Pratylenchus pseudocoffeae n. sp. (Nematoda: Pratylenchidae)
from Composite Plants in Japan

Takayuki MIZUKUBO

*Laboratory of Nematology and Soil Zoology, Department of Environmental Biology,
National Institute of Agro-Environmental Sciences, Kannondai
3-1-1, Tsukuba, Ibaraki 305, Japan*

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Two populations of the lesion nematode collected from the rhizosphere of composite plants, *Chrysanthemum morifolium* (in Miyazaki Pref.) and *Artemisia Feddei* (in Nagasaki Pref.) were described as *Pratylenchus pseudocoffeae* n. sp. The new species closely resembled *P. coffeae*, but was distinguished by its divided pattern in *en face*, short distance from spear base to DGO (1.8–2.3 μm vs. 2.6–3.3 μm in mean), narrower spear knobs (3.4–3.7 μm vs. 3.7–4.2 μm in mean) and frequent 3 lip annuli (28.5% on either side and 5.5% on both sides: $n=91$).

Key words: Taxonomy, lesion nematode

INTRODUCTION

In the previous report (MIZUKUBO, 1992), the 6 populations of *Pratylenchus coffeae* in Japan were comparatively investigated by scanning electron microscope (SEM) and with stepwise linear discriminant function analysis. This study was successful, on both morphological and statistical bases, in discriminating 3 groups among the populations, concluding that 2 of the 3 groups would represent different and new species. Since no systematic action was made in the previous report, a species taken from composite plants is described herein as *Pratylenchus pseudocoffeae* n. sp.

METHODS

SEM examination. Specimens stored in TAF were processed in 30% to 100% ethanol, critical-point dried, coated with platinum-vanadium (20 nm), and examined in a Hitachi X-650 SEM at 10 kV.

Preparation of the specimens for measurement and drawing. Observations were made on the specimens killed by gentle heating, fixed in TAF and mounted in dehydrated glycerin by the quick method of SEINHORST (1959). The specimens were observed and measured for all features with oil immersion at 2,400 $\times$ on an Olympus BH-2 light microscope equipped with Normarski interference contrast.

SYSTEMATICS

Pratylenchus pseudocoffeae n. sp.

(Figs. 1, 2 & 3; Table 1)

Measurements

Measurements of *holotype* female, calculated means and SDs of the measurements in 40 *paratype* females (Miyazaki), 10 *paratype* females (Nagasaki), 11 *paratype* males (Miyazaki) and 1 *paratype* male (Nagasaki) are shown in Table 1. For the ranges of measurements in the *paratype* females, the reader is referred to MIZUKUBO (1992).

Measurement ranges in the paratype males (Miyazaki: n=11): $L=394\text{--}541\text{ }\mu\text{m}$; $a=25.6\text{--}37.0$; $b=4.6\text{--}6.9$; $b'=2.9\text{--}3.8$; $c=18.1\text{--}23.9$; $c'=1.8\text{--}2.9$; $\text{spear}=13.9\text{--}15.5\text{ }\mu\text{m}$ ($n=19$); $m=45.2\text{--}52.4\%$; $\text{DGO}=1.3\text{--}2.6\text{ }\mu\text{m}$; $\text{knob width}=2.8\text{--}3.4\text{ }\mu\text{m}$; $\text{excretory pore}=72\text{--}90\text{ }\mu\text{m}$; $\text{esophagus}=73\text{--}98\text{ }\mu\text{m}$; $\text{tail}=21\text{--}27\text{ }\mu\text{m}$; $T=38.3\text{--}47.8\%$; $\text{spicule}=15.2\text{--}19.1\text{ }\mu\text{m}$ ($n=15$); $\text{gubernaculum}=3.3\text{--}4.6\text{ }\mu\text{m}$; $\text{spicule/spear ratio}=1.02\text{--}1.24$ ($n=15$).

Description

Female ($n=40$ for measurements; $n=91$ for some morphological features based on the Miyazaki population): body curved ventrally when heat-relaxed. Annules

Table 1. Measurements of *Pratylenchus pseudocoffeae* n. sp.

Criteria	Holotype (♀)	Paratypes (♀)		Paratypes (♂)	
	Miyazaki ($n=1$)	Miyazaki ($n=40$)	Nagasaki ($n=10$)	Miyazaki ($n=11$)	Nagasaki ($n=1$)
L (μm)	578	554: 59.9 ^a	471: 56.1	489: 42.7	430
a	30.2	29.9: 2.19	25.1: 2.54	30.6: 3.00	28.3
b	7.1	6.3: 0.71	5.2: 0.50	6.1: 0.72	5.1
b'	3.3	3.1: 0.37	3.0: 0.38	3.3: 0.29	3.0
c	19.0	19.4: 1.41	19.1: 1.04	20.3: 1.61	17.6
c'	2.3	2.3: 0.21	2.0: 0.25	2.4: 0.33	2.3
V (%)	78.5	80.4: 0.89	80.8: 1.28	—	—
spear (μm)	16.5	16.4: 0.46	16.0: 0.84	14.9: 0.45	15.2
m (%)	50.0	50.3: 1.65	50.4: 1.62	50.7: 1.91	52.2
DGO (μm)	2.0	1.8: 0.39	2.3: 0.34	1.8: 0.38	2.0
knob width (μm)	4.0	3.7: 0.25	3.4: 0.26	3.0: 0.20	3.0
excretory pore (μm)	90.4	88: 5.4	85: 5.0	83: 5.7	81.8
esophagus (μm)	82	88: 9.1	91: 6.7	81: 8.2	85
vulva-anus (μm)	94	80: 11.3	65: 8.2	—	—
tail (μm)	30	29: 3.4	25: 3.0	24: 2.4	24
post-uterine branch (μm)	26	28: 5.0	25: 4.8	—	—
U	1.5	1.7: 0.28	1.4: 0.20	—	—
G_2 (%)	4.5	5.0: 0.86	5.4: 0.90	—	—
tail annule	22	20: 1.8	18: 2.5	—	—
T (%)	—	—	—	43: 2.9	35.3
spicule (μm)	—	—	—	16.9: 1.29	16.5
gubernaculum (μm)	—	—	—	4.0: 0.40	5.0
spicule/spear ratio	—	—	—	1.14: 0.057	1.09

^a mean: S.D.

Fig. 1. *Pratylenchus pseudocoffeae* n. sp. Female. A–D: heads (*en face* view); E: head (lateral view); F: lateral field; G: vulva; H: typical tail (lateral view); I: tail (ventral view). Scale bars = 2 μ m.

1.4 μ m $\pm$ 0.26 (1.0–1.9) wide at mid-body. Lip region relatively low, 2.6 μ m $\pm$ 0.19 (2.1–3.0) high, 7.5 μ m $\pm$ 0.32 (6.9–8.1) wide, flattened anteriorly, weakly offset. Labial framework moderately developed, laterally extending into body at 1 annule. Lip with 2 annuli on both sides (66%; $n=91$) (Fig. 1 E; Fig. 2 D–F), 2 and 3 annuli (28.5%) or 3 annuli on both sides (5.5%). SEM *en face* view (Fig. 1 A–D) showed division between sub-median and lateral segments; lateral edges of sub-median segments curving slope, never angular, assigned into “Group 2” of CORBETT and CLARK (1983). Amphid apertures narrow and oblique (Fig. 1 D). Cephalids not seen. Spear 2.2 times $\pm$ 0.10 (2.0–2.5) as long as head diameter. Spear knobs massive, 2.0 μ m $\pm$ 0.21 (1.7–2.4) high, 3.7 μ m $\pm$ 0.27 (3.3–4.3) across; shape variable but mostly broadly rounded or

Fig. 2. *Pratylenchus pseudocoffeae* n. sp.—Female. A: entire; C: esophageal region (normal); D: esophageal region with dorso-laterally overlapping esophageal basal lobe; E–G: heads; H: gonad; I: vulval region; J–L: tails.—Male. B: entire; M: posterior end. Scale bars: A & B = 100 μ m; C–M = 30 μ m.

Fig. 3. Morphological variation of female tails of *Pratylenchus pseudocoffeae* n. sp. Scale bar=30 μ m.

flattened anteriorly (Fig. 2 C-G), i.e., of the 37 individuals, the following shapes were observed: rounded and sloping posteriorly = 1 (2.7%); broadly rounded = 15 (40.5%); flattened anteriorly = 11 (29.7%); slightly indented = 6 (16.2%); indented = 4 (10.8%). Metacarpus oval, $13.1 \mu\text{m} \pm 0.81$ (11.9–15.8) ($n=20$) long, $9.5 \mu\text{m} \pm 0.46$ (8.6–10.6) ($n=20$) across, 1.4 ± 0.1 (1.3–1.7) in length/width ratios, occupying about half of the corresponding body diameter; valve conspicuous, $57 \mu\text{m} \pm 3.0$ (48–62) from anterior body end, or $65.0\% \pm 5.96$ (46.0–79.3) of esophagus length. Esophageal basal lobe (Fig. 2 C-D) extending $182 \mu\text{m} \pm 17.5$ (150–241) from anterior body end, ventrally overlapping intestine $93 \mu\text{m} \pm 17.9$ (64–155) or a distance of 5.1 times ± 1.09 (3.0–9.0) the corresponding body diameter; rarely overlapping dorso-laterally (7.7%; $n=91$) (Fig. 2 D) or laterally (15.4%). Esophageal nuclei usually line up. Excretory pore $100.5\% \pm 9.8$ (78–123) of esophageal length or 63 ± 2.6 (58–68) ($n=22$) body annuli from anterior body end; annuli between anterior body end and excretory pore $1.4 \mu\text{m} \pm 0.09$ (1.2–1.5) in average width. Hemizonid flat, 2 to 3 annuli long, level with or immediately anterior to excretory pore; hemizonion lenticular, 8 ± 1.2 (6–10) annuli posterior to excretory pore.

Gonad outstretched, $159 \mu\text{m} \pm 25.1$ (123–227) ($n=25$) long; ovary with oocytes generally in a row (rarely partially doubled); spermatheca oblong $63 \mu\text{m} \pm 7.5$ (52–77) from vulva, $26 \mu\text{m} \pm 5.8$ (18–38) ($n=35$) long, $11.7 \mu\text{m} \pm 1.48$ (9.2–14.5) ($n=26$) wide,

2.4 ± 0.46 (1.6–3.1) ($n=26$) in length/width ratios, packed with spermatozoa (Fig. 2 H). Post-uterine branch usually less than twice of vulval body diameter, with terminal rudimental ovary (Fig. 2 I). Vagina narrow-walled, perpendicular to body axis, $7.1 \mu\text{m} \pm 0.59$ (5.9–8.3) ($n=23$) long, or $43.6\% \pm 4.62$ (36.4–52.2) of vaginal body diameter; vaginal opening wide slit (Fig. 1 G). Annuli between vulva and anus 57 ± 4.8 (48–66) ($n=23$) in number, $1.4 \mu\text{m} \pm 0.19$ (1.1–1.7) ($n=23$) in average width.

Tail termini with some variation in shape (Fig. 2 J–L; Fig. 3): using tail tip shape codes of FREDERICK and TARJAN (1989), hem (hemispherical)=1 (3%), shm (sub-hemispherical)=16 (52%), blp (bluntly pointed)=14 (45%), but no fnp (finely pointed) in the 31 individuals with exact lateral orientation; terminus smooth, rarely wrinkled, with no evident annuli. Tail terminal cuticle $2.8 \mu\text{m} \pm 0.48$ (1.8–3.6) thick; phasmids pore-like, centered in lateral fields (Fig. 1 H), $13 \mu\text{m} \pm 2.7$ (7–19) ($n=30$) behind anus or $16.0 \mu\text{m} \pm 2.4$ (10.6–21.1) ($n=30$) from tail tip.

Lateral fields $5.4 \mu\text{m} \pm 0.84$ (4.0–7.3) ($n=23$) wide, i.e. $28.8\% \pm 2.94$ (23.1–34.5) of the widest body diameter, consist of 3 bands or 4 incisures; inner band appears plain in transmitted light (Fig. 2 D, C) but is incised by 1 or 2 faint broken lines (Fig. 1 F); outer bands appear almost plain in mid-body region (Fig. 1 F) and areolated at tail region (Fig. 1 H, I). Lateral fields extend at tail tip and continue round the tail terminus (Fig. 1 I).

Males ($n=11$): Body straight to arched when killed by gentle heating (Fig. 2 B). Annuli finer than in female, $1.2 \mu\text{m} \pm 0.17$ (0.9–1.5) wide at mid-body. Lip region relatively low, $2.6 \mu\text{m} \pm 0.25$ (2.2–3.0) high, $6.7 \mu\text{m} \pm 0.32$ (5.9–7.3) wide, flattened anteriorly, weakly offset or continuous to body. Labial framework moderately developed, laterally extending into body at 1 annule. Lip annuli 2 in transmitted light, sometimes 3 annuli can be determined. Cephalids not seen. Spear shorter than in female, 2.2 times ± 0.13 (2.0–2.5) as long as head diameter. Spear knobs smaller than female, $3.0 \mu\text{m} \pm 0.20$ (2.8–3.4) across, $2.0 \mu\text{m} \pm 0.19$ (1.7–2.3) high, 1.5 ± 0.10 (1.4–1.7) in width/height ratios; shape varies from rounded to flattened anteriorly, but mostly broadly rounded. DGO close to spear base. Metacarpus oval, $11.6 \mu\text{m} \pm 0.66$ (10.6–12.5) long, $8.1 \mu\text{m} \pm 0.66$ (7.3–9.6) across, 1.4 ± 0.12 (1.2–1.6) in length/width ratios, occupying about half of the corresponding body diameter; valve conspicuous, $53 \mu\text{m} \pm 2.5$ (47–56) from anterior body end, or $65.9\% \pm 6.86$ (49.3–72.8) of esophagus length. Esophageal basal lobe extending $150 \mu\text{m} \pm 18.8$ (114–169) from anterior body end, ventrally overlapping the intestine $70 \mu\text{m} \pm 17.9$ (40–90) or a distance of 4.4 times ± 1.2 (2.5–6.0) the corresponding body diameter. Esophageal nuclei usually line up. Excretory pore at $104\% \pm 11.0$ (81–115) of esophageal length; body annuli at metacarpus $1.4 \mu\text{m} \pm 0.12$ (1.1–1.5) wide. Hemizonid flat, same level or immediately anterior to excretory pore.

Testis outstretched, with spermatogonia in double or triple rows, $208 \mu\text{m} \pm 21.6$ (165–244) ($n=25$) long. Spicule arch-shaped, slightly longer than spear; gubernaculum crescent-shaped. Bursal alae $39 \mu\text{m} \pm 2.8$ (32–43) long, 3.9 times ± 0.4 (3.4–4.5) of anal body diameter. Tails' terminal cuticle $6.2 \mu\text{m} \pm 0.93$ (4.3–7.9) thick, ending in finely rounded terminus (Fig. 2 M); phasmids $10.6 \mu\text{m} \pm 2.14$ (7.3–13.9) behind anus or $13.3 \mu\text{m} \pm 2.51$ (9.2–17.2) from tail tip.

Lateral fields $3.8 \mu\text{m} \pm 0.37$ (3.0–4.3) wide, i.e. $24.0\% \pm 2.00$ (21.8–27.3) of widest body diameter.

Type specimens

Holotype female (slide no. T32-1) and 136 *paratype* females and 16 *paratype* males (slides nos. T32-2–T32-39) are deposited in the Herbarium and Insect Museum of the National Institute of Agro-Environmental Sciences. Nine *paratype* females and a *paratype* male (slides nos. T32-40, T32-41) and 9 *paratype* females and a *paratype* male (slides nos. T32-42, T32-43) are deposited in the University of California Davis Nematode Collection (UCDNC), Davis, CA, U.S.A. and Department of Nematology, Rothamsted Experimental Station, Harpenden, Herts., England, respectively.

Type host and locality

Specimens collected from rhizosphere of *Chrysanthemum morifolium* RAM. 'Tabiji' at Touganji, Takachiho, Miyazaki Prefecture, Japan by Mr. Nobuyuki INOUE in November, 1988.

Other hosts

Artemisia Feddei LEV. et VAN. (at Tarami-machi, Nagasaki, collected in January, 1991 by T. MIZUKUBO).

Diagnosis and relationships

Pratylenchus pseudocoffeae n. sp. is characterized by its 2- or 3-lip annuli, a vulva of 78.5–82.2%, presence of male, oblong spermatheca, differentiated long post-uterine branch ($U=0.9-2.2$), and generally rounded or subhemispherical smooth tail terminus. There are only 2 species with 2-lip annuli, spear 15–18 μm long, V-value greater than 79% in mean, smooth tail tip and oblong spermatheca with spermatozoa, i.e. *P. coffeae* and *P. loosi*. The new species is distinguished from the others by the high frequency of 3-lip annuli (observed in one third of individuals examined), and divided pattern in *en face* view (Group 2 of CORBETT and CLARK (1983)) by SEM (according to CORBETT and CLARK (1983), *P. coffeae* and *P. loosi* belong to Group 1 with no division between sub-median and lateral sectors). The new species specifically distinguished from *P. loosi* LOOF, 1960 by the shape of the tail tip (more or less rounded vs. finely pointed) and lower V-value (78.5–82.2% (80.4%) vs. 79–85% (82.5%)). *Pratylenchus pseudocoffeae* n. sp. is practically separable from a *Pratylenchus* sp. from Kumamoto (a sample used in the statistics) by the longer spear and the glandular esophageal basal lobe ventrally over-lapped intestine.

GOTOH (1974) denoted an unidentified species taken from chrysanthemum in Nagasaki as "*Pratylenchus* sp. 2", which had 2- or 3-lip annuli (percentage of 3 lip annuli was not described), oblong spermatheca and long post-uterine branch. I felt that it was difficult to compare *P. pseudocoffeae* n. sp. to the latter, since no drawing and only a few dimensions are available. Regrettably, the specimens left are of no use due to heavy coloration and distortion caused after losses of mounting medium. "*Pratylenchus* sp. 2" may represent another species since it differs from *P. pseudocoffeae* n. sp. in the lower V-value (78.0% (75.0–80.2)), longer post-uterine branch ($U=2.2$ (1.8–2.6)) and acute tail termini that often resemble those of *P. vulnus*.

Remarks

In the previous report (MIZUKUBO, 1992), I have showed that *P. coffeae* populations from chrysanthemum or other composite plants in Japan are morphologically and

morphometrically excluded from true *P. coffeae*. Furthermore, I have observed that *P. coffeae* collected from maize in Nagano (with an evident plain first lip annule) failed to reproduce on chrysanthemum. Although it is difficult at present to reach a conclusion on the difference in the host specificity between *P. coffeae* s. str. and other species group, including *P. pseudocoffeae* n. sp., unless carefully designed experimental studies are conducted, these facts suggest that *P. coffeae*, hitherto reported universally as being parasitic to the root of chrysanthemum, may be distinguishable from *P. coffeae* s. str.

There had been some records of *P. coffeae* from chrysanthemum in U.S.A. and Barbados (in SIDDIQI, 1972) and in India (RASHID and KHAN, 1976). *Pratylenchus chrysanthus* from chrysanthemum in India (EDWARD et al., 1969), which has been taken as *species inquirenda* since LOOF (1978), is also a population resembling *P. coffeae*. Morphometric comparison of such characters as *V*-value, spear length, spear knob width, and DGO of a population described by RASHID and KHAN (1976) with Japanese *P. coffeae* complex suggests that *P. coffeae* of RASHID and KHAN is independent of any groups within the Japanese *P. coffeae* complex. *P. chrysanthus* sp. inq. cannot be compared with Japanese *P. coffeae* complex due to its insufficient description, but its acute tail terminus indicates its relationship to *P. loosi* (as suggested by LOOF (1978)) and GOTOH's *Pratylenchus* sp. 2.

It is desirable to re-examine the world *P. coffeae* populations, especially those reproducing on chrysanthemum, with respect to the surface morphology of the first lip annule or their host range properties.

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