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# First report of a cyst nematode, *Heterodera daverti*, from Australia

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## Abstract

Occurrence of cyst nematode *Heterodera daverti* Wouts and Sturhan 1978 (Wouts & Sturhan 1978) is reported from Australia for the first time. Cysts were recovered from soil samples collected from a hop farm in Merriang, Victoria. Morphological and morphometric characteristics of cysts and second stage juveniles match those described for this species reported from other parts of the world. Identification was further supported by molecular evidence through comparison of newly generated sequences of ITS rRNA, CO1 mtDNA and 28S rRNA gene regions with sequences previously available on NCBI GenBank.

**Keywords** *Heterodera daverti* · First report · Cyst nematode · Victoria · Australia

Cyst nematodes are sedentary endoparasites classified within the eight genera of the family Heteroderidae (Handoo and Subbotin 2018). They infect economically important crops such as legumes, cereals, sugar beet, potatoes, carrots etc., as well as certain indigenous vegetation and directly impact host yield (Knoetze and Swart 2014; Moens et al. 2018). The most damaging cyst nematodes belong to the genera *Globodera* and *Heterodera*, with the latter containing more than 80 species (Moens et al. 2018).

*Heterodera daverti* Wouts and Sturhan 1978 belongs to the Schachtii group of *Heterodera* and was first described from the roots of white clover, *Trifolium repens*, from a pasture at Davert, a forest area in Germany (Wouts and Sturhan 1978). It is known to be prevalent in parts of Europe (Germany, Italy, The Netherlands, UK) and Africa (Egypt and Tunisia) (Nordmeyer et al. 1978; Subbotin et al. 2010). *Heterodera daverti* is morphologically very similar to the clover cyst nematode *Heterodera trifolii* (Goffart 1932), the most noticeable difference being that *H. daverti* is bisexual (i.e., males are present in populations) whereas *H. trifolii* is parthenogenic (Mulvey 1958; Vovlas et al. 2015). It can further be differentiated from *H. trifolii* and other members of the Schachtii group by a shorter J2 body and tail as well as a variation in the stylet knob shape (Massoud et al. 1988). In addition, the CO1 gene region of the mitochondrial DNA is an efficient molecular marker for species differentiation between *H. trifolii* and *H. daverti*, whereas the ITS rRNA gene sequence is similar for both species (Huston et al. 2022; Subbotin et al. 2010; Vovlas et al. 2015).

There is limited research on the pathogenicity of *H. daverti*, however, one pot experiment reported severe losses of *T. subterraneum* (subterranean clover) induced by *H. daverti* (Sikora 1977). In another pot trial, Massoud et al. (1988) found that *H. daverti* reproduced on ten different leguminous cultivars and that *T. alexandrinum* (Egyptian clover), *T. pratense* (red clover) and *T. repens* were the most suitable hosts.

In May of 2021, five soil samples were collected from a hop farm located in Merriang, Victoria, Australia. Cysts were extracted using the Cobb's sieve method (Cobb 1918). Vulval cone regions were excised from cysts and mounted in a modified Kaiser's glycerine jelly (Dioni 2003). Second-

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stage juveniles (J2s) recovered from cysts were studied using temporary wet-mounts. Photographs were taken using a ZEISS Axioscope light microscope and mounted camera Axiocam 506 mono, and measurements were taken using ZEISS Blue imaging software (ZEISS, Germany). Whole cyst images were taken using Leica M205C Stereo Microscope with attached Leica DMC 4500 digital camera and associated Leica LAS core application suite software (Leica Biosystems, Germany).

Classical morphology was further verified by molecular assessment. Genomic DNA was extracted from six cysts using DNeasy Blood and Tissue kit (Qiagen®) and three gene regions were amplified: the internal transcribed spacer (ITS) region of rRNA with primer pair TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') (Curran et al. 1994), cytochrome oxidase 1 (CO1) with primers JB3 (5'-TTTTTTGGGCATCCTGAGGTTTAT-3') (Bowles et al. 1992) and JB5 (5'-AGCACCTAAACTTAAAACATAATGAAAATG-3') (Derycke et al. 2005) and the D2 and D3 expansion regions of the large subunit 28S rRNA gene with primer pair D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and reverse D3B (5'-TCGGAAGGAACCAGCTACTA-3') (De Ley et al. 1999). One PCR reaction contained 10 µM (1 µl each) of each primer, 12.5 µl of OneTaq® DNA polymerase (New England BioLabs, Ipswich, Massachusetts, USA) and 5 µl of DNA template with a final volume of 25 µl. PCR cycling included an initial denaturation of four minutes at 94 °C followed by 35 cycles of 94 °C for one minute, annealing at 55 °C for 1 min 30 s, extension at 72 °C for 2 min and a final extension at 72 °C for 10 min. PCR products were sent for purification and Sanger sequencing at Macrogen (Seoul, Rep. of Korea). All resultant sequences were trimmed and analysed using Geneious Prime® 2022.1.1 (www.geneious.com). The new sequences obtained were aligned to reference sequences downloaded from NCBI GenBank using MAFFT alignment v7.450 (Katoh et al. 2017) and Maximum Likelihood (ML) analyses were performed using RAxML (v8) (Stamatakis 2014). The data was analysed in Geneious Prime® 2022.1.1 (www.geneious.com) according to the nucleotide model GTR Gamma I (Rodríguez et al. 1990) with joint branch length optimisation and 1000 rapid bootstrap inferences followed by a thorough ML search to generate phylogenetic trees depicting their relationships with other species of the Heteroderidae family to confirm species identity. Sequence fragments generated for each gene region were compared against the NCBI GenBank database using BLAST (Altschul et al. 1990) to determine percentage similarities with other species.

Cysts were pale to dark brown in colour, thin walled and lemon-shaped with the terminal area protruding (Fig. 1a). A prominent vulval cone with an elongated appearance was observed (Fig. 1b) and fenestrae were ambifenestrate (Fig. 1c). Irregular shaped bullae were observed in some cysts on areas surrounding the anus along with the presence of a well-developed underbridge. J2 juveniles were vermiform (Fig. 2a), and the body possessed a slight ventral curvature along with the presence of a distinct median bulb (Fig. 2b). A slender, well-developed stylet was present with large basal knobs that were slightly concave towards the anterior side (Fig. 2c). Small phasmids were observed towards the end of the hyaline tail region (Fig. 2d). Morphometrics of cysts and second-stage juveniles of *H. daverti* are shown in Table 1. All the characters are consistent with those described for *H. daverti* (Subbotin et al. 2010). Microscopic slides (slide accession numbers 8734–8740) of vulval cone sections and J2 juveniles have been vouchered in the Nematology section of the Australian National Insect Collection, CSIRO, Canberra, ACT, Australia.

Five identical ITS rRNA gene sequence replicates were generated, and a single fragment of 973 bp was deposited in GenBank under the accession number ON870657. This sequence shares a 99.59% similarity with that of *H. trifolii* isolate from Japan (GenBank accession number LC208684.1) (Sekimoto et al. 2017). However, this marker does not clearly differentiate (Fig. 3) between other closely related species of the Schachtii group (Huston et al. 2022). The present study demonstrated a clear differentiation between *H. daverti*, *H. betae* and *H. trifolii* with the alignment of the CO1 mtDNA gene (Fig. 4) including

**Table 1** Morphometrics of vulval region of cysts and second-stage juveniles of *Heterodera daverti* (all measurements are given in µm)

| Characters | Population<br>Victoria, Australia (2021) |
|---|---|
| **Vulval region (n = 5)** | |
| Fenestral length | 36.5 ± 2.9 (33.2–40.4) |
| Semifenestral width | 28.9 ± 2.5 (26.6–33.0) |
| Vulval slit length | 42.9 ± 3.2 (39.5–48.1) |
| Vulval bridge width | 6.2 ± 0.9 (5.1–7.5) |
| Underbridge length | 101.6 ± 11.9 (90.3–120) |
| **Second-stage juvenile (n = 10)** | |
| Body length | 513.2 ± 72.3 (415.3–642.2) |
| Stylet length | 26 ± 1.9 (22.6–29.8) |
| Hyaline tail length | 39.9 ± 4 (33.3–46.8) |
| True tail length | 58.1 ± 5.1 (48.6–62.9) |

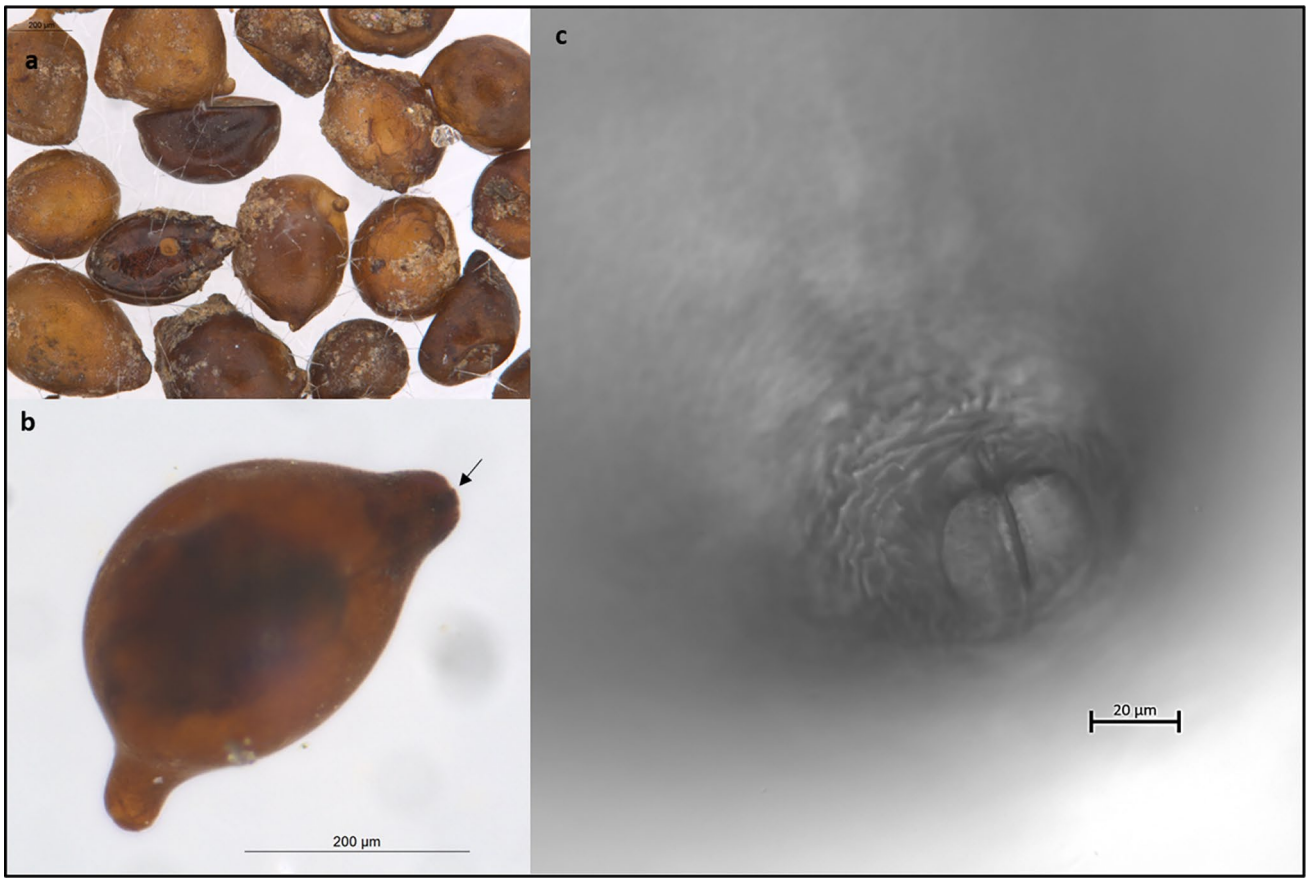


**Fig. 1** Photomicrographs of cysts of *Heterodera daverti*. **a**: cysts of *H. daverti* **b**: a single cyst of *H. daverti* showing the prominent vulval cone (arrow) **c**: Vulval area showing ambifenestrate fenestration and vulval slit along vulval bridge. Scale bar: a, b = 200 µm and c = 20 µm

34 sequences of *Heterodera* species and one sequence of *Globodera rostochiensis* taken as the outgroup. Five identical replicates of 434 bp each were generated, of which one single sequence fragment was submitted to GenBank under the accession number ON926517, sharing a 99.74% identity with that of a *H. daverti* isolate from Germany (GenBank accession number KT163237.1) (Vovlas et al. 2015).

Four identical 28S rRNA gene sequence replicates were generated, of which a single sequence (Fig. 5) of 737 bp was submitted to GenBank under the accession number ON926516 sharing 100% identity with that of *H. daverti* isolate (GenBank accession number KT163230.1) from Italy (Vovlas et al. 2015).

The clover cyst nematode *H. trifolii* is known to have been established in Australia for more than 60 years and is now both common and widespread in white clover-based pastures in southern parts of Australia (Brown 1978; McLeish et al. 1997). *Heterodera trifolii* and *H. daverti* may well have arrived together in Australia, however, due to their close morphological similarity, the potential presence of *H. daverti* could have been overlooked and the incidence of males may not have prompted any suspicion due to the abundant occurrence of suitable host plants, *Trifolium* spp., common weeds in Australian hop gardens.

To our knowledge this is the first report of *Heterodera daverti* from Australia. However, further investigation needs to be done to estimate yield loss incurred by this species.

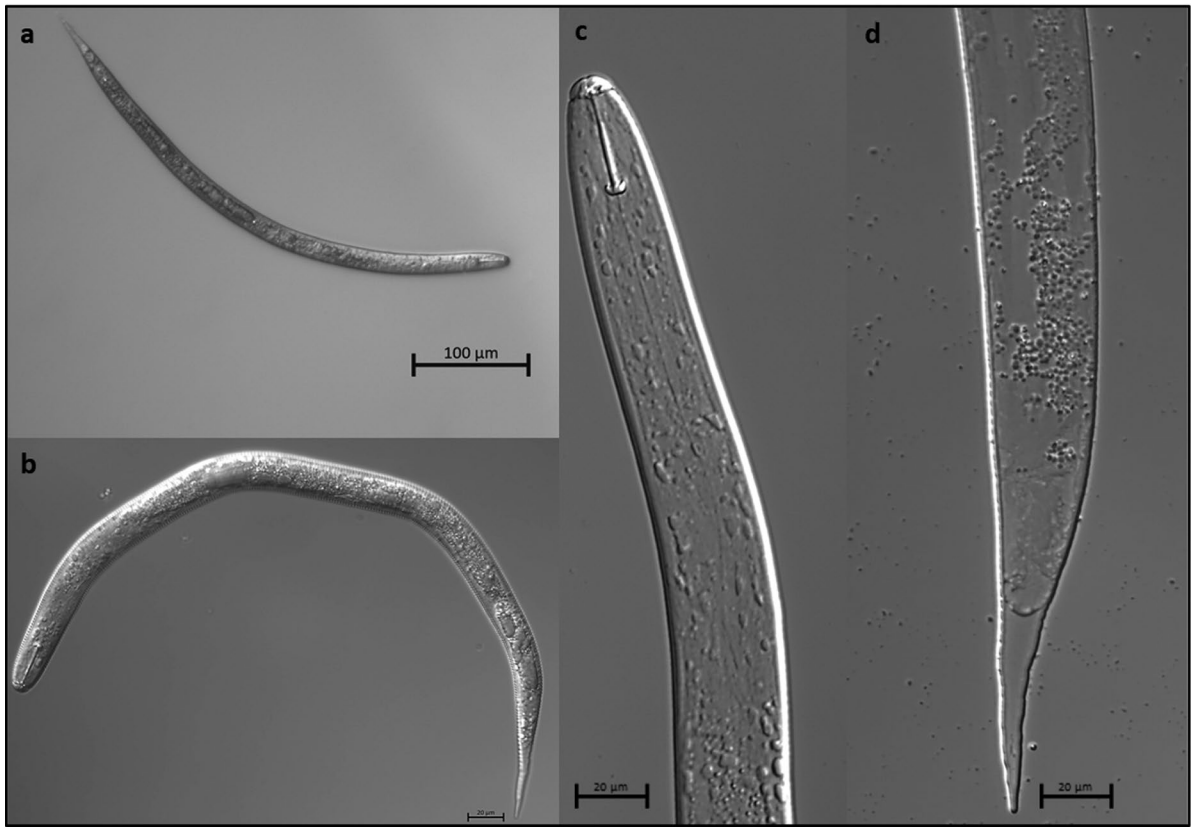


**Fig. 2** Photomicrographs of second-stage juvenile (J2) of *Heterodera daverti*. **a**: J2 juvenile at 20x magnification **b**: J2 juvenile at 40x magnification **c**: head showing stylet and basal knobs **d**: true tail and hyaline tail region of the juvenile. Scale bar: a = 100 µm, b–d = 20 µm

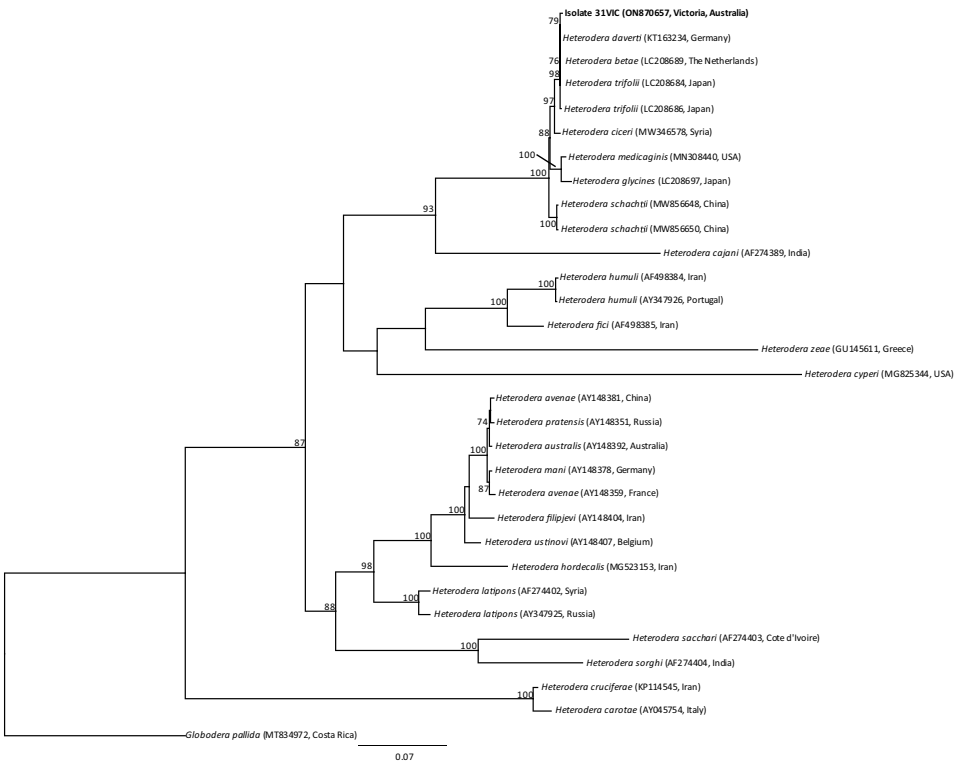


**Fig. 3** Phylogenetic relationship of *Heterodera daverti* among other species of the Heteroderidae family, as inferred from maximum likelihood analysis of the MAFFT alignment of ITS rRNA gene sequences under the GTR Gamma I model. Branch support values above 70% are shown. New sequence is highlighted in bold type on appropriate branch

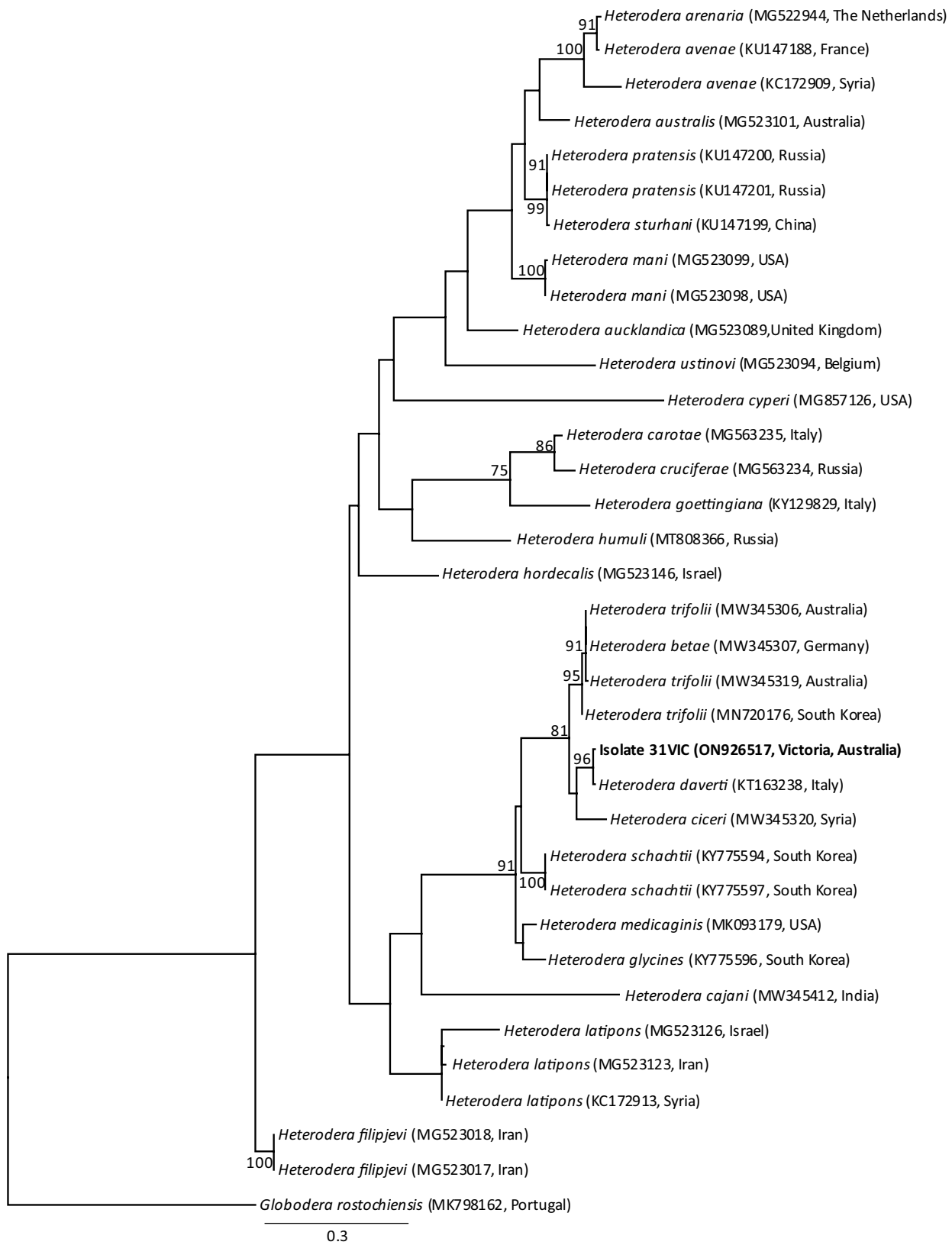


**Fig. 4** Phylogenetic relationship of *Heterodera daverti* among other species of the Heteroderidae family, as inferred from maximum likelihood analysis of the MAFFT alignment of mtDNA CO1 gene sequences under the GTR Gamma I model. Branch support values above 70% are shown. New sequence is highlighted in bold type on appropriate branch

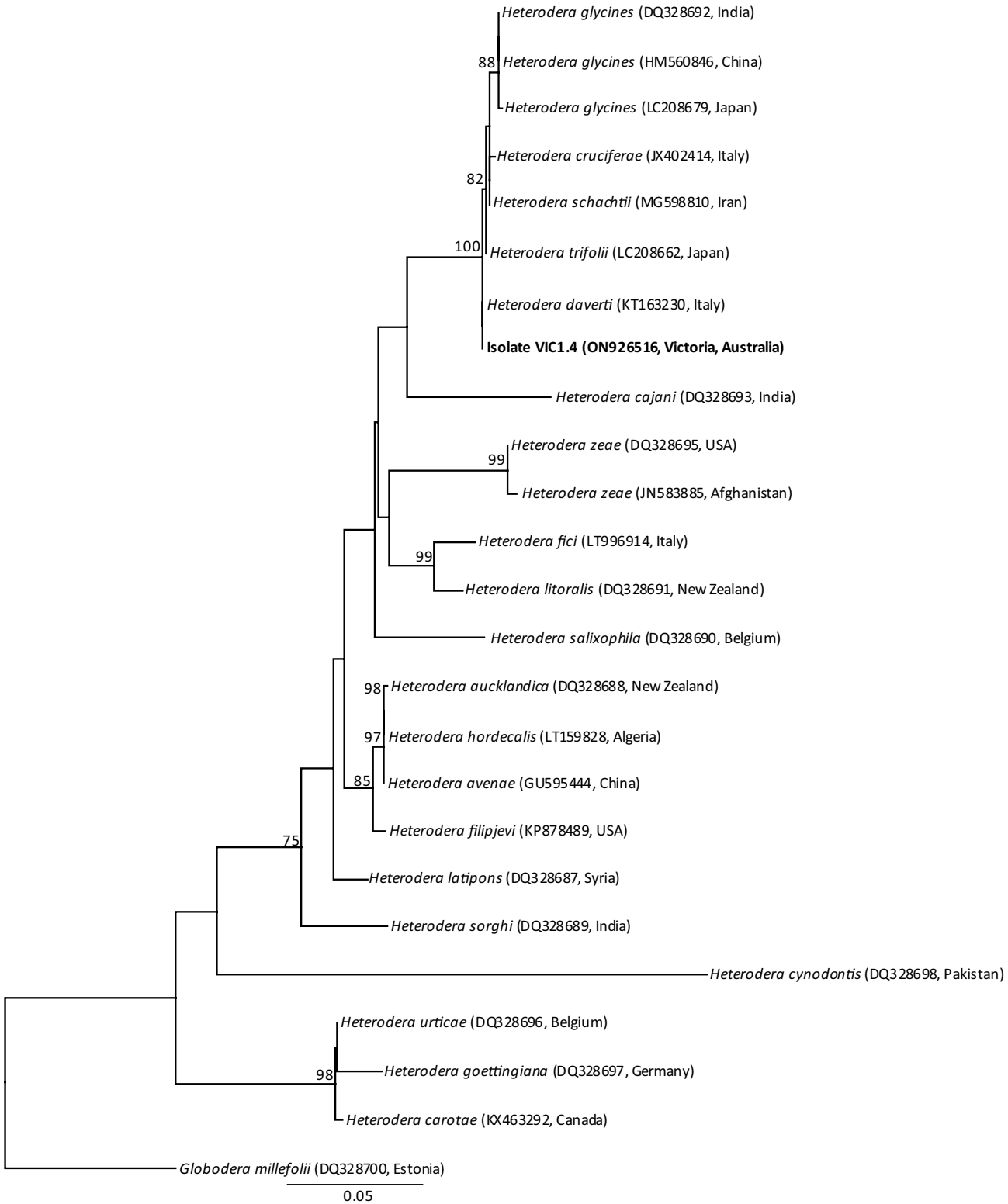


**Fig. 5** Phylogenetic relationship of *Heterodera daverti* among other species of the Heteroderidae family, as inferred from maximum likelihood analysis of the MAFFT alignment of D2-D3 expansion segments of 28S rRNA gene sequences under the GTR Gamma I model. Branch support values above 70% are shown. New sequence is highlighted in bold type on appropriate branch

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## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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