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Phasmarhabditis hermaphrodita (Nematoda: Rhabditidae), a potential biocontrol agent isolated for the first time from invasive slugs in North America

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Summary – *Phasmarhabditis hermaphrodita* is reported for the first time in North America from cadavers of the invasive slug species *Deroceras reticulatum*, *D. laeve* and *Lehmannia valentiana* collected from three different locations in California, USA. Four isolates were identified using combined morphology, morphometrics and molecular sequence data for complete internal transcribed spacer (ITS-1, 5.8S, ITS-2), D2-D3 expansion segments of the large subunit (LSU or 28S) and nearly complete small subunit (SSU or 18S) ribosomal DNA. Extremely low sequence variations in the COI gene of the mitochondria were observed among US isolates as well as between US isolates and the two UK sequences. The occurrence of *P. hermaphrodita* in North America has regulatory implications for potential biological control strategies against non-native gastropod species that are pests in ornamental and agricultural cultivation on this continent. The D2-D3 sequence of the LSU rDNA is new for the species.

Keywords – biological control, *Deroceras laeve*, *Deroceras reticulatum*, gastropods, *Lehmannia valentiana*, molecular, morphology, morphometrics, new record, slugs, systematics, taxonomy.

Phasmarhabditis hermaphrodita (Schneider, 1859) Andrássy, 1983 was first described as *Pelodytes hermaphroditus* Schneider, 1859 from *Arion* sp. in Germany (Schneider, 1859) and was rediscovered in 1988 from *Deroceras reticulatum* O.F. Müller during a search for biological control agents in the UK (Wilson *et al.*, 1993; Glen *et al.*, 1996). Bacterial associates were identified and virulence tested in monoxenic combinations with *D. reticulatum*. Of these bacteria, *Moraxella osloensis* supported the highest production of the nematode (Wilson *et al.*, 1995) and together both have been used in a commercial preparation marketed as 'Nemaslug®' (BASF Agricultural Specialties, formerly by Becker Underwood, Littlehampton, UK) for home gardeners since spring of 1994. This product has been approved for application on vegetables, high-value crops (Rae *et al.*, 2007) and field crops (Brown *et al.*, 2011) and is available in the UK, Ireland, France, The Netherlands, Belgium, Germany, Denmark, Norway, Finland, Poland, Spain, the Czech Republic, Italy and Switzerland (Rae *et al.*, 2007); however, it has not been introduced to the US because *P. hermaphrodita* has not been confirmed to be already present, despite repeated nationwide surveys (Grewal *et al.*, 2000; Kaya & Mitani, 2000; Ross *et al.*, 2010). Interestingly, a species of *Phasmarhabditis* (isolate 434, CGC) has been isolated from earthworms in The Bronx, New York (http://www.nyu.edu/projects/fitch/WSRN/strains/em434.html). The genus was also reported in earthworms (*Lumbricus terrestris* L.) on campus at the University of Illinois at Urbana-Champaign, Illinois (Zaborski *et al.*, 2001), and baited from soil at the Brigham Young University campus in Utah using *Galleria mellonella* L. grubs (http://jur.byu.edu/?p=3491). Both the Utah and New York sequences obtained by the aforementioned authors were similar but did not match partial 28S rDNA sequences available for *P. hermaphrodita* in GenBank. Outside of Europe, *P. hermaphrodita* has also been found in Chile (France & Gerding, 2000), Egypt (Genena *et al.*, 2011), Iran (Karimi *et al.*, 2003) and New Zealand (Wilson *et al.*, 2012).

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The genus *Phasmarhabditis* Andrássy, 1976 ("Phasmarhabditis is characterised by the presence of large rod-like phasmids standing out from the body contour, and the short mouth prisma which is only once to twice as long as wide") was proposed by Andrássy within the subfamily Peloderinae with *Pelodera papillosa* Schneider, 1866 as type species. In 1983, Andrássy published a key to five species based on: *i*) female tail shape and length; *ii*) appearance of the bursa and spicule length; *iii*) number and position of bursal papillae; and *iv*) the frequency of males and host association. Recently, *P. tawfiki* Azzam, 2003 was described in Egypt from the terrestrial snail *Eobania vermiculata* Müller and the slug *Limacus flavus* L. Two undescribed new species were found associated with *D. reticulatum* and *Ariostralis nebulosa* Sergil, in South Africa (Ross *et al.*, 2012).

Sudhaus (2011) published a catalogue of Rhabditidae and proposed *Phasmarhabditis* as a junior synonym of *Pellioditis* Dougherty, 1953. Although the taxon *Pelodytes* Schneider, 1859 with type species *hermaphroditus* (= *hermaphrodita*) precedes *Phasmarhabditis*, the former name is preoccupied by an amphibian genus (Bonaparte, 1838; Herraiz-Sanchez *et al.*, 2000). For the purpose of this paper, and to avoid more taxonomic confusion, we will discuss the species herein as belonging to the genus '*Phasmarhabditis*'.

Species of this genus are mainly associated with gastropods belonging to 16 families (see Grewal *et al.*, 2002; Rae *et al.*, 2007; Ross *et al.*, 2011). *Phasmarhabditis hermaphrodita* has the widest host range and is associated with all the families mentioned.

Herein we report the first confirmed records of *P. hermaphrodita* and its association with slugs in the USA and North America.

Materials and methods

Collection and maintenance of gastropods

From October 2012 to September 2013, 11 gastropod collections were made in nurseries and garden centres throughout California, USA. We collected in Cloverdale, Eureka, Irvine, McKinleyville, Menifee, Moreno Valley, Orange, Riverside, San Diego, San Mateo, Santa Barbara and Sonoma. During the course of these surveys, 956 specimens were collected and brought back to the Insectary Facility at UC Riverside under CDFA Permit 2942 for natural enemy screening. Slugs and snails from each location were sorted by species and grown on organic carrots in plastic containers lined with moist paper towels. The food and towels were replaced twice weekly. Following death of slugs and snails, they were grown on 1% plain agar (PA: see De Ley & Mundo-Ocampo, 2004) and nematodes that emerged were isolated and sub-cultured to, and subsequently maintained on, fresh PA and nutrient agar.

Molecular analyses

Individual nematodes were picked from culture plates and washed in three transfers of sterile water. DESS-preserved (Yoder *et al.*, 2006) *P. hermaphrodita* (UK isolate) specimens were washed of any traces of the preservative and rinsed in three transfers of sterile water. Multifocal video clips were obtained of each of these specimens for morphological archiving (VCE *in* De Ley & Bert, 2002; De Ley *et al.*, 2005). DNA extraction and amplification were performed as described in Tandingan De Ley *et al.* (2007) for the D2-D3 expansion segments of the LSU, ITS and the SSU rDNA (Tandingan De Ley *et al.*, 2002). 2-3 μl of the genomic template DNA was used in a 25 μl PCR reaction using Illustra PuReTaq Ready-To-Go™ PCR beads (GE Healthcare) under the same PCR conditions and using the same amplification and sequencing primers previously described for D2-D3 and SSU (Blaxter *et al.*, 1998; Tandingan De Ley *et al.*, 2002); N93 forward (5′-TTGAACCGGGTAAAAGTCG-3′) and N94 reverse (5′-TTAGTTTCTTTTCCTCCGCT-3′) primers for ITS (Nadler *et al.*, 2005); and forward primer COI-F1 (5′-CCTACTATGATTGGTGGTTTTGGTAATTG-3′) and reverse primer COI-R2 (5-GTAGCAGCAGTAAAA TAAGCACG-3′) for mtCOI (Kanzaki & Futai, 2002).

PCR products were cleaned with QIAquick® PCR Purification Kit (Qiagen) following the manufacturer's protocol. Nucleotide sequences were determined using dye-terminator sequencing chemistry on a 96-capillary ABI 3730xl (Applied Biosystems) at the UCR Core Instrumentation Facility. Contigs were assembled and compared by BLAST with published sequences in GenBank using CodonCode Aligner (CodonCode). *De novo* sequences were submitted to GenBank with accession numbers KM510193-KM510200, KM510206-KM510209, KM510201-KM510202 and KM555038-KM555043 for D2-D3, SSU, ITS and COI, respectively.

Morphology and morphometrics of nematodes

Nematodes were picked from culture plates, fixed in 4% formalin and processed to anhydrous glycerin

(Seinhorst, 1959 as modified by De Grisse, 1969) for light microscopy. Measurements were determined using the 'Measure > active path' function in GIMP v 2.8.0 (Kimball *et al.*, 2012) for the appropriate distances of all pertinent body structures on jpeg images extracted from multifocal HD video clips recorded at highest relevant magnifications with VCE microscopy (De Ley & Bert, 2002).

Scanning electron microscopy was performed at Ghent University, Belgium. Nematodes were placed in an embryo dish with as little water as possible, 700 μl Trump's fixative added, microwaved 'high' for 5 s, and left at room temperature for at least an hour. Specimens were rinsed twice for 10 min with 0.1 M freshly prepared Sorenson buffer, rinsed twice for 10 min with water and sonicated for 8 min. This was followed by a dehydration series in increasing concentrations of ethanol, starting at 30% for 15 min, transferred successively to 50, 75, 95 and 98% each for 20 min; and three times at 100% for 15 min. Dehydrated nematodes were critical point-dried using Balzers Union (CPD020), mounted on stubs (carbon tabs) on the surface of double-sided conductive tape, sputter-coated with 25 nm layer gold (Balzers Union, SCD040) for 3 min and observed on a JEOL JSM-840 at 5 kV.

Results

The following gastropod taxa were collected: *A. hortensis* agg. (species complex comprising *Arion hortensis* A. Férussac, *A. distinctus* J. Mabille and *A. owenii* Davies; diagnostics rely mainly on combined morphological and molecular data (*e.g.*, mitochondrial *cytochrome c oxidase* I and 16S rRNA sequences)), *A. rufus* L., *A. subfuscus* Draparnaud, *Boettgerilla pallens* Simroth, *Cornu aspersum* O.F. Müller, *D. invadens* Reise, Hutchinson, Schunack & Schlitt, *D. laeve* O.F. Müller, *D. reticulatum*, *L. valentiana* A. Férussac, *L. flavus* L., *M. gagates* Draparnaud, *Oxychilus* sp., *Prophysaon andersoni* J.G. Cooper and *Succinea* spp. A total of 693 dead gastropods was transferred to agar in nematode emergence assays, 273 (42%) of which had associated nematodes, with more nematode occurrence in Southern California (57.7%) than in Northern California (27.2%). Of these, ten had *Phasmarhabditis* spp. and only four slug specimens belonging to three slug species had *P. hermaphrodita*. These species were *D. laeve* (DL), *D. reticulatum* (DR) and *L. valentiana* (LV) collected in Sonoma (DL, Sonoma Co.); and in two locations in Eureka (DR and LV, Humbolt Co.).

Preliminary sequences of the D2-D3 domain of the large subunit (LSU) and/or near complete small subunit (SSU) of other nematodes revealed they belong to major groups Rhabditida (*e.g.*, *Alloionema appendiculatum*, *Caenorhabditis elegans*, *C. briggsae*, *Koerneria* sp., *Oscheius dolichura*, *O. tipulae*, *Strongyloides* sp.) and Cosmocercoidea/Ascaridae (*e.g.*, *Nemhelix bakeri*, *Krefftascaris* sp.).

Phasmarhabditis hermaphrodita US isolate ITD272

(Fig. 1)

Measurements

See Table 1.

Description

Female

Body 1.3-1.7 mm long, almost straight or slightly curved when relaxed by heat. Annules fine and less prominent under light microscope. Lateral field with six prominent incisures and on another specimen, as seen on SEM, additional two finer alae on either side, adding three more lines in vulval region. Anterior end bluntly rounded, lip region 18 (14-20) μm broad, continuous with body, six lips grouped in pairs. One labial papilla protruding anteriorly from each lip with a clearly demarcated inner labial dendrite emerging apically. A smaller subapical protrusion of a second dendrite ending visible only on lips with cleanest papillar surfaces, corresponding perhaps to the rootleted inner labial dendrite below the papillar surface in *Caenorhabditis elegans* (Ware *et al.*, 1975). Two less prominent outer cephalic papillae on dorsal lip pair and one each on subventral pairs. Amphid opening a small slit opening laterally near outer margin of each lateral lip. Mouth triangular with slightly convex sides. Stoma 18 μm long, about as long as lip region diam., distinct cheilostom, gymnostom, and stegostom with mean lengths of 4, 3 and 11 μm, respectively. Stegostom ending with well developed, rounded, isomorphic metarhabdions, each with three minute tubercles. Corpus cylindrical, 2.3 times as long as isthmus with slightly enlarged non-valvular metacorpus narrowing into isthmus and a pyriform basal bulb with striated valvular apparatus. Nerve ring surrounding anterior part of isthmus. Deirids prominent. Excretory pore very posterior, opening at middle or near base of terminal bulb. Cardia conoid.

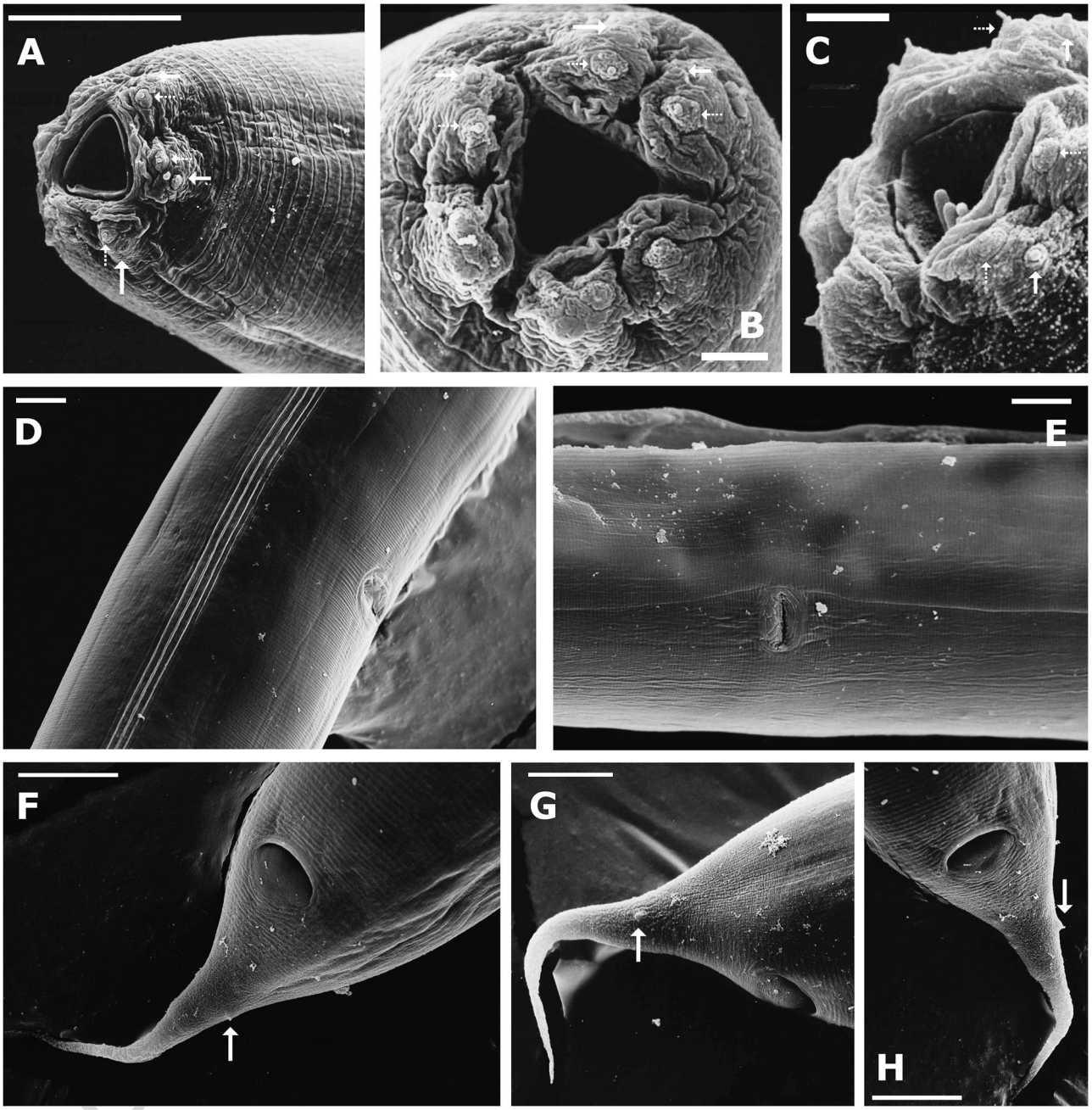

Fig. 1. SEM of *Phasmarhabditis hermaphrodita* US isolate ITD272. A-C: Lip region showing labial papillae (dashed arrows), cephalic papillae (solid arrows) and amphids (large arrow); D, E: Mid-body with lateral field and vulva; F-H: Posterior region showing anus, short tail and posterior phasmids (solid arrows). (Scale bars: A, D-H = 10 μm; B, C = 2 μm.)

Table 1. Comparative morphometrics between *Phasmarhabditis hermaphrodita* US isolate ITD272 from host *Deroceras reticulatum* and grown on xenic cultures with associated bacteria, UK isolates and *P. neopapillosa*. All measurements are in μm and in the form: mean $\pm$ s.d. (range).

Character	*P. hermaphrodita* US isolate ITD272	*P. hermaphrodita*[a]	*P. neopapillosa*[b]
n	10	20	20
L	1542 $\pm$ 161.2 (1284-1721)	1354 $\pm$ 115 (1186-1525)	2227 $\pm$ 190 (1817-2449)
a	17.2 $\pm$ 1.6 (15.1-19.5)	15.2 $\pm$ 1.6 (12.4-17.9)	16.0 $\pm$ 1.8 (14.6-16.2)
b	8.0 $\pm$ 0.6 (6.9-8.9)	5.9 $\pm$ 0.4 (5.1-6.4)	7.7 $\pm$ 0.5 (7.2-8.4)
c	15.6 $\pm$ 1.2 (13.4-17.4)	13.1 $\pm$ 0.7 (11.6-14.3)	14.2 $\pm$ 1.2 (12.1-16.9)
c′	2.5 $\pm$ 0.2 (2.2-3.0)	2.9 $\pm$ 0.2 (2.4-3.2)	3.9 $\pm$ 0.5 (3.3-5.0)
V	50.4 $\pm$ 1.5 (48.2-52.9)	– –	– –
Max. body diam.	90 $\pm$ 8.3 (82-107)	90 $\pm$ 11.2 (75-106)	141 $\pm$ 19.2 (101-174)
Lip region diam.	17.7 $\pm$ 1.9 (14.1-20.4)	18 $\pm$ 0.7 (17-19)	19 $\pm$ 0.5 (18-19)
Stoma length (St L)	18.4 $\pm$ 1.0 (17.0-20.3)	18 $\pm$ 1.3 (16-21)	21 $\pm$ 1.2 (19-24)
Cheilostom	4.0 $\pm$ 0.5 (3.4-4.9)	–	–
Gymnostom	3.2 $\pm$ 0.9 (2.1-4.9)	–	–
Stegostom	11.3 $\pm$ 1.1 (10.1-12.9)	–	–
Promesorhabdion	14.5 $\pm$ 1.1 (13.0-16.6)	11 $\pm$ 0.8 (10-12)	12 $\pm$ 1.1 (11-15)
Procorpus	62.3 $\pm$ 2.5 (57.1-65.6)	–	–
Metacorpus	35.1 $\pm$ 6.4 (25.1-44.8)	–	–
Cardia	8.5 $\pm$ 1.9 (5.1-11.5)	–	–
Corpus length (pro + meta)	97 $\pm$ 6.1 (86-107)	107 $\pm$ 5.2 (96-114)	144 $\pm$ 10.7 (126-168)
Metacorpus diam.	25.1 $\pm$ 7.2 (17.7-44.8)	–	–
Isthmus length	42.7 $\pm$ 3.5 (37.3-48.2)	59 $\pm$ 3.4 (54-63)	76 $\pm$ 6.8 (65-85)
Basal bulb length	36.9 $\pm$ 4.4 (32.1-45.9)	35 $\pm$ 2.2 (31-40)	57 $\pm$ 5.8 (48-62)
Basal bulb diam.	29.6 $\pm$ 1.9 (27.2-32.4)	–	–
Neck length (NL)[4]	193 $\pm$ 9.3 (175-209)	–	–
Nerve ring	139 $\pm$ 7.2 (126-149)	141 $\pm$ 6.9 (131-154)	188 $\pm$ 11.3 168-205

Table 1. (Continued.)

Character	*P. hermaphrodita* US isolate ITD272	*P. hermaphrodita*[a]	*P. neopapillosa*[b]
Excretory pore	–	172 ± 12.5	216 ± 10.6
	–	(157-189)	(199-231)
Deirid	185 ± 16.1	156 ± 11.1	–
	(169-205)	(139-171)	
Lip region to vulva	776 ± 71	–	–
	(673-866)		
Vagina length	30.2 ± 5.9	–	–
	(23.3-33.6)		
Vulva body diam.	89 ± 8.5	–	–
	(80-107)		
G_1%	24.2 ± 1.9	27 ± 1.7	34 ± 2.8
	(20.7-26.5)	(24-30)	(31-39)
G_2%	26.7 ± 2.7	29 ± 2.9	33 ± 2.6
	(22.0-31.7)	(22-34)	(29-37)
Rectum length	39.1 ± 5.3	–	–
	(33.6-52.3)		
Anal body diam. (ABD)	39.5 ± 4.3	–	–
	(34.0-48.2)		
Anus to phasmid	47 ± 9.7	–	–
	(38.0-70.1)		
Wide part of tail	55 ± 12.1	–	–
	(42-83)		
Tail length (TL)	99 ± 10.4	104 ± 8.6	157 ± 15.3
	(85-117)	(82-113)	(141-174)
ABD/TL	40 ± 3.2	–	–
	(33.3-45.0)		
St L/LRW	1.1 ± 0.2	–	–
	(0.9-1.4)		
Corpus L/isthmus L	2.3 ± 0.2	–	–
	(2.0-2.6)		
Nring (% NL)	72.2 ± 2.9	–	–
	(67.2-75.4)		
Deirid (% NL)	97 ± 3.3	–	–
	(92-100)		
G (RTL as % L)	50.9 ± 4.0	–	–
	(46.1-56.8)		
Rectum L/ABD	1.0 ± 0.1	–	–
	(0.8-1.3)		
Phasmid (% TL)	47.1 ± 5.8	–	–
	(41.6-62.9)		
Wide part tail/TL	45.6 ± 25.1	–	–
	(49.8-74.2)		

G_1/G_2: vulva to anterior/posterior flexure of gonad as % of body length in female; CL: corpus length, measured along curvature of the lumen; NL: neck length, from anterior end to the base of the basal bulb, measured along middle of the body; RTL: reproductive tract length, measured along body axis, from anterior-most tip to posterior-most tip, *i.e.*, excluding all flexures; stoma length: from cheilorhabdia to base of the stoma. Stoma terminology (cheilostom, gymnostom and stegostom) was adapted from De Ley *et al.* (1995) and terminology associated with the structures of the nematode anterior is based on Rashid *et al.* (1988).

[a] After Hooper *et al.* (1999).

[b] Equivalent to pharynx measurement in Hooper *et al.* (1999).

Reproductive system didelphic, amphidelphic, ovaries reflexed with tips sometimes reaching level of vulva. Anterior and posterior ovaries, as measured from vulva to anterior/posterior flexure, occupying 21-32% of total body length. Numerous sperm in oviducts despite absence of males. Gonads of mature females often filled with round oocytes commonly hatching inside body. Vulva a transverse slit located halfway along body. Length of vagina variable, extending almost a third of vulval body diam. Intestine ending in a rectum 0.8-1.3 anal body diam. long with three cell bodies of associated sphincters. Anus an arcuate slit. Anal body diam. equivalent to 40% of tail length. Phasmids prominent, position variable, located at 47 (42-63)% of tail length. Tail conoid, wider part forming 47 (50-74)% of tail length, hyaline tail region short.

Male

No males found in four isolates.

SEQUENCE ANALYSIS

The 12-taxa D2-D3 dataset contained 530 positions, 187 of which were variable and 97 parsimony-informative. D2-D3 sequences of all four US isolates were identical to that of the UK isolate (Table 2) which has 5.23%, 4.83%, and 5.8% sequence divergence from undescribed *Phasmarhabditis* sp. 1, *Phasmarhabditis* sp. 2 and *Phasmarhabditis* sp. 3 (CGC 434), respectively. SSU and ITS sequences were also identical. Extremely low sequence divergence in the COI gene of the mitochondria was observed among US isolates as well as between US isolates and the two UK sequences (0.2-0.3%).

Discussion

The association of *P. hermaphrodita* with three invasive slug species (McDonnell *et al.*, 2009) is reported for the first time for the USA and North America (*Deroceras laeve* populations in California are thought to comprise both native and invasive specimens. Native populations of the species are likely found in remote areas, whereas invasive populations probably occur in more synanthropic situations (McDonnell *et al.*, 2009)). This species was reported from Chile in 1996 (France & Gerding, 2000) but it has not been previously found in North or Central America. Molecular tools provide an accurate, fast and efficient technique for species diagnostics and are relevant because of phenotypic plasticity within species and confounding morphological overlap between congeners. For instance,

Table 2. Pairwise distance showing mean (above) and total (below) character differences in D2-D3 sequences between *Phasmarhabditis* species and related taxa.

	1	2	3	4	5	6	7	8	9	10	11	12
Pellioditis marina	–	0.13900	0.14230	0.14230	0.15400	0.16016	0.15595	0.15370	0.15564	0.15820	0.16834	0.17248
AY602177 *Rhabditella axei*	72	–	0.19417	0.19417	0.19806	0.20468	0.20000	0.20349	0.19380	0.19844	0.19246	0.20385
Phasmarhabditis hermaphrodita, US isolate ITD272	73	100	–	0.00000	0.02708	0.05058	0.05233	0.04836	0.05803	0.05814	0.07600	0.20971
Phasmarhabditis hermaphrodita, UK	73	100	0	–	0.02708	0.05058	0.05233	0.04836	0.05803	0.05814	0.07600	0.20971
GQ167725 *Angiostoma limacis*	79	102	14	14	–	0.05058	0.04457	0.04062	0.05416	0.05233	0.06600	0.21165
GQ167726 *Angiostoma dentifera*	82	105	26	26	26	–	0.06214	0.05825	0.06408	0.07198	0.10241	0.23002
Phasmarhabditis n. sp. 1 ITD236	80	103	27	27	23	32	–	0.00774	0.03675	0.05620	0.07800	0.20000
Phasmarhabditis n. sp. 2 ITD510	79	105	25	25	21	30	4	–	0.03282	0.05620	0.07984	0.20349
Phasmarhabditis EM434	80	100	30	30	28	33	19	17	–	0.04457	0.08383	0.21899
FJ949063 *Angiostoma milacis*	81	102	30	30	27	37	29	29	23	–	0.09018	0.22568
GQ167724 *Angiostoma glandicola*	84	97	38	38	33	51	39	40	42	45	–	0.21756
EU195974 *Cruznema tripartitum*	89	106	108	108	109	118	103	105	113	116	109	–

P. hermaphrodita females were found to be morphologically identical to *P. neopapillosa* (Hooper *et al.*, 1999) and *P. tawfiki* (Azzam, 2003). Like other known parasites, *P. hermaphrodita* measurements are also affected by the environment where the nematodes are collected or grown, *e.g.*, those from the host will likely be bigger than those cultured on plain agar with introduced or associated bacteria (Hooper *et al.*, 1999). The presence or absence of the male and papillar arrangement on the male bursa may be useful as diagnostic characters with gonochoristic species (Andrássy, 1983) but are a likely source of taxonomic confusion or misdiagnosis with hermaphrodites, especially as more species are described or found (Azzam, 2003; Ross, *et al.*, 2012). For example, variations exist in the pattern of caudal papillae in certain strains of *C. briggsae* that match the *C. elegans* pattern instead, thus making species distinction even more difficult (Baird, 2001). Reports of known and new species will therefore require a combination of morphology/morphometrics and molecular data analysis.

Morphological and morphometric studies by light microscopy and SEM confirmed the identity of our *P. hermaphrodita* isolate and measurements (Table 1) are within the range of those previously reported (Hooper *et al.*, 1999). It was further observed that nerve ring position was more anterior in *P. hermaphrodita* with a value of 140 (126-149) μm for the USA isolate and 141 (131-154) μm for the UK isolate *vs* 188 (168-205) μm for *P. neopapillosa*. The tail was also shorter with a length of 99 (85-117) μm for the US isolate and 104 (82-113) μm for the UK isolate *vs* 157 (141-174) μm for *P. neopapillosa*.

Molecular sequence data for complete internal transcribed spacer (ITS-1, 5.8S, ITS-2), D2-D3 expansion segment of the LSU and nearly complete SSU ribosomal DNA, as well as the COI gene of the mitochondria further corroborated the identity of our isolates based on morphology and morphometrics. The sequence variations observed in the COI gene of the mitochondrial DNA among isolates from the US, and between those from the UK and US, were extremely low and may be attributed to nucleotide incorporation error during early phases of PCR. Additional sampling and mitochondrial genome sequencing will shed light on the genetics of *Phasmarhabditis*.

Recovery of this nematode was limited to the gastropod species *D. reticulatum*, *D. laeve* and *L. valentiana* collected from December 2012-January 2013 from three nurseries in Northern California, the cooler parts of the state. None has been recovered so far from nurseries in the warmer inland region of southern California. Nurseries were targeted not only because of their relevance to the funding source of our research (California Department of Food and Agriculture), but also because they are hubs for trade and movement of planting materials from international and domestic sources. It is likely that non-native slugs were brought into the country along with some of their natural enemies from overseas, including *P. hermaphrodita*. Preliminary testing of Koch's postulate on *D. reticulatum* and *D. laeve* resulted in host death and reisolation of the nematode. Experiments are underway to elucidate the nature of associations between bacteria and *Phasmarhabditis* spp. recovered in California. Further tests will be performed to determine the specific roles of nematodes and bacteria as possible causal agents of the mortality of invasive slugs.

The occurrence of *P. hermaphrodita* in North America has regulatory implications for potential biological control strategies against non-native slug species that are pests in horticultural and agricultural systems on this continent. A number of arthropod natural enemies have previously been identified as sources of mortality for gastropods in North America (*e.g.*, McDonnell *et al.*, 2007a, b). However, unlike Europe, there are no commercial biological control products currently available for slug and snail management in North America. Identification of *P. hermaphrodita* in California, therefore, opens the opportunity for a new tool in effective biological control.

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Queries to the Authors:

Please check if "(5-GTAGCAGCAGTAAAATAAGCACG-3′)" should be changed to "(5′-GTAGCAGCAGTAAAATAAGCACG-3′)" (page 2, line 81).

There is no explanation for superscript 4 (Table 1, page 5, line 45). Please check.