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## Record of clover cyst nematode *Heterodera trifolii* Goffart, 1932, from Nilgiris Tamil Nadu, Its distribution and morphometrics

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### Abstract

The clover cyst nematode *Heterodera trifolii* has been recorded on white clover *Trifolium repens* from Nilgiri hills, Tamil Nadu. It was observed only at high altitudes of Nilgiris at an altitude above 2500 mSL. The cyst counts ranged from 42-186 cysts/ 200 cc soil. *H.trifolii* was observed only on white clover and other legumes and non legumes were found free from infestation. The morphometric studies indicate the variations in the morphological features are minimum in respect of juveniles, white females and cysts, as compared to the original description by Goffart, 1932 [2].

**Keywords:** *Heterodera trifolii*, *Trifolium repens*, morphometry

### Introduction

Cyst nematodes are considered as the highly specialized and well established parasites of plants and causes considerable yield losses in many cultivated plants in tropics, subtropics and temperate ecosystems.

The clover cyst nematode *Heterodera trifolii* Goffart, 1932<sup>[2]</sup> first described the species on red clover *Trifolium pratense*. It has a worldwide distribution on many leguminous plants including 17 species of *Trifolium*. It is a cosmopolitan species having wide distribution in USA, Europe, Canada, Isrel, Australia and Newzeland. In India Khan *et al.*, (1989)<sup>[5]</sup> recorded *H.trifolii* on blackgram *Vigna mungo* from Kangra region of Himachal Pradesh. On a random survey the clover *trifolium repens* from Nilgiris hills of Tamil Nadu. Its distribution and morphometry has been studied.

### Materials and methods

Random sampling of rhizosphere soil (200cc) and roots (5g) were drawn. The cysts from the soil were separated by using a Fenwick can (Fenwick, 1940)<sup>[1]</sup> and juveniles from the soil were isolated by using Cobb's wet sieving method followed by modified Baermann funnel method (Schindler, 1961)<sup>[9]</sup>. The white females adhering to the roots were removed by using a mounting needle and camel hair brush. The juveniles were fixed in hot 4% Formaldehyde and processed by slow method to glass slides in glycerine medium.

The measurements of morphological characters of juveniles, white females and cysts were taken by using an image analyser. The juvenile characters *viz.*, body length (L), width, spear length, tail length and hyaline portions of tail length and hyaline portions of tail were measured.

The white females and cysts were mounted on a cavity slide and the measurements on length, width and neck length were measured. The vulval cone of adult females and cysts were cut and mounted on glycerine jelly (Kornobis, 1976)<sup>[7]</sup>, after soaking the cysts overnight. The measurements of vulval slit, Fenestral length, width were taken. The vulval bridge and bullae were observed on vulval cone mounts.

### Results

#### Distribution of *H.trifolii* in Nilgiris

Out of 44 locations surveyed in Nilgiris comprising Udthagamandalam, Coonoor and Kotagiri subdivisions, only 10 locations in Udthagamandalam subdivisions, yielded *H. trifolii*. The cyst counts ranged from 92-286 per 200 cc soil. The highest population was recorded in Colegrain farm which lies in high altitude among the locations surveyed (Table.1).

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**Morphometrics of *H. trifolii* juveniles, white females and cysts**

A total of 25 each of juveniles, adult females and cysts were subjected to morphometric studies and the results are as follows.

**Second stage juveniles**

**25J2**

L = 522 (462 – 540)  $\mu\text{m}$ ; width = 23 (21 -24)  $\mu\text{m}$ ; spear = 26 (23 – 31)  $\mu\text{m}$ ; tail length = 58 (56 -69)  $\mu\text{m}$  hyaline area of tail = 32 (29 – 39)  $\mu\text{m}$ .

**25 Adult females**

L = 585 (511 – 665)  $\mu\text{m}$ ; width = 543 (202 – 626)  $\mu\text{m}$ ; vulval anal distance : 59 (61 – 69)  $\mu\text{m}$ ; vulval slit = 38 (36 -55)  $\mu\text{m}$ ; Fenestral length = 55 (42 – 61)  $\mu\text{m}$ ; Fenestral width = 39 (29-48)  $\mu\text{m}$ .

**25 cysts**

L = 886 (316 – 1012)  $\mu\text{m}$ ; width = 516 (185 – 622)  $\mu\text{m}$ ; vulval anal distance = 59  $\mu\text{m}$  (56 -71)  $\mu\text{m}$ ; vulval slit = 55 (38

– 59)  $\mu\text{m}$ ; Fenestral length 56 (46 -62)  $\mu\text{m}$ ; Fenestral width = 46 (32-53)  $\mu\text{m}$ .

**Diagnosis**

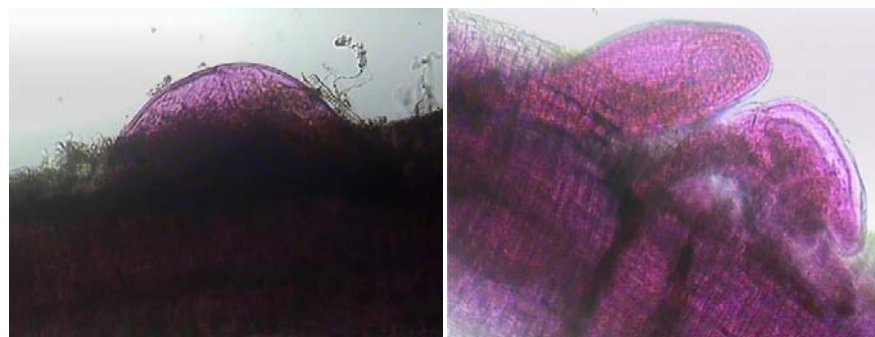
Adult females are lemon shaped with prominent posterior vulval cone. The neck is not on the central axis and lies at an angle in many cysts. Median bulb pyriform with a valve. Adult females with a gelatinous matrix containing around 200 eggs and some eggs are related inside the body. Excretory pore below the neck region.

Cysts larger in size containing more than 200 eggs. Under bridge strong and both the ends were heavily furcated with in the fenestral area the bullae were prominent.

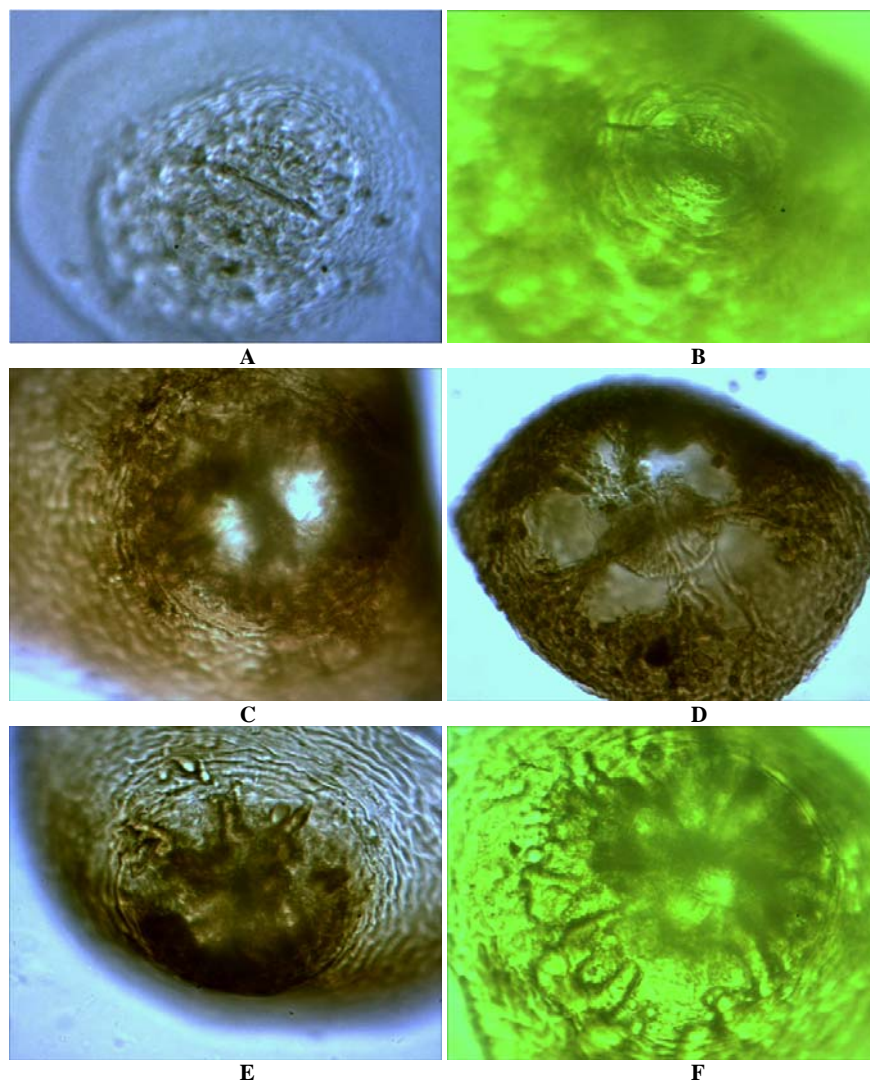
**Juveniles:** Head set off from the body with 3-4 annules. Head stelerotization is strong. Spear 26  $\mu\text{m}$  long with spherical knobs which are indented and concave dorsally. Oesophageal gland overlaps intestine ventrally and ventro laterally, the hyaline portion of the tail is about 32  $\mu\text{m}$  (Table.2).



**Plate 1:** Shapes of adult females of *Heterodera trifolii*



**Plate 2:** Femlaes of *Heterodera trifolii* feeding on white clover roots



**Plate 3:** Vulval cone structures of *H. trifolii* **A** – Adult female cone showing vulva and ambifenestra; **B & C:** Cyst ambifenestra; **D & E** Vulval bridge showing forking on both sides; **F** – Strongly developed bullae

**Table 1:** Distribution of *Heterodera trifolii* in Nilgiris

S.No	Location and GPS	Cysts / 200 cc soil
1.	Conoor	114 (18-155)
2.	Ketti	122 (32 – 168)
3.	K. Palada	142 (18 – 158)
4.	Lovedale	121 (16 – 163)
5.	Colegrain farm	286 (42 – 322)
6.	Nanjanadu	92 (16 – 126)
7.	M. Palada	126 (15 – 136)
8.	Kuruthukuzhi	129 (18 – 132)
9.	Sandynallah	156 (55 – 202)
10.	Muthorai	126 (32 – 168)

**Table 2:** Morphometrics of *Heterodera trifolii*

Characters	Original measurements after Goffari, 1932 n=20	Measurements of Nilgiris population n=25
J <sub>2</sub>		
L	510 μm (490 – 530)	522 μm (462 – 540)
Width	(20 -22) μm	23 μm (21-24)
Spear	27 μ (25 -29)	26 μm (23 – 31)
tail length	59 μm (55 -65)	56 μm (58 – 69)
tail hyaline area	36 μm (30 – 38)	32 μm (29 – 39)
White females		
L	520 – 650 μm	585 μm (511 – 665)
width	190 – 615 μm	542 μm (202 – 626)

vulval anal distance	54 – 68 $\mu\text{m}$	59 $\mu\text{m}$ (61-69)
vulval slit	39 – 58 $\mu\text{m}$	38 $\mu\text{m}$ (36 – 55)
Fenestral length	45 – 60 $\mu\text{m}$	55 $\mu\text{m}$ (42 – 61)
Fenestral width	31 – 49 $\mu\text{m}$	39 $\mu\text{m}$ (29 – 48)
Cysts (After Hirschmann, 1956) excluding neck		
L	310-995 $\mu\text{m}$	886 $\mu\text{m}$ (316 – 1012)
Width	190 – 615 $\mu\text{m}$	516 $\mu\text{m}$ (185 – 622)
vulval anal distance	54 – 68 $\mu\text{m}$	59 $\mu\text{m}$ (56 -71)
vulval slit	39 – 58 $\mu\text{m}$	55 $\mu\text{m}$ (38 – 59)
Fenestral length	45 – 60 $\mu\text{m}$	56 $\mu\text{m}$ (46 – 62)
Fenestral width	31 – 49 $\mu\text{m}$	46 $\mu\text{m}$ (32 – 53)

## Discussion

Cyst nematodes *Heterodera* spp has worldwide distribution and most of the species are distributed in the tropical and some re subtropical and temperate species. *Heterodera trifolii* has been reported in Northern European countries where the temperate climate exists. However it has also been reported in Israel and Australia, where mostly tropical climate prevails In india although Swarup *et al.*, (1964)<sup>[10]</sup> reported *H. trifolii* infecting cowpea *vigna unguiculata* in Delhi, it was later described as *H. cajani* by Koshy, 1967<sup>[6]</sup>. Kaushal *et al.*, (2007a)<sup>[3]</sup> and (2007b)<sup>[4]</sup> reported *H. trifolii* from Kangra region of Himachal Pradesh, Which is a temperate zone. The present findings of observation is a first record from south India. Although Southern India falls under tropical zone, the Nilgiris where it was observed is a temperate zone and further its occurrence was only in high altitudes above 2500 mSL.

The morphometrics showed that not much of variations in the features of juveniles, white females and adults were noticed and the measurements are in close proximity with the original description by Goffart, 1932<sup>[2]</sup>. Ross *et al.*, 1960 Observed *H. trifoli* on the upper and lower surfaces of red clover leaves. However in the present study no cysts were observed on the foliage of white clover.

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