〔SHORT COMMUNICATION〕

Pictogram Analysis of Spear Length, Lip Region Diameter and Tail Morphology in Cohabiting *Pratylenchus penetrans* and *P. vulnus* (Tylenchida: Pratylenchidae)

Takayuki MIZUKUBO

日本線虫研究会誌第20巻別刷
1990年12月

Reprinted from
Japanese Journal of Nematology Vol. 20
December 1990

〔SHORT COMMUNICATION〕

Pictogram Analysis of Spear Length, Lip Region Diameter and Tail Morphology in Co-habiting *Pratylenchus penetrans** and *P. vulnus* (Tylenchida : Pratylenchidae)

Takayuki MIZUKUBO**

Most diagnostic characters for the species of *Pratylenchus* are more or less variable[1, 3, 6, 7, 8, 9, 10, 12, 13, 14]. However, it was demonstrated that spear length and V-values are less variable within a population[10, 14], among the progenies of a single gravid female[7, 9, 12, 13], under the different environmental factors[7, 10, 13] and among the different geographic populations[8]. It is often the case that even such biometrics as spear length and lip width are so similar and their variation so overlapping that a single biometrical character seems not to have absolute diagnostic value. The mechanical reliance on a morphometrical analysis or on straight diagnostic characters is, then, less effective than a purely intuitive approach based on the totality of characters as revealed by inspection. Specimens of *Pratylenchus* found in a soil sample from maple tree (*Acer* sp.) in Tarami-machi, Nagasaki contained some specimens obviously recognizable as *P. penetrans* and *P. vulunus*, but they showed bridged variability in spear length, lip width, body width, post-uterine branch length, V-value and tail tip shapes. It is valuable to demonstrate how the two species are separable from one another, since, through such a process, we can add information on the taxonomy of the species. A combination of characters usually permits the correct assignment of all seemingly intermediate specimens. In order to discriminate the coincident species properly, a pictogram, a type of multivariate scattergram, analysis was conducted.

MATERIALS AND METHODS

Specimens were killed by gentle heating, fixed in TAF and mounted in dehydrated glycerin by the method of SEINHORST[11]. Examination were made using an Olympus BH-2 light microscope equipped with a differential interference contrast system (NOMARSKI) at the highest magnification (× 2,340). Measurements were made using an eyepiece micrometer. For both spear length and head diameter, specimens were measured by five times without referencing previously made measurements. The coefficient of variability (CV) was calculated from the morphometrical data repeatedly obtained from the same specimen. Unless largely different (CV < 3.0), calculated means of these raw values were adopted as operational data. When two or more measurements deviated largely from the others, the specimen of question were eliminated. The above two numerical characters were plotted on a scatter diagram. Each individual was indicated by a different symbol which denoted a particular type of tail, and was plotted where the value of head diameter (read off the ordinate) intersected the value of spear length (read off abscissa). They were further marked by a numeral assignment corresponding to the actual tail shape (Figs. 1 and 2).

RESULTS AND DISCUSSION

Tail variation of all females observed are illustrated (Fig. 1). As it is difficult to express the tail shapes in words, the individual tails are referred to the intuitively determined six types. Tails oriented dorso-ventrally, though insufficient for the purpose, are also included in the classification. Type I (nos. 1-8) comprises those with notched tips (roughly correspond to "bluntly pointed" (blp) and "cleft" (cft) in the recent codes of FREDERICK & TARJAN[5]); Type II (nos. 9-14) comprises those with small projection on truncate or narrowly rounded tips, which often occur on the ventral sides of tails (roughly assigned to "digitate" (dgt), "finely pointed" (fnp), "truncate" (trc), "blp" and "smooth" (smo)); Type III (nos. 15-27) comprises those with more or less acute tips (may correspond to "blp" or between "fnp" and "blp"); Type IV (no. 28) assigned to that with constricted tip (actually correspond to "clavate"); Type V (nos. 29-33) comprises those with narrowly rounded tips (roughly assigned between "blp" and "subhemispherical" (shm)); Type VI (nos. 34-39)

**Pratylenchus penetrans* (COBB, 1917) has recently been synonymized with *P. pratensis* (DE MAN, 1880) by FREDERICK & TARJAN[5], although the author does not accept this revision.

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

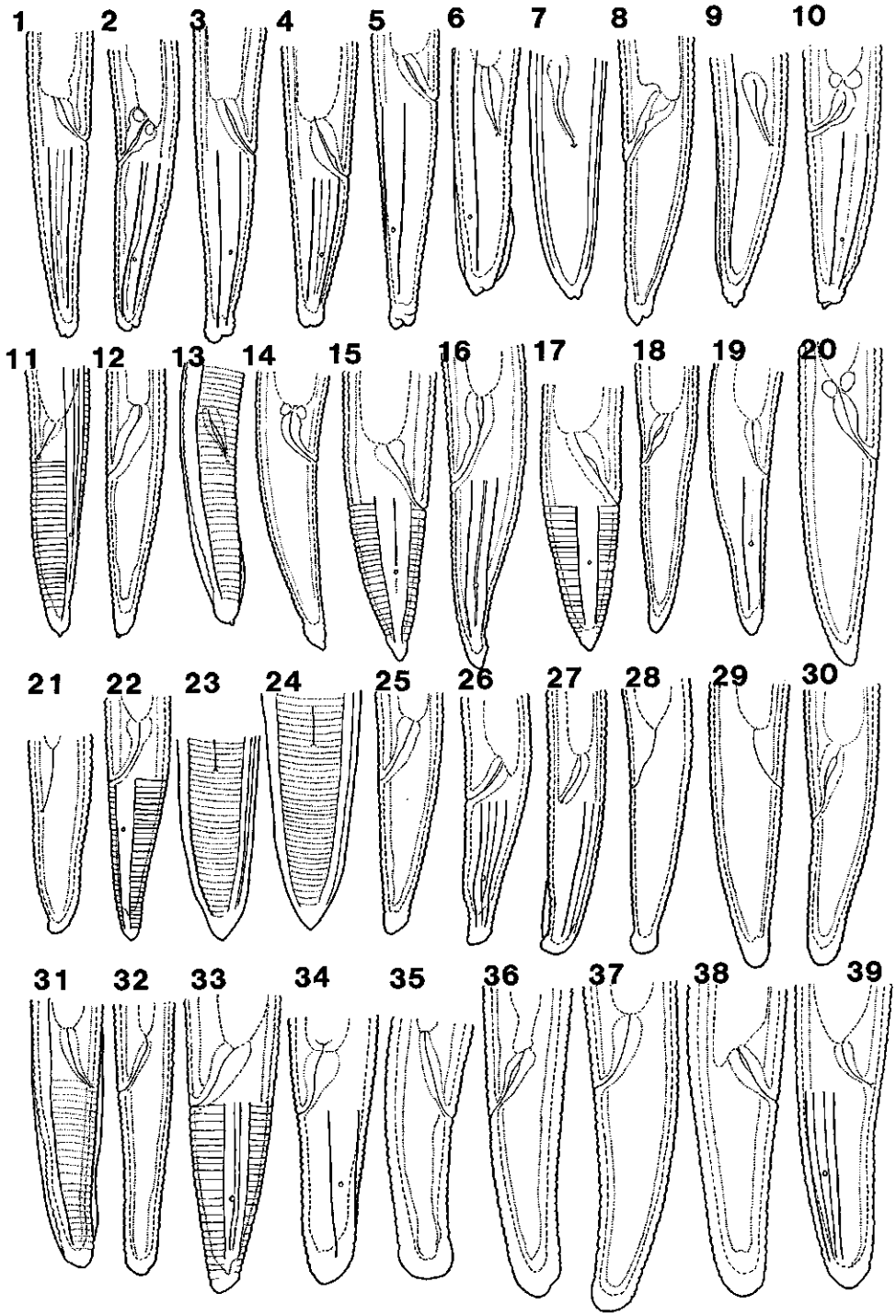

Fig. 1. Tails of females observed in a population. *P. vulnus* (1-33); *P. penetrans* (34-39). Tails assigned to the selected types as: 1-8 (Type I), 9-14 (Type II), 15-27 (Type III), 28 (Type IV), 29-33 (Type V) and 34-39 (Type VI). Numerals identify individual specimens.

comprises those of robust appearance with non-tapering ends (including both "shm" and "hemispherical").

The relationship between the spear length and lip region diameter is represented by a scatter diagram (Fig. 2). The spears of all specimens are short (14.6 μm in mean) for the genus and unusually variable (CV=5.8). The labial regions of the specimens are narrow (7.4 μm in mean) as well for the genus and fairly variable (CV=6.4). There is a very strong correlation between the two morphometrical values ($r=0.837$; n=39; $P<0.001$). A pictogram obtained for the specimens by incorporating tail morphology into the scatter diagram (Fig. 2), gives a good picture of the two clusters of the specimens, though they are not completely separable due to a linking individual (Fig. 1 no. 39). Obviously the individuals occupying the right-upper area in the pictogram (longer spears and wider lips) are accompanied solely by the tails of Type VI. Contrarily the chance distributions of the tails of Types I-V in the pictogram demonstrate that any one of the types do not define the particular clusters, rather they may be the variations within a cluster. It can be concluded that two clusters represent the two forms or species of *Pratylenchus* observed in the specimens. Comparison of dimensions between these forms shows the differences in post-uterine branch (PUB) structures and spermatheca condition and tail annules in addition to a-value and PUB length (Table 1). The first form (the upper right cluster comprising six individuals) agrees with *P. penetrans,* which have apparent stout body (a=25 (23-27)), shorter PUB (24 (18-31) μm) without cellular structures, rounded massive spermatheca filled with smaller spermatozoa and fewer number of tail annules (19 (17-24)). Contrarily the second form (the remainder comprising 33 individuals) may fall into the category of *P. vulnus* ALLEN & JENSEN, 1951[2], possessing slender body (a=32 (27-39)), longer PUB (32 (26-52) μm) with cellular structures, oblong spermatheca often filled with larger sper matozoa, larger number of tail annules (24 (20-30)). These observations confirm the mixture of two distinct species in the specimens. Each species separated by this analysis showed reasonable range and CV in the spear length and lip width measure-

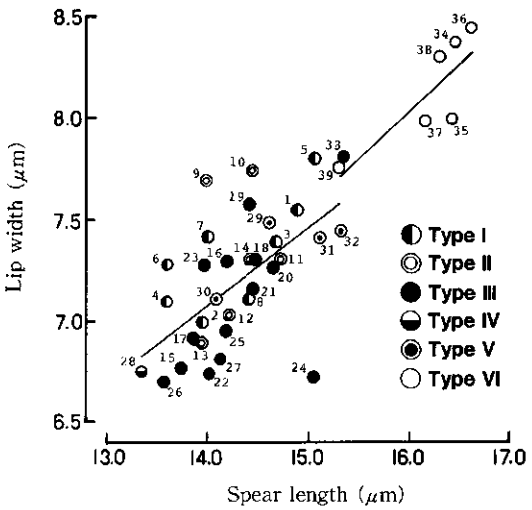

Fig. 2. Pictogram showing distribution of the six tail types on a scatter diagram of spear length and lip width. Numerals identify individual specimens.

ments (Table 1), and between these biometrics, there was no significant correlation ($r=0.798$, n=6; $0.05<P<0.10$) for *P. penetrans* and very strong correlation ($r=0.571$; n=33; $P<0.001$) for *P. vulnus.* The V-values of the two species are similar (Table 1), but the range of the values are wider (76-83 % or CV=2.2) in *P. vulnus* than in *P. penetrans* (80-83 % or CV=1.2). Although in both species the constituents of ratio-V (L and VL) are very strongly correlated (Fig. 3), results in this particular case suggest some difference between the regression lines for the species: regression line of *P. penetrans* passes near the origin, while that of *P. vulnus* passes fairly below the origin. This may account for the higher variability of V-values in *P. vulnus* than in *P. penetrans.*

The main differences in the present population of *P. vulnus* from populations from United States are the much narrower head (7.2 (6.7-7.8) μm vs. 8.4-8.7 (7.8-9.0) μm[10]), shorter body (468 (374-562) μm vs. 620 (500-750) μm) and dorso-ventrally flattened tail. These represent additional new information on the variability of *P. vulnus.*

This study shows the efficacy of the combination of spear length and lip width in this particular case. However, it is unknown if such a combination

can be generally used in separation of the species of this genus, nor is it certain that the ratio given by spear length divided by lip width is a usable diagnostic character. Although many authors commonly pointed out that the spear length of the *Pratylenchus* is less variable, we must pointed out that most of the authors referred to the variability within a population or progenies from a single gravid female, and again it is dangerous to compare directly the measurements between published data, as there is considerable variation in measurements of a single specimen even among experienced

Table 1. Comparison of measurements of *Pratylenchus vulnus* and *P. penetrans* found in a soil sample from Nagasaki, Japan.

Criteria	*P. vulnus*			*P. penetrans*		
	Mean	SD	Range	Mean	SD	Range
female						
n		30			6	
L (μm)	468	38.4	374-562	498	42.9	445-560
a	32.2	2.69	27.4-38.8	25.3	1.34	23.3-27.0
b	6.3	0.59	5.3-7.5	6.5	0.69	5.7-7.6
b′	4.2	0.46	3.5-5.1	4.3	0.36	3.8-4.7
c	20.3	2.22	14.5-25.6	18.8	1.25	17.6-21.1
c′	2.6	0.40	1.9-3.4	2.3	0.15	2.1-2.5
V (%)	79.6	1.77	75.9-82.6	81.3	0.98	80.2-82.6
V′ (%)	83.7	1.72	80.4-86.5	85.9	0.89	84.7-87.2
VL (μm)	373	35.2	290-454	405	33.9	368-455
spear (μm)	14.3	0.54	13.4-15.3	16.2	0.44	15.3-16.5
m	49.9	1.60	45.7-53.8	51.0	1.37	49.0-52.6
lip width	7.2	0.32	6.7-7.8	8.2	0.26	7.8-8.4
knobs width/height	1.5	0.1	1.3-1.8	1.7	0.16	1.5-1.9
esophagus (μm)	75	7.1	63-89	77	4.2	74-85
MB (%)	70	4.8	59-79	66	2.6	63-71
excr. pore (μm)	76	3.9	67-83	80	5.1	71-85
Vulva-anus (μm)	72.1	7.8	57-85	67	7.7	54-76
tail (μm)	23.2	2.40	20-30	26.6	3.12	23-31
tail/V-a (%)	33	5.1	25-45	40	3.0	36-43
post-ut. branch (μm)	32	3.8	26-52	24	7.2	18-38
U	2.4	0.38	1.8-3.1	1.3	0.27	1.1-1.8
G2 (%)	6.9	1.01	5.6-9.0	4.8	1.22	4.0-7.2
tail annules	24.1	2.53	20-30	18.3	0.96	17-19
male						
n		9			1	
L (μm)	436	27.7	407-482	-	-	503
a	34.9	1.76	31.4-36.8	-	-	29.9
b	6.1	0.48	5.6-7.0	-	-	5.8
b′	4.1	0.36	3.5-4.7	-	-	4.3
c	19.6	1.42	17.6-22.6	-	-	21.8
c′	2.7	0.24	2.3-3.1	-	-	2.2
spear (μm)	13.6	0.32	13.2-14.0	-	-	14.5
lip width	6.3	0.19	6.0-6.6	-	-	7.0
m (%)	52.4	1.35	50.0-54.2	-	-	51.8
spicule (μm)	15.1	0.46	14.5-15.5	-	-	16.5
gubernaculum (μm)	5.2	0.71	4.0-5.9	-	-	5.9
spicule/spear	1.11	0.05	1.04-1.18	-	-	1.14

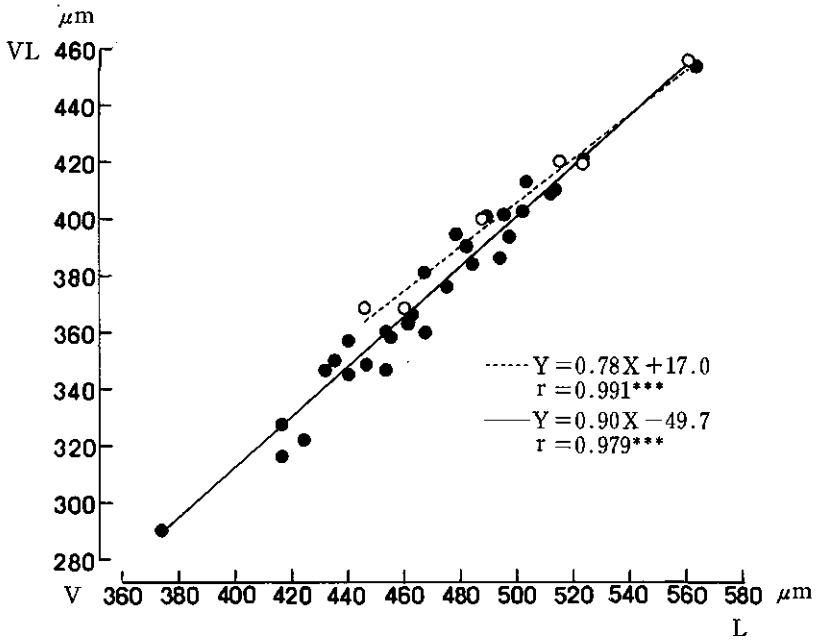

Fig. 3. Graphic representation of ratio V. L: body length; VL: head to vulva distance; (●): *Pratylenchus vulnus*; (○): *P. penetrans*; y=f(x): equation of the regression lines; r: coefficient of correlation; ***: (significant at 0.1 % level).

observers[4]. Labial region diameter has been seldom used in diagnosis nor studied comparatively with respect to variability, probably because of the relatively short distance. ROMAN & HIRSCHMANN[10] noticed that ranges for dimensions in lip width did not overlap between *P. coffeae* (narrower) and *P. vulnus* (wider), while the present results show a much narrower lip for *P. vulnus* which may overlap with that of *P. coffeae*. With the intraspecific variability of spear length, lip width and their combined property, it will be necessary to show how far they vary among the conspecific populations from different geographic sources.

LITERATURE CITED

1) ANDERSON, R. V. & TOWNSHEND, J. L. (1980) Can. J. Zool. **58**, 1336-1340.
2) ALLEN, M. W. & JENSEN, H. J. (1951) Proc. helm. Soc. Wash. **18**, 47-50.
3) CORBETT, D. C. M. & CLARK, S. A. (1983) Revue Nématol. **6**, 85-93.
4) FREDERICK, J. J. & TARJAN, A. C. (1978) Nematologica **24**, 476-478.
5) FREDERICK, J. J. & TARJAN, A. C. (1989) Revue Nématol. **12**, 243-256.
6) LOOF, P. A. A. (1960) Tijdschr. Plantenziekten 66: 29-90.
7) OLOWE, T. & CORBETT, D. C. M. (1984) Indian J. Nematol. **14**, 6-7.
8) OLOWE, T. & CORBETT, D. C. M. (1984) Indian J. Nematol. **14**, 30-35.
9) OLOWE, T. & CORBETT, D. C. M. (1984) Indian J. Nematol. **14**, 97-101.
10) ROMAN, J. & HIRSCHMANN, H. (1969) J. Nematol. **1**, 363-386.
11) SEINHORST, J. W. (1959) Nematologica **4**, 67-69.
12) TARJAN, A. C. & FREDERICK, J. J. (1978) J. Nematol. **10**, 152-160.
13) TARTE, R. & MAI, W. F. (1976) J. Nematol. **8**, 185-195.
14) TAYLOR, D. P. & JENKINS, W. R. (1957) Nematologica **2**, 159-174.

Accepted for publication: September 26, 1990.