. These thickenings are dorsal, ventral and lateral in position. Towards the anterior end of the body these chords are rather poorly defined. In cross section of the body at different levels these chords can be clearly seen bulging out in the body cavity in the dorsal ventral and lateral positions at the junction of esophagus-intestinal junction dividing the visceral cavity, though incompletely into four quadrants in which sets of somatic muscles are arranged.

**Musculature:**

The muscles in the body of the nematodes were studied under two headings:

1. Somatic or unspecialized musculature
2. Specialized musculature
Somatic musculature:

Each quadrant of hypodermis is provided with four cells which are flat at the point of their attachment with the hypodermis. Thus these nematodes are typical meromyarian with platymyarian type of somatic musculature. The number of muscle cells was found to be always constant i.e., four cell for per quadrant but these cells are more prominent towards the middle of the body and less towards both extremities.

Specialized muscles:

In addition to the somatic muscles there are specialized muscles associated with the various organs of the body to help them in their activities. The various body parts such as hooks, esophagus, intestine, anus, vulva etc., have separate sets of muscles attached to them. All the openings are guarded by specialized type of muscles known as sphincters.

Esophageal muscles: The esophagus of *Athusia gracilis* is highly muscular. The lumen of the esophagus is triangular and very narrow. Except for the lumen all the other spaces of esophagus are occupied by radiating muscle fibers which are divided in three sectors and are attached to both sides of the lumen. A pair of muscles present at base of the hooks help to contract and relax them.

Intestinal muscles: The muscles associated with the intestine and hypodermis and maintain the intestine in its position are
known as somato-intestinal muscles.

**Vulval muscles:** There are several sets of muscles which are associated with the vulva helping in the dilation and contraction of the vulva especially at the time of egg laying.

**Anal muscles:** There are several bands of muscles present in the anal region of the female which extend from the lower region of the anus to the subdorsal wall of the body. These muscle bands help in the dilation and contraction of anus at the time of discharge of waste products and also help in the movement of tail.

**Lip region:**

The lip region may be continuous with the esophagus or slightly differentiated by a very short stoma. A pair of crescent-shaped hooks are present just anterior to the lips.

Lips are three in number and are of equal size. One dorsal and two subventral in position. There are four small cephalic papillae present in the inner circle and four large papillae in the outer circle which are supplied by fine nerve endings.

**Digestive system:**

The digestive system consists of the following parts (1) stoma, (2) esophagus, (3) intestine, (4) rectum and (5) anus.
Stoma: A very small stoma is present which is discernible in few specimens only. In majority of the specimens the mouth opens directly into the esophagus without any stoma.

Esophagus:

It is bulbous, oval or somewhat violin-shaped. In some specimens the esophagus is divided into two parts by a slight constriction. The posterior small lobes are less muscular and bulge into the intestine lumen. Valves are absent.

Intestine:

The esophagus directly opens into the intestine. Some glandular cells are present at the esophago-intestinal junction. The intestinal tube is narrow in the beginning but posteriorly it occupies nearly all the available space. It is pushed to dorsal side in mature specimen in order to accommodate the developing genital organs. In the posterior region it somewhat narrows to join the rectum.

Rectum:

The intestine narrows posteriorly and joins the rectum. The rectum is a dorsoventrally bent tube lined by the cuticle. Rectum opens to the outside through the anus.

Anus:

It is a circular opening in the posterior region of the body and is midventral in position. The anal aperture is
surrounded by a set of anal muscles. Through this pore the alimentary canal communicates to the outside.

**Excretory System:**

The excretory system of *Athusia* is of simplest type comprising only of an excretory pore and a vesicle, the excretory vesicle. It lies in the midventral position below the level of nerve ring. The excretory pore is guarded by muscles.

**Nervous System:**

The nervous system is well developed. It consist of a nerve ring, ganglia and the nerve fibres. The nerve ring which is the center of the nervous system is fibrous in nature. The nerves arising from the nerve ring and the associated ganglion to different organs. Two nerves known as cephalic sensory nerve arise from the ganglia and proceed to the anterior end and two lateral nerve arise from the posterior part of the ganglion. From the anterior nerves many delicate branches arise and go to the lip region, amphids, esophagus etc. Same way the posterior part of the body and the middle part is supplied by nerves from the branches of lateral nerve cords.

**Reproductive system:**

Only the females were found and the males were totally absent. The gonads are amphidelphic with outstretched ovaries.
Each sexual branch consist of an ovary, an oviduct, muscular uterus, a short vagina and slit-like vulva. The lengths of the anterior and posterior branches of the ovary are 40-52% and 21-29% respectively of the body length. The vulva is located at 54-69% of the body length from anterior end. The anterior sexual branch is much longer as compared to the posterior one. The former in some older females may also become, reflexed, but the posterior branch is mostly reflexed.

The ovary joins the uterus which is of uniform thickness. The uterus is highly muscular in nature. The anterior uterus is much larger and usually contains more eggs while the posterior one is shorter and has only one or two eggs at a time. The vagina is highly muscular connected to both the uteri. Its muscles help in egg laying. The vaginal walls are also lined with cuticle and open outside through a depressed transverse slit-like vulva which is also cuticularized. The body wall is slightly raised in this region due to the thickening of the cuticle.

Some abnormalities were also found, e.g., the presence of double vulva and the flexure of both the ovaries. The gravid females had two types of egg, one type having well developed juveniles within it while the others without it. In some females both types of eggs were found while in others were either viviparous or oviparous. The eggs without the
juveniles were laid by the females, the eggs having juveniles were retained inside the body and were not laid. The number of eggs present in the uterus varies from 2-11 when there are more than 4-5 eggs in the uterus they are laid at shorter intervals than when the number is less.

The eggs are thin-shelled, and are single layered and measure 70-75 x 22-25 μm. These contain the first stage juvenile which is coiled up within the shell. The other type of eggs which are more common in the population have double-layered cuticular shell and are brownish in appearance, they measure 50 x 30 μ. These eggs are laid in unsegmented stage.
THE JUVENILE STAGES

1st Stage Juvenile:

**Measurement:** Body length 1.04 mm; maximum body width = 56 mm; length of esophagus = 74 μm; tail length = 106 μm.

Body long and slender almost straight. The cuticle is of uniform thickness throughout the body length except at the tail end where it is slightly thickened. The anterior end is bluntly rounded and provided with one pair of hooks just anterior to the lips supported by muscles. Three lips, four small and four large papillae are revealed in the **en face view.** The mouth leads to a muscular esophagus which is followed by a simple glandular intestine. The tail length is about 1/10th of the body length. The anal muscles are poorly developed. The nerve ring encircles the anterior end of the intestine which is just below the esophagus. The excretory vesicle is close to the nerve ring.

2nd Stage Juveniles:

**Measurement:** Body length = 1.4 mm; maximum body width = 59 μm; length of esophagus = 74 μm; tail length = 125 μm.

Body almost straight from anterior to the posterior end. Cuticle thickness uniform throughout the body length except at the anus and tail tips. One pair of hooks can be clearly seen.
Lips and papillae same as in 1st stage juvenile. Amphids are present, but are not as prominent as in adults. Mouth opens into a muscular valve less esophagus which leads to the intestine. Intestine is packed with glandular vacuolated cells, tapers posteriorly to join the rectum. The rudiments of glands can be seen in the posterior half of the body. The future vulva is also indicated at 63% of the body length from anterior end. The nerve ring surrounds the anterior end of intestine. The body muscles are poorly developed.

3rd Stage Juveniles:

Measurements: Body length = 1.427 mm; maximum body width = 96 μm; esophagus = 93 μm; tail length = 112 μm, nerve ring at 9 μm from the anterior end of the body length.

The body of the worm is straight; cuticle is double layered as in adults. One pair of hooks clearly seen at the anterior end. Three lips one dorsal two subventral and 8 papillae in 2 circles can be seen in en face view. Papillae-like amphids are clearly visible at this stage. Esophagus is muscular followed by the intestine which is packed with glandular cells; its anterior end surrounded by nerve ring. The anal muscles are more developed than the former stage. The genital primordia become more differentiated into an anterior and a posterior arm. The developing ovary of both the sides
join a small uterus and then a short vagina. The vagina is connected to the future vulva, situated below the middle of body at 64% of body length from anterior end.

4th Stage Juvenile:

Measurements: Body length = 1.86 mm; maximum body width = 122 μm; esophagus length = 114 μm; tail length = 125 μm; vulva = 1197 μm (64%).

The body is slightly arcuate ventrally upon fixation. The anterior end is blunt and a pair of hooks present near the mouth. Papillated amphids are much more clear than in the former stages. En face view shows lips, papillae, and the amphids as in the adult stage. The esophagus opens directly into the intestine without any valve. The maximum thickness of the intestine is in the middle region of the body, but it tapers posteriorly towards the anus. The anus is surrounded by anal muscles which are not as developed as in the mature worms. The nerve ring is quite prominent. Excretory pore near the nerve ring consisting only of an excretory vesicle. The reproductive organs are more developed than in the former stage, but the anterior one is larger in size than the posterior one. The ovaries open into the muscular uterus, which joins a small vagina, which opens through a vulva.
INTRA-SPECIFIC VARIATIONS IN ATHUSIA GRACILIS

The characters that are used in nematode taxonomy are known to vary among and within the same populations of a single species. These variations may be host induced or due to differences in the environmental conditions or the geographical distribution. The variation may also be due to host species or variety. In the present study intra-specific variations in Athusia gracilis have been studied. The variations in the species were studied within a single local population obtained from the earthworm collected in Jamalpur, University area, Aligarh. The variations were observed in almost every character of Athusia gracilis. These variations are basically different for each character.

**Body length:**

Mostly the body length of the adult ranges from 1.50 mm to 2.66 mm. In exceptional cases the length may be up to 2.8 mm or 3.0 mm, but this is rare.

In juveniles the body length measures from 1.00 mm to 1.35 mm. These variations in the juveniles are according to age and state of development.

**Body width:**

The body width in the adult varies from 0.1 mm to
0.2 mm. Some older specimens measure up to 0.25 mm in thickness.

The body width in juveniles varies from 0.05 mm to 0.1 mm according to their age.

Hooks:

A pair of hooks is present in this species, its maximum length in adults being 5 μm, but it may range from 4.5 μm to 5.3 μm.

The hooks in the juveniles are more clearly visible and the length of hooks is also greater in relation to the body length. The hooks are 4-5 μm long.

Cuticle:

The body cuticle of the mature worms varies in thickness from 4-5 μm. In juveniles, it varies from 2.4 μm. The cuticle of the anal, vulval and tail tips is thicker than on the main body. The thickness is as follows in different regions.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Region</th>
<th>Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>Vulva region</td>
<td>7 - 8 μm</td>
</tr>
<tr>
<td>Adult</td>
<td>Anal region</td>
<td>5 - 7 μm</td>
</tr>
<tr>
<td>Juvenile</td>
<td>Anal region</td>
<td>4 - 5 μm</td>
</tr>
<tr>
<td>Adult</td>
<td>Tail region</td>
<td>5 - 7 μm</td>
</tr>
<tr>
<td>Juvenile</td>
<td>Tail region</td>
<td>7 - 11 μm</td>
</tr>
</tbody>
</table>
The tail tip is comparatively more thicker in the juveniles as compared to adults.

**Esophagus:**

The esophagus is the most variable organ in this nematode. It varies in shape as well as in size. The length of esophagus in mature females varies from 104–170 μm. The esophagus in the juveniles measures 74–95 μm in length.

The esophagus of *Athurta gracilis* is muscular and oval or violin shaped having a narrow lumen and without any valve. The shape of the esophagus is also quite variable. It is divided into two unequal parts by a constriction, the longer anterior part is muscular and the smaller posterior part is less muscular, globe-like. In some specimen, the lower part is again divided into two lobes, a right and a left one. In some specimen the esophagus is slightly constricted in the middle. In most specimen the esophagus is bluntly rounded at the posterior end, but in some it is somewhat narrow. The esophageal lumen is narrow usually present in the center of the muscular part but may be displaced to the left or right side dividing the esophageal mass into two unequal parts.

**Intestine:**

The length and width of the intestine varies according to the length and width of the specimens, so there is no marked
differentiation in the intestine of *Athusia gracilis*.

**Nerve ring:**

It is the least variable character in this nematode. Its position from 12 μm to 17 μm from anterior end of body.

**Reproductive organs:**

The length of anterior and posterior gonads are quite variable in this population. The anterior gonad of the mature females varies from 43-59% and the posterior one from 15-23%. The percentage of vulva ranges from 59-69%. There are only two specimens in this population which have abnormally high vulval percentage, i.e., 75-76% of the body length from anterior end.

**Eggs:**

Eggs not only differ in size but also differ in shape and number. The number of eggs in mature females vary from 2-11 in a single specimen. The shape of the eggs may differ within and between specimens. The eggs possessing a developed juveniles are more elongated and pointed towards their extremities.

The eggs without juvenile are broad at one end and pointed at the other. This type of egg has double-layered shell, the thickness of the shell varies from 2-3 μm.
Tail:

Like esophagus the tail length and shape is also greatly variable. The tail length ranges from 105-140 \( \mu m \) except in one specimen in which the tail was 160 \( \mu m \).

In juveniles the tail varies greatly from 104-125 \( \mu m \). The tail in juveniles is comparatively longer than those of the adult in relation to its body length.

The above observations are based on the study of about 50 specimens both adults and juveniles.
REFERENCES


PLATE I

Fig. A-E. *Athuri gracilis* n. gen. n. sp.

A. Anterior region,

B. Posterior region,

C. Mature female containing developing juveniles inside the eggs,

D. Mature female having eggs with or without juveniles,

E. Mature female having eggs without juveniles.
PLATE II

Fig. A - D. *Athusia setosa* n. sp.

A. Anterior region,
B. Posterior Region,
C. Egg,
D. Mature female.
PLATE III

Fig. A - G. Cross sections through body of *Athusia gracilis*.

A. *En face* view,
B. *Esophagus*,
C. *Intestine*,
D. *Anterior ovary*,
E. *Uterus*,
F. *Egg*;
G. *Posterior reflexed ovary*. 
PLATE IV

Fig. A - F. Juvenile stages of *Athusia gracilis*.

A. First stage,
B. Second stage,
C. Third stage,
D. Fourth stage,
E. Young adult,
F. Developing first stage juvenile inside the egg.
PLATE V

Variability in the shape of esophagus (A - E), reproductive organs (F - M) and tail (N - Q) of Athusia gracilis.