

1 First report of *Heterodera trifolii* on white clover and *Rumex obtusifolius* in Costa
2 Rica.

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7 In 2018, during a survey sampling for cyst-forming nematodes, two populations of
8 *Heterodera* sp. were found in the north region of Cartago (Llano Grande and
9 Oreamuno), Costa Rica. White females and cysts were attached to the plant
10 roots of two weeds, white clover (*Trifolium repens* L.) and *Rumex obtusifolius* L.
11 Plants were asymptomatic above ground. Cysts were extracted from soil samples
12 using the Fenwick method (Fenwick 1940) and cut to release the second-stage
13 juveniles (J2s). Cysts were lemon-shaped with a prominent vulval cone, light to
14 dark brown coloring, and ambifenestrate with developed underbridge and
15 bifurcated at the end. Measurements combining both populations are given in
16 micrometers and provided in the following format: mean $\pm$ SD (range). Cysts ($n =$
17 20): length excluding neck = 744.3 ± 61.6 (648.5 to 837.4); width = 495.9 ± 60.5
18 (421.9 to 621.1); fenestral length = 50.7 ± 6.4 (40.3 to 61.3); and semifenestral
19 width = 36.2 ± 5.6 (24.1 to 45.7). J2s ($n = 60$): length = 541.1 ± 25.6 (492.5 to
20 604.9); width = 22.9 ± 1.7 (20.5 to 27.3); stylet = 27.9 ± 0.9 (26.2 to 29.6); labial
21 region height = 4.7 ± 0.3 (3.8 to 5.5); labial region diameter = 9.4 ± 0.5 (8.5 to
22 10.9); DGO = 5.9 ± 0.5 (4.6 to 7.3); anterior end to excretory pore = 122.2 ± 8.3

23 (106.4 to 148.7) and to median bulb valve = 81.2 ± 5.3 (67.3 to 92.6); tail length =
 24 66.6 ± 4.9 (55.9 to 77.3); and hyaline region of tail length = 37.1 ± 3.7 (27.7 to
 25 44.5). No males were found. The morphological and morphometrical features of
 26 the two *Heterodera* populations overlapped with each other and within other *H.*
 27 *trifolii* populations reported worldwide (Subbotin et al. 2010; Sekimoto et al. 2017).
 28 The primer sets TW81/AB28 (Subbotin et al. 2001), D2A/D3B (De Ley et al. 1999)
 29 and the HTcox1F2 (5'-GCTTCTGATCTTTCCTTCCACGTA-3', this study)/JB4
 30 (Derycke et al. 2005) were used for amplification of the internal transcribed spacer
 31 (ITS) rRNA, the D2-D3 expansion segments of the 28S (28S) rRNA and the partial
 32 mitochondrial *cox1* gene (*cox1*), respectively. The resulting sequences were
 33 submitted to the GenBank (ITS: MT020783, 28S: MT010295, and *cox1*:
 34 MT007852). A BLASTn search of the *ITS*, *28S* and the *cox1* gene sequences of *H.*
 35 *trifolii* from Costa Rica revealed a 100% identity with sequences of *H. trifolii* from
 36 Japan (LC208684), South Korea (MN720070) and USA (MK093174), respectively.
 37 A greenhouse essay was established to confirm the reproduction of *H. trifolii* on
 38 each host. Using ten replicates (individual pots) per treatment (hosts), pots were
 39 inoculated with 1000 individuals (eggs + infective J2s). The experiment was
 40 conducted twice. The average greenhouse temperature was 22.1 ± 3.8 °C and
 41 plants were harvested 75 days after inoculation. Results showed that *H. trifolii* was
 42 able to reproduce successfully on both hosts. Final average population (roots +
 43 soil) in *R. obtusifolius* and *T. repens* were 21,516 and 2,626 nematodes (all stages
 44 and cysts), respectively. These two weed species can be a source of inoculum for
 45 economically important crops. Therefore, studies on life cycle and pathogenicity

assays are being performed on agricultural crops. To our knowledge, this is the first report of *H. trifolii* on white clover and *R. obtusifolius* in Costa Rica.

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>ITS_Heterodera trifolii

TGCTGGATCATTACCCAAGTGATTCTATTACACCTACCTGCTGTCCTGTTGGGCGAG
CGTTGGCACCACCAAATGCCCCGTCGGCTGACGGGCACAGGTCGTTGAGATGACTTGT
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TACGTTGGAGCTGGTGTACCGCTCAGTGCTGCACATGTGAAAGCCTGTGGATGGCTGCTG
TGTGGCAATGTGTCGGTGGCGGGCCGCTCGCTTGGTTGGTTCGCTGCGCCAACGGGGTA
TGCACGCTCGTGGGGCGACCTAACGGCTGTGCTGGCGTCTGTGCGTCGTTGAGCGGTTGT
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>28S_Heterodera trifolii

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AGACTGGGGTTGGCTGTCCTTCTGTTCACTGGCGAATGGGGCATTTCAGGGCGGAGTGCG
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CCAGCTCGGGCTCTCAGTTGTGCGTTCTCGGTGTAAAAGCCGGTCATCTGTTCGACCCGT
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TTGGGGCGCAACATAGTCCCGTCTCGATCGCATGCGATGGGGCGGAGACAGAGCGTATGC
GCTGAGACCCGAAAGATGGTGAACCTATTCCTGAGCAGGATGAAGCCAGAGGAACTCTGG
TGGAAGTCCGAAGCGATTCTGACGTGCAAATCGATCGTCTGACTTGGGTATAGGGGC

>cox1_Heterodera trifolii

TTAATTCCTGAGGTTTTGGTTATTACCGATATCTCTTCTTTCCTTTTTTTAGTTTTT
TGTTGACGAGGGGTTAGGAACTAGTTGAACCCTTTACCCTCCTTTAAGTAGCTTTGCTC
ACCCAGGTTATAGAGTTGATTTTTCAATTCTTCTTTACACCTTGCGGGTATCAGTTCTA
TTGGAGGAAGTTTAAATTTTTGAGTAACTATTAAGAATTTAAAAGTTATAACTTTGAGTT
TTTTTAATCTAAATCTTTTTATTTGAACAATTTAATTTCTATTTTTCTTTAATTTTAA
CCCTTCCTGTTCTAGCTGGAGCTATTACTCTTTTATTGGTGGATCGTAATTTTAATGGAA
ATTTTTTTGATAGTACTGGTGGAGGAAACCCTTTAGTGTTTCAACATCTTTTTTGATTTT
TTGGTCACCCAGAAGTTTATGTTTTAATTTTACCTGCATTCGGTTTAGTTAGTTTAACGA
TTCAATTTTATACAGGTAAAAAATTCTTTTTGGTTATTTAGGTATAATCTATGCTATTA
TTAGAATTGGTTTTATTGGCTGTTTGGTTTGAGCTCACCATATATTTGTGGTTGGAATAG
ATATGGATAGTCGTGCTTATTTTAGAGCAGCAACTATAATTATTGCAATCCCTACAGGTA
TTAAGGTTTTTTCTTGATTAATAAGGGTTTATGGATCTCCTTTTTTTTTTAATTTTTGA
TATATTGAGTTTTTGGTTTTATTTTTCTTTTACAATTGGTGGTTTAAGTGGCTTAATCC
TTAGTAACGCTAGTTTGGATGTAGTCTTACATGATACCTATTATGTTGTAGC