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A comparison of the variation in Indian populations of pigeonpea cyst nematode, *Heterodera cajani* revealed by morphometric and AFLP analysis

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Abstract

The cyst nematode *Heterodera cajani* is one of the major endemic diseases of pigeonpea, an important legume for food security and protein nutrition in India. It occurs in several pulse crops grown over a range of Indian agro climatic conditions but the extent of its intraspecific variation is inadequately defined. In view of this, 11 populations of *H. cajani* were analyzed using morphometrics and the results correlated with those obtained from an AFLP approach using 24 primer pair combinations that amplified a total of 1278 AFLP markers. The cluster solution from this binary data indicated similarities for five populations that differed from those suggested by morphometrics. The differences obtained could not be related to geographic distance between populations. The data suggests that recent and long distance dispersal has occurred whose causes need to be defined to restrict further field introductions. Four AFLP primer pairs clustered the populations similarly to that generated using all 24 primer pairs. This simplified approach may provide a rapid basis for discriminating populations for their future management and help to check further distribution in agricultural trade. It may also have potential to determine differences in populations that relate to host range or virulence to resistance genes.

Keywords

Heterodera cajani, pigeonpea cyst nematode, intraspecific variation, morphometrics, AFLP

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Introduction

The legume pigeonpea, (*Cajanus cajan* [L.] Millsp) is one of the most important pulse crops grown in India which produces 90% of the global production with over 100 cultivars on 2.4 million hectares. Outputs of over 750 Kg/ha about 50 years ago have now declined to 647kg/ha (www.icrisat.org). Diseases (*Fusarium* wilts, sterility mosaic) and pests such as pod borer and nematodes are all assumed to have contributed to the loss of productivity per hectare (www.icrisat.org). *Heterodera cajani* Koshy is the most important nematode pathogen of pigeonpea in India (Sharma 1988). It was first reported on this crop in 1967 in the New Delhi area (Koshy 1967). The economic importance of this nematode in pulse production was first highlighted by Saxena and Reddy (1987), who reported that it causes yield losses of about 30%. It now occurs in all the major pigeonpea producing states of India i.e. Andhra Pradesh, Bihar, Gujarat, Haryana, Karnataka, Maharashtra, Punjab, Rajasthan, Tamil Nadu and Uttar Pradesh. It is particularly widespread on sandy loams in Northern India and vertisols of Southern India (Sharma et al. 1992). The adoption of suitable management practices for control of these important plant parasitic nematodes is essential to curb economic losses.

Knowledge of variability of an economic plant parasitic nematode species is important for the selection of appropriate control strategies (Hyman 1996). Two races of *H. cajani* (race A pigeonpea race and race B clusterbean race) have been reported based on differential hosts for seven populations originating from Ambala (Punjab), Faridabad (Haryana), Bhiwani (Haryana), Ludhaina (Punjab), Delhi (Delhi), Coimbatore (Tamil Nadu), and Hyderabad (Andhra Pradesh) (Walia and Bajaj 1988). A second study discriminated three races among 14 populations from seven districts of just Uttar Pradesh (Siddiqi and Mahmood 1995). Use of the host differentials of cowpea, mungbean, soybean and pigeonpea accessions confirmed the presence of races of *H. cajani* (Mehta and Bajaj 2005). Race identification using differential hosts is time consuming and influenced by many other external factors (Koshy and Swarup 1971, Sharma et al. 1991 and Singh and Sharma 1994). Measurements based on both second stage juveniles (J2) and cyst vulval cones have often proven useful for identifying species of cyst nematodes but similar measurements do not define intraspecific variation in populations of the root knot nematode, *Meloidogyne incognita* that relates to host range (Hirschmann 1985). Analysis of genetic variability in species and populations of the genus *Heterodera* spp has been studied by a wide range of approaches including host preference (Anderson and Anderson 1982), protein analysis (Podzol and Noel 1984, Ferris et al. 1989, Bossis and Rivoal 1996), isozymes (Nobbs et al. 1992), and by random amplified polymorphic DNA analysis (RAPD) (Bendezu et al. 1998, Kaplan et al. 1998, Zhang et al. 1998, Silva et al. 2000, Lax et al. 2004, Umarao et al. 2007). Amplified fragment length polymorphisms (AFLP) also have value as a highly reliable, robust, repeatable approach for studying the genetic structure of populations (Vos et al. 1995).

AFLP is a useful approach for the genetic analysis of nematodes (Grant and Viney 2001). It detected polymorphism in populations of the animal parasitic nematode, *Haemonchus contortus* (Otsen et al. 2001) and intraspecies variation detected by the approach could be correlated with differences in virulence of the potato cyst nematodes *Globodera*

pallida and *G. rostochiensis* (Folkertsma et al. 1996). Similarly, AFLP analysis of 15 populations belonging to *M. arenaria, M. incognita* and *M. javanica* revealed *M. arenaria* and *M. javanica* to be the most and least variable species respectively (Semblat et al. 1998 and 2000). AFLP analysis confirmed the current classification of tobacco cyst nematode complex and specific markers were identified for two of its subgroups i.e. *G. tabacum tabacum* and *G. tabacum solanaceraum* (Marche et al. 2001). Similarly, the normal and giant races of *Ditylenchus dipsaci* could be differentiated by AFLP markers (Esquibet et al. 2003). The approach has also been applied to cyst nematodes of the *Heterodera* genus, including *H. schachtii* (Madani et al. 2007) and *H. trifolii* (Wang et al. 2001).

We have compared standard morphometric measurements for both J2 and the vulval cones of cysts with AFLP analysis for 11 populations of *H. cajani* recovered from across India to determine the comparative utility of the three approaches to discriminate the populations. Cluster analysis was used to compare the relationships defined by the methods used. As a result, we report an AFLP-based approach for identifying major sub groups of *H. cajani* in India. The results suggest that the current wide distribution of the populations in India is recent.

Material and methods

Collection and multiplication of *Heterodera cajani* populations

Soil samples of 11 *H. cajani* populations were collected during surveys, where pigeonpea is cultivated (Table 1). Populations were multiplied on pigeonpea plants growing in pots under glass house conditions to provide continuous stocks of encysted eggs for the work programme. Seventy-five days after adding cysts to plantlets, the soil in the culture pots was processed using Cobb's sieving technique. Cysts were handpicked using a stereo binocular microscope and then processed for detailed morphological and genetic studies. Second stage juveniles (J2s) were expressed from their eggshells after opening cysts with needles.

Morphological studies

The cyst vulval cones and J2 of populations were studied using light microscopy. The J2s were heat killed, fixed in 2% formaldehyde and processed following the method of Seinhorst (1959). Measurements were made using a compound research microscope (Leica) and the characters measured were body length, maximum body width, length of stylet, distance from the head to the excretory pore, distance from the head to the median bulb valve, distance from head to esophageal gland lobe, tail length and hyaline tail length. The morphometric characters for the cysts were vulval slit length, vulval bridge length, vulval bridge breadth, underbridge length, length of fenestra, breadth of fenestra and distance from the anus to fenestra (Koshy 1967).

Table 1. Different *Heterodera cajani* populations collected from various agroclimatic regions of India

Population Number	State	Locality
1	Uttar Pradesh	Allahabad
2	Andhra Pradesh	Hyderabad
3	Uttar Pradesh	Bahadurgarh
4	Uttar Pradesh	Kanpur 1
5	Karnataka	Gulberga
6	Uttar Pradesh	Ghaziabad
7	Haryana	Hisar
8	Uttar Pradesh	Kanpur 2
9	Delhi	Delhi
10	Tamil Nadu	Coimbatore
11	Uttar Pradesh	Meja

Genomic DNA Extraction

Genomic DNA was isolated by using Ultra pure Mammalian Genomic DNA Prep Kit (Bangalore Genei Pvt Ltd, Bangalore, India, Cat # KT-81). The quality and yield of genomic DNA was determined by running samples on 1% agarose gel. DNA concentrations were estimated spectrophotometrically (Perkin Elmer, Lambda-32, UV/visible, USA).

AFLP-PCR

AFLP analysis was performed according to Vos et al. (1995) with modifications in the detection techniques, using radioactivity. Genomic DNA (1 µg) was restricted with *Eco*RI and *Mse*I enzymes (2.5 U each) and linked to adapters (50 and 5 pmols of *Mse*I and *Eco*RI adapters, respectively). Restricted and ligated DNA (50 ng) was pre-amplified using *Eco*RI and *Mse*I primers (50 ng), both with one selective nucleotide. Selective amplifications were performed with a combination of *Eco*RI and *Mse*I primers (15 ng) that had three selective nucleotides each. Twenty four primer pair combinations were used, chosen from the 64 primer pair combinations tested.

PCR conditions were as follows: the preamplification mixture was prepared in a total volume of 50 µl and amplified using 20 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s. The following touchdown protocol was used for selective amplification in a 10-µl volume: 13 cycles of 94°C for 30 s, 65°C for 30 s with a decrease of –0.7°C per cycle, and 72°C for 1 min; followed by 23 cycles at the annealing temperature of 56°C. The PCR products from each primer combination were separated in 6% denaturing polyacrylamide gels in 1× TBE buffer and visualized with autoradiogram. AFLP bands were scored for absence (0) or presence (1) across the analyzed accessions for each primer combination.

Statistical approaches

One-way analysis of variance of variables for cyst cones and J2s were carried out using SPSS version 16.0 with *a priori* contrasts between the reference population (Delhi) and each of the other populations. Cluster and related analyses were completed using a standard package for a portable computer and the recommended analyses provided by this software (Clustan graphics version 7.05, Clustan Ltd, Edinburgh, Scotland; http://www.clustan.com). This involved the morphometric data but not binary data being transformed to z-scores. The steps in the analysis were selected and then conducted automatically generating correlation coefficients, Eigen values and principal component values. The hierarchical cluster method selected for both continuous and binary data was the increase sum of squares method (Ward's method). The upper tail rule was used within the Clustan package for the best cut to generate cluster solutions. This procedure takes the fusion values as a series, computes the mean and standard deviation, and a t-statistic as the standardized deviation from the mean. It then computes the standard deviation for each fusion value on this distribution (assumed normal), and indicates the highest number of clusters that show a significant departure from the distribution of fusion values.

Mantel's tests were used to examine the relationship between distance matrices derived from J2, cyst and AFLP data with that from geographical distances between populations within India. Any trend in the three nematode data matrices with geographical distance was explored using a series of classes representing successively larger geographic distances. Both analyses were carried out using a specialist statistical package for a personal computer (PASSaGE 2).

Results

The geographical origin of the 11 populations of this study is provided in Table 1. Means for nine measurements taken from J2s of the 11 populations of *H. cajani* are given in Table 2. Only the population from Coimbatore had similar means for the all nine measurements with that of Delhi population whereas Meja differed from Delhi in five of its means. Of a total of 27 mean differences from Delhi, only four mean values were higher than the reference population and on each occasion it was different population and character involved. The means for the six measurements taken from cyst cones of the populations are provided in Table 3. The three populations, Allahabad, Hyderabad and Coimbatore, did not differ significantly from the Delhi population for any of the measurements. All other populations showed at least one mean that differed from this reference population with those from Bahadurgarh and Meja having differences for three values. In all cases the Delhi population had higher means than other populations that differed from it, with the sole exception that the length from the anus to the edge of the fenestra was greater for Kanpur 2 relative to the reference population. The mean fenestral width of the Delhi population was also slightly higher than the value provided in its original description.

Table 2. Morphometrics of juveniles (in µm) of 11 populations of *Heterodera cajani* Note: Values are means ± standard error of the mean. ***, P<0.001; **, P<0.01, * P<0.05, for comparisons of each mean with the corresponding value for the Delhi population using Oneway ANOVA with *a priori* contrasts.

Populations	Body length	Body width	Stylet length	Distance from ant. end to median valve	Ant. end to excretory pore	Length from ant end to gland overlapping	Tail length	Hyaline tail length	Length from anterior end to Genital primordia
Allahabad	457.93 ± 4.95	19.73 ±0.12	25.07 ±0.67	71.47 ±1.70*	126.93 ±3.32	206.00 ±4.08	48.53 ±1.10	25.53 ± 0.74	288.13 ± 4.13
Hyderabad	453.07 ±3.88	19.87 ±0.09*	26.07±0.61	67.60 ±1.10	118.27±3.66	216.33 ±5.74	49.60 ±0.96	27.00 ± 0.59	283.73 ± 7.73
Bahadurgarh	442.07 ±7.81	19.73 ±0.12	26.33± 0.41*	65.00 ±1.15	101.73 ±3.45***	160.80 ±3.70***	46.93 ±1.46	26.00 ± 0.89	265.80 ± 5.63**
Kanpur 1	461.13 ±8.12	19.47 ± 0.19	24.47 ±0.80	61.33 ±2.13*	100.47±3.64***	160.93 ±4.60***	48.60 ±1.41	26.73 ± 0.77	276.20 ± 5.97
Gulberga	421.87 ±9.18**	19.27 ± 0.23	25.53± 0.77	64.13 ±1.35	95.67 ±2.35***	152.07 ±6.41***	47.00 ±1.47	27.00 ± 0.77	249.3 ± 6.81***
Ghaziabad	444.00 ±5.03	19.27 ± 0.12	24.53 ±0.58	61.67 ±1.45*	106.33 ±3.15**	184.93 ±5.87***	46.27 ±1.05	25.80 ± 0.65	272.33 ± 6.34*
Hisar	420.00 ±6.64***	19.47 ± 0.19	24.73 ±0.59	63.80 ±1.59	102.33 ±3.38***	149.20 ±4.09***	46.53 ±1.37	24.93 ± 0.94	248.07 ± 7.04***
Kanpur 2	474.33 ±7.35	19.47 ± 0.13	25.60±0.75	66.47 ±1.82	127.27 ±2.95	222.80 ±5.16	49.40 ±2.35	30.53 ± 1.26***	310.27 ± 7.73
Delhi	462.80 ±7.05	19.53 ± 0.13	24.40 ±0.42	66.13 ±1.12	121.87 ±3.23	212.47 ±4.46	47.07 ±1.03	25.67 ± 0.67	293.93 ± 6.45
Coimbatore	462.13 ±4.85	19.73 ±0.21	25.13±0.35	68.13 ±1.50	120.80 ±3.57	199.80 ±4.90	46.67 ±0.93	24.93 ± 0.55	291.47 ± 8.21
Meja	455.87 ±4.55	19.07 ± 0.18*	24.60 ±0.60	59.00 ±1.40**	93.93 ±2.53***	145.33 ±4.28***	48.53 ±1.52	26.00 ± 0.80	270.53 ± 5.46*

Note: Values are means ± standard error of the mean. ***, P<0.001; **, P<0.01, * P<0.05, for comparisons of each mean with the corresponding value for the Delhi population using Oneway ANOVA with *a priori* contrasts.

Table 3. Morphometrics of cyst vulval cones (in µm) of 11 populations of *Heterodera cajani*

Populations	Vulval bridge length	Vulval slit length	Vulval bridge width	Fenestral Length	Fenestral Width	Length from Anus to edge of fenestra
Allahabad	58.20 ±1.530	44.80 ± 1.020	9.60 ± 0.245	55.00 ± 1.732	41.60 ± 0.748	32.00 ± 1.449
Hyderabad	55.20 ± 2.396	44.00 ± 1.643	9.40 ± 0.245	47.00 ± 2.387	37.80 ± 2.583	31.40 ± 2.542
Bahadurgarh	49.20 ± 0.970**	37.60 ± 0.980***	8.40 ± 0.245*	55.80 ± 2.853	40.20 ± 1.114	29.40 ± 2.821
Kanpur 1	52.40 ±1.965	42.60 ± 1.435	9.60 ± 0.245	59.80 ± 4.116	37.20 ± 2.634*	33.40 ± 2.857
Gulberga	48.00 ±0.707**	42.40 ± 0.980	9.20 ± 0.583	55.20 ± 3.878	37.40 ± 0.748*	28.00 ± 1.304
Ghaziabad	52.40 ±1.288	42.00 ± 1.304*	9.80 ± 0.249	58.00 ± 3.240	39.00 ± 2.000	33.80 ± 1.020
Hisar	52.70± 1.126	42.30 ± 1.001*	9.20 ± 0.44	53.80 ± 2.059	37.90 ± 1.716*	29.90 ± 1.345
Kanpur 2	53.60 ±2.379	44.60 ± 0.980	8.40 ± 0.400*	46.00 ± 1.673	38.60 ± 1.536	30.80 ± 2.200*
Delhi	56.00 ± 2.074	46.20 ± 1.960	9.60 ± 0.245	52.60 ± 1.661	43.00 ± 1.924	29.60 ± 2.293
Coimbatore	56.00 ±1.225	44.40 ± 1.364	10.00 ± 0.316	52.40 ± 1.030	38.00 ± 2.846	29.80 ± 2.059
Meja	49.70 ± 0.651**	40.30 ± 1.001**	9.40 ± 0.221	51.30 ± 1.535	35.70 ± 1.075*	30.50 ± 1.515

Note: Values are means ± standard error of the mean. ***, P<0.001; **, P<0.01, * P<0.05, for comparisons of each mean with the corresponding value for the Delhi population using Oneway ANOVA with *a priori* contrasts.

Principal component analysis of the J2 data is presented in Table 4a in ascending order of values for principal component one (PC1). The analysis revealed that 80% and 89% of the data was represented in two and three dimensions respectively (Table 4a). There was no correlation between principal component one (PC1) and PC2 values ($r \leq 0.1$). The data show a widespread range of PC1 values from -2.72 to 3.24 with Kanpur 2 having the most positive values for PC1 and the second most negative for PC2. Principal component scores for the vulval cone data established that 68% and 84% of the variation could be represented in 2 and 3 dimensions respectively (Table 4b). Again there was no correlation between the first and second principal components (PC1 and PC2, $r \leq 0.1$). Five populations had negative PC1 values but differed little in PC2 whereas all other populations had positive PC1 values. Two of these populations with PC1 values close to zero had distinctly positive PC2 values (Ghaziabad and Kanpur 1) whereas Kanpur 2 differed in having a negative PC2 value.

The PC analyses suggested that complex differences between populations occurred and this was explored further using cluster analysis. Data for the 11 populations of J2s in Table 2 were subjected to cluster analysis as described in the methods. The upper tail rule was used for the best cut and it generated the two significant cluster solution as shown in the dendogram (Fig 1a). The populations from Kanpur 2, Hyderabad, Delhi, Allahabad and Coimbatore were in one cluster with the remaining six populations falling under a second cluster. The same cluster approach was adopted for the vulval cone biometrics and three clusters were generated in this case. Cluster 1 had the same members as that for the J2 data. The remaining data subdivided the populations in cluster 2 of the J2 data with Ghaziabad and Kanpur 1 populations separating from the other four populations (Fig 1b). Combining the data resulted in the principal component analysis representing 59% and 74% of total variance in 2 and 3 dimensions respectively. This combined data set provided same two cluster populations as that for the J2 data only. The results for the combined data are therefore not shown in Fig 1 and Table 4.

Molecular Data Analysis

AFLP banding from the two replicate DNA extracts was read for each population but no qualitative difference occurred between them. The eleven nematode populations were each tested with 64 pairs of primers to generate AFLP fingerprints. The number of amplification products ranged from 34 (primer pair +AAA, with +CTA) to 94 (+AAG with +CAG; Figure 2). A subset of 24 primer pair combinations of *EcoR* I and *Mse* I gave a good range of amplification products for all populations shown in Table 5. The fragments generated by the 24 selected primer pairs were recorded in a binary manner.

Principal component analysis revealed that 44% and 33% of the full AFLP data set could be represented in three or two dimensions respectively (Table 6a). The corresponding values for a subset of four primers were 46% and 34% respectively (Table 6b). For both sets of data, four dimensions were required to represent just over 50%

Table 4a. Principal Component scores from cluster analysis for nine J2 morphometrics of *Heterodera cajani*

Populations	Dimensions for J2 morphometrics								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Allahabad	2.10	1.07	-0.38	0.18	1.13	-0.05	-0.26	-0.07	0.08
Hyderabad	2.22	1.14	1.60	-0.38	0.14	0.09	0.59	0.09	-0.05
Bahadurgarh	-1.10	1.56	0.56	-0.43	-0.65	-0.09	-0.49	0.04	-0.02
Kanpur 1	-0.77	-1.15	0.51	-1.10	-0.02	0.51	-0.19	-0.08	0.12
Gulberga	-2.41	0.24	1.04	1.25	0.22	0.21	-0.26	0.10	-0.04
Ghaziabad	-1.34	-0.43	-0.77	0.47	-0.17	0.69	0.35	-0.21	-0.04
Hisar	-2.72	1.77	-0.44	0.04	-0.16	-0.68	0.36	-0.08	0.11
Kanpur 2	3.24	-2.20	0.49	0.58	-0.53	-0.46	-0.11	-0.17	0.01
Delhi	1.72	-0.36	-1.21	0.29	-0.40	0.22	0.08	0.32	0.09
Coimbatore	1.33	1.37	-1.16	-0.44	-0.04	-0.03	-0.16	-0.05	-0.18
Meja	-2.26	-3.01	-0.24	-0.48	0.49	-0.41	0.08	0.12	-0.08
Accumulative % variance		80	89	94	97	99	100	100	100

Table 4b. Principal Component scores from cluster analysis for six vulval cone morphometrics of *Heterodera cajani*

Populations	Dimensions for Vulval cone morphometrics					
	1	**2**	**3**	**4**	**5**	**6**
Allahabad	2.21	0.17	0.94	0.38	-0.19	-0.30
Hyderabad	0.80	-0.73	-1.29	0.66	-0.22	-0.02
Bahadurgarh	-2.76	-0.67	1.67	0.70	-0.47	-0.06
Kanpur 1	-0.01	2.15	-0.12	0.28	0.52	-0.16
Gulberga	-1.79	-0.52	-0.10	-1.38	0.46	0.26
Ghaziabad	0.41	2.05	0.23	0.52	-0.06	0.55
Hisar	-0.69	0.32	0.38	-0.53	0.26	-0.67
Kanpur 2	0.09	-1.82	-0.76	1.16	0.58	-0.03
Delhi	2.11	-1.19	1.18	-0.65	0.11	0.47
Coimbatore	1.24	-0.02	-0.80	-1.07	-0.54	-0.27
Meja	-1.61	0.27	-1.32	-0.07	-0.45	0.23
Accumulative % variance		68	84	95	98	100

of the accumulative variation with 6-7 dimensions representing 75% of the variance. Therefore PC analysis was not used further for this data. As a consequence, all PC data for both morphometric and AFLP analyses are shown in tables rather than two or three-dimensional plots which cannot adequately represent the AFLP data.

Cluster analysis was used for the full AFLP set as described in the statistical methods and the best cut indicated three clusters (Figure 3a). The first cluster consisted of the Hyderabad, Allahabad, Ghaziabad and Bahadurgarh populations, the second included those from Delhi, Kanpur-2 and Meja while, the populations from Coim-

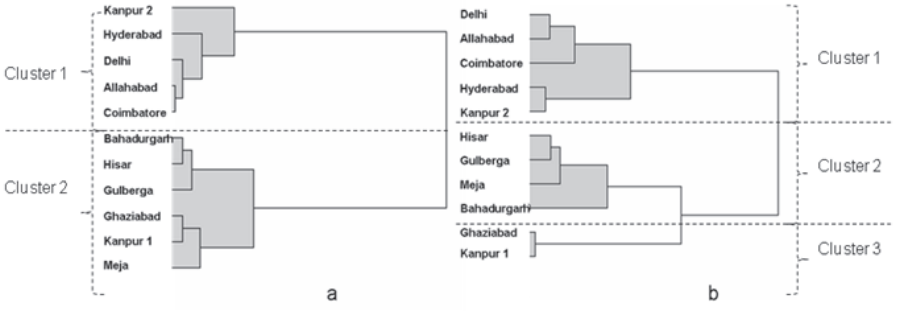

Figure 1. Dendograms from cluster analysis **a)** for the nine biometric measurements made on second stage juveniles of eleven populations of *Heterodera cajani* (see Table 2 for data) **b)** vulval cones of cysts of the same populations. (See Table 3 for data). The using the upper tail rule the best cut procedure indicated the highest number of significant cluster partitions was for a) 2 and for b) 3 with realized deviates and t- statistics respectively of a) 2.71 and 8.56 and b) 1.04 and 3.27.

batore, Kanpur 1, Gulberga and Hisar have been grouped as third cluster. Comparison of this dendogram with cluster trees provided by subsets of the 24 AFLP primer pairs suggested that the four highlighted in Table 5 with an asterisk provided similar dendograms to that generated by all the primer pairs (Figure 3b).

Mantel and partial Mantel tests did not detect a significant relationship between the matrix of geographical distances (see Figure 4 for a map showing locations of the 11 populations) and any of the three genetic distance matrices based on the data for J2s, cysts or AFLP (Table 7). Correlograms did not detect significant mantel r values or trends for the three sets of nematode data using five or more distance classes for the matrix of geographical distance. There was also no significant mantel correlation between the distance matrix for the full AFLP data set and for either set of morphological data.

Discussion

The two-morphometric sets of data and the AFLP approach all recognized significant clusters of populations within the set of eleven tested. All three approaches clustered the Allahabad and Hyderabad populations together and likewise the Gluberga and Hisar populations were similar as were Delhi and Kanpur 2 populations. Morphometrics disagree with AFLP for relationships between populations from Ghaziabad and Bahadurgarh, and for Coimbatore and Kanpur 1. Meja also was not placed with the same populations using both morphology and genetic approaches. This suggests that the morphometric approaches are of limited value for the analysis of intraspecific variation with in *H. cajani*. The 11 populations used in this study have been analyzed before using RAPD analysis when a greater similarity between some of the populations than others was detected (Umarao et al. 2007).

Previous work with *G. pallida* (a potato cyst nematode) using microsatellites in Peru has established high genetic similarly between individuals within a field. The lim-

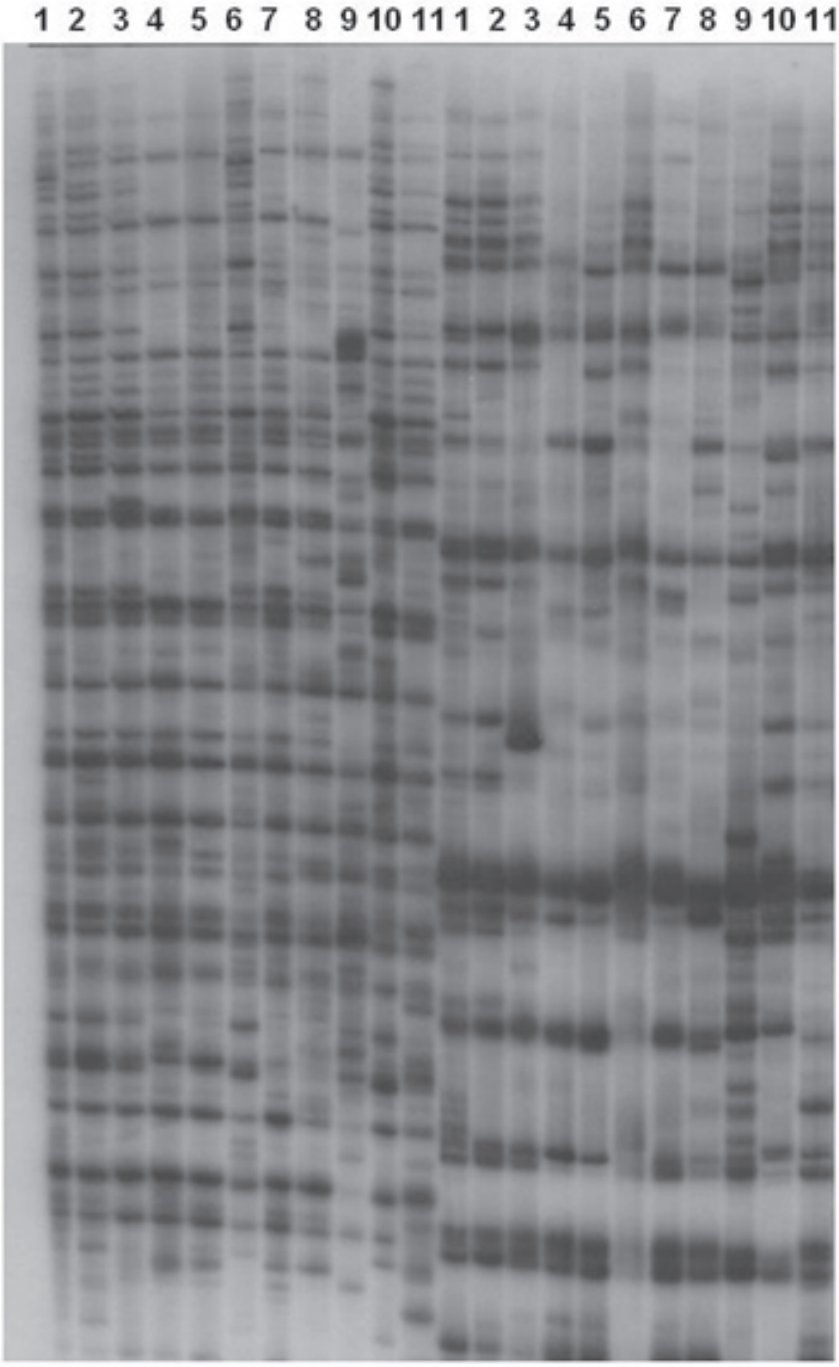

Figure 2. AFLP Autoradiogram of pigeon pea cyst nematode *Heterodera cajani* with *EcoRI* (+AAG) + *MseI*, (+CAG) and *EcoRI* (+AAA) + *MseI* (CTA). **Lane 1 to 11:** *Heterodera cajani* populations from Andhra Pradesh, Allahabad, Bahadurgarh, Coimbatore, Kanpur-1, Ghaziabad, Gilberga, Hisar, Delhi, Kanpur-2, and Meja.

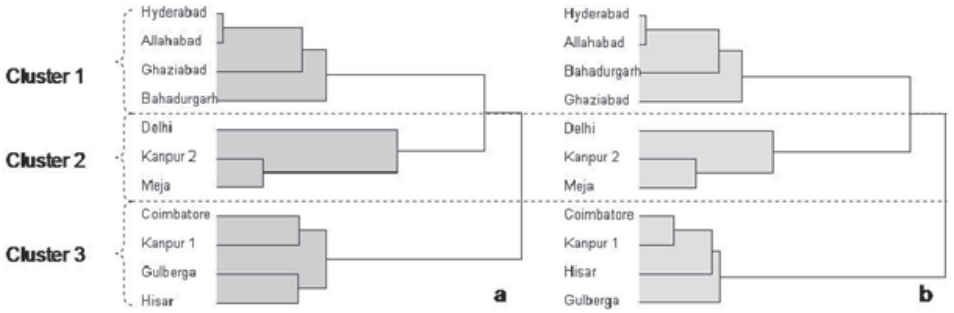

Figure 3. Dendograms from cluster analysis *of Heterodera cajani* **a)** for 1278 amplified restriction fragment digests using 24 primer pairs and **b)** the four primer pairs that suggest a similar dendogram to the full set. The using the upper tail rule the best cut procedure indicated the highest number of significant cluster partitions was 3 as in both cases with realised deviates and t statistics respectively of a) 1.47 and 4.66 and b) 1.59 and 5.04.

Table 5. Characterization of amplification products Obtained with 24 AFLP primer pairs used to analyze the genetic diversity of *Heterodera cajani* populations

Primer EcoR*I*/Mse*I*		**Total number of fragments Obtained**
+AAA,	+CAA	78
	+CAC	71
	+CAG*	80
	+CAT	58
	+CTA	34
	+CTC	44
+AAC,	+CAA	50
	+CAT	73
	+CTA*	64
	+CTC	54
+AAG,	+CAA	58
	+CAC*	44
	+CAG	94
	+CAT*	73
	+CTA	69
	+CTC	69
	+CTG	59
	+CTT	54
+AAT,	+CAA	44
	+CAC	44
	+CAG	44
	+CAT	55
	+CTA	40
	+CTC	43

Table 6a. Principal Component scores from cluster analysis for *Heterodera cajani* AFLP markers generated using 24 set of primer pairs

Populations	Dimensions									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Allahabad	10.75	-2.02	6.90	-1.32	-0.59	6.98	-0.08	-0.38	1.28	7.18
Hyderabad	11.45	-2.46	8.08	-5.01	-2.66	10.63	-0.37	0.90	1.20	-6.59
Bahadurgarh	8.70	2.41	6.41	9.80	1.78	0.93	0.43	0.13	3.06	-2.89
Kanpur 1	4.72	3.56	0.29	-1.84	3.38	5.30	11.10	5.63	0.42	-1.44
Gulberga	-0.32	2.36	-0.44	-2.92	-3.53	-0.31	-5.14	7.86	-0.46	-1.25
Ghaziabad	10.46	-3.95	4.00	-6.50	-2.35	-4.86	3.96	-1.58	6.47	-1.80
Hisar	-2.00	4.44	2.01	1.59	-11.32	5.77	4.68	-2.20	3.85	-1.14
Kanpur 2	6.14	-8.70	-4.41	0.83	-1.59	2.48	2.27	-4.31	-5.39	-2.22
Delhi	-7.54	-10.49	7.62	-0.24	0.76	3.88	2.35	0.86	2.88	-1.47
Coimbatore	-2.15	7.30	0.19	-4.26	5.36	5.08	-0.96	-5.41	3.62	-1.86
Meja	6.42	-7.56	-6.62	1.40	-0.24	7.61	0.22	2.01	9.07	-1.44
Accumulative % variance		33	44	54	64	72	80	88	95	100

Table 6b. Principal Component scores from cluster analysis for *Heterodera cajani* AFLP markers generated using 4 set of primer pairs

Populations	Dimensions									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Allahabad	-2.13	-6.29	-2.54	2.72	-1.53	-0.10	1.15	-1.73	1.24	3.37
Hyderabad	-2.75	-6.23	-3.55	3.22	-4.36	0.19	1.98	-1.87	-0.20	-1.93
Bahadurgarh	-0.71	-5.67	-1.54	4.16	2.84	-0.27	2.04	1.25	0.19	-0.30
Kanpur 1	0.22	-1.70	-0.43	3.20	-2.07	-0.48	2.14	0.11	5.04	-0.60
Gulberga	0.44	0.45	-0.41	0.33	-0.50	2.24	-0.66	-1.48	0.11	-0.08
Ghaziabad	-1.65	-5.22	-0.53	-2.26	-2.03	-2.59	1.03	1.85	0.67	0.08
Hisar	-0.47	-0.58	-0.94	2.52	-3.33	1.08	5.71	2.00	-0.46	0.81
Kanpur 2	-6.42	-2.00	1.84	1.59	-0.06	-1.79	3.72	-2.39	0.56	-0.04
Delhi	-5.68	0.66	-5.80	1.86	-0.52	-1.49	1.46	0.58	0.93	0.18
Coimbatore	2.48	-0.85	-1.13	4.17	-1.92	-4.12	1.21	-0.82	-0.21	0.23
Meja	-5.94	-2.27	1.33	4.95	-2.88	-0.48	-0.79	1.78	0.35	0.23
Accumulative % variance		34	46	57	66	75	83	91	96	100

ited active movement of J2s and males does not move them far in soil but tillage, either transport by surface water, running water and movement of infected potato tubers all contribute to a local homogeneity. Genetic similarity extends to neighboring fields and even those within a region which was defined as a range of about 35km for *G. pallida* in Peru (Piccard et al. 2004). This large passive dispersion is favoured inside an agronomic area where *G. pallida* has a continuous distribution and it is commonly at a high

Table 7. Mantel tests for the relationships between the matrix of geographical distance and the three genetic distance matrices based on the data for J2s, cysts or AFLP.

Type of Comparison (a) with geographical matrix	Mantel test		Partial Mantel	
	r	t-value	r	t-value
Biometric values on J2	-0.08	-0.61	-0.06	-0.43
Biometric values on vulval cones	-0.20	-1.43	-0.18	-1.24
24 AFLP primers	0.13	0.78	0.12	0.73
4 AFLP primers	0.03	0.14	0.02	0.12
(b) with 24 AFLP primer pairs distance matrix				
Biometric values on J2	0.09	0.63	0.10	0.68
Biometric values on vulval cones	-0.05	-0.33	-0.06	-0.44

Partial mantel tests were carried out a) for geographical distance among populations holding matrices not in the comparison constant with the 24 AFLP primer pair matrix being used when the biometric data was considered and b) for the correlations of the 24 AFLP primer pair genetic distance matrix with that for the two sets of biometic data holding that not in the comparison constant.

density (Picard and Plantard 2006). Genetic isolation did occur over large geographic distances which was greater than 50km for this nematode in the mountainous regions of Peru. AFLP has also successfully detected a pattern of isolation by distance for mountain pine bark beetle *Dendroctonus ponderosae* in western North America (Mock et al. 2007). The current results are similar to those for *G. pallida* in that Mantel r tests and correlograms established that genetic divergence did not correlate with geographic distances for the distantly spaced populations studied in this work (Figure 4). Further work may establish that populations of *H. cajani* like those of *G. pallida* share a genetic similarity when many populations in close proximity are analyzed.

Cluster analysis of both the AFLP and morphometric data revealed a similarity between populations belonging to different regions of India. Allahabad and Hyderabad cluster together for all approaches but they are about 2000 km apart as did Gulberga and Hisar which are 2200 km apart. Morphometric analysis on J2 means clustered Bahadurgarh and Meja populations together and Kanpur 1 population with that of Ghaziabad whereas the AFLP approach clustered these populations differently. AFLP analysis detected variation that was not evident in the morphological characters of J2s and vulval cones. Coimbatore is similar to populations in north India that are more than 2500 km from it (Figure 4). The two populations in closest proximity were those from Delhi and Ghaziabad. They are only 35 km apart but they did not cluster together with either AFLP or morphometric measurements. The variation we detected is not consistent with allopathic speciation over the large distances that prevail in India. It differs from the variation in *Wucheria bancroftii* in India which has been correlated with two geographically isolated and ancient introductions to this subcontinent (Thangadurai et al. 2006). Possibly the pattern of variation in *H. cajani* reflects

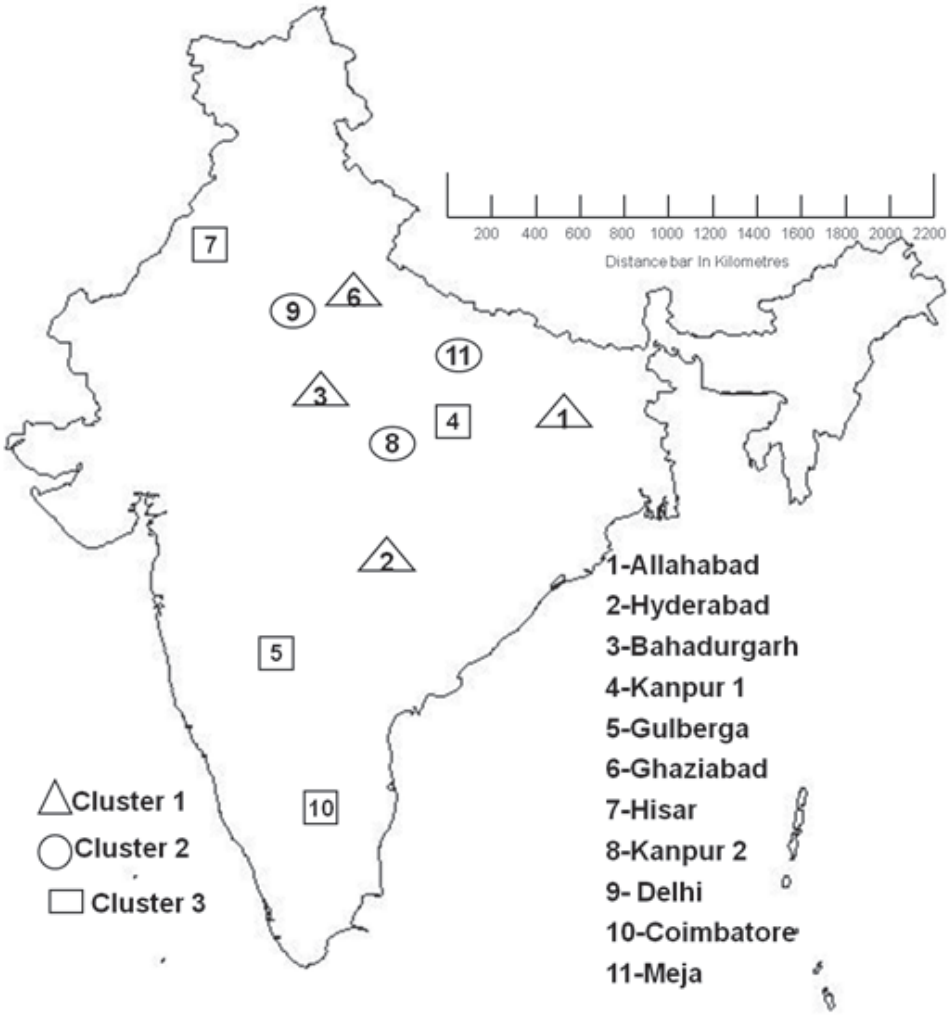

Figure 4. India Map showing distances of collected 11 *H. cajani* populations with distances in (Kilometres)

modern dispersal of cysts. It is noteworthy that *H. cajani* was recorded from only seven out of 471 fields sampled in 1971 (Koshy and Swarup 1971) yet by 1992 it was widespread in many Indian states (Sharma et al. 1992). This situation resembles the rapid dispersal of another cyst nematode (*Heterodera glycines*) which also parasitizes a leguminous seed crop (soybean) after its introduction to USA (Riggs et al. 1977). Cysts are spread less readily by seed crops than by the tubers of the vegetatively propagated potato but transport can occur with soil adhering to all types of planting material not just host crops. There has been ample opportunity for this as pigeonpea may have an Indian origin based on the presence of several wild relatives, the large diversity of the gene pool, ample linguistic evidences, a few archaeological remains and the wide usage

in daily cuisine (van der Maesen 1983). It possibly originated in Africa but if so it has been cropped since at least 2000 B.C in India (van der Maesen 1986). All but two of the 21 hosts of *H. cajani* are legumes (Sharma et al. 1992) suggesting it is a long established parasite of legumes in India. It may however not have been widely present in those fields now used for the expanded Indian pigeonpea crop. The genetic similarity between locations far apart within the sub-continent suggests recent dispersal across India. It would be valuable to determine the most frequent causes of this cyst dispersal to establish if further introductions can be prevented and to reduce the dispersal of virulent populations or those with distinct host ranges.

Analysis of more Indian populations would be of value using the four primer sets that identify members of the three distinct AFLP clusters found in this work. The AFLP approach may correlate population differences with agricultural significant factors such as host range, virulence to pigeonpea resistance genes (Sharma et al. 1993 and Mehta and Bajaj 2005) or persistence of populations in soil to help reduce the current impact of *H. cajani* on this important legume crop in India.

Conclusion

This is a first detailed study correlating morphological with molecular analysis of 11 populations of *H. cajani* representing major pigeonpea growing areas in India. Morphometrics of *H. cajani* though revealed some variation among the 11 populations but not as efficient as genetic analysis by AFLP. AFLP defined genetic variation had no relationship with geographical distance between populations. A sub-set of four AFLP primer sets clustered the 11 populations in the present study similarly to a larger group of 64 primer combinations. It may be useful for rapid, large scale characterization of additional populations. It could be applied to determine the extent of intraspecific variation among Indian populations of *H. cajani* which occur on a range of legumes in the 20 agroclimatic zones and 60 sub regions in India (Anonymous 2009). The present findings suggest that a comprehensive AFLP based program on genetic variation of *H. cajani* may assist future management of this nematode if the detected differences could be correlated with important factors such as agricultural trade activities that favor dispersal, host ranges and virulence to major legume crops in India.

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References

Anderson S, Anderson K (1982) Suggestions for determination and terminology of pathotypes and genes for resistance in cyst-forming nematodes, especially *Heterodera avenae*. EPPO Bulletin 12: 379–386. doi: 10.1111/j.1365-2338.1982.tb01819.x

Anonymous (2009) Handbook of Agriculture: Directorate of Information and Publications of Agriculture: Indian Council of Agriculture (ICAR), Sixth Edition, 1617.

Bendezu IF, Evans K, Burrows PR, De Pomerai D, Canto-Saenz M (1998) Inter and Intra-Specific Genomic Variability of the Potato Cyst Nematodes *Globodera pallida* and *G. rostochiensis* from Europe and South America Using RAPD-PCR. Nematologica 44: 49–61. doi: 10.1163/005225998X00064

Bossis M, Rivoal R, (1996) Protein variability in cereal cyst nematodes from different geographic regions assessed by two-dimensional gel electrophoresis. Fundamental and Applied Nematology 19: 25–34.

Esquibet M, Grenier E, Plantard O, Andaloussi FA, Caubel G (2003) DNA polymorphism in the stem nematode *Ditylenchus dipsaci*: development of diagnostic markers for normal and giant races. Genome 46: 1077–83. doi: 10.1139/g03-072

Ferris VR, Faghihi J, Ireholm A, Ferris JM (1989) Two–dimensional protein patterns of cereal cyst nematodes. Phytopathology 79: 927–933. doi: 10.1094/Phyto-79-927

Folkertsma RT, Rouppe van der voort JN, De Groot KE, Van Zandvoort P M, Schots A, Gommers FJ, Helder J, Bakker J (1996) Gene pool similarities of potato cyst nematode populations assessed by AFLP analysis. Molecular Plant Microbe Interactions 9: 47–54. doi: 10.1094/MPMI-9-0047

Grant WN, Viney ME (2001) Post-genomic nematode parasitology. International Journal for Parasitology 31: 879–888. doi: 10.1016/S0020-7519(01)00205-3

Hirschmann H (1985) The genus *Meloidogyne* and morphological characters differentiating its species. In: Sasser J N and Carter C C (Eds) An advanced treatise on *Meloidogyne,* Biology and control, 79–93

http://www.clustan.com

http://www.icrisat.org

Hyman BC, Whipple LE (1996) Application of mitochondrial DNA polymorphism to *Meloidogyne* molecular population biology. Journal of Nematology 28: 268–276.

Kaplan M, Caswell-Chen EP, Williamson VM (1998) Assessment of Host-Induced Selection on Three Geographic Isolates of *Heterodera schachtii* Using RAPD and AFLP Markers, Phytopathology 89: 68–73. doi: 10.1094/PHYTO.1999.89.1.68

Koshy PK (1967) A new species of *Heterodera* from India. *Indian* Phytopatholgy 20: 272–274.

Koshy PK, Swarup G (1971) Investigations on the life history of the pigeon pea cyst nematode. International Journal of Nematology 1: 44–51.

Lax P, Rondan JC, Dueñas, Gardenal CN, Doucet ME (2004) Genetic variability estimated with RAPD-PCR markers in two populations of *Heterodera glycines*-Ichinohe, 1952 (Nematoda: *Heteroderidae*) from Argentina. Nematology 6: 13–21.

Madami M, Tina K, Nathalie C, Subbotin SA, Gheysen G, Moens M (2007) Polymorphism among sugar beet cyst nematode *Heterodera schachtii* populations as inferred from AFLP and ITS rDNA gene analyses. Russian Journal of Nematology 15: 117–128.

Marché L, Valette S, Grenier E, Mugniéry D (2001) Intra-species DNA polymorphism in the tobacco cyst – nematode complex (*Globodera tabacum*) using AFLP. Genome 44: 941–946.

Mehta SK, Bajaj HK (2005) Response of Resistant Germplasm to Different Races/Populations of Pigeonpea Cyst Nematode, *Heterodera cajani.* International Chick pea and pigeon pea news letter 12: 47–49.

Mock KE, Bentz BJ, Neill MO, Chong JP, Orwin J, Pfrender ME (2007) Landscape-scale genetic variation in a forest outbreak species, the mountain pine beetle (*Dendroctonus ponderosae*). Molecular Ecology 16: 553–568. doi: 10.1111/j.1365-294X.2006.03158.x

Nobbs CM, Ibrahim K, Rowe J (1992) A morphological and biochemical comparison of the four cyst nematode species, *Heterodera elachista, H. oryzicola, H. oryzae* and *H. sacchari* (Nematoda: *Hetetoderidae*) known to attack rice (*Oryzae sativa*). Fundamental Applied Nematology 15: 551–562.

Otsen M, Hoekstra R, Martijn P, Jacob B, Johannes L, Marleen R (2001) Amplified fragment length polymorphism analysis of genetic diversity of *Haemonchus contortus* during selection for drug resistance. International Journal for Parasitology 31: 1138–1143. doi: 10.1016/S0020-7519(01)00225-9

Picard D, Plantard O, Scurrah M, Mugniéry D (2004) Inbreeding and population structure of the potato cyst nematode (*Globodera pallida*) in its native area (Peru). Molecular Ecology 13: 2899–2908. doi: 10.1111/j.1365-294X.2004.02275.x

Picard D, Plantard O (2006) What constitutes a population for the plant parasitic nematode *Globodera pallida* in its native area (Peru)? International Journal for Parasitology 36: 115–122. doi: 10.1016/j.ijpara.2005.08.015

Pozdol RF, Noel GR (1984) Comparative electrophoretic analyses of soluble proteins from *Heterodera glycine* races 1–4 and three other *Heterodera* species. Journal of Nematology 25: 1–6.

Riggs RD (1977) Worldwide distribution of soybean cyst nematode and its economic importance. Journal of Nematology 9: 34–39.

Saxena R, Reddy DDR (1987) Crop losses in pigeonpea and mungbean by pigeonpea cyst nematodes, *Heterodera cajani*. Indian Journal of Nematology 17: 91–94.

Seinhorst JW (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4: 67–69. doi: 10.1163/187529259X00381

Semblat JP, Wajnberg E, Dalmasso A, Abad P, Castagnone-Sereno P (1998) High resolution DNA fingerprinting of parthenogenetic root-knot nematodes using AFLP analysis. Molecular Ecology 7: 119–125. doi: 10.1046/j.1365-294x.1998.00326.x

Sharma HC (1998) Bionomics, host plant resistance, and management of the legume pod borer, *Maruca vitrata* — a review. Crop Protection 17: 373–386. doi: 10.1016/S0261-2194(98)00045-3

Sharma SB, Smith DH, McDonald D (1992) Nematode constraints of chickpea and pigeon pea production in the semi-arid tropics. Plant Disease 76: 868–874. doi: 10.1094/PD-76-0868

Sharma SB, Kumar AP, Mc Donald D (1991) A green house technique to screen pigeon pea for resistance to *Heterodera cajani*. Annals of Applied biology 118: 351–356. doi: 10.1111/j.1744-7348.1991.tb05635.x

Sharma SB, Remananden PR, Jain KC (1993). Resistant to cyst nematode (*Heterodera cajani*) in pigeon pea cultivars and in wild relatives of *cajanus*. Annals of Applied Biology 123: 75–81. doi: 10.1111/j.1744-7348.1993.tb04074.x

Siddiqi ZA, Mahmood I (1995) Occurrence of races of *Heterodera cajani* in Uttar Pradesh, India. Nematologia Mediterranean 21: 185–186.

Da Silva AT, Penna JCV, Goulart LR, Dos Santos MA, Arantes NE (2000) Genetic variability among and within races of *Heterodera glycines* Ichinohe assessed by RAPD markers. Genetics and Molecular Biology 23: 323–332.

Singh M, Sharma SB (1994) Temperature effects on development and reproduction of *Heterodera cajani* on pigeon pea. Journal of Nematology 26, 241–248.

Thangadurai R, Hoti SL, Pradeep Kumar N, Das PK (2006) Phylogeography of human lymphatic filarial parasite, *Wuchereria bancrofti* in India. Acta Tropica 98: 297–304. doi: 10.1016/j.actatropica.2006.06.004

Umarao, Rao SB, Gothalwal R, Gaur H.S. Bhat KV (2007) Variation of RAPD profiles in different geographical Indian populations of *Heterodera cajani* (Koshy 1967). International Journal of Nematology 17: 21–28.

van der Maesen, LJG (1983). World distribution of pigeonpea. Information Bulletin no. 14. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 42 pp

van der Maesen LJG, Cajanus DC, Alylosia WA (1986) Leguminosae: Agric. Univ. of Wageningen Papers 85–4.

Vos P, Hogers R, Bleeker M, Rijans M, Van den Lee T, Hornes M, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acid Research 23: 4407–4414. doi: 10.1093/nar/23.21.4407

Walia RK, Bajaj HK (1988) Further studies on existence of races in Pigeon pea cyst nematode *Heterodera cajani*. International Journal of Nematology 18: 269–272.

Wang S, Riggs RD, Yang Y (2001) Grouping of populations of *Heterodera trifolii* by host preference and AFLP pattern. Nematology 3: 667–674. doi: 10.1163/156854101753536037

Zhang L, Dean RA, Knap HT (1998) Diversity among a *Heterodera glycines* field isolate and derived inbreds based on RAPD analysis and reproduction on soyabean genotypes. Journal of Nematology 30: 477–484.

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First report of *Xiphinema brevicolle* Lordello et Costa, 1961 (Nematoda, Longidoridae) in Japan

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Abstract

Mixed populations of *Xiphinema americanum*-group species were detected from a root zone soil sample of Japanese holly, *Ilex crenata*, during a survey for plant-parasitic nematodes of commercial ornamental plant nurseries in Chiba Prefecture, Japan. From the result of the morphological study, the species were identified as *X. brevicolle* and *Xiphinema* sp. This is the first record of *X. brevicolle* in Japan. Morphometrics of *X. brevicolle* generally agree with those of the type specimens and the topotype specimens. *Xiphinema* sp. morphometrically resembles *X. paramonovi* except for tail length. The mitochondrial COI region, the nuclear 18S rDNA and the nuclear large subunit rDNA D2/D3 region of the species were sequenced and compared in the molecular study. For the COI region, PCR primers were newly designed to obtain longer sequences, ca. 900 bp, than previously used. Sequence identities of COI, 18S and D2/D3 regions between these two populations were 84.0-84.1%, 99.9% and 98.1-98.2%, respectively. Phylogenetic analyses of maximum likelihood trees were carried out to compare genetic relationships among the group and some suggestions were made on the *X. brevicolle*-subgroup.

Keywords

COI, *Ilex crenata*, Japanese holly, rDNA, *Xiphinema americanum*-group, *Xiphinema brevicolle*-subgroup

Introduction

During a survey for plant-parasitic nematodes of commercial ornamental plant nurseries in Chiba Prefecture, Japan, we detected mixed populations of *Xiphinema americanum*-group species from a root zone soil sample of Japanese holly, *Ilex crenata* Thunb.,

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one of the major garden tree species in Japan. This study was conducted using morphological characters of females and DNA sequences of the mitochondrial COI region and the nuclear ribosomal RNA (rDNA) regions to identify and characterize the species in the mixed populations.

Methods

Collection of the nematode specimens

The soil sample containing the *Xiphinema* spp. (No. 001-001) was taken from the root zone of Japanese holly growing in a commercial plant nursery at Sosa City, Chiba, Japan. Nematodes were extracted with Cobb's wet-sieving technique. Material collected on a 75 μm mesh sieve was placed on a Baermann funnel and nematodes were collected after one day at room temperature.

Morphological observation

Females of *Xiphinema* spp. were removed from the nematode suspension using a dissecting microscope and nematode pick. Female nematodes were transferred to a small amount of water then killed by either heating at 60°C for 2 min or by adding hot FP4:1 (Netscher and Seinhorst 1969). Killed nematodes were fixed with FG4:1 (De Grisse 1969). The specimens were fixed for more than one week, processed into glycerin by the ethanol/glycerin method (Seinhorst 1959, De Grisse 1969), and mounted in dehydrated glycerin supported with both minute glass beads and paraffin on glass slides. Morphological observations were made using a DIC microscope (BX51, Olympus Co., Japan). A digital camera, Olympus DP20 or DP21 was used for measuring and taking photo images. The body length and position of vulva were measured with a digital curvimeter (CV-9 Jr., Koizumi Sokki Mfg. Co. Ltd., Japan) on nematode line drawings prepared using a drawing tube. Illustrations of nematodes were sketched directly on highly transparent tracing film (No. 200Z-A4 (S): Tochiman Technical Paper Co. Ltd., Japan) by tracing DP21 digital camera images on a panel-protected LC-display. Sketched images were converted to digital images using the software Adobe Illustrator CS4 (Adobe Systems Inc., USA) and a pen tablet (Intuos4: Wacom Co., Ltd., Japan).

Molecular study

DNA extractions from single female specimens were carried out according to Sakai (2010), which yielded 200 μL lysate for each specimen. Before DNA extraction, the specimens were tagged, killed by gentle heat, prepared as temporary water mounts,

digital images of mounts were photographed, and then images were measured for body and odontostyle length, respectively.

DNA fragments of the mitochondrial COI region were amplified by PCR using the set of primers, CO1-F1 and CO1-R1 (Table 1), which were originally designed from sequence comparison of the COI region between *X. americanum* (He et al. 2005a: GenBank accession AY382608; NCBI Reference Sequence NC_005928) and *Caenorhabditis elegans* (Howe and Denver 2008: GenBank accession EU407804). CO1-R1 is virtually identical to COIR (He et al. 2005a), with the latter having only a difference in degeneracy. The PCR reaction mixture consisted of 0.2 mM dNTPs, 0.3 μM of each primer, 0.5 U PrimeSTAR HS DNA Polymerase with PrimeSTAR Buffer (5 mM Mg_2^+ plus) (Takara Bio Inc., Japan), and 10 μL of DNA lysate as PCR template, in a total volume of 20 μL. The reaction conditions were as follows: a single step of pre-denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 40°C for 15 s, 0.5°C/s ramp up to extension temperature, and extension at 72°C for 1 min.

DNA fragments of the nuclear 18S rDNA region were amplified using the set of primers, 18S 39 and 18S 1573R (Mullin et al. 2005). The PCR reaction mixture consisted of 0.2 mM dNTPs, 0.2 μM of each primer, 0.5 U TaKaRa Ex Taq Hot Start Version with Ex Taq Buffer (Mg_2^+ plus) (Takara Bio), and 2 μL of DNA lysate as PCR template, in a total volume of 20 μL. The reaction conditions were as follows: a single step of pre-denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 55°C for 30s, and extension at 72°C for 1 min.

DNA fragments of the nuclear large subunit rDNA D2/D3 region were amplified using the set of primers, D2Ab (De Ley et al. 1999) and D3B (Thomas et al. 1997). The PCR reaction conditions were the same as those for 18S rDNA.

These PCR products were purified with QIAquick PCR Purification Kit (Qiagen K.K., Japan), subjected to cycle sequencing with BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies Japan), purified with either DyeEx 2.0 Spin Kit (Qiagen) or Agencourt CleanSEQ (Beckman Coulter Inc., USA), and sequenced on the ABI PRISM 3100 Genetic Analyzer (Life Technologies Japan Ltd., Japan). Primers 18S 599R, 18S 550, 18S 977R, 18S 965 (Mullin et al. 2005), and D3B (Thomas et al. 1997) were also used as inner primers for sequencing as well as those designed by us (Table 1). The DNA sequences were aligned by MUSCLE (Edgar 2004) and arranged using BioEdit (Hall 1999). Multiple alignments were manually refined where necessary.

Phylogenetic analyses including substitution model selections were performed using MEGA5 (Tamura et al. 2011) to compare the obtained sequences with homologous sequences of the *X. americanum*-group in GenBank searched via BLAST. A maximum likelihood (ML) tree was constructed using 1,578 sites for multiply aligned sequences of the 18S rDNA region, where the model K2 + G, the heuristic search with the Close-Neighbor-Interchange (CNI) method, bootstrapping with 500 replications, gaps treatment using all sites, and the neighbor-joining (NJ) tree as the initial tree were employed. A ML tree was constructed using 676 sites for multiply aligned sequences of the D2/D3 region with gaps and an ambiguous site deleted, where the model K2 +

Table 1. Primers designed and employed originally in this study for PCR amplification and sequencing of mitochondrial COI region and rDNA D2/D3 region.

primer name	Sequence	Note
CO1-F1	5'-ATAATTTTTTTTATGGTAATACC-3'	PCR, sequencing
CO1-R1	5'-ACTACATAATAAGTATCATG-3'	PCR, sequencing
CO1-F2	5'-TATATTTTAATTTTACCTGG-3'	sequencing
CO1-R2	5'-CCAGGTAAAATTAAAATATA-3'	sequencing
D3A-R	5'-AGACTCCTTGGTCCGTGTTTC-3'	sequencing

G, the CNI method, bootstrapping with 500 replications, and a maximum parsimony (MP) tree as the initial tree were employed. A ML tree was constructed using 342 sites for multiply aligned sequences of the COI region, where the model T92 + G + I, the CNI method, bootstrapping with 500 replications, and a MP tree as the initial tree were employed. Among COI homologous sequences of the *X. americanum*-group in the database, those including alignment gaps were excluded from the analysis because they possibly represent pseudogenes. Sequences of *Longidorus* species available in GenBank were used to root those trees.

Results

The DNA sequence analysis of the mitochondrial COI region for 9 specimens suggested two different *X. americanum*-group species were present. Morphometric data were used to identify them as *Xiphinema brevicolle* Lordello et Costa, 1961, and an undescribed *X. americanum*-group species.

Morphological observations

***Xiphinema brevicolle* Lordello & Costa, 1961**
http://species-id.net/wiki/Xiphinema_brevicolle
Figs 1A, 1C, 1E, 1G, 2, 4A, 5A, 6A

Measurements. See Tables 2, 3.

Remarks. Morphometrics of the specimens obtained here generally agree with those of the type specimens and topotype specimens (Lamberti et al. 1992, Luc et al. 1998) of the species (Table 2). No male was detected.

Nomenclatorial note. The emended name of this species, *Xiphinema brevicollum*, was proposed by Luc et al. (1998) and used in many works to date. Monteiro (2010) claimed that the correct species name is *Xiphinema brevicolle* Lordello et Costa, 1961, and should have been preserved unaltered. We support the claim by Monteiro (2010) and *X. brevicolle* is used here.

***Xiphinema* sp.**
Figs 1B, 1D, 1F, 1H, 3, 4B, 5B, 6B

Measurements. See Tables 2, 3.

Remarks. These specimens morphometrically resemble *Xiphinema paramonovi* Romanenko, 1981, except for the clearly different tail length (Table 2). The morphometrics of the specimens partly overlap those of *X. brevicolle*. General morphology and DNA information addressed below suggest that these specimens belong to some species related to *X. brevicolle*, though a specific species accommodating them was not found. We finally regarded these specimens as an unidentified *X. americanum*-group species. Further information is required to identify the specimens as a new species or determine if they represent intra-specific variation of species previously described. No male was detected.

Molecular study. DNA sequences of 886 bp except for primer regions were obtained for the mitochondrial COI region. The five *X. brevicolle* specimens observed had identical sequences, whereas a single nucleotide in the sequence differed among the four specimens of *Xiphinema* sp. though this variation resulted in no difference between the translated amino acid sequences within the specimens. Sequence identity between these two species was 84.0-84.1%, whereas the sequences of *X. brevicolle* and *Xiphinema* sp. were 80.7 and 80.4-80.5% identical to that of *X. americanum* (He et al. 2005a) respectively, with no gap found among them (Fig. 7). Putative amino acid sequences were available without any stop codon when translations were made from the second base of the obtained sequences. DNA sequences of 1,566 bp except for primer regions were obtained for the 18S rDNA region from one specimen for each species. The difference between sequences of the two species studied here was only a single nucleotide, resulting in 99.9% identity without any gap. DNA sequences of 788-791 bp except primer regions were obtained for the D2/D3 region from four specimens for each species. A single nucleotide variation of sequence among four specimens of *X. brevicolle* was observed, whereas no variation of sequence was observed among four specimens of *Xiphinema* sp. Sequence identity between these two species was 98.1-98.2%, with gaps found.

ML trees inferred for the 18S rDNA and D2/D3 regions placed our specimens in similar clades, which include *X. brevicolle* and its junior synonyms by Luc et al. (1998) such as *X. diffusum* Lamberti et Bleve-Zacheo, 1979, *X. incognitum* Lamberti and Bleve-Zacheo, 1979, *X. taylori* Lamberti et al., 1992, as well as other different species like *X. inaequale* (Khan et Ahmad, 1975) and *X. lamberti* Bajaj et Jairajpuri, 1977 (Figs 8, 9). On the other hand, the ML tree inferred for the COI region didn't show strong support for such a clade because of a low bootstrap value though several subclades were strongly supported by high bootstrap values (Fig. 10).

Discussion

This study reports the occurrence of *X. brevicolle* in Japan for the first time. The only member species of the *Xiphinema americanum*-group recorded to date in Japan was *X.*

Table 2. Morphometrics of *Xiphinema brevicolle*, *Xiphinema* sp., *X. paramonovi* and *X. diffusum* (female). Mean ± standard deviation (range) in µm, except for L in mm, and ratios.

	Xiphinema brevicolle				*Xiphinema* sp. (this study)	*Xiphinema paramonovi* Paratypes (Romanenko 1981)	*Xiphinema diffusum* Paratypes (Lamberti and Bleve-Zacheo 1979)
	Population in this study	Topotypes (Luc et al. 1998)	Topotypes (Lamberti et al.1992)	Types (Lordello and Costa 1961)			
n	22	25	17	-	13	27	10
L	1.93 ± 0.11 (1.71-2.10)	1.92 ± 0.122 (1.7-2.16)	2.1 ± 0.1 (1.8-2.2)	(1.82-2.20)	2.30 ± 0.12 (2.08-2.47)	2.1 (2.0-2.3)	1.7 (1.6-1.8)
a	46.1 ± 2.6 (40.5-50.5)	46.1 ± 1.72 (44-51)	44.5 ± 2.3 (40.7-50.1)	(36.0-42.2)	48.8 ± 2.4 (45.0-52.3)	49.6 (44-54.2)	47 (46-51)
b	6.6 ± 0.6 (5.5-8.2)	5.92 ± 0.37 (5.1-6.7)	6.4 ± 0.6 (5.6-7.7)	(7.0-10.5)	7.0 ± 0.4 (6.5-8.1)	6.1 (4.8-7.1)	6.9 (5.3-8.9)
c	69.8 ± 4.4 (60.5-79.1)	76.9 ± 5.64 (67.6-89.9)	77.8 ± 0.6 (60.3-94.0)	(62.5-93.0)	79.6 ± 8.5 (67.5-94.5)	60.5 (49.1-68.5)	72 (63-84)
c'	1.0 ± 0.1 (0.9-1.2)	0.96 ± 0.06 (0.89-1.10)	1.0 ± 0.06 (0.9-1.1)	-	0.9 ± 0.1 (0.8-1.2)	1.1 (0.9-1.2)	0.9 (0.8-1.1
V	50.5 ± 1.2 (48.1-53.6)	53 ± 1.9 (50-55)	53 ± 0.9 (51.0-54.0)	(50.0-54.0)	52.4 ± 1.1 (50.9-54.0)	52.1 (50.8-55.0)	50 (47-52)
Total stylet	149.0 ± 4.4 (144-161)	159 ± 8.05 (144-173)	-	(156.0-168.3)	166.5 ± 2.6 (161-171)	-	-
Odontostyle	94.1 ± 3.3 (88-102)	101 ± 6.14 (89-110)	101.9 ± 7.2 (84.7-108.2)	-	107.3 ± 2.7 (103-111)	103.5 (88.5-120.0)	87 (84-89)
Odontophore	54.9 ± 2.1 (51-59)	59 ± 3.43 (50-64)	57.0 ± 2.9 (48.8-60.0)	(61.2-62.7)	59.2 ± 1.6 (57-62)	56.7 (53.1-60.0)	50 (48-51)
Oral aperture to guide ring	76.5 ± 3.8 (67-83)	86.0 ± 4.23 (77-92)	86.3 ± 5.6 (72.3-92.3)	-	85.5 ± 5.0 (78-93)	79.6 (66.0-103.0)	62 (60-64)
Pharyngeal bulb length	79.3 ± 4.0 (73-86; n = 10)	-	-	(61-79)	84.9 ± 2.7 (82-90; n = 7)	94.2 (65.0-117)	-
Pharyngeal bulb width	21.2 ± 1.2 (18-23; n = 21)	-	-	(12-19)	22.9 ± 1.7 (20-26)	21.1 (18.0-24.0)	-

Tail	27.8 ± 2.0 (25-32)	26.0 ± 1.71 (23-28)	26.8 ± 2.0 (24.1-31.2)	(24.5-29.0)	29.2 ± 2.8 (24-34)	36.1 (33.0-47.0)	24 (21-28)
Hyaline portion of tail (J)	10.4 ± 1.5 (8-13)	-	8.0 ±0.9 (5.9-9.4)	-	11.4 ± 1.5 (9-14)	9 (7-11)	12 (10-14)
Body diam. at lip region	12.5 ± 0.4 (12-13)	11.5 (11-13)	11.5 ± 0.5 (10.6-12.3)	-	12.4 ± 0.4 (11-13)	14.6 (13.5-15.0)	11 (10-12)
Body diam. at guide ring	30.4 ± 0.8 (29-32)	-	29.8 ± 1.5 (27.1-31.8)	-	32.6 ± 0.8 (31-34)	31.7 (30.0-36.0)	26 (26-27)
Body diam. at base of pharynx	37.9 ± 1.2 (35-40)	-	39.4 ± 2.9 (35.3-45.3)	-	41.3 ± 2.2 (38-45)	40.1 (36.0-42.0)	33 (31-35)
Body diam. at vulva	42.0 ± 2.0 (38-46)	-	46.6 ± 3.4 (39.4-50.0)	(49.0-59.7)	47.2 ± 2.5 (44-52)	43.4 (39.0-47.2)	36 (33-38)
Body diam. at anus	27.0 ± 1.6 (24-30)	26 (22-29)	26.6 ± 1.7 (21.8-29.4)	(30.6-36.7)	29.7 ± 1.1 (28-32)	32.4 (27.0-41.0)	25 (23-28)
Body diam. at beginning of J	16.2 ± 2.2 (12-20)	-	13.7 ± 1.1 (11.2-14.7)	-	18.4 ± 2.2 (15-22)	7 (6-8)	17 (15-20)

Table 3. Uterine and vaginal region lengths of *Xiphinema brevicolle* and *Xiphinema* sp. measured in this study (female). Mean ± standard deviation (range; number of specimens) in μm, except where indicated.

	Xiphinema brevicolle	***Xiphinema* sp.**
Anterior uterus length	44.3 ± 4.8 (34-54; n = 14)	35.1 ± 2.9 (32-40; n = 9)
Posterior uterus length	41.1 ± 2.9 (37-46; n = 9)	32.7 ± 2.7 (29-37; n = 9)
Pars proximalis vaginae	5.9 ± 0.7 (5-7; n = 22)	7.6 ± 1.5 (5-10; n = 13)
Pars distalis vaginae	10.5 ± 0.7 (9-12; n = 22)	11.3 ± 1.0 (9-13; n = 13)
Vagina length	16.4 ± 1.0 (14-18; n = 22)	18.9 ± 1.5 (17-22; n = 13)
Vagina length / body diam. at vulva (%)	39.2 ± 3.2 (31.3-44.6; n = 22)	40.1 ± 2.9 (34.8-44.2; n = 13)

incognitum Lamberti & Bleve-Zacheo, 1979, described as a new species for nematode specimens detected from soil of bonsai trees exported from Japan to England (Lamberti and Bleve-Zacheo 1979).

The specimens of this study were obtained from mixed populations of the *X. americanum*-group. Information on juveniles was not included because the coexistence of the two closely related nematodes requires special care to separate juvenile specimens of the different species. Situations like this will make matters worse if one is to identify the species, since it is difficult enough to identify even a single population of the group in many cases. Detection of mixed populations of *X. americanum* Cobb, 1913 and *X. rivesi* Dalmasso, 1969 were reported (Vrain et al. 1992, Vrain 1993), and such coexistence of multiple populations and/or species of the *X. americanum*-group may be common. Therefore, it is strongly recommended to check the genetic uniformity of a given population to be identified. The mitochondrial COI region has been recently used to examine variations in populations of *Xiphinema* species including members of the *X. americanum*-group (Lazarova et al. 2006, Kumari et al. 2010a, 2010b), where ca 400 bp sequences were examined. This genetic region has much more information to differentiate between populations than 18S rDNA and D2/D3 regions. As shown above, we developed new primers and examined longer sequences of the COI region than previously used. Examination of longer sequences provides not only more reliable comparative results but also another option to develop a more specific primer to the species to be tested since high variability of this region may contribute to some unfitness of reported primers. Among options available at present, sequences of the COI region can be efficiently used to examine the diversity of *Xiphinema* specimens.

ML phylogenetic analyses of 18S rDNA and D2/D3 regions moderately supported the clade including *X. brevicolle*, *X. diffusum*, *X. inaequale*, *X. incognitum*, *X. lamberti*, and *X. taylori* with our materials (Figs 8, 9), whereas such a clade was not so highly supported in the ML tree of COI (Fig. 10). Member species of *X. americanum*-group harbor endosymbionts (Vandekerckhove et al. 2000). The

difference in phylogenetic inference between nuclear and mitochondrial genetic regions may result from the symbionts' effect on mitochondrial DNA since unreliable results of phylogenetic inference based on mitochondrial DNA due to the presence of a symbiont are known (Hurst and Jiggins 2005). Furthermore, the ML tree of COI brought another problem to us. It showed closer relationship of our *X. brevicolle* population to *X. diffusum* than to other *X. brevicolle* populations. Our specimens identified as *X. brevicolle* were reasonably larger than type specimens of *X. diffusum* (Table 2), and our identification was made considering more overlapping morphometrics of type specimens of *X. brevicolle*. If COI sequences can differentiate species of *X. americanum*-group, our specimens may be identified as *X. diffusum* rather than *X. brevicolle*. In such a situation, morphological features used to identify species should be reconsidered since a single species like *X. diffusum* can have a wide range of morphometrics which may result in more difficult diagnoses without reducing the number of species by synonymizing them intensively. In any case, it should be desirable to collect much more COI sequences of other species and populations, such as the sequence data of topotype specimens of *X. diffusum*, which is unavailable at present. Our results of phylogenetic analyses, however, may be helpful to refine the concept of a *X. brevicolle*-subgroup, which was previously discussed in some works (Romanenko and Stegaresku 1985, Lamberti and Ciancio 1993, He et al. 2005b). Taking our results of phylogenetic analyses into consideration, we suggest that the *X. brevicolle*-subgroup includes at least the five species of *X. brevicolle*, *X. diffusum*, *X. inaequale*, *X. incognitum*, and *X. taylori*, and our specimens also belong to the subgroup, though the validity of respective species is a different matter. Appropriate establishment of subgroups within the *X. americanum*-group will contribute to a more feasible identification process of the member species and requires further research.

Conclusion

In this study, specimens from mixed populations of the *X. americanum*-group, present in the root zone of Japanese holly in Japan, were identified as *X. brevicolle* and *Xiphinema* sp. This record of *X. brevicolle* is the first for Japan. PCR primers to amplify longer sequences of the mitochondrial COI regions were originally designed and used to efficiently differentiate the specimens. Phylogenetic analyses using 18S rDNA, D2/D3, and COI regions supported a close relationship among our specimens and species related to *X. brevicolle* or the *X. brevicolle*-subgroup.

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References

De Grisse AT (1969) Redescription ou modifications de quelques techniques utilisées dans l'étude des nématodes phytoparasitaires. Mededelingen van de Rijksfaculteit Landbowwetenschappen Gent 34: 351–369.

De Ley P, Félix M-A, Frisse LM, Nadler SA, Sternberg PW, Thomas K (1999) Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). Nematology 1: 591–612.

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797. doi: 10.1093/nar/gkh340

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

He Y, Jones J, Armstrong M, Lamberti F, Moens M (2005a) The mitochondrial genome of *Xiphinema americanum sensu stricto* (Nematoda: Enoplea): considerable economization in the length and structural features of encoded genes. Journal of Molecular Evolution 61: 819–833. doi: 10.1007/s00239-005-0102-7

He Y, Subbotin SA, Rubtsova TV, Lamberti F, Brown DJF, Moens M (2005b) A molecular phylogenetic approach to Longidoridae (Nematoda: Dorylaimida). Nematology 7: 111–124. doi: 10.1163/1568541054192108

Howe DK, Denver DR (2008) Muller's Ratchet and compensatory mutation in *Caenorhabditis briggsae* mitochondrial genome evolution. BMC Evolutionary Biology 8: 62. doi: 10.1186/1471-2148-8-62

Hurst GDD, Jiggins FM (2005) Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proceedings of the Royal Society B 272: 1525–1534. doi: 10.1098/rspb.2005.3056

Kumari S, Decraemer W, De Luca F (2010a) Molecular characterization of *Xiphinema brevicollum* (Nematoda: Longidoridae) from the Czech Republic. European Journal of Plant Pathology 128: 243–250. doi: 10.1007/s10658-010-9651-8

Kumari S, Decraemer W, De Luca F, Tiefenbrunner W (2010b) Cytochrome c oxidase subunit 1 analysis of *Xiphinema diversicaudatum*, *X. pachtaicum*, *X. simile* and *X. vuittenezi* (Nematoda, Dorylaimida). European Journal of Plant Pathology 127: 493–499. doi: 10.1007/s10658-010-9614-0

Lamberti F, Bleve-Zacheo T (1979) Studies on *Xiphinema americanum sensu lato* with descriptions of fifteen new species (Nematoda, Longidoridae). Nematologia Mediterannea 7: 51–106.

Lamberti F, Ciancio A (1993) Diversity of *Xiphinema americanum*-group species and hierarchical cluster analysis of morphometrics. Journal of Nematology 25: 332–343.

Lamberti F, Ciancio A, Agostinelli A, Coiro MI (1992) Relationship between *Xiphinema brevicolle* and *X. diffusum* with a redescripiton of *X. brevicolle* and descriptions of three new species of *Xiphinema* (Nematoda: Dorylaimida). Nematologia Mediterranea 19 (1991): 311–326.

Lazarova SS, Malloch G, Oliveira CMG, Hübschen J, Neilson R (2006) Ribosomal and mitochondrial DNA analyses of *Xiphinema americanum*-group populations. Journal of Nematology 38: 404–410.

Lordello LGE, Costa CP (1961) A new nematode parasite of coffee roots in Brazil. Revista Brasileira de Biologia, 21: 363–366.

Luc M, Coomans A, Loof PAA, Baujard P (1998) The *Xiphinema americanum*-group (Nematoda: Longidoridae). 2. Observations on *Xiphinema brevicollum* Lordello & da Costa, 1961 and comments on the group. Fundamental and Applied Nematology 21: 475–490.

Monteiro AR (2010) Nomenclatorial note on *Xiphinema brevicolle* Lordello & Costa, 1961 (Nematoda: Longidoridae). Nematologia Brasileira 34: 1–2.

Mullin PG, Harris TS, Powers TO (2005) Phylogenetic relationships of Nygolaimina and Dorylaimina (Nematoda: Dorylaimida) inferred from small subunit ribosomal DNA sequences. Nematology 7: 59–79. doi: 10.1163/1568541054192199

Netscher C, Seinhorst JW (1969) Propionic acid better than acetic acid for killing nematodes. Nematologica 15: 286.

Romanenko ND (1981) [Discovery of a new species of nematode *Xiphinema paramonovi* n. sp. (Nematoda: Longidoridae) in the territory of the soviet Union]. Tezisy Dokladov, Pervaya Konferentsiya po Nematodam Rastenii, Nasekomykh, Pochvy i Vod, 68–69.

Romanenko ND, Stegaresku OP (1985) [Taxonomic problem in nematodes of the *Xiphinema americanum* group (Nematoda, Dorylaimoidea)]. Parazitologiya 19: 434–442.

Sakai H (2010) A DNA extraction method with SDS from single nematodes for direct application to PCR amplification. Nematological Research 40: 13–14. doi: 10.3725/jjn.40.13

Seinhorst JM (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4: 67–69. doi: 10.1163/187529259X00381

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution. doi: 10.1093/molbev/msr121

Thomas WK, Vida JT, Frisse LM, Mundo M, Baldwin JG (1997) DNA Sequences from formalin-fixed nematodes: integrating molecular and morphological approaches to taxonomy. Journal of Nematology 29: 250–254.

Vandekerckhove TTM, Willems A, Gillis M, Coomans A (2000) Occurrence of novel verrucomicrobial species, endosymbiotic and associated with parthenogenesis in *Xiphinema americanum*-group species (Nematoda, Longidoridae). International Journal of Systematic and Evolutionary Microbiology 50: 2197–2205. doi: 10.1099/00207713-50-6-2197

Vrain TC (1993) Restriction fragment length polymorphism separates species of the *Xiphinema americanum* group. Journal of Nematology 25: 361–364.

Vrain TC, Wakarchuk DA, Lévesque, AC, Hamilton RI (1992) Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. Fundamental and Applied Nematology 15: 563–573.

Figures

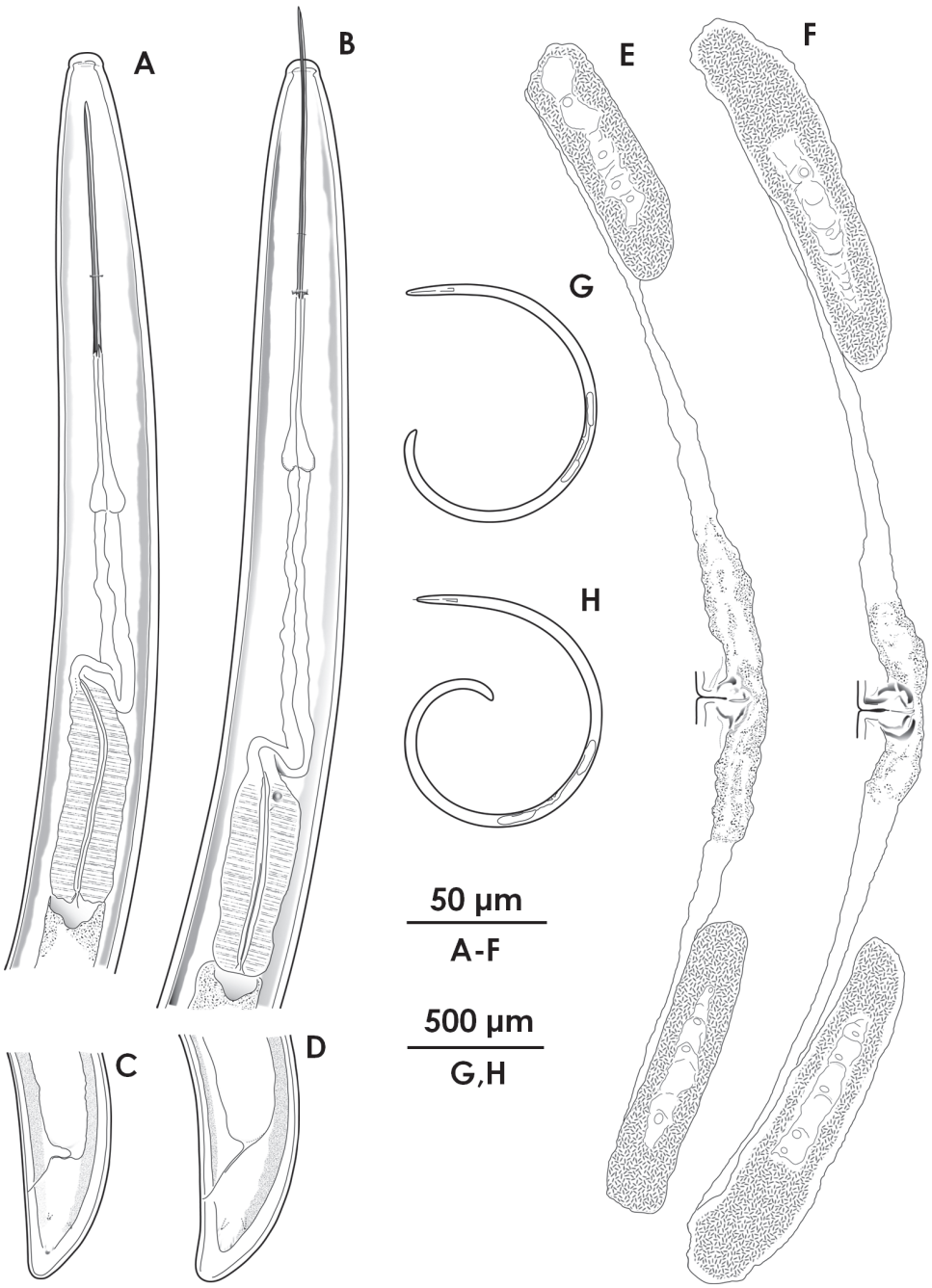

Figure 1. Female morphology of *Xiphinema brevicolle* **(A, C, E, G)** and *Xiphinema* sp. **(B, D, F, H) A–B** Anterior region **C–D** Tail **E–F** Reproductive system **G–H** Entire body.

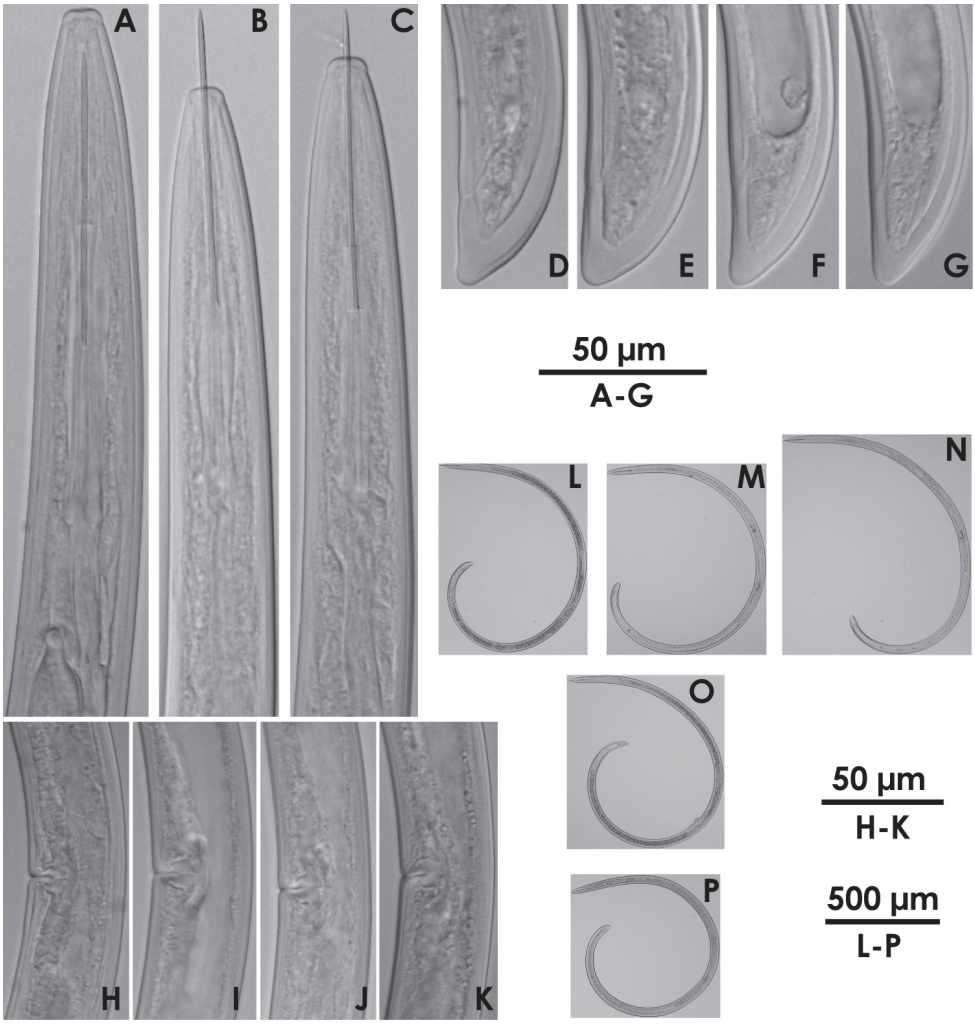

Figure 2. *Xiphinema brevicolle* (female) **A–C** Anterior region **D–G** Tail **H–K** Vulval region **L–P** Entire body.

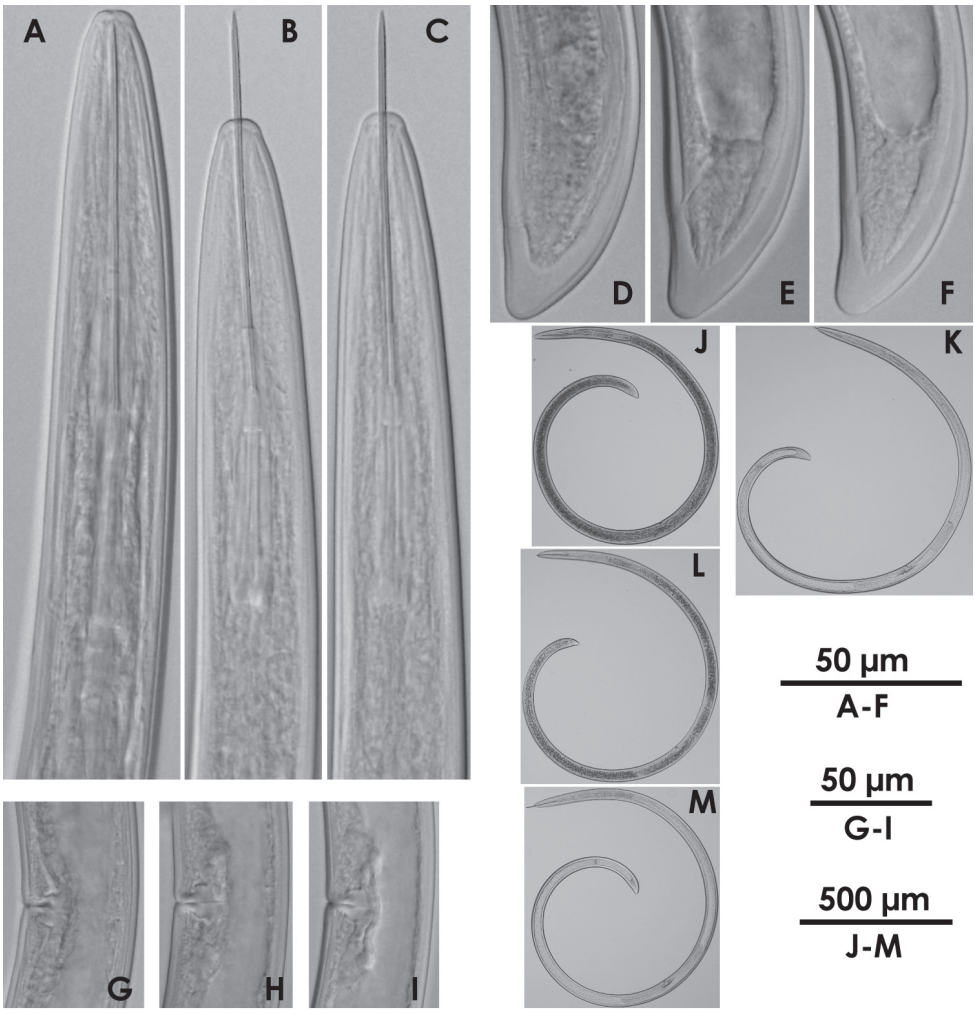

Figure 3. *Xiphinema* sp. (female) **A–C** Anterior region **D–F** Tail **G–I** Vulval region **J–M** Entire body.

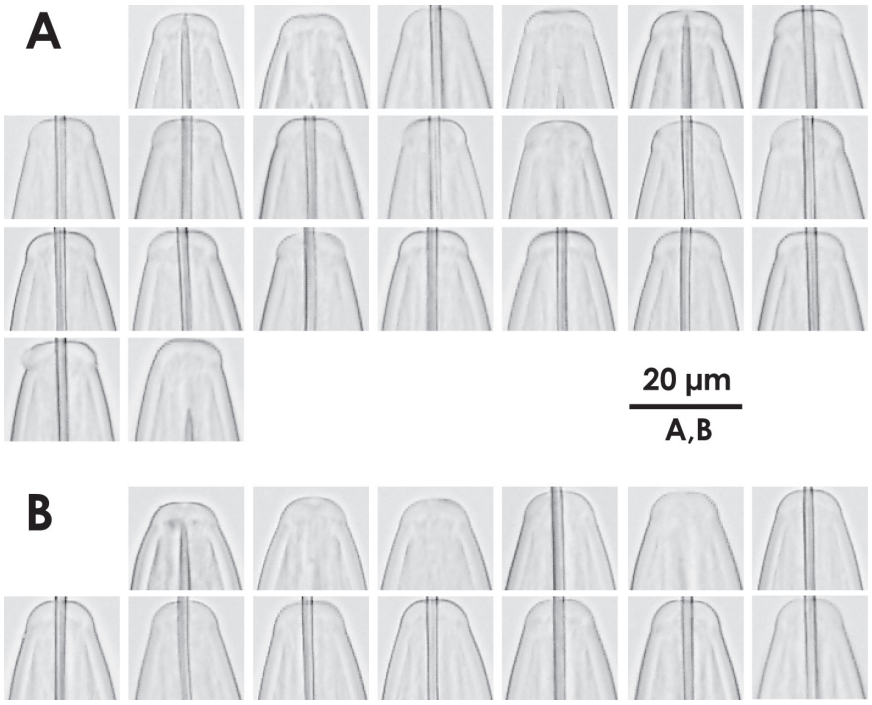

Figure 4. Intra-population variation of lip region in females **A** *Xiphinema brevicolle* **B** *Xiphinema* sp.

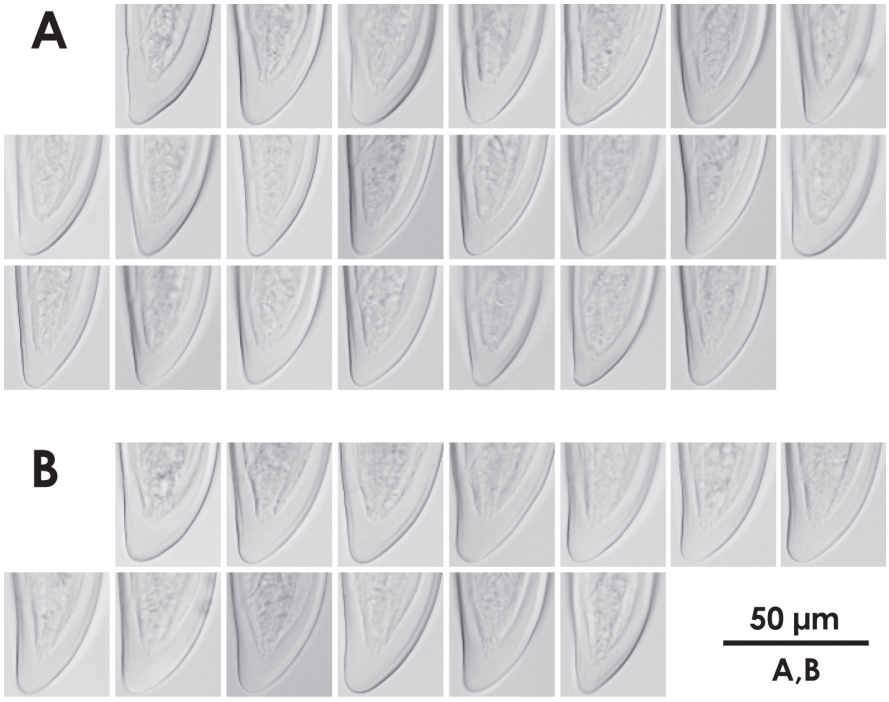

Figure 5. Intra-population variation of tail in females **A** *Xiphinema brevicolle* **B** *Xiphinema* sp.

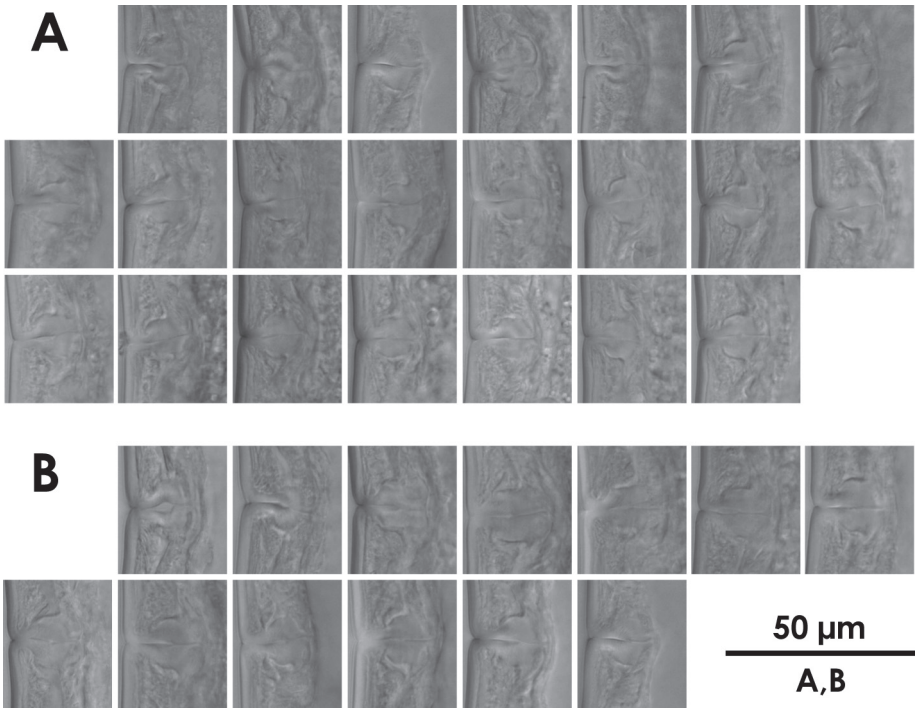

Figure 6. Intra-population variation of vagina in females **A** *Xiphinema brevicolle* **B** *Xiphinema* sp.

```
Xbre      1 AATTTTAATTGGAGGATTTGGTAATATTTTATTGCCGCTGATACTAGGAGCCCAAGATATGTGTTTTCCTCGTTTAAATA 80
Xsp1      1 G........C.......................A..A..A.....T.....A.....C..A...........A....... 80
Xsp2      1 G........C.......................A..A..A.....T.....A.....C..A...........A....... 80
Xame   9214 ....C.......G...................A..CTC....T.......A.....C.....C..C..C..C..G..C. 9293

Xbre     81 ATTTTAGATTTTGGCTTTTACCGATCAGAGGATTGTTTATATTAAGCTCTATATTTATAGGTTGTGGCGCTGGAACCGGA 160
Xsp1     81 ............A........AG.A........A...T..A...................A.....A.....G..T... 160
Xsp2     81 ....C........A........AG.A........A...T..A...................A.....A.....G..T... 160
Xame   9294 .C.....G.....AT.A......G....G..G..AC.....A....T......C..C.G..A..C..T.....T..T..T 9373

Xbre    161 TGAACTATTTATCCTCCGCTTAGAGGTATTGCAGGTCATTCTAATTGAAGTGTAGACTTAGTAATTTTTAGTCTACACTT 240
Xsp1    161 .......................TA.A..CA....A..C..A..C.....G...................AT....... 240
Xsp2    161 .......................TA.A..CA....A..C..A..C.....G...................AT....... 240
Xame   9374 ..........C.....T......A.........A.....G..C.....A..G........G.........T.G..TC. 9453

Xbre    241 AGCTGGAGTAAGATCGATTGCTGGATCCATTAATTTTTTGTGTACTATTAATAATTTAAAAAACAGTGCTATTTCATGAA 320
Xsp1    241 ..............A........T..............A.......................T.....C........G. 320
Xsp2    241 ..............A........T..............A.......................T.....C........G. 320
Xame   9454 ......T..T.....A..C.....T..T.............C...........CC.T..GTCT..AT.A..C..T.... 9533

Xbre    321 TGTCAATACCTTTATTTTTGATTAGGGTATGAGTAACCGCTTTTTTGTTAGTTCTTAGATTACCAGTATTGGCAGGTGGA 400
Xsp1    321 .A.....G........CC.T.....A..T..G..T..T......C.TC.....T.A...C....T..GC.A........G 400
Xsp2    321 .A.....G........CC.T.....A..T..G..T..T......C.TC.....T.A...C....T..GC.A........G 400
Xame   9534 .A.GT.....G..G...........A..T.....T..T...........G...T.A........T..T..A..T...... 9613

Xbre    401 ATTACGATGCTTTTGTTTGATCGAAATTTAAACACTTCTTTCTTTGATCCTTTAGGAGGGGGGGATCCAATTTTATTCCA 480
Xsp1    401 .....T..AT.A..A............C....T.....G..T........C........A..T........C.....T.. 480
Xsp2    401 .....T..AT.A..A............C....T.....G..T........C........A..T........C.....T.. 480
Xame   9614 .....T..A.....A..C........C..G........C........C..AC....T..A........C..C.....T.. 9693

Xbre    481 ACATTTATTCTGATTTTTTGGGCACCCAGAAGTATATATTTTAATTTTACCTGGGTTTGGATTAGTTAGGCATGCTGTTA 560
Xsp1    481 ...CC.C........C.....T..T..............................A........G..A..A.........AG 560
Xsp2    481 ...CC.C........C.....T..T..............................A........G..A..A.........AG 560
Xame   9694 G...C.T..T.....C.....A.....G..G..G........G.....................A..A..C..C.... 9773

Xbre    561 TAGTTTCTAGAGGAAAGCCATCTCCGTTTGGTGTTCCAGGTATGTTTTTAGCTATTACTAGGATTGGTGTCTTAGGTTGT 640
Xsp1    561 .......C........AA.......T.....AA.C..G.............C........A........A.....A... 640
Xsp2    561 .......C........AA.......T.....AA.C..G.............C........A........A.....A... 640
Xame   9774 ..A....C...........T.....A.....G..A.....C..A..C.............A.....A..A..G..A..C 9853

Xbre    641 GTGGTCTGAGCGCATCATATATTTAGAGTAGGATTAGACATAGATACTCGACTGTATTTTACAGCGGCCACTATAATTAT 720
Xsp1    641 ..A..T.....T........G........T..T.....T.....C..A...T.A..............T........... 720
Xsp2    641 ..A..T.....T........G........T..T.....T.....C..A...T.A..............T........... 720
Xame   9854 ..A..A.....A.....C..G........G..T.....T..............T...........A.............. 9933

Xbre    721 TGCTGTTCCAACTGGGATTAAGGTATTCAGATGATTGGTATCCTTTAGAGGAAGAAAAATATTATTGGCCCCGCTCCAAT 800
Xsp1    721 C.....A..T..A..A.....................................................GA.A..A..TACT.... 800
Xsp2    721 C.....A..T..A..A.....................................................GA.A..A..TACT.... 800
Xame   9934 ..............A...........T.....G..A.C...T........G........G...A..ATA..A..A.... 10013

Xbre    801 TGTGGATTTTAGGCTTTTGTTTTTATTTACAGTGGGAGGACTTACTGGCATTGTATTAGCCAACGGGACTTTAGATTTA 880
Xsp1    801 .A..AG.AC.T..T..C..A.....................T.A.....T.....T.....T..T..A............ 880
Xsp2    801 .A..AG.AC.T..T..C..A.....................T.A.....T.....T.....T..T..A............ 880
Xame  10014 .A..A...C.T..T..C..A.....G.....G..T..T...T.A.....T.....G.....T..T.............. 10093

Xbre    881 TTATAT 886
Xsp1    881 C.T... 886
Xsp2    881 C.T... 886
Xame  10094 ..G..C 10099
```

Figure 7. Sequence comparison of mitochondrial COI region. Xbre: *X. brevicolle* (this study: GenBank accession AB604337); Xsp1, Xsp2: *Xiphinema* sp. (this study: AB604338 and AB604339 respectively); Xame: *X. americanum* (He et al. 2005: usAY382608).

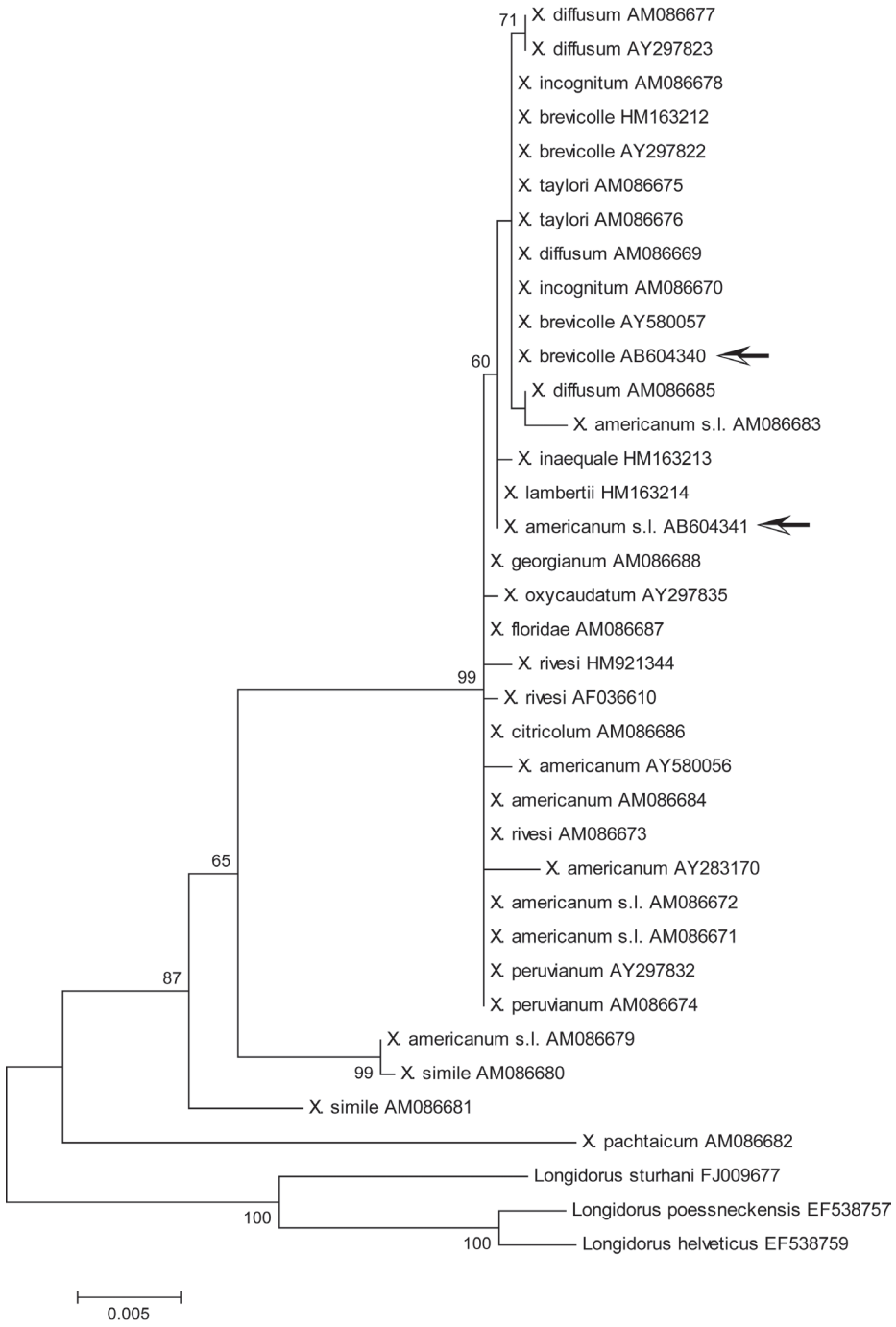

Figure 8. Maximum likelihood tree for 18S rDNA region. Bootstrap values higher than 50 are shown. Arrows indicate specimens examined in this study: *Xiphinema brevicolle* GenBank accession AB604340; *Xiphinema* sp. AB604341. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

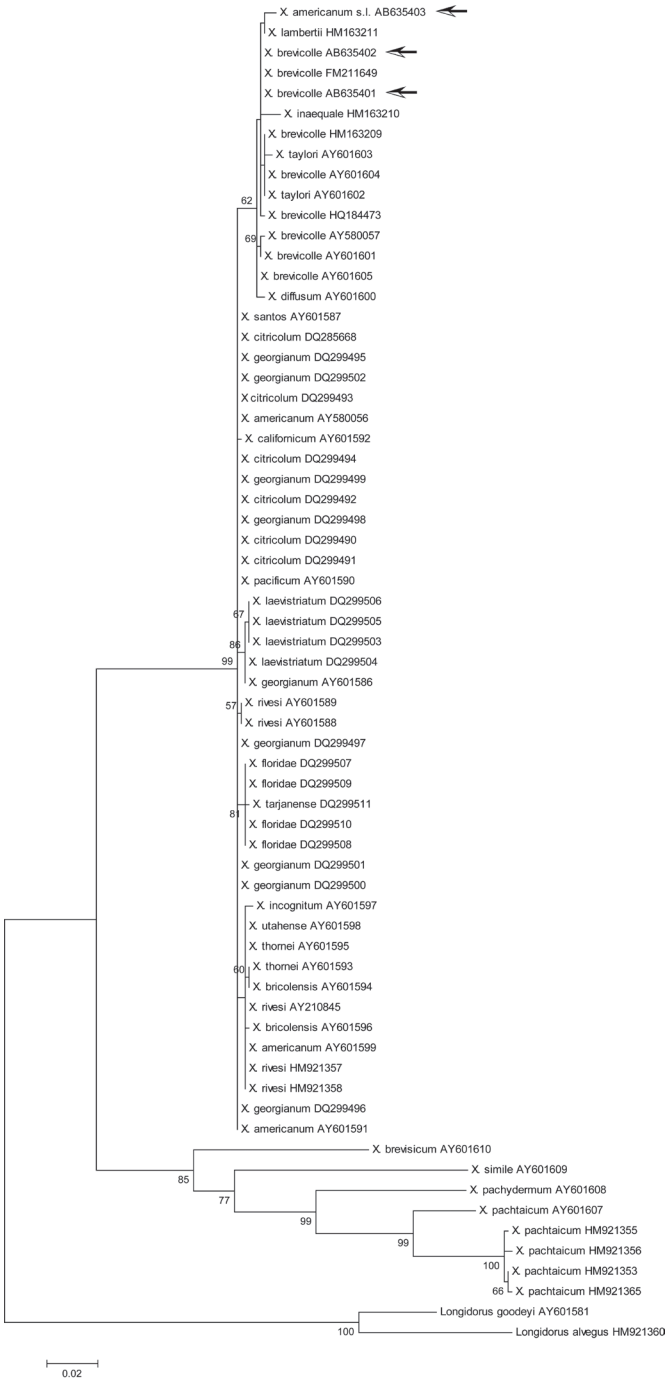

Figure 9. Maximum likelihood tree for D2/D3 region. Bootstrap values higher than 50 are shown. Arrows indicate specimens examined in this study: *Xiphinema brevicolle* GenBank accession AB635401 and AB635402; *Xiphinema* sp. AB635403. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

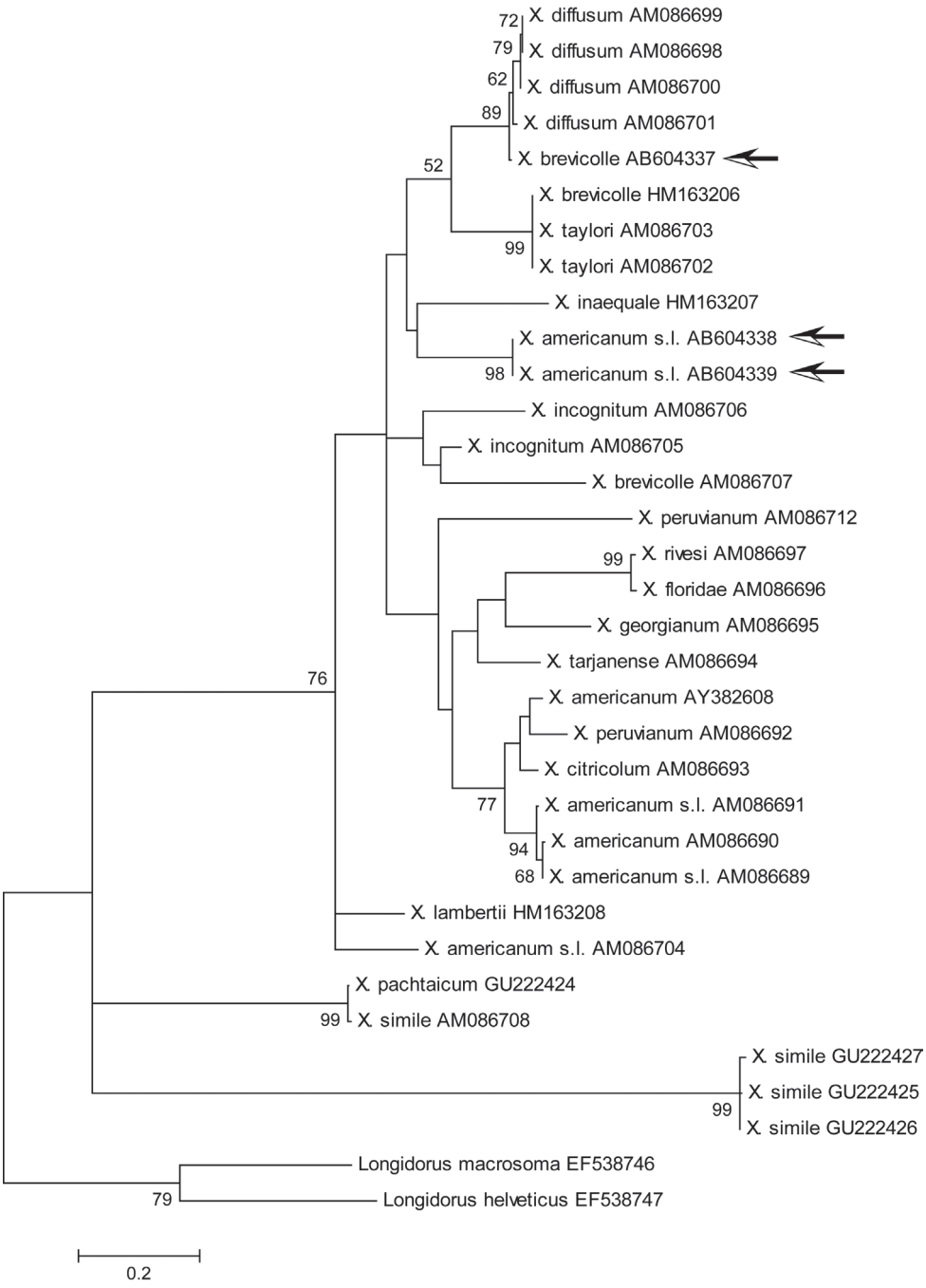

Figure 10. Maximum likelihood tree for COI region. Bootstrap values higher than 50 are shown. Arrows indicate specimens examined in this study. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

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A review of *Aleurodaphis* (Hemiptera, Aphididae, Hormaphidinae) with the description of one new species and keys to species

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Abstract

The genus *Aleurodaphis* van der Goot is reviewed. One new species *Aleurodaphis sinojackiae* Qiao & Jiang, **sp. n.** on *Sinojackia xylocarpa* from Jiangsu and Zhejiang, China is described. *Aleurodaphis sinisalicis* Zhang, 1982 is synonymised with *A. blumeae* van der Goot, 1917. Keys to species, morphological description and features of the new species, host plants, and distribution are provided. The specimens including types are deposited in British Natural History Museum, London (BMNH), Kôgakkan University, Japan and the National Zoological Museum of China, Institute of Zoology, Chinese Academy of Sciences, Beijing, China (NZMC).

Keywords

Hormaphidinae, *Aleurodaphis*, new species, synonym

Introduction

Aleurodaphis is erected in 1917 by van der Goot. He described the species *Aleurodaphis blumeae* as the type of the genus, from *Blumea*. Its remarkable characters are the followings, body of apterae is aleyrodiform, frontal horn is absent and wax glands are arranged along crenulated margin of body. Takahashi studied the specimens from East

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Asia, and described two species A. *mikaniae* Takahashi, 1925 and *A. asteris* Takahashi & Sorin, 1958. More than 30 years later, one new species was found from India, *A. antennata* Chakrabarti & Maity, (1980) 1982 and one new species was reported in China, *A. sinisalicis* Zhang, 1982. Sorin and Miyazaki (2004) reviewed the genus from Japan with descriptions of three new species, *A. impatientis*, *A. ligulariae* and *A. stewartiae*. After identifying the specimens from China and checking the specimens of the genus in British Natural History Museum, one new species, *A. sinojackiae* Qiao & Jiang, sp. n. is found, and *A. sinisalicis* Zhang, 1982 is synonymised with *A. blumeae* van der Goot, 1917 here. Therefore, the genus has eight known species in the world (Remaudière and Remaudière 1997; Sorin and Miyazaki 2004), including the new species described here.

Material and methods

Aphid terminology in this paper follows Sorin and Miyazaki (2004) and Ghosh (1988). The unit of measurements in this paper is millimeters (mm).

In Tables 1-2, the following abbreviations have been used: Ant. I – IV = antennal segments I – IV; Ant. V b = base of antennal segment V; pt = processus terminalis; URS = ultimate rostral segment; Hind T & F = hind trochanter & femur; 2HTs = second hind tarsal segment.

Specimen depositories. The holotype and some paratypes of the new species are deposited in British Natural History Museum, London (BMNH), while the other paratypes in the National Zoological Museum of China, Institute of Zoology, Chinese Academy of Sciences, Beijing, China (NZMC) and Kôgakkan University, Japan. All the other specimens studied are deposited in BMNH and NZMC.

Systematics

Aleurodaphis van der Goot, 1917

http://species-id.net/wiki/Aleurodaphis

Aleurodaphis van der Goot, 1917: 239.

Aleurodaphis van der Goot: Baker, 1929: 86; Takahashi, 1931: 92; Takahashi and Sorin, 1958: 31; Raychaudhuri, Ghosh, Pal and Ghosh, 1980: 36; Ghosh, 1988: 249; Noordam, 1991: 47; Tao, 1990: 58; Blackman and Eastop, 1994: 551; Remaudière and Remaudière, 1997: 181; Tao, 1999: 17.

Type species. *Aleurodaphis blumae* van der Goot, 1917.

Diagnosis. Body oval and flat. In apterous females: body aleyrodiform, absence of frontal horns, and wax glands arranged along the crenulated margin of body. Head and prothorax, meso- and metathorax, abdominal tergites I–VII fused, respectively; only abdominal tergite VIII free; antennae 4 or 5-segmented, primary rhinaria

small and ciliated; eyes with 3 facets. Dorsal setae fine and sparse. Rostrum reaching mid-coxae, at most hind coxae. Ultimate rostral segment obviously longer than second hind tarsal segment. Legs short; first tarsal chaetotaxy: 2–4, 2–4, 2–4; dorsal-apical setae on second hind tarsal segments with funnel-shaped apex. Siphunculi ring-shaped. Cauda knobbed and anal plate bilobed. In alate viviparous females: antennae 5-segmented, with secondary rhinaria near ring-shaped, without cilia; eyes normal; first tarsal chaetotaxy: 4, 4, 4, sometimes 3 or 2; fore wings with media once branched, pterostigma extended and two cubitus fused or separated at base; hind wings with two obliques.

Host plants. The range of host plants in *Aleurodaphis* is quite wide, including Compositae (*Aster*, *Blumea*, *Carpesium*, *Chrysanthemum*, *Kalimeris*, *Ligularia*, *Parasenecio*, *Senecio*), Balsaminaceae (*Impatiens*), Gramineae (*Bambusa*), Moraceae (*Ficus*), Plantaginaceae (*Plantago*), Scrophulariaceae (*Mazus*), Styracaceae (*Sinojackia*), Theaceae (*Stewartia*), Verbenaceae (*Callicarpa*) and Violaceae.

Biology. Five species, *A. asteris*, *A. blumeae*, *A. impatientis*, *A. ligulariae* and *A. mikaniae*, mainly feeding on Compositae species, have monoecious and anholocyclic life cycle. *A. sinojackiae* Qiao & Jiang, sp. n. and *A. stewartiae* can form galls on the leaves of the primary host plants, but their secondary hosts are unknown. The details of *A. antennata* were unreported (Ghosh 1988; Blackman and Eastop 1994, 2006; Sorin and Miyazaki 2004).

Distribution. China, Japan, India and Indonesia.

Keys to species of *Aleurodaphis*

Apterous viviparous females

1 Body without marginal wax glands; on *Stewartia*, in curled leaves..... ..... ***A. stewartiae***

– Body with marginal wax glands **2**

2 Marginal wax glands arranged in each segment, not connecting with each other (Fig. 20); on *Sinojackia*, in curled leaves .... ***A. sinojackiae* Qiao & Jiang, sp. n.**

– Marginal wax glands arranged consecutively along the crenulated margin of body (Figs 1, 2), not in curled leaves..... **3**

3 Ultimate rostral segment slender and long, 4.60–5.67 times as long as its basal width (Fig. 2) ..... ***A. blumeae***

– Ultimate rostral segment stout and short, less than 3.30 times as long as its basal width..... **4**

4 Each first tarsal segment with 2 setae; marginal wax glands along the crenulated margin of body with 120 wax facets at most..... ***A. asteris***

– Each first tarsal segment with more than 2 setae; marginal wax glands along the crenulated margin of body with 150 wax facets at least **5**

5 Ultimate rostral segment 1.16–1.40 times as long as second hind tarsal segment..... **6**

– Ultimate rostral segment 1.41–2.02 times as long as second hind tarsal segment **7**

6 Ultimate rostral segment 1.16–1.30 times as long as second hind tarsal segment; dorsal of body without obvious mastoid process; on *Bambusa*..... ***A. antennata***

– Ultimate rostral segment 1.40 times as long as second hind tarsal segment; dorsal of body with obvious mastoid process; on Compositae and Balsaminaceae (Impatiens) ..... ***A. mikaniae***

7 First tarsal chaetotaxy: 3 or 4, 3 or 4, 3 or 4; triommatidia elongate, with the outer-most facet placed widely apart from the other two; on *Impatiens*..... ***A. impatientis***

– First tarsal chaetotaxy: 2 or 3, 2 or 3, 2; triommatidia thickset, with the outermost facet placed close to the other two; on *Ligularia*..... ***A. ligulariae***

Alate viviparous females

1 Antennae 4-segmented, with more than 30 setae on each of segments III and IV ..... ***A. ligulariae***

– Antennae 5-segmented, with 2–5 setae on each of segments III and IV **2**

2 Ultimate rostral segment less than 3.00 times as long as its basal width..... **3**

– Ultimate rostral segment more than 3.00 times as long as its basal width.... **4**

3 Antennal segment III with 10–14 secondary rhinaria; first tarsal chaetotaxy: 4, 4, 4, sometimes 3, 3, 3..... ***A. sinojackiae*** **Qiao & Jiang, sp. n.**

– Antennal segment III with 24–27 secondary rhinaria; first tarsal chaetotaxy: 3, 3, 3, sometimes 2, 2, 2..... ***A. mikaniae***

4 Ultimate rostral segment 4.60–7.50 times as long as its basal width, and 1.96–2.32 times as long as second hind tarsal segment ..... ***A. blumeae***

– Ultimate rostral segment 3.38–4.00 times as long as its basal width, and 1.19–1.80 times as long as second hind tarsal segment **5**

5 First tarsal chaetotaxy: 3, 3, 3; antennal segment III with 25–29 secondary rhinaria..... ***A. stewartiae***

– First tarsal chaetotaxy: 4, 4, 4 or 3–4, 3–4, 2–4; antennal segment III with 9–12 secondary rhinaria..... ***A. impatientis***

Aleurodaphis antennata Chakrabarti & Maity, (1980) 1982

http://species-id.net/wiki/Aleurodaphis_antennata

Aleurodaphis antennata Chakrabarti & Maity, (1980) 1982: 56.

Aleurodaphis antennata Chakrabarti & Maity: Ghosh, 1988: 252; Blackman and Eastop, 1994: 551; Remaudière and Remaudière, 1997: 179.

Host plants. *Bambusa* sp.

Distribution. India (Ghosh 1988) .

***Aleurodaphis asteris* Takahashi & Sorin, 1958**
http://species-id.net/wiki/Aleurodaphis_asteris

Aleurodaphis asteris Takahashi & Sorin, 1958: 31.
Aleurodaphis asteris Takahashi & Sorin: Zhang, Zhong and Zhang, 1992: 142; Remaudière and Remaudière, 1997: 179; Tao, 1999: 17; Sorin and Miyazaki, 2004: 166.

Material examined. CHINA (NZMC): 2 apterous viviparous females, 15 April 1991, Jiangle, Fujiang, No. 10054, on Violaceae, coll. W. Y. Zhang; 8 apterous viviparous females, 13 August 2003, Motuo, Tibet, No. 15371, host plants unknown, coll. G. X. Qiao and X. L. Huang; **JAPAN** (BMNH): 24 apterous viviparous females, 5 August 1966, Osaka, Chihaya, on *Aster* sp., coll. M. Sorin.; 9 apterous viviparous females, 29 May 1964, Osaka, Kongo Mt., on *Aster* sp., coll. v. d. Bosch; 4 apterous viviparous females, 7 June 1966, Kyushu, Hikosan, on *Kalimeris* sp., coll. H. Takada; 8 apterous viviparous females, 6 August 1980, Kyoto, Kibune Mt., on *Aster yomena*, coll. R. L. Blackman; **KOREA** (BMNH): 2 apterous viviparous females, 15 September 1963, Ulnungdo, on *Aster incisus*, coll. W. H. Paik; 1 apterous viviparous female, 14 September 1963, Pusan, on *Chrysanthemum zawidskii*, coll. W. H. Paik.

Host plants. *Carpesium abrotanoides, Aster yomena*, *A. incisus*, *Chrysanthemum zawaidskii*, *Kalimeris* sp. and Violaceae.

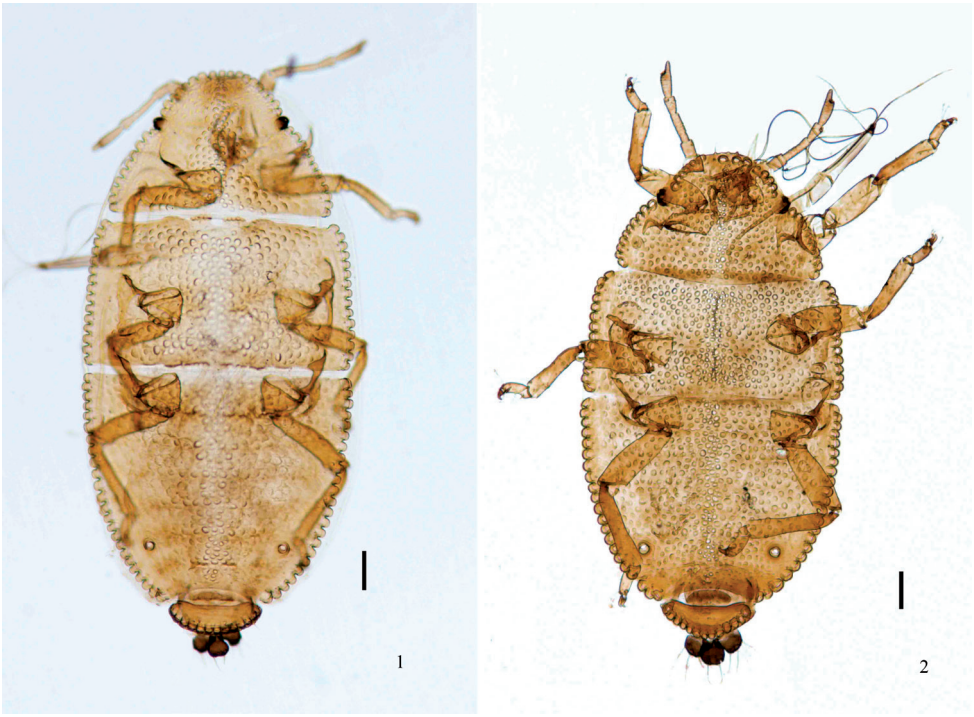
Biology. The species feed on the stems, leafstalks, flower stalks and leaves of the host plants.

Distribution. China, Japan and Korea.

***Aleurodaphis blumeae* van der Goot, 1917**
http://species-id.net/wiki/Aleurodaphis_blumeae
Figs 1–2

Aleurodaphis blumeae van der Goot, 1917: 240.
Aleurodaphis nobukii Shinji, 1923: 301.
Astegopteryx japonica Takahashi, 1923: 150.
Aleurodaphis sinisalicis Zhang, 1982: 20. syn. n.
Aleurodaphis blumeae van der Goot: Takahashi, 1921: 92; Takahashi, 1923: 150; Takahashi, 1924: 98; Raychaudhuri, Ghosh, Pal and Ghosh, 1980: 362; Ghosh, 1988: 256; Noordam, 1991: 47; Tao, 1990: 59; 1999: 17; Remaudière and Remaudière, 1997: 179; Sorin and Miyazaki, 2004: 166.

Comments. The type specimens of *Aleurodaphis sinisalicis* Zhang, 1982 were checked, including 48 apterous viviparous females, 25 July 1963, Sichuan (Guanxian County), No. Y0399, on *Salix* sp., coll. G. X. Zhang and T. S. Zhong. The result confirmed the queries of Blackman and Eastop (1994) and Remaudière and Remaudière (1997) that *A. sinisalicis* (Fig. 2) was a synonym of A. *blumeae* (Fig. 1).



Figures 1–2. Apterous viviparous female. **1** dorsal view of body, *Aleurodaphis blumeae* van der Goot **2** dorsal view of body, syntype of *Aleurodaphis sinisalicis* Zhang. Scale bars = 0.10 mm.

The original descriptions of *Aleurodaphis sinisalicis* Zhang, 1982 were accurate, but the morphological characters of *A. blumeae* in his diagnosis were wrong. Perhaps, this is the main reason why Zhang (1992) described it as a new species. In the original descriptions of Aleurodaphis sinisalicis Zhang, 1982, the diagnosis was: the ratio of body length to antennae length was 4.70 (*A. blumeae*: 2.70), the base of cauda restricted (*A. blumeae*: not restricted), and the anal plate bilobed (*A. blumeae*: not bilobed). Actually, the morphological characters of *A. blumeae* in this diagnosis were inaccurate. In A. blumeae, the ratio of body length to antennae length was 4.80 instead of 2.70, the base of cauda restricted instead of not restricted, and the anal plate bilobed instead of not bilobed.

The host plant of *A. sinisalicis*, *Salix* sp., is perhaps mis-recorded.

Material examined. CHINA (NZMC): 6 apterous viviparous females, 17 August 2004, Guizhou (Daozhen County), No. 15597, host plants unknown, coll. J. Y. Yang; 6 apterous viviparous females, 17 July 2001, Shaanxi (Nanzheng County), No. Y8606, host plants unknown, coll. S. H. Wang; 7 apterous viviparous females, 8 September 1995, Jiangxi (Jinggangshan City), No. 10852, on Compositae, coll. G. X. Zhang; 9 apterous viviparous females and 7 alate viviparous females, 25 April 1984, Shaanxi (Yangling County), No. 64, on *Carpesium cernuum*, coll. X. F. Dai; 5 alate viviparous females, May 1984, Shaanxi (Yangling County), No. Y6227, host plants unknown, coll. X. F. Dai; 3 apterous viviparous females, 26 June 1983, Zhejiang (Lin'an City),

No. Y2692, on *Carpesium abrotanoides*, collector unknown; 5 apterous viviparous females, 8 April 1998, Guangxi (Napo County), No. 11772, on *Callicarpa bodinieri*, coll. G. X. Qiao; 14 apterous viviparous females, 21 April 1998, Guangxi (Fangchenggang City), No. 11840, on Senecio *scandens*, coll. G. X. Qiao; 16 apterous viviparous females, 22 March 1998, Guangxi (Pingxiang City), No. 11580, on *Plantago asiatica*, coll. G. X. Qiao; 7 apterous viviparous females, Hunan, No. 8887, on Compositae, the collector unknown; 3 apterous viviparous females and 2 nymphs, Feburary 1925, Taiwan (Taihoku), on *Ficus* sp., coll. R. Takahashi (BMNH); 107 apterous viviparous females, 1 alate viviparous female and 24 nymphs, 21 May 1985, Zhejiang (Hangzhou City), on *Carpesium abrotanoides*, coll. V. F. Eastop (BMNH); **JAPAN** (BMNH): 2 apterous viviparous females and 4 nymphs, 29 August 1913, Kumamoto, on *Blumea* sp., coll. Theobald; 10 alate viviparous females, 22 September 1957, Osaka, on *Carpesium abrotanoides var. tumbergianum*, coll. M. Sorin; 2 apterous viviparous females, 2 alate viviparous females and 4 nymphs, 30 July 1957, Tokyo, Takao Mt., on *Blumea* sp., coll. R. Takahashi; 7 apterous viviparous females and 12 nymphs, 16 August 1991, Chiba, Sayama, on *Carpesium* sp., coll. D. L. Stern; **KOREA** (BMNH): 2 apterous viviparous females, 15 September 1963, Ulnungdo, on *Mazus miguelii*, coll. W. H. Paik; 1 apterous viviparous female, July 1969, Lri, host plants unknown, coll. W. H. Paik; **INDONESIA** (BMNH): 6 apterous viviparous females and 2 alate viviparous females, 13 July 1916, Garoet, on Compositae, coll. D. van der Goot; **MALAYSIA** (BMNH): 3 apterous viviparous females and 2 nymphs, 23 September 1944, Cameron Highlands, on *Blumea* sp., coll. R. Takahashi; **PHILIPPINES** (BMNH): 1 alate viviparous female, September 1962, Davao Exp. Station, trap in Abacca grove, coll. M. R. Gavarra; 1 alate viviparous female, July 1963, Davao Exp. Station, host plants unknown, coll. M. R. Gavarra; 1 alate viviparous female, January 1964, Davao Exp. Station, host plants unknown, coll. M. R. Gavarra; 1 alate viviparous female, March 1964, Davao Exp. Station, host plants unknown, coll. M. R. Gavarra; 3 apterous viviparous females and 2 nymphs, 13 September 1964, Makiling, on *Blumea* sp., coll. V. S. Calilung.

Host plants. *Carpesium cernuum*, *C. abrotanoides*, *C. abrotanoides* var. *tumbergianum*, *Senecio scandens*, *Blumea chinensis*, *Callicarpa bodinieri*, *Mazus miguelii*, *Ficus* sp. and *Plantago asiatica*. The common hosts are various Compositae.

Biology. This species feeds on the lower surface of leaves, along the main veins. It can infest *Blumea* on stems and undersides of young leaves, causing slight leaf-curl (Calilung 1967).

Distribution. China, Japan, Korea, Indonesia, Malaysia and Philippine.

***Aleurodaphis impatientis* Sorin & Miyazaki, 2004**

http://species-id.net/wiki/Aleurodaphis_impatientis

Aleurodaphis impatientis Sorin & Miyazaki, 2004: 167.

Host plants. *Impatiens textori* and *I. noli-tangere*.

Biology. The species is viviparous throughout the year on *Impatiens* spp. Alate viviparous females appear in the latter part of September. Adult apterous viviparous females pass the winter on the stalks near and just below the ground level. The hibernated adult females move to the seedlings and start to feed on the leaves and stalks in mid-April (Sorin and Miyazaki 2004).

Distribution. Japan (Sorin and Miyazaki 2004).

Aleurodaphis ligulariae Sorin & Miyazaki, 2004

http://species-id.net/wiki/Aleurodaphis_ligulariae

Aleurodaphis ligulariae Sorin & Miyazaki, 2004: 170.

Host plant. *Ligularia fischeri*.

Biology. The aphid lives on the lower side of the leaves and the apical part of the stem, as well as on the flower stalk at the tips of the host plant shoots. The alate viviparous females appear in the latter part of September. Adult apterous viviparous females pass the winter on the basal part of the stem just below the ground level and on fallen leaves in the ground litter (Sorin and Miyazaki 2004).

Distribution. Japan (Sorin and Miyazaki 2004).

Aleurodaphis mikaniae Takahashi, 1925

http://species-id.net/wiki/Aleurodaphis_mikaniae

Aleurodaphis mikaniae Takahashi, 1925: 51.
Aleurodaphis mikaniae Takahashi: Remaudière and Remaudière, 1997: 179; Tao, 1999: 17.

Material examined. CHINA (NZMC): 6 apterous viviparous females and 3 alate viviparous females, 24 August 2004, Guizhou (Daozhen County), No. 15638, host plants unknown, coll. J. Y. Yang; 4 apterous viviparous females, 15 August 2003, Sichuan (Baoxing County), No. 15017, on *Parasenecio* sp., coll. K. Guo; 7 apterous viviparous females, 27 June 1999, Shaanxi (Foping County), No. 12336, on Compositae, coll. T. L. He; 20 apterous viviparous females, 12 October 1988, Hunan (Zhangjiajie City), No. 8962, on *Impatiens* sp., coll. T. S. Zhong and G. X. Zhang; 4 apterous viviparous females, 26 September 1974, Guizhou (Guiyang City), No. Y2123, on *Senecio scandens*, coll. Y. Y. Rao; 10 apterous viviparous females, 31 March 1982, Yunnan (Kunming City), No. 7373, on *Senecio scandens*, coll. G. X. Zhang; 6 apterous viviparous females, 12 October 1996, Shaanxi (Zhouzhi County), No. 11096, host plants unknown, coll. G. X. Qiao; 4 apterous viviparous females, 18 August 2003, Sichuan (Baoxing County), host plants unknown, coll. K. Guo; 10 apterous viviparous females, 12 July 2002, Shaanxi (Meixian County), No. 13559, host plants unknown, coll. E. B. Ma; 14 apterous viviparous females, July 1936, Taiwan (Shinkwan),

host plants unknown, coll. R. Takahashi; **JAPAN** (BMNH): 41 apterous viviparous females, 6 August 1980, Kyoto, Kibune Mt., on *Impatiens* sp., coll. R. L. Blackman.

Host plants. *Parasenecio* sp., *Impatiens* sp., and *Senecio scandens*.

Distribution. China and Japan.

***Aleurodaphis sinojackiae* Qiao & Jiang, sp. n.**

urn:lsid:zoobank.org:act:4FE949A7-BF92-425C-BEDC-176EB4CA495A

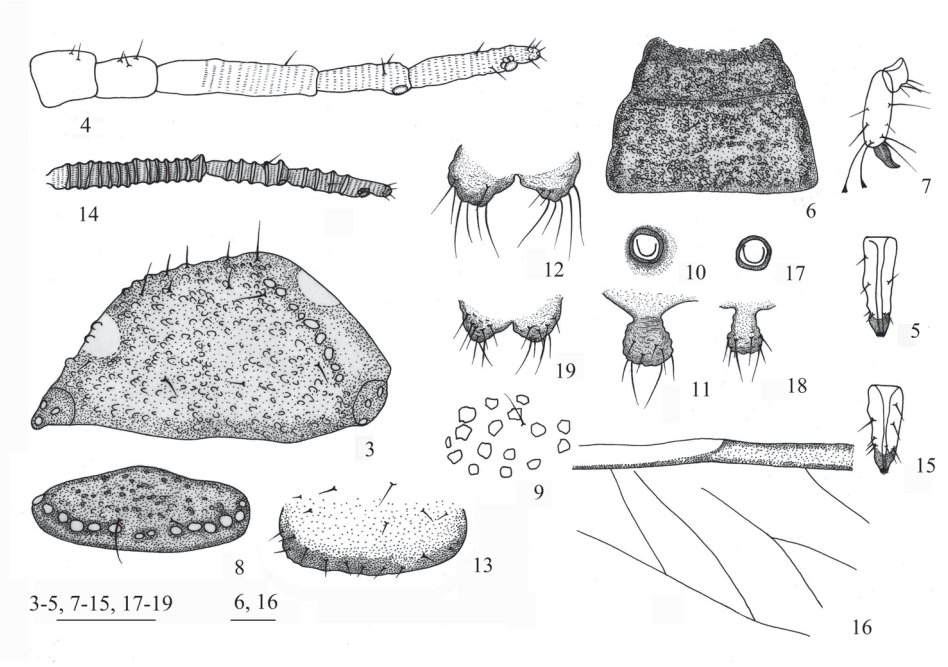
http://species-id.net/wiki/Aleurodaphis_sinojackiae

Figs 3–36

Locus typicus. China (Jiangsu and Zhejiang).

Etymology. The new species is named after its host plant, *Sinojackia xylocarpa*.

Description of mounted specimens. *Apterous viviparous females* (Table 1; Figs 3–13, 20–28). Body oval (Fig. 20). Measurements: body 1.86–2.09 long, 1.01–1.24 wide. Cephalic setae, marginal setae on abdominal tergite I and dorsal setae on abdominal tergite VIII 0.04–0.06, 0.04, 0.06–0.07 long. Antennae 0.61–0.68 long, segment III 0.19–0.23 long. Setae on segment III 0.03 long. Ultimate rostral segment



Figures 3–19. *Aleurodaphis sinojackiae* Qiao & Jiang, sp. n. **3–13** Apterous viviparous female. **3** dorsal view of head **4** antennae **5** ultimate rostral segment **6** dorsal view of thorax **7** hind tarsal segments **8** abdominal tergite VIII **9** dorsal setae and sculptures on abdominal tergite VI **10** siphunculus **11** cauda **12** anal plate **13** genital plate. **14–19** Alate viviparous female. **14** antennae 15 ultimate rostral segment **16** basal half of fore wing **17** siphunculus **18** cauda **19** anal plate. Scale bars = 0.10 mm.

Table 1. Measurements of apterous viviparous females of *Aleurodaphis sinojackiae* Qiao & Jiang, sp. n. (mm)

No.	Body length	Body width	Ant. I	Ant. II	Ant. III	Ant. IV	Ant. V b	Ant. V pt	URS	Hind T & F	2HTs	Cauda length
1	1.92	1.15	0.07	0.08	0.21	0.12	0.13	0.04	0.13	0.36	0.10	0.10
2	1.98	1.13	0.07	0.07	0.20	0.10	0.12	0.03	0.15	0.36	0.10	0.09
3*	2.05	1.21	0.07	0.08	0.23	0.12	0.13	0.03	0.14	0.38	0.10	0.09
4	1.89	1.07	0.07	0.08	0.19	0.12	0.12	0.03	0.12	0.33	0.10	0.09
5	1.86	1.09	0.08	0.07	0.21	0.11	0.12	0.04	0.14	0.37	0.11	0.08
6	2.09	1.01	0.08	0.08	0.23	0.12	0.14	0.04	0.13	0.39	0.11	0.09
7	2.05	1.24	0.07	0.08	0.22	0.12	0.13	0.03	0.15	0.37	0.10	0.09
8	2.03	1.05	0.07	0.08	0.22	0.11	0.13	0.04	0.14	0.37	0.10	0.09
Average	1.98	1.12	0.07	0.08	0.21	0.11	0.13	0.03	0.14	0.37	0.10	0.09

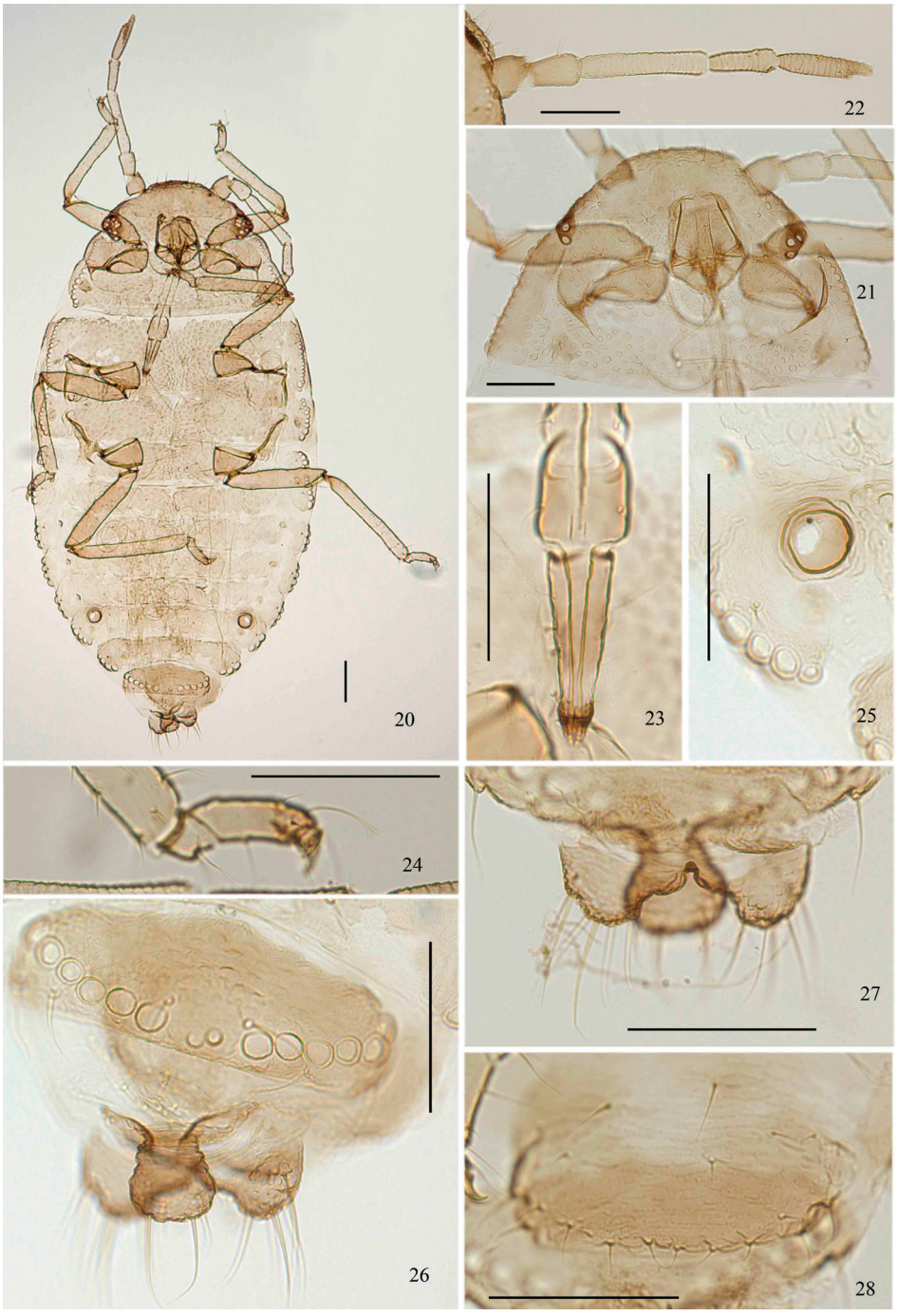
Remark. ***** Holotype; for abbreviations see Materials and Methods.

Table 2. Measurements of alate viviparous females of *Aleurodaphis sinojackiae* Qiao & Jiang, sp. n. (mm)

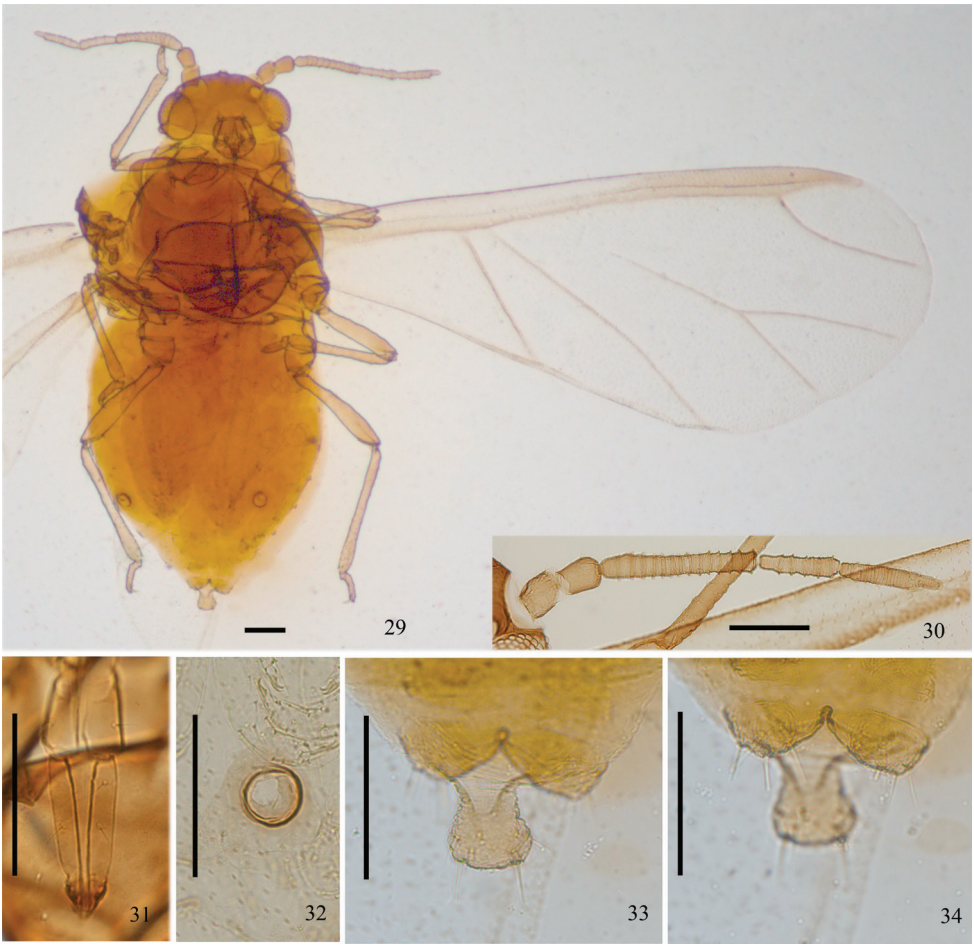
No.	Body length	Body width	Ant. I	Ant. II	Ant. III	Ant. IV	Ant. V b	Ant. V pt	URS	Hind femur	2HTs	Cauda length
1	1.84	0.81	0.08	0.07	0.28	0.14	0.14	0.04	0.14	0.40	0.09	0.08
2	2.00	0.82	0.07	0.07	0.25	0.14	0.13	0.04	0.13	0.39	0.11	0.08
4	1.49	0.66	0.06	0.06	0.18	0.11	0.09	0.02	0.10	0.29	0.08	0.06
5	1.45	0.63	0.05	0.05	0.19	0.09	0.08	0.02	0.10	0.30	0.08	0.06
6	1.32	0.64	0.05	0.05	0.21	0.11	0.11	0.03	0.11	0.31	0.08	0.05
7	1.42	0.56	0.06	0.05	0.18	0.11	0.09	0.02	0.11	0.31	0.07	0.06
8	1.30	0.58	0.06	0.05	0.19	0.10	0.09	0.03	0.11	0.26	0.08	0.06
Average	1.45	0.64	0.06	0.05	0.20	0.11	0.10	0.03	0.11	0.30	0.08	0.06

0.12–0.15 long. Hind trochanter and femur 0.33–0.39 long, hind tibia 0.38–0.44 long, second hind tarsal segment 0.10–0.11 long. Setae on hind tibia 0.04–0.06 long. Apical diameter of siphunculi 0.04–0.05. Cauda 0.08–0.10 long.

Head and pronotum (Figs 3, 21), mesonotum and metanotum (Fig. 6), abdominal tergites I–VII fused (Fig. 20), respectively; tergite VIII free (Fig. 8). Antennae, rostrum and legs brown; cauda, anal plate and genital plate dark brown. Dorsum of body rough, covered with dense sculptures on dorsum of head and thoracic notums, and with sparse sculptures on abdominal tergites (Fig. 9). Dorsum of body with round marginal wax glands, composited with big facets (Figs 8, 25, 26). Pro- and metanotum each with 13 wax glands, mesonotum with 8 wax glands, abdominal tergites I–VII each with 3–6 pairs of wax glands; tergite VIII with 10–13 wax glands. Dorsal setae of body fine and short (Fig. 9). Head with 2 pairs of cephalic and spinal setae, 3 pairs of marginal setae; pronotum with 2 pairs of spinal, 1 pair of pleural and 2 pairs of marginal setae; mesonotum with 2 pairs of spinal, 3 pairs of pleural and 2 pairs of marginal setae; metanotum with 1 pair of spinal, 3 pairs of pleural and 2 pairs of marginal setae; abdominal tergite I with 1 pair of spinal, pleural and marginal setae; tergites II–VII each with 1 pair of spinal and marginal setae; tergite VIII with 1 pair of spinal and 5 marginal setae.



Figures 20–28. *Aleurodaphis sinojackiae* Qiao & Jiang, sp. n. Apterous viviparous female. **20** dorsal view of body **21** dorsal view of head and pronotum **22** antenna **23** ultimate rostral segment **24** fore tarsal segments **25** siphunculus **26** abdominal tergite VIII and cauda **27** anal plate **28** genital plate. Scale bars = 0.10 mm.



Figures 29–34. *Aleurodaphis sinojackiae* Qiao & Jiang, sp. n. Alate viviparous female. **29** dorsal view of body **30** antennae **31** ultimate rostral segment **32** siphunculus **33** cauda **34** anal plate. Scale bars = 0.10 mm.

Cephalic setae, marginal setae on abdominal tergite I, setae on abdominal tergite VIII 1.20–1.60, 0.45–1.20 and 1.09–2.00 times as long as widest diameter of antennal segment III, respectively. Spiracles oval, closed, on brown oval spiracular plates.

Head: Front flat and straight. Eyes with 3 facets. Antennae 5-segmented (Figs 4, 22), with spinulose imbrications on segments III–V, 0.33–0.38 times as long as body. Length in proportion of segments I–V: 31–38 : 34–42 : 100 : 50–62 : 56–65+13–21, respectively. Processus terminalis 0.25–0.33 times as long as base of the segment V. Segments I–V each with 2–4, 2 or 3, 0 or 1, 1 or 2, 2+0 setae, respectively. Processus terminalis with 5 or 6 apical setae. Setae on segment III 0.58 times as long as widest diameter of the segment. Primary rhinaria small and round. Rostrum short, reaching mid-coxae. Ultimate rostral segment acute wedge-shaped (Figs 5, 23), 2.67–3.33 times as long as its basal width, 1.14–1.43 times as long as second hind tarsal segment; with 2 pairs of primary setae and 1 or 2 pairs of secondary setae.

Thorax: Mesosternal furca with two separated arms, each arm 1.21–1.41 times as long as widest diameter of antennal segment III. Legs normal. Trochanter and femora fused, hind trochanter and femur 1.63–1.85 times as long as antennal segment III, hind tibia 0.20–0.22 times as long as body; setae on hind tibia 0.88–0.94 times as long as its mid-diameter. First tarsal chaetotaxy: 4, 4, 4, sometimes 3, 3, 4 or 4, 4, 3. Second hind tarsal segment with 2 setae between claws and each seta with funnel-shaped apex (Figs 7, 24).

Abdomen: Siphunculi pore-like (Figs 10, 25), on abdominal tergite VI, apical diameter 1.00–1.40 times as long as widest diameter of antennal segment III. Cauda, anal plate and genital plate with spinulose imbrications. Cauda knobbed (Figs 11, 26), constricted in middle, 0.55–0.68 times as long as its basal width, with 9 or 10 apical setae. Anal plate bilobed (Figs 12, 27), each with 6–8 setae. Genital plate broad band-shaped (Figs 13, 28), with 3 or 4 anterior setae and 14–23 middle and posterior marginal setae. Two gonapophyses each with 5 short setae.

Alate viviparous females (Table 2; Figs 4–19, 29–34). Body oval (Fig. 29). Measurements: body 1.30–2.00 long, 0.56–0.82 wide. Cephalic setae, marginal setae on abdominal tergite I and dorsal setae on abdominal tergite VIII 0.016–0.021, 0.015–0.017, 0.020–0.026 long, respectively. Antennae 0.49–0.74 long, segment III 0.18–0.28 long. Ultimate rostral segment 0.10–0.14 long. Hind femur 0.26–0.40 long, hind tibia 0.36 long, second hind tarsal segment 0.07–0.11 long. Setae on hind tibia 0.030 long. Fore wing 1.64–1.74 long. Apical diameter of siphunculi 0.04–0.05. Cauda 0.05–0.08 long.

Dorsum of body dark brown, antenna, apex of rostrum, legs, cauda, anal plate and genital plate brown. Dorsal setae of body fine, short and pointed, slightly longer than ventral setae. Head with 2 pairs of cephalic setae, 2 pairs of setae between antennae and 2 pairs of setae between eyes; abdominal tergites I–VII each with 1 pair of spinal and marginal setae; tergite VIII with 1 pair of spinal setae. Cephalic setae, marginal setae on abdominal tergite I, setae on abdominal tergite VIII 0.51–0.67, 0.50–0.54 and 0.64–0.83 times as long as widest diameter of antennal segment III, respectively.

Head: Front rounded. Antennae 5-segmented (Figs 14, 30), with sparse imbrications on segments I–II and dense spinulose imbrications on segments III–V. Whole length of antennae 0.37–0.43 times as long as body, length in proportion of segments I–V: 21–32 : 24–33 : 100 : 49–60 : 41–53+10–15, respectively. Processus terminalis 0.29–0.37 times as long as base of the segment V. Segments I–V each with 3–5, 2 or 3, 0 or 1, 1, 1 or 2+0 setae, respectively. Processus terminalis with 5 apical setae. Primary rhinaria irregular ring-shaped. Segments III, IV and base of Segment V each with 10–14, 3–6 and 2–4 secondary rhinaria, respectively. Rostrum short, reaching mid-coxae. Ultimate rostral segment (Figs 15, 31) 2.50–2.86 times as long as its basal width, 1.20–1.54 times as long as second hind tarsal segment; with 2 or 3 pairs of primary setae and 1 or 2 pairs of secondary setae.

Thorax: Legs normal. Hind femur 1.50–1.62 times as long as antennal segment III, hind tibia 0.25–0.28 times as long as body; setae on hind tibia 0.91–1.20 times as long as its mid-diameter. First tarsal chaetotaxy: 4, 4, 3. Fore wing (Figs 16, 29) 1.17–1.34 times as long as body, 2.00–2.42 times as long as width of the wing. Media

once branched. Pterostigma long and curved to the apex of the wing. Hind wings with one thick longitudinal vein and two oblique veins.

Abdomen: Siphunculi pore-like (Figs 17, 32), apical diameter 1.33–1.51 times as long as widest diameter of antennal segment III. Cauda knobbed (Figs 18, 33), constricted in middle, 0.68–0.86 times as long as its basal width, with 6–8 apical setae. Anal plate bilobed (Figs 19, 34), each with 6 or 7 setae. Genital plate broad band-shaped, with 3 anterior setae and 12–15 posterior marginal setae. Two gonapophyses each with 5 or 6 setae.

Embroys. Body oval, with wax glands arranged along crenulated margin in both apterae and alatae. Cephalic setae short and pointed. Antennae 4-segmented. Rostrum and legs well developed. Legs covered with dense setae. Siphunculi visible.

Type material examined. Holotype, 1 apterous viviparous female, **CHINA:** Zhejiang (Hangzhou City), 21 May 1985, on *Sinojackia xylocarpa*, coll. V. F. Eastop (BMNH). Paratypes, 28 apterous viviparous females, 2 alate viviparous females and 8 nymphs, with the same collection data as holotype (BMNH); 9 apterous viviparous females and 11 alate viviparous females, **CHINA:** Jiangsu (Zhongshan Botanic Garden, Nanjing City, Alt. 100m), No. Y7116, 10 June 1987, on *Sinojackia xylocarpa*, coll. T. S. Zhong (NZMC); 1 apterous viviparous female and 1 alate viviparous female, **CHINA:** Jiangsu (Zhongshan Botanic Garden, Nanjing City, Alt. 100m), No. Y7116, 10 June 1987, on *Sinojackia xylocarpa*, coll. T. S. Zhong (Kôgakkan University, Japan).

Host plants. *Sinojackia xylocarpa*.

Biology. The species induced the leaves of host plants to curl and form boat-shaped leaf galls.

Diagnosis. The new species differs from the other known species as follows: in apterous viviparous female: wax glands arranged in each segment, not connecting with each other (the other species: arranged continuously along the edge of body as a crenulation, or without wax glands); in alate viviparous female compared to the most similar species *A. mikaniae*: antennal segment III with 10–14 secondary rhinaria (*A. mikaniae*: 24–27); first tarsal chaetotaxy: 4, 4, 4, sometimes 3, 3, 3 (*A. mikaniae*: 3, 3, 3, sometimes 2, 2, 2).

Remark. As the detailed biological information is very important to research the taxonomic position of the genus and species identification, the life cycle of the new species will receive further study in future.

Aleurodaphis stewartiae Sorin & Miyazaki, 2004

http://species-id.net/wiki/Aleurodaphis_stewartiae

Aleurodaphis stewartiae Sorin & Miyazaki, 2004: 174.

Host plants. Primary host: *Stewartia monadelpha*. Secondary hosts unknown.

Biology. The aphid induces a leaf gall, which is formed by rolling the marginal part of the leaf upwards. The gall is about 47.5 long and 7.2 wide, with a surface rough to the touch. The alate viviparous females emerge in early August, and then disappear

from the host tree, probably emigrating to some unknown secondary host (Sorin and Miyazaki 2004).

Distribution. Japan (Sorin and Miyazaki 2004).

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References

Baker AC (1929) Generic classification of the Hemipterous family Aphididae. Bulletin, United States, Department of Agriculture 826: 1–109.

Blackman RL, Eastop VF (1994) Aphids on the World's Trees. An Identification and Information Guide. CAB International in Association with the Natural History Museum, Wallingford, UK, 987 pp.

Blackman RL, Eastop VF (2006) Aphids on the World's Herbaceous Plants and Shrubs. The Natural History Museum, John Wiley & Sons Ltd, Chichester, UK, 1439 pp.

Calilung VJ (1967) A faunistic study of Philippine Aphids. Philippine Agricultural Scientist 51: 93–170.

Chakrabarti S, Maity SP (1982) New genus, new species and new records of Cerataphidine aphids Homoptera: Aphididae) from northwest Himalaya. Proceedings of the Zoological Society (Calcutta) 33(1–2): 55–63.

Eastop VF, Hille Ris Lambers D (1976) Survey of the World's Aphids. Dr. W. Junk b.v., Publishers, The Hauge, 573 pp.

van der Goot P (1917) Zur Kenntnis der Bhattlause Javeis. Contrib. Faune Indes Neerl 1(3): 1–301.

Ghosh AK (1988) The Fauna of India and the Adjacent Countries (Homoptera: Aphidoidea) Part 4 Subfamilies: Phloeomyzinae, Anoeciinae and Hormaphidinae. Zoological Survey of India, Calcutta, 429 pp.

Noordam D (1991) Hormaphidinae from Java (Homoptera: Aphididae). Zoologische Verhandelingen (Leiden) 270: 1–525.

Raychaudhuri DN, Ghosh MR, Pal PK, Ghosh AK (1980) Taxonomy of aphids of north-east India and Bhutan. Taxonomic accounts of the subfamilies. Subfamily Hormaphidinae. 358–394. In Raychaudhuri DN. Aphids of north-east India and Bhutan. The Zoological Society, i–viii, Calcutta, 521pp.

Remaudière G, Remaudière M (1997) Catalogue of the World's Aphididae. Institut National de la Recherche Agronomique, Paris, 473 pp.

Shinji GO (1923) One new species of aphids from Saitama and Maroika. Doubts. Zasshi. 35(417): 301–309.

Sorin M, Miyazaki M (2004) *Aleurodaphis* of Japan (Hemiptera, Aphididae). Japanese Journal of Systematic Entomology 10(2), 165–177.

Takahashi R (1921) Aphididae of Formosa. Part I. Agriculture Experiment Station Government of Formosa, Report 20: 1–97.

Takahashi R (1923) Aphididae of Formosa. Part 2. Department of Agriculture Government Research Institute Formosa, Report 4: 1–173.

Takahashi R (1924) Aphididae of Formosa. Part 3. Department of Agriculture Government Research Institute Formosa, Report 10: 1–121.

Takahashi R (1925) Aphididae of Formosa. Part 4. Department of Agriculture Government Research Institute Formosa, Report 16: 1–65.

Takahashi R (1931) Aphididae of Formosa. Part 6. Department of Agriculture Government Research Institute Formosa, Report 53: 1–127.

Takahashi R, Sorin M (1958) Notes on the aphid of *Aleurodaphis* in Japan (Homoptera). Akitu, Kyoto 7: 31–32.

Tao CC (1990) Aphid-fauna of Taiwan Province, China. Taiwan Provincial Museum Press, Taibei, 327pp.

Tao CC (1999) List of Aphidoidea (Homoptera) of China. Taiwan Agricultural Research Institute Special Publication 77: 1–144.

Zhang GX, Zhong TS (1982) New species and new subspecies of Chinese Aphidoidea (Homoptera). Sinozoologia 2: 19–28.

Zhang GX, Zhong TS, Zhang WY (1992) Aphidoidea. 137–175. In: Peng JW, Liu YQ. Eds. 1992. Iconography of forest insects in Hunan China. Academia Sinica and Hunan Forestry Institute, Hunan, China. Hunan Science and Technology Press, Changsha, 473pp.

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First record of *Neolindus* Scheerpeltz from French Guiana (Coleoptera, Staphylinidae, Paederinae), with a key to males

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Abstract

The genus *Neolindus* Scheerpeltz, 1933, of the tribe Cylindroxystina Bierig, 1943, is recorded from French Guiana for the first time. Two new species, *Neolindus irmleri* **sp. n.** and *Neolindus hermani* **sp. n.**, are described and illustrated. A key to males of *Neolindus* is provided.

Keywords

Coleoptera, Staphylinidae, Paederinae, *Neolindus*, key, French Guiana, new species

Introduction

The genus *Neolindus* Scheerpeltz, 1933 was revised by Herman (1991) in his extensive phylogenetic work and revision of the tribe Cylindroxystina. In that publication he described three characters that support the monophyletic status of the genus and described 27 species, principally from South America.

More recently, Irmler (2011) described two new species from the Peruvian Amazon. *Neolindus* is currently the larger of the two Neotropical genera (*Neolindus* and

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Cylindrxystus) of Cylindroxystina, represented by 35 species distributed from northern Costa Rica to southern Brazil (Herman 2001, Irmler 2011), 29 of which are known only from South America. *Neolindus* species are typically found in forest leaf litter at altitudes ranging from 50 to 2500 m.

In this paper, two new species of *Neolindus* are described and illustrated and the genus is recorded for the first time from French Guiana, increasing the number of known species to 37. A key to males of the genus is presented based mainly on characters of the genitalia. *Neolindus amazonicus* Irmler 1981, *N. peruvianus* Irmler 1981 and *N. hanagarthi* Irmler 1981 are excluded from the key because they are known only from females.

Methods

Specimens. Specimens were collected via flight interception traps (FIT or window traps), an especially useful capture method which has resulted in the discovery of many new species of multiple taxa. The specimens studied here were collected by SEAG (Société Entomologique Antilles-Guyane) with FITs hung approximately 1.50 m above ground level (Fig. 1 in Degallier et al. 2011). Traps consisted of an acrylic panel hung vertically in the forest and a gutter at the base of the panel containing a preservative fluid (water, salt and detergent, or propylene glycol).

To study morphological characters, dried specimens were macerated in boiling water for five minutes and then cleared in 10% KOH overnight. Dissections were carried out under a Carl Zeiss Stemi SV6 stereoscopic microscope and drawings made with the same equipment. Photographic illustrations were done using IM 50 (Image Manager) software and combined using Auto-Montage Pro (Syncroscopy) software. Measurements were made with an ocular micrometer in the SV6 microscope.

For the type label data, quotation marks " " separate different labels and a slash / separates different lines. Text within square brackets [ ] is explanatory and was not included in the original labels.
The following abbreviations are used:

BL body length (from anterior margin of clypeus to posterior margin of tergite IX)
BW body width (maximum width of elytra)
EL elytral length (maximum)
EW elytral width (maximum)
HL head length (from anterior margin of clypeus to posterior margin of head disc)
HW head width (maximum)
PL pronotum length (maximum)
PW pronotum width (maximum)

All measurements are in millimeters and are based on the holotypes. The terminology adopted for the descriptions follows Irmler (1981, 2011) and Herman (1991).

Depositories

All specimens are deposited in the following collections:

DZUP Coleção de Entomologia Pe. J. S. Moure, Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Brazil (Lucia M. de Almeida).
MNHN Muséum National d'Histoire Naturelle, Paris, France (Thierry Deuve).
MUSM Colección Entomológica del Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos, Lima, Peru (Gerardo Lamas).

Results

***Neolindus irmleri* Asenjo, sp. n.**
urn:lsid:zoobank.org:act:DF665432-A4A7-4173-B61B-166F17AADBFD
http://species-id.net/wiki/Neolindus_irmleri
Figs 1–7

Type material. FRENCH GUIANA: Holotype male, with labels: "GUYANA FRANCESA: / Montagne des chevaux, / 04°43'N,52°25'W, 90m, / flight intercept trap(glass), / 9.v.2009, S. Brûlé, / P.H. Dalens, E. Poirier" "Holotype / *Neolindus* / *irmleri* Asenjo / Desig. Asenjo, 2011" (MNHN).

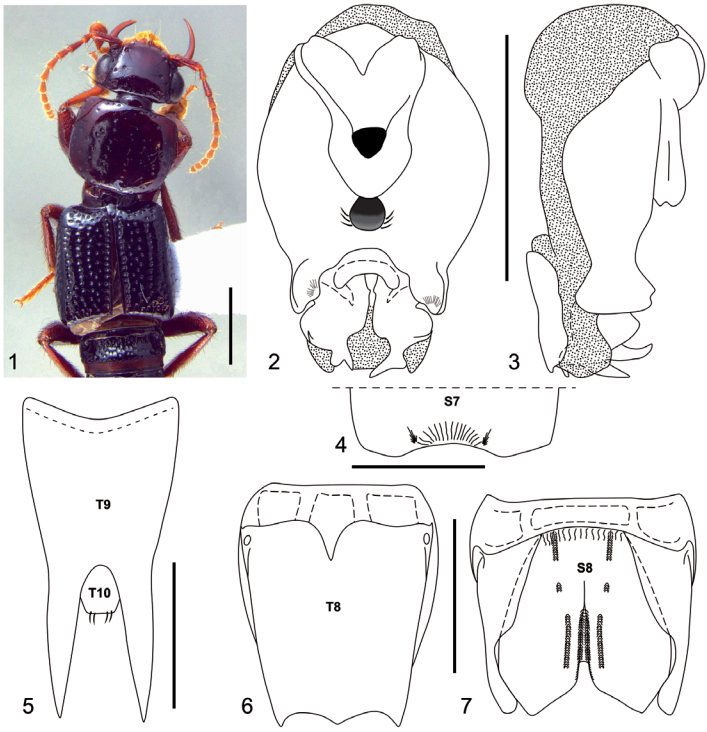
Paratype. 1 male with labels: "GUYANA FRANCESA: / Montagne des chevaux, / 04°43'N, 52°25'W, 90m, / flight intercept trap(glass), / 13.vi.2009, S. Brûlé, / P.H. Dalens, E. Poirier" "Paratype / *Neolindus* / *irmleri* Asenjo / Desig. Asenjo, 2011" (DZUP).

Diagnosis. *Neolindus irmleri* sp. n. can be distinguished from other *Neolindus* species by the sternum VIII divided into one central and two lateral plates (Fig. 7).

Description. Holotype male, BL: 12.36.

Body dark brown (Fig. 1). Mandibles, femora and tibiae dark reddish brown; antennal segments 1–3 dark reddish brown, segments 4–11 and all tarsi paler.

Head and pronotum moderately flattened dorsoventrally. Head (Fig. 1) wider (HW: 1.61) than long (HL: 1.02), with acute hind angles. Head disk with umbilicate punctures each carrying a black macroseta and one trichobothrium on lateral side of vertex near anterior third of eye, the umbilicate punctures mainly distributed at posterior edge in transversal line. Epicranium shiny without microsculpture and with micropunctures between umbilicate punctures, micropunctures denser anteriorly. Gula with two long setae near anterior margin. Labrum with large, apically rounded lobe near middle of anterior margin and with smaller, apically rounded lobe near lateral edge of anterior margin. Antennae with scape gradually thickened, pedicel (0.24) shorter than 2.6 times the length of scape (0.63), pedicel and segment 3 similar in width (0.12), segment 3 longer (0.33) than pedicel (0.24), segment 4 (length 0.22 : width 0.14) longer than wide, segment 5 (0.24 : 0.14) longer than wide, segments 6 to



Figures 1–7. *Neolindus irmleri* Asenjo sp. n. holotype male. **1** Adult habitus **2** aedeagus, ventral view **3** aedeagus, lateral view **4** apex of sternum VII (S7), setae omitted **5** tergum IX (T9) and tergum X (T10), setae omitted **6** tergum VIII (T8), setae omitted **7** sternum VIII (S8), setae omitted. Scale bars= 1mm.

8 longer than wide and identical measurements (0.20 : 0.14), segment 9 longer than wide (0.16 : 0.13), segment 10 quadrate (0.14 : 0.14), segment 10 longer than wide (0.22 : 0.12); segments 4–11 densely covered with microsetae; scape to segment 3 with black macrosetae lacking a defined pattern, on segment 4 to 10 arranged in one ring in the apical region, on segment 11 in a ring in the middle region and one tuft in the apical region.

Pronotum (Fig. 1) wider than long (PL: 1.53; PW: 1.86), with anterior margin straight, lateral margin slightly concave and hind angles rounded. Disk polished and shiny without microsculpture, with longitudinal row of 7-9 punctures on each side of midline; several punctures on lateral to paramedial row of punctures; rare micropunctures homogeneously distributed. Elytra (Fig. 1) slightly wider than pronotum (EL: 2.08; EW: 1.96) with epipleural ridge; surface polished and shiny, with irregular rows; with black macrosetae.

Legs uniformly covered with glossy black macrosetae; segments 1–4 of protarsus strongly bilobate and with yellowish pale setae ventrally.

Abdomen polished and shiny, uniformly punctate; the first segments more strongly punctate than the last.

Male with broad and moderately deep, median apical emargination on sternum VII (Fig. 4), posterior margin with small carina on lateral edge of emargination. Segment VIII (Figs. 6–7) with four internal canals at base of tergum and sternum. Tergum VIII (Fig. 6) with trilobed posterior margin; basal ridge with short median carina. Sternum VIII (Fig. 7) constituted by two lateral plates and one central plate, fused at the base. Central plate with broad, median emargination; the emargination wide apically and strongly narrowed basally, depression margined laterally by longitudinal carinae; each side of depression with additional lateral carinae; basal ridge with longitudinal small grooves and pair of central carinae; between basal and apical carinae is a small carina. Tergum IX (Fig. 5) fused medially and with long black setae. Aedeagus as in Figs. 2–3; parameres symmetric, fused around basal foramen; with broad, deep median apical emargination; ventral side with median cavity between median depression and basal foramen; median depression with small cluster of setae on lateral margin.

Female not known.

Habitat. From window traps in rainforest.

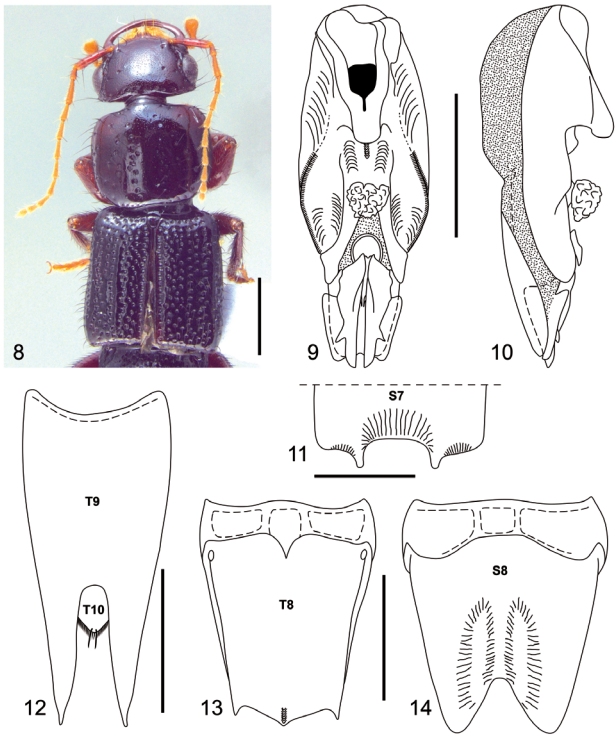
Distribution. Known from Montagne des Chevaux, French Guiana, 90 m.

Etymology. This species is named in honor of Dr. Ulrich Irmler of the Institute of Ecosystem Research, Germany.

***Neolindus hermani* Asenjo sp. n.**
urn:lsid:zoobank.org:act:2B271F06-AAF9-48EC-B0B7-E904AFA5A527
http://species-id.net/wiki/Neolindus hermani
Figs 8–14

Type material. FRENCH GUIANA: Holotype male with labels:"GUYANA FRANCESA: / Montagne des chevaux, / 04°43'N,52°25'W, 90m[altitude in relation to sea level], / flight intercept trap(glass), / 8.iii.2009, S. Brûlé, / P.H. Dalens, E. Poirier" "Holotype / *Neolindus* / *hermani* Asenjo / Desig. Asenjo, 2011" (DZUP).

Paratypes. 5 males with labels: "GUYANA FRANCESA: / Montagne des chevaux, / 04°43'N,52°25'W, 90m[altitude in relation to sea level], / flight intercept trap(glass), / 2.v.2009, S. Brûlé, / P.H. Dalens, E. Poirier" [without right elytron] (MNHN); "GUYANA FRANCESA: / Montagne des chevaux, / 04°43'N,52°25'W, 90m[altitude in relation to sea level], / flight intercept trap(glass), / 10.iii.2009, S. Brûlé, / P.H. Dalens, E. Poirier" [without left elytron] (DZUP); "GUYANA FRANCESA: / Montagne des chevaux, / 04°43'N,52°25'W, 90m[altitude in relation to sea level], / flight intercept trap(glass), / 8.iii.2009, S. Brûlé, / P.H. Dalens, E. Poirier" [without right elytron] (MUSM); "GUYANA FRANCESA: / Réserve Trésor, [around]~225m[altitude in relation to sea level], 04°36'37.6"N,52°16'44.5"W, / flight intercept trap (glass), / 1.xi.2009, S. Brûlé, P.H. / Dalens, E. Poirier" (MUSM); "GUYANA FRANCESA: The / Nouragues natural reserve, / Saut Pararé, 04°02'17.1"N, / 52°40'22.3"W, 80m[altitude in relation to sea level], flight" "intercept trap (glass), /



Figures 8–14. *Neolindus hermani* Asenjo sp. n. holotype male. **8** Adult habitus **9** aedeagus, ventral view **10** aedeagus, lateral view **11** apex of sternum VII (S7), setae omitted **12** tergum IX (T9) and tergum X (T10), setae omitted **13** tergum VIII (T8), setae omitted **14** sternum VIII (S8), setae omitted. Scale bars= 1mm.

20.x.2009, S. Brûlé, P.H. / Dalens, E. Poirier" [without elytra] (MUSM). 2 female with labels: "GUYANA FRANCESA: / Montagne des chevaux, / 04°43'N,52°25'W, 90m[altitude in relation to sea level], / flight intercept trap(glass), / 20.vi.2009, S. Brûlé, / P.H. Dalens, E. Poirier" (MUSM); "GUYANA FRANCESA: The / Nouragues natural reserve, / Saut Pararé, 04°02'17.1"N, / 52°40'22.3"W, 80m[altitude in relation to sea level]," "vii.2009, flight intercept trap / (glass), S. Brûlé, P.H. / Dalens, E. Poirier" (MUSM); all paratypes with label "Paratype / *Neolindus* / *hermani* Asenjo / Desig. Asenjo, 2011".

Diagnosis. Among *Neolindus* species, *N. hermani* sp. n. is similar to *N. pastazae*, in having the three triangular lobes on the posterior margin of tergum VIII (Fig. 13) and antennal segment 10 shorter than 9. *Neolindus hermani* sp. n. differs from it by the acute lobe on each side of median apical emargination on sternum VII (Fig. 11) and sternum VIII with a large pair of depressions on each side of the central emargination of the apex (Fig. 14).

Description. Holotype male, BL: 13.75.

Body dark brown (Fig. 8). Mandibles, femora, tibiae and antennal segments 1–2 dark reddish brown; antennal segments 3–11 reddish brown to yellow; all tarsi paler.

Head and pronotum moderately flattened dorsoventrally. Head (Fig. 8) wider (HW: 1.90) than long (HL: 1.22), with acute hind angles. Head disk with umbilicate punctures each carrying a black macroseta and one trichobothrium on lateral side of vertex near anterior third of eye. The umbilicate punctures mainly distributed at posterior edge in transversal line. Epicranium shiny without microsculpture and with micropunctures between umbilicate punctures, micropunctures homogeneously distributed. Gula with transverse cluster of numerous setae near anterior margin. Labrum with large, apically rounded lobe near middle of anterior margin and with smaller, apically rounded lobe near lateral edge of anterior margin. Antennae with scape gradually thickened, pedicel (0.20) shorter than 3.8 times the length of scape (0.76), scape (0.14) wider than pedicel (0.11), segments 3–11 longer than wide and with identical width (0.10), segment 3 (0.47) longer than pedicel (0.20), length segments 4 and 8 (0.31), length segments 5 and 6 (0.35), length segment 7 (0.33), length segment 9 (0.24), length segments 10 and 11 (0.20); segments 3–11 densely covered with microsetae; scape and pedicel with black macrosetae lacking a defined pattern, on segment 3 to 10 arranged in one ring in the apical region, on segment 11 in a ring in the middle region and one tuft in the apical region.

Pronotum (Fig. 8) wider than long (PL: 1.82; PW: 2.12), with anterior margin straight, lateral margin slightly concave and hind angles rounded. Disk polished and shiny without microsculpture; with longitudinal row of 8–11 punctures on each side of midline; several punctures on lateral to paramedial row of punctures; micropunctures homogeneously distributed. Elytra (Fig. 8) slightly wider than pronotum (EL: 2.45; EW: 2.33) with epipleural ridge; surface polished and shiny, with irregular rows; with black macrosetae.

Legs uniformly covered with glossy black macrosetae; segments 1–4 of protarsus strongly bilobate and with yellowish pale setae ventrally.

Abdomen polished and shiny, uniformly punctate; the first segments more strongly punctate than the last. Segments VII and VIII with microsculpture between punctures.

Male with broad and deep median apical emargination on sternum VII (Fig. 11), posterior margin with lobe on lateral edge of emargination; surface adjacent to emargination with shallow median depression. Segment VIII (Figs. 13–14) with four internal canals at base of tergum and sternum. Tergum VIII (Fig. 13) with three triangular, apically acute lobes on posterior margin, apex of central lobe longer than lateral lobes; basal ridge with short median carina; surface with slightly midlongitudinal carina on apical portion of median lobe; Sternum VIII (Fig. 14) with large pair of depressions on each side of central emargination of apex. Tergum IX (Fig. 12) fused medially and with long black setae. Aedeagus as in Figs. 9–10; parameres symmetric and fused to median lobe; with broad, deep median apical emargination; ventral side with median carina in front of basal foramen; apex of median lobe with many sclerites exposed.

Female with characters of head, pronotum, and elytra as described for male. Abdominal sterna VII and VIII with posterior margin emarginated.

Habitat. From window traps in rainforest.

Distribution. Known from Montagne des Chevaux (90m), Nouragues natural reserve-Saut Pararé (80m) and Réserve Trésor (225m) from French Guiana.

Etymology. This species is named in honor of Dr. Lee Herman of the American Museum of Natural History, USA.

Key to the males of *Neolindus* species

The Peruvian species *N. amazonicus*, *N. hanagarthi* and *N. peruvianus* described for Irmler 1981 are excluded from the key because they are known only from females.

1	Head with one pair of trichobothria (Fig. 64 in Herman 1991)	**2**
–	Head with two pairs of trichobothria (Fig. 78 in Herman 1991)	**26**
2(1)	Pronotum longer than wide	**3**
–	Pronotum wider than long (Fig. 64 in Herman 1991)	**10**
3(2)	Tergum VIII with posterior margin rounded or truncate (Fig. 122 & 148 in Herman 1991)	**4**
–	Tergum VIII with posterior margin emarginate (Fig. 136 in Herman 1991)	**7**
4(3)	Antennal segments 3 to 11 with dense pubescence. Peru (Huánuco)	***N. verhaaghi***
–	Antennal segments 4 to 11 with dense pubescence	**5**
–	Antennal segments 5 to 11 with dense pubescence	**6**
5(4)	Abdominal tergum VIII (Fig. 122 in Herman 1991) with posterior margin rounded and without internal canals at base, segment III without paratergites; aedeagus with apex of median lobe pointed (Fig. 119 in Herman 1991). Costa Rica (Puntarenas)	***N. incanalis***
–	Abdominal tergum VIII (Herman 1991; Fig. 140) with posterior margin truncate and with internal canals at base, segment III with one pair of paratergites; aedeagus with apex of median lobe emarginate (Fig. 141 in Herman 1991). Peru (Cuzco)	***N. cephalochymus***
6(4)	Tergum VIII with posterior margin rounded (Fig. 127 in Herman 1991). Bolivia (Beni), Brazil (Pará)	***N. brewsterae***
–	Tergum VIII with posterior margin sinuotruncate (Fig. 144 in Herman 1991). Brazil (Distrito Federal)	***N. agilis***
7(3)	Head without midlongitudinal carina at anterior margin	**8**
–	Head with midlongitudinal carina at anterior margin	**9**
8(7)	Antennal segment 2 longer than 3, segments 5–11 with dense pubescence. Colombia (Amazonas), Brazil (Pará)	***N. densus***
–	Antennal segment 2 shorter than 3, segments 4–11 with dense pubescence. Peru (Ucayali, Madre de Dios)	***N. punctiventris***

9(7) Body length about 9.00 mm; tergum VIII with moderately deeply to shallowly emarginate posterior margin (Fig. 159 in Herman 1991). Ecuador (Napo, Pichincha) ..... ***N. retusus***

– Body length about 6.00 mm; tergum VIII with feebly emarginate posterior margin (Fig. 165 in Herman 1991). Ecuador (Pichincha) ... ***N. procarinatus***

10(2) Tergum VIII with posterior margin rounded or truncate (Fig. 178 & 215 in Herman 1991) **11**

– Tergum VIII with posterior margin emarginate, lobed or trilobed (Figs. 203, 157 & 206 in Herman 1991) **18**

11(10) Tergum IX with base fused medially (Fig. 180 in Herman 1991) **12**

– Tergum IX with base divided medially (Fig. 171 in Herman 1991) **13**

12(11) Antennal segment 2 longer than 3. Costa Rica (Puntarenas) ..... ***N. cuneatus***

– Antennal segment 2 shorter than 3. Ecuador (Napo) ..... ***N. milleri***

13(11) Antennal segments 3 to 11 with dense pubescence; median orifice of median lobe of aedeagus with the sclerites hidden (Fig. 170 in Herman 1991) **14**

– Antennal segments 4 to 11 with dense pubescence; median orifice of median lobe of aedeagus with the sclerites exposed (Fig. 214 in Herman 1991) **15**

14(13) Aedeagus on ventral surface with median apical carina on median lobe (Fig. 170 in Herman 1991). Panama (Panama) ..... ***N. campbelli***

– Aedeagus on ventral surface without median apical carina on median lobe (Fig. 174 in Herman 1991). Panama (Panama) ..... ***N. apiculus***

15(13) Tergum VIII with posterior margin rounded (Fig. 183 in Herman 1991); aedeagus without median hole on ventral surface (Fig. 186 in Herman 1991); antennal segment 2 shorter than 3 ..... **16**

– Tergum VIII with posterior margin truncate (Fig. 215 in Herman 1991); aedeagus with median hole on ventral surface (Fig. 214 in Herman 1991); antennal segment 2 and 3 subequal in length. Ecuador (Napo) ..... ***N. dichymus***

16(15) Aedeagus without setae on ventral surface (Fig. 186 in Herman 1991). Brazil (Pará) ..... ***N. lodhii***

– Aedeagus with setae on ventral surface (Fig. 198 in Herman 1991) **17**

17(16) Sternum VIII with shallow apical emargination; emargination about one-fifteenth of length of sternum (Fig. 199 in Herman 1991). Brazil (Pará) ..... ***N. sinuatus***

– Sternum VIII with deep apical emargination; emargination about one-fifth of length of sternum (Fig. 195 in Herman 1991). Costa Rica (Heredia), Panama (Panama, Canal Zone) ..... ***N. basisinuatus***

18(10) Aedeagus without median hole on ventral surface (Fig. 156 in Herman 1991) **19**

– Aedeagus with median hole on ventral surface (Fig. 210 in Herman 1991) **22**

19(18) Antennal segment 2 longer than 3; gula with two setae **20**

– Antennal segment 2 shorter than 3; gula with transverse cluster of setae ... **21**

20(19) Tergum VIII with posterior margin lobed and middle of basal ridge carinated (Fig. 203 in Herman 1991); tergum IX with the base divided medially (Fig. 201 in Herman 1991). Brazil (São Paulo) ..... ***N. unilobus***

– Tergum VIII with posterior margin emarginated and middle of basal ridge not carinated (Fig. 157 in Herman 1991); tergum IX with the base fused medially (Fig. 155 in Herman 1991). Ecuador (Pichincha)..... ***N. bullus***

21(19) Tergum VIII without carina in middle of basal ridge (Fig. 2F in Irmler 2011); antennal segment 10 shorter than 9, segments 3–11 with dense pubescence (Fig. 2A in Irmler 2011). Ecuador (Tungurahua)..... ***N. pastazae***

– Tergum VIII with middle of basal ridge pointed (Fig. 206 in Herman 1991); antennal segments 9 and 10 subequal in length, segments 4–11 with dense pubescence. Panama (Chiriqui)..... ***N. punctogularis***

22(18) Tergum VIII with middle of basal ridge carinate (Fig. 221 in Herman 1991) ...**23**

– Tergum VIII with middle of basal ridge pointed **24**

23(22) Gula with two setae; antennal segments 2 and 3 subequal in length. Ecuador (Pichincha) ..... ***N. bidens***

– Gula with four setae; antennal segment 2 shorter than 3. Brazil (São Paulo, Rio de Janeiro)..... ***N. schubarti***

24(22) Sternum VIII not divided (Fig. 14).....**25**

– Sternum VIII divided into three plates, one central and two lateral (Fig. 7). French Guiana..... ***N. irmleri* sp. n.**

25(24) Gula with two setae; antennal segments 9 and 10 subequal in length and 4 to 11 with dense pubescence; aedeagus with setae on ventral surface (Fig. 224 in Herman 1991). Brazil (Pará)..... ***N. religans***

– Gula with transverse cluster of setae; antennal segment 10 shorter than 9 and 3 to 11 with dense pubescence; aedeagus without setae on ventral surface (Fig. 9). French Guiana..... ***N. hermani* sp. n.**

26(1) Tergum VIII with posterior margin rounded (Fig. 82 in Herman 1991).....**27**

– Tergum VIII with posterior margin emarginate, trilobed (Fig. 109 & 117 in Herman 1991).....**31**

27(26) Aedeagus with apex of median lobe pointed (Fig. 81 in Herman 1991) **28**

– Aedeagus with apex of median lobe emarginate (Fig. 85 in Herman 1991)..**30**

28(27) Sternum VIII with surface of apex of internal canals unmodified. Venezuela (Trujillo)..... ***N. plectrus***

– Sternum VIII with depressions in the surface of apex of internal canals (Fig. 92 in Herman 1991) **29**

29(28) Aedeagus distinctive and without transversal carina or process on ventral surface (Fig. 105 in Herman 1991). Venezuela (Aragua)..... ***N. rudiculus***

– Aedeagus distinctive and with transversal carina or process on ventral surface (Fig. 93 in Herman 1991). Colombia (Valle del Cauca)..... ***N. pumicosus***

30(27) Antennal segment 2 longer than 3. Ecuador (Napo) ..... ***N. parallelus***

– Antennal segments 2 and 3 subequal in length. Venezuela (Aragua) .... ***N. brachiatus***

31(26) Pronotum longer than wide; aedeagus with apex of median lobe pointed (Fig. 113 in Herman 1991) **2**

– Pronotum wider than long; aedeagus with apex of median lobe emarginate (Fig. 152 in Herman 1991). Brazil (Pará)..... ***N. hamatus***

32(31) Elytra shorter than pronotum; tergum VIII with posterior margin emarginate (Fig. 109 in Herman 1991). Ecuador (Aragua) ..... ***N. lirellus***

– Elytra longer than or as long as pronotum; tergum VIII with posterior margin trilobed (Fig. 117 in Herman 1991). Ecuador (Napo) ..... ***N. prolatus***

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References

Degallier N, Leivas FWT, Moura DP (2011) Histerid beetles of French Guiana. V. Revision of the genus *Ebonius* Lewis (Coleoptera, Histeridae, Omalodini). Zootaxa 2824: 44–52.

Herman LH (1991) Revision of the subtribe Cylindroxystina (Coleoptera: Staphylinidae: Paederinae). Bulletin of the American Museum of Natural History 203: 1–83.

Irmler U (1981) Neue Arten der neotropischen Gattung *Neolindus* Scheerpeltz (Coleoptera, Staphylinidae). Studies on Neotropical Fauna and Environment 16: 209–215. doi: 10.1080/01650528109360595

Irmler U (2011) Two new species and a new record of the genus *Neolindus* Scheerpeltz, 1933 (Coleoptera: Staphylinidae: Paederinae). Bonn Zoological Bulletin 60(1): 103–107.

Scheerpeltz O (1933) Staphylinidae VII. In: Schenkling S (Ed) Coleoptorum Catalogus. W. Junk, Berlin, pars 129: 989–1500.

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The genus *Camptochaeta* in Nearctic caves, with the description of *C. prolixa* sp. n. (Diptera, Sciaridae)

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Abstract

Camptochaeta prolixa **sp. n.** (Diptera, Sciaridae) is described from caves in Nevada, and three other congeneric species are recorded from caves in Nevada and Arkansas, United States. The new species shows some indication to a subterranean mode of life, including long antenna and legs, and in some specimens, reduction of the eye bridge.

Keywords

Diptera, Sciaridae, *Camptochaeta,* new species, new records, caves, USA

Introduction

The genus *Camptochaeta* Hippa & Vilkamaa, 1994 includes three species which have been found in caves: *C. ofenkaulis* (Lengersdorf, 1925) and *C. scanica* Hippa & Vilkamaa, 1994 and *C. subcamptochaeta* (Mohrig, 1992) (see Mohrig and Eckert 1992). Of these, *C. scanica* has been found also outside caves.

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Regarding the Nearctic Sciaridae, only specimens of '*Corynoptera* sp.' have been recorded from a cave in Arkansas (Graening et al. 2003).

Between May 2006 and July 2007, a bioinventory of caves at Great Basin National Park, White Pine County, Nevada was undertaken, and that study has already led to the description of several new species, including two millipeds (Shear 2007, Shear et al. 2009), a springtail (Zeppilini et al. 2009) and an amphipod (Taylor and Holsinger 2011), with additional material still undescribed.

Material and methods

Though a variety of sampling methods have been implemented in the Great Basin cave bioinventory, sciarids of the genus *Camptochaeta* were only obtained by hand collections, often using an aspirator. The sampling effort included entrance, twilight and dark zone sampling in 21 caves over 64 visits, and most collections are associated with data on light, temperature, and relative humidity, all collected with handheld meters, as well as data on subtrate, cave zone and elevation. Samples were preserved in the field in 70% ethanol, and later sorted and curated in the laboratory, with select material being distributed to appropriate taxonomic experts.

The material, kept in alcohol, was slide-mounted in "Euparal" after desiccation in absolute ethanol. The morphological terminology used and methods of measuring structures follow mainly Hippa and Vilkamaa (1991, 1994). The material is deposited in National Museum of Natural History, Washington, D.C (USNM), Illinois Natural History Survey Insect Collection, Champaign (INHS) and Zoological Museum, Finnish Museum of Natural History, Helsinki (MZH).

Taxonomy

***Camptochaeta prolixa* sp. n.**
urn:lsid:zoobank.org:act:9289EB2A-6160-49E2-B2E4-69E67037868B
http://species-id.net/wiki/Camptochaeta_prolixa

Type locality. USA, Nevada, White Pine County, Root Cave [39°00'N, 114°13'W], hand collection, 25.v.2006, S.J. Taylor, J.K. Krejca, M.E. Slay, G.M. Baker & B. Roberts.

Type material. *Holotype male,* dissected, and mounted on microscope slide in Euparal. Original label: USA, Nevada, White Pine County, Root Cave, hand collection, 25.v.2006, S.J. Taylor, J.K. Krejca, M.E. Slay, G.M. Baker & B. Roberts (in USNM). *Paratypes*, dissected and mounted as the holotype: 1 male, same data but dry calcite floor, G.M. Baker, S.J. Taylor & J.K. Krejca (in USNM); 1 male, Nevada, White Pine County, Lehman Cave, 25.vi.2006, G.M. Baker, M.A. Horner & B. O'Doan (in INHS); 1 male, same locality but dry calcite wall, hand collection, 26.v.2006, S.J. Taylor, J.K. Krejca, M.E. Slay & G.M. Baker (in MZH); 2

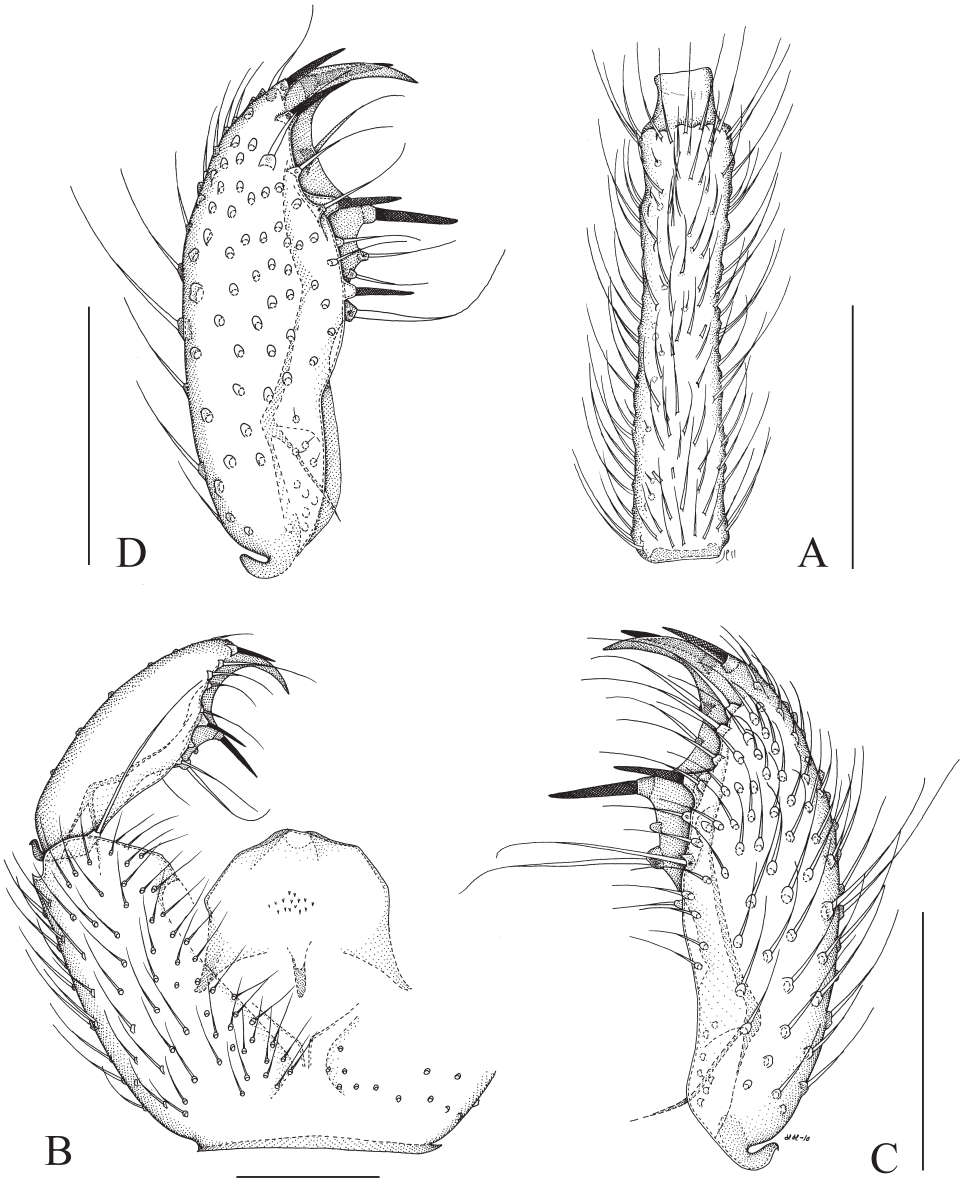

Figure 1. *Camptochaeta prolixa* sp. n. **A** Antennal flagellomere 4, lateral view **B** part of hypopygium, ventral view **C** and **D** Gonostylus, ventral view. **A–C** holotype, **D** paratype from Lehman Cave annex. Scale 0.1 mm.

males, Lehman Cave Annex, under rocks, hand collection, 25.v.2006, S.J. Taylor, J.K. Krejca, M.E. Slay B. Roberts, & M. Horner (in MZH); 1 male, Nevada, White Pine County, Cave 24, hand collection, 17.vii.2007, G.M. Baker & S.J. Taylor (in MZH). *Other material*. 1 female, Nevada, White Pine County, Lehman Cave,

under rocks, hand collection, 25.v.2006, S.J. Taylor, J.K. Krejca, M.E. Slay, B. Roberts, & M. Horner (in MZH).

Description. Male. **Head.** Brown, maxillary palpus very pale brown, antenna concolorous with face at base but paler towards apex. Eye bridge 1–2 facets wide, medially lacking ommatidia, sometimes narrowed into stripe. Face with 5–8 scattered setae. Clypeus with 1 seta. Maxillary palpus with 3 palpomeres; palpomere 3 longer than palpomere 1, palpomere 2 shortest; palpomere 1 with one (rarely 2) long sharp seta, with a dorsal pit with long sensilla; palpomere 2 with 3 (rarely 4) long sharp setae, 7–9 shorter truncate setae, palpomere 3 with 8–12 short truncate setae. Antenna long, antennal flagellomere 4, Fig. 1A, 4.0–5.8x as long as wide, the neck shorter than the width of flagellomere, the longest setae longer than the width of flagellomere. **Thorax.** Concolorous brown, setae pale. Anterior pronotum with 3–6 setae. Episternum 1 with 5–8 setae. **Wings.** Length 2.4–2.8 mm. Width/length 0.35–0.40. R1/R 0.85–1.05. c/w 0.65–0.70. r-m with 2–5 setae, bM non-setose. Halter pale brown. **Legs.** Yellowish, long. Metatarsus long, probasitarsomere as long as profemur; the modified vestiture of protibia pale, in patch in shallow depression. Protibial spur slightly longer than tibial width. **Abdomen.** Pale brown. Setae pale and long. **Hypopygium,** Fig. 1B–D. Brown, as abdomen. Gonocoxa slightly longer than gonostylus. The ventral setosity of gonocoxa sparse. Gonostylus narrow, the mesial side impressed on apical third; the setosity sparse, apicomesially with a few elongated setae; with long apical tooth, with 2–3 megasetae in two groups, apically and subapically, megasetae straight, one of the subapical ones larger than others; mesially with 1–2 long flagellate setae. Tegmen simple, with indistinct subapical lateral shoulders, laterally slightly sclerotized. Aedeagal apodeme short, aedeagal teeth minute.

Female. Slightly larger than male, wing length 3.0 mm, without diagnostic characters.

Discussion. *Camptochaeta prolixa* isvery similar to *C. subcamptochaeta* found in central European caves, and the very similar *C. pentacantha* found in the Altay, Russia. *C. prolixa* differs from both by having a more slender gonostylus and by having the antennal flagellomeres longer, in most specimens more than five times as long as wide, with longer setosity; and by having a narrower eye bridge. *C. prolixa* and resembles the European cave-dwelling *C. ofenkaulis* by having long antenna and legs, but the gonostylus is remarkably different. Some specimens of C. *prolixa* show a tendency to a reduced eye bridge, which in addition to the long legs and antenna may be an accommodation to the subterranean mode of life.

Ten of the 11 specimens of *C. prolixa* were collected in the dark zones of caves, and one in the twilight zone. Based on microhabitat-specific data for ten specimens: air temperature ranged from 6.6 to 13.0 C, average 10.9 C; relative humidity ranged from 82.6 to 92.4 %, average 87.7 %; light ranged from 0 to <1 lux, average 0.0 lux; and elevation ranged from 2089 to 2013 m, average 2228 m. All *C. prolixa* specimens were collected in May (9 specimens) and July (2 specimens), even though sampling was carried out monthly in Lehman Caves. Most specimens were associated with bedrock or calcite walls or ceilings.

Etymology. The name is Latin (adjective), *prolixa*, streched out, referring to the very long extremities of the fly.

Records of *Camptochaeta* species in caves

Camptochaeta mutua (Johannsen, 1912)

9 males, USA, Arkansas, Stone County, Blanchard Springs Caverns, 27.iv.2002, M. Slay, G. Graening & K. Tinkle (in MZH).

Camptochaeta mutua was described from Ithaca, New York (Johannsen 1912) and is recorded from eastern USA and Canada (Newfoundland, Ontario, Quebec and also from Yukon (Hippa and Vilkamaa 1994).

Camptochaeta pellax Hippa & Vilkamaa, 1994

1 male, Nevada, White Pine County, Lehman Cave Annex, dry ceiling, hand collection, 25.v.2006, S.J. Taylor, J.K. Krejca, M.E. Slay B. Roberts, & M. Horner (in MZH); 1 male, White Pine County, Root Cave, calcite wall, hand collection, 25.v.2006, S.J. Taylor, G.M. Baker & B. Roberts (in INHS).

Two specimens of *C. pellax* were collected from the dark zone of caves, with the following microhabitat-specific data: air temperature ranged from 10.9 to 13.0 C, average 11.95 C; relative humidity ranged from 84.4 to 88.6 %, average 86.5 %; light 0 lux; elevation ranged from 2089 to 2235 m, average 2162 m. *Camptochaeta pellax* is earlier known only from the type material from Colorado (Hippa and Vilkamaa 1994).

Camptochaeta spicigera Hippa & Vilkamaa, 1994

1 male, Nevada, White Pine County, Lincoln Mine, hand collection, 15.vii.2007, S.J. Taylor, J.K. Krejca, M.E. Slay, C.M. Slay (in MZH).

The Nevada specimen of *C. spicigera* was collected in the entrance zone of a mine on wet rocks above water on the mine floor, with the following microhabitat-specific data: air temperature 9.7 C; relative humidity 52.5%; light 1755 lux; elevation 2621 m. *Camptochaeta spicigera* is earlier known only from the type material from Colorado (Hippa and Vilkamaa 1994).

Discussion

The distributions of temperature and humidity data for the new species are consistent with the morphological evidence that this species is a troglobite. Only one specimen was found in the twilight zone, and with the exception of that specimen, the species was always associated with elevated relative humidity and stable temperatures consistent with deep-cave habitats.

Our description of a new *Camptochaeta* brings the number of recently described cave organisms from Great Basin National Park to five. The sampled caves span a range of 1724 to 3413 meters in elevation, crossing a variety of vegetation zones from to above timberline. Within the caves, there are a variety of microhabitats with varying levels of nutrient input and habitat stability. In addition, the Park is located in a relatively sparsely populated area, with few entomologists. A combination of these factors may account for the relatively high number of new species recently described from this area.

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References

Graening GO, Slay ME, Tinkle KK (2003) Subterranean diversity of Arkansas, Part 1: Bioinventory and bioassessment of caves in the Sylamore Ranger District, Ozark National Forest, Arkansas. Journal of the Arkansas Academy of Sciences 57: 44–58.

Johannsen OA (1912) The fungus gnats of North America. Part IV (Conclusion). Bulletin of Maine Agricultural Experimental Station 200: 57–146.

Hippa H, Vilkamaa P (1991) The genus *Prosciara* Frey (Diptera, Sciaridae). Entomologica Fennica 2: 113–155.

Hippa H, Vilkamaa P (1994) The genus *Camptochaeta* gen. nov. (Diptera, Sciaridae). Acta Zoologica Fennica 194: 1–85.

Komarova LA, Hippa H, Vilkamaa P (2007) A review of the sciarids species of the genus *Camptochaeta* Hippa et Vilkamaa, 1994 (Diptera: Sciaridae) of the Altai fauna. Far Eastern Entomologist 171: 1–9.

Lengersdorf F (1925) Beitrag zur Höhlenfauna Siebengebirges unter besonderer Berücksichtigung der Dipteren. Speleologische Jahrbuch 1924/1925: 16–22.

Mohrig W, Eckert R (1992) Trauermücken aus Naturhöhlen des Harzes, Deutschland (Insecta, Diptera, Sciaridae). Mitteilungen aus dem Zoologischen Museum in Berlin 68: 295–298. doi: 10.1002/mmnz.19920680210

Shear WA (2007) Cave millipeds of the United States. V. The genus *Idagona* Buckett & Gardner (Chordeumatida, Conotylidae, Idagoninae). Zootaxa 1463: 1–12.

Shear WA, Taylor SJ, Wynne JJ, Krejca JK (2009) Cave millipeds of the United States. VIII. New genera and species of polydesmidan millipeds from caves in the southwestern United States (Diplopoda, Polydesmida, Macrosternodesmidae). Zootaxa 2151: 47–65.

Taylor SJ, Holsinger JR (2011) A new species of the subterranean amphipod crustacean genus *Stygobromus* (Crangonyctidae) from a cave in Nevada, USA. Subterranean Biology 8: 39–47. doi: 10.3897/subtbiol.8.1230

Zeppelini D, Taylor SJ, Slay ME (2009) Cave *Pygmarrhopalites* Vargovitsh, 2009 (Collembola, Symphypleona, Arrhopalitidae) in United States. Zootaxa 2204: 1–18.