©CAB International 2018 – for Sergei A. Subbotin

# 16 Molecular Taxonomy and Phylogeny

**Sergei A. Subbotin[1,2] and Andrea M. Skantar[3]**

[1]*Plant Pest Diagnostic Center, California Department of Food and Agriculture, California, USA;* [2]*Center of Parasitology of A.N. Severtsov Institute of Ecology and Evolution, Moscow, Russia;* [3]*USDA, ARS, Beltsville, Maryland, USA*

| | | |
|---|---|---|
| 16.1 | Introduction | 399 |
| 16.2 | Nuclear Ribosomal RNA Genes | 400 |
| 16.3 | Nuclear Protein-coding Genes | 400 |
| 16.4 | Mitochondrial DNA Genome Organization | 403 |
| 16.5 | Origin and Phylogeny of Heteroderidae | 407 |
| 16.6 | Phylogeny and Phylogeography of Punctoderinae | 409 |
| 16.7 | Phylogeny and Phylogeography of *Globodera* | 410 |
| 16.8 | Co-evolution of Cyst Nematodes with their Host Plants | 412 |
| 16.9 | Conclusions and Future Prospects | 413 |
| 16.10 | References | 414 |

## 16.1 Introduction

For many years evolutionary relationships among species and genera of cyst nematodes were estimated by classical comparison of morphological characters. Since the 1990s molecular information, such as nucleotide, amino acid sequences and restriction fragment length polymorphism (RFLP), has become increasingly available for inferring phylogenetic relationships and for reconstructing phylogenetic trees of cyst nematodes.

The availability of molecular data has had a significant impact on the systematics of cyst nematodes, reshaping concepts of their relationships at both the species and genus level. Phylogenetic analysis of sequences has allowed the validity of some taxa to be tested, has supported or rejected species synonymization or the erection of several new genera and, finally, has helped to arrange all taxa in a natural classification. In recent years, molecular phylogenetic trees have become increasingly valuable to taxonomists as the information is integrated with morphological characters and biological particularities in evolutionary analysis.

Although molecular studies of cyst nematodes have yielded a substantial amount of data on their genomes, transcriptomes and proteomes, at present only small fractions of these datasets have been used for phylogenetic reconstructions. Molecular data currently used for cyst nematode phylogeny have come primarily from nuclear ribosomal RNA genes, and nuclear and mitochondrial protein-coding genes. Reconstructing the phylogeny from genes is not straightforward and requires several methodical steps to be followed with certain assumptions and further

©CAB International 2018 – for Sergei A. Subbotin


careful verification of results. Phylogenetic trees that illustrate the relationship among the aligned gene sequences are considered as gene trees. Whether these gene trees can be interpreted as representing the relationship among species depends on whether the genes provided for the alignment are truly orthologous, having evolved from a common ancestral gene by speciation.

## 16.2 Nuclear Ribosomal RNA Genes

The rRNA genes are the main genes traditionally used for phylogenetic studies of cyst nematodes. These include 18S, 28S and, especially, the internal transcribed spacer 1 (ITS1) and internal transcribed spacer 2 (ITS2), which are interspersed between the 18S, 5.8S and 28S rRNA genes, respectively. The ITS regions are subjected to a higher mutation rate, thus containing sufficient signals to resolve phylogenies within subfamilies or genera of cyst nematodes better than, for example, the 18S rRNA gene. The partial and whole ITS rRNA gene phylogenies of cyst nematodes have been published in many articles (Ferris, 1998; Ferris *et al*., 1993, 1995, 1998, 1999a, b, 2004; Sabo *et al*., 2001, 2002; Subbotin *et al*., 2001, 2006, 2010, 2017; Tanha Maafi *et al*., 2003; Madani *et al*., 2004; Sturhan *et al*., 2007; Mundo-Ocampo *et al*., 2008; Bernard *et al*., 2010; De Luca *et al*., 2013; Knoetze *et al*., 2013; Wang *et al*., 2013; Zhuo *et al*., 2013, 2014 and others) (Fig. 16.1). Phylogenies reconstructed using 18S rRNA gene sequences (Ferris *et al*., 2004; Mundo-Ocampo *et al*., 2008; van Megen *et al*., 2009; Bernard *et al*., 2010; De Luca *et al*., 2013) and the D2-D3 expansion fragment of 28S rRNA gene sequences (Subbotin *et al*., 2006, 2010; Mundo-Ocampo *et al*., 2008; Bernard *et al*., 2010; De Luca *et al*., 2013; Wang *et al*., 2013; Zhuo *et al*., 2013, 2014) were more appropriate to study relationships among subfamilies and genera of Heteroderidae.

## 16.3 Nuclear Protein-coding Genes

Nuclear protein-coding genes can serve as a rich source of genetic data for phylogenetic analysis of many organisms, including nematodes. Genomic, cDNA or amino acid sequences from the same gene locus provide flexibility in the information content that is available for phylogenetic reconstruction of cyst nematode relationships at the genus and species levels. Several nuclear protein-coding genes, including heat shock protein 90 (Skantar and Carta, 2004; Mundo-Ocampo *et al*., 2008), actin (Kovaleva *et al*., 2005a; Mundo-Ocampo *et al*., 2008; Toumi *et al*., 2013a), fructose-bisphosphate aldolase (Kovaleva *et al*., 2005b) and beta-tubulin (Sabo and Ferris, 2004) have also been used for characterization and reconstruction of phylogenies of cyst nematodes. Phylogenetic analysis of combined 47 protein-coding gene sequences recovered from the EST data of *Heterodera glycines*, *Globodera pallida* and *G. rostochiensis* with several species of root-knot nematodes was conducted by Scholl and Bird (2005). This study illustrated, for the first time, that genomic analyses using EST data mining methods can lead to interesting and useful results.

### 16.3.1 Heat shock protein 90

Heat shock protein 90 (*hsp90*) encodes a molecular chaperone protein that assists other proteins to fold properly, and serves as a buffer against deleterious variation, particularly under heat stress. Skantar and Carta (2004) used degenerate primers to obtain partial *hsp90* gene sequences from several plant-parasitic nematodes including *H. glycines*, and demonstrated that this gene was present in a single copy in the genome of this species. This is also the case for the model nematode *Caenorhabditis elegans*, in which *hsp90* is encoded by the *daf-21* gene (Birnby *et al*., 2000), as well as the animal-parasitic nematode *Brugia pahangi* (Devaney *et al*., 2005) and the pine wood nematode *Bursaphelenchus xylophilus* (Wang *et al*., 2012). Moreover, *hsp90* EST sequences were included in the multigene phylogeny of Scholl and Bird (2005), which was based upon several single-copy genes. *Hsp90* protein sequence alignments were used to construct higher order taxonomic trees, whilst genomic DNA and coding region nucleotide alignments resolved several species with high bootstrap support (Skantar and Carta, 2004). Mundo-Ocampo *et al*. (2008) and Skantar *et al*. (2012) characterized partial *hsp90* from several *Heterodera* species,

©CAB International 2018 – for Sergei A. Subbotin


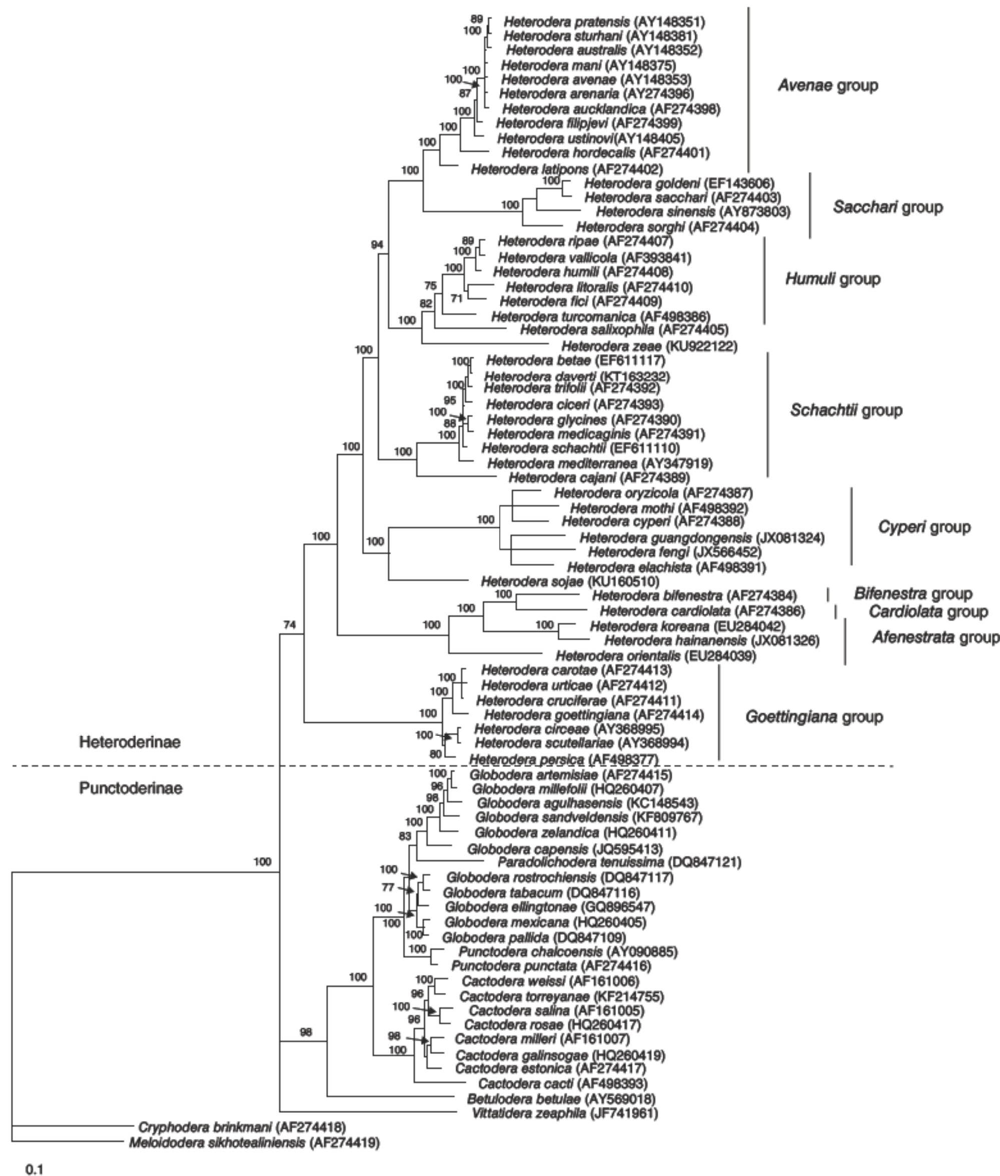


**Fig. 16.1.** Phylogenetic relationships within cyst nematodes as inferred from Bayesian analysis of the ITS1-5.8S-ITS2 rRNA gene sequence alignment under the GTR + I + G model. Posterior probabilities more than 70% are given for appropriate clades.

and Madani *et al.* (2011) provided some sequences for several *Globodera* species. Phylogenetic trees based on *hsp90* sequences showed equal resolution and in most cases were congruent with those inferred from ribosomal markers. The phylogenetic relationships of cyst nematodes are given in Figure 16.2.

### 16.3.2 Actin

Actin is a highly conserved structural, multifunctional protein that forms microfilaments and is ubiquitously expressed in eukaryotic cells. Multicellular eukaryotes contain multiple actin genes, the sequences of which are

©CAB International 2018 – for Sergei A. Subbotin


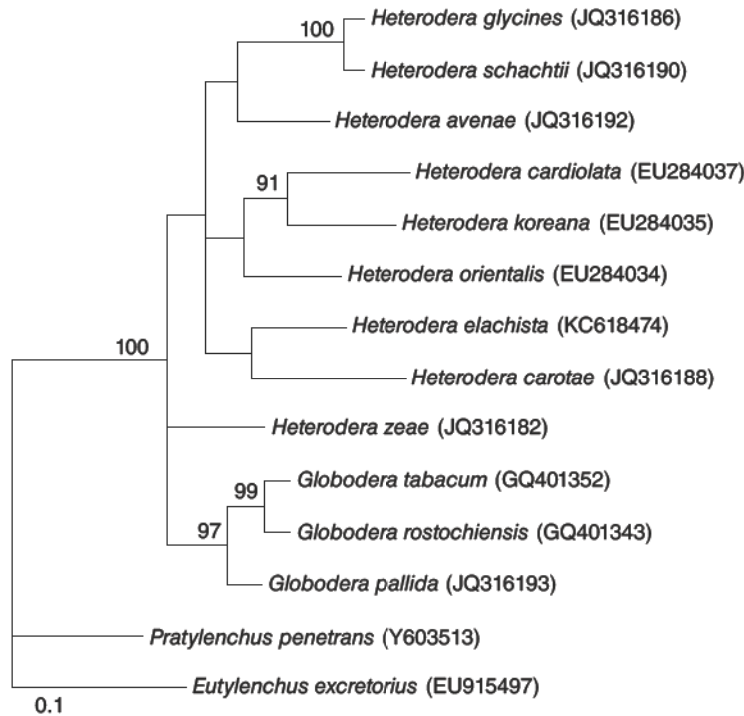


**Fig. 16.2.** Phylogenetic relationships within cyst nematodes as inferred from Bayesian analysis of the *hsp90* gene sequence alignment under the GTR + I + G model. Posterior probabilities more than 70% are given for appropriate clades.

highly conserved, with pairwise percentage identities of actins in the range of 88–98%. The existence of multiple actin genes in eukaryotes is thought to provide a higher copy number to cope with the demands for actin. The *C. elegans* genome encodes five actin genes (*act-1*, *act-2*, *act-3*, *act-4* and *act-5*). Kovaleva *et al*. (2005a) characterized three genes encoding actin from *H. glycines* and *G. rostochiensis*, and partial sequences of this gene from six other cyst nematodes. Actin sequence variation within the Heteroderidae ranged from 0.3 to 13.0%, allowing separation of species similar to that achieved by other ribosomal markers and *hsp90* (Mundo-Ocampo *et al*., 2008). Partial sequences of actin genes from several species were also published by Toumi *et al*. (2013a). The actin phylogenetic tree for *Heterodera* is similar with those for ribosomal RNA genes (Fig. 16.3). Amino acid sequences were similar for the cyst nematodes, ranging from 99–100% identity, and apparently do not contain phylogenetic signals.

### 16.3.3 Fructose-bisphosphate aldolase

Fructose-bisphosphate aldolase, often just named as aldolase, is an enzyme catalysing a reversible cleavage of fructose 1,6-bisphosphate into the triose phosphates dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. Two distinct types of cDNAs for aldolase, *ce-1* and *ce-2*, have been isolated from *C. elegans* (Inoue *et al*., 1997). Kovaleva *et al*. (2005b) were the first to characterize full cDNA and genomic aldolase sequences for *H. glycines* and *G. rostochienis* and fragments of this gene from several other cyst nematodes. Based upon intron characteristics, it appeared that *ce-2* was more similar to the cyst nematodes aldolase genes than *ce-1*. In addition, *C. elegans ce-2* aldolase had a higher amino acid similarity to the cyst nematodes (72%) than does *ce-1* (60%). Whilst it has not been used extensively to date, the aldolase gene can be considered as a prospective candidate marker for reconstruction of phylogeny within cyst nematode subfamilies.

©CAB International 2018 – for Sergei A. Subbotin


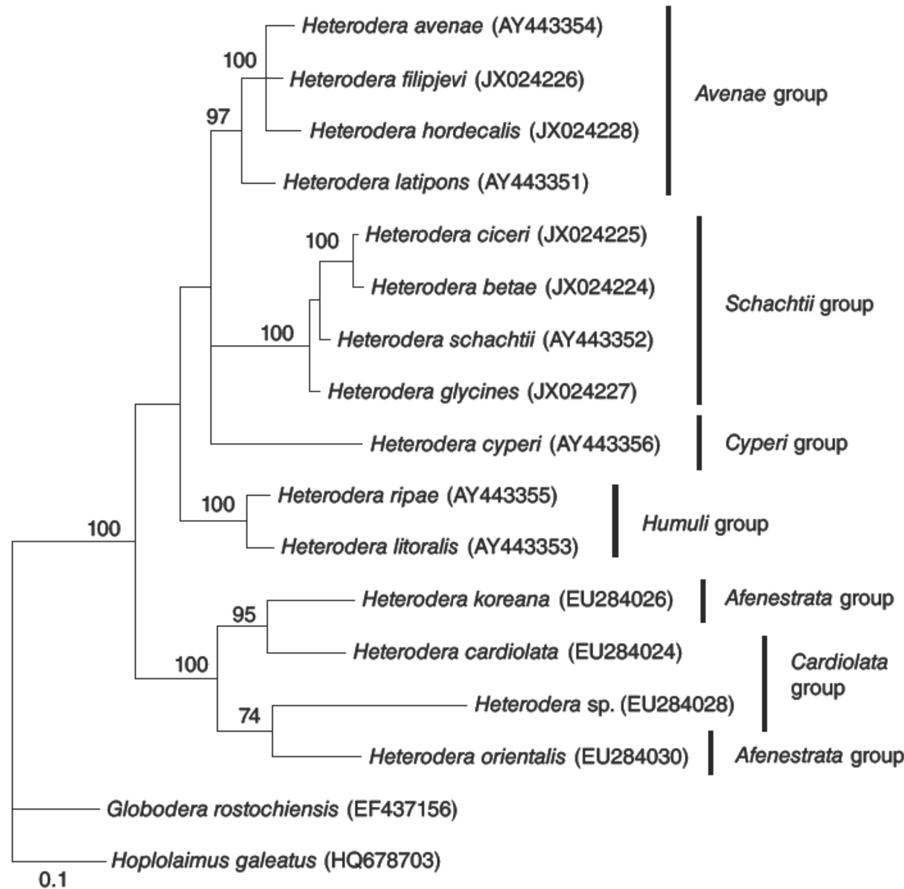


**Fig. 16.3.** Phylogenetic relationships within the genus *Heterodera* as inferred from Bayesian analysis of the actin gene sequence alignment under the GTR + I + G model. Posterior probabilities more than 70% are given for appropriate clades.

### 16.3.4 β-tubulin gene

Sabo and Ferris (2004) attempted to reconstruct the cyst nematode phylogeny using nuclear and amino acid sequences of *β*-tubulin gene. These studies resulted in an unresolved tree reconstructed from six putative paralogues of this gene, thus leading to the conclusion that this gene is not suitable for phylogenetic study of this nematode group.

## 16.4 Mitochondrial DNA Genome Organization

At present, mitochondrial genomes have been characterized in detail for only four cyst nematode species: *G. pallida* (Armstrong *et al.*, 2000; Gibson *et al.*, 2007a), *G. rostochiensis* (Gibson *et al.*, 2007b), *G. ellingtonae* (Phillips *et al.*, 2016) and *H. glycines* (Gibson *et al.*, 2011). The studies revealed two types of mitochondrial DNA genome organizations for cyst nematodes: (i) one circular, double-stranded DNA molecule presently found in *H. glycines*; and (ii) several circular, double-stranded DNA molecules with different gene contents found in representatives of the genus *Globodera*. The mechanism by which the multipartite genome in *Globodera* arose is still uncertain.

The mitochondrial genome of *H. glycines* was characterized by Gibson *et al.* (2011), who estimated that the entire single circular mtDNA should be around 21–22 kb containing 12 protein-coding genes, two rRNA genes and 22 tRNA genes (Fig. 16.4A) The genome contained a major

Fig. 16.4. Mitochondrial genome organization: *Heterodera glycines* (A) and chromosome I and II of *Globodera ellingtonae* (B and C, respectively).

©CAB International 2018 – for Sergei A. Subbotin


non-coding and unsequenced region (between *trnG* and *trnN*), which was estimated to be 7–8 kb long. There were also smaller non-coding tracts between the *atp6* and *nad5* genes (330 nucleotides), the *trnR* and *trnD* genes (335 nucleotides), and between the *nad4L* and *trnG* genes (130 nucleotides). The organization of the mitochondrial genome of *Radopholus similis*, a migratory endoparasitic nematode, showed the highest level of similarity to that of *H. glycines*. Only minor differences were found when the *H. glycines* mtDNA genome was compared with partly sequenced mtDNA genomes of *H. cardiolata* and *Punctodera chalcoensis*.

Armstrong *et al.* (2000) were the first to analyse mitochondrial DNA genome organization for *G. pallida* mtDNA. The researchers provided evidence that this species was unusual among the metazoa and had a multipartite mtDNA structure or subgenomes. Small, circular mitochondrial DNAs (*Gpa*-scmtDNAs I-VI) ranging from 6.3 to 9.5 kb, have been discovered by polymerase chain reaction (PCR) in a British population of *G. pallida*, although additional components of the *G. pallida* mtDNA still remain uncharacterized. All of these *G. pallida* mtDNAs contained sequences similar to known mitochondrial genes, with most containing sequences that showed highest sequence similarity to previously described nematode mitochondrial genes. Armstrong *et al.* (2000) also discovered that these *Gpa*-scmtDNAs were present together in populations of *G. pallida*, although their relative frequencies may vary considerably between populations. The analysis revealed a degree of redundancy in gene content, suggesting that not all *Gpa*-scmtDNAs may be required to compose a functional mtDNA. For example, *Gpa*-scmtDNA I (*coxII, nad4, coxIII, nad6, nad1, nad3*) was found to include genes duplicated on *Gpa*-scmtDNAs II (*nad1, coxII*) and III (*nad3, cytb*). This was consistent with the observation that the Luffness population of *G. pallida* was found to contain only *Gpa*-scmtDNA I, and not *Gpa*-scmtDNA II and III, whereas the Gourdie population was found to contain *Gpa*-scmtDNAs II and III, with *Gpa*-scmtDNA I being detectable only by PCR (Armstrong *et al.*, 2000). Later studies also revealed evidence for recombination of sequence variants of *Gpa*-scmtDNA IV for South American P4A population (Armstrong *et al.*, 2000). Gibson *et al.* (2007a) also found that three of the *Gpa*-scmtDNAs (I, II and III) of *G. pallida* were mosaics, composed predominantly of multigenic fragments found on other scmtDNA molecules, and suggested that this mosaic pattern was an indication of the operation of inter-mt recombination.

Subgenomic organization was also found to occur in *G. rostochiensis*, which is a close relative of *G. pallida* (Gibson *et al.*, 2007b). A comparison of subgenomic organization between these two *Globodera* species revealed a considerable degree of overlap between them, showing that the two subgenomes were identical to that reported for *G. pallida*. However, other subgenomes were unique to *G. rostochiensis* (*Gro*-scmtDNA VI and VII), although some of these have blocks of genes comparable to those in *G. pallida*. Comparisons of pairs of subgenomes from *G. rostochiensis* indicated that the different subgenomes shared fragments with high sequence identity. Gibson *et al.* (2007b) interpreted this as evidence that recombination is operating in the mitochondria of *G. rostochiensis*.

Several genes, *nad2, nad4L, nad5*, 12S rRNA and two tRNA, normally found in nematode mitochondrial genomes, were not identified in any of the five subgenomes completely sequenced in *G. pallida* (Armstrong *et al.*, 2000; Gibson *et al.*, 2007a). Similarly, *nad2, nad6*, 12S rRNA and two tRNA were also not found in any of the subgenomes sequenced from *G. rostochiensis* (Gibson *et al.*, 2007b). These missing genes may therefore reside on other as yet uncharacterized subgenomes.

A recent study of *G. ellingtonae* revealed that its mitochondrial genome is unique and distinct from other multipartite mitochondrial genomes. The genetic content of the genome was disproportionately divided between the two circles, although they shared a ~6.5 kb non-coding region. The 17.8 kb circle (*Gel*-mtDNA-I) contained ten protein-coding genes and two tRNA genes, whereas the 14.4 kb circle (*Gel*-mtDNA-II) contained two protein-coding genes, 20 tRNA genes and both rRNA genes (Fig. 16.4B, C). The copy number of mtDNA-II was more than fourfold that of mtDNA-I in individual nematodes. The difference in copy number increased between second-stage and fourth-stage juveniles (Phillips *et al.*, 2016).

The representatives of the genus *Punctodera* are closely related to those from the genus

©CAB International 2018 – for Sergei A. Subbotin



*Globodera*. *Punctodera chalcoensis* was studied by Gibson *et al*. (2011), who found that its genome was larger than the multipartite mitochondrial genomes of *G. pallida* and *G. rostochiensis* and might also have minicircular mitochondrial genome organization.

### 16.4.1 Mitochondrial DNA genes

MtDNA genes have advantages over rRNA genes for studies of phylogenetic relationships at the genus and species level, due in part to the higher possibility of discovering intraspecific polymorphism resulting from faster accumulation of substitutions within mtDNA genes. The cytochrome c oxidase subunit I, or *coxI* gene, was successfully used by several researchers (Toumi *et al*., 2013a, b; De Luca *et al*., 2013; Vovlas *et al*., 2015; Subbotin, 2015; Sekimoto *et al*., 2017) for diagnostics and reconstruction of phylogenetic relationships within several groups of cyst nematodes. This gene is known as the standard molecular barcode for many animals. The *coxI* gene and other mtDNA genes are also presently used as markers for phylogeographical analysis of cyst nematode populations. For phylogeographical studies of *G. pallida* another mtDNA gene, cytochrome b, or *cytb* was also successfully applied (Picard *et al*., 2007; Plantard *et al*., 2008; Madani *et al*., 2010; Eves-van den Akker *et al*., 2015) (Fig. 16.5). It is interesting to note that intraspecific divergence in mtDNA sequences for cyst nematodes can reach 10%, which is higher than for other organisms. Such a high degree of polymorphism may also limit the ability of researchers to develop sets of universal primers for amplification of mtDNA genes in cyst nematodes.

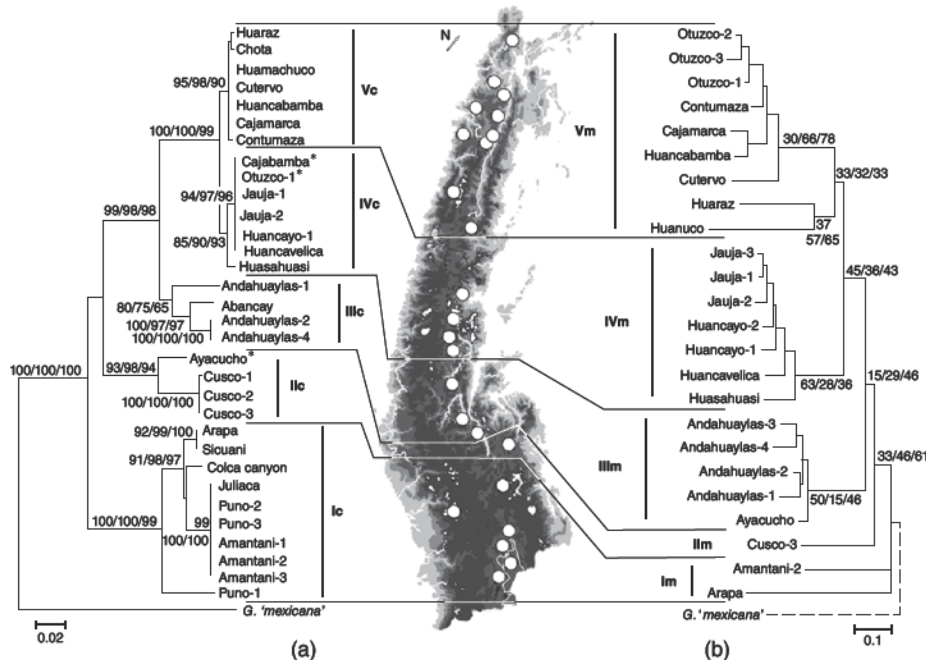


**Fig. 16.5.** Relationships between populations of *Globodera pallida* along the Andean Cordillera as inferred from the analysis of cytochrome *b* partial gene (a) and eight microsatellite loci (b). Asterisks (*) symbolize haplotypes located outside the clade corresponding to their geographical origin; bootstrap values are indicated for the nodes common to the tree analyses (neighbour-joining/maximum parsimony/maximum likelihood). (b) Neighbour-joining analysis, with an Infinite alleles model (IAM) genetic distance $D_A$, or Nei *et al*. (1983) distance, based on eight microsatellite loci; numbers by nodes represent the node between clades common to the three IAM genetic distance methods. (After Picard *et al*., 2007.)

©CAB International 2018 – for Sergei A. Subbotin


## 16.5 Origin and Phylogeny of Heteroderidae

The relationships of cyst nematodes with other Tylenchida have been the subjects of several studies. Paramonov (1967) was the first to propose that cyst nematodes might represent a particular phylogenetic lineage of hoplolaimids based upon feeding adaptations on host plant roots that appears to suggest a transition of this group to sedentary endoparasitism. This idea was later supported by Wouts and Sher (1971) and Krall and Krall (1978). Several evolutionary schemes that represent historical developments for cyst nematodes were proposed by Stone (1975, 1979), Krall and Krall (1978), Ferris (1979, 1985), Coomans (1979, 1983) and Baldwin and Schouest (1990) using morphological characters. Wouts (1985) was the first to develop a detailed phylogenetic classification of the family Heteroderidae, in which a putative cladogram consisted of six main lineages corresponding to six subfamilies: Verutinae, Meloidoderinae, Cryphoderinae, Heteroderinae, Ataloderinae and Punctoderinae. According to Wouts (1985), the hypothetical heteroderid ancestor developed from a nematode that resembled modern members of the family Hoplolaimidae and was possibly closely related to *Rotylenchulus*. The genus *Verutus* was considered the most primitive genus within heteroderids and was characterized by a very large equatorial vulval slit. The genus *Meloidodera* was considered to be further derived by a reduction in the length of the vulva. Genera, interpreted as developing later, exhibited a subterminal vulval slit and progressive loss of annulation of the female cuticle. Wouts (1985) also proposed that genera that developed later exhibit a subterminally located vulval slit and progressively lost the annulation of the female cuticle. In this process four evolutionary lines emerged: (i) a posterior shift of the vulva and the formation of more or less distinct vulval lips gave rise to the genera *Zelandodera* and *Cryphodera*; (ii) changes in the lip configuration of the second-stage juvenile gave rise to the genera *Hylonema* and *Heterodera*; (iii) changes in the composition of the female cuticle resulted in the genera *Thecavermiculatus*, *Atalodera*, *Sherodera*, *Sarisodera* and *Bellodera*; and (iv) a reduction in vulval slit size led to the development of the genera *Dolichodera*, *Globodera*, *Cactodera* and *Punctodera*. Wouts's phylogeny proposes independent development of cysts in two different lineages.

New insight into the phylogeny of Heteroderidae has been provided by the analyses of ribosomal RNA gene sequences as represented by Ferris (1998), Ferris *et al.* (1999a, b), Sabo *et al.* (2001), Subbotin *et al.* (2001, 2006, 2010), Tanha Maafi *et al.* (2003), Sturhan *et al.* (2007), Mundo-Ocampo *et al.* (2008), Bernard *et al.* (2010), De Luca *et al.* (2013), Zhuo *et al.* (2014) and others. Molecular phylogeny strongly supports close relationships between Heteroderidae, Hoplolaimidae and Rotylenchulidae and the hypothesis of Wouts (1985) that *Verutus* and *Rotylenchulus* are placed in the basal positions to all sedentary nematodes of Heteroderidae (Subbotin *et al.*, 2010). Molecular phylogenies also support: (i) the division of cyst nematodes into two subfamilies, the Heteroderinae and Punctoderinae; (ii) the monophylies of *Cactodera*, *Punctodera*, *Globodera* and *Heterodera*; and (iii) the validities of *Paradolichodera*, *Betulodera* and *Vittatidera*. The molecular analysis indicates that cyst formation appeared only once during the evolution of Heteroderidae.

### 16.5.1 Phylogeny of Heteroderinae

The subfamily Heteroderinae includes only a single genus *Heterodera*. Historically, species of the genus *Heterodera* were separated into three groups based on vulval cone structures, that is, the *Schachtii*, *Goettingiana* and *Avenae* groups (Mulvey and Golden, 1983; Baldwin and Mundo-Ocampo, 1991). Stone (1975) also recognized several groups based on juvenile lip morphology. Several additional, partly overlapping, groups have also been proposed: *Humuli* (Mathews, 1971; Subbotin *et al.*, 1997), *Fici-humuli* (Ferris, 1979), *Oryzae*, *Cruciferae* and *Graminis* (Stone, 1979), *Latipons* (Wouts and Sturhan, 1995) and *Bifenestra* (Sturhan, 2006). Subbotin *et al.* (2001), considering a combination of molecular data with the morphology of vulval structures and the number of incisures in the lateral field of juveniles, supported the recognition of the *Schachtii*, *Goettingiana*, *Avenae* and *Humuli* groups, albeit with a modified species composition and the erection of two new groups, *Cyperi* and *Sacchari*.

©CAB International 2018 – for Sergei A. Subbotin


After synonymization of the genus *Afenestrata* with the genus *Heterodera*, the *Afenestrata* group was also proposed (Mundo-Ocampo *et al*., 2008). Morphological and molecular analysis further supports the division of *Heterodera* into more or less distinct species groups recognized as follows: *Afenestrata*, *Avenae*, *Bifenestra*, *Cardiolata*, *Cyperi*, *Goettingiana*, *Humuli*, *Sacchari* and *Schachtii*. Although most valid species can be accommodated in one of the above-mentioned groups, the position of some *Heterodera* species (e.g. *H. salixophila* and *H. zeae*) remains undefined or uncertain (Subbotin *et al*., 2010).

There are several hypotheses proposed about the earliest group to arise within the genus *Heterodera*: amphimictic species *H. schachtii*, *H. glycines* and, perhaps, *H. salixophila* (Krall and Krall, 1978); or a *H. cruciferae*-like form parasitizing dicots (Stone, 1979); or the cyst-forming species previously placed in *Afenestrata* (Ferris, 1979; Wouts, 1985). The phylogenetic analyses presented by Subbotin *et al*. (2001, 2010), Tanha Maafi *et al*. (2003) and De Luca *et al*. (2013) resolved the basal relationships within *Heterodera*, and usually placed the *Goettingiana* group (*H. goettingiana*, *H. carotae*, *H. urticae*, *H. cruciferae*, *H. scutellariae*, *H. circeae* and *H. persica*) as an earliest diverged group within the genus. Obviously, the molecular data lend significant support to Stone's (1979) hypothesis. Although relationships between heteroderid groups are not well resolved, nevertheless four groups, *Avenae*, *Sacchari*, *Schachtii* and *Humuli*, are often placed in derived positions in most phylogenetic trees. The *Avenae* and *Sacchari* groups have a strong sister relationship in all trees. The relationships between *Avenae* + *Sacchari*, *Schachtii* and *Humuli* groups remain unresolved, indicating the possible rapid evolutionary radiation of these nematode lineages.

Some inconsistencies between molecular phylogeny and previously proposed phylogenetic hypotheses or groupings may be attributed to homoplastic evolution. For example, according to molecular analysis a bifenestral vulval cone developed independently in three groups, *Avenae*, *Humuli* and *Bifenestra*, during the evolution of cyst nematodes. Likewise, the presence of three incisures in the lateral field of second-stage juveniles of the cyst nematodes seems to have also arisen three times independently (*Afenestrata*, *Cyperi* and *Sacchari* groups). Finally, a short vulval slit developed independently in the *Avenae* and *Bifenestra* groups within Heteroderinae.

The molecular data suggest an early divergence between tropical and temperate heteroderid species. Krall and Krall (1978) stated that the centre of origin for any organism group is likely to be the extant area with the highest species diversity. On this basis they suggested that the Mediterranean and Central Asia regions could be the centre of origin for the genus *Heterodera*. However, the analysis of presently known patterns of geographical distributions of the species occupying basal positions (*H. cajani*, *H. mothi*, *H. zeae*, *H. latipons* and *H. persica*) in phylogenetic trees from different groups suggest that most probably a centre of origin of *Heterodera* is in south and western Asia, although the *Afenestrata* group may have originated from the east Asia region.

Phylogenetic relationships of species from the genus *Heterodera* have also been intensively studied within the groups, especially within *Avenae* and *Schachtii* groups, to which the most agricultural important species belong. Phylogenetic relationships with the *Avenae* group were analysed using the ITS-rRNA gene sequences described in detail by Subbotin *et al*. (2003). When *H. latipons* and *H. hordecalis* were used as outgroup taxa, the species having cysts with an underbridge (*H. ustinovi* and *H. filipjevi*) occupied basal positions in the tree. Other species were distributed into two main clades: (i) *H. avenae*, *H. arenaria*, *H. aucklandica* and *H. mani*; and (ii) *H. australis*, *H. pratensis* and *H. sturhani*. However, the ITS-rRNA gene sequences did enable *H. avenae* to be distinguished from *H. arenaria*, and *H. pratensis* from *H. sturhani*. Partial *coxI* mtDNA gene sequences provided better discrimination and resolution for species relationships. Phylogenetic relationships between cyst nematode species of the *Avenae* group as inferred from the analysis of *coxI* mtDNA gene sequences were recently analysed by Subbotin (2015). This gene revealed distinct differences between *H. australis* and *H. avenae*, between *H. pratensis* and *H. sturhani*, and between European and Asian populations of *H. avenae* (type A and type B). The partial *coxI* mtDNA gene sequences also discriminate *H. avenae* type A from *H. arenaria*.

Vovlas *et al*. (2015) recently presented phylogenetic relationships between several species of the *Schachtii* group as inferred from the

©CAB International 2018 – for Sergei A. Subbotin


analysis of the ITS-rRNA gene sequences. The tropical species *H. cajani* is distributed in India, Pakistan and Egypt, and occupies a basal position in the *Schachtii* group. *Heterodera glycines* had sister relationships with *H. medicaginis*, whereas relationships between *H. trifolii*, *H. daverti*, *H. betae* and *H. ciceri* were not well resolved. It is interesting that some ITS-rRNA clones of *H. schachtii* were also clustered with these species, possibly indicating incomplete lineage sorting within species of this group. Madani *et al.* (2007) showed that intraspecific divergence for the ITS-rRNA gene sequence can reach 2.5% for *H. schachtii*, whereas for other cyst nematode species it does not exceed 1.6–1.8% (Subbotin *et al.*, 2000; Tanha Maafi *et al.*, 2003; Madani *et al.*, 2004). Recent analysis showed that the *coxI* mtDNA gene sequences provided better resolution for the study of relationships within this group (Vovlas *et al.*, 2015; Sekimoto *et al.*, 2017).

Phylogenetic relationships within species of other groups were analysed by Mundo-Ocampo *et al.* (2008) for *Afenestrata* group, Tanha Maafi *et al.* (2007) for *Sacchari* group, Eroshenko *et al.* (2001) for *Humuli* group and Zhuo *et al.* (2014) for *Cyperi* group.

## 16.6 Phylogeny and Phylogeography of Punctoderinae

The subfamily Punctoderinae constitutes six genera, *Betulodera*, *Cactodera*, *Dolichodera*, *Globodera*, *Paradolichodera* and *Punctodera*, which share the character of possessing a vulval circumfenestra. Although Siddiqi (1986, 2000) and Luc *et al.* (1988) did not accept the subdivision of cyst nematodes into two subfamilies, Heteroderinae and Punctoderinae, the phylogenetic analyses of rRNA gene sequences gave evidence that circumfenestrate nematodes represent one of two separate major lineages within cyst nematodes (Subbotin *et al.*, 2001, 2010, 2011; Tanha Maafi *et al.*, 2003).

Phylogenetic relationships among circumfenestrate cyst nematodes were inferred from analyses of ITS-rRNA gene sequences in studies conducted by several researchers (Ferris *et al.*, 1999a, b, 2004; Subbotin *et al.*, 2000, 2001; Sabo *et al.*, 2002; Skantar *et al.*, 2007; Bernard *et al.*, 2010; Madani *et al.*, 2010; Handoo *et al.*, 2012; Lax *et al.*, 2014). The molecular phylogenetic tree of Punctoderinae consists of six major clades corresponding to the division into genera (Fig. 16.1). Monophylies of *Punctodera*, *Cactodera* and *Betulodera* were always highly supported, whereas monophyly of *Globodera* was not always evident. It has been previously remarked that the observed paraphyly of *Globodera* may simply reflect the general tendency of phylogenetic algorithms to produce unbalanced trees rather than to define any true evolutionary history of a group with high rates of evolution. Genetic divergence within this genus is reflected by the species groupings based on geographical origin and host plant speciation (Subbotin *et al.*, 2001). *Betulodera* with *Vittatidera* clades occupy basal positions on all trees. Relationships of *Paradolichodera* and *Punctodera* with other genera are not well resolved and may vary among trees (Subbotin *et al.*, 2011).

The phylogeographic analysis using rRNA gene sequences was used to test possible origin and patterns of dispersal of circumfenestrate cyst nematodes. Subbotin *et al.* (2011, 2017) suggested that North America appeared to be a centre of early evolution and the area from which subsequent dispersal of Punctoderinae occurred. Indeed, in North America the greatest number of related circumfenestrate cyst nematode species and genera occur: 12 of the 13 known *Cactodera* species, three of four known *Punctodera*, known *Betulodera*, *Dolichodera* and *Vittatidera* species and several *Globodera* species. Of six valid Punctoderinae genera, only *Paradolichodera* was described from New Zealand and has never been recorded in North America or in any other region of the world.

Another proposed approach to determine a centre of origin is to identify the area in which the most evolutionarily primitive representatives, closest in form to the supposed ancestral group, occur, with the assumption that they are not likely to have dispersed far from the centre of origin. Phylogenetic analyses using morphological (Krall and Krall, 1978; Wouts, 1985; Baldwin and Schouest, 1990) and molecular (Subbotin *et al.*, 2006, 2010) datasets revealed that extant Ataloderinae and Meloidoderinae share common ancestors with cyst nematodes. The species of these subfamilies parasitize a rather wide spectrum of plants, including gymnosperms and angiosperms. The ability of some species of *Meloidodera*, *Cryphodera* (Meloidoderinae) and

©CAB International 2018 – for Sergei A. Subbotin


*Rhizonemella sequoiae* (Ataloderinae) to parasitize plants from *Pinus* and *Sequoia*, respectively, is consistent with the argument of Krall and Krall (1978) that suggests an ancient origin for these genera in the New World. The distribution of extant Meloidoderinae includes North America, Europe, Asia and Oceania, whilst most representatives of Ataloderinae are found in North America (*Ekphymatodera*, *Sarisodera*, *Rhizonemella*, *Bellodera* and *Atalodera*). However, some species of *Atalodera* are not restricted to North America and have been described from South America and Asia. In addition, only two Ataloderinae genera, *Hylonema* and *Camelodera*, are reported from Africa and Asia, respectively. Thus, the general pattern of modern distribution of non-cyst nematodes sharing a common ancestor with Punctoderinae favours the hypothesis of a North American origin of circumfenestrate cyst nematodes. Although *Cactodera* and *Punctodera* putatively originated and began to diversify in North America, results of the phylogeographical analysis, in which several new findings of undescribed species of *Globodera* from New Zealand and South Africa were included, revealed that South America or Africa appears to be a centre of origin of *Globodera*. Thus, Stone's (1979) hypothesis of an 'out-of-the-west' Gondwana origin of *Globodera* with subsequent dispersal of the species of this genus to Europe, North America, Asia and Oceania found some support from the rRNA datasets.

The absence of a fossil record together with unequal rates of rRNA gene evolution do not allow reliable application of molecular clock approaches to estimate divergence time for different cyst nematode lineages. The ages of cyst nematode divergences have been estimated using different approaches. Picard *et al.* (2008) considered the age of the *Cactodera* – (*Punctodera* + *Globodera*) divergence to be close to the Heteroderinae–Punctoderinae divergence, which might have occurred after separation of Laurasia and Gondwana, that is, 173–130 million years ago. The divergence of the South American and Laurasian *Globodera* lineages was considered to have occurred between 80 and 60 million years ago after the break of a temporary connection between North and South America in the Palaeocene. However, if we consider the estimated ages of host plants presently associated with non-cyst and cyst nematodes, slightly younger dates of divergence between nematode lineages could be suggested. For example, the age of Meloidoderinae associated with *Pinus* could be proposed as the time when *Pinus* diverged from the other genera, that is, 140 million years ago. For Ataloderinae parasitizing *Sequoia*, it might be the late Cretaceous (99–65 million years ago) and Tertiary, periods from which *Sequoia* fossil records are primarily known. The late Cretaceous was also considered as a possible time period for origin, diversification and spread of various Poaceae (Prasad *et al.*, 2005), which are reported to be hosts for some Ataloderinae. From this line of reasoning one could speculate on the Heteroderinae–Punctoderinae divergence occurring between 90 and 70 million years ago. The divergence of the two main *Globodera* lineages is associated with the time of origin for the Solanaceae, that is, 65–51 million years ago (Wikström *et al.*, 2001; Paape *et al.*, 2008). Thus, the split of the two *Globodera* lineages might have occurred subsequent to the breakup of Gondwana and the Africa and South America split in the Mid-Cretaceous, and it seems that the evolution of Punctoderinae cannot be explained solely by the separation of the continents and diffusion expansion. Recent advances in phylogenetics and, in particular, molecular dating, indicate that transoceanic dispersal has played an important role in shaping plant and animal distributions, thereby obscuring any effect of tectonic history (Vanderpoorten *et al.*, 2010). Subbotin *et al.* (2011) also suggested that 'jump dispersal' might play a significant role in the biogeographic history of cyst nematodes and hypothesized that long-distance dispersal might even have occurred repeatedly during cyst nematode evolution. This idea suggested a scenario whereby a modern *Globodera* lineage arose from introduction of the ancestral Punctoderinae to Gondwana via long-distance dispersal from North America.

## 16.7 Phylogeny and Phylogeography of *Globodera*

The genus *Globodera* displays two main clades in phylogenetic trees: (i) *Globodera* from South and North America parasitizing plants from Solanaceae; and (ii) *Globodera* from Africa, Europe,

©CAB International 2018 – for Sergei A. Subbotin


Asia and New Zealand parasitizing plants from Asteraceae and other families. The first main clade includes the first subclade with *G. pallida* and *G. mexicana* and the second one with *G. ellingtonae*, *G. tabacum* and *G. rostochiensis*. The second main clade consists of three subclades: (i) *G. capensis* from South Africa; (ii) *G. zelandica* and two undescribed species from New Zealand; and (iii) *G. artemisiae*, *G. millefolii* from Europe and Asia, two recently described species from South Africa and one undescribed species from Portugal. It has been hypothesized that centres of diversification and speciation in each main clade are associated with mountain regions (Subbotin *et al.*, 2016). The centre of diversification for *Globodera* parasitizing Solanaceae occurs in the Andes (Grenier *et al.*, 2010), known as one of 35 world biodiversity hotspots. The *Globodera* belonging to the second main clade have several centres of diversification: mountains of the Western Cape in South Africa, South Island mountains in New Zealand and, perhaps, mountain ranges of Portugal (Subbotin *et al.*, 2016).

The pale potato cyst nematode, *G. pallida*, is considered a major worldwide pest of potatoes. Several studies revealed that this species originated from South America, from where it has been introduced to many parts of the world, particularly to Europe, Asia, and also to the USA, Canada and New Zealand. In South America, this cyst nematode is mainly found between 2000 and 4000 m above sea level, with the heaviest infestations between 2900 and 3800 m elevation (Franco, 1977). Distinct genetic differences among *G. pallida* populations spanning Europe and those found in South America were revealed by sequence and phylogenetic analyses of the ITS-rRNA (Blok *et al.*, 1998; Subbotin *et al.*, 2000, 2011; Madani *et al.*, 2010), *cytb* (Picard *et al.*, 2007; Plantard *et al.*, 2008; Pylypenko *et al.*, 2008; Madani *et al.*, 2010), PCR-ITS-RFLP (Ayub and Rumpenhorst, 2000; Grenier *et al.*, 2001), simple sequence repeat primer analysis (Blok and Phillips, 1995), PCR-RAPD (Blok *et al.*, 1997; Bendezu *et al.*, 1998; Grenier *et al.*, 2001; Rumpenhorst and Ayub, 2001), isoelectric focusing (IEF) of proteins (Rumpenhorst and Ayub, 2001), 2-DGE of proteins (Grenier *et al.*, 2001) and satellite DNA analysis (Grenier *et al.*, 2001; Plantard *et al.*, 2008). The analysis of partial *cytb* gene sequences of *G. pallida* collected in different regions allowed Plantard *et al.* (2008) to identify the origin of western European populations with a high degree of certainty (Fig. 16.5). This analysis showed that all of these populations originated from a single restricted area in the extreme south of Peru, located between the north shore of the Lake Titicaca and Cusco. Plantard *et al.* (2008) found that only four *cytb* haplotypes are reported in western Europe, one of them also being found in some populations of this area of southern Peru. After studying the USA and Canadian *G. pallida*, Madani *et al.* (2010) concluded that they belong to the western European haplotype and, thus, North American populations resulted from the continued spread of *G. pallida* from western Europe to other countries and continents, and were unlikely to be the result of a separate introduction to North America directly from South America.

The highest ITS-rRNA and *cytb* gene sequence diversity for *G. pallida* was reported in Peru and the Andes. Picard *et al.* (2007) used 42 populations sampled along a 1500-km north–south transect in Peru genotyped with a partial *cytb* and seven nuclear microsatellite loci, and described a clear phylogeographical pattern among Peruvian populations revealing five distinct clades (Fig. 16.5). The clade containing the southern populations was genetically more diverse and forms the most basal branch. The large divergence among *cytb* haplotypes suggested that they diverged before human domestication of potato. Investigations by Picard *et al.* (2007) also clearly illustrated a northward expansion of populations from the south of Peru (around Lake Titicaca) to the north. It has been hypothesized that this south-to-north pattern took place during the uplift of the Andes beginning 20 million years ago and followed the same direction, and reflected the colonization of progressively emerging favourable areas of the Andes by wild potatoes and the co-evolution of *G. pallida* with its host plant (Picard *et al.*, 2007; Grenier *et al.*, 2010). Grenier *et al.* (2010) also suggested that northward from Peru (i.e. in Ecuador and Columbia) this pattern of decreasing genetic variability and speciation might continue. It is also noted that *G. mexicana*, exhibiting a lower genetic variability, was only reported in Mexico. Under the assumption of a northward expansion from south Peru of *G. pallida* populations on wild solanaceous hosts, Grenier *et al.* (2010) hypothesized that *G. mexicana* would

©CAB International 2018 – for Sergei A. Subbotin


represent a distinct speciation event that was initiated through *G. pallida*. Close relationships of *G. mexicana* with representatives of one of the *G. pallida* clade have been strongly supported by the phylogenetic analysis of the ITS-rRNA gene sequences (Subbotin *et al*., 2011).

Phylogenetic analysis with limited samplings for representatives of the second subclade of *Globodera* parasitizing Solanaceae also shows a pattern of their northward expansion in the Andes. The potato cyst nematode *G. ellingtonae*, recently described from the USA and evidently introduced from a central Andes location (Grenier *et al*., 2010; Handoo *et al*., 2012; Lax *et al*., 2014), occupies a basal position in the subclade, whereas *G. tabacum* and *G. rostochiensis*, having a sister relationship, are distributed more in the north.

The golden cyst nematode *G. rostochiensis*, native to South America, has been introduced in many parts of the world, including Europe and North America. Boucher *et al*. (2013) analysed 12 new microsatellite markers in order to characterize the genetic links between 15 globally distributed *G. rostochiensis* populations, including the populations found in Quebec. The results revealed that the five populations from South America, especially those from Bolivia, had a higher genetic diversity than those originating from Europe and North America. The results also indicated that the Bolivian populations were distinct from other populations and suggested that a minimum of two introductions with different origins would have to have occurred in North America: (i) one in Quebec and/or Newfoundland directly or indirectly from European populations; and (ii) one in British Columbia and/or New York from a currently unidentified location, as inferred by the fact that these populations make up an independent genetic cluster.

The tobacco cyst nematode *G. tabacum* is a polytypic species containing the following subspecies: *G. tabacum tabacum*, *G. tabacum virginiae* and *G. tabacum solanacearum*, which are poorly differentiated by morphology although separable by host preference. Relationships within this complex were not resolved using the ITS-rRNA gene sequence analysis; however, PCR-RAPD (Thiéry *et al*., 1997) and AFLP (Marché *et al*., 2001) techniques, and sequences of genes coding CLE peptides and cell wall degrading enzymes (Alenda *et al*., 2013) were able to differentiate the subspecies. Moreover, Marché *et al*. (2001) recognized a fourth subspecies within *G. tabacum* from Mexico, named *G. tabacum* '*azteca*'; however, Alenda *et al*. (2013) later discarded this subspecies. The analysis of six genes did not support the fourth subspecies as all the Mexican populations clustered either with *G. tabacum virginiae* or *G. tabacum solanacearum*.

Picard *et al*. (2008) estimated divergence dates using nuclear ribosomal (ITS1-5.8S-ITS2) and *cytb* sequences, and proposed that *G. tabacum* most probably diverged from *G. rostochiensis* 24.7–18.5 million years ago (Late Oligocene–Early Miocene), and that *G. pallida* diverged from *G. mexicana* 20.6–15.4 million years ago (Early Miocene). Within *G. pallida*, basal divergence of clades of the *cytb* dendrogram occurred at some time in the 17.8–13.4 million years ago (Middle Miocene) and 11.8–8.8 million years ago (Late Miocene) intervals. Alenda *et al*. (2013) assumed that subspecies of *G. tabacum* appeared less than 15 million years ago.

One of the important practical implications of these phylogeographical studies is that the reference sequence databases of *cytb*, ITS-rRNA and other genes for *Globodera* populations parasitizing Solanaceae have been created. These databases comprise a valuable resource for accurate identification of suspected new *Globodera* from quarantine samples, providing early detection that could prevent introduction and spread of new nematode isolates or species. As was the case for *G. ellingtonae*, it is possible that new nematodes would be genetically very divergent from known species, requiring additional plant-resistance sources to control them, and would enhance the already high adaptive potential of established local *Globodera* populations.

## 16.8 Co-evolution of Cyst Nematodes with their Host Plants

Krall and Krall (1978) and Krall (1990) argued the importance of a comparative ecological approach to study the evolution of cyst nematodes. They proposed a hypothesis of co-evolution of heteroderids with plants using the concept of flowering plant evolution developed by

©CAB International 2018 – for Sergei A. Subbotin


Grossheim (1945). Additional aspects of hypotheses of co-evolution of cyst nematodes and their plant hosts were developed and discussed by Stone (1979, 1985) and Sturhan (2000). Molecular phylogeny of cyst nematodes allowed Subbotin *et al*. (2001, 2010) to evaluate the hypothesis of co-evolution of heteroderids with their hosts as proposed by Krall and Krall (1978) and Stone (1979). The results tended to support the idea that different cyst nematode groups co-evolved with hosts belonging to single or closely related families of plants. Nevertheless, some species were able secondarily to colonize ecologically convergent plant species from unrelated families (Subbotin *et al*., 2010).

In relation to the taxonomy according to current plant classification (Angiosperm Phylogeny Group, 2016), cyst nematode species have been described from the type plant hosts belonging to the following orders: the genus *Heterodera* – Poales (46 species), Fabales (10), Caryophyllales (5), Rosales (7), Lamiales (4), Mapighiales (3), Apiales (2), Brassicales (2), Saxifragales (1), Myrtales (1), Fagales (1), Sapindales (1), Asterales (1); the genus *Globodera* – Solanales (7), Asterales (2), Rosales (1), Myrtales (1), Caryophyllales (1); the genus *Cactodera* (14 species) – Caryophyllales (11), Asterales (1), Poales (1); the genus *Punctodera* – Poales (4); the genus *Dolichodera* – Poales (1); the genus *Paradolichodera* – Poales (1); the genus *Betulodera* – Fagales (1) and *Vittatidera* – Poales (1). Thus, half of known cyst nematode species have been described from plants in the order Poales, one of the largest orders of flowering plants. Six main lineages are evident within *Heterodera* – three of them with monocotyledons and three with dicotyledons. The *Goettingiana* group includes species parasitizing plants belonging to various taxonomic groups of dicotyledons. The *Sacchari*, *Avenae*, *Afenestrata*, *Bifenestra*, *Cardiolata* and *Cyperi* groups all co-evolved with Poales. The *Schachtii* group primarily co-evolved with plants from the order Fabales and then colonized Lamiales, Caryophyllales and Asterales. Another lineage includes species of the *Humuli* group associated with hosts from Caryophyllales and Rosales. Two species related to this group but different in morphology, *H. salixophila* and *H. zeae*, parasitize plants from Malpighiales and Poales, respectively. It is plausible to hypothesize that some of these species are associated with changes in host specialization relatively recently, jumping to phylogenetically unrelated but ecologically similar host plants

Molecular phylogenetics further suggest that within Punctoderinae there are several distinct lineages: (i) basally derived *Betulodera* having hosts from Fagales; (ii) *Vittatidera* parasitizes Poales; (iii) *Cactodera* associating with Caryophyllales (Amaranthaceae, Caryophyllaceae, Portulacaceae) and, perhaps, relatively recently with Asterales and Poales; (iv) *Punctodera* and *Paradolichodera* parasitizing Poales; (v) *Globodera* having hosts from Asterales and Myrtales; and (vi) *Globodera* parasitizing Solanales (Subbotin *et al*., 2010).

## 16.9 Conclusions and Future Prospects

In recent years, genome-scale data or phylogenomics has become an increasingly powerful tool for phylogenetic inference of many organisms and for resolving difficult phylogenetic and phylogeographic questions; however, these approaches have not yet found widespread practical application for the study of cyst nematode evolution. The main strength of phylogenomics is the drastic reduction of errors in tree inference that may arise from a single gene dataset when replaced by the use of large multigene datasets. Genome data afford a much broader selection of genes that are appropriate for phylogenetic analysis, and reduction of non-phylogenetic signals from the large multigene datasets is a challenging area of future research, which enables difficult issues of relationships in these nematodes to be resolved.

Another topic of molecular phylogenetics that has been recently influenced by population genetics is species delimitation by tree-based and non-tree-based methods that rely upon molecular sequence data. A robust hypothesis testing for species limits that incorporates information on the rRNA and mtDNA genes together with application of coalescent theory, which deals with complex models incorporating phenomena such as migration, selection and recombination (Rosenberg and Nordborg, 2002), has enormous promise.

©CAB International 2018 – for Sergei A. Subbotin


## 16.10 References


Armstrong, M.R., Blok, V.C. and Phillips, M.S. (2000) A multipartite mitochondrial genome in the potato cyst nematode *Globodera pallida. Genetics* 154, 181–192.

Alenda, C., Gallot-Legrand, A., Fouville, D. and Grenier, E. (2013) Sequence polymorphism of nematode effectors highlights molecular differences among the subspecies of the tobacco cyst nematode complex. *Physiological and Molecular Plant Pathology* 84, 107–114. DOI: 10.1016/J.PMPP.2013.08.004

Angiosperm Phylogeny Group (2016) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* 181, 1–20. DOI: 10.1111/boj.12385

Ayub, M. and Rumpenhorst, H.J. (2000) Genetic variation in potato cyst nematode (*Globodera pallida*) from Europe and South America as revealed by PCR-RFLP of ITS regions. In: *Abstracts der 28 Tagung des Arbeitskreises Nematologie, 1–2 März 2000, Veitshöchheim*, pp. 36–37.

Baldwin, J.G. and Mundo-Ocampo, M. (1991) Heteroderinae, cyst and non-cyst-forming nematodes. In: Nickle, W.R. (ed.) *Manual of Agricultural Nematology.* Marcel Dekker Inc., New York, pp. 275–362.

Baldwin, J.G. and Schouest Jr, L.P. (1990) Comparative detailed morphology of the Heteroderinae Filipjev and Schuurmans Stekhoven, 1941, *sensu* Luc *et al*. (1988): phylogenetic systematics and revised classification. *Systematic Parasitology* 15, 81–106.

Bendezu, I.F., Evans, K., Burrows, P.R., De Pomerai, D. and Canto-Saenz, M. (1998) Inter and intraspecific 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

Bernard, E.C., Handoo, Z.A., Powers, T.O., Donald, P.A. and Heinz, R.D. (2010) *Vittatidera zeaphila* (Nematoda: Heteroderidae), a new genus and species of cyst nematode parasitic on corn (*Zea mays*). *Journal of Nematology* 42, 139–150.

Birnby, D.A., Link, E.M., Vowels, J.J., Tian, H., Colacurcio, P.L. and Thomas, J.H. (2000) A transmembrane guanylyl cyclase (DAF-11) and Hsp90 (DAF-21) regulate a common set of chemosensory behaviors in *Caenorhabditis elegans*. *Genetics* 155, 85–104.

Blok, V.C. and Phillips, M.S. (1995) The use of repeat sequence primers for investigating genetic diversity between populations of potato cyst nematodes with differing virulence. *Fundamental and Applied Nematology* 18, 575–582.

Blok, V.C., Phillips, M.S. and Harrower, B.E. (1997) Comparison of British populations of potato cyst nematodes with populations from continental Europe and South America using RAPDs. *Genome* 40, 286–293. DOI: 10.1139/g97-040

Blok, V.C., Malloch, G., Harrower, B., Phillips, M.S. and Vrain, T.C. (1998) Intraspecific variation in ribosomal DNA in populations of the potato cyst nematode *Globodera pallida*. *Journal of Nematology* 30, 262–274.

Boucher, A.C., Mimee, B., Montarry, J., Bardou-Valette, S., Bélair, G., Moffett, P. and Grenier, E. (2013) Genetic diversity of the golden potato cyst nematode *Globodera rostochiensis* and determination of the origin of populations in Quebec, Canada. *Molecular Phylogenetics and Evolution* 69, 75–82. DOI: 10.1016/j.ympev.2013.05.020

Coomans, A. (1979) General principles of systematics with particular reference to speciation; 1–19. In: Lamberti, F. and Taylor, C.E. (eds) *Root-knot Nematodes* (Meloidogyne *Species*). *Systematics, Biology and Control*. Academic Press, London, pp. 1–19.

Coomans, A. (1983) General principles for the phylogenetic systematics of nematodes. In: Stone, A.R., Platt, H.M. and Khalil, L.F. (eds) *Concepts in Nematode Systematics*. Academic Press, New York, pp. 1–10.

Devaney, E., O'Neill, K., Harnett, W., Whitesell, L. and Kinnaird, J.H. (2005) Hsp90 is essential in the filarial nematode *Brugia pahangi. International Journal of Parasitology* 35, 627–636.

De Luca, F., Vovlas, N., Lucarelli, G., Troccoli, A., Radicci, V., Fanelli, E., Cantalapiedra-Navarrete, C., Palomares-Rius, J. and Castillo, P. (2013) *Heterodera elachista* the Japanese cyst nematode parasitizing corn in Northern Italy: integrative diagnosis and bionomics. *European Journal of Plant Pathology* 136, 857–872. DOI: 10.1007/s10658-013-0212-9

Eroshenko, A.S., Subbotin, S.A. and Kazachenko, I.P. (2001) *Heterodera vallicola* sp. n. (Tylenchida: Heteroderidae) from elm trees, *Ulmus japonica* in the Primorsky territory, the Russian Far East, with rDNA identification of closely related species. *Russian Journal of Nematology* 9, 9–17.

Eves-van den Akker, S., Lilley, C.J., Reid, A., Pickup, J., Anderson, E., Cock, P.J.A., Blaxter, M., Urwin, P.E., Jones, J.T. and Blok, V.C. (2015) A metagenetic approach to determine the diversity and distribution

©CAB International 2018 – for Sergei A. Subbotin


of cyst nematodes at the level of the country, the field and the individual. *Molecular Ecology* 24, 5842–5851. DOI: 10.1111/mec.13434

Ferris, V.R. (1979) Cladistic approaches in the study of soil and plant parasitic nematodes. *American Zoologist* 19, 1195–1215. DOI: 10.1093/icb/19.4.1195

Ferris, V.R. (1985) Evolution and biogeography of cyst-forming nematodes. *EPPO Bulletin* 15, 123–129. DOI: 10.1111/j.1365-2338.1985.tb00211.x

Ferris, V.R. (1998) Evolution, phylogeny and systematics. In: Sharma, S. (ed.) *The Cyst Nematodes.* Dordrecht, The Netherlands, Kluwer Academic Publishers, pp. 57–82.

Ferris, V.R., Ferris, J.M. and Faghihi, J. (1993) Variation in spacer ribosomal DNA in some cyst-forming species of plant-parasitic nematodes. *Fundamental and Applied Nematology* 16, 177–184.

Ferris, V.R., Miller, L.I., Faghihi, J. and Ferris, J.M. (1995) Ribosomal DNA comparisons of *Globodera* from two continents. *Journal of Nematology* 27, 273–283.

Ferris, V.R., Riggs, R.D., Sabo, A., Faghihi, J. and Ferris, J.M. (1998) Relationships of *Cactodera betulae* to other cyst nematodes based on ribosomal DNA. *Phytopathology* 88, S28.

Ferris, V.R., Krall, E., Faghihi, J. and Ferris, J.M. (1999a) Relationships of *Globodera millefolii*, *G. artemisiae* and *Cactodera salina* based on ITS region of ribosomal DNA. *Journal of Nematology* 31, 498–507.

Ferris, V.R., Subbotin, S.A., Ireholm, A., Spiegel, Y., Faghihi, J. and Ferris, J.M. (1999b) Ribosomal DNA sequence analysis of *Heterodera filipjevi* and *H. latipons* isolates from Russia and comparisons with other nematode isolates. *Russian Journal of Nematology* 7, 121–125.

Ferris, V.R., Sabo, A., Baldwin, J.G., Mundo-Ocampo, M., Inserra, R.N. and Sharma, S. (2004) Phylogenetic relationships among selected Heteroderoideae based on 18S and ITS ribosomal DNA. *Journal of Nematology* 36, 202–206.

Franco, J. (1977) *Studies on the Taxonomy and Biology of Potato Cyst Nematodes* Globodera *spp.* Ph.D. Dissertation, University of London, London.

Gibson, T., Blok, V.C., Phillips, M.S., Hong, G., Kumarasinghe, D., Riley, I.T. and Dowton, M. (2007a) The mitochondrial subgenomes of the nematode *Globodera pallida* are mosaics: evidence of recombination in an animal mitochondrial genome. *Journal of Molecular Evolution* 64, 463–471. DOI: 10.1007/s00239-006-0187-7

Gibson, T., Blok, V.C. and Dowton, M. (2007b) Sequence and characterization of six mitochondrial subgenomes from *Globodera rostochiensis*: multipartite structure is conserved among close nematode relatives. *Journal of Molecular Evolution* 65, 308–315. DOI: 10.1007/s00239-007-9007-y

Gibson, T., Farrugia, D., Barrett, J., Chitwood, D., Rowe, J., Subbotin, S.A. and Dowton, M. (2011) The mitochondrial genome of the soybean cyst nematode, *Heterodera glycines*. *Genome* 54, 565–574. DOI: 10.1139/g11-024

Grenier, E., Bossis, M., Fouville, D., Renault, L. and Mugniéry, D. (2001) Molecular approaches to the taxonomic position of Peruvian potato cyst nematodes and gene pool similarities in indigenous and imported populations of *Globodera*. *Heredity* 86, 277–290. DOI: 10.1046/j.1365-2540.2001.00826.x

Grenier, E., Fournet, S., Pettit, E. and Anthoine, G. (2010) A cyst nematode 'species factory' called the Andes. *Nematology* 12, 163–169. DOI: 10.1163/138855409X12573393054942

Grossheim, A.A. (1945) [On question of graphical image of the system of flowering plants.] *Sovetskaya Botanika* 13, 3–27.

Handoo, Z.A., Carta, L.K., Skantar, A.M. and Chitwood, D.J. (2012) Description of *Globodera ellingtonae* n. sp. (Nematoda: Heteroderidae) from Oregon. *Journal of Nematology* 44, 40–57.

Inoue, T., Yatsuki, H., Kusakabe, T., Joh, K., Takasaki. Y., Nikoh, N., Miyata, T. and Hori, K. (1997) *Caenorhabditis elegans* has two isozymic forms, CE-1 and CE-2, of fructose-1,6-bisphosphate aldolase which are encoded by different genes. *Archives of Biochemistry and Biophysics* 339, 226–234.

Knoetze, R., Swart, A. and Tiedt, L.R. (2013) Description of *Globodera capensis* n. sp. (Nematoda: Heteroderidae) from South Africa. *Nematology* 15, 233–250. DOI: 10.1163/15685411-00002673

Kovaleva, E.S., Subbotin, S.A., Masler, E.P. and Chitwood, D.J. (2005a) Molecular characterization of the actin gene from cyst nematodes in comparison to those from other nematodes. *Comparative Parasitology* 72, 39–49. DOI: 10.1654/4138

Kovaleva, E.S., Masler, E.P., Subbotin, S.A. and Chitwood, D.J. (2005b) Molecular characterization of aldolase from *Heterodera glycines* and *Globodera rostochiensis*. *Journal of Nematology* 37, 292–296.

Krall, E. (1990) Different approaches to and recent developments in the systematics and co-evolution of the family Heteroderidae (Nematoda: Tylenchida) with host plants. *Proceedings of the Estonian Academy of Sciences, Biology* 39, 259–270.

©CAB International 2018 – for Sergei A. Subbotin


Krall, E.L. and Krall, H.A. (1978) [Revision of the plant nematodes of the family Heteroderidae on the basis of trophic specialization of these parasites and their co-evolution with their host plants.] In: *Fitogel'mintologicheskie Issledovaniya.* Nauka, Moscow, USSR, pp. 39–56.

Lax, P., Rondan Dueñas, J.C., Franco-Ponce, J., Gardenal, C.N. and Doucet, M.E. (2014) Morphology and DNA sequence data reveal the presence of *Globodera ellingtonae* in the Andean region. *Contributions to Zoology* 83, 227–243.

Luc, M., Maggenti, A.R. and Fortuner, R. (1988) A reappraisal of Tylenchina (Nemata). 9. The family Heteroderidae Filipjev and Schuurmans Stekhoven, 1941. *Revue de Nématologie* 11, 159–176.

Madani, M., Vovlas, N., Castillo, P., Subbotin, S.A. and Moens, M. (2004) Molecular characterization of cyst nematode species (*Heterodera* spp.) from the Mediterranean basin using RFLPs and sequences of ITS-rDNA. *Journal of Phytopathology* 152, 229–234. DOI: 10.1111/j.1439-0434.2004.00835.x

Madani, M., Kyndt, T., Colpaert, N., Subbotin, S.A., Gheysen, G. and Moens, M. (2007) Polymorphism among sugar beet cyst nematode *Heterodera schachtii* populations as inferred from AFLP and ITS-rRNA gene analyses. *Russian Journal of Nematology* 15, 117–128.

Madani, M., Subbotin, S.A., Ward, L.J. and De Boer, S.H. (2010) Molecular characterization of Canadian populations of potato cyst nematodes, *Globodera rostochiensis* and *G. pallida* using ribosomal nuclear RNA and cytochrome b genes. *Canadian Journal of Plant Pathology* 32, 252–263.

Madani, M., Ward, L.J. and De Boer, S.H. (2011) Hsp90 gene, an additional target for discrimination between the potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*, and the related species, *G. tabacum tabacum*. *European Journal of Plant Pathology* 130, 271–285. DOI: 10.1007/s10658-011-9752-z

Marché, L., Valette, S., Grenier, E. and Mugniéry, D. (2001) Intraspecies DNA polymorphism in the tobacco cyst nematode complex (*Globodera tabacum*) using AFLP. *Genome* 44, 941–946. DOI: 10.1139/g01-091

Mathews, H.J.P. (1971) Two new species of cyst nematode, *Heterodera mani* n. sp. and *H. iri* n. sp., from Northern Ireland. *Nematologica* 17, 553–565. DOI: 10.1163/187529271X00279

Mulvey, R.H. and Golden, A.M. (1983) An illustrated key to the cyst forming genera and species of Heteroderidae in the western hemisphere with morphometrics and distribution. *Journal of Nematology* 15, 1–59.

Mundo-Ocampo, M., Troccoli, A., Subbotin, S.A., Del Cid, J., Baldwin, J.G. and Inserra, R.N. (2008) Synonymy of *Afenestrata* with *Heterodera* supported by phylogenetics with molecular and morphological characterisation of *H. koreana* comb. n. and *H. orientalis* comb. n. (Tylenchida: Heteroderidae). *Nematology* 10, 611–632. DOI: 10.1163/156854108785787190

Nei, M., Tajima, F. and Tateno, Y. (1983) Accuracy of estimated phylogenetic trees from molecular data. *Journal of Molecular Evolution* 19, 153–170.

Paape, T., Igic, B., Smith, S.D., Olmstead, R., Bohs, L. and Kohn, J.R. (2008) A 15-myr old genetic bottleneck. *Molecular Biology and Evolution* 25, 655–663. DOI: 10.1093/molbev/msn016

Paramonov, A.A. (1967) [A critical review of the suborder Tylenchina (Filip'jev, 1934) (Nematoda: Secernentea).] *Trudy Gel'mintologicheskoi Laboratorii Akademii Nauk SSSR* 18, 78–101.

Phillips, W.S., Brown, A.M.V., Howe, D.K., Peetz, A.B., Blok, V.C., Denver, D.R. and Zasada, I.A. (2016) The mitochondrial genome of *Globodera ellingtonae* is composed of two circles with segregated gene content and differential copy numbers. *BMC Genomics* 17, 706. DOI: 10.1186/s12864-016-3047-x

Picard, D., Sempere, T. and Plantard, O. (2007) A northward colonisation of the Andes by the potato cyst nematode during geological times suggests multiple host-shifts from wild to cultivated potatoes. *Molecular Phylogenetics and Evolution* 42, 308–316. DOI: 10.1016/j.ympev.2006.06.018

Picard, D., Sempere, T. and Plantard, O. (2008) Direction and timing of uplift propagation in the Peruvian Andes deduced from molecular phylogenetics of highland biotaxa. *Earth and Planetary Science Letters* 271, 326–336. DOI: 10.1016/j.epsl.2008.04.024

Plantard, O., Picard, D., Valette, S., Scurrah, M., Grenier, E. and Mugniéry, D. (2008) Origin and genetic diversity of Western European populations of the potato cyst nematode *Globodera pallida* inferred from mitochondrial sequences and microsatellite loci. *Molecular Ecology* 17, 2208–2218. DOI: 10.1111/j.1365-294X.2008.03718.x

Prasad, V., Stromberg, C.A.E., Alimohammadian, H. and Sahni, A. (2005) Dinosaur coprolites and the early evolution of grasses and grazers. *Science* 310, 1177–1180. DOI: 10.1126/science.1118806

Pylypenko, L.A., Phillips, M.S. and Blok, V.C. (2008) Characterisation of two Ukrainian populations of *Globodera pallida* in terms of their virulence and mtDNA, and the biological assessment of a new resistant cultivar Vales Everest. *Nematology* 10, 585–590. DOI: 10.1163/156854108784513798

©CAB International 2018 – for Sergei A. Subbotin



Rosenberg, N.A. and Nordborg, M. (2002) Genealogical trees, coalescent theory and the analysis of genetic polymorphisms. *Nature Reviews Genetics* 3, 380–390. DOI: 10.1038/nrg795

Rumpenhorst, H.J. and Ayub, M. (2001) Gibt es eine dritte Art beim Kartoffelnematoden? Die 29 Tagung des Arbeitskreises Nematologie, Monheim, Germany, 14–15 March 2001.

Sabo, A. and Ferris, V.R. (2004) β-tubulin paralogs provide a qualitative test for a phylogeny of cyst nematodes. *Journal of Nematology* 36, 440–448.

Sabo, A., Vovlas, N. and Ferris, V.R. (2001) Phylogenetic relationships based on ribosomal DNA data for four species of cyst nematodes from Italy and one from Syria. *Journal of Nematology* 33, 183–190.

Sabo, A., Reis, L.G.L., Krall, E., Mundo-Ocampo, M. and Ferris, V.R. (2002) Phylogenetic relationships of a distinct species of *Globodera* from Portugal and two *Punctodera* species. *Journal of Nematology* 34, 263–266.

Scholl, E.H. and Bird, D.M.K. (2005) Resolving tylenchid evolutionary relationships through multiple gene analysis derived from EST data. *Molecular Phylogenetics and Evolution* 36, 536–545. DOI: 10.1016/j.ympev.2005.03.016

Sekimoto, S., Uehara, T. and Mizukubo, T. (2017) Characterisation of populations of *Heterodera trifolii* Goffart, 1932 (Nematoda: Heteroderidae) in Japan and their phylogenetic relationships with closely related species. *Nematology* 19 543–558. DOI: 10.1163/15685411-00003067

Siddiqi, M.R. (1986) *Tylenchida: Parasites of Plants and Insects.* Commonwealth Agricultural Bureaux, Farnham Royal, Farnham, UK.

Siddiqi, M.R. (2000) *Tylenchida: Parasites of Plants and Insects*, 2nd edn. CAB International, Wallingford, UK. DOI: 10.1079/9780851992020.0000

Skantar, A.M. and Carta, L.K. (2004) Phylogenetic evaluation of nucleotide and protein sequences from the heat shock protein 90 gene of selected nematodes. *Journal of Nematology* 36, 136–145.

Skantar, A.M., Handoo, Z.A., Carta, L.K. and Chitwood, D.J. (2007) Morphological and molecular identification of *Globodera pallida* associated with potato in Idaho. *Journal of Nematology* 39, 133–144.

Skantar, A.M., Handoo, Z.A., Zanakis, G.N. and Tzortzakakis, E.A. (2012) Molecular and morphological characterization of the corn cyst nematode, *Heterodera zeae*, from Greece. *Journal of Nematology* 44, 58–66.

Stone, A.R. (1975) Head morphology of second-stage juveniles of some Heteroderidae (Nematoda: Tylenchoidea). *Nematologica* 21, 81–88. DOI: 10.1163/187529275X00374

Stone, A.R. (1979) Co-evolution of nematodes and plants. *Symbolae Botanicae Upsaliensis* 22, 46–61. DOI: 10.1163/187529275X00374

Stone, A.R. (1985) Co-evolution of potato cyst nematodes and their hosts: implications for pathotypes and resistance. *EPPO Bulletin* 15, 131–137.

Sturhan, D. (2000) Wirts-Spezifität bei Zystennematoden und anderen Heteroderiden. *Mitteilungen aus der Biologischen Bundesanstalt für Land und Forstwirtschaft* 376, 299.

Sturhan, D. (2006) Zystenbildende Nematoden und andere Heteroderiden in Deutschland. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* 404, 18–30.

Sturhan, D., Wouts, W.M. and Subbotin, S.A. (2007) An unusual cyst nematode from New Zealand, *Paradolichodera tenuissima* gen. n., sp n. (Tylenchida: Heteroderidae). *Nematology* 9, 561–571. DOI: 10.1163/156854107781487314

Subbotin, S.A. (2015) *Heterodera sturhani* sp. n. from China, a new species of the *Heterodera avenae* species complex (Tylenchida: Heteroderidae). *Russian Journal of Nematology* 23, 145–152.

Subbotin, S.A., Sturhan, D., Waeyenberge, L. and Moens, M. (1997) *Heterodera riparia* sp. n. (Tylenchida: Heteroderidae) from common nettle, *Urtica dioica* L., and rDNA-RFLP separation of species from the *H. humuli* group. *Russian Journal of Nematology* 5, 143–157.

Subbotin, S.A., Halford, P.D., Warry, A. and Perry, R.N. (2000) Variations in ribosomal DNA sequences and phylogeny of *Globodera* parasitising Solanaceae. *Nematology* 2, 591–604.

Subbotin, S.A., Vierstraete, A., De Ley, P., Rowe, J., Waeyenberge, L., Moens, M. and Vanfleteren, J.R. (2001) Phylogenetic relationships within the cyst-forming nematodes (Nematoda, Heteroderidae) based on analysis of sequences from the ITS regions of ribosomal DNA. *Molecular Phylogenetics and Evolution* 21, 1–16. DOI: 10.1006/mpev.2001.0998

Subbotin, S.A., Sturhan, D., Rumpenhorst, H.J. and Moens, M. (2003) Molecular and morphological characterisation of the *Heterodera avenae* complex species (Tylenchida: Heteroderidae). *Nematology* 5, 515–538. DOI: 10.1163/156854103322683247

Subbotin, S.A., Sturhan, D., Chizhov, V.N., Vovlas, N. and Baldwin, J.G. (2006) Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. *Nematology* 8, 455–474. DOI: 10.1163/156854106778493420

©CAB International 2018 – for Sergei A. Subbotin


Subbotin, S.A., Mundo-Ocampo, M. and Baldwin, J.G. (2010) *Systematics of Cyst Nematodes (Nematoda: Heteroderinae). Nematology Monographs and Perspectives 8A* (series editors: Hunt, D.J. and Perry, R.N.). Brill, Leiden, The Netherlands.

Subbotin, S.A., Cid Del Prado Vera, I., Mundo-Ocampo, M. and Baldwin, J.G. (2011) Identification, phylogeny and phylogeography of circumfenestrate cyst nematodes (Nematoda: Heteroderidae) as inferred from analysis of ITS-rDNA. *Nematology* 13, 805–824. DOI: 10.1163/138855410X552661

Subbotin, S.A., Knoetze, R. and Cid Del Prado Vera, I. (2016) *Globodera* species: current systematics and phylogeography. *Journal of Nematology (Abstract)* 48, 373–374.

Subbotin, S.A., Akanwari, J., Nguyen, C.N., Cid del Prado Vera, I., Chitambar, J.J., Inserra, R.N. and Chizhov, V.N. (2017) Molecular characterization and phylogenetic relationships of cystoid nematodes of the family Heteroderidae (Nematoda: Tylenchida). *Nematology* 19, 1065–1081. DOI: 10.1163/15685411-00003107

Tanha Maafi, Z., Subbotin, S.A. and Moens, M. (2003) Molecular identification of cyst-forming nematodes (Heteroderidae) from Iran and a phylogeny based on the ITS sequences of rDNA. *Nematology* 5, 99–111. DOI: 10.1163/156854102765216731

Tanha Maafi, Z., Sturhan, D., Handoo, Z., Mordehai, M., Moens, M. and Subbotin, S.A. (2007) Morphological and molecular studies of *Heterodera sacchari*, *H. goldeni* and *H. leuceilyma* (Nematoda: Heteroderidae). *Nematology* 9, 483–497. DOI: 10.1163/156854107781487242

Thiéry, M., Fouville, D. and Mugniéry, D. (1997) Intra- and interspecific variability in *Globodera*, parasites of solanaceous plants, revealed by Random Amplified Polymorphic DNA (RAPD) and correlation with biological features. *Fundamental and Applied Nematology* 20, 495–504.

Toumi, F., Waeyenberge, L., Viaene, N., Dababat, A., Nicol, J.M., Ogbonnaya, F. and Moens, M. (2013a) Development of two species-specific primer sets to detect the cereal cyst nematodes *Heterodera avenae* and *Heterodera filipjevi*. *European Journal of Plant Pathology* 136, 613–624. DOI: 10.1007/s10658-013-0192-9

Toumi, F., Waeyenberge, L., Viaene, N., Dababat, A., Nicol, J.M., Ogbonnaya, F. and Moens, M. (2013b) Development of a species-specific PCR to detect the cereal cyst nematode, *Heterodera latipons*. *Nematology* 15, 709–717. DOI: 10.1163/15685411-00002713

van Megen, H., van den Elsen, S., Holterman, M., Karssen, G., Mooyman, P., Bongers, T., Holovachov, O., Bakker, J. and Helder, J. (2009) A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. *Nematology* 11, 927–950. DOI: 10.1163/156854109X456862

Vanderpoorten, A., Gradstein, S.R., Carine, M.A. and Devos, N. (2010) The ghosts of Gondwana and Laurasia in modern liverwort distributions. *Biological Reviews of the Cambridge Philosophical Society* 85, 471–487. DOI: 10.1111/j.1469-185X.2009.00111.x

Vovlas, N., Vovlas, A., Leonetti, P., Liébanas, G., Castillo, P., Subbotin, S.A. and Palomares-Rius, J.E. (2015) Parasitism effects on white clover by root-knot and cyst nematodes and molecular separation of *Heterodera daverti* from *H. trifolii*. *European Journal of Plant Pathology* 143, 833–845. DOI: 10.1007/s10658-015-0735-3

Wang, F., Wang, Z., Li, D. and Chen, Q. (2012) Identification and characterization of a *Bursaphelenchus xylophilus* (Aphelenchida: Aphelenchoididae) thermotolerance-related gene: Bx-HSP90. *International Journal of Molecular Sciences* 13, 8819–8833.

Wang, H.H., Zhuo, K., Ye, W., Zhang, H.L., Peng, D. and Liao, J.L. (2013) *Heterodera fengi* n. sp. (Nematoda: Heteroderinae) from bamboo in Guangdong Province, China – a new cyst nematode in the *Cyperi* group. *Zootaxa* 3652, 179–192. DOI: 10.11646/zootaxa.3652.1.7

Wikström, N., Savolainen, V. and Chase, M.W. (2001) Evolution of the angiosperms: calibrating the family tree. *Proceedings of the Royal Society of London, B, Biological Sciences* 268, 2211–2220. DOI: 10.1098/rspb.2001.1782

Wouts, W.M. (1985) Phylogenetic classification of the family Heteroderidae (Nematoda: Tylenchida). *Systematic Parasitology* 7, 295–328. DOI: 10.1007/BF00009997

Wouts, W.M. and Sher, S.A. (1971) The genera of the subfamily Heteroderinae (Nematoda: Tylenchoidea) with a description of two new genera. *Journal of Nematology* 3, 129–144.

Wouts, W.M. and Sturhan, D. (1995) *Heterodera aucklandica* sp. n. (Nematoda: Heteroderidae) from a New Zealand native grass, with notes on the species of the *H. avenae* group. *New Zealand Journal of Zoology* 22, 199–207.

Zhuo, K., Wang, H.H., Ye, W., Peng, D.L. and Liao, J.L. (2013) *Heterodera hainanensis* n. sp. (Nematoda: Heteroderinae) from bamboo in Hainan Province, China – a new cyst nematode in the *Afenestrata* group. *Nematology* 15, 303–314. DOI: 10.1163/15685411-00002678

Zhuo, K., Wang, H.H., Zhang, H.L. and Liao, J.L. (2014) *Heterodera guangdongensis* n. sp. (Nematoda: Heteroderinae) from bamboo in Guangdong Province, China – a new cyst nematode in the *Cyperi* group. *Zootaxa* 3881, 488–500. DOI: 10.11646/zootaxa.3881.5.4