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Supplemental description of *Paraphelenchus acontioides* (Tylenchida: Aphelenchidae, Paraphelenchinae), with ribosomal DNA trees and a morphometric compendium of female *Paraphelenchus*

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Summary – Nematodes were isolated from surface-sterilised stems of cheatgrass, *Bromus tectorum* (Poaceae), in Colorado, grown on *Fusarium* (Hypocreaceae) fungus culture, and identified as *Paraphelenchus acontioides*. Morphometrics and micrographic morphology of this species are given to supplement the original description and expand the comparative species diagnosis. A tabular morphometric compendium of the females of the 23 species of *Paraphelenchus* is provided as the last diagnostic compilation was in 1984. Variations in the oviduct within the genus are reviewed to evaluate the taxonomic assignment of *P. deckeri*, a morphologically transitional species between *Aphelenchus* and *Paraphelenchus*. Sequences were generated for both 18S and 28S ribosomal DNA, representing the first identified species within *Paraphelenchus* so characterised. These sequences were incorporated into phylogenetic trees with related species of Aphelenchidae and Tylenchidae. *Aphelenchus avenae* isolates formed a well supported monophyletic sister group to *Paraphelenchus*. The ecology of *Paraphelenchus*, cheat grass and *Fusarium* is also discussed.

Keywords – fungivorous nematode, invasive species, key, morphology, molecular, phylogeny, taxonomy.

Nematodes of the genus *Paraphelenchus* Micoletzky, 1922 are generally regarded as fungivorous (Hunt, 1993), and are frequently found in association with plants. Twenty-three species are known, including *Paraphelenchus acontioides* Taylor & Pillai, 1967; *P. alii* (Ali, Farooqui & Suryawanshi, 1970) Fortuner, 1985 (= *P. afsi* Hunt, 1993) and *P. micoletzkyi* Ali, Farooqui & Suryawanshi, 1970 (= junior homonym) *nec P. micoletzkyi* Steiner, 1941 (= junior synonym of *Aphelenchus avenae*); *P. amblyurus* Steiner, 1934; *P. basili* Das, 1960; *P. batavicus* Filipjev, 1934; *P. crenatus* Das & Singh, 1968; *P. deckeri* (Zeidan & Geraert, 1989) Andrássy, 2007; *P. fidicaudatus* Eroshenko, 1966; *P. goodeyi* Tandon & Singh, 1970; *P. heterolineatus* Haque, 1967; *P. intermedius* Thorne & Malek, 1968; *P. myceliophthorus* J.B. Goodey, 1958; *P. obscurus* Muchina, 1988; *P. octolineatus* Shavrov, 1968; *P. orientalis* Muchina, 1988; *P. paramonovi* Haque 1967; *P. porrectus* Eroshenko, 1966; *P. pseudoparietinus* Micoletzky, 1922; *P. sacchari* Husain & Khan, 1967; *P. tritici* Baranovskaya, 1958; *P. ussuriensis* Eroshenko, 1966; *P. zeae* Romaniko, 1968; and *P. zicsii* Andrássy, 1989.

Many of the morphometric values of the species overlap, so qualitative morphology is particularly important for diagnosis. The most important of these include the presence and shape of the mucro on the female tail, the number of lines in the lateral field (typically 4-9), and continuous or indented lip region. Unfortunately, molecular data appear to be confined to an unknown species for which the small subunit rDNA has been derived (Holterman *et al.*, 2009).

Many of the species have been described from Asia, part of their proposed land origin from eastern Gondwana in the Devonian epoch (Ryss, 2007), including descriptions of life stages and female tail papillae of *P. myceliophthorus* (Ryss & Chernetskaya, 2010). Seven species have been found in Europe (Andrássy, 2007). Within the USA, numerous specimens of *P. pseudopari-*

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etinus and *P. intermedius* have been deposited into the United States Department of Agriculture Nematode Collection (USDANC) database. One notable species is *P. acontioides* which was discovered in soil around Kentucky bluegrass (*Agrostis palustris* Huds.) in Urbana, Illinois. The original population of this nematode species was initially raised for at least 5 years on the fungus *Pyrenochaeta terrestris* (Hansen) Gorenz, J.C. Walker & Larson, before actually being described (Taylor & Pillai, 1967). Subsequently, morphometrics for this nematode were taken for material cultured on the same fungus and seven others, resulting in expanded ranges (Pillai & Taylor, 1967a), especially for the b ratio.

This paper reports the discovery of a *Paraphelenchus* population in stems of cheatgrass, *Bromus tectorum* L., in Colorado during a survey of endophytic fungi as possible biocontrol agents within eight mid-western and western United States and British Columbia, Canada. Because many other *Paraphelenchus* species descriptions are incomplete and not readily comparable, detailed morphometrics of the new population were compiled and contrasted with other isolates of *P. acontioides* and the other species of the genus to enhance the diagnostic information available.

Materials and methods

Nematode and fungus isolation

Sixty-three populations of cheatgrass, *B. tectorum*, were sampled throughout North America (Idaho, Washington, Nevada, Colorado, New Mexico, Iowa, Illinois and British Columbia). Populations were typically about five miles apart, but others were 1-20 or more miles apart. Twenty plants from each population were collected. A 2 cm segment was clipped around the lowest culm node (solid region on the shoot central axis that may generate a leaf sheath or adventitious bud) of each plant. Segments were surface-sterilised in 50% ethanol for 5 min and rinsed in sterile deionised water for 1 min (Luginbuhl & Muller, 1980; Schulz *et al.*, 1993). To facilitate isolation of fungi, culm segments from each population were placed onto potato dextrose agar plates. Imprint plates were made to ensure surface sterilisation was successful. Fungi were identified morphologically. Cultures were placed on laboratory benches at ambient room temperature and subcultured as needed. Two grass populations had nematodes and endophytic *Fusarium* sp., and nematodes and fungi were not found independently of each other. The cheatgrass population with *P. acontioides* was isolated from near Piney River, CO, USA (39°50′24.99″N, 106°38′26.85″W), south of Yampa, CO, USA, off Highway 131.

Nematode preservation and imaging

Nematodes were rinsed from fungal plates, placed in 4% formalin for 24 h, rinsed in 1.5 ml plastic tubes with water and sent to Beltsville, MD, USA. From here they were placed in 4% formalin for another 24 h, dehydrated in alcohol and glycerin, mounted in glycerin, and sealed. Images and measurements were made with a Zeiss Ultraphot II microscope (Carl Zeiss, Jena, Germany, and Baltimore Instrument Company, Baltimore, MD, USA) equipped with differential interference contrast (DIC) optics. Photographs were taken of formalin-fixed specimens rinsed in water. Females were measured with an ocular micrometer. Fixed specimens used to evaluate relationships were observed from the USDANC. These included type and paratype slides of *P. intermedius* from the Thorne collection (USDANC Entries 31046, 29744, 28745, 31049 slides 1a, 1b (type, five females), 1c, 1i, for different South Dakota sites of G. Thorne 28744), and *P. pseudoparietinus* Micoletzky, 1922 (USDANC Entry 21084, slide G11751 from soil of Chewings fescue grass, *Festuca rubra commutate*, W.B. Courtney, Astoria, Oregon, 1941) identified as *P. intermedius* here based on six lateral incisures and morphometrics, and four female paratypes of *P. deckeri* (slide T-4299p from shrubs of Abuttaraz, Sudan). Type material from the old Nematode Slide Collection of the Department of Plant Pathology (now Crop Sciences), University of Illinois, Urbana, IL, USA, and *P. amblyurus* from the Steiner collection of the USDANC, were unavailable. Reproductive system terminology of Triantaphyllou and Fisher (1976) as used for *A. avenae* was applied to a single gonad arm going in an anterior direction: vulva, vagina, gonoduct (uterus, spermatheca, fertilisation chamber (eight columns of two rows of tightly packed oval cells not contained in a membrane), sphincter – (three rows of globular cells not contained in a membrane)), and ovary. The oviduct comprises a fertilisation chamber and sphincter cells (Triantaphyllou & Fisher, 1976), but the term has also been used to describe the sphincter cells only (Geraert, 1981).

PCR and sequencing

Nematodes were rinsed from fungal plates, placed in 70% alcohol in 1.5 ml plastic tubes and sent to Beltsville,

MD, USA. They were rinsed in water, mounted on slides for imaging, recovered and individually processed. Nematodes were mechanically disrupted in 20 μl of extraction buffer as described by Thomas *et al.* (1997) and then stored in PCR tubes at −80°C until needed. Extracts were prepared by incubating the tubes at 60°C for 60 min, followed by 95°C for 15 min to deactivate the proteinase K and centrifuged briefly prior to use in PCR. Each 25 μl PCR reaction contained 1 unit Platinum *Taq* (Invitrogen, Carlsbad, CA, USA), 1× reaction buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl, 2.5 mM $MgCl_2$), 0.2 mM dNTP mix, 0.3 μM of each primer, and 2 μl nematode extract. 28S reaction contained primers D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) (Nunn, 1992). Cycling was performed as described by De Ley *et al.* (2005) and Ye *et al.* (2007). The partial 18S sequence was amplified in two overlapping segments, using primers 550F (5′-GGCAAGTCTGGTGCCAGCAG CC-3′) with 1108R (5′-CCACTCCTGGTGGTGCCCTT CC-3′) and 18s1.2 (5′-GGCGATCAGATACCGCCCTA GTT-3′) with 18sr2b (5′-TACAAAGGGCAGGGACGT AAT-3′). Cycling conditions for the 550F/1108R PCR reaction were: 1 cycle of 94°C for 2 min, followed by 40 cycles of 94°C for 20 s, 65°C for 30 s, and 72°C for 30 s, finishing with 1 cycle of 72°C for 5 min. For the 18s1.2/18sr2b primer pair cycling conditions were the same as above except the annealing temperature was 59°C. PCR products were analysed by electrophoresis on 1% agarose/1× SB (sodium borate-EDTA). Gels were stained with ethidium bromide and visualised using the U:Genius gel documentation system (Syngene, Frederick, MD, USA). DNA was excised from the gels and purified with the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA). PCR products were quantified using a Nanodrop 8000 spectrophotometer (Thermo Fisher Scientific, Pittsburgh, PA, USA) and sequenced directly at the University of Maryland Center for Biosystems Research. DNA sequences were assembled using Sequencher 4.10.1 (Genecodes, Ann Arbor, MI, USA). DNA sequences were analysed using the BLASTN megablast program optimised for highly similar sequences, http://www.ncbi.nlm.nih.gov/blast/Blast.cgi. Sequences were submitted to GenBank under accession numbers HQ218322 for 28S and HQ218323 for 18S.

Phylogenetic methods

Nematode sequences for *P. acontioides* were combined with other GenBank sequences of Tylenchidae and Aphelenchidae, namely 18S: *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 AY593906, *Tylenchus arcuatus* Siddiqi, 1963 EU306349, *A. avenae* EU306347, *A. avenae* 1 AY284639, *A. avenae* 2 AY284640, *A. avenae* AB368918, *A. avenae* AF036586, *Aphelenchus* sp. AY284641, *Paraphelenchus* sp. (JH-2004 isolate) AY284642, and new *P. acontioides* HQ218323; and 28S: *Coslenchus costatus* (de Man, 1921) Siddiqi, 1978 DQ328719, *Ditylenchus halictus* Giblin-Davis, Erteld, Kanzaki, Ye & Center, 2010 AY589364, *D. destructor* Thorne, 1945 DQ328727, *A. avenae* AB368536, *A. avenae* EU325683, *Aphelenchus* sp. DQ145664, and new *P. acontioides* HQ218323.

Alignments were made with ClustalW2 (Larkin *et al.*, 2007) and checked by eye for consistency of conserved positions. The alignment was run through PAUP* 4b10 (Swofford, 2002). Maximum Parsimony (MP) bootstrapped searches with 1000 replicates were conducted employing tree bisection-reconnection (TBR) branch swapping, and accelerated transformation (ACCTRAN) character state optimisation. PAUP* was also used for generating sequence and tree statistics. Geneious Pro v. 5.0.3 (Biomatters, Auckland, New Zealand; Drummond *et al.*, 2009) was used to examine apomorphic characters of *Paraphelenchus*. Maximum likelihood (ML) trees are presented in the figures because the computationally-intensive, probabilistic ML method is less affected by sampling error and infers better trees than distance or parsimony methods (Swofford *et al.*, 1996). Alignments in PHYLIP format were run in web-based RAxML (Stamatakis *et al.*, 2008) with 100 bootstrap runs and ML estimate of 25 per site rate categories. Branch support values above 50% given for ML followed by those for MP, and ML parameters given in figure legends.

Description

Paraphelenchus acontioides Taylor & Pillai, 1967
(Figs 1, 2A)

Measurements

See Table 1.

Description

Body spiral to crook-shaped after death. Non-indented lip region *ca* 15% stylet length high, 3.5 μm high × 9 μm wide. Stylet without swellings at base, although

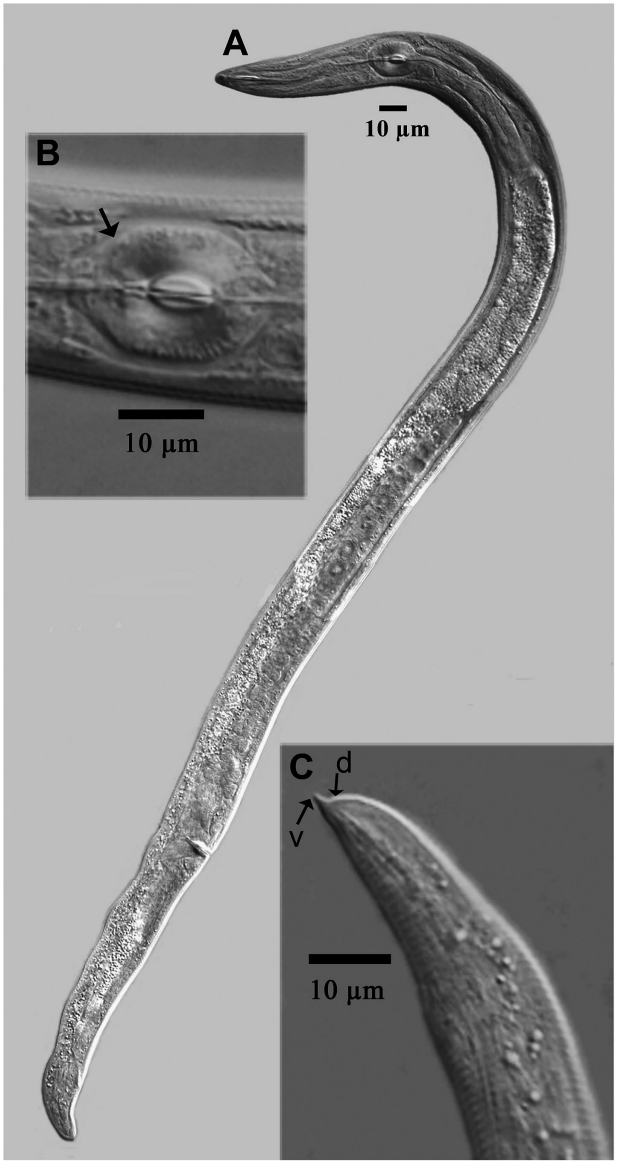

Fig. 1. Paraphelenchus acontioides *Colorado population female. A: Body; B: Median bulb with arrow pointing at constriction; C: Tail with mucro/ventral tuberculus (v arrow) and obscure dorsal tuberculus (d arrow).*

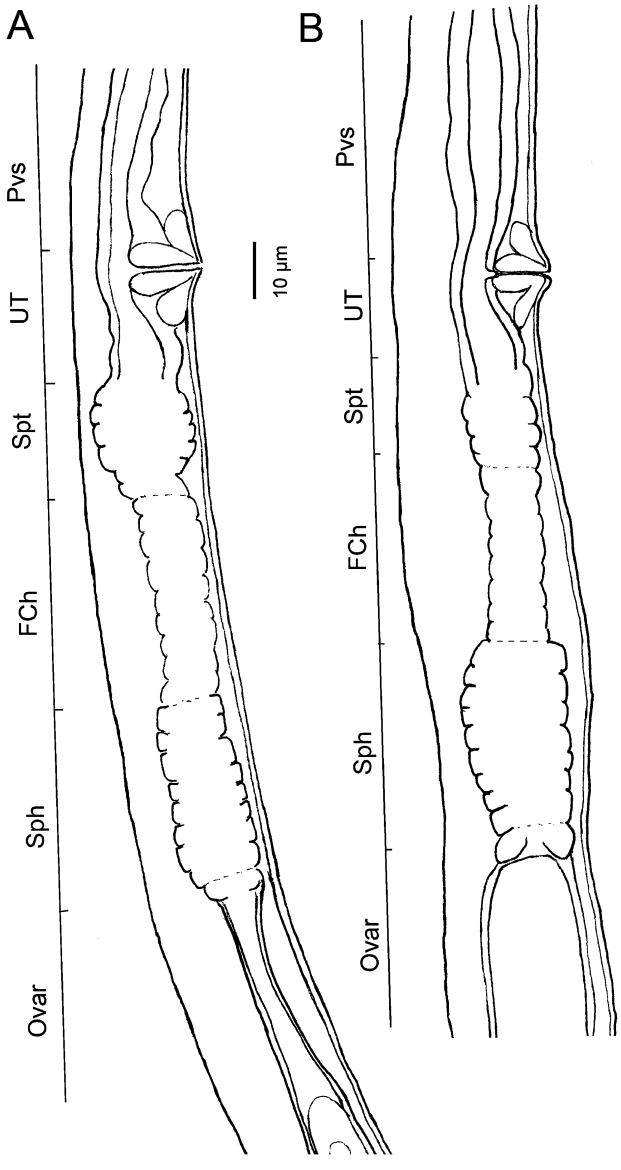

Fig. 2. Paraphelenchus *spp. gonoducts. A:* P. acontioides *female post-vulval uterine sac (Pvs), uterus (UT), spermatheca (Spt), fertilisation chamber (Fch), sphincter (Sph) and ovary (Ovar); B:* P. intermedius *female post-vulval uterine sac, uterus, spermatheca, fertilisation chamber, oviduct and ovary with developing oocyte, from USDANC slide G11751 of G. Thorne, Astoria, OR, USA, 1941.*

basal lumen sometimes slightly inflated, surrounded by prominent muscles. Corpus length *ca* 2.7 times isthmus length, slightly longer than isthmus, pharyngeal glands abutting intestine. Median bulb with constriction anteriorly and high, collar-like sheath surrounding anterior muscle. Nerve ring just posterior to median bulb. Excretory pore opening posterior to nerve ring. Anterior gonad length highly variable, not reaching pharyngo-intestinal junction. Post-vulval uterine sac (Pvs) length variable, sometimes reaching nearly half the vulval-anal distance (VAD). VAD/tail = 4-4.5. Lateral incisures beginning at level of median bulb, increasing to eight at mid-body, ending at mid-tail. Rectum slightly shorter than tail length. Small pair of ventro-lateral papillae discernible between

Table 1. *Morphometrics of* Paraphelenchus acontioides *and* P. intermedius *females. All measurements are in* μm *and in the form: mean* $\pm$ *s.d. (range).*

Character	*P. acontioides* Piney River, CO, n = 20	*P. intermedius* South Dakota, G. Thorne, n = 6 Astoria, OR, W. Courtney, n = 4 Combined populations, n = 10
L	842 ± 118 (540-1059)	937 ± 84 (835-1110)
a	29.4 ± 5 (22.6-37.4)	27.8 ± 2.8 (21.7-32)
b	4.8 ± 0.6 (3.3-5.8)	5.3 ± 0.4 (4.5-5.9)
c	23.1 ± 2.2 (16.1-27.7)	25.3 ± 1.7 (22.6-27.9)
c′	2.1 ± 0.3 (1.7-2.6)	2.3 ± 0.4 (1.6-2.7)
V	75 ± 2 (69-81)	76 ± 1 (73-78)
Max. body diam.	29 ± 6 (23-43)	34 ± 4 (28-40)
Pharynx length	176 ± 9 (153-199)	177 ± 17 (153-216)
Tail length	37 ± 5 (28-45)	37 ± 3 (31-44)
Anal body diam.	17 ± 1 (15-20)	17 ± 3 (15-20)
Stylet length	17 ± 2 (13-20)	17 ± 1 (16-19)
Vulva-anal distance (VAD)	164 ± 29.4 (119-224)	185 ± 17.5 (159-214)
Anterior gonad	278 ± 32 (185-321)	456 ± 72 (364-571)
Post-vulval uterine sac (Pvs)	55 ± 10 (23-65)	58 ± 11 (37-74)
Pvs/VAD%	35 ± 7 (17-55)	35 ± 4 (29-39)
Pvs/vulval body diam.	2 ± 0.4 (0.9-2.5)	2.5 ± 1.1 (1.1-4.3)
Rectum length	27 ± 3 (22-33)	34 ± 3 (31-37)
Lateral incisures	8	6

ca 60-70% of tail length. Dorsal paired papillae located just anterior to ventral base of asymmetrical, ventrally directed tail mucro, also known as a ventral tuberculus (d, v in Fig. 1C). In mature gonoducts, 8-10 globular columns of two cells, squared at their edges, visible on oviduct sphincter, and eight columns of two, thinner-walled cells comprising the fertilisation chamber. Uterus and spermatheca about equal in length to fertilisation chamber and to oviduct sphincter length. Egg (n = 30) dimensions = 58.9 ± 5.8 (51-77.5) × 32.4 ± 6.6 (24-52.5) μm. Smallest egg 51 × 30 μm, largest 77.5 × 52.5 μm in size.

Host and locality

Isolated from the lowest node of *B. tectorum* (cheat grass) from Piney River, CO, USA (39°50′24.99″N 106°38′26.85″W), *ca* 30 miles south of Yampa, CO, USA, and *ca* 1.5 miles south of County Road 1 on Highway 131.

Bionomics

This Colorado population of *P. acontioides* was located *ca* 150 miles northwest of Colorado Springs, which was where *P. pseudoparietinus* and *P. intermedius* were collected from native prairie (USDANC entries 28758 and 31067, deposited by Gerald Thorne, 1966). *Paraphelenchus intermedius* could grow and reproduce at 30°C in fungal culture (Thorne & Malek, 1968).

Phylogeny

18S sequences

For 18S sequences a ClustalW alignment of 1195 alignment positions was used for a Maximum Parsimony tree (TL = 175, CI = 0.914, and HI = 0.086) of which 5.6% of positions were parsimony informative. It was also used for a Maximum Likelihood tree bootstrapped 100 times using RAxML with GTR matrix. Gamma model parameters were estimated by the RAxML program and there were 115 alignment patterns. Slightly different MP bootstrap values are indicated also on the ML tree (Fig. 3). This tree shows *Paraphelenchus* basal to the clade of fairly cohesive *Aphelenchus* populations that form a monophyletic group with high bootstrap support. The distance matrix 'p' value between *Aphelenchus* and *Paraphelenchus* (0.042), also visualised with relative branch lengths, was greater than the value between the most divergent *Aphelenchus* populations (0.011).

A Geneious nucleotide alignment of 1198 total alignment positions for all included taxa, including the *P. acon-*

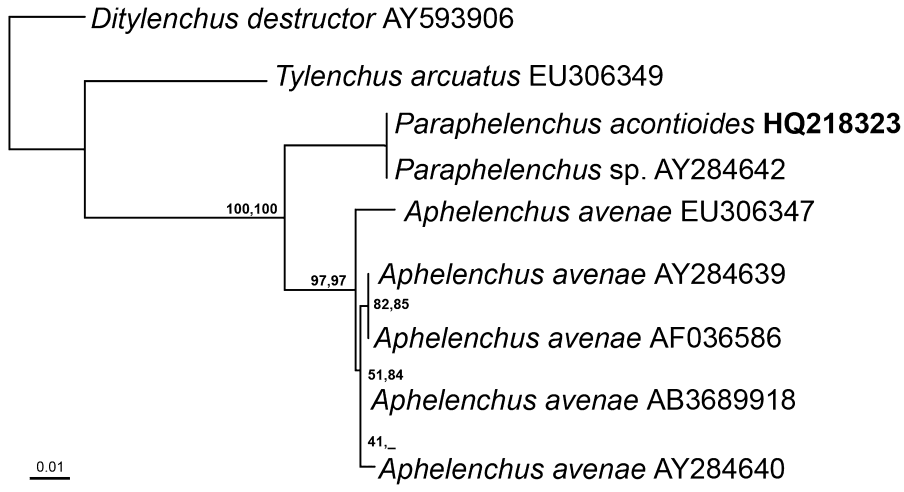

Fig. 3. *18S rDNA Maximum Likelihood Tree of Aphelenchidae implemented in RAxML. Proportion of gaps and completely undetermined characters in alignment: 0.038808, Model parameter alpha: 0.020014, Tree Length: 0.188690, Final ML Optimisation Likelihood: −2582.824816. Bootstrap values are listed for 100 ML replicates, and 1000 MP replicates.*

tioides 1061 bp sequence within a 1075 bp segment of the total alignment (available on request), gave the same tree topology using MrBayes as the Clustal alignment. There were 18 *Paraphelenchus*-specific apomorphic nucleotides in this alignment (position# – bp change), most of which occurred between positions 98 and 176: 98 – A *vs* C; 105 – T *vs* G; 106 – C *vs* T; 124 – T *vs* C, A; 159 – A *vs* T, C; 166 and 175 – C *vs* T; 176 – A *vs* G; 286 – A *vs* C, T; 490 – T *vs* C; 501 – A *vs* T; 510 – A *vs* G; 793 – T *vs* G; 934 – G insertion; differences for *Paraphelenchus* sp. only, after the *P. acontioides* sequence ran out, included: 1109 – A *vs* T; 1150 – A *vs* G; 1161 – A *vs* G, T; 1182 – T *vs* A. There was only one minor A *vs* R (= A or G) difference at position 861 for *P. acontioides* compared to *Paraphelenchus* sp.

Outgroup *Tylenchus* and *Ditylenchus* 18S sequences had intermediate GC sequence content values of 49.2 and 49.3%, respectively. Sequences for *Aphelenchus avenae* isolates had 49.6-50.0% GC base content, whilst the more divergent *P. acontioides* and *Paraphelenchus* sp. had 48.1-48.5% GC.

28S sequences

For 28S sequences a ClustalW alignment (available on request) of 745 alignment positions was used for a Maximum Parsimony tree (TL = 626, CI = 0.821, HI = 0.179) of which 32% of characters were parsimony informative. It was also used for a ML tree bootstrapped 100 times using RAxML with GTR matrix. Gamma model parameters were estimated by the RAxML program, and there were 300 alignment patterns on the ML tree (Fig. 4). All MP bootstrap values were 100% and not indicated on the tree.

The GC content for *Coslenchus* 28S sequences was 51.3%, for *D. destructor* 54.4%, for *Ditylenchus* sp. 46.8%, for two *A. avenae* 50.1%, for *Aphelenchus* sp. 51.1%, and for *P. acontioides* 48.2%.

Diagnosis

See Tables 1, 2, Figure 5A, B. Morphometrics for this Colorado population of *P. acontioides* are similar to *P. amblyurus* and *P. myceliophthorus*, except for the lower b ratio and longer stylet. In a second paper by the authors of *P. acontioides*, the b ratio was considerably lower when nematodes were grown on several different fungi (Pillai & Taylor, 1967a). The post-vulval uterine sac length/vulval anal distance (Pvs/VAD) was 39-47 *vs* 50% for *P. amblyurus* and 66% for *P. myceliophagus.* The Pvs length is similar for *P. acontioides* (55 ± 10, 23-65) and *P. intermedius* (58 ± 11, 37-74 μm). The Pvs/vulval body diam. (Vbd) was also somewhat shorter in *P. acontioides* compared to *P. intermedius* (2 ± 0.4, 0.9-2.5 *vs* 2.5 ± 1.1, 1.1-4.3) (Table 1), and *P. pseudoparietinus* (Pvs/Vbd = 2-3, Andrássy, 2007). The *P. intermedius* oviduct had 8-12 cell columns, and the fertilisation chamber had eight columns of cells (Fig. 2B), whilst 8-12 cells were observed in the fertilisation chamber of *P. deckeri*. Although *P. acontioides* has eight lateral incisures, it is otherwise similar to species with only six lines, such as *P. inter-*

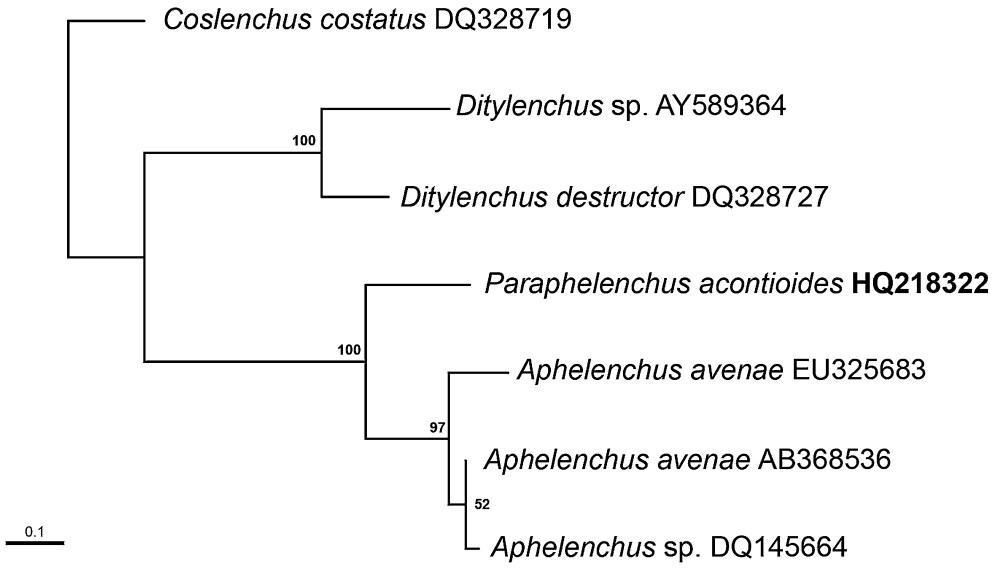

Fig. 4. *28S Maximum Likelihood Tree of Aphelenchidae implemented in RAxML. Proportion of gaps and completely undetermined characters in alignment: 0.288448, Model parameter alpha: 0.428791, Tree Length: 1.791170, Final ML Optimisation Likelihood: −3800.974955. Bootstrap values are listed for 100 ML replicates.*

medius and *P. amblyurus*, for which males are described. Unfortunately, the lateral field and other features were especially difficult to see in putative *P. pseudoparietinus* from old specimens in the USDANC. *Paraphelenchus amblyurus* was described with what appeared at the time to be a unique differentiation of the anterior median bulb (Steiner, 1934), similar to the bulb in the Colorado population of *P. acontioides.* This median bulb differentiation of less striated tissue was also visible in *P. intermedius* slides (Fig. 5A). The tail of *P. intermedius* was more attenuated toward the distal end (Fig. 5B) than in *P. acontioides* (Fig. 1C).

Discussion

Taxonomy

This population of *P. acontioides* extended the reported size range with a longer body and stylet, lower V and slightly lower b value. The b values from the first description were reduced substantially in a second paper describing basic morphometrics on various fungal cultures (Pillai & Taylor, 1967a). The b values of this Colorado population are more similar to those in the second paper *vs* the original. Therefore, relying primarily on morphometrics could lead an identifier astray, especially with species having few described populations or few replicates of a single population. As with any identification of a nematode outside the type locality, definitive assignment of this isolate to *P. acontioides* is a hypothesis. If molecular sequences from this population are shown to be somewhat different than specimens from the type locality in the future, this redescription could serve as a framework for the proposal of a possible new cryptic or complementary species.

Egg measurements extended the original range for length and diam. of *P. acontioides* eggs ((64-74) × (28-32) μm) (Taylor & Pillai, 1967) at either end of the range ((51-78) × (24-52) μm). *Paraphelenchus intermedius* had a similar sized egg (69×27 μm) to *P. acontioides*, whereas *P. myceliophthorus* had larger (78×32 μm) and *A. avenae* somewhat smaller ((60-88) × (20-30) μm) eggs.

Of the 23 *Paraphelenchus* species, only eight lack a mucro and nine lack males. Absence of males is sometimes a result of the small number of specimens available, but may also be related to temperature threshold. Among 39 populations of *A. avenae*, most were parthenogenetic and males were inducible in many populations only above 30°C (Ali *et al.*, 1999a). Both *A. avenae* and *P. acontioides* had minimum generation times of 5 and 6 days at 35°C (Pillai & Taylor, 1967b). Genetic diversity of *A. avenae* populations did not correlate with geography, fungal host, thermal preference or presence/absence of males (Ali *et al.*, 1999b). *Paraphelenchus acontioides* may be a complementary species (Osche, 1954) of *P. pseudopari-*

Table 2. Paraphelenchus *female morphometrics.*

Species	L	a	b	c	V	Stylet	>1 mucro	c′	Males	Li	Pvs/Vbd
Tail mucro											
P. acontioides I[a]	710-880	25-30	4.4-5.3	20-30	73-77	14-16	–	2.4	–	8	2
P. acontioides II[b]	510-960	15.9-35.6	3.5-6.2	14.5-30	71-80	–	–	–	+	–	–
P. acontioides CO	540-1059	23-37	3.4-5.8	16-33.7	69-78.3	13-23	–	1.7-2.4 (2.1)	+	8	1.7-2.3
P. alii	790-980	23.5-29.6	5.2-6.5	14.7-22.2	73-76	13.6-15.2	–	3.1	+	6	1.8
P. amblyurus	540-930	23-33	3.7-5.9	17-24	76	17-19	–	2.5	+	6	2.1
P. basili	550-590	23.4-23.9	5-5.3	15.6-18	70-74	16	–	3.2	+	4	2.1
P. crenatus	680-740	27.6-30.8	4.8-5.1	19.2-23.7	74.6-74.7	13.8-15	–	3.4	+	8	2.3
P. fidicaudatus	676-759	38-39	3.1-5.3	24.6-27.4	75-79.8	13	2	2.1	–	8	2.8
P. goodeyi	715-766	20	9-10	18-20	74	12	–	2.1	–	6	3.2
P. heterolineatus	615-803	19.6-21.6	4.0-4.8	17.1-29.2	75.1-77.9	15.1	–	2	–	8	1
P. intermedius	700-1000	24-28	4.3-5.6	23-26	76	15-19	–	2.4	–	6	1.1-4.3
P. myceliophthorus	580-820	22-34	4.1-6.6	13-24	71-78	6-19	–	1.4-2.7	+	6	3.2
P. paramonovi	587-937	18.9-26	4.4-5.5	18.1-25.2	75.6-77.8	12.8-13.9	–	1.5	+	6	2.7
P. porrectus	704-770	30.8-31.2	4.8	20.7-23.3	76.4-78.9	13	–	1.8	+	8	–
P. sacchari	590-880	30-39	5.1-6.6	20-21	68-77	11-16	–	2.9	+	4	3
P. pseudoparietinus	380-910	25-36 (30)	4.1-6.3 (5)	14-24 (20)	68-78 (74)	13-14	–	2.7	+	9	2-3
P. zicsii	970	36	5.7	14	72	14-15	2	4.3	+	4 + 2	3.2
No tail mucro											
P. batavicus	750	32-37	7-8	27	75	12-13	–	2.2	+	12	4.3
P. deckeri	695-855	37-39	5.2-6	22-25	75-78	14.5-17.5	–	2.3-3.2	–	10-12	2-3
P. obscurus	560-630	26-42	4.2-5.2	20-24	72-74	12-13	–	2	+	5	3.6
P. octolineatus	512-597	27.4-34.2	3.7-4.6	17.1-21.6	75-78.9	13-14	–	2.9	+	8	3.6
P. orientalis	666-712	25-37	5-6	29-37	73-77	12-13	–	1	–	8	3.5
P. tritici	442-851	26.8-32	3.4-5.9	16.7-21.2	77-78	12-13	–	2.0	+	–	2
P. ussuriensis	830-841	30.2-37.4	4.8-5.6	20.2-28	73.8-76.1	15	–	2.4	–	6	–
P. zeae	658-700	24.4	8.15	31.3	76.5	16-17	–	2.0	–	–	4.4

Tail mucro present in first 15 species. –/+, absence/presence of males; Li, lateral incisures; Pvs, post-vulval uterine sac; Vbd, vulval body diam. Measurements in the form: range and/or (mean).

[a] From Taylor & Pillai (1967).

[b] From Pillai & Taylor (1967a).

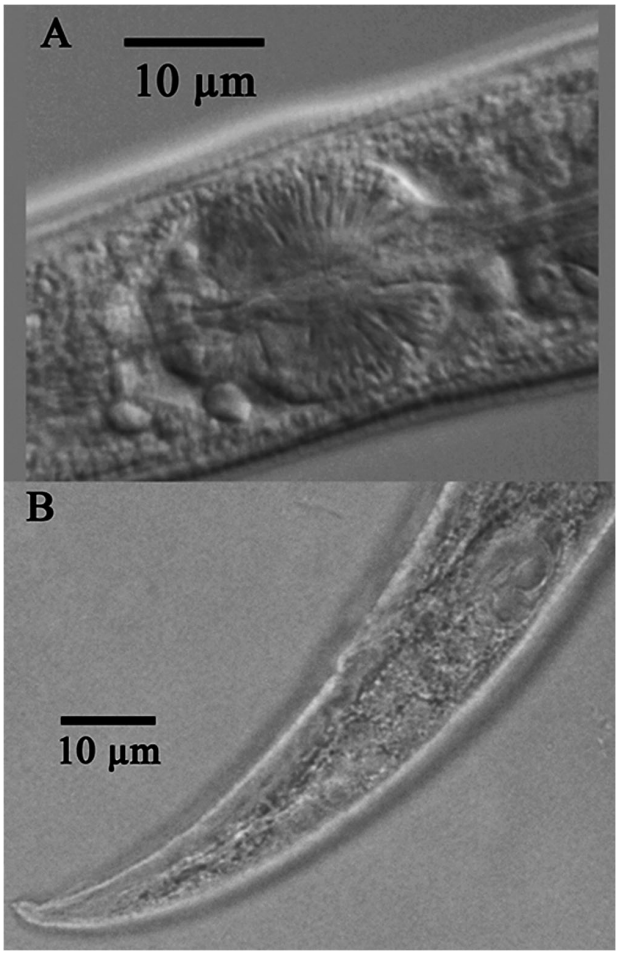

Fig. 5. Paraphelenchus intermedius. *A: Median bulb; B: Tail from USDANC slide G11751 of G. Thorne, Astoria, Oregon, 1941.*

etinus since *P. acontioides* lacks males, an indented lip region and a ninth lateral incisure. Otherwise, these species geographically overlap and have relatively unique plant stem habitats in grasslands.

The molecular sequence gap between *Paraphelenchus* and *Aphelenchus* seems appropriate for these genera within taxonomic subfamilies (Hunt, 2008) rather than the more distant families of a previously taxonomic framework (Hunt, 1993). Paraphelenchinae, and its only genus *Paraphelenchus*, is distinguished from similarly monotypic Aphelenchinae/*Aphelenchus* (Hunt, 2008) by a pharyngeal bulb *vs* pharyngeal gland overlapping the intestine, a vulval slit *vs* a pore, male tail with 4-5 pairs of papillae but no bursa *vs* a bursa containing four papillae, and lateral field with 4-9 lines *vs* lateral field with more than six and typically 10-12 incisures (Hunt, 1993).

The original description of *A. deckeri* Zeidan & Geraert, 1991 mentioned transitional characters between the two families, including a pharyngeal bulb and vulval slit as in *Paraphelenchus*, but 10-12 incisures in the lateral field and an oviduct sphincter (5-9 cell columns) slightly modified from that seen in a *Paraphelenchus* sp. (6-10 columns, although nine was most common) (Geraert, 1981). This pattern was different from that drawn for *A. avenae* in another study showing eight fertilisation chamber cell columns + four globular columns of oviduct cells (Triantaphyllou & Fisher, 1976). There was uncertainty about its generic identity in the description based on this obscure and untested character since the oviduct was rarely and inconsistently characterised in *Paraphelenchus* spp. and the diagnosis was uncharacteristically made with other *Paraphelenchus* species rather than with *Aphelenchus* species (Zeidan & Geraert, 1991). Hodda considered this species a *Paraphelenchus* by generic definition (www.ento.csiro.au/science/nematodes/checklist, 2003), a notion formalised by Andrássy (2007) and noted in a recent checklist of the group (Hunt, 2008). One suggestion for resolution was to find or generate males (Hunt, 1993). Another alternative would be to update the range of variation of the oviduct in *Paraphelenchus.* Considerable variation has been reported or illustrated in the literature (Table 3) and many drawings lack clear regional limits for cell types. The eight columns of fertilisation chamber cells plus four columns of globular oviduct sphincter cells posterior to the ovary in a population of *A. avenae* (Triantaphyllou & Fisher, 1976) differed from the 5-9 oviduct sphincter cell columns reported in another population of *A. avenae* (Geraert, 1981). Unfortunately, it was not possible to count the number of cells in the obscure fertilisation chamber of a *Paraphelenchus* sp. in that comparative study (Geraert, 1981). Most species of *Paraphelenchus* have six or more oviduct cell columns (Table 3), so the 12 oviduct sphincter cells of *P. deckeri* were considerably more numerous than those of either *Paraphelenchus* or any *A. avenae* population. Most drawings and descriptions are not definitively clear about the number of cells in the fertilisation chamber especially, so the values in the table should be considered estimates. The relative length of the uterus/spermatheca, fertilisation chamber and oviduct sphincter cell columns may be somewhat more useful when characterising species. In *P. acontioides*, these were equivalent in length in three specimens, but in *P. intermedius*, the uterus and spermatheca extended to about half the total length of the three regions in two of three specimens, whilst the fertilisation chamber and oviduct were

Table 3. Paraphelenchus *spp. and* Aphelenchus avenae *female reproductive system cells interpreted from published drawings or observations.*

Species	Fertilisation chamber cell number	Oviduct sphincter cell number	Reference
P. deckeri	7-8	10-12	Zeidan & Geraert (1991)
	8-12	12	This study
P. batavicus	10	10	Filipjev (1934)
P. acontioides	8	8-10	This study
P. intermedius	8	8-10	This study
P. orientalis	8[a]	8	Muchina (1988)
P. crenatus	–	8	Das & Singh (1968)
P. myceliophthorus	–	6-8	Ryss & Chernetskaya (2010); Goodey (1958)
P. alii (= *P. micoletzkyi*)	8	6-8	Ali *et al*. (1970)
	8-16[b]	8	Zeidan & Geraert (1991)
P. tritici	8	5-6	Baranovskaya (1958)
P. obscurus	20	4	Muchina (1988)
P. zeae	–	4	Romaniko (1968)
P. octolineatus	8-10	2	Shavrov (1968)
A. avenae	8	4	Triantaphyllou & Fisher (1976)
	5-7	5-9	Geraert (1981)

[a] Ambiguous cell boundaries; the number represents two small, rounded transitional cells and six hatched, cross-sectioned cells.
[b] *Paraphelenchus micoletzkyi* was described with "oviduct composed of fertilisation chamber only with 16-18 packed glandular cells", without a sphincter. Instead, we interpreted the drawing to include a clearly defined sphincter of eight cells and a fertilisation chamber that probably had only eight cells. The discrepancy is based on an ambiguous spermathecal boundary.

about equal in length. Both species had the same number of cells in the fertilisation chamber and oviduct (Table 3). Between specimens of the same species, the length of the fertilisation chamber was somewhat variable, although the relatively narrow diameter cells could curve obliquely to the body axis and bunch together. Also the oviduct/sphincter region had atypical cells with a transitional appearance at either end. More complete descriptions and variation within species may yet show the utility of these differences. It will be especially interesting to determine where *P. deckeri* resides within molecular phylogenetic trees.

Ecology

Paraphelenchus pseudoparietinus was found in swellings on the stem base of cocklebur (*Xanthium* sp. and *X. americanum* Walter = *X. strumarium* L. var. *glabratum* (DC.) Cronquist) in Fayetteville, AR, USA and Alexandria, VA, USA. While this Colorado population was not associated with stem swelling, it represents one of only a few reports of related nematodes within plant tissue. *Aphelenchus avenae* was originally isolated by Bastian from oat leaf sheaths (Hunt, 1993) and was observed feeding on plant roots (Decker, 1962).

Fusarium solani (Mart.) Sacc. and *F. moniliforme* were excellent hosts of *P. acontioides*, although *F. moniliforme* was preferred to *F. solani*, even though the population was at least four times greater on *F. solani* after two generations (Pillai & Taylor, 1967a). Recently it was demonstrated that *F. mangiferae* Britz, Wingfield & Marasas spores were carried by the eriophyoid mite, *Aceria mangiferae* Sayed, in the epidemiology of mango malformation disease (Gamliel-Atinsky *et al.*, 2010). Adding to speculations on evolutionary trends in aphelench-host associations (Ryss, 2007), it is conceivable that *Paraphelenchus* could also vector pathogenic *Fusarium* to crop plants as well. The only demonstrated nematode vector of a fungal pathogen is that of the seed-gall nematode *Anguina tritici*, which was reported to vector spores of the fungus *Dilophosphora alopecuri* to cereals (Atanasoff, 1925). It is more common for migratory endoparasitic nematodes within Pratylenchidae, Anguinidae and Aphelenchoididae to vector bacteria (Moens & Perry, 2009), such as *Anguina tritici* carrying *Rathayibacter* (= *Clavibacter*) *toxicus* (Riley & Ophel) Sasaki, Chi-

jimatsu & Suzuki to wheat (Riley, 1992), and other nematode-bacteria combinations (Dorofeeva *et al*., 2002). However, most nematode-microbial associations involve wounding or host predisposition rather than a strict vector relationship (Powell, 1963).

Cheatgrass is an invasive species that is especially common in western crops such as winter wheat and alfalfa (Young, 2000) and, like many weeds, may be capable of harbouring various plant diseases. Alternatively, like *A. avenae* (Rhoades & Linford, 1959), *Paraphelenchus* may be an opportunist fungivore scavenging fungi under a variety of conditions.

We look forward to future research involving more detailed SEM images of various *Paraphelenchus* species so as to reveal clearer profiles and variability of the lateral field in various species, and of the presence of other female tail papillae homologous to those in males as reported in *P. myceliophthorus* (Ryss & Chernetskaya, 2010). More molecular sequences and descriptions of the oviduct in more species will undoubtedly improve the systematic knowledge of this group.

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