

## First Report of Estonian Cyst Nematode *Cactodera estonica* from India

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Among plant parasitic nematodes, cyst-forming nematodes (*Heteroderidae*) are the most notorious pathogens of agriculture and horticulture crops, and most species are restricted to cool climates (Baldwin and Mundo-Ocampo, 1991; Madani et al., 2004; Ma et al., 2008). In November 2016, a survey for identification and characterization of cyst nematodes was carried out in the Nilgiri hills, South India. During the survey, a large number of cysts were recovered from the rhizosphere and from the root system of *Polygonum nepalense* (*Polygonaceae*). Soil samples were subjected to Fenwick can method for cyst extraction, and the cysts were handpicked using a sterilized forceps under a stereo zoom microscope (Fenwick, 1940). The collected cysts were subjected to morphological and molecular characterization. The cysts were characterized by their dark brown color, lemon shape, as well as distinct neck and vulval cones. The vulval cone of the cyst was excised using the method described by Mulvey, 1972 and observed under a compound microscope. A comprehensive study of the vulval cone showed the typical circumfenestrate pattern. The cyst measurements ( $n = 5$ ) were length  $550.45 \pm 12$  (470.72–687.64)  $\mu\text{m}$ , width  $454.41 \pm 9$  (391.51–541.88)  $\mu\text{m}$ , neck length  $97.76 \pm 4.1$  (93.85–102.77)  $\mu\text{m}$ , length-to-width ratio  $1.4 \pm 0.16$  (1.2–1.6), and vulval cone length  $47.44 \pm 3.9$  (40.73–53.63)  $\mu\text{m}$ . The eggs recovered from these cysts were covered with small protuberances/tubercles. The second-stage juveniles were tapering posteriorly with a well-developed stylet and round basal knobs; tail tapering with a hyaline tail terminal. Second-stage juveniles ( $n = 15$ ) showed the following morphometric characters: body length  $469 \pm 15$  (418–526)  $\mu\text{m}$ ,  $a = 25.37 \pm 2.8$  (21.23–28.4),  $b = 4.14 \pm 0.5$  (0.4–4.8),  $b' = 4.5 \pm 0.6$  (3.8–5.5),  $c = 11.33 \pm 1.1$  (9.54–12.16),  $c' = 2.24 \pm 0.4$  (1.56–2.98), body width  $18.73 \pm 8$  (17.43–19.59)  $\mu\text{m}$ , stylet length  $21.21 \pm 2.2$  (19.6–23.16)  $\mu\text{m}$ , tail length  $42.5 \pm 3.6$  (34.8–45.57)  $\mu\text{m}$ , hyaline tail length  $18.30 \pm 1.08$  (16.2–19.6)  $\mu\text{m}$ , lip height  $3.58 \pm 0.3$  (3.4–4.1)  $\mu\text{m}$ , and lip width  $8.83 \pm 0.9$  (7.5–9.7)  $\mu\text{m}$ . The observations of cyst shape, eggs, vulval cone, and second-stage juveniles were typical of the genus *Cactodera* Krall & Krall, 1978 (Golden and Mulvey, 1983). For further confirmation, the cysts were subjected to molecular analysis.

DNA was isolated from individual cyst by using the protocol described by Sambrook et al. (1989) with slight modifications. Genomic DNA was amplified using ITS-rDNA. A fragment containing the partial 18S gene, complete ITS1, 5.8S, and ITS2, and the partial 28S rRNA gene was amplified using forward (CGTAA-CAAGGTAGCTGTAG) and reverse (TCCTCCGCTA-AATGATAT) primer (Ferris et al., 1993). Amplicon of ITS-rDNA yielded a single fragment of approximately 735 bp; the obtained sequence was deposited into the GenBank database (Accession no. MF537581) and was compared with published sequences by means of BLAST search in the database. The comparison revealed 100% similarity, with sequence corresponding to *C. estonica* isolates from Turkey (KX579922), 99% with Belgium population (AF274417) and 98% with China population (KY475583, HM560730, KC771888, KC771889, JN684904, EU106164). Although three species of *Cactodera* viz. *C. cacti*, *C. Chaubattia*, and *C. jahanseni* have already been reported from India, (Kaushal et al., 2007) this is the first report of *C. estonica* from India. In previous studies, *C. estonica* has been mainly reported on common weed *Polygonum* species of the family *Polygonaceae*, which is a well-known host for *C. estonica* (Subbotin et al., 2001; Norgren, 1986). Cyst forming nematodes are highly specialized and economically important pathogens and easily dispersed to new area by wind, water, and human and animals activities. If *C. estonica* increases in a new habitat, it will be the major threat to the biodiversity of the southern hills of India.

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