Morphological and Statistical Differentiation of *Pratylenchus coffeae* Complex in Japan (Nematoda: Pratylenchidae)

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Japanese populations ascribed to *Pratylenchus coffeae* were comparatively investigated by scanning electron microscope (SEM) and with stepwise linear discriminant function analysis (SLDA). SEM observation of the first lip annule showed 2 different *en face* patterns among the 6 populations; 3 populations with undivided faces and the remainder with the face incised to demarcate sub-median and lateral sectors. SLDA was successful in discriminating 6 populations into 3 groups. This analysis indicated that Japanese *P. coffeae* populations could, at least, be divided into 3 distinct species.

Key words: Morphology, Taxonomy, *Pratylenchus coffeae* complex

INTRODUCTION

LUC (1987) discussed the *Pratylenchus* as “stenomorphic genus in which the species are difficult to separate because of the small number of characters diagnostic at species level, and because of the intraspecific variability of some of these characters”, accepting 60 valid species for the genus. Similar considerations may be shared by several taxonomists who accepted quite different numbers of valid species as: 45 spp. (RYSS, 1988), 46 spp. (LOOF, 1991), 49 spp. (FREDERICK and TARJAN, 1989), 63 spp. (HANDOO and GOLDEN, 1989) and 69 spp. (SIDDIQI, 1986). The difference in the numbers reflects, at least partially, the different number of synonymy, which indicates that the authors do not agree on the diagnostic characters and that they even assign various weights to the same diagnostic characters, which, in turn, suggests an uncertainty among the taxonomists as to the diagnostic characters for the species of the genus. Variability studies on the diagnostic characters made so far with *Pratylenchus* species exemplified the vast variability, within a species, of the many diagnostic characters influencing the reduction of a valid species. These situations call for careful examination of many characters, including those with current diagnostic values or those hitherto not considered when discriminating among closely allied species. Some nematode species have not been clearly differentiated by the evident gaps in the common diagnostic characters but were recognized by such minor characters that are normally overlooked in the routine comparisons. Such separation has also been justified in the taxonomy of Pratylenchidae nematodes (FORTUNER and MAGGENTI, 1991).

There are a few Japanese populations that show some character deviations from *P. coffeae* but that are not easily distinguished from each other because of the overlapping

variability of most diagnostic character-states. This suggests that either: 1) there is a vast variability in the characters among *P. coffeae* populations, or 2) *P. coffeae* is a heterogeneous species complex. In the present work, the surface morphology of the first lip region in the 6 Japanese populations attributed to *P. coffeae* was studied first on a SEM and then the morphometrics of the 6 populations were examined using a multivariate technique in a search for objective differences between these populations.

MATERIALS AND METHODS

Specimens. Six populations of the root lesion nematodes have been studied (Table 1), including a Miyazaki population which was suggested to be a new species by MIZUKUBO et al. (1989) (population code 1), 3 populations from Nagasaki, Nagano and Okinawa (codes 2, 3 and 4), 2 populations from Saitama (code 5) and 1 population from Kumamoto (code 6). The code-5 population collected from upland rice fields had been continuously maintained for several years on upland rice or sweet potato in the greenhouse of the National Institute of Agro-Environmental Sciences, Tsukuba. Their surface features were examined to determine the effects of hosts on nematode morphology. For statistical analysis, the populations maintained on these plants were mixed, since there were no morphometric differences between them.

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 specimens that were killed by gentle heating, fixed in TAF and mounted in dehydrated glycerin by the quick method of SEINHORST (1959). The specimens from Kumamoto were mounted in glycerin by the slow glycerol-ethanol method devised by MINAGAWA (unpublished) which is similar to the GOLDEN's method described by HOOPER (1970), except that SEINHORST's solution I was used instead of formalin-glycerol fixative. The specimens were observed and measured for all features at oil immersion 2,400 X on an Olympus BH-2 light microscope equipped with Normarski interference contrast.

Characters selected for analysis. Ten characters (Table 2) were selected for statistical analysis. Such characters as body length (L), esophagus length, ratio-a, ratio-b, ratio-b', spermatheca length/width ratio, ratio-c' and tail shape were not used in the analysis for the following reasons: 1) body length is largely affected by the fixation and mounting methods and is often affected by environmental conditions including host plants; 2) accurate esophagus length (and hence, ratio b) is difficult to obtain because

Table 1. Origin of the specimens

Code of population	n	Locality	Host
1	25	Takachiho, Miyazaki	<i>Chrysanthemum morifolium</i>
2	10	Tarami, Nagasaki	<i>Artemisia Feddei</i>
3	20	Iiyama, Nagano	<i>Zea mays</i>
4	25	Iriomote Is., Okinawa	<i>Bambusa</i> sp.
5a	17	Kitamoto, Saitama	<i>Ipomoea batatas</i>
5b	4	Kitamoto, Saitama	<i>Oryza sativa</i>
6	25	Nishigoshi, Kumamoto	<i>Artemisia princeps</i>

Table 2. Variables used in the analyses, codes and character names

SPEAR:	Spear length
LIPW:	Lip region width
DGO:	Distance spear to DGO
EXPORE:	Position excret. pore
KNOBW:	Spear knobs width
V:	Ratio V
C:	Ratio c
U:	Ratio U (post. ut. length divided by vaginal body width)
LIPANN:	Number of lip annuli
TANN:	Number of tail annuli

of difficulty in determining esophago-intestinal junction; 3) ratio-a differs between mature (pregnant) and immature females and is subject to preparation artifact; 4) ratio-b' is affected by the length of glandular esophageal basal lobe which is considered to be influenced by the host plant; 5) spermatheca of some specimens are often empty and are insufficient for the measurements; 6) ratio-c' is highly variable and is affected by maturity and preparation artifact as in ratio-a; 7) tail shapes were considerably different among populations but are difficult to be coded for statistical purposes.

Statistical analysis. The data were analyzed with the aid of the statistical package Seto/B of Kyoritsu Shuppan Co., Ltd. on a NEC PC-9801 series personal computer. Raw measurements or values were used as descriptive statistics, but the data on LIPANN were modified before application, weighing them as "2" for 2-lip annuli on both side, "5" for 2 and 3 annuli on either sides and "10" for 3 annuli on both sides. Then, Stepwise Linear Discriminant Function Analysis (SLDA) and Canonical Discriminant Function Analyses (CDFA) were performed, using Seto/B programs DISC34 for the analyses and display of the data. In the SLDA, the program DISC34 generates a statistics according to WILKS' lambda criterion (WILKS, 1932) and a variance ratio or *F* statistics calculated according to a formula introduced by RAO (1951), which will be mentioned in the following section on results. These were elegantly accounted by RAO (1973, in p. 556) and were further summarized by TANAKA et al. (1984, in p. 113), which is roughly followed below.

WILKS' lambda statistics is generated to diagnose the total difference among groups in the SLDA. Considering *g*, *q*-variate populations, this statistics is defined as a ratio of the total sum of squares and products matrix (*T*) and the within-groups sum of squares and products matrix (*W*):

$$T = \sum_{k=1}^g \sum_{i=1}^{n_k} (x_i^{(k)} - \bar{x})(x_i^{(k)} - \bar{x})', \left(\bar{x} = \frac{1}{n} \sum_{k=1}^g \sum_{i=1}^{n_k} x_i^{(k)} \right)$$

$$W = \sum_{k=1}^g \sum_{i=1}^{n_k} (x_i^{(k)} - \bar{x}^{(k)})(x_i^{(k)} - \bar{x}^{(k)})'$$

$$A = |W|/|T|$$

The lambda distribution can be approximated as a variance ratio [*F*]:

$$F = \frac{f_2}{f_1} \frac{1 - A^{1/c}}{A^{1/c}}$$

on f_1 and f_2 degrees of freedom, where

$$\begin{aligned} f_1 &= qa; f_2 = bc + 1 - qa/2; \\ a &= g - 1; b = n - 1 - (q + g)/2; \\ c &= \begin{cases} \{(q^2 a^2 - 4)/(q^2 + a^2 - 5)\}^{1/2}, & (q^2 + a^2 \neq 5) \\ 1, & (q^2 + a^2 = 5) \end{cases} \end{aligned}$$

Hence, the lambda is ranging from 0 to 1, and small lambda close to 0 was successful in discriminating the groups. The null hypothesis that no differences exist between groups, or that q variables do not contribute to the discrimination, can be tested using the F distribution.

RESULTS

SEM observation

Surface structures obtained using the SEM were studied (Fig. 1 A–L). The morphology of the first lip annule of specimens taken from Nagano (Fig. 1 A), Okinawa (Fig. 1 B) and Saitama (Fig. 1 C, D) were identical to those shown for *P. coffeae* by MOMOTA (1978) and CORBETT and CLARK (1983): there is no differentiation in the first lip annule and it can be referred to Group 1 (CORBETT and CLARK, 1983) and further referred to 'i)' of Group 1 (BAUJARD et al., 1990). The Group 1 of CORBETT and CLARK is characterized as "a plain undivided face with no division between sub-median and lateral segments, presumably due to fusion of the first head annule to the oral disc". The subdivision 'i)' of the Group 1 by BAUJARD et al. (1990) is designated for the condition that the first cephalic annule is without any differentiation along with conditions 'ii)' and 'iii)', which show slight differentiation around the oral disc. The face pattern was not affected by different host plants; the Saitama populations maintained on upland rice (Fig. 1 C) and on sweet potato (Fig. 1 D) did not show any difference in their head annuli.

Specimens from Miyazaki (Fig. 1 E) and Nagasaki (Fig. 1 F) had the first lip annule with a division between sub-median and lateral sectors. This pattern was identical to Group 2 of CORBETT and CLARK (1983), which was characterized as "outer part of the face subdivided into sub-median and lateral sectors. . . . submedian segments are fused to the oral disc and are narrower at their inner extremity widening toward the outer edge of the face". The face view of the specimens from Kumamoto, which will be described in detail by MINAGAWA, was identical to the Group 2 of CORBETT and CLARK (1983) as well.

Number of lip annuli was 2 (Fig. 1 G–L) in the lateral aspects of the specimens examined. The occurrence of 3-lip annuli was not ascertained in this observation.

Correlation and normality of the characters

The matrix of correlations for all the characters in all populations was studied but is not shown here to save space. Modes of correlation between these characters were different among populations, although there was no significant correlation between the characters except between C and TANN. The C and TANN showed a relatively high negative correlation of $-0.6826 < r < -0.5712$ in the 5 populations except for a population from Okinawa with $r = -0.2921$. For 8 variables or characters, the ranges,

Fig. 1. Lip surface structures of 5 populations of *Pratylenchus coffeae* complex. A–D G–J: *P. coffeae*; E, F, K & L: *Pratylenchus* sp. A & G: Nagano (from maize); B & H: Okinawa (from bamboo); C & I: Saitama (from up-land rice); D & J: Saitama (from sweet potato); E & K: Miyazaki (from chrysanthemum); F & L: Nagasaki (from *Artemisia Feddei*). (Scale bars = 3 μ m)

means, SDs and 95% confidence ranges were compared among the 6 groups (Fig. 2), which roughly showed the statistical structure of the characters. Most characters, with a few exceptions, showed normality and each population was considered to be monospecific through the Principal Component Analysis. To save space, analysis data is not given here.

Multivariate analyses

At first, the SLDA was performed on all the selected variables of the 6 populations.

Fig. 2. Comparisons of morphometrical characters among 6 populations of *Pratylenchus coffeae* complex. Boxes: standard deviation; Bars: 95% confidence ranges. Codes: 1: Miyazaki; 2: Nagasaki; 3: Nagano; 4: Okinawa; 5: Saitama; 6: Kumamoto.

The F statistics for each variable in the final model is shown in Table 3. When the partial F statistics of a variable is greater than the given F statistics at 5% level, the variable contributes significantly to the discrimination of the groups. At this step, each variable had the partial F with d.f.=(5, 111). This indicated that all variables, except C, contributed significantly (at the 5% level) to the discrimination of the groups because the F statistics of variables (except C) were greater than 2.329 ($F_{111}^5(0.05) < F_{80}^5(0.05) = 2.329$). The F statistics of C was as small as 1.3894 and did not contribute to the discrimination. The total group differences were diagnosed by Wilks' lambda statistics =0.0103991 or $F=19.8871$ with d.f.=(45, 504). This was significant at 1% level since the Wilks' lambda statistics was as small as 0.0103991 and was tested as: $F_0=$

Table 3. Discriminant function in the 6 populations of *P. coffeae* complex

Group	Partial F	1	2	3	4	5	6
SPEAR	36.7374	40.5380	38.7549	42.9409	43.5498	38.5581	29.2335
DGO	30.8731	12.4401	15.8872	21.0018	19.7886	25.5440	19.3785
KNOBW	9.9773	81.8626	76.3148	85.9229	90.7607	82.6613	78.3976
EXPORE	8.4400	-0.4861	-0.5466	-0.7892	-1.1336	-0.7286	-0.2425
U	7.4409	-42.7229	-45.8988	-44.8060	-42.6919	-44.7957	-34.5228
LIPW	5.6492	63.1661	69.8831	67.1848	66.4557	64.9411	69.4848
TANN	5.3073	2.8339	2.4522	2.9354	2.7953	3.4573	2.9213
V	4.5653	56.4066	56.6525	56.2566	55.8217	56.5051	54.8094
LIPANN	3.4937	-5.9128	-5.9803	-6.6343	-6.7807	-6.4656	-4.9484

Table 4. Results of discrimination among 6 populations of *P. coffeae* complex

Code of specimens	No. of cases	No. correct	Percent correct	Number of cases classified					
				1	2	3	4	5	6
1	25	22	88.0	22	3	0	0	0	0
2	10	8	80.0	2	8	0	0	0	0
3	20	16	80.0	0	0	16	3	1	0
4	25	22	88.0	0	0	3	22	0	0
5	21	21	100.0	0	0	0	0	21	0
6	25	25	100.0	0	0	0	0	0	25

Table 5. Canonical variates in the populations of *P. coffeae* complex

Variate	CAN1	CAN2	CAN3	CAN4	CAN5
SPEAR	1.9048	-0.3352	0.0953	-0.1853	0.0980
DGO	0.0791	2.5599	-0.6610	-0.0659	0.6427
KNOBW	1.3473	0.8428	2.4400	1.0542	0.1158
EXPORE	-0.0971	-0.0659	-0.0843	0.0445	0.1862
U	-1.2555	0.0578	1.8622	0.7663	0.7817
LIPW	-0.4627	0.4163	0.4391	-3.1899	0.2983
TANN	-0.0028	0.1118	-0.1021	0.2886	0.0957
V	0.1746	-0.0827	-0.4024	0.0499	-0.1648
LIPANN	-0.2373	-0.1087	0.0207	0.0345	0.0935
constant	-35.3302	2.4609	25.6618	8.3411	-11.9105

$19.8871 > F_{240}^{40}(0.01) = 1.677 > F_{504}^{45}(0.01)$. The result (Table 4) showed that the discrimination by the 9 characters was incomplete between G1 and G2, G3 and G4, and G3 and G5, while G5 and G6 were fully discriminated. The CDFA-mediated canonical structures (Table 5) of all the specimens were plotted on the CAN1 (X) and CAN2 (Y) axes (Fig. 3). These results and observations of *en face* morphology suggested that the 6 populations could be classified into 3 taxonomic groups, and this idea was tested as follows:

Six groups represented by each population were combined into 3 groups: 1 and 2 into G1 ($n=35$); 3, 4 and 5 into G2 ($n=66$); and 6 into G3 ($n=25$); the SLDA and CDFA analyses were performed. The F statistics for each variable in the final model by SLDA is given in Table 6. Variables at this stage had the partial F with d.f. = (2,

Fig. 3. Plot of the 6 populations of *Pratylenchus coffeae* complex using 9 selected variables. Symbols represent populations: $\times$ = 1, $+$ = 2, $\square$ = 3, $\triangle$ = 4, $*$ = 5, $\circ$ = 6. CAN1, CAN2: Canonical discriminant functions 1 and 2.

Table 6. Discriminant function in the 3 groups of *P. coffeae* complex

Group	Partial F	G1	G2	G3
SPEAR	61.1625	38.2095	40.3940	30.6045
DGO	64.6728	23.1795	31.6005	26.1687
KNOBW	10.1817	89.1303	95.5239	90.9743
U	18.9656	-25.5290	-25.3299	-14.9761
V	11.4415	55.7475	55.3833	53.7283
EXPORE	11.3287	0.1586	-0.1640	-0.4364
LIPANN	9.3762	-5.2810	-5.9721	-4.3180

Table 7. Results of discrimination among 3 groups of *P. coffeae* complex

Code of group	No. of case	No. correct	Percent correct	Number of cases classified		
				1	2	3
G1	35	35	100.0	35	0	0
G2	66	66	100.0	0	66	0
G3	25	25	100.0	0	0	25

116). This indicated that 7 characters (SPEAR, DGO, KNOBW, U, V, EXPORE and LIPANN) contributed significantly at the 5% level to discriminate the 3 groups as these F statistics were greater than 3.111 ($F_{116}^2(0.05) < F_{80}^2(0.05) = 3.111$). The total group differences were diagnosed by the WILKS' lambda statistics to be as small as 0.0506102 or $F=57.5823$ with d.f.=(14, 234). This was significant at 1% level as $F_0=57.5823 > F_{120}^{12}(0.01)=2.336 > F_{234}^{14}(0.01)$. The population discrimination by using the 7 characters was successfully adopted for all the groups (Table 7). The C DFA-mediated canonical structures (Table 8) of all the specimens were plotted on CAN1 (X) and CAN2 (Y) (Fig. 4). The results verified the assumption that the 6 populations were successfully classified into 3 taxonomic groups.

DISCUSSION

Practical identification

The morphometrics of 6 Japanese populations are given in Table 9. This study has suggested that it is possible to characterize representative samples of 3 taxonomic groups in Japan by a combination of the following 7 characters (given with mean value):

SPEAR: spear length (G1: 16.0–16.4 μm ; G2: 15.9–17.0 μm ; G3: 14.1 μm);

Table 8. Canonical variates in the 3 groups of *P. coffeae* complex

Variate	CAN1	CAN2
SPEAR	1.5480	−0.3781
DGO	0.9649	2.1513
KNOBW	0.7894	1.5859
U	−1.6003	1.2218
V	0.2509	−0.3042
EXPORE	−0.0977	−0.0386
LIPANN	−0.2373	−0.0416
constant	−38.7370	20.1419

Fig. 4. Plot of the 3 groups of *Pratylenchus coffeae* complex using 7 selected variables. Symbols represent groups (populations): $\times$ = G1 (1 and 2), $+$ = G2 (3, 4 and 5), $\square$ = G3 (6). CAN1, CAN2, Canonical discriminant functions 1 and 2.

Table 9. Measurements of 6 populations of *Pratylenchus coffeae* complex in Japan

Criteria	Locality	G1		G2		G3	
		Miyazaki	Nagasaki	Nagano	Okinawa	Saitama	Kumamoto
females (<i>n</i>)		40	10	20	25	21	25
L (μm)		554:59.9 ^a (438–658) ^b	471:56.1 (363–544)	524:36.2 (448–585)	479:37.3 (405–532)	511:41.8 (458–583)	534:43.3 (452–653)
a		29.9:2.19 (25.2–34.5)	25.1:2.54 (22.1–29.7)	26.4:2.5 (22.2–30.4)	27.1:1.69 (24.2–30.6)	25.4:2.32 (20.3–30.0)	25.3:2.66 (19.3–29.7)
b		6.3:0.71 (5.2–8.0)	5.2:0.50 (4.1–5.9)	6.5:0.45 (5.8–7.3)	6.0:0.39 (5.2–6.7)	6.2:0.57 (5.0–7.5)	6.7:0.70 (5.7–7.8)
b'		3.1:0.37 (2.3–3.8)	3.0:0.38 (2.6–3.9)	4.5:0.51 (3.0–5.4)	3.7:0.37 (2.8–4.2)	3.7:0.29 (3.3–4.2)	4.5:0.42 (3.6–5.5)
c		19.4:1.41 (16.7–22.2)	19.1:1.04 (17.3–20.3)	18.6:1.4 (16.7–21.7)	18.8:1.68 (16.0–23.6)	18.4:1.93 (15.7–22.5)	19.5:1.73 (16.6–23.2)
c'		2.3:0.21 (2.0–2.8)	2.0:0.25 (1.7–2.6)	2.3:0.23 (2.0–2.8)	2.3:0.19 (2.0–2.7)	2.2:0.26 (1.6–2.7)	2.3:0.23 (1.9–2.8)
V (%)		80.4:0.89 (78.5–82.2)	80.8:1.28 (78.7–82.4)	79.7:1.1 (77.4–82.1)	79.0:1.24 (75.7–81.1)	80.1:1.19 (77.7–82.5)	78.3:1.69 (73.6–81.1)
V' (%)		84.8:0.96 (82.2–87.0)	85.3:1.27 (83.4–86.8)	84.3:1.1 (81.9–86.1)	83.4:1.33 (79.7–85.8)	84.8:1.25 (81.9–87.3)	82.5:1.71 (77.8–85.5)
VL (μm)		445:47.3 (354–528)	381:47.7 (293–448)	419:31.7 (347–469)	379:30.8 (318–417)	409:34.4 (365–475)	418:37.4 (333–513)
spear (μm)		16.4:0.46 (15.5–17.8)	16.0:0.84 (14.9–17.2)	17.0:0.4 (16.2–17.8)	16.9:0.50 (15.8–17.8)	15.9:0.40 (15.2–16.5)	14.1:0.47 (13.2–14.9)
m (%)		50.3:1.65 (46.9–55.6)	50.4:1.62 (48.6–53.3)	49.7:1.45 (46.2–52.0)	50.9:1.21 (48.1–52.9)	50.2:1.45 (48.0–53.2)	50.7:1.44 (47.6–54.6)
DGO (μm)		1.8:0.39 (0.7–2.3)	2.3:0.34 (1.7–2.8)	2.8:0.30 (2.3–3.3)	2.6:0.33 (2.0–3.3)	3.3:0.30 (2.6–4.0)	2.5:0.38 (1.7–3.3)
esophagus (μm)		88:9.1 (73–119)	91:6.7 (79–103)	81:6.5 (68–94)	80:5.1 (73–89)	83:7.3 (63–94)	80:6.2 (70–93)
excre. pore (μm)		88:5.4 (76–98)	85:5.0 (78–90)	81:6.5 (68–94)	81:3.0 (76–89)	81:4.5 (72–88)	82:4.9 (70–90)
vulva-anus (μm)		80:11.3 (59–104)	65:8.2 (51–76)	78:5.6 (67–87)	75:8.0 (63–90)	74:9.3 (55–95)	88:9.9 (69–110)
tail (μm)		29:3.4 (22–36)	25:3.0 (19–28)	28:2.6 (23–33)	26:2.8 (21–30)	28:2.7 (22–34)	28:2.4 (24–32)
spermatheca L/W		2.4:0.46 (1.6–3.1)	1.9:0.58 (1.1–2.6)	2.1:0.53 (1.3–3.3)	1.6:0.29 (0.9–2.0)	1.4:0.37 (1.1–2.6)	2.6:0.92 (1.4–5.3)
post-ut. branch (μm)		28:5.0 (17–44)	25:4.8 (16–31)	26:4.2 (20–34)	26:3.9 (19–35)	27:4.0 (20–37)	41:9.8 (25–76)
U (%)		1.7:0.28 (0.9–2.2)	1.4:0.20 (1.1–1.8)	1.5:0.27 (1.0–2.0)	1.6:0.22 (1.2–2.2)	1.5:0.21 (0.8–1.8)	2.1:0.44 (1.1–3.4)
G2 (%)		5.0:0.86 (3.0–7.0)	5.4:0.9 (4.2–7.3)	4.9:0.8 (3.8–6.2)	5.4:0.7 (4.3–7.6)	5.3:0.7 (3.6–6.7)	7.6:1.5 (4.2–12.6)
tail annuli		20:1.8 (16–24)	18:2.5 (16–23)	21:1.9 (18–25)	21:2.2 (17–26)	23:3.5 (15–32)	22:2.9 (17–29)
knobs width (μm)		3.7:0.25 (3.3–4.3)	3.4:0.26 (3.0–3.8)	4.0:0.22 (3.6–4.5)	4.2:2.22 (3.6–4.6)	3.7:0.26 (3.0–4.1)	3.4:0.25 (3.2–4.3)
3-lip annuli (%)		34 (<i>n</i> =91)	30 (<i>n</i> =10)	11 (<i>n</i> =28)	8 (<i>n</i> =25)	5 (<i>n</i> =21)	48 (<i>n</i> =44)

^a mean: SD; ^b minimum-maximum.

DGO: distance of the dorsal gland opening to spear knobs (G1: 1.8–2.3 μm ; G2: 2.6–3.3 μm ; G3: 2.5 μm);

KNOBW: width of spear knobs (G1: 3.4–3.7 μm ; G2: 3.7–4.2 μm ; G3: 3.4 μm);

U: ratio-U (G1: 1.4–1.7; G2: 1.5–1.6; G3: 2.1);

V: ratio-V (G1: 80.4–80.8%; G2: 79.0–80.1%; G3: 78.3%);

EXPORE: distance from anterior end to excretory pore (G1: 85–88 μm ; G2: 81 μm ; G3: 82 μm);

LIPANN: percentage of 3-lip annuli (G1: 30–34%; G2: ca. 5–11%; G3: ca. 48%).

For practical identification, these characters can be assessed by comparing the data from the unknown sample with the values indicated above.

The position of the unknown specimens can be ascertained by using 2 functions, which were used for plotting (Table 8).

$$\text{CAN1} = (\text{SPEAR} \times 1.5480) + (\text{DGO} \times 0.9649) + (\text{KNOBW} \times 0.7984) - (\text{U} \times 1.6003) + (\text{V} \times 0.2509) - (\text{EXPORE} \times 0.0977) - (\text{LIPANN} \times 0.2373) - 38.7370$$

$$\text{CAN2} = -(\text{SPEAR} \times 0.3781) + (\text{DGO} \times 2.1513) + (\text{KNOBW} \times 1.5859) + (\text{U} \times 1.2218) - (\text{V} \times 0.3042) - (\text{EXPORE} \times 0.0386) - (\text{LIPANN} \times 0.0416) + 20.1419$$

Morphology of the first lip annule is thought to be a critical characteristic for separating groups 1 and 2, although the use of this character is not practical. Specimens from Kumamoto are also distinctive by having frequent dorsal overlap of the esophageal basal lobe (more than 50% of the individuals).

Systematic position of P. coffeae complex in Japan

The present SEM observations and statistical analysis strongly suggest that *P. coffeae* complex in Japan is composed of at least 3 independent species (G1, G2 and G3: Fig. 4). A population from Kumamoto is rather distinctive, with its shorter spear and higher frequency of dorsal overlap of esophageal basal lobe. However, differences between groups 1 and 2 are dependent on some minor characters which have not been used in the routine identification of the species. A different surface features of the first lip annule is the most critical and is suggested to be a constant feature not affected by different host plants. This study further demonstrated that specimens with different surface features of the first lip annule also showed differences in some small morphometrics. CORBETT and CLARK (1983) concluded that the *en face* pattern was less variable than the other diagnostic characters and was usable for *Pratylenchus* taxonomy. The present study supports their conclusion as far as *P. coffeae* is concerned. The *P. coffeae* complex may be diagnosed by the surface feature of the first lip annule.

Based on the present morphological and statistical investigations, I concluded to separate *P. coffeae* complex into 3 categories: 1) the specimens from Miyazaki and Nagasaki (G1) represent a new species, which will be described in a subsequent report; 2) a specimen from Kumamoto (G3) is another representative of a distinct species, which will be described in detail by N. MINAGAWA in a separate report; 3) the specimens from Nagano, Okinawa and Saitama (G2) represent the true *P. coffeae*.

The methods of SLDA and CDFA employed in the present study to make the groups were originally devised to search for gaps between predetermined groups by exaggerating the existing slight differences, and therefore they must be used with caution. Some geographic populations can be perfectly separated as shown in

the first step of the SLDA; the specimens from Saitama were readily separated from the specimens from Nagano and Okinawa. The present study is limited by the small number of samples. In order to verify the separation, further study should be done on a larger number of samples than those used in this study.

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