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Pseudaphelenchus vindai n. sp. (Tylenchomorpha: Aphelenchoididae) associated with termites (Termitidae) in Barro Colorado Island, Panama

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Summary – In 2008, a field survey of termite-associated nematodes was conducted on Barro Colorado Island, Smithsonian Tropical Research Institute (BCI, STRI), Panama. During that survey, an undescribed *Pseudaphelenchus* species was isolated from three species of subterranean termites, *Amitermes beaumonti*, *Microcerotermes exiguous* and *Obtusitermes panamae*. The nematode is described and figured herein as *P. vindai* n. sp. The new species is morphologically similar to its only congener, *P. yukiae*, *i.e.*, these two species share a thin stylet with small and clear basal knobs, a true bursa supported by three bursal limb-like genital papillae and a nerve ring surrounding the anterior clear region of the pharyngeal gland lobe and intestine. The molecular phylogenetic analysis based upon near full length (*ca* 1.7 kb) SSU ribosomal DNA sequence suggested that the new species forms a well supported clade with *P. yukiae*, at the basal position of the family Aphelenchoididae. The new species is distinguished from *P. yukiae* by possessing a clear condylus and rostrum of the capitulum and arcuate calomus/lamina complex of the spicules *vs* no condylus and rostrum and a relatively straight calomus/lamina complex, long and tapering female tail without small mucro *vs* blunt with small mucro present and possession of lateral field with three incisures *vs* four incisures.

Keywords – description, molecular, morphology, morphometrics, new species, phylogeny, taxonomy, termites

Until recently, biotic surveys of termite-associated nematodes have been relatively rare with only a few random reports of several rhabditid nematodes (*e.g.*, Poinar, 1975; Wang *et al.*, 2002). However, in the past few years, several research groups have begun describing a number of termite-associated nematodes (Fürst von Lieven, 2003; Sudhaus & Koch, 2004; Carta & Osbrink, 2005; Fürst von Lieven & Sudhaus, 2008; Kanzaki *et al.*, 2009a, b). Fürst von Lieven and Sudhaus (2008) intensively sampled a single species of subterranean termite, *Reticulitermes lucifugus* (Rossi), on the island of Corsica. They reported 20 nematode species with casual to tight associations and demonstrated the potential richness of termite-associated nematode faunas. From 2005 to the present, as part of a National Science Foundation (NSF) Biotic Surveys and Inventories (BSI) project using molecular operational taxonomic unit (MOTU) barcoding for species diversity estimates, we have been surveying termite species in the American tropics and subtropics and Asian subtropical islands (Giblin-Davis *et al.*, 2007; Powers *et al.*, 2009) and have described several termite-associated nematodes (Kanzaki *et al.*, 2009a, b, d).

During a 2008 field survey of termite-associated nematodes on Barro Colorado Island, Panama, an undescribed species of the currently monotypic genus *Pseudaphelenchus* Kanzaki & Giblin-Davis *in* Kanzaki, Giblin-Davis, Scheffrahn, Center & Davies, 2009b was isolated from subterranean termites and successfully cultured. This new nematode species is described and figured herein as *P. vindai* n. sp.

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Materials and methods

Nematode isolation and morphological observation

Field colonies of termites were collected from wood and soil along trails at the Barro Colorado Island (BCI), Smithsonian Tropical Research Institute (STRI), Panama (9°10′N latitude, 79°50′W longitude, 0 m elevation) on 15 May of 2008, and a total of nine species (25 colonies) of termites from two families was obtained; namely, three colonies of *Cornitermes walkeri* Snyder, two colonies of *Nasutitermes guayanae* (Holmgren), one colony of *Obtusitermes panamae* Snyder, four colonies of *Amitermes beaumonti* Banks, five colonies of *Microcerotermes exiguous* (Hagen), one colony of *Cylindrotermes macrognathus* Snyder, one colony of undescribed *Anoplotermes* sp. (Termitidae) and three colonies of *Heterotermes convexinotatus* (Snyder) and five colonies of *H. tenuis* (Hagen) (Rhinotermitidae). Twenty individual termites were randomly chosen from each colony, placed in a water droplet for several minutes and observed for external/casual associations. The termites were then squashed on a 60 mm diam. 1.5% water agar plate, kept at room temperature and examined daily. Termite subsamples with workers, soldiers, and alates (if present) were stored in 100% ethanol, brought back to the laboratory and identified by the fourth author (RHS).

Successful cultures of *P. vindai* n. sp. were obtained from three species of Termitidae; *O. panamae* (culture code: RGD880), *A. beaumonti* (RGD881, RGD882) and *M. exiguous* (RGD883), which were maintained in the laboratory on a mycelial lawn of *Monilinia fructicola* grown on LGPDA with biweekly subculturing at 27°C. *Pseudaphelenchus vindai* n. sp. was preliminarily identified by molecular barcode sequences obtained from cultured isolates according to the molecular operational taxonomic unit (MOTU) methods provided in Mullin *et al.* (2003) and Powers *et al.* (2009), and confirmed using general morphology and reverse taxonomy.

Morphological observation of *Pseudaphelenchus vindai* n. sp.

Adults of *P. vindai* n. sp. (RGD881) from 14-day-old cultures were killed by heat (65°C), fixed in TAF (triethanolamine/formalin/distilled water = 2:7:91), processed through a glycerin-ethanol series using Seinhorst's method (see Hooper, 1986) and mounted in glycerin according to the method of Maeseneer and d'Herde (see Hooper, 1986).

We employed the terminology in Kanzaki *et al.* (2009b) for the morphological description of the new species, and followed Hunt (2008) for the superfamily and family taxonomic scheme.

Molecular characterisation and phylogeny of *Pseudaphelenchus vindai* n. sp.

Adults of *P. vindai* n. sp. were hand-picked from a culture plate and rinsed with deionised water. Nematodes were processed with IsoHair® (Nippon Gene, Tokyo, Japan) for preparation of a PCR template according to the methods described in Kikuchi *et al.* (2009). The DNA base sequences of partial ribosomal DNA (= *ca* 1.7 kb of SSU and *ca* 0.7 kb of LSU) were determined following the methods of Ye *et al.* (2007) and Kanzaki *et al.* (2008) and deposited in the GenBank database with accession numbers AB537559 and AB537560, respectively.

The inferred molecular phylogenetic status of *P. vindai* n. sp. was determined for the partial sequence of SSU ribosomal DNA. The compared sequences were aligned with ClustalW and the model of base substitution was evaluated using MODELTEST version 3.7 (Posada & Crandall, 1998). The Akaike-supported model, the log likelihood (lnL), the Akaike information criterion (AIC), the proportion of invariable sites and the gamma distribution shape parameters and substitution rates were used in phylogenetic analyses. Bayesian analysis was performed to confirm the tree topology for each gene separately using MrBayes 3.1.0 (Huelsenbeck & Ronquist, 2001) running the chain for 10^6 generations and setting the 'burn in' at 1000. We used MCMC (Markov Chain Monte Carlo) methods within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees (Larget & Simon, 1999) using the 50% majority-rule. We chose the species for molecular phylogenetic comparisons with *P. vindai* n. sp. according to the inferred molecular phylogenies of Kanzaki *et al*. (2009b).

Pseudaphelenchus vindai* Kanzaki & Giblin-Davis n. sp.

(Figs 1-3)

* Specific epithet derived from the name of Mr Boudanath (Vinda) Maharajh, University of Florida, in honour of his life and dedication to termite biology.

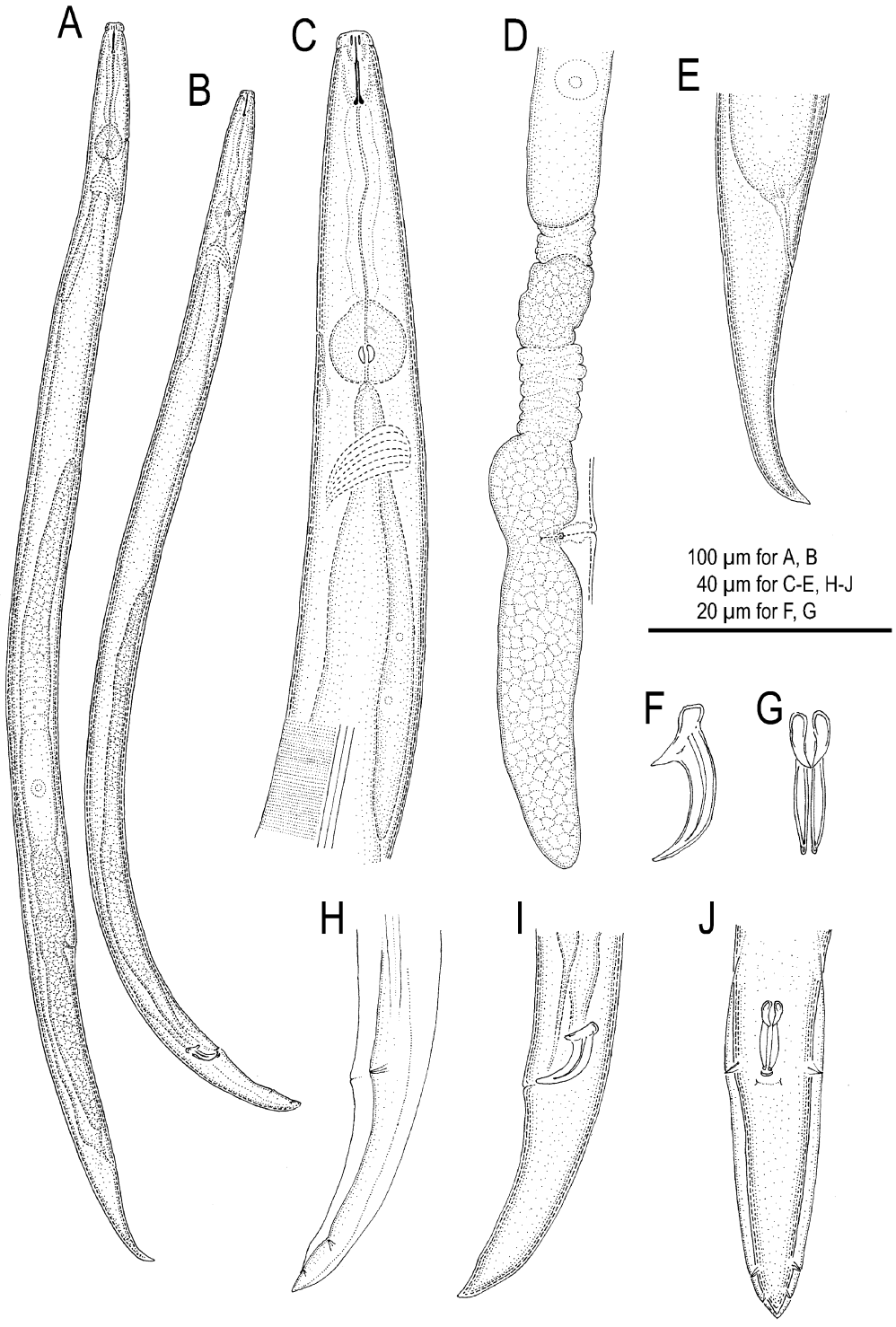

Fig. 1. Pseudaphelenchus vindai *n. sp. A: Female; B: Male; C: Anterior part of female; D: Female reproductive tract; E: Lateral view of female tail; F: Lateral view of spicule; G: Ventral view of spicule; H: Lateral view of male tail surface; I: Lateral view of male tail; J: Ventral view of male tail.*

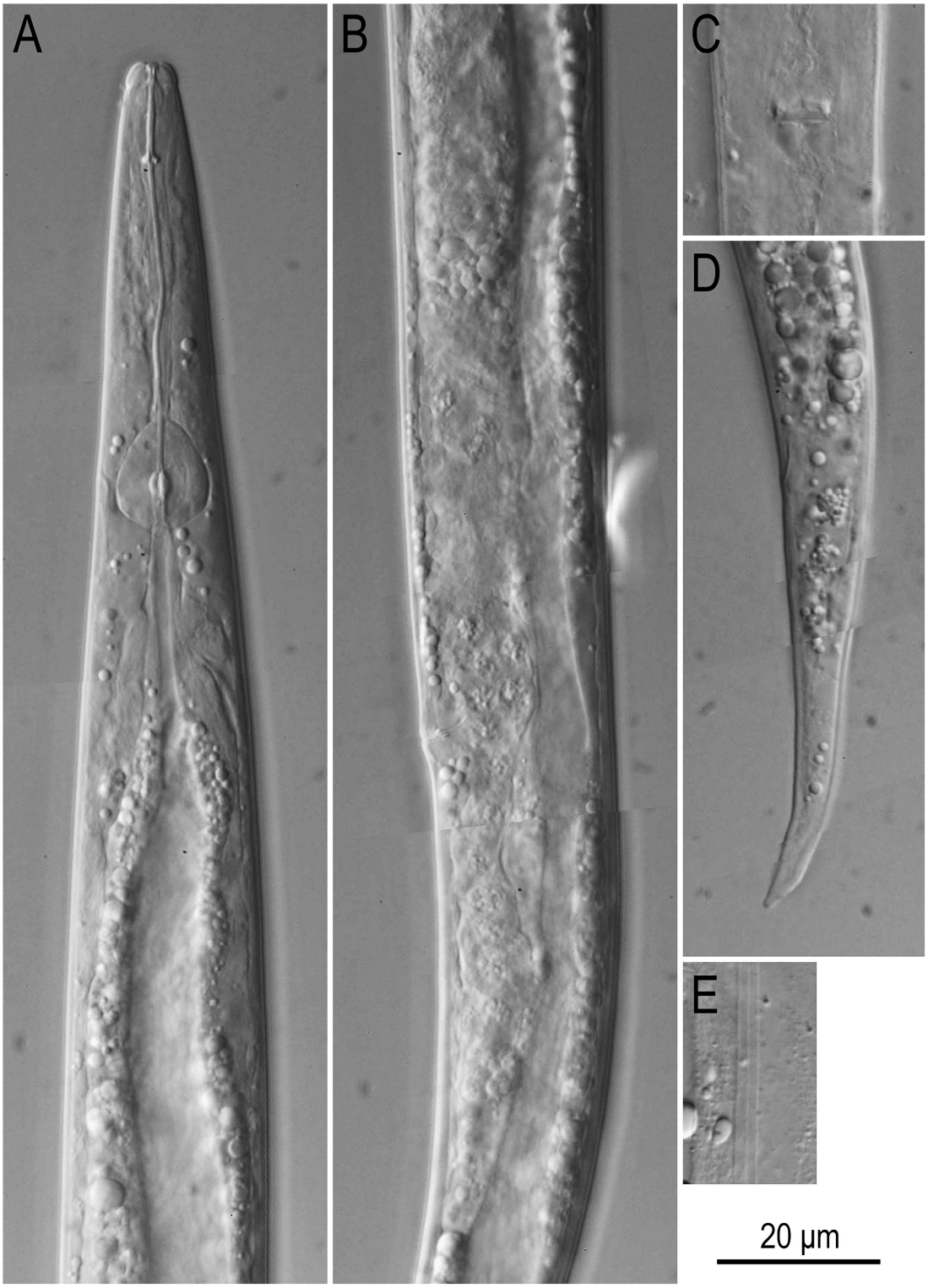

Fig. 2. Pseudaphelenchus vindai *n. sp. A: Anterior region; B: Female reproductive tract (vulval region); C: Vulval region; D: Female tail; E: Lateral field.*

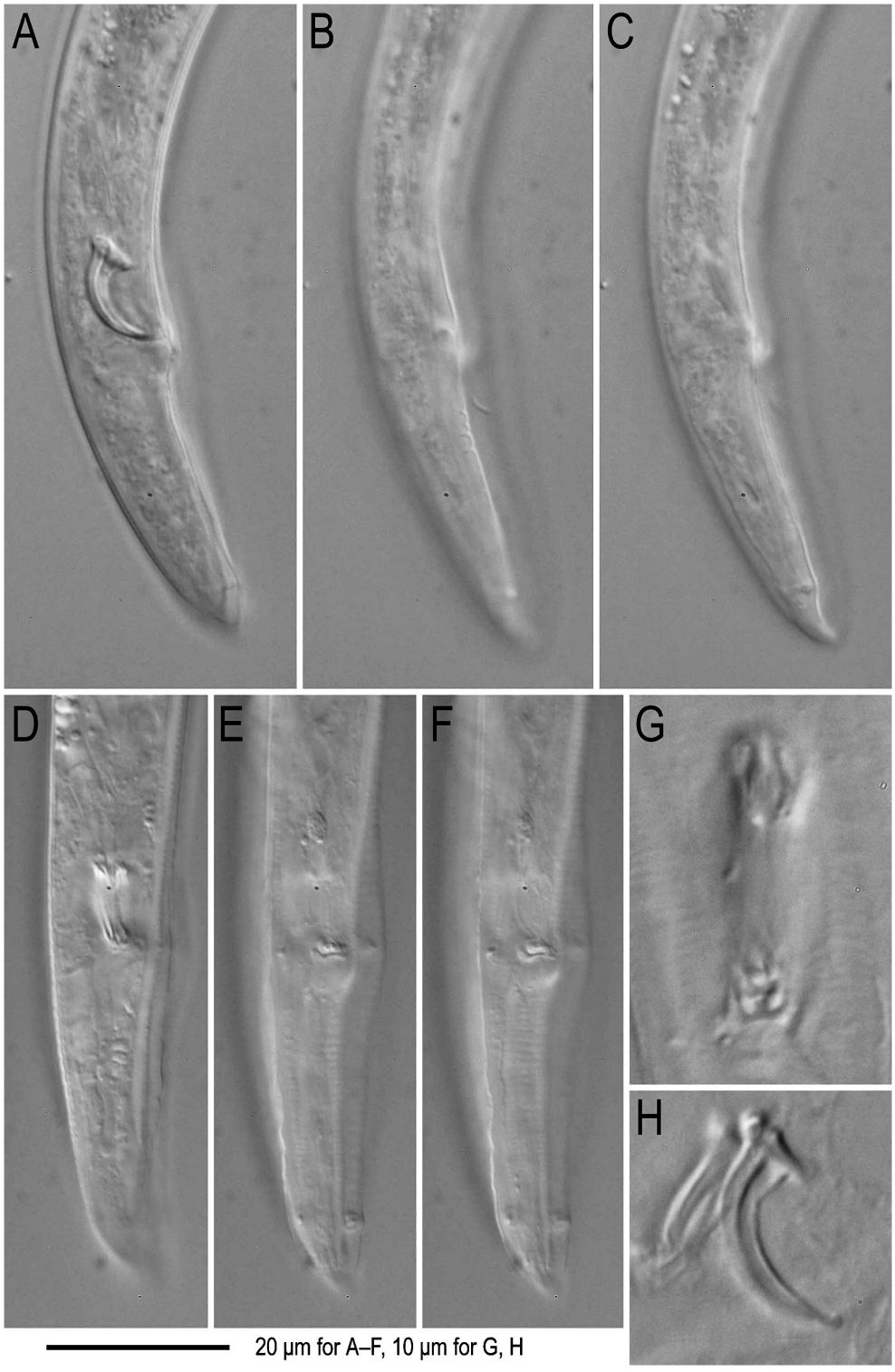

Fig. 3. Pseudaphelenchus vindai *n. sp. A-C: Lateral view of male tail in different focal plane; D-F: Ventral view of male tail in different focal plane; G: Ventral view of spicule; H: Lateral view of spicule (squashed to observe from right lateral).*

MEASUREMENTS

See Table 1.

DESCRIPTION

Adults

Small to medium-sized species, *i.e.*, 300-400 μm and 400-500 μm in male and female, respectively. Body cylindrical, ventrally arcuate or straight when killed by heat treatment. Cuticle thin, annulated. Lateral field with three clear incisures. Head distinctly offset from body, lip region in lateral view truncate, less than twice as broad as high. Stylet two part, cone short, *ca* one-third of total stylet length, shaft with small and conspicuous basal knobs. Procorpus cylindrical, *ca* 2.5 stylet lengths long, ending in well developed, pear-shaped to spherical metacorpus (= median bulb). Metacorpal valve plate located slightly posterior to middle of metacorpus. Dorsal pharyngeal gland orifice opening into lumen of metacorpus *ca* one metacorpal valve length anterior to valve, subventral pharyngeal glands open into lumen one metacorpal valve length posterior to valve. Pharyngo-intestinal junction immediately posterior to, or sometimes partially embedded in, posterior end of metacorpus. Postcorpus glandular, overlapping intestine dorsally, *ca* five to six metacorpal lengths long. Three nuclei observed in gland in several individuals. Excretory pore *ca* at level of metacorpus, *i.e.*, from near centre of metacorpus to base of metacorpus. Nerve ring *ca* one stylet length posterior to metacorpus, surrounding dorsal pharyngeal gland and intestine. Hem-

Table 1. *Morphometrics of holotype and paratype specimens of* Pseudaphelenchus vindai *n. sp. All measurements are in μm and in the form: mean ± s.d. (range).*

Character	Male		Female
	Holotype	Paratypes	Paratypes
n	–	15	15
L	370	352 ± 15 (330-380)	463 ± 30 (415-510)
a	31.4	27.4 ± 2.0 (24.1-31.7)	27.4 ± 1.2 (25.7-30.0)
b	8.7	8.4 ± 0.4 (7.7-9.4)	10.3 ± 0.5 (9.6-11.1)
c	12.3	11.8 ± 0.6 (11.2-13.1)	12.1 ± 0.8 (10.7-13.9)
c′	3.3	2.9 ± 0.2 (2.6-3.3)	4.4 ± 0.4 (3.6-5.0)
T or V	48.6	54.3 ± 7.2 (38.3-68.8)	72.1 ± 1.6 (68.2-75.6)
Max. body diam.	12.0	13.0 ± 0.9 (11.5-15.0)	17.0 ± 1.0 (15.5-19.0)
Lip diam.	5.0	5.0 ± 0.2 (4.5-5.5)	5.5 ± 0.3 (5.0-6.0)
Lip height	2.5	2.5 ± 0.2 (2.0-3.0)	3.0 ± 0.2 (2.5-3.0)
Stylet length	6.0	6.0 ± 0.4 (5.0-6.5)	6.0 ± 0.5 (5.0-6.5)
Excretory pore[1)]	39	35 ± 2.1 (32-40)	38 ± 2.2 (34-42)
Excretory pore[2)]	4.0	6.0 ± 2.1 (2.0-9.0)	7.5 ± 2.2 (4.0-11.0)
Base of median bulb	43	42 ± 1.7 (39-45)	45 ± 2.1 (42-48)
Hemizonid	67	66 ± 3.61 (59-70)	75 ± 3.2 (70-79)
Spicule length[3)]	14.0	13.0 ± 0.7 (11.5-14.5)	–
Spicule length[4)]	13.0	12.5 ± 0.7 (11.0-13.5)	–
Testis or ovary length	162	169 ± 27 (114-217)	228 ± 28 (176-286)
Vulval body diam.	–	–	16.0 ± 0.9 (15.0-18.0)
Post-uterine sac length	–	–	41 ± 5.1 (35-56)
Vulva-anus distance	–	–	84 ± 10 (70-106)
Post-uterine sac/vulva to anus (%)	–	–	48.7 ± 5.0 (39.0-59.0)
Tail length	30	30 ± 1.2 (28-32)	38 ± 2.9 (33-43)
Anal or cloacal body diam.	9.0	10.0 ± 0.7 (9.0-11.0)	9.0 ± 0.6 (8.0-10.0)

[1)] From anterior end.

[2)] Anteriorly from the base of median bulb.

[3)] Measured as arc from bottom of capitulum depression to distal end.

[4)] Measured as straight line from condylus tip to distal end.

izonid *ca* two stylet lengths posterior to base of metacorpus.

Male

Lateral field extending posteriorly, overlapping with anterior part of bursa. Gonad outstretched, reflexed in some individuals, sperm amoeboid. *Vas deferens* and intestine separated at level of anterior end of spicule in some individuals, probably fused to form cloacal tube at middle or lower level of spicule. Tail weakly arcuate, *ca* 2.5-3.5 cloacal body diam. long. Spicules paired, separate, condylus truncate, rostrum conical with bluntly pointed tip, calomus/lamina complex smoothly arcuate, tapering towards bluntly pointed distal end, cucullus lacking. Gubernaculum absent. Bursa present, long, conspicuous, starting *ca* one body diam. anterior to cloacal aperture, surrounding entire tail, supported by six (= three pairs) precloacal and postcloacal limb-like genital papillae. Genital papillae distributed as one pair subventral precloacal papillae (P2) slightly anterior to cloacal slit and other two pairs (P3 and P4) close to each other near tail tip. No single midventral precloacal papilla (P1) observed.

Female

Reproductive tract arranged as ovary, oviduct, spermatheca, crustaformeria, uterus, vagina + vulva and postuterine sac. Ovary single, anteriorly outstretched, anterior end reflexed in some individuals, oocytes in triple to double and single files in anterior and posterior halves of ovary, respectively. Oviduct tube-like, connecting ovary and spermatheca, sometimes occupied by well developed oocyte. Spermatheca irregular rectangular in shape, sometimes filled with well developed sperm. Crustaformeria not conspicuous, mosaic-like pattern (presumably typical cell arrangement) observed in some individuals. Uterus with thick wall. Vagina straight, almost perpendicular to body surface, surrounded by prominent muscle-like tissue. Vulva a simple slit in ventral view, without any vulval flap in lateral view, anterior and posterior vulval lips slightly protuberant. Small sclerotised structure, probably with a valve-like function, surrounding end of vagina (= vagina-uterus junction). Postuterine sac *ca* four to five vulva body diam. long, extending for *ca* 80% of vulval-anus distance, sometimes filled with sperm. Sperm in post-uterine sac morphologically identical to those in spermatheca. Anus a small dome-shaped slit in ventral view. Tail *ca* five to six times anal body diam. long, ventrally reflexed when heat-killed, smoothly tapering to bluntly pointed tail tip.

TYPE HOST AND LOCALITY

Pseudaphelenchus vindai n. sp. (culture code RGD881) was isolated from the bodies of *Amitermes beaumonti* workers collected at Barro Colorado Island, Smithsonian Tropical Research Institute, Panama (9°10′N latitude, 79°50′W longitude, 0 m elevation), on 15 May 2008.

TYPE MATERIAL

Type material was obtained from a 2-week-old culture of RGD881, originally isolated from *A. beaumonti*. Holotype male (slide number *Pseudaphelenchus vindai* M-01), two paratype males (slide number M-02-03) and three paratype females (slide numbers F-01-03) deposited in Smithsonian Tropical Research Institute Synoptic Insect Collection and the Fairchild Museum, University of Panama, Panama; three paratype males (slide numbers *Pseudaphelenchus vindai* M-04-06) and three paratype females (slide numbers F-04-06), USDA Nematode Collection (USDANC), Beltsville, MD, USA; three paratype males (slide numbers *Pseudaphelenchus vindai* M-07-09) and three paratype females (slide numbers F-07-09), deposited at Fort Lauderdale Research and Education Center (FLREC), University of Florida, Fort Lauderdale, FL, USA; three paratype males (slide numbers *Pseudaphelenchus vindai* M-10-12) three five paratype females (slide numbers F-10-12), deposited in the Harold W. Manter Laboratory of Parasitology, University of Nebraska State Museum, Lincoln, NE, USA; three paratype males (slide numbers *Pseudaphelenchus vindai* M-13-15) and three paratype females (slide numbers F-13-15), deposited in Forest Pathology Laboratory collection, Forestry and Forest Products Research Institute (FFPRI), Tsukuba, Japan.

In addition to the above-mentioned type specimens, mass fixed material in formalin-glycerin or dehydrated glycerin is available at USDANC, FLREC, FFPRI, Canadian National Collection, Ottawa, ON, Canada and FERA, Sand Hutton, York, UK.

DIAGNOSIS AND RELATIONSHIPS

Pseudaphelenchus vindai n. sp. is characterised by its thin stylet with small and conspicuous basal knobs, pharynx lacking a clear isthmus with the nerve ring surrounding the dorsal pharyngeal lobe and intestine, true bursa on male tail, and male spicules with conspicuous capitulum truncate condylus and conical rostrum with bluntly pointed tip and smoothly arcuate calomus/lamina complex tapering to bluntly pointed distal end, long female tail tapering to a bluntly pointed distal end and lateral field with three incisures.

The genus *Pseudaphelenchus* was previously monotypic, comprising only the type species, *P. yukiae*. Thus, *P. vindai* n. sp. becomes a putative sister species that is distinguished from *P. yukiae* by its lateral field with three *vs* four incisures, paired and separate male spicules each possessing a conspicuous capitulum with truncate condylus and conical rostrum and smoothly arcuate calomus/lamina complex *vs* without clear capitulum and calomus/lamina complex which is relatively straight but has a strong ventral bend at the anterior part, bursal initiation point one *vs* two cloacal body diam. anterior to the cloacal aperture and conical to bluntly pointed female tail tip without mucro *vs* blunt with a small mucro (Kanzaki *et al.*, 2009b).

Molecular characterisation and phylogeny of *Pseudaphelenchus vindai* n. sp.

The inferred molecular phylogenetic status of *P. vindai* n. sp. is shown in Figure 4. The new species formed a well supported clade with its putative sister species, *P. yukiae* Kanzaki & Giblin-Davis *in* Kanzaki, Giblin-Davis, Scheffrahn, Center & Davies, 2009b.

In the previous study, we established *Pseudaphelenchus* as a monotypic genus based upon its unique morphological characters and molecular phylogenetic position using 1.2 kb of SSU (Kanzaki *et al.*, 2009b). In the present study, we updated the molecular phylogenetic tree employing near full length SSU and confirmed the status of the genus, *i.e.*, the genus is not monotypic and forms a basal clade of the family Aphelenchoididae.

Biology

Pseudaphelenchus vindai n. sp., which feeds and propagates on fungi, *e.g.*, *Botrytis cinerea* and *M. fructicola*, was isolated from three subterranean termite species in the family Termitidae; *i.e.*, *A. beaumonti*, *M. exiguous* and *O. panamae*, which inhabit moist soil. The nema-

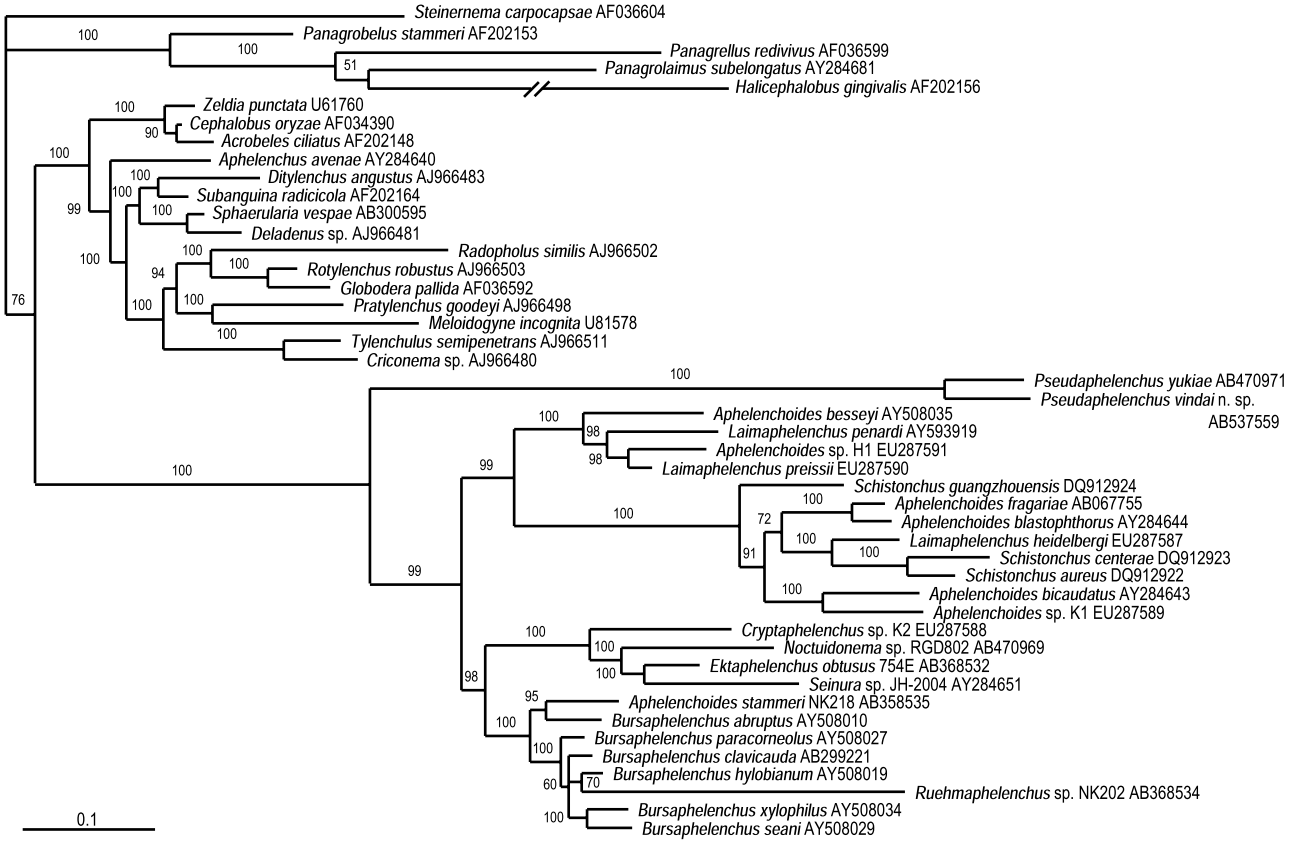

Fig. 4. *Molecular phylogenetic relationship among 45 species of nematodes. The 10001st Bayesian tree inferred from near full length SSU was generated under the GTR + I + G model. Posterior probability values exceeding 50% are given on appropriate clades.*

tode probably survives in termite galleries and nest environs where it feeds and propagates on various species of soil fungi. The dispersal mode of the nematode, *e.g.*, the type of dispersal stage (dauer) and the associated termite organ, is unknown. However, *P. vindai* n. sp. is hypothesised to use alates for vertical transmission, as documented for other recently discovered termite-nematode associates (Kanzaki *et al.*, 2009b, c).

Generic definition of the genus *Pseudaphelenchus*

The genus *Pseudaphelenchus* is morphologically intermediate between the families Aphelenchidae and Aphelenchoididae (Hunt, 2008; Kanzaki *et al.*, 2009b). In the previous study, we defined the then monotypic genus with two important generic characters: *i*) the pharyngo-intestinal junction being located immediately posterior to the median bulb and the nerve ring surrounding the intestine and pharyngeal glands; and *ii*) males with a true bursa (with three pairs of limb-like genital papillae in *P. yukiae*). We also suggested: *iii*) a thin stylet with distinct small basal knobs; *iv*) the low number (four in *P. yukiae*) of lateral incisures; and *v*) a long and tapering female tail with small mucro, as possible generic characters.

Pseudaphelenchus vindai n. sp. mostly fits these generic characters. Therefore, we suggest the following characters for an emended generic definition: aphelenchoidid-like pharyngeal structure (see above); aphelench-like bursa with limb-like genital papillae (see above); conspicuous and small stylet knobs clearly distinguished from stylet shaft; low number of lateral incisures (probably at most six); and long and tapering female tail.

Acknowledgements

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