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Pelodera termitis sp. n. and two other rhabditid nematode species associated with the Turkestan termite *Anacanthotermes turkestanicus* from Uzbekistan

L. K. Carta*, Z. A. Handoo*, N. I. Lebedeva, A. K. Raina***, T. I. Zhuginisov** and A. Sh. Khamraev****

***USDA-ARS, Nematology Laboratory, 10300 Baltimore Avenue, Beltsville, MD 20705, USA**
****Institute of Zoology of Uzbek Academy of Sciences, Tashkent, Uzbekistan**
*****USDA-ARS Formosan Subterranean Termite Research Unit, 1100 Robert E. Lee Blvd., New Orleans, LA 70124, USA**
E-mail: lynn.carta@ars.usda.gov

Abstract. Biological, ecological and faunistic studies were conducted on three nematodes, one of which was associated with significant mortality in nature of the Turkestan termite, *Anacanthotermes turkestanicus* in Uzbekistan. *Pelodera termitis* sp. n. was discovered from the Nurabad district, Samarkand province. It is morphologically similar to members of the *Pelodera strongyloides* species group, differing from these species by presence of a prerectum, male 'a' ratio (17.4-21.0), and female tail shape and 'c' ratio (15.7-31.6). *Panagrolaimus* sp. cf. *spondyli* was the second nematode found in these termites from the Khiva region of Khorezm Province. *Acrobeloides* sp. cf. *amurensis* was also found from the Nurabad district. It was identified through morphometrics and the D3 region of the large subunit (28S) rDNA. Its sequence was identical to that of a hermaphroditic culture of *Acrobeloides* sp. cf. *amurensis* Truskova, 1971 (PS1146), originally from Blythe, California, but with a longer body (608-710 µm) and shorter 'V' ratio (59-64%).

Keywords. *Acrobeloides*, *Anacanthotermes turkestanicus*, description, morphology, nematode, new species, *Panagrolaimus*, pathogen, *Pelodera termitis*, phoresy, termite, Uzbekistan.

INTRODUCTION

Termites of the genus *Anacanthotermes* found in Central Asian states are important pests of wooden structures (Luppova, 1958), and dead and even live trees and shrubs (Luppova and Luppov, 1968). No effective way of eliminating these termites within their range has been developed yet. Chemical treatments are not always satisfactory, and to reduce the negative impact of chemicals on the environment it is important to discover and develop biological methods for termite control. A variety of parasitic and phoretic nematodes have been found in termites (Carta and Osbrink, 2005; Gouge, 2006; Kanzaki et al., 2009). Discovery of other new termite-associated nematodes is valuable since commercial entomopathogenic nematodes are not highly effective against termites (Gouge, 2006).

This research focused on the discovery and description of three apparently phoretic nematodes of the Rhabditida, at least one of which was naturally pathogenic to termites in Uzbekistan.

MATERIALS AND METHODS

Sampling and isolation

Field surveys and collection of the Turkestan termite, *Anacanthotermes turkestanicus*, was conducted in the foothill area of Djam farm in Nurabad district, Samarkand province of Uzbekistan between 2004 and 2007. Initial surveys were also made in Surkhandarya and Khoresm provinces, and in the city of Nukus of the Republic Karakalpakstan. Laboratory studies were conducted at the Laboratory of General Entomology and Arachnology of the Institute of Zoology of Uzbek Academy of Sciences in Tashkent. Termites from living quarters, auxiliary rooms and sheds for cattle and poultry were collected in polyethylene containers.

Initial identification of nematodes parasitizing termites was done using the MBS-1 microscope at a magnification of 16X, according to routine techniques (Pavlovsky, 1957; Lazarevskaya, 1962). When nematodes were observed in the heads of termites, the latter were dissected and separate organs were carefully studied. Of the nematodes thus isolated, some specimens were placed in 3% formaldehyde, alcohol-glycerin and pure glycerin for fixation and dehydration (Golden, 1990; Hooper, 1970). Other specimens were placed in plastic tubes in 1 M NaCl for *Pelodera* in 2004, and 1 M NaCl and DESS (Yoder *et al.*, 2006) for *Acrobeloides* in 2007, for morphometric and molecular characterization in the USA. Based on individual dissections of termites, the extent and intensity of the infection in each sample and individuals was determined, as well as the localization of nematodes in termites.

Nematode measurements and images

Initial measurements were made for some *Caenorhabditis* specimens with Leica Wild MPS48 or Leitz DMRB compound microscope with Differential Interference Contrast (DIC) optics and ocular micrometer. Measurements and images for the tables and figures were made for the nematodes on a Zeiss UltraPhot III light microscope with DIC optics and ocular micrometer. High magnification composite images of female (Figs. 1, 2, 5) and male (Fig. 4) bodies were contrast enhanced and stitched in Adobe PhotoShop CS (Adobe Systems Inc., San Jose, CA, USA). Nematodes were identified with relevant literature for *Pelodera* (Belogurov et al., 1977; Boström, 1996; Kiontke and Fitch, 2005; Sudhaus et al., 1987; Sudhaus and Schulte, 1986, 1988) *Panagrolaimus* (Abolafia and Peña-Santiago, 2005; Andrássy, 1984), and *Acrobeloides* (Abolafia and Peña-Santiago, 2002). Four *Acrobeloides* females and one male were received in DESS in July 2007, imaged (Fig. 5), measured and identified (Andrássy, 1984; Truskova, 1971b; Paul DeLey, personal communication).

Molecular characterization

The *Pelodera* material from 1 M NaCl was too degraded to use, and subsequent sampling yielded information only about *Acrobeloides*. Individual specimens were mechanically disrupted with an eye-knife or sharp-tipped forceps in 20 µl nematode extraction buffer as described by Thomas et al. (1997) and stored at –80°C. For preparation of nematode extracts, samples were thawed, an additional 1 µl proteinase K (from 2 mg/ml stock solution) was added, and the tubes were incubated at 60°C for 60 min, followed by 95°C for 15 min to deactivate the proteinase K. The ribosomal LSU D3 expansion segment was amplified with primers D3A 5'-GACCCGTCTTGAAACACGGA-3' and D3B 5'TCGGAAGGAACCAGCTACTA-3' (Nunn *et al.*, 1996) using an amplification procedure modified from Al-Banna et al. (1997) and W. K. Thomas (personal communication), where the cocktail for 50 µl reactions employed 4 µl (*vs.* 2 µl) template in 0.2 ml tubes in a hot-start reaction. Cycling conditions were 1x 95° C, 30 sec; 35 x (93° C, 30 sec; 55° C 40 sec; 72° C, 120 sec); 1x 72° C, 10 min. The PCR reaction was sequenced directly using M13F, M13rev, D3A, and D3B primers to obtain complete sequence coverage in both directions for 4 sequencing runs (Veritas, Inc., Rockville, MD).

RESULTS

Termites parasitized by nematodes were observed in the village of Djam in the Nurabad district, and the nematodes were found in termite heads, abdomens and legs. The rate of natural infection of termites was as high as 22.3%, with 1 to 26 adult individuals and infective larvae found in each termite worker. In addition to the private farm, infected termites were also found in different parts of the village situated at significant distances from each other. Dissections of seventeen samples consisting of 840 individuals of termites collected from different locations between May 19 and June 15, 2003 revealed that the extent of infection ranged from 2.7 to 100% and the intensity of infection from 1 to more than 50 individuals per termite, including nematode eggs and larvae. No nematodes were observed in April, August and November. No nematodes were found in any of the 13 specimens of termites from Surkhandarya province and 30 specimens collected from Nukus (Karakalpakstan) on January 3, 2004. In 161 specimens collected in Khoresm province on July 28, 2003, the incidence of infection reached 9.5%, while the intensity was 1 to 6 individuals per termite. However, no nematodes were recorded in January, February and early July in termites from this location. Adult nematodes collected in April 2004, July 2005 and May 2007, and sent to Beltsville, MD for identification were not mixed species but either homogeneous individuals of genus *Pelodera, Panagrolaimus* or *Acrobeloides*, respectively.

Although nematodes were widespread in Djam, they were generally sparse in Uzbekistan. The eggs, juveniles and adult nematodes were first found in the head; nematodes were recorded in legs of termites in later samplings where *Pangrolaimus* and *Acrobeloides* were recovered. The infection of nematodes in termite heads appears to take place orally, through food. The body colouration of termites is usually light-yellow, while that of those infected by the nematodes is yellowish-gray, or gray to steel-blue color. Workers of mainly mid- and old ages were found to be infected. The larvae, nymphs, soldiers, alates and secondary females were free from nematodes.

DESCRIPTIONS

Pelodera termitis sp. n.

(Figs. 1, 2; Table 1)

Female: Females larger than males (Fig 1. A, B). Lips of

Table 1. *Pelodera termitis* sp. n. Range, Mean ± S.D. in parentheses, fixed specimens.

Measure (µm)	Female (n=12)	Male (n=7)
Body length (L)	953-1627 (1326±254)	888-1321 (1104±136)
Max. Body diameter (D)	60-95 (76±13)	45-71 (57 ± 8)
Pharynx (L)	190-261 (231±23)	179-226 (198±19)
Tail (L)	48.8-65.5 (55.2±5.7)	33.3-47.6 (42.7±5.8)
a	12.9-21.9 (17.4±2.3)	17.4-21 (19.4±1.3)
b	4.2-6.8 (5.7±0.8)	4.9-7.2 (5.6±0.8)
c	15.7-31.6 (24.3±5.6)	19.6-32.1 (26.2±4.4)
c'	1.3-2.2 (1.7±0.25)	0.9-1.5 (1.3±0.2)
V %	55-58 (57±1)	-
Rectum (L)	23.8-45.2 (35.1±6.2)	-
Prerectum	60-119 (92±18)	83-119 (98±12)
Spicule (L)	-	70-77 (75±3)
Gubernaculum (L)	-	45.2-53.6 (50.2±3.3)
Anal body (D)	28.6-36.9 (32.6±3)	29.8-38.1 (34±3.4)
Stoma (L)	26.2-33.3 (29.8±2.4)	21.4-29.8 (25.8±3.1)
Stoma (D)	3-4.8 (3.9±0.5)	3-4.2 (3.7±0.4)
Excretory pore to lips	129-252 (199±44)	157-212 (183±18.5)
Phasmid to anus	14.3-23.8 (18.9±3.1)	-
Anterior gonad L	190-583 (429±137)	576-982 (766±121)
Posterior gonad L	190-565 (400±128)	-

stoma slightly to moderately indented, about 25% of stoma length. Pharyngeal sleeve about 25% of stoma length in males and females. Three elongated, slightly curved teeth per glottoid swelling of stoma. Base of stoma slightly expanded. Nerve ring between half and posterior third of isthmus. Length ratio of pharyngeal corpus to isthmus to basal bulb about 2:1.5:1 (2.1 : 1.4 : 1). Median bulb twice the width of isthmus width. Excretory pore positions range from centre of isthmus to just below basal bulb (Fig. 2C). Four incisures in lateral field. Amphidelphic gonads with dorsally reflexed arm nearly as long as, to slightly longer than, proximal gonad extension from vulva. Mating plug on vulva of gravid females present. Prerectum present in males and females, with constriction slightly anterior or just posterior of the posterior gonad arm (Fig. 2E). Female tail conical to nearly cupola-shaped, tapering to relatively short spicate termini in most specimens (Fig. 2F), but sometimes with longer termini (Fig. 2G). Phasmid openings at anterior 26-46% of tail length.

Male: Stoma as in female, except male lips slightly narrower than female (14.8±0.9 *vs.* 16.8±0.2). Testis ½ to 2 body widths behind pharyngeal-intestinal junction. Length of distal testis overlaps 30-70% of proximal gonad arm. Male tail fan with cuticular "washboard pattern". Male tail (Figs 1C, 1D, for papillae 1-6) with two preanal papillae and tips of papillae 3, 7 and 10 bent dorsally out of fan. Papilla 5 slightly longer than papilla 4, with tip bent ventrally at the outermost edge of fan. Fan pattern with 2 + 5 + 3 or 2 + 4 + 4 papillae on the open bursa (Figs 2H, 2I). Gubernaculum shaped like a slender hunting bow, slightly curved at both ends (Fig. 2H). Spicule relatively long, slender, straight in side view (Fig. 2H), fused about 65% of total length as seen in face view (Fig. 2I), with small trapezoidal head and acute, gently curved, tips. Amorphous material from mating plug often on spicule tips.

Habitat and locality: Within heads of termites *Anacanthotermes turkestanicus* on farm in the village of Djam, Nurabad district, Samarkand province, Uzbekistan, April, 2004.

Type specimens: Three slides, G-14900-14902, with female and male paratypes deposited in the USDA Nematode Collection, Beltsville, MD, USA.

Diagnosis and relationships: The closest relatives were *P. arnbomi* Boström, 1996, *P. comandorica* Belogurov *et al.*, 1977, *P. strongyloides* Schneider, 1860 and *P. cutanea* Sudhaus *et al.*, 1987 (Table 2.). The new species differed from all others by presence of a prerectum. Females of the new species had a longer, more conical tail, and smaller 'c' ratio compared to *P. strongyloides* and *P. cutanea*. Males had a much larger 'a' ratio than *P. strongyloides* or *P. cutanea*. Male 'a' ratios were similar to those of *P. arnbomi* and *P. comandorica* that also had more conical tails. *Pelodera termitis* sp. n. males had a shorter body length (888-1321 *vs.* 1600 µm), body width (45-71 *vs.* 83 µm),

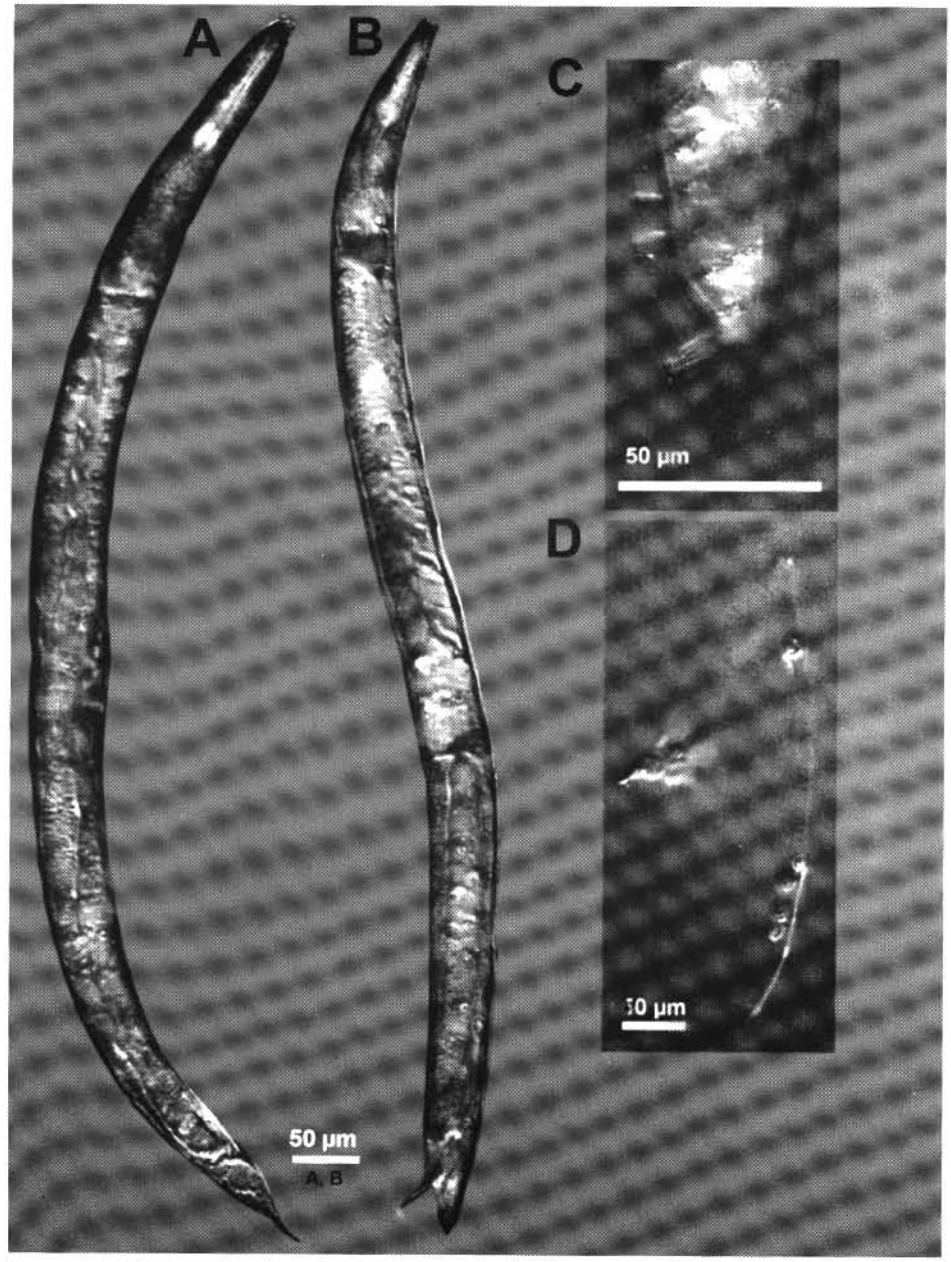


Fig. 1. *Pelodera termitis* sp. n. formalin fixed from termites. A. Female body. Scale bar – 50 µm. B. Male body. Scale bar – 50 µm. C. Lateral male tail showing anterior rays 1-6 and fan. Scale bar – 50 µm. D. Lateral edge of male fan with tip of papilla 2 broadly separated from 4 clustered papillae: terminal papilla 3 bent dorsally out of fan, papillae 4, 5 tips within fan, papilla 5 bent ventrally out of fan. Scale bar – 10 µm.

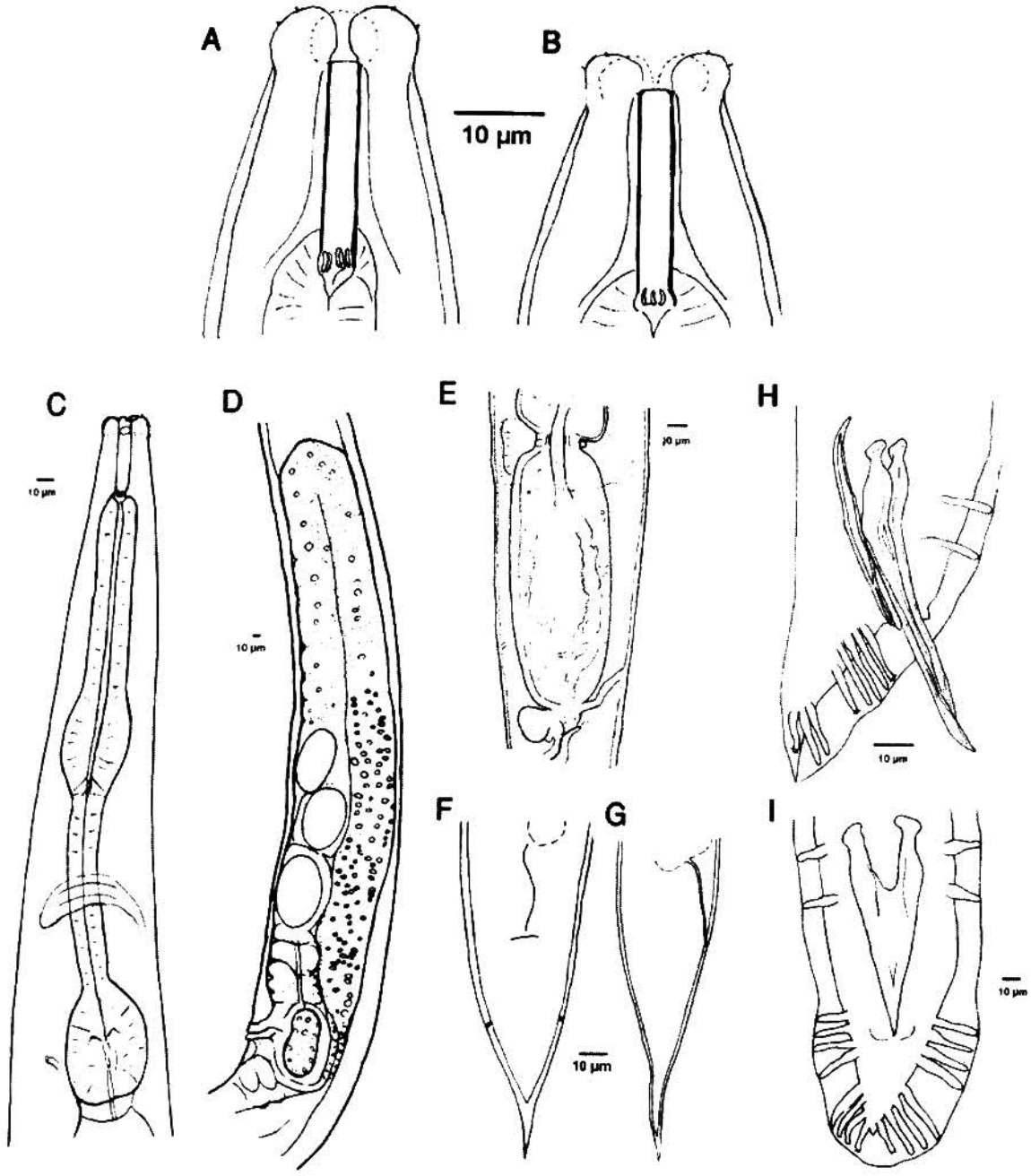


Fig. 2. *Pelodera termitis* sp. n. A-G. Female: A. dorsal view of stoma. B. lateral view of stoma, C. Pharynx, nerve ring, excretory pore. D. Anterior gonad, Vulva. E. Prerectum. F. Ventral view of tail, with typical spicate terminus. G. Lateral view of tail with more elongate terminus. Male: H. Lateral view of tail fan, papillae, spicule, gubernaculum. I. Face view of tail fan, papillae, spicule, gubernaculum. Scale bars – 10 µm.

Table 2. Female measurements (in µm) of *Pelodera strongyloides, P. cutanea* (Sudhaus and Schulte, 1986; Sudhaus, Schulte and Hominick, 1987), *P. arnbomi* Boström, 1996 and *P. comandorica* Belogurov, Muchina & Churikova, 1977 for diagnostic comparison.

Character	*P. strongyloides* (n=11)	*P. cutanea* (n=11)	*P. arnbomi* (n=1)	*P. comandorica* (n=3)
L	1142-2149 (1629)	1003-1780 (1423)	881	1260-1500 (1280)
w	80-132 (107)	60-115 (86)	48	50-90 (75)
pharynx				263-293 (280)
Stoma L	30-38 (33)	26-34 (30)	30-35	31
Stoma w		5	6-7	4.8
Sto/phar		12-14 %		10%
a	12-18.7 (15.1)	14.8-18.4 (16.7)	18	14.6-16.7 (17.6)
b	4.9-7.6 (6.2)	5.2-7.3 (6.4)	4.2	4.1-5.3 (4.6)
c	26.3-37.9 (33)	22.7-31 (26.6)	15	12-30 (22.4)
c'			2.8	1.4*-1.5 (1.45)
V%	55-64 (58)	57-59 (58)	57	50-56 (52.7)
G1 + G2				420 + 380 = 800
Gonads	653-1157 (890)	418-956 (706)	327*	
Gonads/L%	46-63	39-56	38	53
Exp	183-295 (235)	161-241 (208)	165	190
Exp/Phar%			78.5%	65%
rectum		31-40	27	42
Tail (T) L				50-90 (63.3)
aPh - an			8	5.5
aphs % T			13%	11%
pPh-Tt pphs % T		27-36 (32)	56	42
	32-61%		86.6%	79%

* derived from drawing.

pharynx length (179-226 *vs.* 271 µm) and 'c' ratio (20-32 *vs.* 44) than *P. comandorica* males. *Pelodera termitis* sp. n. females had a shorter pharynx (190-261 *vs.* 260-270 µm), larger 'V' ratio (55-58 *vs.* 50-56%) and a phasmid more posteriorly situated on the tail (phasmid – tail tip/tail length: 54-74 *vs.* 79%) than *P. commandorica*. Compared to *P. arnbomi* and *P. comandorica*, *Pelodera termitis* sp. n. had only slightly offset lips *vs.* moderately offset lips.

Remarks: In other *Pelodera* species, male papilla 7 is also the phasmid (Kiontke and Sudhaus, 2000). *Pelodera termitis* sp. n. is one of a few members of the Rhabditidae that have a prerectum besides *Rhabditis silvatica* Volz, 1951 from beetles and beech forest litter (Zell, 1983), and *Rhabditis rainai* Carta and Osbrink, 2005 from Formosan termites (Carta and Osbrink, 2005). Possibly some ecological factor internal or external to termites may be influencing the unusual convergent morphology in these species. This new *Pelodera* species was a seasonal associate of *A. turkestanicus* and caused up to 100% mortality in laboratory culture.

Panagrolaimus sp.

(Figs 3, 4)

Female measurements: (n=8) Body length=394-586 µm, Body width=15 – 18 µm, 'a' ratio=25.5-38.6, 'b' ratio=3.5-4.6, 'c' ratio=12-17.8, 'V' ratio=53-60%

Male measurements: (n=2) Body length=549-668 µm, Body width=19.5-20.7 µm, 'a'=26-27, 'b'=4.3-5.8, 'c'=10.7-14, Spicule=21.9-24.5 µm, Gubernaculum=11.5-12.1 µm

Habitat and locality: Nematodes were in and on termites from Khiva District, Khorezm Province, Uzbekistan in July, 2005.

Remarks: The Khiva population is like *P. spondyli* except for 'a' ratios in males (Fig. 4) and females, which are more

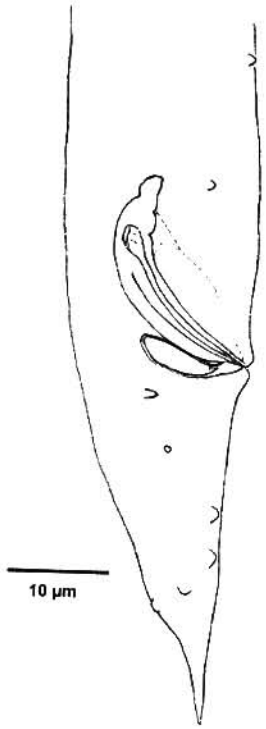


Fig. 3. *Panagrolaimus* sp., Male tail with papillae, phasmid (lateral circle), spicule, gubernaculum. Scale bar 10 µm.

slender like *P. australis* and *P. multidentatus*. *P. australis* was considered a valid species distinct from *P. multidentatus* with the only differences being the larger spicule and gubernaculum sizes in the male, and female phasmid at the level of the anus *vs.* on the tail. Both species are distinct from *P. spondyli* in having a post-vulval sac greater than the body diameter, and 7 rather than 6 male tail papillae (Andrássy, 1984). The Khiva population male (Fig. 3) had a slightly smaller spicule than *P. australis* (one of its specific features), and overlapping but slightly larger spicule size than *P. spondyli*. This population also had different tail papillae positions than *P. australis* (papillae 2 at level of spicule head not shown for *P. australis*). The male body size is slightly larger than *P spondyli*. The 'c' ratio in males overlaps and barely reaches the lower range of *P. australis*. This species was not compared in the diagnosis within Yeates, 1969, nor was its original description of *Rhabditis australis* Cobb, 1893, with the coincidentally same species name as that given in its later rediscovery and redescription. This Uzbek *Panagrolaimus* sp. population had male tail features and most morphometrics like *P. spondyli* but female post vulval sac length, phasmid position, 'a' ratios and larger male body length like *P. australis*. These mosaic characters do not make this species assignment clear, but qualitative male features are often given precedence over other features that may be related to nutritional status. Furthermore, *P. spondyli* is one of the oldest of the complex of similar species. A number of these morphologically similar species represent a complex needing a systematic study of more specimens, since assignment of tail papillae is especially difficult.

Acrobeloides sp.

(Fig. 5)

Female measurements: (n=4) Body length=608-710 µm, 'a' ratio=15-18.5, 'b' ratio=5-5.5, 'c' ratio=18-22, 'V' ratio=59-64%, Body width=37-40 µm, Tail length=31-40 µm, conical with acute terminus.

Male measurement: (n=1) Body length=602 µm, a=19, b=5, c=19, Spicule length=28 µm, tail length=31 µm.

Besides a somewhat more anterior vulva position, females differed from *A. amurensis* Truskova, 1971b and *A.* cf. *amurensis* PS 1146 by smaller body length and more acute tail tip, although both these features can be highly variable within the same species of *Acrobeloides* (Anderson, 1968).

Habitat and locality: Within heads and legs of termites *Anacanthotermes turkestanicus* Djam, Nurabad district, Samarkand province, Uzbekistan, May, 2007.

Remarks: This species was also a seasonal parasite of *A. turkestanicus* and was associated with some mortality in laboratory culture.

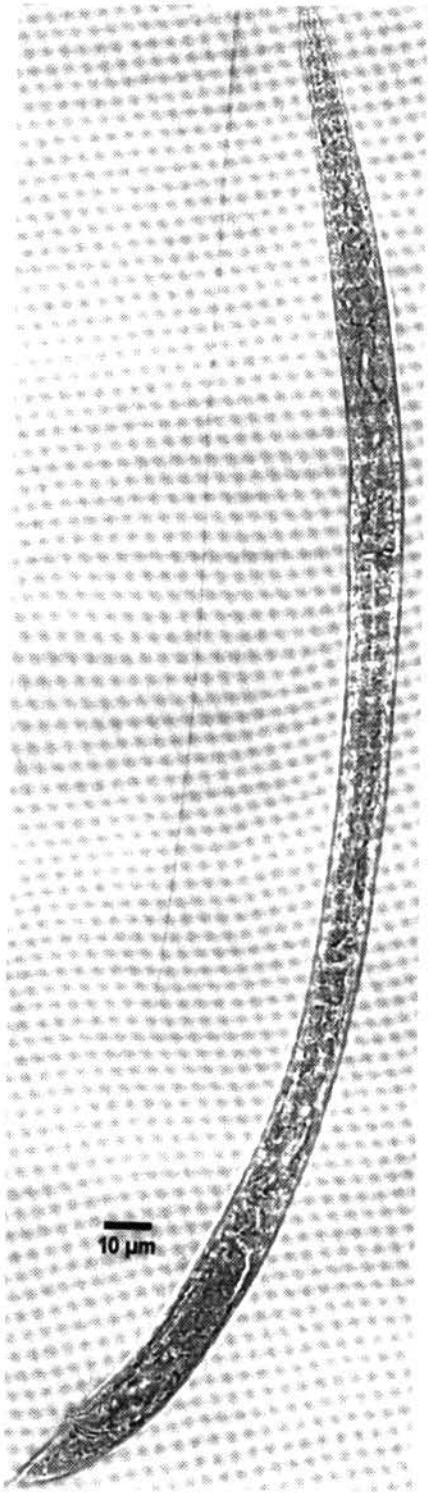
Molecular sequencing

We were unable to get good quality DNA to amplify from *Pelodera* in saline collections of April and October, 2004, or from *Panagrolaimus* sp. in 2005, but were able to amplify that of *Acrobeloides* from May, 2007. The D3LSU rDNA sequence of the *Acrobeloides* from Uzbekistan was identical to that from *Acrobeloides* sp. cf. *amurensis* PS1146 (GenBank DQ03101), demonstrating a fairly close genetic relationship of these populations:

>*Acrobeloides* sp. D3 rDNA_DQ03101
TTCGGAAGGAACCAGCTACTAGATGGTTCGATTAGT
CTTTCGCCCCTATACCCAAGTCAGACGATCGATTTG
CACGTCAGAAGCGCTTCGGACCTCCaCCAGAGTTTC
CTCTGGCTTCATCCTGCTCAGGCATAGTTCACCATCT
TTCGGGTACCAGCGAATACGCTCTACCTCCGCCCCA
CTGCAAGCAGTCGAGACGGGGCTATGCTGCTCCGCC
ACACCGAAGTGCAACGGATCGCATATCAGCCTCCA
GAAGAGACCTTCACTTTCATTACGCCTATGGGTTTTC
CACCCAATGACTCGCGCATACGCTAGACTCCTTGGT
CCGTGTTTCAAGACGGGTC

DISCUSSION

The first report of a nematode parasitizing Turkestan termites was made by Lebedeva (1994), but identification could not be performed at that time. This finding represents



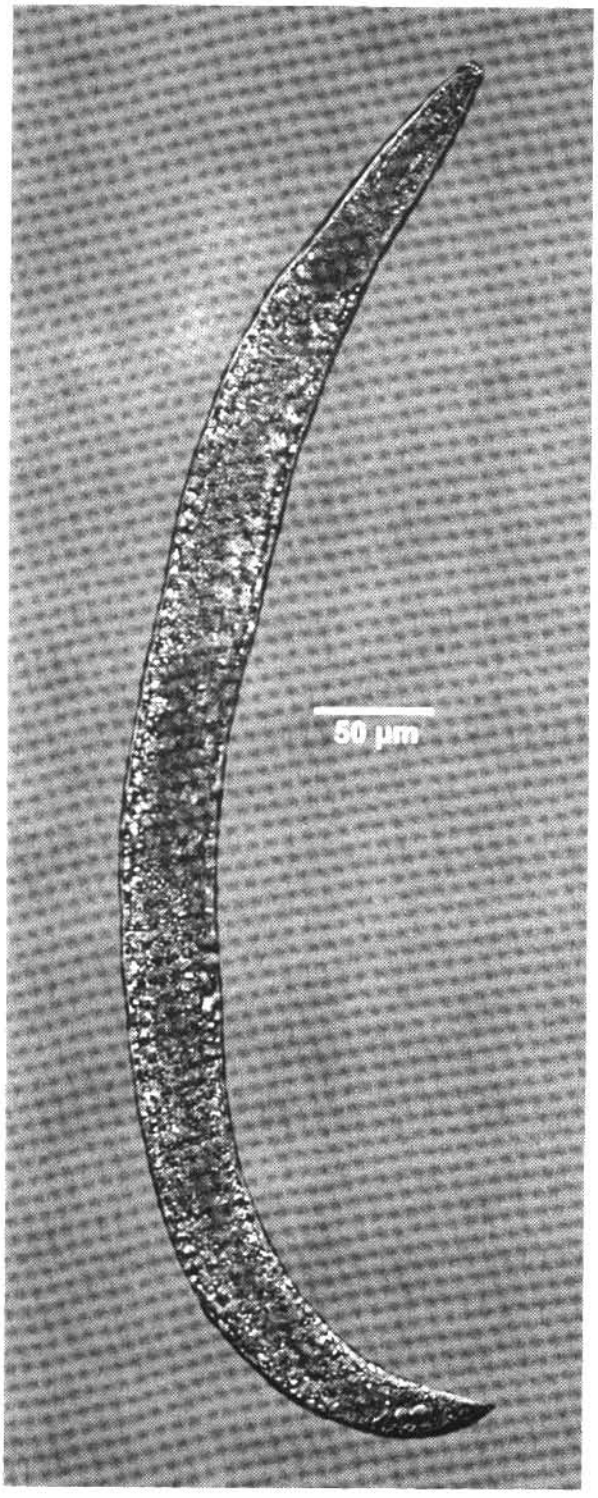


Fig. 4. *Panagrolaimus* sp., Male Body. Scale bar 10 µm.

Fig. 5. *Acrobeloides* sp., Female Body. Scale bar 50 µm.

the first report of *Pelodera* in termites anywhere in the world, and a new host and country record for Uzbekistan. Nematodes of the genus *Pelodera* have three teeth on each stoma glottoid swelling, ten papillae on the male tail and fused male spicules (Andrássy, 1984). *Pelodera termitis* sp. n. has morphological features most similar to members of the *Pelodera strongyloides* group of nematodes that also lack a stomatal collar unlike most species of the genus (Sudhaus and Fitch, 2001). *Pelodera strongyloides* and *P. cutanea* have developed associations with vertebrates (Sudhaus and Schulte, 1986; Sudhaus and Schulte, 1988). Similar adaptations may have allowed *P. termitis* sp. n. to develop a close association with termites.

This *Pelodera* appears to be a seasonal parasite of the Turkestan termite *A. turkestanicus* with a patchy distribution throughout Uzbekistan. This nematode is oviparous, dioecious and localized in the heads and legs but was occasionally found in the abdomen. The infection of termites by nematodes may have taken place orally through food, as the nematodes were first recorded in heads of termites. In May, the extent of the infection of termites by nematodes reached 2.7 to 100%, while the intensity ranged from 1 to 50 individuals, including eggs and larvae of different ages per infected worker. However it was not clear whether the termites were directly killed by *Pelodera* or some cryptic microbial agent, or whether the nematodes were feeding on the termites after their death from another cause.

This is also the first record of an *Acrobeloides* within a termite. A detailed taxonomic and morphological description of this *Acrobeloides* could be compared to the related isolate first described in the Amur province of Siberia (Truskova, 1971a, b) and the similar culture from California. Nomenclatural issues for these populations are complex, requiring separate treatment with more specimens.

Two species very similar to this *Panagrolaimus* sp., *P. spondyli* and *P. multidentatus*, were previously found in Uzbekistan (Andràssy, 1984), in the Khorezm region (Khamraev, 2003). A revision of this *Panagrolaimus* species complex is needed before this Khiva population can be distinctly classified.

Although we do not know the full extent of association in the field for both these species of nematodes, the obvious potential for biological control of termites will add a new component to the diverse ecology of *Pelodera*, *Panagrolaimus* and *Acrobeloides* species. Additional information regarding the distribution of these nematodes within the region and especially in termites throughout the world is needed. Further studies are warranted to consider the use of these nematode species in the control of the Turkestan and other termites.

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