Morphological and morphometrical characterisation of ten *Pratylenchus coffeae* populations from Vietnam

Nguyen Thi Tuyet[1], Annemie Elsen[2, 5], Ho Huu Nhi[3] and Dirk De Waele[4, 5]

[1] **Agro-biodiversity Department, Plant Resources Center, Vietnam Academy of Agricultural Sciences (VAAS), An Khanh, Hoai Duc, Hanoi, Vietnam.**

[2] **Bodemkundige Dienst van België, Herentsesteenweg 42, B-3012 Wilsele, Belgium.**

[3] **Laboratory of Basic Research, Hybrid Rice Research and Development Center, Food Crops Research Institute, Vietnam Academy of Agricultural Sciences (VAAS), Van Dien, Thanh Tri, Hanoi, Vietnam.**

[4] **Laboratory of Tropical Crop Improvement, Department of Biosystems, Faculty of Bioscience Engineering, University of Leuven (KU Leuven), Kasteelpark Arenberg 13, B-3001 Leuven, Belgium, e-mail: dirkdewaele@pandora.be**

[5] **School of Environmental Sciences and Development, North-West University, Private Bag X6001, 2520 Potchefstroom, South Africa.**

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Summary. Ten *Pratylenchus coffeae* populations collected from different crops in different agro-ecological regions in Vietnam were compared on the basis of both light microscope and scanning electron microscope observations (morphological and morphometrical characters) to assess the intraspecific variability of these characters. The results revealed the presence of substantial variability in morphology and morphometry within and between these populations. However, these differences fall within the range of the morphological and morphometrical variability described previously in *P. coffeae* populations from other parts of the world. Our scanning electron microscopy observations further confirm that in *P. coffeae* there is a complete fusion of the 1st (lip) annulus with the oral disc resulting in an undivided *en face* view with no division between the lateral and median (sub-dorsal and sub-ventral) segments of the 1st (lip) annulus. Although canonical discriminant analysis enabled the separation of the ten *P. coffeae* populations from Vietnam examined in three groups based on a combination of five morphological characters for the males, there was no relationship between these groups and their geographic origin or between these groups and the host plants from which they were originally isolated.

Key words: Canonical discriminant analysis, morphology, morphometrics, SEM, variability.

The genus *Pratylenchus* comprises 76 described species (De Waele & Elsen, 2007). Up to now, 139 original descriptions, among them four subspecies, were published (Munera Uribe, 2008). The taxonomy of this genus has been the subject of many publications (Sher & Allen, 1953; Loof, 1960, 1978, 1991; Corbett, 1969; Romann & Hirschmann, 1969; Corbett & Clark, 1983; Bajaj & Bhatti, 1984; Geraert & Raski, 1987; Café Fihlo & Huang, 1989; Frederick & Tarjan, 1989; Handoo & Golden, 1989; Siddiqi, 2000; Hernandez *et al.*, 2001; Ryss, 2002; Castillo & Vovlas, 2007; Mizukubo *et al.*, 2007). The small number of diagnostic morphological characters at species level and the overlapping of morphometrical characters among species make it difficult to separate the species. Because several *Pratylenchus* species are among the economically most damaging plant-parasitic nematodes and are found on a wide variety of crops (Duncan & Moens, 2006), and some *Pratylenchus* species are pathogens of world quarantine importance (Ryss, 2002), the correct identification up to species level is of vital importance to the prevention, locally and internationally, of their spread, and the development and success of effective nematode management strategies.

Up to now, nine *Pratylenchus* identification keys have been published (Sher & Allen, 1953; Corbett, 1969; Café Filho & Huang, 1989; Frederick & Tarjan, 1989; Handoo & Golden, 1989; Loof, 1991; Siddiqi, 2000; Ryss, 2002; Castillo & Vovlas, 2007). The morphological and morphometrical characters most commonly used to separate *Pratylenchus* species are: presence/absence of

males; body length; de Man ratio's (especially vulva position or V value); shape of lip region; number of lip annuli; stylet length; shape of stylet; shape of stylet knobs; length of pharyngeal overlap; number of lateral field lines at vulval region; presence or absence of areolated bands on the lateral fields at vulval region; length and structure of post-vulval uterine sac; shape of spermatheca; shape of female tail and tail tip (Castillo & Vovlas, 2007; Munera Uribe, 2008). Up to now, the morphometrics of 41 *P. coffeae* populations collected from 18 different plants in 30 localities in 14 countries have been published (Mizukubo, 1992; Duncan *et al.,* 1999; Munera Uribe, 2008).

The objectives of our study were to compare, on the basis of both light microscope and scanning electron microscope observations, the morphological and morphometrical characters of ten *P. coffeae* populations collected from different crops in different agro-ecological regions in Vietnam *i*) among each other; *ii*) with the *Pratylenchus* species descriptions published in the literature; and *iii*) to assess the intraspecific variability of some of these characters.

MATERIALS AND METHODS

Ten *P. coffeae* populations collected from banana, coffee and an ornamental tree in different agro-ecological regions in Vietnam were used for the morphological and morphometrical study (Table 1). The specimens studied were obtained from carrot disc cultures (Moody *et al.*, 1973). *Pratylenchus coffeae* females were extracted from infected roots using the sieving-Baermann funnel method (Hooper *et al*., 2005), sterilised in 0.01% $HgCl_2$ and 2,000 ppm streptomycin sulphate (Myers *et al*., 1965) and 25 females were placed on the margin of each carrot disc which were incubated at 25°C in the dark. Carrot disc cultures with vigorous developing nematode populations (many active nematodes) were selected. The nematodes were collected by rinsing the Petri dishes with sterile water. The Seinhorst method (1959) as modified by De Grisse (1969) was used to kill and fix the nematodes. Cobb slides were used to mount the nematodes in glycerin (Cobb, 1917).

Morphological characters studied were based on Coomans (1978) and on the morphological characters used in the taxonomic studies of *Pratylenchus* by Roman & Hirschmann (1969), Geraert and Raski (1987), Handoo & Golden (1989), Duncan *et al.* (1999), Ryss (2002) and Mizukubo *et al.* (2007).

Fifteen females and 15 males of each *P. coffeae* population were measured. Measurements were taken using a camera lucida drawing tube attached to a light microscope (Zeiss Axioskop 2 Plus) which was equipped with a Canon D30 digital camera for photography. The mean, standard deviation and coefficient of variation (CV) were calculated for all characters measured. To assess the variation in tail and tail tip shape, at least 50 females of each *P. coffeae* population were examined. The morphometrical data were analysed statistically using GenStat Release 9.1. Canonical discriminant analysis (CDA) was used to make an objective assessment of the relative similarity among the populations based on 14 morphometrical characters for the females and five morphometrical characters for the males.

After light microscope observation and measuring, the slides were broken open with a scalpel and the glycerin-embedded nematodes transferred to a drop of pure glycerin in a small embryo dish to which distilled water was added gradually until the nematodes were almost in pure distilled water. To remove particles adhering to the surface of the nematodes, the specimens were exposed to ultrasonic treatment for about 10 to 15 minutes. The nematodes were then dehydrated by passing them through a series of 25, 50, 75 and 100% ethanol at intervals of 2 h, and left overnight

Table 1. Origin and population codes of the ten *Pratylenchus coffeae* populations from Vietnam used in the study.

No.	Host plant	Province	Agro-ecological region	Population code
1	Banana	Dien Bien	Northwest	NW
2	Coffee	Yen Bai	Northeast	NE1
3	Banana	Yen Bai	Northeast	NE2
4	Banana	Phu Tho	Northeast	NE3
5	Banana	Bac Kan	Northeast	NE4
6	Banana	Ha Tay	Red River Delta	RRD1
7	Ornamental tree	Hung Yen	Red River Delta	RRD2
8	Banana	Thanh Hoa	North Central Coast	NCC1
9	Coffee	Nghe An	North Central Coast	NCC2
10	Coffee	Dak Lak	Central Highlands	CH

in 100% ethanol. For drying, the standard critical point drying procedure with CO_2 as drying liquid was used. Finally, the dried specimens were mounted on stubs and coated with gold-palladium (Eisenback, 1985). The nematodes were examined with a JEOL LSM-840 scanning electron microscope at 15 kV.

RESULTS

Morphological observations

Female. Body rather slender in young females, thick in old ones. Body width increases from the level of the median pharyngeal bulb towards the vulva and decreases from the level of the vulva towards the tail tip. Cuticular annulation fairly conspicuous.

Lip region usually bearing two distinct annuli and not set off from the rest of the body by a constriction but often showing a small discontinuity in body outline at its base (see arrow in Fig. 1B). When viewed by light microscope, outer margins of the heavily sclerotised labial framework extending into the body about one body annulus. Occasionally, three annuli on one side of the lip region were observed in some specimens of the Northwest, Red River Delta 2 and Central Highlands populations (see arrow in Fig. 1I). When viewed by scanning electron microscope, a minute or incomplete initiation of a 3rd lip annulus visible in a specimen of the Northeast 3 and Red River Delta 1 populations (see arrows in Figs 4B & D). *En face* view characterised by a complete fusion of the 1st lip annulus and the oral disc, resulting in a plain undivided *en face* view with no division between the sub-median and lateral segments (Figs 4A & B). There are six small pores very close to the oral aperture. Amphid opening wide, slightly triangular (Figs 4A & B). The anterior surface of the oral disc low-conical or flat with rounded edges (Figs 4C & D).

Stylet strong, basal knobs of stylet are round to oblong (Figs 1B, C & F). Median pharyngeal bulb broadly oval to nearly round (Fig. 1A). Glandular lobe of pharyngeal glands overlapping anterior end of the intestine ventrally and slightly laterally (Figs 1E & H). Hemizonid just anterior to the excretory pore, about two body annuli long (Fig. 1H). Excretory pore and canal located slightly posterior to the pharyngo-intestinal junction except in the Red River Delta 1 population. In this population, the excretory pore is located slightly anterior to the pharyngo-intestinal junction.

Lateral fields distinct with four lines forming three equal or unequal bands (Figs 2E, H-I & M-O and Fig. 4E-H). Outer lines of lateral fields crenated in the tail region (Fig. 2N). Occasionally the internal band can be sculptured by striae, oblique or parallel to the main bands (Figs 2E & M). When viewed by scanning electron microscope, areolation of the outer bands was observed in all populations (Fig. 4E-H). Some specimens of the Northeast 1 and Red River Delta 2 populations have punctuations scattered in the outer bands when viewed by light microscope (Fig. 2O). When viewed by scanning electron microscope, the beginning of the lines of the lateral fields at the 9th or 10th annulus appear as two lines (Figs 4C & D), widening to three lines at the following annuli and to four lines at the level of the median pharyngeal bulb (Fig. 2D) that extend to the phasmid. Only the outer bands extend till the tail tip in all populations examined except in the Red River Delta 2 population. At the level of the 10th annulus anterior to the tail tip, the outer bands fuse into one band (Fig. 5G). At the level of the vulva, the structure of the lateral field is similar as at the mid-body level (Fig. 4H).

Vulva with well-developed lips, often protruding (Figs 2F & J-K and Fig. 4H). In young specimens, the ovary extends over 1/4th of the body length; in old specimens, over more than one-half of the body length. The ovary does not extend to the pharyngeal glands, except in the North Central Coast 1 population in which the ovary extends to the median pharyngeal bulb in some specimens. Ovary consists of a single row of oocytes, except a double row near the anterior end (Fig. 2A). A reflexed ovary (Fig. 2B) was found in specimens from the Northeast 1 and Red River Delta 1 populations. Spermatheca variable in size, nearly round to broadly oval in all populations (Figs 2C, F-G & J-L), except in the Northeast 1 population in which the spermatheca is small and round. Spermatheca usually contains spermatozoa. Post-vulval uterine sac generally short. Sometimes cellular tissue is present at the distal end of the post-vulval uterine sac. Phasmids slightly posterior to the middle of the tail.

A higher frequency of tails tapering strongly with narrow tip was observed in most populations (Figs 3 & 5), except in the Red River Delta 1 and North Central Coast 2 populations. Tail shapes of the females examined from the Red River Delta 1 and North Central Coast 2 populations tapering slightly with broad tail tip were recorded in 63 and 40.3%, respectively, (Figs 3: RRD1a-c & NCC2a-d, respectively). Tail tip variable in shape: smooth or crenated, truncated or obliquely truncated, broadly rounded, hemispherical or sub-hemispherical, bluntly pointed or pointed, convex, digitated, cleft or bilobed (Table 2 and Fig. 3A-L). A smooth tail tip

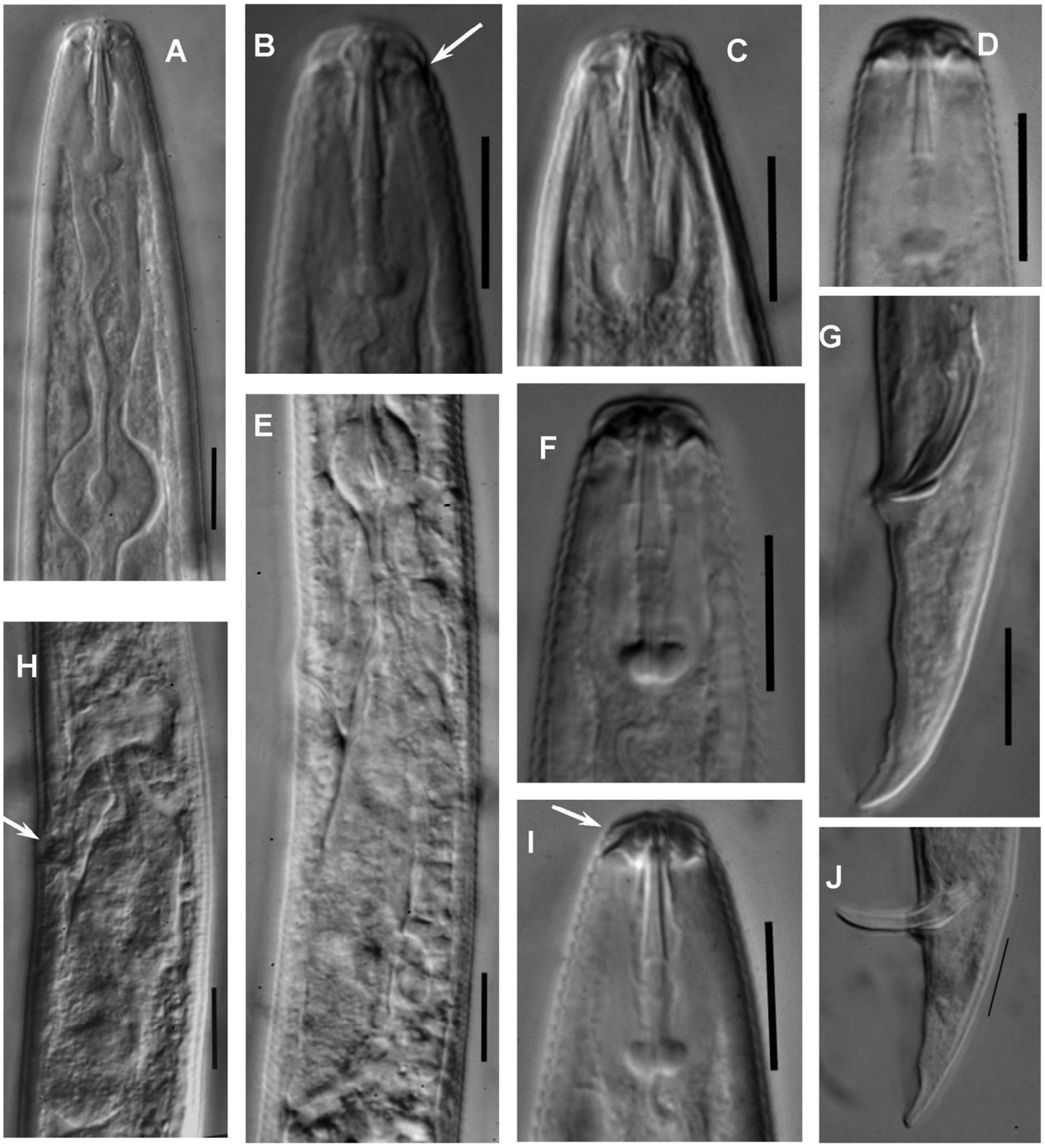

Fig. 1. Light microscope photographs of *Pratylenchus coffeae* populations from Vietnam. A-C, F & I: female anterior region; D: male anterior region; E: female pharyngo-intestinal junction; H: female hemizonid and excretory pore; G & J: male tail region. Scale bars: 10 µm. Populations from Northwest (I), Northeast 2 (A, C), Northeast 3 (H), Northeast 4 (J), Red River Delta 1 (E), Red River Delta 2 (B), North Central Coast 1 (F) and North Central Coast 2 (G).

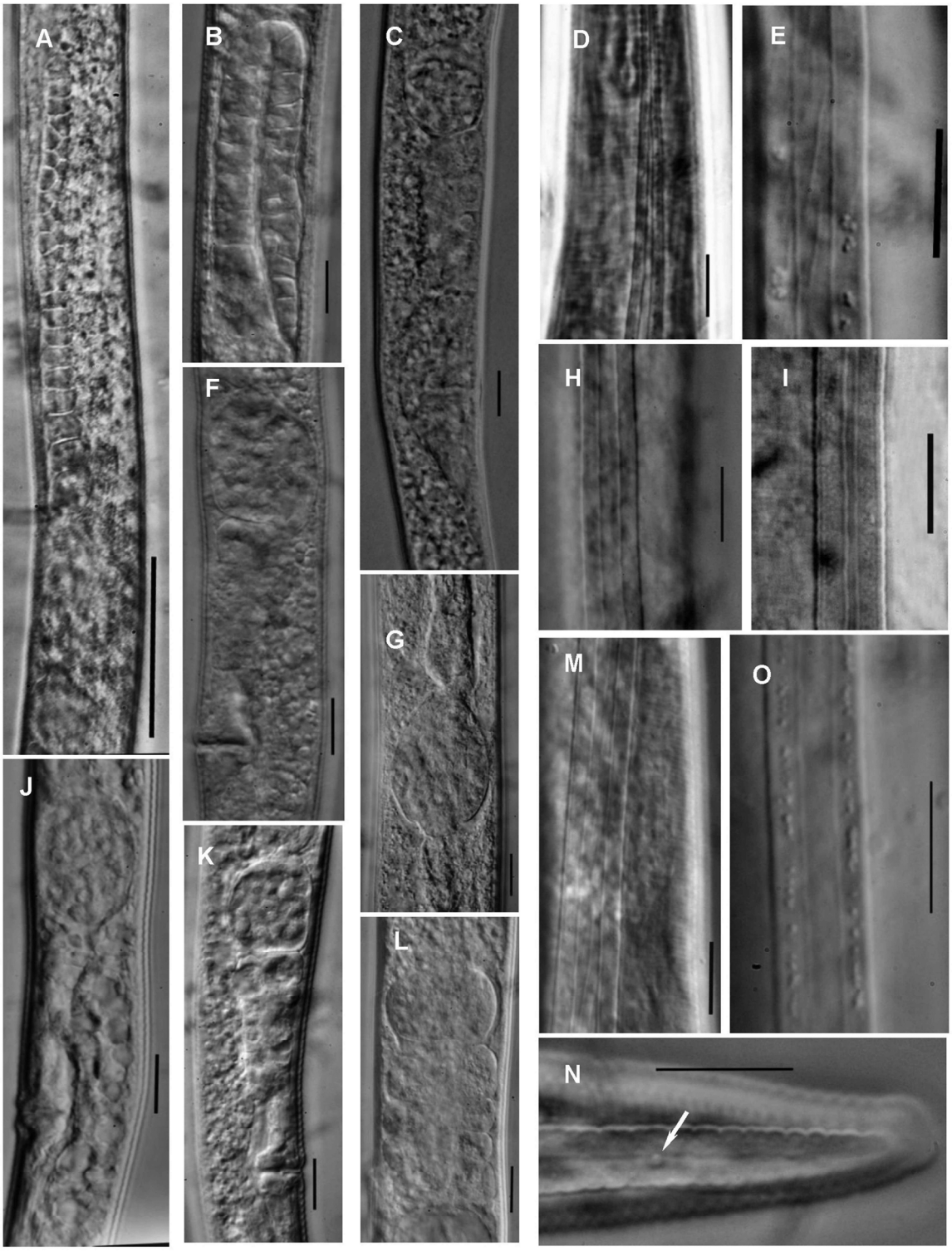

Fig. 2. Light microscope photographs of *Pratylenchus coffeae* populations from Vietnam. A-B: female anterior genital reproductive system branch; C: female post-vulval uterine sac; D: female lateral field at level of the median pharyngeal bulb; E, H-I & M-O: female lateral fields at mid-body; F-G, J-L: female spermatheca; N: female lateral fields at the tail region (arrow shows phasmid position). Scale bars: A: 50 µm; B-M: 10 µm. Populations from Northwest (C, H), Northeast 1 (B, N), Northeast 2 (A, G, L), Northeast 3 (M), Northeast 4 (J, K), Red River Delta 1 (E), Red River Delta 2 (D, F, O) and Central Highlands (I).

was more common than the other tail tip shapes. The Northwest and Central Highlands populations were the most variable in tail tip shape with 11 out of a total of 12 tail tip shapes (Table 3). An obliquely truncated tail tip shape was observed with the highest frequency (22.5-41%) in most of the populations, except in the Red River Delta 1 and 2, and Central Highlands populations. In these three populations, a hemispherical tail tip was observed with the highest frequency (24-31%).

Male. Males as abundant as females in all populations examined. Morphologically similar to the females, but body length is smaller and slender than in the females. Stylet knobs smaller than in females (Fig. 1D). Reproductive system with a single anteriorly outstretched testis, extending over about one-half of the body length, not extending to the pharyngeal glands. Testis shorter than vas deferens, spermatocytes in single or multiple rows. Spicules curved ventrally, gubernaculum plain and non-protrusible (Fig. 1J). Male tail pointed, bursal edges faintly crenated (Figs 1G & J). Bursa arising from a little anterior to the level of the head of the spicules and surrounding the tail tip (Fig. 5L-U). Phasmids slightly posterior to the middle of the tail.

Morphometrical observations

Female. The morphometrics of the females of the *P. coffeae* populations collected in Vietnam are presented in Table 4. The body length ranged from 465 to 765 µm (mean: 601 µm). The females of the Red River Delta 1 population had the shortest average body length (553 µm) while the females of the North Central Coast 2 population had the largest average body length (650 µm). The 'a' ratio ranged from 19.0 to 35.6 (mean: 26.8). The females of the Northeast 4 population had the lowest average 'a' ratio (24.6) while the females of the Central Highlands population had the highest 'a' ratio (28.5). The average body width of the former population was 23.8 µm *vs* 19.8 µm of the latter population. The 'b' ratio ranged from 4.1 to 9.7 (mean: 7.3). The females of the Red River Delta 1 population had the lowest average 'b' ratio (6.6) while the females of the North Central Coast 1 population had the highest average b ratio (8.1). The 'c' ratio ranged from 13.1 to 26.3 (mean: 20.1). The females of the Central Highlands population had the lowest average c ratio (18.3) while the females of the Northeast 3 population had the highest average 'c' ratio (21.3). The average tail length of the former population was 31.5 µm *vs* 29.1 µm of the latter population. The pharynx length ranged from 65.5 to 130 µm (mean: 83.1 µm). The females of the Northeast 1 and North Central Coast 1 populations had the shortest average pharynx length (79.9 µm) while the females of the Northwest population had the largest average pharynx length (85.8 µm). The length of the pharyngeal overlap ranged from 29.5 to 79.5 µm (mean: 51.1 µm). The females of the Central Highlands population had the shortest average length of the pharyngeal overlap (41.4 µm) while the females of the North Central Coast 2 population had the largest average length of the pharyngeal overlap (62.3 µm). The V value ranged from 71.5 to 92.3 % (mean: 80.1%). The females of the Northeast 2 population had the lowest average V value (79.2%) while the females of the North Central Coast 2 population had the highest V value (81.9%). The stylet length ranged from 13.5 to 17.7 µm (mean: 15.6 µm). The shortest average stylet length was observed in the females of the Central Highlands population (15.3 µm) while the longest average stylet length was observed in the females of the Northwest population (16.1 µm). The length of the post-vulval uterine sac ranged from 19.7 to 54.5 µm (mean: 30.1 µm). The post-vulval uterine sac was on average shortest in the females of the North Central Coast 2 population (25.7 µm) and longest in the females of the Northeast 3 (33.3 µm). The distance of the excretory pore from the anterior end ranged from 63.3 to 98.5 µm (mean: 84.1 µm). This distance was on average shortest in the Red River Delta 1 population (78.9 µm) and longest in the North Central Coast 2 population (89.9 µm).

Male. The morphometrics of the males of the *P. coffeae* populations collected in Vietnam are presented in Table 5. The body length ranged from 422 to 673 µm (mean: 532 µm). The males of the Red River Delta 1 population had the shortest average body length (497) while the males of the North Central Coast 1 population had the longest average body length (551 µm). The 'a' ratio ranged from 20.7 to 38 (mean: 28.4). The males of the Red River Delta 2 population had the lowest average a ratio (24.6) while the males of the North Central Coast 2 population had the highest 'a' ratio (32.4). The average body width of the former population was 22.2 µm *vs* 16.8 µm of the latter population. The 'b' ratio ranged from 5.8 to 8 (mean: 6.8). The males of the Red River Delta 1 population had the lowest average 'b' ratio (6.4) while the males of the North Central Coast 1 population had the highest average 'b' ratio (7.2). The 'c' ratio ranged from 14.6 to 23.4 (mean: 18.4). The males of the Northeast 2 population had the lowest average 'c' ratio (17.1) while the males of the Northeast 3 population had the highest average 'c' ratio (19.4). The average tail length of the former population was 29.6 µm *vs* 28.3 µm of the latter population. The pharynx length ranged

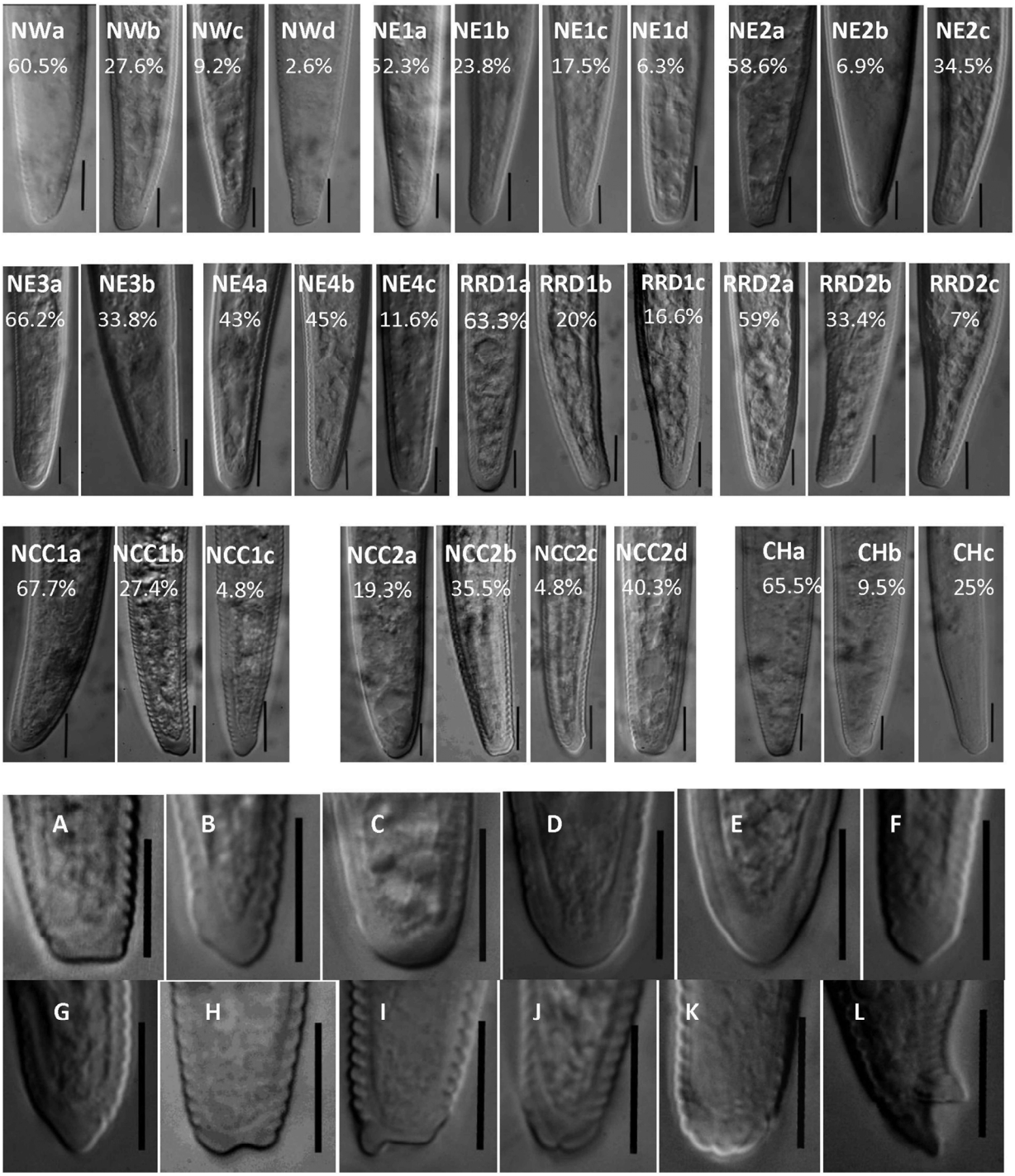

Fig. 3. Light microscope photographs of the tail and tail tip shapes of *Pratylenchus coffeae* females from Vietnam. Population Northwest (NW), Northeast 1 (NE1), Northeast 2 (NE2), Northeast 3 (NE3), Northeast 4 (NE4), Red River Delta 1 (RRD1), Red River Delta 2 (RRD2), North Central Coast 1 (NCC1), North Central Coast 2 (NCC2) and Central Highlands (CH). %: frequency of occurrence of that specific tail shape in the population ($n \geq 50$). Tail tip shapes: truncated (A); obliquely truncated (B); broadly rounded (C); hemispherical (D); sub-hemispherical (E); bluntly pointed (F); pointed (G); convex (H); digitated (I); cleft (J); crenated (K); bilobed (L). Scale bars: 10 µm.

Table 2. Frequency of occurrence (in %; n ≥ 50) of the tail tip shapes of *Pratylenchus coffeae* females from Vietnam.

Population	A	B	C	D	E	F	G	H	I	J	K	L
Northwest (NW)	8	31	2	16	14	16	6	2	2	1	2	0
Northeast 1 (NE1)	1	29	2	20	8	24	14	0	1	1	0	0
Northeast 2 (NE2)	4	30	0	8	5	4	3	0	20	26	0	0
Northeast 3 (NE3)	0	41	10	14	9	0	0	12	14	0	0	0
Northeast 4 (NE4)	10	26	2	12	4	12	4	0	4	26	0	0
Red River Delta 1 (RRD1)	7	20	0	25	11	14	7	2	2	10	2	0
Red River Delta 2 (RRD2)	6	18	5	31	13	8	8	0	10	1	0	0
North Central Coast 1 (NCC1)	4.8	22.5	3.2	9.7	12.9	17.7	6.4	4.8	12.9	4.8	0	0
North Central Coast 2 (NCC2)	20	13.3	25.3	9.3	0	8	12	8	4	0	0	0
Central Highlands (CH)	10	10	4	24	0	14	4	4	4	12	2	12

A: truncated; B: obliquely truncated; C: broadly rounded; D: hemispherical; E: sub-hemispherical; F: bluntly pointed; G: pointed;H: convex; I: digitated; J: cleft; K: crenated; L: bilobed.

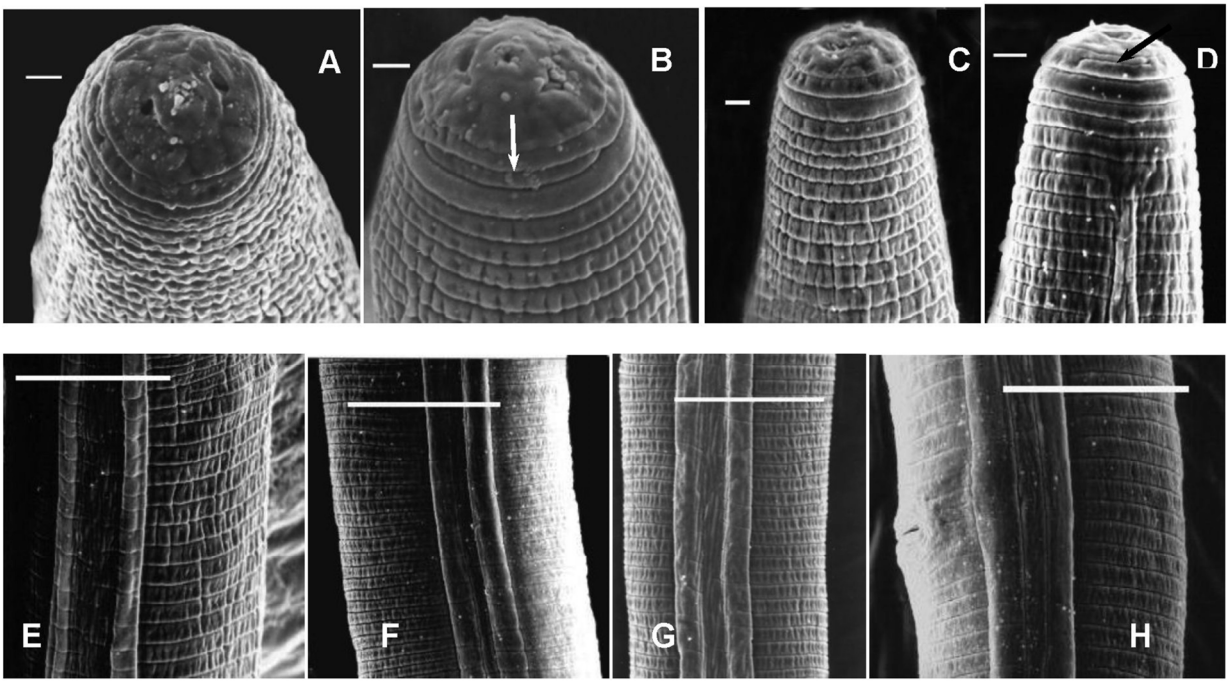

Fig. 4. *En face* (A-B), lateral lip region (C-D) and lateral field at mid-body (E-G) and at vulva level (H) scanning electron microscope photographs of *Pratylenchus coffeae* specimens from Vietnam. Populations from Northeast 2 (A), Red River Delta 1 (B), Nortwest (C and E), Northeast 3 (D), Northeast 4 (F and H) and Central Highlands (G). Scale bars: A, B, C, D = 1 µm; E, F, G, H = 10 µm.

from 66.2 to 94.5 µm (mean: 78.3 µm). The males of the Northeast 2 population had the shortest average pharynx length (76.4 µm) while the males of the Red River Delta 2 population had the longest average pharynx length (83.9 µm). The length of the pharyngeal overlap ranged from 30 to 67.3 µm (mean: 45 µm). The males of the Northeast 1 population had the shortest average pharyngeal overlap (37.7 µm) while the males of the North Central Coast 2 population had the longest average pharyngeal overlap (51.2 µm). The T value ranged from 18.9 to 59.3 % (mean: 44.1%). The males of the Red River Delta 2 population had the lowest average T value (38%) while the males of the Northwest population had the highest T value (50.7%). The stylet length ranged from 13.1 to 16.7 µm (mean: 14.5 µm). The males of the North Central Coast 1 population had on average the shortest stylet (14 µm) while the males of the Northwest, Northeast 2, Northeast 4 and North Central Coast 2 had on average the longest stylet (14.8 µm). The distance of the excretory pore from the anterior end ranged from 63.6 to 95.1 (mean: 77.9 µm). This distance was on average the shortest

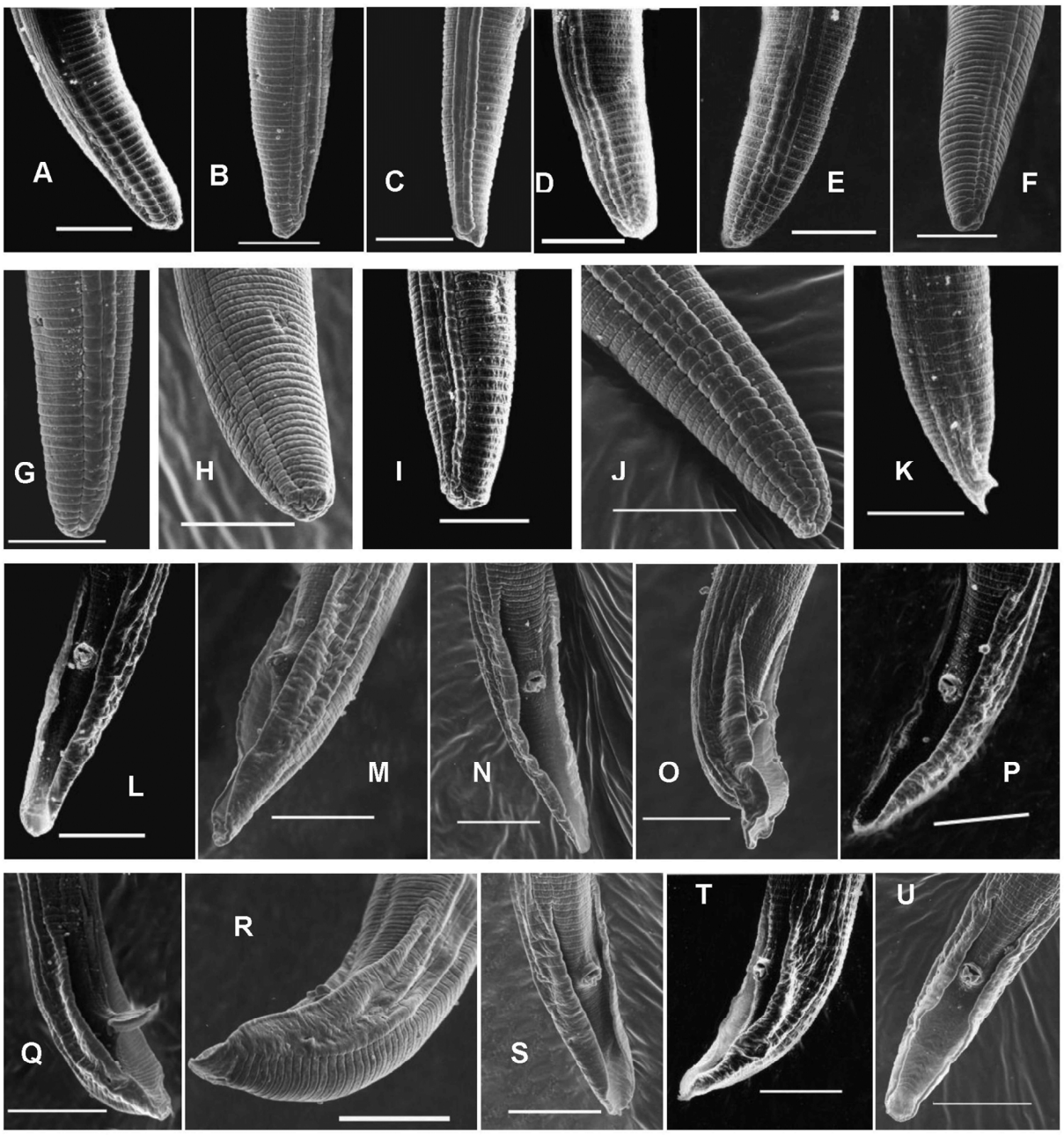

Fig. 5. Female and male tail scanning electron microscope photographs of *Pratylenchus coffeae* specimens from Vietnam. Populations from Northwest (A and L), Northeast 1 (B and M), Northeast 2 (C and N), Northeast 3 (D and O), Northeast 4 (Eand P), Red River Delta 1 (F and Q), Red River Delta 2 (G and R), North Central Coast 1 (H and S), North Central Coast 2 (I and T) and Central Highlands (J-K and U). Scale bars: 10 µm.

in the males of the Red River Delta 1 population (73.2 µm) and the longest in the males of the North Central Coast 2 population (81.7 µm). The spicule length of the Vietnamese males ranged from 14 to 20.7 µm (mean: 17.3 µm). The shortest average spicule length was observed in the males of the Northeast 2 and North Central Coast 1 populations (16.7 µm) while the longest average spicule length was observed in the males of the Northeast 1, Northeast 3 and Northeast 4 populations (17.6 µm).

In the females of the Vietnamese *P. coffeae* populations, the coefficient of variation was the lowest for the V value (3.1%) and stylet length (4.6%). The coefficient of variation was the highest for the length of the ovary (22.1%) and the distance from the anterior end to the dorsal pharyngeal gland orifice (17.8%).

Table 3. Morphometrics of the females of the *Pratylenchus coffeae* populations collected in Vietnam (n = 15)

Population	L*	a	b	b'	c	c'	stylet*	DGO*	body width*	excr. pore*
NW	605.6 ± 43.9	26.4 ± 1.9	7.1 ± 0.3	4.2 ± 0.3	19.1 ± 1.3	2.4 ± 0.4	16.1 ± 0.7	2.9 ± 0.4	23.0 ± 2.1	89.3 ± 6.4
	(519-664)	(21.9-29.0)	(6.6-7.8)	(3.8-4.7)	(17.1-22.2)	(1.9-3.2)	(14.6-17.7)	(2.1-3.9)	(20.2-27.8)	(76.3-98.5)
NE1	630.3 ± 36.4	27.6 ± 1.8	7.9 ± 0.5	5.2 ± 0.4	20.8 ± 2.0	2.1 ± 0.2	15.4 ± 0.8	3.1 ± 0.4	22.9 ± 2.1	80.5 ± 5.8
	(580-706)	(22.8-29.5)	(7.2-8.8)	(4.4-5.8)	(17.0-25.2)	(1.7-2.4)	(13.5-17.1)	(2.6-3.6)	(20.2-27.0)	(71.2-89.1)
NE2	582.5 ± 55.0	25.6 ± 2.3	7.0 ± 1.0	4.5 ± 0.4	19.1 ± 1.8	2.1 ± 0.4	15.6 ± 0.7	2.6 ± 0.6	22.8 ± 2.0	83.7 ± 7.1
	(467-760)	(22.7-31.6)	(4.1-7.9)	(3.9-5.2)	(16.4-22.0)	(1.4-2.6)	(14.5-16.7)	(1.6-4.2)	(18.3-26.5)	(71.2-97.0)
NE3	607.6 ± 40.9	28.1 ± 1.8	7.4 ± 0.7	4.6 ± 0.4	21.4 ± 2.1	2.0 ± 0.2	15.5 ± 0.7	3.0 ± 0.6	21.7 ± 2.0	84.6 ± 4.2
	(501-676)	(23.6-30.5)	(6.3-8.8)	(4.1-5.3)	(17.6-25.9)	(1.8-2.7)	(14.5-16.5)	(2.1-4.2)	(17.0-25.2)	(76.9-91.7)
NE4	573.4 ± 29.0	24.6 ± 3.3	7.1 ± 0.3	4.2 ± 0.3	19.9 ± 1.9	2.2 ± 0.2	15.4 ± 0.6	3.1 ± 0.4	23.8 ± 3.9	84.3 ± 5.0
	(534-633)	(19.0-30.3)	(6.5-7.7)	(3.8-4.9)	(16.3-22.4)	(1.9-2.6)	(14.1-16.7)	(2.6-3.6)	(18.7-30.3)	(75.8-93.4)
RRD1	552.9 ± 39.4	26.1 ± 2.9	6.6 ± 0.5	4.0 ± 0.3	20.2 ± 2.2	1.9 ± 0.2	15.4 ± 0.6	3.1 ± 0.4	21.3 ± 2.2	78.9 ± 4.9
	(465-610)	(22.0-30.9)	(5.7-7.4)	(3.6-4.5)	(17.2-26.3)	(1.6-2.3)	(14.0-16.4)	(2.6-3.6)	(18.3-25.2)	(69.0-86.0)
RRD2	588.8 ± 42.9	26.4 ± 3.8	6.9 ± 0.8	4.6 ± 0.3	20.0 ± 3.1	2.2 ± 0.3	15.7 ± 0.7	2.9 ± 0.4	22.3 ± 3.2	84.4 ± 5.3
	(534-664)	(21.5-34.6)	(5.8-8.5)	(4.2-5.1)	(13.4-24.6)	(1.7-3.0)	(14.5-17.0)	(2.3-3.6)	(18.3-29.3)	(77.4-92.6)
NCC1	646.9 ± 51.0	27.3 ± 2.0	8.1 ± 0.7	4.9 ± 0.5	20.6 ± 2.6	2.3 ± 0.2	15.4 ± 0.6	2.7 ± 0.5	23.8 ± 2.4	84.4 ± 9.4
	(567-765)	(24.4-31.2)	(7.0-9.7)	(3.8-5.8)	(15.8-24.9)	(1.8-2.7)	(14.5-16.4)	(1.9-3.1)	(19.8-27.7)	(63.3-92.6)
NCC2	649.6 ± 34.6	26.9 ± 3.1	7.6 ± 0.3	4.4 ± 0.2	21.3 ± 1.9	2.2 ± 0.3	15.9 ± 0.6	3.0 ± 0.7	24.4 ± 2.6	89.9 ± 5.2
	(571-706)	(21.8-32.7)	(6.9-8.0)	(4.0-4.8)	(18.4-25.4)	(1.6-2.9)	(14.6-16.7)	(2.1-5.2)	(19.7-27.3)	(81.8-98.0)
CH	568.7 ± 42.9	28.5 ± 3.3	6.8 ± 0.6	4.6 ± 0.5	18.3 ± 2.5	2.3 ± 0.3	15.3 ± 0.8	3.2 ± 0.5	19.8 ± 1.8	80.7 ± 3.5
	(508-647)	(23.4-35.6)	(5.5-7.8)	(3.8-5.2)	(13.1-22.4)	(1.7-2.8)	(13.8-16.5)	(2.6-4.2)	(17.6-22.6)	(72.5-87.6)
CV (%)	8.6	10.7	10.3	10.7	11.7	14.3	4.6	17.8	12.1	7.9

Table 3. Morphometrics of the females of the *Pratylenchus coffeae* populations collected in Vietnam (n = 15), **(continued).**

Population	Pharynx length*	Pharyngeal overlap*	L/excr. pore	V	ovary*	post-vulval uterine sac*	tail*	vulva-anus*	lip width*	lip height*	pharyngeal bulb
NW	85.8 ± 5.9	58.1 ± 8.9	6.7 ± 0.2	80.0 ± 1.6	238.1 ± 46.2	31.2 ± 3.5	31.9 ± 3.2	90.5 ± 12.5	7.8 ± 0.5	2.4 ± 0.3	0.58 ±0.03
	73.7-96.0	41.1-69.5	(6.4-7.1)	(77.3-83.0)	(171.7-314.9)	(24.2-35.4)	(26.3-36.4)	(60.6-121.2)	(6.8-8.3)	(2.1-3.1)	(0.4-0.7)
NE1	79.9 ± 4.9	42.1 ± 7.4	7.8 ± 0.6	80.6 ± 1.7	218.8 ± 57.4	30.8 ± 7.9	30.7 ± 3.2	92.3 ± 10.4	7.6 ± 0.6	2.3 ± 0.3	0.56 ±0.05
	71.8-87.5	32.9-58.1	(6.8-8.8)	(78.5-84.8)	(149.4-382.9)	(22.2-54.5)	(23.0-36.5)	(76.2-107.1)	(6.5-9.1)	(1.9-2.9)	(0.5-0.6)
NE2	83.3 ± 16.3	49.9 ± 10.4	7.0 ± 0.5	79.2 ± 3.8	197.1 ± 50.6	29.9 ± 4.0	30.9 ± 3.9	89.7 ± 10.0	8.2 ± 0.4	2.3 ± 2.1	0.51 ±0.05
	68.7-130	35.2-66.2	(6.2-8.1)	(73.0-85.7)	(102.5-279.1)	(24.2-37.9)	(21.2-35.9)	(74.5-102.7)	(7.3-8.9)	(2.1-2.6)	(0.4-0.6)
NE3	82.7 ± 5.5	48.5 ± 9.2	7.2 ± 0.4	80.1 ± 1.1	211.1 ± 42.9	33.3 ± 5.3	29.1 ± 3.5	87.0 ± 8.1	8.2 ± 0.6	2.3 ± 0.3	0.55 ± 0.03
	71.8-94.5	29.7-61.6	(6.5-7.9)	(77.9-82.0)	(156.8-284.1)	(27.3-41.4)	(24.7-35.4)	(73.7-106.6)	(7.3-9.4)	(2.0-3.1)	(0.5-0.6)
NE4	81.1 ± 3.8	55.6 ± 9.3	6.8 ± 0.3	81.2 ± 3.3	265.4 ± 47.2	29.2 ± 5.2	27.5 ± 2.9	81.2 ± 17.2	8.2 ± 1.0	2.3 ± 0.6	0.53 ± 0.03
	75.8-88.4	36.8-71.4	(6.1-7.4)	(78.4-92.3)	(165.5-333.5)	(24.2-39.4)	(22.9-33.8)	(24.2-99.0)	(6.8-10.4)	(1.6-2.3)	(0.5-0.6)
RRD1	83.9 ± 7.1	52.7 ± 5.6	7.0 ± 0.6	81.2 ± 3.3	265.4 ± 47.2	31.2 ± 3.8	27.5 ± 2.9	81.2 ± 17.2	8.2 ± 1.0	2.3 ± 0.6	0.53 ± 0.03
	73.7-103.1	39.5-59.8	(5.9-7.9)	(78.4-92.3)	(165.5-333.5)	(27.7-41.9)	(22.9-33.8)	(24.2-99.0)	(6.8-10.4)	(1.6-2.3)	(0.5-0.6)
RRD2	85.6 ± 10.2	43.8 ± 7.4	6.9 ± 0.4	80.6 ± 1.7	186.7 ± 45.6	28.3 ± 4.1	30.0 ± 4.6	88.1 ± 9.3	7.9 ± 0.5	2.2 ± 0.3	0.53 ± 0.03
	72-114.7	34.7-66.1	(6.1-7.4)	(71.5-82.6)	(49.4-255.6)	(22.2-36.4)	(23.3-40.0)	(75.0-105.2)	(7.3-8.9)	(1.9-2.6)	(0.5-0.6)
NCC1	79.9 ± 7.7	53.9± 13.8	7.8 ± 0.9	80.4 ± 1.9	227.8 ± 35.6	30.5 ± 4.6	31.8 ± 4.2	98.3 ± 15.1	7.8 ± 0.9	2.1 ± 0.2	0.58 ± 0.03
	65.5-91.9	39.0-79.5	(6.8-10.1)	(75.7-83.1)	(179.1-290.2)	(21.7-38.9)	(23.3-40.9)	(74.9-125.7)	(6.2-9.4)	(1.7-2.6)	(0.5-0.6)
NCC2	85.3 ± 4.9	62.3 ± 5.4	7.2 ± 0.3	81.9 ± 1.3	212.0 ± 33.5	25.7 ± 4.1	30.7 ± 3.1	88.8 ± 8.2	8.4 ± 0.7	2.5 ± 0.4	0.59 ± 0.05
	77.3-93.9	52.4-72.5	(6.6-7.6)	(78.9-84.0)	(151.9-281.6)	(19.7-34.8)	(23.2-35.4)	(77.8-110.6)	(7.3-9.9)	(1.9-3.1)	(0.5-0.7)
CH	83.7 ± 6.3	41.4 ± 7.3	7.1 ± 0.6	81.5 ± 3.4	199.3 ± 41.1	30.9 ± 4.5	31.5 ± 3.4	88.9 ± 9.0	8.0 ± 0.5	2.3 ± 0.3	0.55 ± 0.03
	73.7-96.1	29.5-58.7	(5.8-8.1)	(78.0-89.6)	(129.7-249.5)	(24.2-37.9)	(23.7-38.7)	(75.6-105.8)	(7.3-8.9)	(1.9-2.9)	(0.5-0.6)
CV%	9.3	17.1	8.4	3.1	22.1	17.0	12.0	13.1	8.3	15.1	7.8

*: measurements in µm. Mean ± standard deviation; (range).
For the list with the nematode population codes see Table 1.
b': body length/distance from anterior end to posterior end of pharyngeal gland; c: tail length/body width at anus; L/excr. pore: body length/distance from anterior end to excretory pore; V: distance of vulva from anterior end x 100/body length (%); ovary: distance from vulva to anteriormost part of ovary; vulva-anus: distance from vulva to anus. For the other abbreviations see Table 5.
CV: coefficient of variation (%).

Table 4. Morphometrics of the males of the *Pratylenchus coffeae* populations collected in Vietnam (n = 15)

Population	L*	a	b	b'	c	c'	stylet*	DGO*	body width*	excr. pore*
NW	540.2 ± 22.1	29.0 ± 2.1	7.0 ± 0.4	4.4 ± 0.3	17.5 ± 1.8	2.7 ± 0.4	14.8 ± 0.5	2.4 ± 0.2	18.7 ± 1.2	80.8 ± 4.5
	(498.6-571.3)	(24.6-32.3)	(6.3-7.7)	(3.8-5.0)	(15.6-23.4)	(2.0-3.3)	(14.1-15.7)	(2.0-2.5)	(16.1-20.7)	(72.2-88.9)
NE1	530.1 ± 17.0	27.6 ± 2.0	6.8 ± 0.5	4.6 ± 0.3	19.1 ± 0.9	2.5 ± 0.2	14.1 ± 0.4	2.7 ± 0.2	19.3 ± 1.5	75.8 ± 4.2
	(508.0-567.0)	(23.4-30.0)	(6.3-7.8)	(4.1-5.1)	(17.1-20.0)	(2.2-2.8)	(13.5-15.1)	(2.5-2.8)	(17.6-22.0)	(67.4-81.3)
NE2	503.2 ± 35.8	26.8 ± 2.4	6.6 ± 0.4	4.2 ± 0.4	17.1 ± 1.6	2.6 ± 0.3	14.8 ± 0.6	2.6 ± 0.3	19.0 ± 1.5	76.7 ± 3.0
	(441.5-561.0)	(22.6-30.3)	(5.8-7.4)	(3.6-4.8)	(14.6-20.4)	(1.9-3.0)	(13.2-15.7)	(2.2-3.2)	(15.5-20.7)	(73.7-83.8)
NE3	541.7 ± 16.4	29.9 ± 3.5	6.8 ± 0.3	4.4 ± 0.3	19.4 ± 2.3	2.6 ± 0.4	14.2 ± 0.6	3.0 ± 0.2	18.4 ± 2.2	75.8 ± 3.1
	(515.0-574.0)	(22.9-36.0)	(6.1-7.4)	(3.9-4.9)	(15.5-22.7)	(2.0-3.4)	(13.2-14.8)	(2.8-3.2)	(15.4-23.7)	(70.6-83.2)
NE4	543.3 ± 21.9	29.2 ± 3.3	7.0 ± 0.3	4.4 ± 0.4	18.8 ± 1.2	2.7 ± 0.2	14.8 ± 0.5	2.3 ± 0.3	18.8 ± 1.6	81.5 ± 4.1
	(503.8-576.5)	(25.3-34.9)	(6.5-7.6)	(3.8-5.1)	(17.3-20.6)	(2.3-2.9)	(13.6-15.2)	(1.5-2.5)	(16.1-20.7)	(74.2-88.4)
RRD1	497.1 ± 23.0	27.1 ± 2.1	6.4 ± 0.2	4.0 ± 0.3	18.5 ± 1.5	2.4 ± 0.2	14.4 ± 0.7	2.7 ± 0.6	18.3 ± 1.7	73.2 ± 4.4
	(468.0-554.0)	(23.6-30.2)	(6.0-6.9)	(3.5-4.5)	(16.0-20.7)	(2.0-2.7)	(13.2-15.7)	(2.0-3.8)	(15.7-20.8)	(66.2-81.8)
RRD2	540.8 ± 32.3	24.6 ± 2.5	6.5 ± 0.4	4.2 ± 0.3	18.4 ± 1.0	2.5 ± 0.2	14.2 ± 0.5	2.7 ± 0.2	22.2 ± 1.9	78.2 ± 5.1
	(468.0-580.0)	(20.7-28.9)	(5.9-7.1)	(3.6-4.7)	(15.8-20.2)	(2.0-2.9)	(13.2-15.1)	(2.5-3.2)	(17.6-25.2)	(64.3-84.1)
NCC1	551.0 ± 34.4	27.5 ± 2.5	7.2 ± 0.6	4.5 ± 0.3	18.9 ± 1.9	2.5 ± 0.2	14.0 ± 0.5	2.9 ± 06	20.2 ± 1.6	78.6 ± 5.4
	(502.0-633.0)	(24.1-33.2)	(6.3-8.0)	(4.1-5.2)	(15.7-21.8)	(2.1-2.9)	(13.2-14.8)	(1.9-3.7)	(18.3-23.3)	(65.5-88.0)
NCC2	542.3 ± 23.2	32.4 ± 2.4	7.1 ± 0.3	4.3 ± 0.3	17.6 ± 1.2	2.7 ± 0.3	14.8 ± 1.2	2.6 ± 0.2	16.8 ± 1.0	81.7 ± 2.3
	(472.7-571.3)	(28.5-38.0)	(6.4-7.5)	(3.9-5.0)	(15.5-19.6)	(2.2-3.0)	(13.1-16.7)	(2.5-3.0)	(15.0-18.1)	(78.8-86.4)
CH	534.9 ± 45.4	29.5 ± 3.0	6.7 ± 0.2	4.4 ± 0.4	18.5 ± 1.6	2.7 ± 0.2	14.5 ± 0.6	2.4 ± 0.4	18.3 ± 1.8	76.3 ± 7.1
	(482.0-673.0)	(26.1-37.5)	(6.3-7.1)	(3.9-5.1)	(16.3-21.0)	(2.2-3.1)	(13.5-15.8)	(1.9-3.2)	(13.9-20.8)	(63.6-95.1)
CV (%)	6.1	11.5	6.3	8.1	9.1	11.0	4.8	14.6	10.9	6.6

Table 4. Morphometrics of the males of the *Pratylenchus coffeae* populations collected in Vietnam (n = 15), **(continued)**.

Population	L/excr. pore	Pharynx length	Pharyngeal overlap	T	testis*	tail*	lip width*	lip height*	spicule length*
NW	6.7 ± 0 .3	77.7 ± 4.3	44.8 ± 6.9	50.7 ± 4.4	271.7 ± 25.3	31.2 ± 3.3	6.2 ± 0.5	1.6 ± 0.2	17.3 ± 1.5
	(6.0-7.2)	66.2-84.3	34.6-57.3	(43.3-56.5)	(225-304)	(21.7-36.2)	(5.6-7.1)	(1.5-2.0)	(14 - 18.6)
NE1	7.0 ± 0.5	77.8 ± 5.0	37.7 ± 3.7	40 ± 10.4	210.3 ± 50.9	27.7 ± 1.1	6.7 ± 0.3	1.7 ± 0.2	17.6 ± 1.0
	(6.3-8.4)	68.0-85.7	31.8-43.9	(18.9-57.5)	(107-296)	(25.8-30.2)	(6.3-7.0)	(1.5-1.9)	(16.6 - 19.7)
NE2	6.6 ± 0.4	76.4 ± 4.9	44.8 ± 6.3	43.6 ± 7.5	218.8 ± 35.1	29.6 ± 3.1	6.6 ± 0.4	1.7 ± 0.2	16.7 ± 1.1
	(5.9-7.2)	70.7-89.5	35.2-55.3	(32.6-58.1)	(153-278)	(23.3-34.6)	(6.1-7.2)	(1.5-2.0)	(15.5 - 19.2)
NE3	7.1 ± 0.3	79.93 ± 4.8	42.2 ± 8.1	43.8 ± 5.2	237.6 ± 30.0	28.3 ± 3.5	6.8 ± 0.6	1.8 ± 0.2	17.6 ± 1.2
	(6.6-7.5)	74.3-94.5	30.0-56.7	(34.8-57.3)	(193-314)	(23.3-36.2)	(6.1-7.5)	(1.5-1.9)	(15.5 - 19.7)
NE4	6.7 ± 0.3	77.9 ± 4.2	47.1 ± 8.2	46.3 ± 4.2	251.4 ± 23.9	29.1 ± 1.4	6.3 ± 0.4	1.5 ± 0.2	17.6 ± 1.5
	(6.2-7.2)	71.2-86.4	31.6-67.3	(39.3-54.4)	(222-294)	(26.8-31.8)	(5.6-7.1)	(1.0-1.5)	(15 - 20.7)
RRD1	6.8 ± 0.4	77.3 ± 4.0	48.4 ± 8.9	44.9 ± 9.0	223.1± 45.2	26.8 ± 2.4	7.1 ± 0.7	1.6 ± 0.2	17.5 ± 0.8
	(6.2-7.9)	73.0-86.9	30.4-61.4	(24.6-59.3)	(120-280)	(23.9-32.8)	(6.1-8.2)	(1.4-2.0)	(16.1 - 18.6)
RRD2	6.9 ± 0.3	83.9 ± 3.1	46.3 ± 5.7	38 ± 6.6	204.8 ± 38.0	29.4 ± 1.7	7.1±0.7	1.8 ± 0.2	17 ± 1.1
	(6.4-7.3)	78.1-90.4	36.4-55.4	(21.4-50.3)	(113-275)	(27.0-32.1)	(6.1-7.7)	(1.5-2.0)	(15 - 19.2)
NCC1	7.0 ± 0.7	77.5 ± 4.9	44.4 ± 6.9	43.9 ± 6.2	241.9 ± 37.7	29.3 ± 2.3	7.4 ± 0.4	2.0 ± 0.2	16.7 ± 1.0
	(6.0-8.3)	69.9-86.9	32.1-53.5	(29.0-53.3)	(159-299)	(25.0-35.0)	(6.6-7.9)	(1.5-2.4)	(15.5 - 18.1)
NCC2	6.6 ± 0.3	76.5 ± 2.6	51.2 ± 7.6	44.3 ± 4.9	239.8 ± 23.1	30.9 ± 2.7	6.4 ± 0.5	1.5 ± 0.2	17 ± 1.3
	(5.5-7.1)	73.2-81.8	38.6-63.8	(36.0-53.0)	(196.4-289)	(27.3-35.9)	(5.6-7.6)	(1.3-2.0)	(14.5 - 19.2)
CH	7.0 ± 0.3	78.9 ± 4.4	41.5 ± 7.1	45.1 ± 4.7	241.5 ± 28.6	29.1 ± 2.7	7.2 ± 0.8	1.7 ± 0.2	17.2 ± 0.7
	(6.4-7.6)	70.6-87.6	30.4-50.6	(36.4-52.6)	(192-288)	(23.9-33.4)	(6.1-8.2)	(1.3-2.0)	(16.1 - 18.6)
CV (%)	6.1	5.9	15.9	16.4	16.4	9.5	9.1	13.9	6.8

*: Measurements in µm. Mean ± standard deviation; (range).
For the list with the nematode population codes see Table 1.
b': body length/distance from anterior end to posterior end of pharyngeal gland; c: tail length/body width at anus; L/excr.pore: body length/distance from anterior end to excretory pore; T: distance from cloaca to anteriormost part of testis x 100/body length (%); testis: distance from cloaca to anteriormost part of testis. For the other abbreviations see Table 5.
CV: Coefficient of variation (%).

In the males of the Vietnamese *P. coffeae* populations, the coefficient of variation was the lowest for the stylet length (4.8%) and the pharynx length (5.9%). The coefficient of variation was the highest for the T value and the length of the testis (both 16.4%), and the pharyngeal overlap length (15.9%).

Table 5. Standardized coefficients for canonical variates of females and males of the *Pratylenchus coffeae* populations from Vietnam.

	Females		Males	
	Root 1	Root 2	Root 1	Root 2
% of variation	44.7	22.1	81.57	9.62
Selected characters	Vector loadings			
L	0.9690	0.2831	-1.3796	-1.2341
a	0.0784	0.8185		
b			1.7399	0.5770
c’	0.2800	-0.4652		
Stylet	0.1019	-0.1957	0.2669	0.5761
V	0.3434	0.3977		
Spicule length			0.0394	0.2695
Post-vulval uterine sac	-0.0340	0.5899		
Tail	-0.2947	0.6838		
DGO	-0.1586	-0.1068		
Lip width	0.2933	0.0928		
Lip height	-0.0940	-0.3505		
Pharyngeal bulb	0.8123	0.1580		
Excr. pore	0.5492	-0.7876	2.0118	-0.2585
Pharynx length	0.9582	-0.2664		
Pharyngeal overlap	0.0413	-0.1260		

L: total body length; a: total body length/maximum body width; b: total body length/distance from anterior end to pharyngo-intestinal junction; c’: tail length/body width at anus; V: distance of vulva from anterior end x 100/total body length (%); DGO: distance from basal knobs of stylet to the dorsal pharyngeal gland orifice; excr.pore: distance from anterior end to excretory pore.

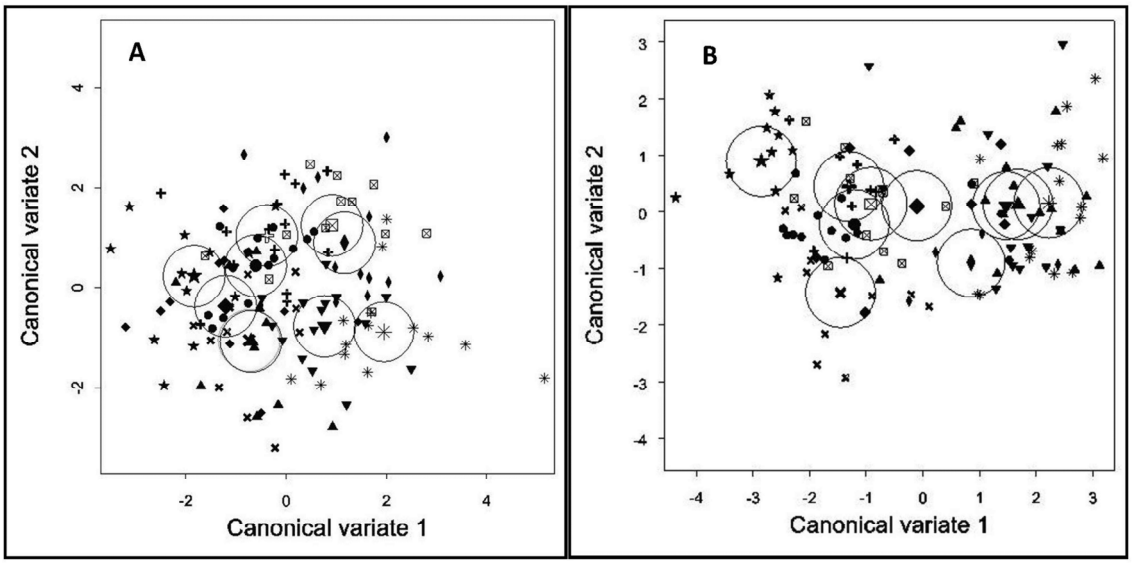

Fig. 6. Canonical discriminant analysis of ten *Pratylenchus coffeae* populations from Vietnam for females (A) and males (B) performed with 14 female variables and 5 male variables (see Table 5). The circles display 95% confidence regions. Nematode populations: Northwest (▼), Northeast 1 (W), Northeast 2 (◆), Northeast 3 (●), Northeast 4 (▲), Red River Delta 1 (★), Red River Delta 2 (**x**), North Central Coast 1 (⧫), North Central Coast 2 (*) and Central Highlands (+).

The results of the canonical discriminant analysis are presented in Table 6. Using a combination of 14 morphometrical characters for the females, it was not possible to separate the ten *Pratylenchus* populations from Vietnam by canonical discriminant analysis (Fig. 6A). However, a combination of five morphometrical characters for the males enabled the separation of the ten *P. coffeae* populations from Vietnam in three groups by canonical discriminant analysis (Fig. 6B). One group (on the right of Fig. 6B) includes the Northwest, Northeast 4 and North Central Coast 1 populations (the populations collected from banana) and the North Central Coast 2 population (a population collected from coffee). Another group (on the middle of Fig. 6B) clusters the other *P. coffeae* populations with the exception of the Red River Delta 1 population (a population originally isolated from banana), which stands out from the two other groups. The body length, the 'b' ratio and the distance from the anterior end to the excretory pore were the best morphometrical male characters for the separation of the populations

DISCUSSION

In the ten populations of *P. coffeae* from Vietnam examined the presence of substantial variability in morphology and morphometry within and between the populations was observed.

***En face* view of the lip (= 1st (lip) annulus).** Observed by scanning electron microscope, the *en face* view of the head (= 1st (lip) annulus) is similar in all *P. coffeae* populations from Vietnam examined: complete fusion of the 1st (lip) annulus with the oral disc resulting in an undivided *en face* view with no division between the lateral and median (sub-dorsal and sub-ventral) segments of the 1st lip annulus. According to Geraert (2006), the *en face* views of the lip region in the genus *Pratylenchus* as observed by scanning electron microscope are characterised by the fusion of the median segments with each other and the oral disc. The large lateral segments are either also fused with the median segments and the oral disc or are completely separated. The *en face* view of the *P. coffeae* populations from Vietnam is similar to the *en face* views of *P. coffeae* populations from citrus in Florida, USA, described and illustrated by Corbett and Clark (1983). By contrast, the *en face* views of a *P. coffeae* population from coffee (type host) in Java (near the type locality), Indonesia, show a distinct oral disc separated from the lateral and median segments of the 1st (lip) annule (Corbett & Clark, 1983; Golden *et al.*, 1992; Inserra *et al.*, 1998).

Pratylenchus coffeae was described in 1898 by Zimmermann from coffee in Java, Indonesia. Zimmermann did not designate a type location when he described *P. coffeae*, specifying only that it caused a decline of coffee in eastern Java. In 1953, the type material was re-examined by Sher and Allen and *P. coffeae* re-described. They synonomised *P. musicola* from banana and *P. mahogani* from mahogany with *P. coffeae*. Subsequently, lesion nematodes found on coffee, banana, citrus, yam and other crops have been identified as *P. coffeae*. Zimmermann, Sher and Allen were unable to use a scanning electron microscope to examine the *en face* views of the lip regions of the type specimens of *P. coffeae*. The only type material of *P. coffeae* preserved is a neotype designated by Sher and Allen in 1953 but there is ambiguity about the geographical origin of this neotype (Duncan *et al*., 1999). In addition to this neotype, specimens collected in 1952 from coffee in Djember near the type locality are also available in two permanent slides. This neotype and the Djember specimens have a divided *en face* view (Inserra *et al*., 1998). By contrast, *Pratylenchus* populations identified as *P. coffeae* from citrus in Florida (USA), Brazil and Oman, from yam in Puerto Rico (USA), Martinique and Brazil, from banana in Costa Rica, Honduras, Ghana and Malaysia, from cocoyam and Diffenbachia (both in Brazil), *Aglaonema* (Florida, USA) and ficus (China), and from coffee in Brazil and Indonesia have an undivided *en face* view (Duncan *et al*., 1999). In fact, of the 25 *P. coffeae* populations examined by Duncan *et al*. (1999), only the preserved neotype of *P. coffeae* has a divided *en face* view. Among these 25 *P. coffeae* populations were five populations collected from coffee in five different provinces of eastern Java which is the type locality of *P. coffeae*. Corbett and Clark (1983) suggested that this morphological character is a reliable taxonomic character for the identification of *Pratylenchus* populations at species level and this is confirmed by our observations. Therefore, we agree with Inserra *et al*. (2001) that the Kalwing (K6) *P. coffeae* population from eastern Java is a reliable standard for the morphological comparison with other *Pratylenchus* populations. In 2002, Ryss presented a tabular multi-entry key to the genus *Pratylenchus* and a computerised multi-entry image-operating key developed on the basis of the stepwise computer diagnostic system BIKEY-PICKEY using 26 morphological and morphometrical characters.

However, the *en face* view of the lip region was not included as a diagnostic morphological character.

Number of lip annuli. With the exception of some specimens of the Red River Delta 2 and Central Highlands populations, which have three annules on one side and two annules on the other side, all specimens of all *P. coffeae* populations from Vietnam examined have two lip annules. Román and Hirschmann (1969) also observed that in *Pratylenchus* species characterised by the presence of two lip annuli, such as *P. coffeae*, individuals were sometimes found with three annuli on one side and two annuli on the other side. Corbett and Clark (1983) remarked that in the genus *Pratylenchus* the number of lip annuli varied between species and somewhat within species but that the variability was never so large as to invalidate the number of lip annuli as a reliable diagnostic character. The number of lip annuli was included as a diagnostic morphological character in the multi-entry keys of Ryss (2002).

Structure of the lateral field. In all the *P. coffeae* populations from Vietnam examined the basic pattern of the structure of the lateral field can be described as follows: beginning at the 9^{th} or 10^{th} annulus as two lines, widening to three lines at the following annuli and to four lines at the level of the median pharyngeal bulb that extend to the phasmid; only the outer bands extend until the tail tip where they fuse to one band; outer lines crenate in the tail region; occasionally the internal band sculptured by striae; viewed with the light microscope in some specimens punctuations scattered in the outer bands were observed; viewed with the scanning electron microscope in all populations areolation of the outer bands was observed. Similar deviations from the basic pattern were reported by Román and Hirschmann (1969). They studied six *Pratylenchus* species (*P. penetrans*, *P. vulnus*, *P. scribneri*, *P. zeae*, *P. brachyurus* and *P. coffeae*) but found punctuations scattered in the outer bands of the lateral field only in *P. coffeae.* In Tylenchida, the structure of the lateral field is considered a useful taxonomic character (Siddiqi, 2000). But in the genus *Pratylenchus* variability, especially of the ornamentation of the lateral field, was found in so many species as to make the structure of the lateral field an unreliable diagnostic character according to Román and Hirschmann (1969), and Corbett and Clark (1983). Nevertheless, Ryss (2002) included three features of the lateral field (lateral field incisures at mid-body; lateral field areolation; lateral field incisures between phasmid and tail tip) in his multi-entry keys. At mid-body, all *P. coffeae* populations from Vietnam examined in our study had four lines.

Shape of the spermatheca. According to Ryss (2002), the best diagnostic morphological character to separate species of the genus *Pratylenchus* is the shape of the spermatheca in females. In his view, the shape of the spermatheca represents a sequence of (five) stages of reduction which corresponds with the transition of *Pratylenchus* species from an amphimictic to a parthenogenic mode of reproduction. Parthenogenesis is typical for more than 60% of the *Pratylenchus* species. *Pratylenchus coffeae* is amphimictic. Ryss (2002) distinguishes the following five stages: a) spermatheca filled with sperm, round, b) spermatheca filled with sperm, oval, c) spermatheca without sperm, distinct, offset, with round or oval cavity, d) spermatheca without sperm, distinct, offset, with slit-like cavity and e) spermatheca indistinct, not offset from outline of female genital branch. Separation of *Pratylenchus loosi* from *P. coffeae* was only based on the difference of the structure of the spermatheca between these two species (Pourjame *et al*., 1997). For *P. loosi*, the spermatheca was described as long, oval to almost rectangular, filled with rounded sperms. In the *P. coffeae* populations from Vietnam examined the shape of the spermatheca varied from small rounded to large oval, usually filled with sperms.

Shape of female tail and tail tip. In some *Pratylenchus* species, including *P. coffeae*, the intraspecific variability in tail and, especially, tail tip shape can be very high (Román & Hirschmann, 1969; Tarjan & Frederick, 1978; Corbett & Clark, 1983; Bajaj & Bhatti, 1984). Ryss (2002) included the shapes of the tail tip and the tail tip annulation as two different morphological characters of the tail in his multi-entry keys. In our study of the *P. coffeae* populations from Vietnam we distinguish 12 types (Table 3.3) based on a combination of the tail tip and tail tip shape following the descriptions by Román & Hirschmann (1969), Tarjan & Frederick (1978), Corbett & Clark (1983) and Bajaj & Bhatti (1984).

Based on the coefficient of variation (CV), the position of the vulva (V value; CV = 3.1%) in the females and the stylet length in both females (4.6%) and males (4.8%) of the *P. coffeae* populations from Vietnam examined are the least variable morphometrical characters. This observation confirms earlier reports (Román & Hirschmann, 1969; Tarjan & Frederick, 1978; Bajaj & Bhatti, 1984; Café Filho & Huang, 1989) that in *P. coffeae* the intraspecific variability of the position of the vulva and the stylet length is low and stable and thus of diagnostic value. The V value observed in the *P.*

coffeae populations from Vietnam examined ranged on average from 79.2 to 81.9%. The stylet length of the *P. coffeae* populations from Vietnam examined ranged on average from 15.3 to 16.1 µm (females) and from 14 to 14.8 µm (males). These values are similar to values reported for *P. coffeae* populations from the Americas (Duncan *et al.* 1999; Inserra *et al.* 2001), Africa (Van den Berg, 1971; Duncan *et al.*, 1999) and Asia (Sher & Allen, 1953; Rashid & Khan, 1976; Bajaj & Bhattti, 1984; Inserra *et al.*, 1998; Xiuhua, 2006).

Based on the coefficient of variation (CV), the length of the ovary in females (CV = 22.1%) and the length of the testis in males (T value; CV = 16.4%) of the *P. coffeae* populations from Vietnam examined are the highest variable morphometrical characters. This is not unexpected since the length of these structures will depend upon the stage of reproductive development of the nematodes measured. In the females, the coefficient of variation of the length of the post-vulval uterine sac was also very high (CV = 17%) and ranged on average from 25.5 to 31.2 µm. This observation confirms earlier reports (Román & Hirschmann, 1969; Tarjan & Frederick, 1978; Bajaj & Bhatti, 1984). All other morphometrical characters, including those commonly used in nematode taxonomy, have relatively high coefficients of variation.

Canonical discriminant analysis enabled the separation of the ten *P. coffeae* populations from Vietnam in three groups based on a combination of five morphological characters for the males but there was no relationship nor between these groups and their geographic origin or between these groups and their host plants.

The best morphometrical male characters for the separation of the populations (body length, 'b' ratio and distance from the anterior end to the excretory pore) correspond partly with the male morphometrical characters with the lowest coefficient of variation and partly with the morphometrical characters most commonly used to separate *Pratylenchus* species. de La Peña *et al.* (2007) reported that canonical discriminant analysis enabled discrimination of four *Pratylenchus* species: *P. pratensis*, *P. dunensis*, *P. brzeskii* and *P. penetrans*. For the females and males 62.8% and 65.2%, respectively, of the variation of the characters selected could be explained by differences in 12 morphometrical characters. However, in their analysis, body length and distance from the anterior end to the excretory pore were not included. Also in their analysis, the b ratio was an important discriminating morphometrical character. Discriminant analysis also differentiated several *Pratylenchus* species from the three valid species of *Zygotylenchus* (Palomares-Rius *et al*., 2010). Seven characters accounted for 71.1% of the variation of the characters selected. The morphometric characters with the greatest discriminating power were: length of the post-vulval uterine sac; body length/length of the post-vulval uterine sac ratio, number of female tip annuli and the 'b'ratio.

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Nguyen Thi Tuyet, A. Elsen, Ho Huu Nhi, D. De Waele. Морфологическая и морфометрическая характеристика десяти популяция *Pratylenchus coffeae* из Вьетнама.

Резюме. Для оценки уровня внутривидовой вариабельности морфологических и морфометрических признаков десяти популяций *Pratylenchus coffeae,* собранных от различных культур в различных агро-экологических регионах Вьетнама, было проведено сравнение этих популяций по данным световой и сканирующей электронной микроскопии. Выявлена существенная изменчивость по морфологическим и морфометрическим признакам как в пределах отдельных популяций, так и между ними. Тем не менее, наблюдаемые различия попадают в пределы изменчивости этих признаков, описанные ранее для популяций *P. coffeae* из различных регионов мира. Наблюдения в сканирующем электронном микроскопе подтвердили полное слияние 1-го (губного) кольца кутикулы с оральным диском, что проявляется в отсутствии границ раздела на апикальном (*en face*) виде головного конца, а также в отсутствии разделения между латеральным и медианными (субдорсальными и субвентральными) сегментами 1-го (губного) кольца. Хотя канонический дискриминантный анализ позволил разделить десять вьетнамских популяций *P. coffeae* на три отдельные группы по совокупности пяти морфологических признаков, не удалось выявить какой-либо связи этих групп с растениями-хозяевами или их географическим происхождением.