

Studies on the occurrence and diversity of Heteroderidae and Meloidogynidae (Nematoda: Tylenchida) in natural forests of Vietnam

Chau N. Nguyen¹, Dieter Sturhan² and Sergei A. Subbotin³

¹Department of Nematology, Institute of Ecology and Biological Resources, VAST,
18 Hoang Quoc Viet, Hanoi, Vietnam; e-mail: nchaulinh@yahoo.com

²Arnethstr. 13D, 48159 Münster, Germany; Formerly: Biologische Bundesanstalt
für Land- und Forstwirtschaft, Institut für Nematologie und Wirbeltierkunde, Münster, Germany

³Plant Pest Diagnostic Center, California Department of Food and Agriculture,
3294 Meadowview Road, Sacramento, CA 95832-1448, USA

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Summary. In a survey of the occurrence and distribution of members of the families Heteroderidae and Meloidogynidae in 425 soil samples from 16 natural forests in different regions of Vietnam, Heteroderidae were found in about 40% of all samples and root-knot nematodes (*Meloidogyne*) in about 22.5% of the samples. At least five unidentified *Heterodera* species were distinguished, which can all be ascribed to the *afenestrata* and *bifenestral/cyperi* or *sacchari* species groups. The genus *Cryphodera* proved to be more common and widespread than *Heterodera*; it was observed in ten of the forests studied. Three *Cryphodera* species could be distinguished by molecular data. Differences in morphometrics and other characters indicate the presence of even more different species of this genus in Vietnam, possibly all new to science. Representatives of two obviously undescribed genera were recovered, which could not be attributed to any of the currently known Heteroderidae genera. Based on morphological characters of second-stage juveniles, about six *Meloidogyne* species could be distinguished. The related genus *Meloinema* was found in two of the forests; the species appears to be still undescribed. The various taxa distinguished are briefly characterised and a tentative key for identification is presented. Because of limited material available and mostly only second-stage juveniles were found, the precise identification of the *Meloidogyne* and Heteroderidae species present in forest soils in Vietnam has not been attempted in this first survey.

Key words: *Cryphodera*, *Heterodera*, *Meloidogyne*, *Meloinema*, morphology, ITS analysis, molecular phylogeny, natural forests, plant-parasitic nematodes, Vietnam.

Root-knot nematodes are relatively common in agricultural soils in Vietnam and they are known to cause damage to many crops, such as black pepper, vegetables, tobacco, banana, rice etc. Five *Meloidogyne* species have been recorded for Vietnam: *Meloidogyne incognita* (Kofoid & White, 1919), *M. javanica* (Treub, 1885), *M. arenaria* (Neal, 1889), *M. cynariensis* Pham, 1988 and *M. graminicola* Golden & Birchfield, 1965 (Eroshenko *et al.*, 1985; Pham, 1988; Nguyen, 1993; Nguyen & Nguyen, 1993, 2000). Among these species, *M. incognita* and *M. javanica* are most widely distributed in Vietnam. Nothing is known so far about the occurrence of root-knot nematodes in non-agricultural soils and in natural biotopes.

Cyst nematodes and genera of related non-cyst forming Heteroderidae have not been recorded for

Vietnam (Nguyen, 1993; Nguyen & Nguyen, 1993, 2000), apart from a report of Sharma *et al.* (1994) on the occurrence of *Heterodera* sp. on groundnut in Nghe An province, but data on morphological characters were not provided. A survey by the senior author at localities indicated in the report mentioned above revealed no heteroderids. In addition, recent surveys of cyst nematodes in crops such as soybean, groundnut, tobacco and corn were 'negative' for any members of the family Heteroderidae (N.C. Nguyen, unpubl.), but – surprisingly and unexpectedly – heteroderids were quite commonly found in soil samples collected in natural forests in Vietnam.

Observations of *Meloidogyne* spp., *Heterodera* sp., *Cryphodera* spp. and unidentified heteroderids from natural forest soils in Vietnam as hosts of the bacterial parasites *Pasteuria* spp. have already been

reported (Sturhan & Nguyen, 2005). Detailed results of a survey on the occurrence, distribution and morphology of members of the families Heteroderidae and Meloidogynidae in woodland areas from all over Vietnam are presented in this paper. A remarkable high number of taxa could be differentiated, most of which are briefly morphologically characterised.

MATERIAL AND METHODS

A total of 425 soil samples were collected in 16 natural forests in different parts of Vietnam (Table 1). Most of these samples were taken in the period 1997-2000. The locations of the sampling sites are shown in Fig. 1. At each site at least 3-4 cores per sample were taken around the base of a tree or from around the stem of neighbouring trees of the same species. The soil samples were stored in polyethylene bags and moved to the laboratory for processing. Nematodes were extracted from 250 ml soil samples by a modified Cobb's sieving-decanting technique with final use of filter paper, as described by Nguyen & Nguyen (1993). The coarse particles from the first filtering were checked frequently for the presence of nematode cysts and other immobile nematode stages. In addition, for a limited number of samples containing infective heteroderid juveniles, a Fenwick can was used for recovery of cysts. The nematodes and nematode suspensions obtained were mostly fixed with hot TAF and subsequently processed to glycerin. Selected specimens were mounted on permanent microscopical slides for morphological studies following the procedure of Seinhorst (1959), but some specimens were processed to dehydrated glycerin *via* warm lactophenol (Siddiqi, 1986).

Voucher specimens on permanent microscopical slides are deposited in the German Nematode Collection (DNST) at Julius Kühn-Institut (formerly: Biologische Bundesanstalt) in Münster, Germany, and in the nematode collection at Department of Nematology, Institute of Ecology and Biological Resources, VAST, Hanoi, Vietnam.

From a few samples viable, unfixed nematodes were hand-selected for subsequent molecular studies. Molecular analysis has been made only with *Cryphodera* samples from Cuc Phuong NFP and Cat Ba NFP. DNA was extracted from several juveniles from each sample using proteinase K protocol. Nematode specimens were transferred to an Eppendorf tube containing 16 µl double distilled

water, 2 µl 10X PCR buffer and 2 µl proteinase K (600 µg ml⁻¹) (Promega); specimens were crushed during 3 min with an ultrasonic homogeniser. The tubes were incubated at 65°C (1 h) and then at 95°C (15 min). Detailed protocols for PCR, cloning and sequencing were as described by Tanha Maafi *et al.* (2003). The forward primer TW81 (5'-GTT TCC GTA GGT GAA CCTGC-3') and the reverse primer 5.8SM5 (5'-GGC GCA ATG TGC ATT CGA-3') (Zheng *et al.*, 2000) were used for amplification of the internal transcribed spacer 1 of rRNA gene in PCR. The newly obtained sequences have been submitted to GenBank database under the numbers JF894388-JF894402 as indicated in Fig. 5.

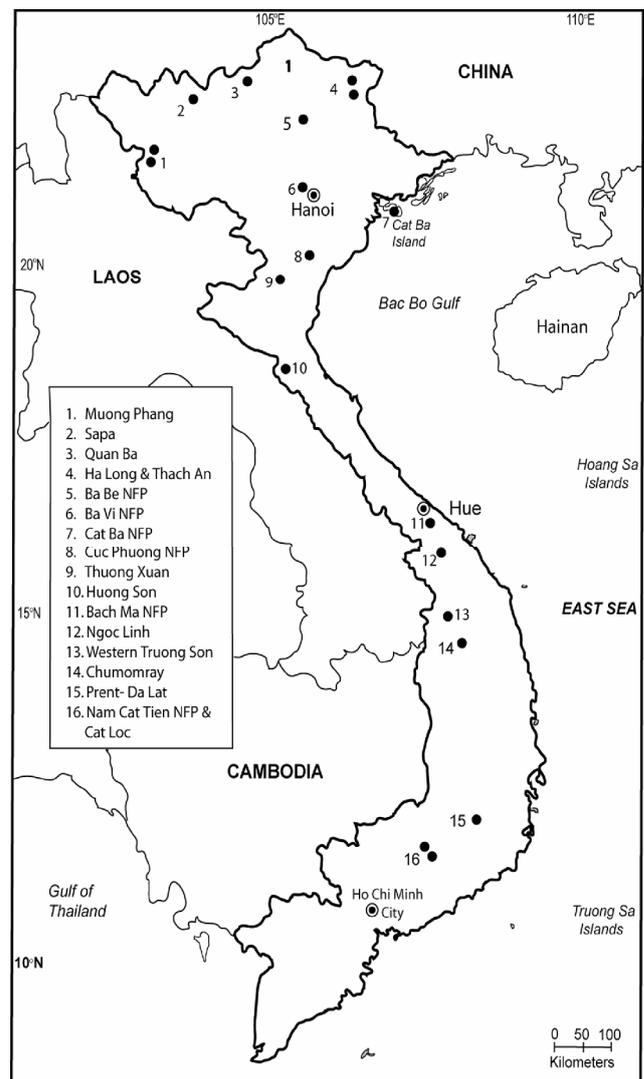


Fig. 1. Map of Vietnam showing distribution of forest sampling sites for heteroderids and meloidogynids.

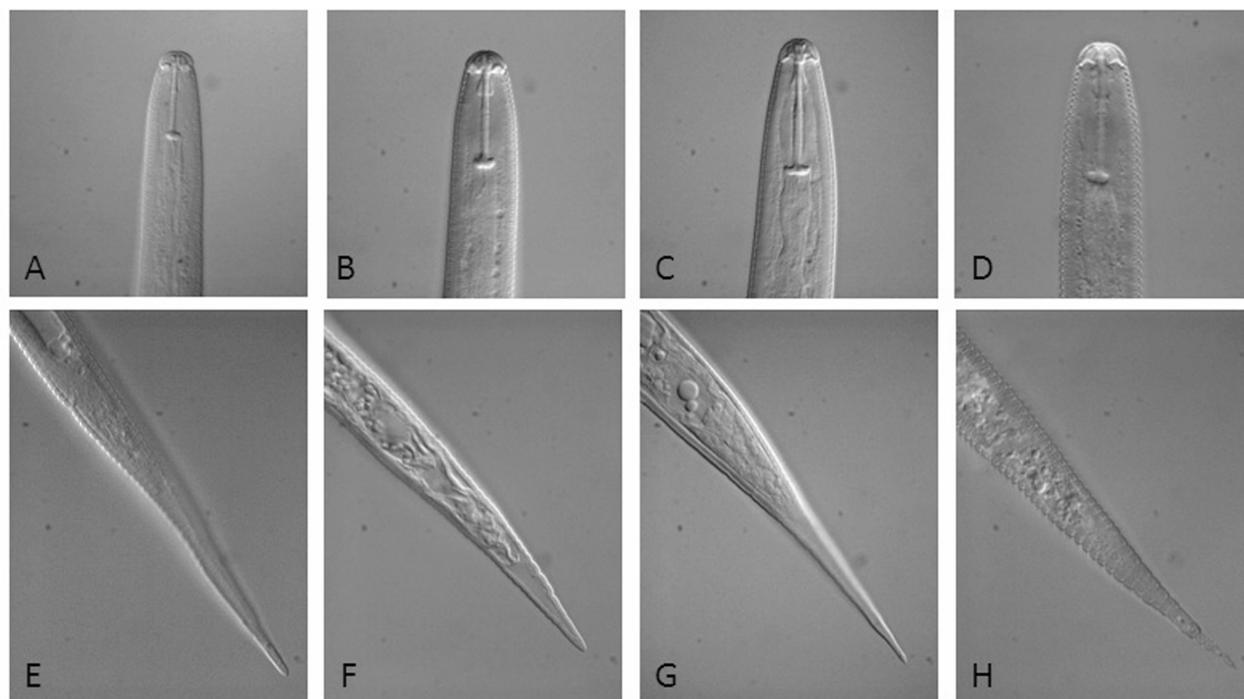


Fig. 2. Anterior ends and tails of *Heterodera* spp. A (A, E), *Heterodera* sp. C (B, F), *Heterodera* sp. D (C, G) and *Heterodera* sp. E (D, H). (Scale bar = 15 μ m).

The ITS1 sequences of *Cryphodera* spp. were aligned using ClustalX 1.83 with default parameters with published ITS sequences of non-cyst nematode species of the family Heteroderidae (Subbotin *et al.*, 2001; Ferris *et al.*, 2004) and two hoplolaimid species, chosen as outgroup taxa. Sequence alignment was analysed with Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). BI analysis under the GTR+I+G model was initiated with a random starting tree and was run with four chains for 1.0×10^6 generations. The Markov chains were sampled at intervals of 100 generations. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analysis to generate a 50% majority rule consensus tree.

RESULTS

Mobile stages of plant-parasitic nematodes, in which mature females and advanced juvenile stages are sedentary, were commonly present in the nematode suspensions. Besides second-stage juveniles (J2) and a few males of root-knot nematodes and heteroderids, juveniles, immature females and males of other sedentary plant-parasitic nematodes, such as *Rotylenchulus* were encountered in three of the samples, and juveniles and males of unidentified Sphaeronematidae (*Sphaeronema*, *Trophotylenchulus* and *Tylenchulus*) were observed in at least 30 samples. Cysts or cyst-like bodies and

swollen females were found in a few samples processed by the second extraction method employed. From this material, which had been kept in dry condition, occasionally juveniles could be released by squashing.

Based on the material obtained by both extraction methods, the genera and species of Heteroderidae and Meloidogynidae detailed below could be differentiated. Because of too little material available and/or presence of certain developmental stages only, species identification has not been attempted. The results of the preliminary survey are detailed below. Each taxon distinguished is characterised in brief and data on their occurrence at the various forest sites studied are given.

HETERODERIDAE

J2 of heteroderids and occasionally also a few males were found in 171 of the 425 soil samples, which is equivalent to 40 % of all samples particularly examined for mobile nematodes from forest soil in Vietnam. Except for Prent forest, Lam Dong province (No. 15), all sampling sites revealed Heteroderidae, often far more than half of all samples, and in Thuong Xuan forest, Thanh Hoa province (No. 9), even all samples taken (Table 1).

Based mainly on juvenile morphological characters at least five *Heterodera* species could be distinguished, including species of the former genus *Afenestrata*, which has recently been synonymised

with *Heterodera* (Mundo-Ocampo *et al.*, 2008). J2 characters indicated that the majority of heteroderid findings could be assigned to the genus *Cryphodera* (54 samples, against 31 samples with *Heterodera*); correctness of *Cryphodera* identification could be confirmed by females isolated from a few soil samples and males found at some of the sites. Morphometrics indicate that at least five *Cryphodera* species might be present, and molecular studies conducted with a few populations only, revealed three distinct *Cryphodera* species. Surprisingly, two different heteroderid forms were found that could not be assigned to any of the presently known genera in the family Heteroderidae. One of these forms, which much resembled *Cryphodera* species in J2 morphology, was detected only by diverging molecular data.

Simultaneous occurrence of two heteroderids in a single sample was observed 21 times and three species co-occurring in a sample was found once, which contributed to difficulties in species differentiation. In particular, the simultaneous occurrence of different *Cryphodera* species may occasionally have been overlooked.

***Heterodera* sp. A (Fig. 2, A, E; Table 2)**

Second-stage juvenile: Body of heat-relaxed specimens straight or slightly curved to the ventral side, in particular the posterior end. Stylet short, knobs rather small (3.5 µm in diam.), rounded with anterior faces mostly inclined. Dorsal gland orifice 4-4.5 µm posterior to stylet knobs. Lip region hemispherical, offset, with two annuli and labial disc, but only posterior annulus wide and distinct. Pharynx well developed, with long gland lobes. Lateral fields with three incisures, forming two irregularly areolated bands. Tail long and slender, terminus narrowly rounded; hyaline tail portion distinctly longer than half total tail length. Phasmid with distinct opening, 5-11 annuli posterior to anus, with weak lens-like extension in the cuticle.

Male body slightly twisted. Lip region high, with two or three annuli in lateral view. Stylet length 17.5-23 µm, basal knobs rounded, 4-5 µm in diam., anterior faces mostly inclined. Lateral fields with three incisures, but inner incisure occasionally divided into two closely arranged lines; irregular areolation present. Spicules slender, 32-42 µm long, distal ends pointed. Gubernaculum 10-14 µm long. Tail almost absent, distinct cloacal tubus present, phasmids lacking.

From a young, light-brown cyst or female with elevated vulva region, long vulval slit and no fenestrae and bullae developed, two poorly preserved J2 were isolated, which closely agreed in morphology with the J2 described above. The soil sample had been collected from around an unidentified timber

tree from Truong Son mountain forest (No. 13).

Heterodera sp. A was isolated from rhizosphere soil samples of bamboo (*Bambusa procera*) in Nam Cat Tien NFP and adjacent Cat Loc forest (No. 16), from three sites in Thuong Xuan forest (No. 9); soil samples from bamboo and the timber trees *Castanopsis* sp. and *Photinia* sp. and from a soil sample from cane brake (Bambuseae) in Muong Phang forest (No. 1).

Juvenile measurements of the two populations *Heterodera* sp. A (1) and *Heterodera* sp. A (2) given in Table 2 show differences in measurements, in particular, in tail length, which may indicate the presence of two different species, but other distinguishing morphological characters were not observed. A population from Nam Cat Tien NFP (no measurements given) showed some differences in certain morphological details from both other populations studied and may eventually represent a third species within a complex of three close species with extremely short J2 stylet present in Vietnam. This species complex occurred more or less widely from the north to the south of the country. *Heterodera* sp. A probably belong to the *afenestrata* group of *Heterodera*. From the two species in this group described from bamboo, *H. bamboosi* (Kaushal & Swarup, 1988) and *H. koreana* (Vovlas, Lamberti & Choo, 1992), *Heterodera* spp. A differ from the first species in shorter body and stylet, from the second species in presence of males, which were found in all three populations studied by us in more detail.

***Heterodera* sp. B (Tables 2, 3)**

A few cysts were isolated from two samples collected from the rhizosphere of bamboo (*Dendrocalamus hamiltonii*) at two sites in Ba Be NFP (No. 5). The cysts were very small, lemon-shaped with slightly projecting vulva, dark to reddish-brown in colour and the cyst wall pattern was rugose. Fenestrae and bullae were lacking. within eggs and a single J2 released from eggs by squashing had a 20-23 µm long stylet, 3-4 distinct lip annuli plus labial disc, lateral field with three well developed lateral lines and areolation, a slender conical tail (50 µm long, H = 20-25 µm) with pointed terminus and small phasmids with slightly lens-like swelling, located 12 annuli posterior to the anus. This species is also considered to be a member of the *afenestrata* species group.

***Heterodera* sp. C (Fig. 2, B, F; Table 2)**

In a soil sample collected around a flowering plant (*Dichroa* sp., Hydrangeaceae family) in Huong Son forest (No. 10), a number of cysts were found partially filled with eggs, from which J2 could be released. The

Table 1. Heteroderidae and Meloidogynidae (excl. *Meloinema*) isolated from soil samples from natural forests in Vietnam.

No.	Forest (Province)	Total number of samples	Samples positive for	
			Heteroderidae	<i>Meloidogyne</i>
1	Muong Phang (Dien Bien)	88	34	17
2	Sapa (Lao Cai)	19	4	3
3	Quan Ba (Ha Giang)	15	3	3
4	Ha Long & Thach An (Cao Bang)	6	3	3
5	Ba Be NFP (Bac Kan)	63	25	27
6	Ba Vi NFP (Hanoi)	8	5	3
7	Cat Ba NFP (Hai Phong)	6	5	1
8	Cuc Phuong NFP (Ninh Binh)	80	45	19
9	Thuong Xuan (Thanh Hoa)	3	3	2
10	Huong Son (Ha Tinh)	28	7	4
11	Bach Ma NFP (Thua Thien-Hue)	12	2	1
12	Ngoc Linh (Quang Nam)	8	6	1
13	Western Truong Son (Kon Tum)	22	5	1
14	Chumomray (Kon Tum)	32	10	8
15	Prent-Da Lat (Lam Dong)	6	0	2
16	Nam Cat Tien NFP & Cat Loc (Dong Nai)	29	14	1
Total		425	171	96

cysts were lemon-shaped with slightly projecting vulval cone and laterally directed neck region, light brown, with zig-zag cuticular pattern, vulval cone ambifenestrate, almost hemispherical semifenestrae, vulva slit longer than width of fenestra, fragile vulva bridge, high underbridge present, bullae absent. J2 had a short body, an offset, rounded lip region, with posterior annulus distinct and two indistinct anterior annuli, a stylet base 5 μm wide with anterior face of knobs flat to slightly concave, lateral fields with three distinct incisures and irregular areolation, a straight conical tail with finely rounded terminus, punctiform phasmids situated 8-9 annuli posterior to the anus. J2 isolated from a soil sample from the rhizosphere of an unidentified Vitaceae species in Ba Vi NFP (No. 6) were similar in morphology and measurements (Table 2).

Heteroderids from Ba Be NFP (No. 5) may belong to the same species. Among cysts of *Heterodera* sp. B in a sample from around the bamboo, *Dendrocalamus hamiltonii* (see above), one small lemon-shaped light-brown cyst was present with ambifenestrate vulval cone, weak vulva bridge, distinct underbridge and a few small bullae. Juveniles still within eggs had a 21-25 μm long stylet, three lip annules, three incisures in the lateral field and a conical tail with a more or less offset terminal mucron (no measurements given in Table 2).

Heterodera sp. D (Fig. 2, C, G; Table 2)

Second-stage juveniles. Body of heat-relaxed specimens slightly curved, lip region weakly offset, with four to five annuli plus labial disc. Stylet with flat and anterior distinct concave knobs, stylet base 5.5-6 μm in dia. Median bulb and pharyngeal glands well developed. Lateral field with three distinct incisures, irregularly areolated. Tail slender with pointed terminus, which is mostly bearing a small offset mucron. Phasmids distinct, but without lens-like structure, 5-8 annuli posterior to the anus.

Males. Stylet 30-32 μm long, knobs rounded, laterally directed. Lip region with 3-4 annuli. Lateral fields with three incisures. Spicules about 22 μm long, stout, gubernaculum 5 μm long, cloacal tubus absent.

Cysts. From a *Heterodera* cyst isolated from a soil sample from around an unidentified timber tree in Ba Be NFP (No. 5), a single poorly preserved juvenile was released with morphological characters as described above. The brown cyst was lemon-shaped, the cyst wall pattern rugose, the type of fenestration could not be ascertained; bullae were absent. Unhatched J2 within eggs, isolated from a single lemon-shaped brown cyst with more or less round fenestra (vulval bridge lost?) and with remnants of an underbridge also closely agreed in morphology with

Table 2. Morphometrics of second-stage juveniles of heteroderid species and populations and of *Meloinema* sp. from forests in Vietnam (all measurements in μm , mostly $n = 10$).

Species/population	Forest (site number)	Body length	Stylet length	Tail length	Hyaline tail length	Phasmid to tail terminus
<i>Heterodera</i> sp. A (1)	Nam Cat Tien NFP (16)	405 (373-427)	16.7 (16.2-17.6)	54 (49-61)	35 (28.5-40)	47 (42-51)
<i>Heterodera</i> sp. A (2)	Thuong Xuan (9)	420 (396-434)	17.1 (16.5-17.7)	68 (65-74)	42 (38-45)	57 (55-62)
<i>Heterodera</i> sp. B	Ba Be NFP (5)	-	20-23	50	20-25	-
<i>Heterodera</i> sp. C (1)	Huong Son (10)	335 (320-380)	24 (23-25)	47 (45-51)	24 (22-26)	32 (25-36)
<i>Heterodera</i> sp. C (2)	Ba Vi NFP (6)	364 (350-381)	24 (23.5-25)	51 (49-54)	27 (25-30)	40 (36-43)
<i>Heterodera</i> sp. D	Quan Ba (3)	447(427-470)	27.4 (26-29)	60(53-63.5)	38 (33-44)	50 (44-55)
<i>Heterodera</i> sp. E	Ngoc Linh (12)	551 (540-559)	27.3 (27-28.4)	62 (66.5-70.5)	44 (43-45)	56 (52-60)
<i>Cryphodera</i> pop. 1	Thach An (4)	414 (369-458)	28.2 (26-30.5)	50 (45-53)	27 (23.5-31)	44 (40-49)
<i>Cryphodera</i> sp. A	Cuc Phuong NFP (8)	423 (385-470)	28.2 (26-29)	53.5 (49-56)	29 (26-31)	49.5 (44-59)
<i>Cryphodera</i> sp. C	Cat Ba NFP (7)	437 (395-480)	29.5 (28-31)	52 (47.5-60)	29.5 (25-32.5)	46 (42.5-51)
<i>Cryphodera</i> pop. 2	Nam Cat Tien NFP (16)	445 (424-474)	27.3 (26-29)	52 (44-59)	26 (20-30)	47 (39-54)
<i>Cryphodera</i> sp. B	Cuc Phuong NFP (8)	453 (430-500)	28.6 (27-30)	54 (49-61)	29.5 (25-34)	47 (43-53)
<i>Cryphodera</i> pop. 3	Thach An (4)	490 (438-539)	29.8 (28-32)	57 (51-68)	31 (26-36)	51 (45-59)
<i>Cryphodera</i> pop. 4	Cuc Phuong NFP (8)	504 (470-575)	30.2 (29-32)	56 (52-72.5)	31.5 (27.5-45.5)	50 (45-62.6)
<i>Cryphodera</i> pop. 5	Muong Phang (1)	579 (505-644)	31.1 (29.5-32.3)	63 (54-71)	35 (27-40)	57 (48-65)
Genus indet. A	Ba Vi NFP (6)	464 (425-495)	36 (35-37.5)	66 (59-76)	39 (34-42.5)	55 (46.5-64)
Genus indet. B	Ngoc Linh (12)	638 (608-665)	26.7 (26-28)	83 (77-86)	57 (54-59)	76 (73-78)
<i>Meloinema</i> sp.	Ba Be NFP (5) + Chumomray (14)	453 (430-475)	25 (24.5-26.5)	64 (58-69)	41 (35-47)	52 (46-58)

those of the J2 described in detail above. Eggs from this cyst were covered by a thick granular layer. The cyst was collected from around a timber tree (*Dillenia* sp.) in Cuc Phuong NFP (No. 8).

Besides the places mentioned above, *Heterodera* sp. D was identified at several sites in Muong Phang forest (No. 1), Quan Ba forest (No. 3), Cat Ba NFP (No. 7) and in Ngoc Linh mountain forest (No. 12). Most of the samples were taken from around various monocotyledon plants (cane brake, bamboo and wild banana).

***Heterodera* sp. E (Fig. 2, D, H; Table 2)**

Second-stage juveniles with long body; offset lip region with three (exceptionally four) distinct annuli plus wide labial disc; stylet with rather thick knobs, rounded and anterior face flattened; stylet base 5.0-5.5 μm in dia. Nucleus and nucleolus of dorsal pharyngeal gland large. Lateral field with three lines and irregular areolation. Tail coarsely annulated with slender and pointed terminus,

without offset mucron. Phasmids small, 7-9 annuli posterior to the anus. *Males* were not found.

This species, which is also considered as a member of the above mentioned species groups, was found at one locality only: Ngoc Linh mountain forest (No. 12), around roots of *Melastoma* sp., Melastomataceae family in the order Myrtales.

***Cryphodera* spp. (Fig. 3; Table 2)**

Nematodes identified as members of the genus *Cryphodera* were found in 11 of the 16 forests studied, but not in forest numbers 2, 5, 11, 12 and 14 listed in Table 1. J2 and males of different origin were very similar in their general morphological characters, but morphometrics showed some variation among different populations (Table 2), which appears to indicate the presence of different species.

Morphological characters of *Cryphodera* spp. were:

Second-stage juveniles. Body of heat-relaxed specimens ranging from slightly to strongly curved to

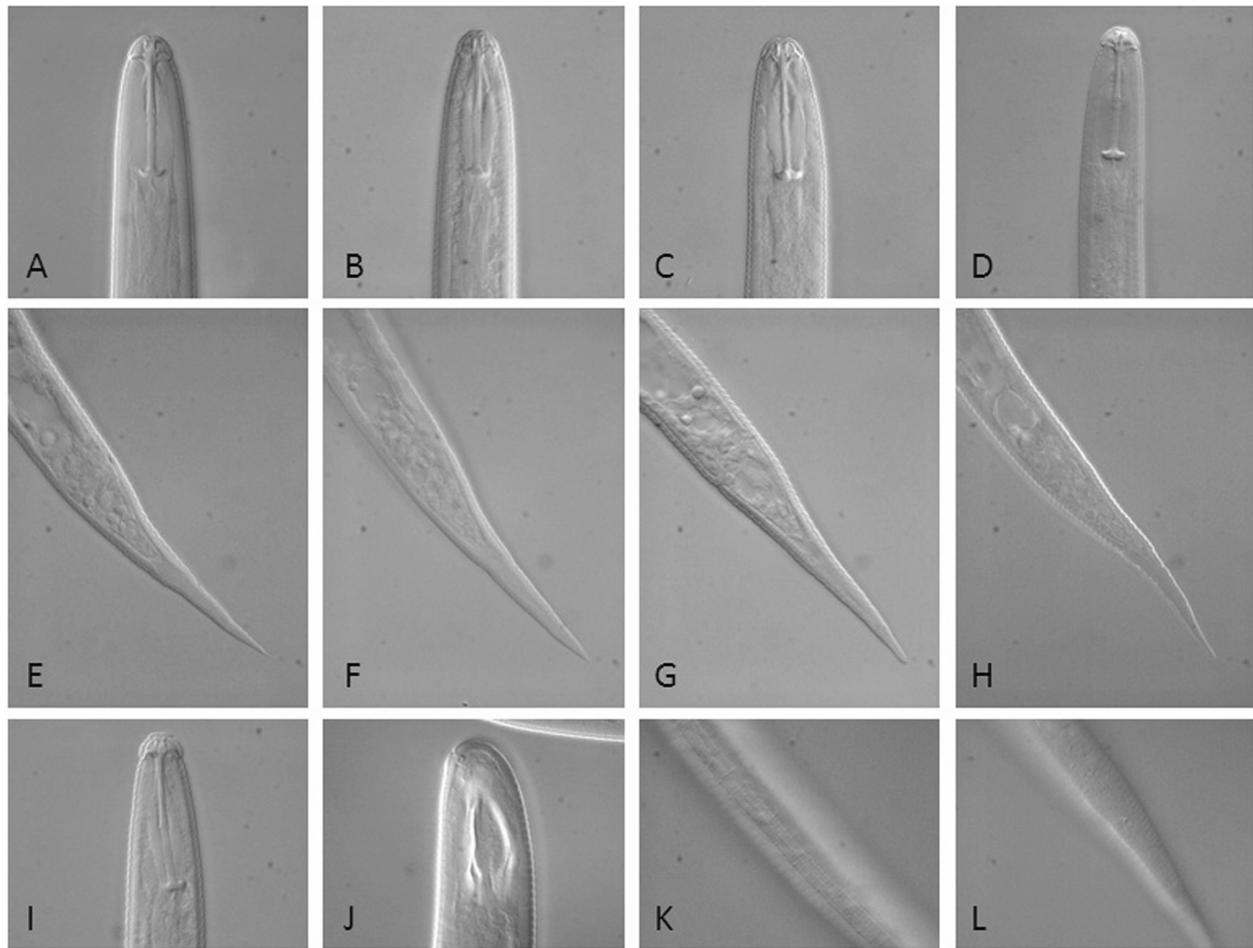


Fig.3. Photomicrographs of *Cryphodera* sp. A (A: J2 anterior end, E: J2 tail, I: male anterior end, J: male posterior end showing phasmid in lateral position), *Cryphodera* sp. B (B: J2 anterior end, F: J2 tail), *Cryphodera* sp. C (C: J2 anterior end, G: J2 tail, K: J2 lateral field), *Cryphodera* pop. 2 (D: J2 anterior end, H: J2 tail, L: J2 tail region showing large phasmid). (Scale bar = 15 μ m).

the ventral side. Stylet long, with wide and flat, anteriorly directed knobs, stylet base 5-7 μ m in diam. (Fig. 3, A-D). Lip region slightly offset, with (mostly) three distinct annuli plus weakly offset labial disc, vestibule extension distinct; basal plate of head skeleton with high arches. Median bulb well-developed; pharyngeal glands filling body cavity, rather short (extending 2-3 corresponding body widths posterior to the pharyngo-intestinal junction, which is generally indistinct); subventral gland lobes bluntly rounded distally. Lateral fields with three incisures and areolation (Fig. 3, K). Tail conoid, with slender posterior part and finely blunt or pointed terminus (Fig. 3, E-H). Phasmid opening large with lens-like extension in cuticle, situated 2-6 annuli posterior to the anus (Fig. 3, L).

Males. Body turned around its axis, particularly the posterior part. Lip region distinctly offset, with prominent labial disc, narrow first lip annulus and considerably wider posterior annulus, which is

longitudinally indented (Fig. 3, I). Stylet 26-33 μ m long, with rounded, laterally directed or slightly indented knobs; stylet base 5 μ m in diam. Excretory pore 3-4 annuli behind hemizonid. Lateral field with three indistinct incisures, but often only a single incisure visible. Spicules 25-28 μ m long, with blunt tip, gubernaculum straight. Tail very short and hemispherical, short cloacal tubus occasionally present, phasmids more or less distinct, with lens-like extension (Fig. 3, G).

Molecular data with specimens of very few populations allowed distinction of three separate species, which are tentatively named here *Cryphodera* sp. A, *Cryphodera* sp. B and *Cryphodera* sp. C. Sequence alignment of the ITS1 rRNA gene used in the study contained 21 sequences and was 774 positions in length. Sixteen sequences of *Cryphodera* were included in the alignment and fifteen of them were newly obtained in this study. The ITS1 sequences clearly distinguish *Cryphodera*

species A, B, and C from each other and from *C. brinkmani*. The BI majority consensus tree is given in Fig. 5. Four distinct clades representing four *Cryphodera* species are presented in a phylogenetic tree. Relationships between these taxa are not well resolved. Sequence analysis revealed that interspecific variation within *Cryphodera* species was 19.7 % (123 nucleotides) and intraspecific variations for *Cryphodera* sp. A was 2.5 % (16), *Cryphodera* sp. B, 0.7 % (5) and *Cryphodera* sp. C, 0.7 % (5).

The three species distinguished by molecular analysis showed only slight differences in morphometrics, and only other minor distinguishing morphological characters were found. The additional five populations listed in Table 2 as *Cryphodera* pop. 1, *Cryphodera* pop. 2, *Cryphodera* pop. 3, *Cryphodera* pop. 4 and *Cryphodera* pop. 5 mainly differ in certain measurements (Table 2).

Cryphodera sp. A (Fig. 3, A, E, I, J; Table 2): J2 with a sharply pointed or finely rounded tail terminus mostly carrying an offset mucron; body mostly C-shaped; stylet knobs in general flat cup-shaped. Lateral fields with three incisures and partially areolated. Phasmids 3-5 annuli posterior to the anus. Males present, with characters mentioned above; three lateral incisures mostly indistinct; stylet length 26-32 μm , stylet knobs roundish and slightly flattened anterior, 4.5-5 μm in diam. and 2 μm high; spicules 24-28 μm long, gubernaculum 7-7.5 μm long. Specimens identified as sp. A by molecular methods were found around wild bananas at two sites in Cuc Phuong NFP (No. 8), at one of the sites co-occurring with *Cryphodera* sp. B. J2 with similar morphological characteristics were found in 15 additional samples in Cuc Phuong NFP, from around various trees, *Fuchsia* sp., *Sapindus* sp., *Polypodium vulgare*, a palm tree and several times again around wild bananas. Males were occasionally found. Juvenile specimens from a sample from Cat Ba NFP (No. 7) and four samples from the Thach An limestone forest (No. 4) were similar in morphological characters and in measurements (see *Cryphodera* pop. 1 in Tab. 2).

Cryphodera sp. B (Fig. 3, B, F; Tab.2): J2 only slightly longer than those of *Cryphodera* sp. A. Heat-relaxed specimens bow-shaped with uniformly conoid tail with slender posterior end and finely rounded terminus. Lateral fields more or less uniformly areolated. Phasmids situated 4-6 annuli posterior to the anus. The species was found in three samples from Cuc Phuong NFP (No. 8) where it co-occurred at one site with *Cryphodera* sp. A. Males were not found.

Cryphodera sp. C (Fig. 3, C, G, K; Tab. 2): J2 with ventrally bent body, straight or slightly bent tail

with blunt, finely rounded and not offset terminus. Lateral fields with three incisures and irregular areolation. Phasmids situated four annuli posterior to the anus. Measurements are close to those of *Cryphodera* sp. A and *Cryphodera* sp. B. A single male was found showing the general characteristics mentioned above; stylet length 32 μm , stylet knobs laterally directed with weakly concave anterior surfaces. The species was found in a single sample collected in Cat Ba NFP (No. 7).

Cryphodera pop. 1 (Table 2) from Thach An limestone forest (No. 4) may be identical with *Cryphodera* sp. A (see above).

Cryphodera pop. 2 (Fig. 3, D, H, L; Table 2) was found in several soil samples collected in Nam Cat Tien forest (No. 16) from around various trees, bamboo and *Zingiber* sp. The J2 are medium-sized with the shortest stylets among the *Cryphodera* species and populations measured. The body of heat-relaxed specimens is mostly weakly bow-shaped, the lip region is slightly demarcated, the 3-4 lip annuli are often indistinct (in contrast to the well demarcated annuli in the three species mentioned above), but the labial disc is mostly well separated. The stylet knobs are rounded with anterior faces flat or slightly concave, 5 μm in diam. The three lateral incisures are mostly indistinct. The tail is conoid with slender hyaline portion and finely rounded terminus; the large phasmids are situated 2-3 annuli posterior to the anus. Males could not be isolated from any of the samples.

Cryphodera pop. 3 (Table 2) was isolated from two samples from around *Ficus glomerata* in Thach An limestone forest (No. 4). The tail terminus of J2 is slender and mostly finely pointed. The stylet base measured 6-7.5 μm in diam.

Cryphodera pop. 4 (Table 2) from a soil sample around an unidentified tree had J2 with the longest body and stylet among the populations found in Cuc Phuong NFP (No. 8).

Cryphodera pop. 5 (Table 2) with the longest body and stylet of all *Cryphodera* J2 studied from the Vietnam forests was found in two samples from canebrake and an unidentified tree from Muong Phang forest (No. 1). Heat-relaxed specimens are almost straight; the conoid tail with slender hyaline portion has a bluntly rounded terminus.

A number of other *Cryphodera* populations found at various sampling sites could not be assigned to the species and populations mentioned above, among others, J2 specimens from Cuc Phuong NFP with a stylet length of 34 μm , 4(5) lip annuli and remarkably large pharyngeal gland nuclei.

Genus indet. A (Fig. 4, A, E, I; Tab.2)

Second-stage juveniles. Heat-relaxed specimens

mostly straight or slightly curved to ventral side. Stylet very long, with well separate knobs with anterior faces cupped, measuring 6.5-7.0 x 2.5 μm ; dorsal gland orifice 4-5 μm behind stylet knobs. Lip region rounded, slightly offset, with 5-6 rather indistinct labial annuli. Pharyngeal glands filling body cavity. Lateral fields with four equally spaced incisures, starting anterior to the median bulb and the inner two amalgamating at anus level. Tail uniformly conical with pointed to finely rounded terminus. Phasmids situated 7-10 annuli posterior to the anus, with large lens-like extension in cuticle. Males and females were not found.

According to the morphological characters of the J2 this species cannot be allocated to any of the known genera in Heteroderidae.

The unidentified heteroderid genus was isolated from a soil sample collected in Ba Vi NFP (No. 6) from the rhizosphere of an unidentified Fagaceae tree growing beside the way to Den Thuong, and from soil taken around wild banana in Ba Be NFP (No. 5).

Genus indet. B (Fig. 4, B, F, J; Table 2)

Second-stage juveniles. Body long and slender, heat-relaxed specimens almost straight. Stylet with rounded knobs, anteriorly flattened and measuring 4.8-5.5 μm in diam.; dorsal gland orifice 8-9 μm posterior to the stylet base. Lip region continuous with body contour, with 5 annuli plus labial disc. Pharyngeal glands long, dorsal gland generally distinct, narrow; subventral glands mostly indistinct, filling body cavity. Lateral field with three incisures, areolated. Tail long and slender, hyaline part around two thirds of total tail length, terminus pointed. Phasmids large, with lens-like structure, situated 3-4 annuli posterior to the anus.

Male. Body only slightly twisted. Stylet 26 μm long, knobs small, 4 μm in diam. Lip region high, rounded, not offset, with six annuli plus labial disc. Lateral field areolated, with four indistinct incisures. Testis well developed. Spicules slender, 40 μm long, with bifid tips; gubernaculum 12 μm long. Long cloacal tubus present. Phasmids not observed.

The continuous, not demarcated lip region is a peculiar character distinguishing this heteroderid from most of the genera in Heteroderidae. Additional 'essential' characters are the presence of large phasmids and three lateral incisures in the J2, long spicules, obvious absence of phasmids and only slightly twisted body in the males. The species cannot be placed in any of the presently recognised genera of Heteroderidae and may be a representative of a separate, still undescribed heteroderid genus.

The species was isolated from three soil samples collected from around bamboo, *Melastoma* sp. and

dead timber trees in Ngoc Linh mountain forest (No. 12).

MELOIDOGYNIDAE

Second-stage juveniles of *Meloidogyne* were observed in 96 of the total 425 soil samples checked for the presence of root-knot nematodes and heteroderids, which is nearly one fifth of all samples. Differences in frequency of occurrence between the sampling sites were observed (Table 1). The population density was low in the majority of positive samples, but occasionally several hundred juveniles per 250 ml sample were encountered. By the usual method employed only J2 were recovered, but occasionally also a few males were found. From soil samples taken in two forest parks nematodes were isolated and were identified as members of the genus *Meloinema*, a genus mostly grouped in the Meloidogynidae subfamily Nacoboderinae.

Meloidogyne spp.

Based on morphological characters of the J2, at least six different species of root-knot nematodes could be distinguished, which differed mainly in their body habitus ranging from straight to C-shaped, appearance of the lip region, shape and size of the stylet knobs, lateral field (4 or 6 incisures), appearance of rectum (swollen vs not swollen), and length and shape of the tail. Among these *Meloidogyne* species, several were morphologically distinctly different from the five species already reported for Vietnam. In particular, specimens from the rhizosphere of bamboo and timber tree (*Castanopsis* sp.) from Thuong Xuan forest (No. 9) were unusual by their subcylindrical tail shape (Fig. 4, D, H, L). J2 from around the timber tree, *Dracontomelum* sp., at Thach An (No. 4) showed six distinct lines in their lateral fields.

Because females were in general not available by the extraction method employed, no attempt was made to make species identification. The only females isolated from a timber tree roots at Chumomray natural forest (No. 14), did not show unique morphological characters, which might have allowed species identification. In at least one soil sample two *Meloidogyne* species occurred simultaneously.

Meloinema sp. (Fig. 4, C, G, K; Tab. 2)

Among cysts collected from soil samples, which had been particularly extracted for recovery of immobile swollen nematodes, a few not well-preserved cyst-like females were found showing the following main characteristics: more or less globular

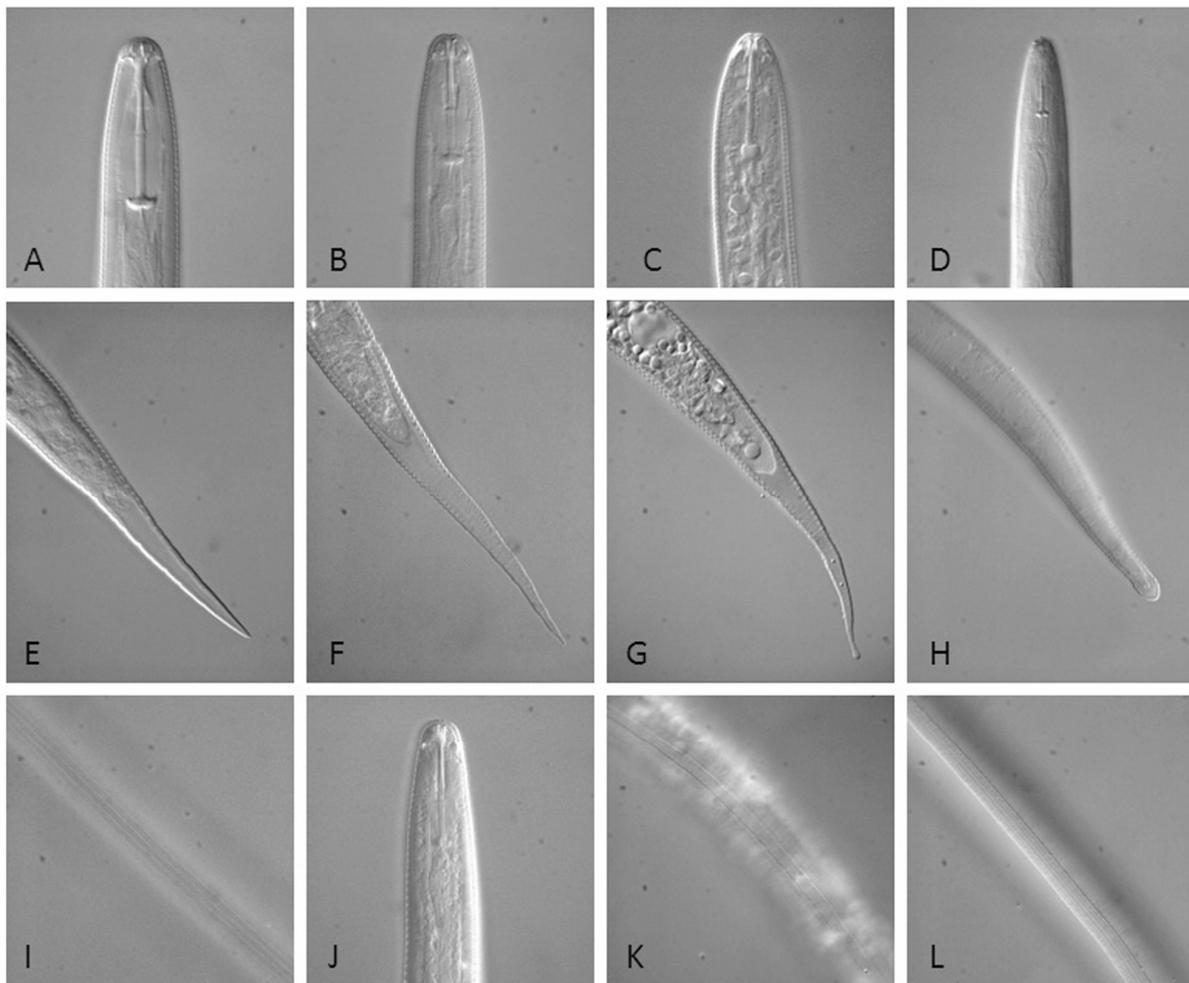


Fig. 4. Photomicrographs of the unidentified heteroderid genus A (A: J2 anterior end, E: J2 tail, I: J2 lateral field in mid-body region), the unidentified heteroderid genus B (B: J2 anterior end, F: J2 tail, J: male anterior end), *Meloinema* sp. (C: J2 anterior end, G: J2 tail, K: J2 lateral field in mid-body region), *Meloidogyne* sp. from Thuong Xuan forest (D: J2 anterior end, H: J2 tail, L: J2 lateral field in mid-body region). (Scale bar = 15 μ m.).

shape, with projecting neck and tail region, light brown in colour and filled with eggs containing well developed J2, vulva in subterminal position, cuticle finely annulated and very distinct lens-like phasmids present, no bullae and underbridge.

J2 released from eggs resembled heteroderid juveniles: Head skeleton heavily sclerotised, lip region almost continuous, conical and anterior slightly flattened, with 5-6 fine annuli, stylet strong, with thick rounded knobs, lateral field with four incisures, but marginal lines only weakly developed, inner lines widely spaced. Tail long and slender with weakly clavate terminus and distinctly demarcated hyaline portion; phasmids distinct but punctiform, 8-9 annules posterior to the anus.

Morphological characters of the females and J2 suggest that the species appears to belong to the subfamily Nacoboderinae in Meloidogynidae. It is tentatively considered as a representative of the

genus *Meloinema* but may eventually be placed in the related genus *Bursadera*. Males, which would allow correct placement in one of the two Nacoboderinae genera known, have not been available. The species found is not identical with any of the five Nacoboderinae species described so far.

The new species was found in three samples from Ba Be NFP (No. 5) around wild bananas, unidentified bushes and timber trees and in one sample from an unidentified timber tree in Chumomray forest (No. 14).

DISCUSSION

Meloidogyne and heteroderid species have occasionally been reported from forests from all over the world, and several species of the families Meloidogynidae and Heteroderidae, which have been described from woodland, have trees as hosts

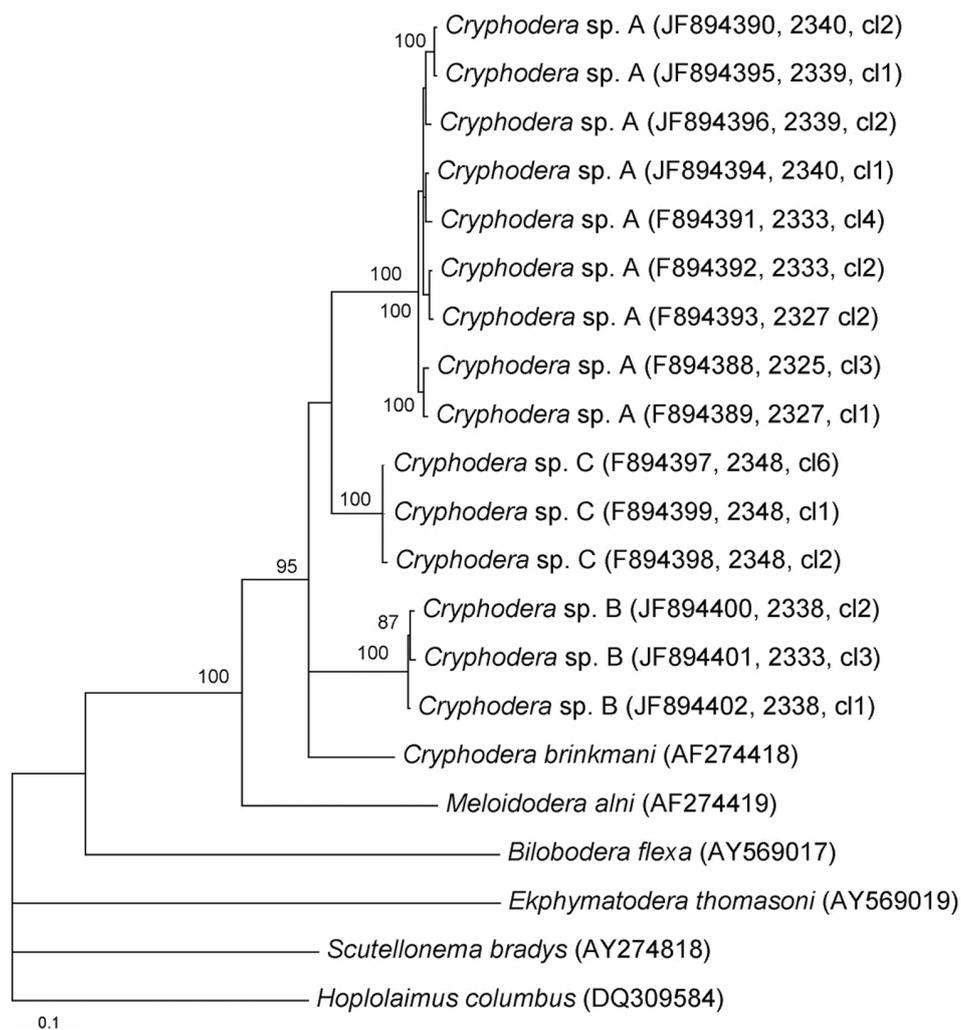


Fig. 5. Phylogenetic relationships within *Cryphodera* species and related genera: Bayesian 50 % majority rule consensus tree from two runs as inferred from analysis of ITS1 rRNA gene sequence alignment under the GTR+I+G model. Posterior probabilities equal or more than 70 % are given for appropriate clades (*Cryphodera* samples 2325-2340 originated from Cuc Phuong NFP, sample 2348 from Cat Ba NFP).

and have not been found in other biotopes. A particular survey for occurrence of root-knot nematodes and of cyst-forming and non-cyst forming heteroderids in natural forests, however, has obviously not been conducted so far. Thus, the present study appears to be a first contribution on the occurrence, distribution and diversity of these nematodes in primary and secondary forests in the subtropical-tropical region. Moreover, only a low number of members of the family Heteroderidae have been reported so far from South-East Asia.

The common occurrence of *Meloidogyne* in forests in Vietnam (22.5% of all samples studied) and the presence of at least six species is remarkable but not surprising for a tropical-subtropical region.

A root-knot nematode species with a subcylindrical tail appears to be unique for the genus. However, the presence of a nematode species, which is tentatively considered by us as a member of the genus *Meloinema*, is of particular interest. The four species of this genus described so far were found in Korea, Eastern Siberia, South Africa and the USA, and the related genus *Bursaderea* with the only species *B. longicollum* Ivanova & Krall, 1985, was described from Kirghizia.

The common and widespread occurrence in woodland in Vietnam of the genus *Cryphodera* is particularly remarkable, with the presence of several obviously still undescribed species of these non-cyst-forming nematodes. Three of the currently known

Table 3. Tentative polytomic key for identification of heteroderid taxa and *Meloinema* sp. found in Vietnam forests, mainly based on second-stage juvenile characters (all measurements in μm)

Lateral incisures	Phasmids	Stylet length	Body length	Taillength	Hyaline tail length	Lip annuli	Other characters	Nematode genera/species
4	small	24.5–26.5	430–475	58–69	35–47	5–6	♀ globular, light brown, cuticle annulated, phasmids distinct	<i>Meloinema</i> sp.
3	medium	16–18	373–434	49–74	29–45	2	Cysts without fenestrae and bullae, ♂ with cloacal tubus	<i>Heterodera</i> spp. A
3	medium	20–23	?	50	20–25	3–4	Cysts without fenestrae and bullae	<i>Heterodera</i> sp. B
3	small	23–25	320–381	45–54	22–30	3	Cysts ambifenestrate, underbridge present, bullae absent or few	<i>Heterodera</i> sp. C
3	small	26–29	427–470	53–64	33–44	4–5	Cysts lemon-shaped, J2 tail mostly with terminal mucro	<i>Heterodera</i> sp. D
3	small	27–28.5	540–559	66.5–70.5	43–45	3(–4)	Nucleolus of J2 dorsal pharyngeal gland large	<i>Heterodera</i> sp. E
3	large	26–32	369–644	44–72.5	20–45.5	3	Basal plate of head skeleton in J2 with high arches, ♂ with phasmids	<i>Cryphodera</i> spp.
3	large	26–28	608–665	77–86	54–59	5	Lip region in J2 and ♂ not offset, J2 almost straight	Genus indet. B
4	large	35–37.5	425–495	59–76	34–42.5	5–6	J2 mostly straight, phasmids 7–10 annuli posterior to anus	Genus indet. A

six species of the genus have been described from New Zealand and one species each from Australia, India and Japan. There is no indication that the species and populations distinguished in Vietnam may be representatives of any of these species. The presence of a particular *Cryphodera* form (*e.g.*, *Cryphodera* sp. A in Cuc Phuong NFP) at several sites and in many samples from a single forest with its extremely high diversity of trees, shrubs and other plants, may indicate a wider host range, which is in general not common among heteroderid species.

Cyst-forming *Heterodera* species were less common in Vietnam forest soils than representatives of the genus *Cryphodera*. Only species, which could be attributed to the *afenestrata* and *bifenestra/cyperii* or *sacchari* species groups, were found, all with three incisures in the lateral fields of the J2. All currently known *Heterodera* species sharing this character are specialised to monocotyledonous hosts, mostly grasses and sedges but including also banana and members of other related families. Most of the soil samples containing *Heterodera* spp. were taken from around monocotyledonous plants, but at

majority of sampling sites the root systems of many different plants were intermixed.

The finding of two heteroderid species of two different genera, which cannot be placed in any of the presently recognised 18 genera of Heteroderidae, is of particular interest. It is assumed that these two forms are also representatives of non-cyst-forming taxa within the family.

The most essential distinguishing morphological characters of the heteroderid taxa and of *Meloinema* sp. found in the Vietnam forests are summarised in the tentative polytomic key presented in Table 3.

Overall, the preliminary results obtained through this first survey revealed a quite unexpected high diversity of Heteroderidae and Meloidogyndae species present in the forests of Vietnam. It is assumed that the subtropical and tropical woodland biotopes of South-East Asia, which remained rather stable over long geological periods and are characterised by a high number and diversity of ancient plant families, represent important areas of development and preservation of heteroderids and other nematodes.

Detailed studies with collection of more nematode material, including the adult stages, are required to enable precise identification of the species present and eventually describe species and genera still unknown to science. Future morphological studies should be supported by molecular characterisation of the species and populations found. Identification and knowledge of the plant hosts of each species found are also essential, because the majority of the taxa in question are assumed to be more or less host specific.

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Chau N. Nguyen, Dieter Sturhan, Sergei A. Subbotin. Исследования по разнообразию и встречаемости Heteroderidae и Meloidogynidae (Nematoda: Tylenchida) в лесах Вьетнама.

Резюме. В рамках обследования лесов Вьетнама с целью выявления нематод сем. Heteroderidae и Meloidogynidae было собрано и обработано 425 образцов почвы из 16 лесов и национальных парков Вьетнама. Heteroderidae были обнаружены в приблизительно 40% всех образцов а галлообразующие нематоды (*Meloidogyne*) – в 22.5% образцов. По крайней мере, были обнаружены пять неопределенных пока видов *Heterodera*. Все они принадлежат к группам видов *afenestrata*, *bifenestra/cyperii* или *sacchari*. Как показало обследование, 10 из 14 лесов род *Cryphodera* оказывается более обычным и широко распространенным, чем *Heterodera*. Три вида *Cryphodera* могут быть дифференцированы по молекулярным данным. Морфометрические различия и другие признаки указывают на присутствие во Вьетнаме даже большего числа неописанных видов. Обнаружены также представители двух не описанных родов, поскольку некоторые найденные нематоды не могут быть отнесены ни к одному известному роду Heteroderidae. Основываясь на морфологических признаках личинок 2-й стадии, можно различить около 6 видов рода *Meloidogyne*. Представители двух, по-видимому, неописанных видов родственного рода *Meloinema* были обнаружены в 2-х исследованных лесах. Дается краткая характеристика и временный ключ для определения найденных форм. Не было сделано даже попытки провести точное определение или описать новые виды *Meloidogyne* и Heteroderidae Вьетнама, поскольку материал по этим формам весьма ограничен и представлен в основном личинками 2-й стадии.
