

# **-SUPPLEMENTARY MATERIAL-**

## **Description of *Heterorhabditis americana* n. sp. (Rhabditida, Heterorhabditidae), a new entomopathogenic nematode species isolated in North America**

Ricardo A. R. Machado<sup>1\*</sup>, Joaquín Abolafia<sup>2+</sup>, María-Cristina Robles<sup>2+</sup>, Alba N. Ruiz-Cuenca<sup>2+</sup>, Ebrahim Shokoohi<sup>3+</sup>, Aashaq Hussain Bhat<sup>1,4+</sup>, Vladimír Půža<sup>5,6</sup>, Xi Zhang<sup>7</sup>, Matthias Erb<sup>7</sup>, Christelle A. M. Robert<sup>7</sup>, Bruce Hibbard<sup>8</sup>

<sup>1</sup>*Experimental Biology Research Group. Institute of Biology. Faculty of Sciences. University of Neuchâtel. Rue Emile–Argand 11, 2000 Neuchâtel, Switzerland.*

<sup>2</sup>*Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus ‘Las Lagunillas’ s/n, Edificio B3, 23071 Jaén, Spain.*

<sup>3</sup>*Department of Biochemistry, Microbiology and Biotechnology, University of Limpopo, Private Bag X1106, Sovenga 0727, South Africa.*

<sup>4</sup>*University Centre for Research and Development and Department of Bioscience, Chandigarh University, Mohali 140413, Punjab, India*

<sup>5</sup>*Institute of Entomology, Biology centre of the Czech Academy of Sciences, CAS, 37005 České Budějovice, Czech Republic.*

<sup>6</sup>*Faculty of Agriculture and Technology, University of South Bohemia, 37005 České Budějovice, Czech Republic.*

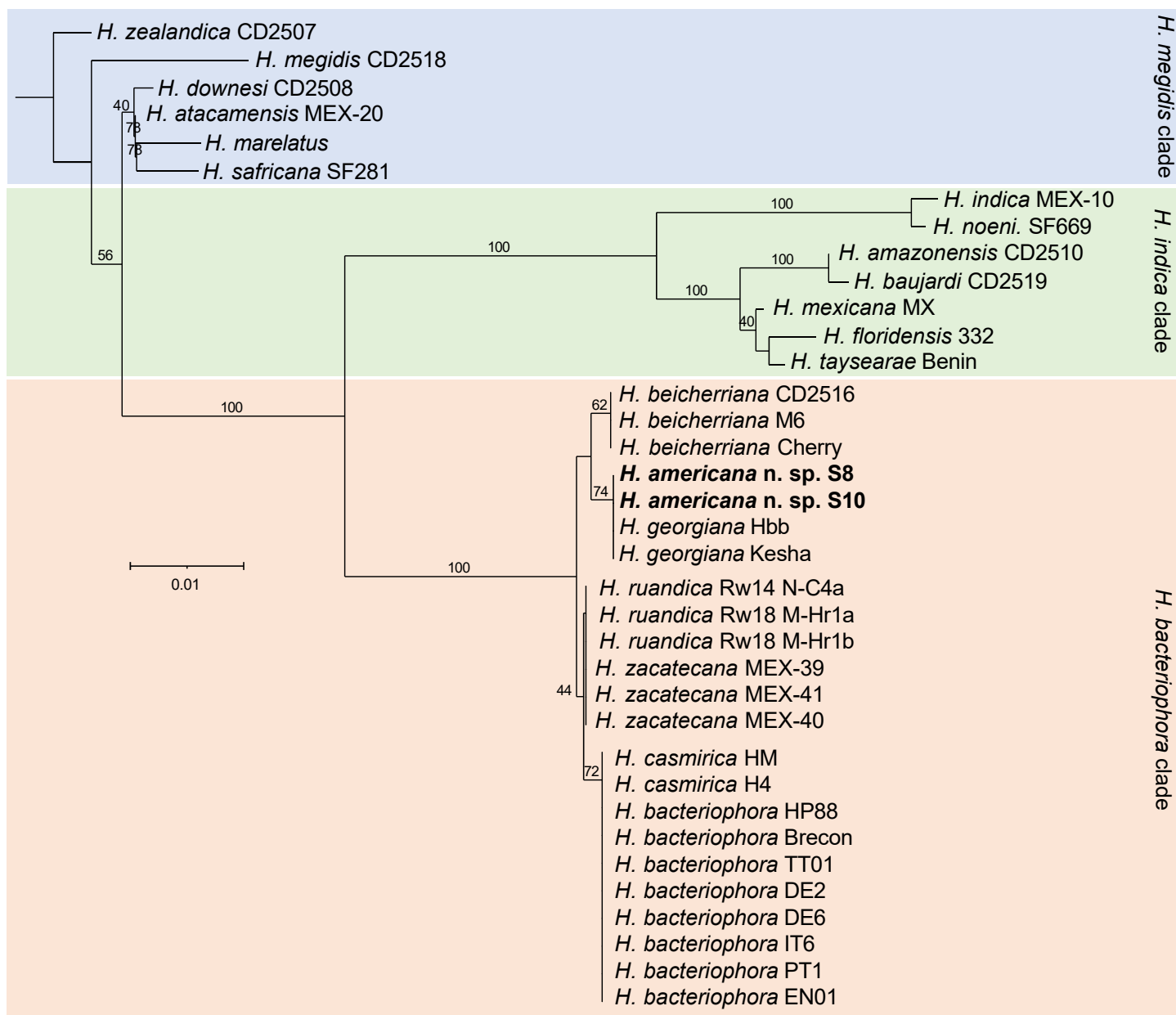
<sup>7</sup>*Institute of Plant Sciences, University of Bern, Altenbergrain 21, 3013 Bern, Switzerland*

<sup>8</sup>*Plant Genetics Research Unit, United States Department of Agriculture (USDA)-Agricultural Research Service, Columbia, MO, United States.*

<sup>+</sup>: These authors contributed equally to this study.

<sup>\*</sup>*Correspondence:* Ricardo A. R. Machado. Emile–Argand 11, 2000 Neuchâtel, Switzerland. Email address: rarm.machado@gmail.com

# **SUPPLEMENTARY FIGURES**



**Figure S1.** Maximum-likelihood phylogenetic tree reconstructed from the nucleotide sequences of the D2-D3 expansion segments of the 28S rRNA gene. A total of 554 nucleotide positions, flanked by primers D2A and D2B, were analyzed. Accession numbers of the nucleotide sequences used for the analyses are shown in Table S2. Numbers at nodes represent bootstrap values based on 100 replications. Bars represent average nucleotide substitutions per sequence position.



|                         |       |       |    |       |       |    |       |       |    |       |       |    |       |    |       |    |       |    |    |    |    |    |      |    |    |    |    |    |    |    |    |    |
|-------------------------|-------|-------|----|-------|-------|----|-------|-------|----|-------|-------|----|-------|----|-------|----|-------|----|----|----|----|----|------|----|----|----|----|----|----|----|----|----|
| H. zealandica CD2507    | ID 34 | 24    | 30 | 29    | 34    | 46 | 46    | 35    | 41 | 39    | 40    | 41 | 29    | 29 | 30    | 29 | 26    | 26 | 26 | 31 | 32 | 33 | 31   | 31 | 30 | 30 | 32 | 31 | 31 | 31 | 31 | ID |
| H. downesi CD2508       | 34    | ID 21 | 34 | 38    | 37    | 53 | 47    | 35    | 42 | 41    | 41    | 41 | 30    | 30 | 27    | 26 | 31    | 30 | 30 | 30 | 31 | 31 | 32   | 32 | 42 | 42 | 36 | 35 | 35 | 35 | 35 | ID |
| H. megidis CD2518       | 24    | ID 28 | 31 | 33    | 62    | 41 | 35    | 42    | 39 | 41    | 41    | 41 | 27    | 27 | 24    | 23 | 27    | 27 | 28 | 29 | 29 | 32 | 31   | 31 | 35 | 35 | 33 | 32 | 32 | 32 | 32 | ID |
| H. marelatius           | 30    | 34    | 28 | ID 29 | 26    | 50 | 48    | 42    | 47 | 45    | 48    | 47 | 32    | 32 | 32    | 30 | 31    | 31 | 35 | 36 | 37 | 37 | 37   | 42 | 42 | 40 | 41 | 40 | 40 | 40 | 40 | ID |
| H. safricana SF281      | 29    | 38    | 31 | 29    | ID 29 | 49 | 51    | 39    | 41 | 43    | 43    | 42 | 35    | 35 | 34    | 35 | 33    | 34 | 34 | 35 | 36 | 37 | 37   | 39 | 40 | 40 | 41 | 41 | 41 | 41 | 41 | ID |
| H. atacamensis MEX-20   | 34    | 37    | 33 | 26    | ID 46 | 47 | 38    | 43    | 47 | 49    | 46    | 35 | 35    | 33 | 33    | 36 | 37    | 37 | 39 | 40 | 40 | 41 | 41   | 41 | 42 | 42 | 43 | 42 | 42 | 42 | 42 | ID |
| H. noenieputensis SF669 | 46    | 53    | 52 | 50    | 49    | 46 | ID 37 | 45    | 48 | 45    | 47    | 43 | 52    | 52 | 50    | 48 | 49    | 49 | 50 | 51 | 51 | 51 | 51   | 52 | 52 | 44 | 45 | 44 | 44 | 44 | 44 | ID |
| H. indica LN2           | 46    | 47    | 41 | 48    | 51    | 47 | 37    | ID 40 | 49 | 45    | 43    | 49 | 47    | 47 | 47    | 47 | 49    | 50 | 50 | 54 | 55 | 55 | 56   | 54 | 57 | 57 | 50 | 50 | 50 | 50 | 50 | ID |
| H. bajardi CD2519       | 35    | 35    | 35 | 42    | 39    | 38 | 45    | ID 40 | 27 | 19    | 25    | 19 | 41    | 41 | 37    | 37 | 41    | 40 | 40 | 44 | 45 | 45 | 46   | 46 | 47 | 39 | 39 | 39 | 39 | 39 | 39 | ID |
| H. amazonensis CD2510   | 41    | 42    | 42 | 47    | 41    | 43 | 48    | 49    | 27 | ID 26 | 32    | 30 | 44    | 44 | 39    | 38 | 47    | 46 | 46 | 48 | 49 | 48 | 48   | 48 | 46 | 46 | 42 | 42 | 41 | 41 | 41 | ID |
| H. mexicana             | 39    | 41    | 39 | 45    | 43    | 47 | 45    | 45    | 19 | 26    | ID 10 | 12 | 43    | 43 | 40    | 40 | 44    | 43 | 43 | 44 | 45 | 45 | 44   | 44 | 44 | 44 | 44 | 44 | 44 | 44 | 44 | ID |
| H. floridensis 332      | 40    | 41    | 41 | 48    | 43    | 49 | 47    | 43    | 25 | 32    | ID 10 | 16 | 46    | 46 | 43    | 43 | 44    | 43 | 43 | 47 | 48 | 48 | 47   | 47 | 47 | 46 | 46 | 41 | 41 | 41 | 41 | ID |
| H. taysearae Benin      | 41    | 41    | 41 | 47    | 42    | 46 | 43    | 49    | 19 | 30    | ID 12 | 16 | ID 45 | 45 | 41    | 41 | 41    | 40 | 40 | 43 | 44 | 44 | 43   | 45 | 44 | 44 | 44 | 44 | 44 | 44 | 44 | ID |
| H. americana n. sp. S8  | 29    | 30    | 27 | 32    | 35    | 35 | 52    | 47    | 41 | 44    | 43    | 46 | ID 0  | 11 | 10    | 22 | 23    | 23 | 27 | 28 | 28 | 27 | 26   | 26 | 29 | 29 | 26 | 26 | 25 | 25 | 25 | ID |
| H. americana n. sp. S10 | 29    | 30    | 27 | 32    | 35    | 35 | 52    | 47    | 41 | 44    | 43    | 46 | ID 0  | 11 | 10    | 22 | 23    | 23 | 27 | 28 | 28 | 27 | 26   | 26 | 29 | 29 | 26 | 26 | 25 | 25 | 25 | ID |
| H. georgiana Kesha      | 30    | 27    | 24 | 32    | 34    | 33 | 50    | 47    | 37 | 39    | 40    | 43 | 41    | 11 | ID 1  | 23 | 22    | 22 | 24 | 25 | 25 | 26 | 25   | 26 | 26 | 23 | 25 | 24 | 24 | 24 | 24 | ID |
| H. georgiana Hbb        | 29    | 26    | 23 | 32    | 35    | 33 | 50    | 47    | 37 | 38    | 40    | 43 | 41    | 10 | ID 10 | 1  | ID 22 | 21 | 21 | 23 | 24 | 24 | 25   | 24 | 24 | 27 | 24 | 24 | 23 | 23 | 23 | ID |
| H. beicherriana M6      | 26    | 31    | 27 | 30    | 33    | 36 | 48    | 49    | 41 | 47    | 44    | 44 | 41    | 22 | ID 2  | 2  | ID 15 | 16 | 16 | 20 | 21 | 21 | 23   | 23 | 22 | 22 | 21 | 21 | 21 | 21 | 21 | ID |
| H. beicherriana Cherry  | 26    | 30    | 27 | 31    | 34    | 37 | 49    | 50    | 40 | 46    | 43    | 43 | 40    | 23 | ID 2  | 2  | ID 17 | 18 | 18 | 21 | 22 | 22 | 22   | 23 | 23 | 23 | 22 | 22 | 22 | 22 | 22 | ID |
| H. beicherriana CD2516  | 26    | 30    | 27 | 31    | 34    | 37 | 49    | 50    | 40 | 46    | 43    | 43 | 40    | 23 | ID 2  | 2  | ID 17 | 18 | 18 | 21 | 22 | 22 | 22   | 23 | 23 | 23 | 22 | 22 | 22 | 22 | 22 | ID |
| H. ruandica Rw14_N-C4a  | 31    | 30    | 28 | 35    | 35    | 39 | 50    | 54    | 44 | 48    | 44    | 47 | 43    | 27 | ID 1  | 6  | ID 7  | 7  | 7  | 7  | 7  | 7  | 7    | 19 | 19 | 19 | 18 | 18 | 18 | 18 | 18 | ID |
| H. ruandica Rw18_M-Hr1b | 32    | 31    | 29 | 36    | 36    | 40 | 51    | 55    | 45 | 49    | 45    | 48 | 44    | 28 | ID 0  | 7  | ID 7  | 8  | 8  | 18 | 18 | 20 | 20   | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | ID |
| H. ruandica Rw18_M-Hr1a | 32    | 31    | 29 | 36    | 36    | 40 | 51    | 55    | 45 | 49    | 45    | 48 | 44    | 28 | ID 0  | 7  | ID 7  | 8  | 8  | 18 | 18 | 20 | 20   | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | ID |
| H. zacatecana MEX-41    | 33    | 31    | 32 | 37    | 38    | 41 | 51    | 56    | 46 | 48    | 44    | 47 | 43    | 27 | ID 3  | 3  | ID 22 | 21 | 21 | 20 | 20 | 20 | 20   | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | ID |
| H. zacatecana MEX-40    | 31    | 32    | 31 | 37    | 36    | 41 | 51    | 54    | 46 | 48    | 44    | 47 | 45    | 26 | ID 3  | 3  | ID 21 | 21 | 20 | 20 | 19 | 19 | 19   | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | ID |
| H. zacatecana MEX-39    | 31    | 32    | 31 | 37    | 36    | 41 | 51    | 54    | 46 | 48    | 44    | 47 | 45    | 26 | ID 3  | 3  | ID 21 | 21 | 20 | 20 | 19 | 19 | 19   | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | ID |
| H. casmirica HM         | 30    | 42    | 35 | 42    | 38    | 42 | 52    | 57    | 47 | 46    | 44    | 46 | 44    | 29 | ID 0  | 16 | ID 16 | 18 | 18 | 22 | 21 | 21 | ID 0 | 16 | 18 | 17 | 17 | 17 | 17 | 17 | 17 | ID |
| H. casmirica H4         | 30    | 42    | 35 | 42    | 38    | 42 | 52    | 57    | 47 | 46    | 44    | 46 | 44    | 29 | ID 0  | 16 | ID 16 | 18 | 17 | 17 | 17 | 17 | 17   | 17 | 17 | 17 | 17 | 17 | 17 | 17 | 17 | ID |
| H. bacteriophora Brecon | 32    | 36    | 33 | 40    | 40    | 42 | 44    | 50    | 39 | 42    | 36    | 41 | 36    | 26 | ID 2  | 1  | ID 2  | 1  | 1  | 1  | 1  | 1  | 1    | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | ID |
| H. bacteriophora HP88   | 31    | 35    | 32 | 40    | 41    | 42 | 43    | 45    | 50 | 39    | 42    | 36 | 41    | 36 | ID 1  | 1  | ID 1  | 1  | 1  | 1  | 1  | 1  | 1    | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | ID |
| H. bacteriophora DE2    | 31    | 35    | 32 | 40    | 41    | 42 | 44    | 50    | 39 | 41    | 36    | 41 | 36    | 25 | ID 0  | 0  | ID 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | ID |
| H. bacteriophora DE6    | 31    | 35    | 32 | 40    | 41    | 42 | 44    | 50    | 39 | 41    | 36    | 41 | 36    | 25 | ID 0  | 0  | ID 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | ID |
| H. bacteriophora EN01   | 31    | 35    | 32 | 40    | 41    | 42 | 44    | 50    | 39 | 41    | 36    | 41 | 36    | 25 | ID 0  | 0  | ID 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | ID |
| H. bacteriophora IT6    | 31    | 35    | 32 | 40    | 41    | 42 | 44    | 50    | 39 | 41    | 36    | 41 | 36    | 25 | ID 0  | 0  | ID 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | ID |
| H. bacteriophora TT01   | 31    | 35    | 32 | 40    | 41    | 42 | 44    | 50    | 39 | 41    | 36    | 41 | 36    | 25 | ID 0  | 0  | ID 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | ID |
| H. bacteriophora PT1    | 31    | 35    | 32 | 40    | 41    | 42 | 44    | 50    | 39 | 41    | 36    | 41 | 36    | 25 | ID 0  | 0  | ID 0  | 0  | 0  | 0  | 0  | 0  | 0    | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | ID |

**Figure S3.** Pairwise comparisons of the number of nucleotide differences in the sequences of the cytochrome c oxidase I (*cox-1*) gene of different *Heterorhabditis* species. A total of 344 nucleotide positions, flanked by primers HCF and HCR, were analyzed. Accession numbers of gene sequences used are shown in Table S2.

|                         |    |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|-------------------------|----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| H. zealandica CD2507    | ID | 84.1 | 88.2 | 88.5 | 87.8 | 88.5 | 67.6 | 67.7 | 69.1 | 68.8 | 68.0 | 68.0 | 68.2 | 74.2 | 74.2 | 74.2 | 74.1 | 74.1 | 74.0 | 74.0 | 74.0 |
| H. megidis CD2518       | ID | 92.7 | 98.4 | 89.8 | 90.2 | 66.4 | 66.5 | 67.9 | 67.8 | 67.0 | 66.8 | 67.5 | 74.3 | 74.3 | 74.3 | 74.2 | 74.1 | 74.1 | 74.0 | 74.0 | 74.0 |
| H. downesi CD2508       | ID | 94.2 | 94.7 | 95.3 | 69.5 | 69.6 | 70.8 | 70.8 | 69.7 | 69.7 | 69.7 | 70.2 | 77.7 | 77.7 | 77.7 | 77.7 | 77.7 | 77.7 | 77.7 | 77.7 |      |
| H. marelatus OH10       | ID | 96.6 | 97.0 | 70.1 | 70.1 | 72.2 | 71.9 | 70.7 | 70.9 | 71.0 | 78.5 | 78.5 | 78.5 | 78.5 | 78.2 | 78.2 | 78.3 | 78.3 | 78.3 | 78.3 |      |
| H. safricana SF281      | ID | 98.1 | 70.5 | 71.4 | 72.6 | 71.4 | 70.2 | 70.3 | 70.7 | 78.2 | 78.2 | 78.2 | 78.2 | 77.9 | 77.9 | 78.0 | 78.0 | 77.9 | 77.9 | 77.9 |      |
| H. atacamensis MEX-20   | ID | 71.4 | 71.4 | 72.6 | 72.3 | 71.1 | 71.3 | 71.7 | 78.8 | 78.8 | 78.8 | 78.8 | 78.8 | 78.4 | 78.4 | 78.4 | 78.6 | 78.6 | 78.5 | 78.5 |      |
| H. noenieputensis SF669 | ID | 98.0 | 84.7 | 84.9 | 84.2 | 84.3 | 84.1 | 71.0 | 71.0 | 70.8 | 70.8 | 70.8 | 70.8 | 70.8 | 70.8 | 70.8 | 70.8 | 70.8 | 70.8 | 70.8 |      |
| H. indica CD2525        | ID | 85.0 | 85.1 | 84.5 | 84.3 | 84.3 | 70.8 | 70.8 | 70.8 | 70.8 | 70.8 | 70.8 | 70.8 | 70.8 | 70.8 | 70.8 | 70.8 | 70.8 | 70.8 | 70.8 |      |
| H. amazonensis CD2510   | ID | 98.0 | 96.5 | 96.9 | 96.8 | 72.0 | 72.0 | 72.2 | 72.1 | 71.9 | 71.9 | 71.9 | 71.9 | 71.9 | 71.9 | 71.9 | 71.9 | 71.9 | 71.9 | 71.9 |      |
| H. baujardi CD2519      | ID | 96.4 | 97.2 | 96.5 | 71.9 | 71.9 | 71.9 | 71.9 | 71.9 | 71.9 | 71.8 | 71.9 | 71.8 | 71.9 | 71.8 | 71.9 | 71.9 | 71.9 | 71.9 | 71.9 |      |
| H. mexicana Mexican     | ID | 98.2 | 98.7 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 |      |
| H. floridensis 332      | ID | 98.2 | 98.2 | 70.7 | 70.7 | 70.7 | 70.7 | 70.7 | 70.7 | 70.7 | 70.7 | 70.7 | 70.7 | 70.7 | 70.7 | 70.7 | 70.7 | 70.7 | 70.7 | 70.7 |      |
| H. tayserae Benin       | ID | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 | 70.6 |      |
| H. beicherriana CD2516  | ID | 100  | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 |      |
| H. beicherriana M6      | ID | 100  | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 |      |
| H. beicherriana Cherry  | ID | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 |      |
| H. georgiana Kesha      | ID | 99.8 | 99.8 | 99.8 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 |      |
| H. georgiana Hbb        | ID | 99.8 | 99.8 | 99.8 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 |      |
| H. americana n. sp. S10 | ID | 99.8 | 99.8 | 99.8 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 | 97.6 |      |
| H. americana n. sp. S8  | ID | 97.8 | 97.8 | 97.8 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 |      |
| H. zacatecana MEX-40    | ID | 100  | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 |      |
| H. zacatecana MEX-39    | ID | 100  | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 |      |
| H. zacatecana MEX-41    | ID | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 |      |
| H. ruandica Rw14_N-C4a  | ID | 100  | 100  | 99.4 | 99.4 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 |      |
| H. ruandica Rw18_M-Hr1b | ID | 100  | 99.4 | 99.4 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 |      |
| H. ruandica Rw18_M-Hr1a | ID | 100  | 99.4 | 99.4 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 | 99.1 |      |
| H. casmrica H4          | ID | 100  | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 |      |
| H. casmrica HM          | ID | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 |      |
| H. bacteriophora TT01   | ID | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  |      |
| H. bacteriophora HP88   | ID | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  |      |
| H. bacteriophora Brecon | ID | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  |      |
| H. bacteriophora DE2    | ID | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  |      |
| H. bacteriophora DE6    | ID | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  |      |
| H. bacteriophora IT6    | ID | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  |      |
| H. bacteriophora EN01   | ID | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  |      |
| H. bacteriophora PT1    | ID | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  | 100  |      |

**Figure S4.** Pairwise comparisons of the nucleotide similarity (%) values for the sequences of the internal transcribed spacer (ITS) region of the rRNA gene of different *Heterorhabditis* species. A total of 775 nucleotide positions, flanked by primers TW81 and AB28, were analyzed. Accession numbers of gene sequences used are shown in Table S2.



[illegible]

**Figure S5.** Pairwise comparisons of the number of nucleotide differences in the sequences of the internal transcribed spacer (ITS) region of the rRNA gene of different *Heterorhabditis* species. A total of 765 nucleotide positions, flanked by primers TW81 and AB28, were analyzed. Accession numbers of gene sequences used are shown in Table S2.

|                         |    |      |      |      |      |      |      |      |      |      |     |      |     |      |     |      |      |     |     |     |
|-------------------------|----|------|------|------|------|------|------|------|------|------|-----|------|-----|------|-----|------|------|-----|-----|-----|
| H. zealandica CD2507    | ID | 97.6 | 98.7 | 98.5 | 97.9 | 97.9 | 90.7 | 90.5 | 91.8 | 89.1 | 6.9 | 1.4  | 9.1 | 2.2  | 9.1 | 2.9  | 1.2  | 9.5 | 0.9 | 5.0 |
| H. megidis CD2518       | ID | 98.1 | 97.9 | 99.7 | 4.9  | 4.9  | 4.9  | 2.9  | 0.0  | 0.0  | 9.0 | 7.0  | 9.0 | 5.0  | 4.9 | 0.4  | 9.0  | 4.9 | 3.9 | 9.3 |
| H. downesi CD2508       | ID | 99.8 | 98.1 | ID   | 99.8 | 99.2 | 99.2 | 9.1  | 6.1  | 4.9  | 2.7 | 9.2  | 5.9 | 2.3  | 9.2 | 3.9  | 1.9  | 1.9 | 5.5 | 5.5 |
| H. atacamensis MEX-20   | ID | 98.5 | 97.9 | 99.8 | ID   | 99.4 | 99.4 | 9.1  | 8.9  | 1.6  | 9.2 | 8.9  | 2.3 | 9.2  | 1.9 | 2.0  | 9.2  | 0.9 | 5.7 | 9.5 |
| H. marelatus            | ID | 97.9 | 97.4 | 9.9  | 2.9  | 9.9  | 4.   | ID   | 98.8 | 9.1  | 2.9 | 0.9  | 1.8 | 9.1  | 4.9 | 1.4  | 9.1  | 4.9 | 5.5 | 2.9 |
| H. safricana SF281      | ID | 97.9 | 97.4 | 9.9  | 2.9  | 9.9  | 4.9  | ID   | 92.1 | 9.2  | 0.9 | 1.9  | 8.9 | 1.6  | 9.1 | 4.9  | 1.4  | 9.1 | 4.9 | 5.5 |
| H. indica MEX10         | ID | 90.7 | 90.2 | 9.1  | 6.1  | 4.9  | 1.8  | 9.1  | 2.9  | 2.1  | ID  | 99.6 | ID  | 96.1 | 1.9 | 6.1  | 9.5  | 8.9 | 5.6 | 9.1 |
| H. noenieputensis SF669 | ID | 90.5 | 90.0 | 9.1  | 4.9  | 1.6  | 9.1  | 1.9  | 2.0  | 9.6  | ID  | 96.1 | 1.9 | 6.1  | 9.5 | 8.9  | 5.6  | 9.1 | 4.9 | 1.4 |
| H. amazonensis CD2510   | ID | 91.8 | 90.9 | 9.2  | 7.9  | 9.2  | 5.9  | 9.2  | 0.9  | 1.9  | 1.6 | 1.9  | ID  | 99.8 | 9.8 | 7.9  | 9.9  | 9.9 | 9.9 | 9.9 |
| H. baujardi CD2519      | ID | 91.6 | 90.7 | 9.2  | 5.9  | 9.2  | 3.9  | 1.8  | 9.2  | 0.9  | 1.6 | 1.9  | ID  | 99.8 | ID  | 98.7 | 9.8  | 9.9 | 9.8 | 9.9 |
| H. mexicana MX          | ID | 91.4 | 90.5 | 9.2  | 3.9  | 1.9  | 1.6  | 9.1  | 8.9  | 9.5  | 8.9 | 9.8  | 7.9 | 9.8  | ID  | 99.0 | 99.8 | 9.1 | 8.9 | 1.8 |
| H. floridensis 332      | ID | 91.2 | 90.4 | 9.2  | 1.9  | 1.9  | 0.9  | 1.4  | 9.1  | 6.9  | 9.5 | 8.9  | 9.8 | 9.9  | 9.9 | ID   | 99.2 | 9.2 | 0.9 | 2.0 |
| H. taysearae Benin      | ID | 91.2 | 90.4 | 9.2  | 1.9  | 1.9  | 0.9  | 1.4  | 9.1  | 6.9  | 9.5 | 9.8  | 9.9 | 9.9  | 9.9 | ID   | 99.2 | 9.2 | 0.9 | 2.0 |
| H. beicherriana CD2516  | ID | 95.0 | 93.9 | 9.5  | 9.5  | 7.9  | 9.5  | 2.9  | 9.1  | 4.9  | 1.3 | 9.2  | 1.9 | 2.0  | 9.1 | 8.9  | 2.0  | 9.1 | 6.  | ID  |
| H. beicherriana M6      | ID | 95.0 | 93.9 | 9.5  | 9.5  | 7.9  | 9.5  | 2.9  | 9.1  | 4.9  | 1.3 | 9.2  | 1.9 | 2.0  | 9.1 | 8.9  | 2.0  | 9.1 | 6.  | ID  |
| H. beicherriana Cherry  | ID | 95.0 | 93.9 | 9.5  | 9.5  | 7.9  | 9.5  | 2.9  | 9.1  | 4.9  | 1.3 | 9.2  | 1.9 | 2.0  | 9.1 | 8.9  | 2.0  | 9.1 | 6.  | ID  |
| H. americana n. sp. S8  | ID | 94.6 | 93.5 | 9.5  | 2.9  | 9.5  | 4.9  | 4.8  | 9.4  | 8.9  | 1.1 | 9.0  | 9.1 | 8.9  | 1.6 | 9.1  | 4.9  | 1.6 | 9.1 | 2.9 |
| H. americana n. sp. S10 | ID | 94.6 | 93.5 | 9.5  | 2.9  | 9.5  | 4.9  | 4.8  | 9.4  | 8.9  | 1.1 | 9.0  | 9.1 | 8.9  | 1.6 | 9.1  | 4.9  | 1.6 | 9.1 | 2.9 |
| H. georgiana Hbb        | ID | 94.6 | 93.5 | 9.5  | 2.9  | 9.5  | 4.9  | 4.8  | 9.4  | 8.9  | 1.1 | 9.0  | 9.1 | 8.9  | 1.6 | 9.1  | 4.9  | 1.6 | 9.1 | 2.9 |
| H. georgiana Kesha      | ID | 94.6 | 93.5 | 9.5  | 2.9  | 9.5  | 4.9  | 4.8  | 9.4  | 8.9  | 1.1 | 9.0  | 9.1 | 8.9  | 1.6 | 9.1  | 4.9  | 1.6 | 9.1 | 2.9 |
| H. ruandica Rw14_N-C4a  | ID | 95.0 | 93.9 | 9.5  | 9.5  | 7.9  | 9.5  | 2.9  | 9.1  | 4.9  | 1.3 | 9.2  | 1.9 | 2.0  | 9.1 | 8.9  | 2.0  | 9.1 | 6.  | ID  |
| H. ruandica Rw18_M-Hr1a | ID | 95.0 | 93.9 | 9.5  | 9.5  | 7.9  | 9.5  | 2.9  | 9.1  | 4.9  | 1.3 | 9.2  | 1.9 | 2.0  | 9.1 | 8.9  | 2.0  | 9.1 | 6.  | ID  |
| H. ruandica Rw18_M-Hr1b | ID | 95.0 | 93.9 | 9.5  | 9.5  | 7.9  | 9.5  | 2.9  | 9.1  | 4.9  | 1.3 | 9.2  | 1.9 | 2.0  | 9.1 | 8.9  | 2.0  | 9.1 | 6.  | ID  |
| H. zacatecana MEX-39    | ID | 95.0 | 93.9 | 9.5  | 9.5  | 7.9  | 9.5  | 2.9  | 9.1  | 4.9  | 1.3 | 9.2  | 1.9 | 2.0  | 9.1 | 8.9  | 2.0  | 9.1 | 6.  | ID  |
| H. zacatecana MEX-40    | ID | 95.0 | 93.9 | 9.5  | 9.5  | 7.9  | 9.5  | 2.9  | 9.1  | 4.9  | 1.3 | 9.2  | 1.9 | 2.0  | 9.1 | 8.9  | 2.0  | 9.1 | 6.  | ID  |
| H. zacatecana MEX-41    | ID | 95.0 | 93.9 | 9.5  | 9.5  | 7.9  | 9.5  | 2.9  | 9.1  | 4.9  | 1.3 | 9.2  | 1.9 | 2.0  | 9.1 | 8.9  | 2.0  | 9.1 | 6.  | ID  |
| H. casmirica HM         | ID | 94.8 | 93.7 | 9.5  | 4.9  | 5.5  | 9.5  | 0.9  | 9.5  | 0.1  | 2.9 | 1.1  | 9.2 | 3.9  | 1.2 | 9.2  | 0.9  | 1.9 | 1.8 | 9.9 |
| H. casmirica H4         | ID | 94.8 | 93.7 | 9.5  | 4.9  | 5.5  | 9.5  | 0.9  | 9.5  | 0.1  | 2.9 | 1.1  | 9.2 | 3.9  | 1.2 | 9.2  | 0.9  | 1.9 | 1.8 | 9.9 |
| H. bacteriophora HP88   | ID | 94.8 | 93.7 | 9.5  | 4.9  | 5.5  | 9.5  | 0.9  | 9.5  | 0.1  | 2.9 | 1.1  | 9.2 | 3.9  | 1.2 | 9.2  | 0.9  | 1.9 | 1.8 | 9.9 |
| H. bacteriophora Brecon | ID | 94.8 | 93.7 | 9.5  | 4.9  | 5.5  | 9.5  | 0.9  | 9.5  | 0.1  | 2.9 | 1.1  | 9.2 | 3.9  | 1.2 | 9.2  | 0.9  | 1.9 | 1.8 | 9.9 |
| H. bacteriophora TT01   | ID | 94.8 | 93.7 | 9.5  | 4.9  | 5.5  | 9.5  | 0.9  | 9.5  | 0.1  | 2.9 | 1.1  | 9.2 | 3.9  | 1.2 | 9.2  | 0.9  | 1.9 | 1.8 | 9.9 |
| H. bacteriophora DE2    | ID | 94.8 | 93.7 | 9.5  | 4.9  | 5.5  | 9.5  | 0.9  | 9.5  | 0.1  | 2.9 | 1.1  | 9.2 | 3.9  | 1.2 | 9.2  | 0.9  | 1.9 | 1.8 | 9.9 |
| H. bacteriophora DE6    | ID | 94.8 | 93.7 | 9.5  | 4.9  | 5.5  | 9.5  | 0.9  | 9.5  | 0.1  | 2.9 | 1.1  | 9.2 | 3.9  | 1.2 | 9.2  | 0.9  | 1.9 | 1.8 | 9.9 |
| H. bacteriophora IT6    | ID | 94.8 | 93.7 | 9.5  | 4.9  | 5.5  | 9.5  | 0.9  | 9.5  | 0.1  | 2.9 | 1.1  | 9.2 | 3.9  | 1.2 | 9.2  | 0.9  | 1.9 | 1.8 | 9.9 |
| H. bacteriophora PT1    | ID | 94.8 | 93.7 | 9.5  | 4.9  | 5.5  | 9.5  | 0.9  | 9.5  | 0.1  | 2.9 | 1.1  | 9.2 | 3.9  | 1.2 | 9.2  | 0.9  | 1.9 | 1.8 | 9.9 |
| H. bacteriophora EN01   | ID | 94.8 | 93.7 | 9.5  | 4.9  | 5.5  | 9.5  | 0.9  | 9.5  | 0.1  | 2.9 | 1.1  | 9.2 | 3.9  | 1.2 | 9.2  | 0.9  | 1.9 | 1.8 | 9.9 |

**Figure S6.** Pairwise comparisons of the nucleotide similarity (%) values for the sequences of the D2-D3 expansion segments of the 28S rRNA gene of different *Heterorhabditis* species. A total of 554 nucleotide positions, flanked by primers D2A and D3B, were analyzed. Accession numbers of gene sequences used for the analyses are shown in Table S2.



[illegible]

**Figure S7.** Pairwise comparisons of the number of nucleotide differences in the sequences of the D2-D3 expansion segments of the 28S rRNA gene of different *Heterorhabditis* species. A total of 554 nucleotide positions, flanked by primers D2A and D3B, were analyzed. Accession numbers of gene sequences used for the analyses are shown in Table S2.



# **SUPPLEMENTARY TABLES**

**Table S1.** Sequences of the primers used to amplify the genes/genetic regions of different *Heterorhabditis* species used for the phylogenetic analyses in this study.

| Genetic region | Primers | Orientation | Sequence 5' to 3'        | Description            |
|----------------|---------|-------------|--------------------------|------------------------|
| ITS            | TW81    | F           | GTTTCCGTAGGTGAACCTGC     | Joyce et al. (1994)    |
|                | AB28    | R           | ATATGCTTAAGTTCAGCGGGT    |                        |
| D2-D3          | D2A     | F           | ACAAGTACCGTGAGGGAAAGTTG  | Subbotin et al. (2006) |
|                | D3B     | R           | TCGGAAGGAACCAGCTACTA     |                        |
| COI            | HCF     | F           | TTACATGATACTTATTATG      | Kuwata et al. (2007)   |
|                | HCR     | R           | CTGATAACTGTGACCAAATACATA |                        |

**Table S2.** National Center for Biotechnology Information (NCBI) accession numbers of the nucleotide sequences used for the phylogenetic analyses in this study. In bold font are the sequences (22 in total) newly generated in this study.

| Organism                          |                        | Genetic Region  |                 |                 |
|-----------------------------------|------------------------|-----------------|-----------------|-----------------|
| Species                           | Strain designation (s) | ITS             | D2-D3           | COI             |
| <i>H. amazonensis</i>             | CD2510, MC01           | MT372499        | MT372502        | MT372499        |
| <b><i>H. americana</i> n. sp.</b> | <b>S8</b>              | <b>PQ483104</b> | OK646639        | <b>PQ483125</b> |
|                                   | <b>S10</b>             | <b>PQ483105</b> | MW817559        | <b>PQ483126</b> |
| <i>H. atacamensis</i>             | MEX-20                 | MK421485        | MW817561        | MW817979        |
| <i>H. bacteriophora</i>           | Brecon                 | <b>PQ483106</b> | <b>PQ492375</b> | <b>PQ483127</b> |
|                                   | DE2                    | MZ326036        | MW817552        | MW817970        |
|                                   | DE6                    | MZ326037        | MW817553        | MW817971        |
|                                   | EN01                   | MZ326038        | MW817554        | MW817972        |
|                                   | IT6                    | MZ326039        | MW817555        | MW817973        |
|                                   | PT1                    | MZ326040        | MW817556        | MW817974        |
|                                   | TT01                   | MZ326041        | MW817557        | MW817975        |
|                                   | CD2504, HP88           | MT372491        | MT372509        | MT373729        |
| <i>H. baujardi</i>                | CD2519, F10            | MT372500        | MT372503        | MT373736        |
| <i>H. beicherriana</i>            | M6                     | <b>PQ483107</b> | <b>PQ492376</b> | <b>PQ483128</b> |
|                                   | CD2516, QZL-2011       | MT372490        | MT372511        | MT373730        |
|                                   | Cherry                 | <b>PQ483108</b> | <b>PQ492377</b> | <b>PQ483129</b> |
| <i>H. casmirica</i>               | H4                     | OQ517949        | OQ517940        | OQ517980        |
|                                   | HM                     | OQ517947        | OQ517936        | OQ517975        |
| <i>H. downesi</i>                 | CD2508, 23.9           | MT372494        | MT372507        | MT373732        |
| <i>H. floridensis</i>             | 332                    | <b>PQ483109</b> | <b>PQ492378</b> | <b>PQ483130</b> |
| <i>H. georgiana</i>               | Hbb                    | MZ326042        | MW817558        | MW817976        |
|                                   | Kesha                  | <b>PQ483110</b> | <b>PQ492379</b> | <b>PQ483131</b> |
| <i>H. indica</i>                  | CD2525                 | MT372498        | -               | -               |
|                                   | MEX-10                 | -               | MK421439        | -               |
|                                   | LN2                    | -               | -               | AB355853        |
| <i>H. marelatus</i>               | OH10                   | AY321479        | -               | -               |
|                                   | No information         | -               | EU100412        | -               |
|                                   | No information         | -               | -               | EF043419        |
| <i>H. megidis</i>                 | CD2518, DV             | MT372495        | MT372506        | MT373733        |
| <i>H. mexicana</i>                | Mexican                | AY321478        | -               | -               |
|                                   | MX                     | -               | EU100414        | -               |
|                                   | No information         | -               | -               | EF043422        |
| <i>H. noenieputensis</i>          | SF669                  | JN620538        | MT372505        | MT373728        |
| <i>H. ruandica</i>                | Rw18_M-Hr1a            | MZ326033        | MW817549        | MW817967        |
|                                   | Rw18_M-Hr1b            | MZ326034        | MW817550        | MW817968        |
|                                   | Rw14_N-C4a             | MZ326035        | MW817551        | MW817969        |
| <i>H. safricana</i>               | SF281                  | EF488006        | EU100416        | OR835850        |
| <i>H. taysearae</i>               | Benin                  | <b>PQ483111</b> | <b>PQ492380</b> | <b>PQ483132</b> |
| <i>H. zacatecana</i>              | MEX-39                 | MZ326030        | MW817546        | MW817964        |
|                                   | MEX-40                 | MZ326031        | MW817547        | MW817965        |
|                                   | MEX-41                 | MZ326032        | MW817548        | MW817966        |
| <i>H. zealandica</i>              | CD2507                 | MT372493        | MT372508        | MT373734        |



**Table S3.** National Center for Biotechnology Information (NCBI) accession numbers of the genomic sequences of different *Photorhabdus* species used in this study.

|                                                                           | NCBI Accession numbers |
|---------------------------------------------------------------------------|------------------------|
| <i>P. aegyptia</i> BA1 <sup>T</sup>                                       | JFGV01                 |
| <i>P. akhurstii</i> subsp. <i>akhurstii</i> DSM 15138 <sup>T</sup>        | RCWE01                 |
| <i>P. akhurstii</i> subsp. <i>bharatensis</i> H3 <sup>T</sup>             | PUWU01                 |
| <i>P. asymbiotica</i> DSM 15149 <sup>T</sup>                              | RBLJ01                 |
| <i>P. australis</i> subsp. <i>australis</i> DSM 17609 <sup>T</sup>        | JONO01                 |
| <i>P. australis</i> subsp. <i>thailandensis</i> PB68.1 <sup>T</sup>       | LOMY01                 |
| <i>P. bodei</i> LJ24-63 <sup>T</sup>                                      | NSCM01                 |
| <i>P. caribbeanensis</i> DSM 22391 <sup>T</sup>                           | RCWB01                 |
| <i>P. cinerea</i> DSM 19724 <sup>T</sup>                                  | PUJW01                 |
| <i>P. hainanensis</i> DSM 22397 <sup>T</sup>                              | RCWD01                 |
| <i>P. heterorhabditis</i> subsp. <i>heterorhabditis</i> SF41 <sup>T</sup> | RCWA01                 |
| <i>P. heterorhabditis</i> subsp. <i>aluminescens</i> Q614 <sup>T</sup>    | JABBCS01               |
| <i>P. hindustanensis</i> H1 <sup>T</sup>                                  | PUWT01                 |
| <i>P. kayaii</i> DSM 15194 <sup>T</sup>                                   | JAJAFZ01               |
| <i>P. kharii</i> subsp. <i>kharii</i> DSM 3369 <sup>T</sup>               | AYSJ01                 |
| <i>P. kharii</i> subsp. <i>guanajuatensis</i> MEX20-17 <sup>T</sup>       | PUJY01                 |
| <i>P. kleinii</i> DSM 23513 <sup>T</sup>                                  | JAJAFY01               |
| <b><i>P. kleinii</i> S8-52</b>                                            | <b>NSCL01</b>          |
| <b><i>P. kleinii</i> S10-54</b>                                           | <b>NSCJ01</b>          |
| <i>P. laumondii</i> subsp. <i>clarkei</i> BOJ-47 <sup>T</sup>             | NSCI01                 |
| <i>P. laumondii</i> subsp. <i>laumondii</i> TT01 <sup>T</sup>             | WSFH01                 |
| <i>P. luminescens</i> subsp. <i>luminescens</i> ATCC 29999 <sup>T</sup>   | FMWJ01                 |
| <i>P. luminescens</i> subsp. <i>mexicana</i> MEX47-22 <sup>T</sup>        | PUJX01                 |
| <i>P. namnaonensis</i> PB45.5 <sup>T</sup>                                | LOIC01                 |
| <i>P. noenieputensis</i> DSM 25462 <sup>T</sup>                           | RCWC01                 |
| <i>P. stackebrandtii</i> DSM 23271 <sup>T</sup>                           | PUJV01                 |
| <i>P. tasmaniensis</i> DSM 22387 <sup>T</sup>                             | PUJU01                 |
| <i>P. temperata</i> DSM 15550 <sup>T</sup>                                | JAJAFX01               |
| <i>P. thracensis</i> DSM 15199 <sup>T</sup>                               | CP011104               |