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Biodiversity and systematics of nematode–bacterium entomopathogens

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Abstract

Nematodes are one of the most abundant animals on earth, and bacteria comprise the most biologically and phylogenetically diverse domains of organisms. On at least two separate occasions a soil dwelling nematode and a bacterium have entered into a mutualistic, insecticidal association. From such origins arose two distinct lineages of nematode–bacterium entomopathogens, *Steinernema–Xenorhabdus* and *Heterorhabditis–Photorhabdus*. Herein, we present a summary and discussion of the known evolutionary diversity and systematics of these two groups relative to other nematodes and bacteria, and their shared evolutionary history.
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1. Introduction

Nematodes are the most abundant animals on earth, and bacteria are the most biologically and phylogenetically diverse (Bongers and Ferris, 1999; Curtis and Sloan, 2004; Curtis et al., 2002; Rappe and Giovannoni, 2003; Torsvik et al., 2002). Cyanobacterial fossils date to 2.9 billion years ago (Noffke et al., 2003), whereas nematodes most likely arose slightly prior to or during the Cambrian explosion (Ayala and Rzhetsky, 1998; Rodriguez-Trelles et al., 2002; Wray et al., 1996). Discovering the full extent of biodiversity of these two clades is one of the greatest challenges facing modern science. Technological and analytical challenges faced by nematode and bacterial systematists are similar, yet the greatest dilemma may be the overwhelming discrepancy between the number of systematists working on the problem and the estimated number of species needing description (Wheeler et al., 2004). The number of nematode taxonomists has diminished to critical levels, with extinction looming on the horizon (Ferris, 1994). Institutional support for bacterial culture collections is at present incapable of accommodating even a small fraction of the yet to be catalogued specimens, and dollar estimates to see such projects to completion involve multiples of billions. But despite the unknown systematic status for the majority of nematode and bacterial taxa, nematode–bacterium entomopathogens are some of the best-studied members of these tremendously diverse groups of organisms.

It has been speculated that in the mid-Paleozoic (approximately 350 million years ago) ancestors of the Heterorhabditidae and Steinernematidae began to independently explore mutualistic relationships with Gram-negative enteric bacteria (Enterobacteriaceae), the respective lineages of which would evolve to comprise *Photorhabdus* and *Xenorhabdus* (Poinar, 1993). The resulting bacterium–nematode complex comprises

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a formidable biological control weapon against insect pests, which probably best explains the accelerated pace of systematic activity that has focused on these groups over the last 15 years. Our knowledge of bacterial and nematode biodiversity at the present is so poor that even the most sophisticated bootstrapping estimates are at best a modest grope towards reality (Boucher and Lambshead, 1995; Lambshead, 1993). In sharp contrast are the entomopathogenic bacterium–nematode complexes, which have been the subject of substantial efforts to reveal their true biological diversity and place them in a meaningful systematic framework. As dynamic processes of discovery, these research programs include sampling efforts that extend to previously unexplored regions of the globe, and evolutionary studies of their historical lineages, from their position within the tree of life, to population genetic structure. Acknowledging that the discovery of each new species renders previous statements of biodiversity obsolete and that there are considerable technological and analytical challenges and gaps that need further scrutiny, the goal of this paper is to summarize entomopathogenic bacterial–nematode biodiversity within an increasingly consistent phylogenetic, systematic framework.

2. Bacterial biodiversity

2.1. Phylogenetic diversity and distribution of major bacterial lineages

The recognition of the major lineages (named "phyla" in the domain Bacteria and "kingdoms" in the domain Archaea; only Bacteria are covered here) is mainly based on the comparative analyses of genes coding for the RNA of the small subunit of ribosomes (16S rDNA of Prokaryotes, 18S rDNA of Eukaryotes). The most comprehensive database of these sequences, ARB (Ludwig et al., 2004), contains about 70,000 aligned sequences from strains, including type material, and from DNA extracted from environmental samples of uncultured organisms. During the past 20 years, about 44 bacterial phyla have been recognized, mostly as a result of gene sequence analyses (Rappe and Giovannoni, 2003). The vast majority of described species are found in only six phyla, Proteobacteria (the majority of Gram-negative organisms), Firmicutes (Gram-positives with low mol% G + C), Actinobacteria (Gram-positives with high mol% G + C), Cyanobacteria, Flavobacteria/Bacteroides, and Spirochaeta [nomenclature according to Garrity et al. (2003)]. These lineages not only contain the most well known organisms of medical, biotechnological, and industrial interest but also the majority of as yet uncultured isolates from terrestrial and marine environments. Ten to hundred species are members of 12 additional phyla. Among these are the lineages containing the deeply rooting Thermotogales and Aquifica, the budding Planctomycetes and Verrucomicrobia, Fusobacteria, and *Deinococcus*/*Thermus* to name only a few phyla. Another six lineages contain as few as one to six mostly recently described species, whereas the majority of phyla do not contain cultured species at all. These lineages, worthy of phylum rank, emerged from molecular environmental studies, embracing putative species because of their low degree of relatedness to any described species. As nothing more than the sequence of a single gene is known (sometimes detection of cells by in situ hybridization with fluorescently labeled oligonucleotide probes allow recognition of morphologies), information on cultural and metabolic properties has not yet been elucidated. It can be assumed that the biotechnological potential of the novel strains is high, considering the phylogenetically isolated position and the sometimes-extreme environments the organisms inhabit (hot springs, peat, and alkaliphilic and acidic soils and waters, soda lakes, and anaerobic sediments).

The 16S rRNA gene sequence-based phylogeny is strongly supported by analysis of other genes with similar features which define a valuable phylogenetic marker: ubiquitous distribution, stable function, and conservative primary structure, including a mixture of variable and less variable sequence stretches. Most of these genes, coding for proteins, are named "housekeeping" genes, as they maintain basic functions in anabolic and catabolic cell processes. While some of them are truly ubiquitous and can be used to evaluate the 16S rDNA-based gene tree topology, others are phylum-specific, to be used in the evaluation of lower ranks (classes, families). The advantages of working with rRNA genes rather than genes coding for proteins are given in Table 1.

2.2. Delimitation of bacterial species

The term "species" has two levels of understanding. The first one is the concept, referring to the theoretical framework (evolution, speciation mechanisms), generality, operationality, and applicability. The second refers to the provision of practical or applied definitions. At present, a unified species concept is missing in bacteriology. Because the boundaries of bacterial species, should they exist in nature, cannot be recognized between closely related and strain-rich entities, bacteriologists have agreed to work with a definition of the taxonomic unit "species" that is arbitrary, artificial, and pragmatic (Stackebrandt, 1999). Thus, a species is defined as a genomically coherent cluster

Table 1
Comparison of properties of 16S rRNA genes and genes coding for proteins used in phylogenetic studies

Property	16S rRNA gene	Protein-coding genes
Universal	+	Some
Intracellular amplification	Up to 14 copies	one copy
Degeneration of the code	Not applicable	+
(Universal) PCR primers available	+	Rarely
Database	Large	Rare (few are substantial)
Conservative evolution	Orthologous[a]	Orthologous or paralogous[a]

[a] Orthology describes genes in different species that derive from a common ancestor, paralogy describes similar phenotype expressed by genes having a different genealogic origin.

of individuals that show a high degree of overall similarity in many independent characters and is diagnosable by a discriminative phenotype. The main criterion to delineate among such clusters is a DNA–DNA reassociation value of about 70% (Rosselló-Mora and Amann, 2001; Stackebrandt, 1999; Vandamme et al., 1996). Recent advances in molecular methodologies (discussed above) make it possible to affiliate an unknown strain with its phylogenetic relatives. Whether or not members of a species as defined today are actually monophyletic and genomically coherent units depends on the DNA similarities of neighboring species. Although genomic cohesion is obvious for single-strain species that are well separated genomically, this is not so clear for "clouds" of closely related species when the phenotypic and molecular properties may overlap. An example is given by the five subspecies of *Photorhabdus luminescens* (Thomas and Poinar) (see Section 3.3, and Hazir et al., 2004), which could also be described as individual species, based on genomic and/or phenotypic differences.

The conceptual and technical bottleneck in the artificial species definition is the DNA–DNA hybridization method in which two denatured (homologous or heterologous) DNA strands are reassociated with the hope that the degree of reassociation is indicative of genetic relatedness. The mechanism is strongly influenced by genome size, temperature, salt content, G + C content, formamide, dimethyl sulfoxide (DMSO), and intrinsic method-related factors. Evolutionary relationships cannot be given directly as reduced hybridization cannot distinguish between losses of genes in one partner and sequence divergence of genes in both partners.

Alternative methods have been introduced recently, such as DNA restriction profiling [amplified fragment length polymorphism (AFLP), multiple-locus variable number of tandem repeat analysis (MLVA)], multi-locus-sequence-typing (MLST, Maiden et al., 1998) and multiple-locus sequencing analysis (MLSA). These methods have been discussed as potential methods to replace DNA–DNA reassociation techniques in the future, once a correlation with DNA hybridization values has been established (Stackebrandt et al., 2002). Even though the genomic structure at the intra-specific level can be elucidated today, the decision at which subgroups are delineated is still arbitrary. For example, in MLST, partial sequences of seven housekeeping genes are generated and the evolutionary distance among strains quantified as the number of loci that are different. All strains that are identical with a particular strain at five or more loci are considered members of a taxonomic unit, or "ecotype". An "ecotype" is usually a genomically coherent subset of strains of a species. Species of medical interest that have been investigated with the MLST approach differ in their genomic complexity. One of the most well known studies is that on species of *Escherichia* and *Shigella*. The similarity between *Escherichia coli* (Migula) and strains of *Shigella* species was recognized earlier by DNA–DNA binding studies but sequence analysis of selected regions of housekeeping genes revealed that *Shigella* strains (except for *Shigella bodyii* Ewing) are members of *E. coli* (Lan and Reeves, 2001) allowing for a genomic affiliation of strains with a significantly higher level of confidence than by the "black-box" approach, DNA–DNA reassociation.

The recognition of a phylogenetic substructure does not immediately imply that the description of new species is a straightforward process. Complete genomes of strains are being sequenced, at least partially, at an unprecedented rate, but it will be many years before systematists will be in a position to use all of this information. Indeed, many molecular biologists and taxonomists believe that bacterial systematics will one day be based solely on the recognition of molecular patterns. However, the time has not yet come to discard morphology, metabolic properties, and other traditional approaches that have served systematists well in the last decades.

3. Biodiversity, phylogeny, and systematics of Xenorhabdus and Photorhabdus

3.1. The life cycle of Xenorhabdus and Photorhabdus

Bacteria of the genera *Xenorhabdus* and *Photorhabdus*, with the exception of *Photorhabdus asymbiotica* Fischer-Le Saux, Viallard, Brunel, Normand and Boemare associate mutualistically with nematodes of the families Steinernematidae and Heterorhabditidae, respectively. Nematodes belonging to these families are obligate insect pathogens in nature and only the third stage infective juveniles (IJs) are able to persist for a while in the environment outside of a host cadaver. The IJs carry cells of their bacterial symbiont within their intestinal tract. In *Steinernema* species, the bacteria are contained inside a specialized intestinal vesicle (Bird and Akhurst, 1983; Bovien, 1937; Forst and Clarke, 2002), whereas the bacteria of *Heterorhabditis* colonize the entire intestine (Ciche and Ensign, 2003). The bacteria persist in a quiescent state while within the nematode. However, some bacterial growth occurs inside *Steinernema* during colonization of the intestinal vesicle (Martens et al., 2003).

The IJs search or wait for a suitable insect host and gain entry into the hemocoel by invading through natural openings (mouth, spiracles, and anus) or, in *Heterorhabditis*, enter the hemocoel directly through the insect's integument (Wang and Gaugler, 1998). Upon entry into the host, the IJs recover from developmental arrest and liberate their bacterial symbionts. *Xenorhabdus* are released from the nematodes by defecation (Martens et al., 2003; Wouts, 1991), whereas *Photorhabdus* exit through the mouth (Ciche and Ensign, 2003). The bacteria and nematodes cooperate to overcome the host's immune response, allowing the bacteria to proliferate vegetatively.

Steinernema species are able to suppress the host's immune response by periodically releasing enzymes, which precede and may facilitate the release of their symbionts (Boemare and Akhurst, 1999; Wang and Gaugler, 1998).

It is unknown if *Heterorhabditis* produce similar proteins (Forst and Clarke, 2002). However, *P. luminescens* bacteria are capable of inhibiting their own phagocytosis by secreting an antiphagocytic factor (Silva et al., 2002). The bacterial symbionts multiply and colonize the host, while producing toxins and exoenzymes that result in septicemia and bioconversion of the insect cadaver (Forst and Clarke, 2002). During early infection, *Photorhabdus* specifically proliferates in the hemolymph, destroying the immune system, and in the midgut, where the bacteria release toxins and a metalloprotease (late in infection) that destroy the midgut epithelium and may facilitate bioconversion of the tissue (Bowen et al., 1998; Silva et al., 2002). *Photorhabdus* secrete a toxin encoded by the *mcf* (makes caterpillars floppy) gene that also destroys the insect's midgut and hemocytes (Daborn et al., 2002). The colonization process of host tissues for *Xenorhabdus* is not yet known, but the bacteria do lyse hemocytes by releasing endotoxins that are lipopolysaccharide (LPS) components of the cell outer membrane (Brillard et al., 2001; Dunphy and Thurston, 1990). The LPS serves a dual purpose in that it also inhibits the opsonic properties of the phenoloxidase system.

Near the end of bacterial proliferation, the symbionts produce a variety of antimicrobial compounds that protect the cadaver from colonization by other organisms. These compounds include antibiotics that are active against other bacteria, fungi, and yeasts (Akhurst, 1982; Boemare et al., 1997) and bacteriocins such as xenorhabdicin (Thaler et al., 1995) and lumicins (Sharma et al., 2002) that are active against bacteria closely related to *Photorhabdus*. Lumicins also have demonstrated activity against more distantly related enteric taxa and may play a role during infection (Sharma et al., 2002). The developing nematodes feed on the bacteria and bioconverted host tissue and reproduce in the cadaver for 1–3 generations. Once the food resources in the cadaver are exhausted, the nematodes develop a new generation of IJs that recruit bacterial cells and emerge from the host cadaver in search of a new host.

3.2. Taxonomic characterization

Poinar and Thomas (1965) described the first bacterial symbiont as a new species, *Achromobacter nematophilus*. *Achromobacter* was not accepted, and *A. nematophilus* did not fit into any of the accepted genera. This led the authors to establish a new genus, *Xenorhabdus*, to accommodate the symbionts *X. nematophilus* and *X. luminescens* (Akhurst, 1983; Thomas and Poinar, 1979). *X. luminescens* was unmistakably distinct from other *Xenorhabdus* strains by both phenotypic and molecular characters (Akhurst and Boemare, 1988), and the lack of DNA homology to other *Xenorhabdus* species supported the proposal of *Photorhabdus* as a new genus for the symbionts of *Heterorhabditis* (Boemare et al., 1993). Euzeby and Boemare (2000) revised the bacterial nomenclature of *Xenorhabdus* by feminizing the species name to correspond to the feminine *rhabdus* (i.e., *X. nematophila*). Currently, 12 species of *Xenorhabdus* and *Photorhabdus* are recognized. Additional subspecies have been identified (Akhurst et al., 2004; Hazir et al., 2004) and many isolates from recently described entomopathogenic nematode species have yet to be examined. There are nine species of *Xenorhabdus*: *X. beddingii*, *X. bovienii*, *X. budapestensis*, *X. innexua*, *X. japonica*, *X. ehlersii*, *X. nematophila* (type species) and *X. poinarii* (Akhurst and Boemare, 1988; Lengyel et al., 2005; Nishimura et al., 1994). Three species have been described for *Photorhabdus*, which include two nematode-symbiotic species and one opportunistic clinical species: *P. luminescens* subsp. *luminescens* (type species), *P. luminescens* subsp. *akhurstii*, *P. luminescens* subsp. *laumondii*, *P. luminescens* subsp. *kayaii*, *P. luminescens* subsp. *thracensis*, *P. temperata*, *P. temperata* subsp. *temperata*, and *P. asymbiotica* (clinical strain) (Fischer-Le Saux et al., 1999; Hazir et al., 2004).

3.2.1. Phenotypic characterization

Xenorhabdus and *Photorhabdus* are assigned to the family Enterobacteriaceae, which belongs to the gamma subdivision of the Proteobacteria. Members of this family are Gram-negative rods, facultatively anaerobic, negative for oxidase, nonsporeforming, chemoorganic heterotrophs with respiratory and fermentive metabolisms and with the exception of a few taxa, are motile by peritrichous flagella (Brenner, 1999). *Xenorhabdus* and *Photorhabdus* are atypical of the Enterobacteriaceae, and no other member of the family is phenotypically similar (Holt et al., 1994). For example, *Xenorhabdus* are negative for catalase, similar only to *Shigella dysenteriae* Castellani and Chalmers O group 1, and most *Xenorhabdus* and *Photorhabdus* are negative for nitrate reductase, a trait shared only by some strains of *Erwinia* and *Yersinia* (Boemare, 2002; Brenner, 1999). Primary differences between the two genera are that most *Photorhabdus* isolates are bioluminescent and catalase positive while all *Xenorhabdus* are negative for both traits (Forst et al., 1997).

Both genera produce phenotypic variant forms. The primary form (phase I) is the form naturally associated with the nematodes, whereas the secondary form (phase II) can arise spontaneously when the bacteria are in culture. There are morphological and physiological differences between the two phases. Phase I bacteria produce antibiotics, adsorb certain dyes and have intracellular inclusions composed of crystalline proteins, whereas phase II do not adsorb dyes, do not produce antibiotics, and produce intracellular inclusions inefficiently. Phases I and II have distinctly different colony morphologies. Differences in pathogenicity between the phases have been observed in some hosts (Volgyi et al., 1998). Phase I is claimed to be superior to phase II in its ability to support nematode propagation in vitro, although recent evidence suggests that this is not always the case (Ehlers et al., 1990; Volgyi et al., 1998). Reversion from phase II to phase I has only been documented with *Xenorhabdus* spp., but it has not been observed in *Photorhabdus*. Detailed information on the phenetically based taxonomy of the symbiotic bacteria is discussed in Akhurst and Boemare (1990), Forst et al. (1997), Boemare and Akhurst (1999), and Boemare (2002).

3.2.2. Molecular characterization

The determination of DNA:DNA homology values remains the cornerstone for the delineation of bacterial species, where strains of a species are recommended to share DNA:DNA relatedness values of 70% or higher and a ΔT_m of 5 °C or less (Section 2.2; Stackebrandt et al., 2002; Wayne et al., 1987). Other molecular methods may be used as long as there is a sufficient degree of congruence with DNA:DNA reassociation (Stackebrandt et al., 2002).

By using both the S1 nuclease and hydroxylapatite methods for determination of DNA similarity, Grimont et al. (1984) separated *Xenorhabdus* into 3 DNA relatedness groups, which include the present *Photorhabdus* genus and two species within *Xenorhabdus*. These findings were supported by DNA relatedness studies (Boemare et al., 1993). Species which had been determined by phenotypic data (Akhurst and Boemare, 1988) were confirmed with DNA:DNA homology values, and the new genus *Photorhabdus* was proposed (Boemare et al., 1993).

Subsequently, based on DNA similarity of numerous strains, the genus *Photorhabdus* was found to be a more homogenous genus than the more species-rich *Xenorhabdus*. *Photorhabdus* forms two distinct groups: *Photorhabdus* clinical strains and *Photorhabdus* nematode-symbiotic strains (Akhurst et al., 1996). However, the symbiotic group appeared to be fairly heterogeneous (Akhurst et al., 1996; Forst et al., 1997), and the phylogenetic analyses of 16S rRNA gene sequences provided additional evidence for heterogeneity among the symbionts (Szállás et al., 1997).

Fischer-Le Saux et al. (1999) conducted a polyphasic, comprehensive approach for the description of species within *Photorhabdus*, which included phenotypic characterization, 16S rRNA analysis and examination of DNA relatedness. It was determined that *Photorhabdus* consists of three species: two symbiotic species, *P. luminescens* and *P. temperata*, and one clinical species, *P. asymbiotica*. The symbiotic species were subdivided into subspecies *P. luminescens luminescens*, *P. luminescens akhurstii*, *P. luminescens laumondii*, and *P. temperata temperata*. Although DNA similarity values persist as the main criterion for determining bacterial species, a polyphasic approach is a superior method for species descriptions. All species descriptions should now include the 16S rDNA sequence and phenotype, including chemotaxonomic characters (Stackebrandt et al., 2002).

Molecular methods can be employed to determine diversity among bacteria or used for rapid identification of a bacterium in question so as to avoid laborious phenotypic characterization. Restriction analysis of PCR amplified gene products and riboprinting are methods that are often used for these purposes (i.e., Szállás et al., 2001). *Xenorhabdus* and *Photorhabdus* can be quickly and accurately identified on the basis of restriction fragment length polymorphisms of the 16S rRNA gene sequence (Bonifassi et al., 1999; Brunel et al., 1997; Fischer-Le Saux et al., 1998). These data also support the taxonomic descriptions of the bacteria. Phylogeny based on 16S rRNA sequences has also been used to identify *Xenorhabdus* strains (Liu et al., 2001).

3.3. Phylogenetic systematics

Fig. 1 depicts the evolutionary relationships of members of the family Enterobacteriaceae (Francino et al., 2003). The phylogenetic tree was constructed using the 16S small subunit rDNA sequences and rooted using the sequence from *Vibrio cholerae* Pacini, a member of the family Vibrionaceae, also part of the gamma subclass of Proteobacteria. *X. nematophila* and *P. asymbiotica* form a monophyletic group with *Proteus* being their closest sister taxon. *X. nematophila* and *P. asymbiotica* have long branch lengths relative to the other taxa that comprise the phylogeny, which is supported by phenotypic characterization. Francino et al. (2003) provide more information on the evolutionary relationships among additional taxa within the Enterobacteriaceae.

To date, only phylogenies constructed using 16S rRNA gene sequence data have been published for analyzing the inter- and intrageneric relationships of *Xenorhabdus* and *Photorhabdus* (Fischer-Le Saux et al., 1999; Hazir et al., 2004; Lengyel et al., 2005; Liu et al., 2001; Liu et al., 1997; Marokhazi et al., 2003; Rainey et al., 1995; Suzuki et al., 1996; Szállás et al., 1997). The phylogenetic tree in Fig. 2 displays the relationships among species of *Xenorhabdus* (Lengyel et al., 2005). This tree is unrooted, making evolutionary inference tenuous. *Xenorhabdus* are distinguished from their neighbor, *Photorhabdus*, by the sequence TTCG at positions 208–211 of the 16S rDNA sequence (Boemare and Akhurst, 1999). Fig. 3 presents the phylogenetic relationships among the presently recognized species and subspecies of *Photorhabdus* (Hazir et al., 2004). Although branch support is weak for the majority of nodes in the

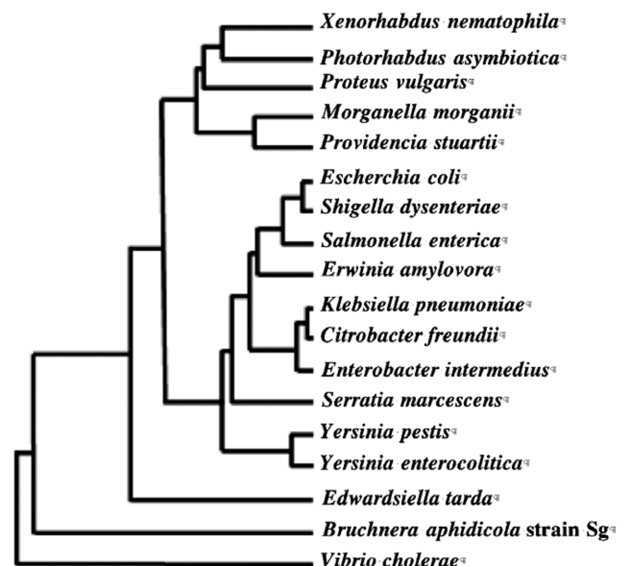

Fig. 1. Phylogenetic tree of members of the family Enterobacteriaceae based on 16S (small subunit) rDNA sequences. Modified after Francino et al. (2003).

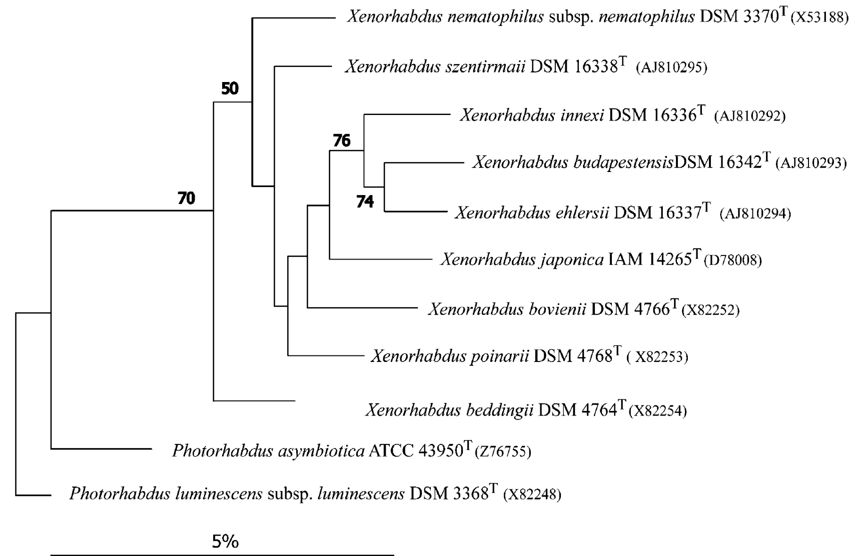

Fig. 2. Dendrogram of *Xenorhabdus*16S rRNA gene sequence similarities generated by distance matrix analysis on the basis of Jukes and Cantor corrections. Bootstrap values (100 replicates) where greater than 50% shown are shown at nodes.

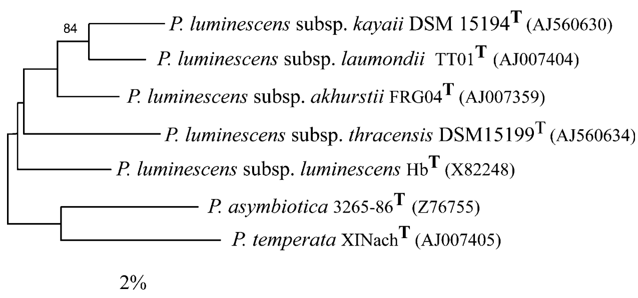

Fig. 3. Neighbor joining dendrogram of 16S rRNA gene similarities between type strains of the genus *Photorhabdus* species and subspecies. Numbers at nodes indicate percentage of bootstrap resampling, derived from 1000 replicates (only values >80% shown). Bar indicates 2% sequence divergence. Accession numbers of 16S rRNA gene sequences appear in parentheses.

tree, the terminal taxa correspond closely with DNA homology groups (Fischer-Le Saux et al., 1999). Although the tree is unrooted, in this scenario *P. asymbiotica* diverges from the other *Photorhabdus* as sister taxon to *P. temperata*, suggesting that the asymbiotic species may have arisen from a nematode-associated ancestor.

Use of ribosomal subunit sequences for determining phylogenetic relationships has limitations. These genes can undergo lateral gene transfer across taxonomic groups or can be recombined, which could provide false evolutionary data (Yap et al., 1999). Therefore, bacterial phylogeny based solely on 16S rDNA sequence should be regarded as preliminary (Lerat et al., 2003). Dauga (2002) demonstrated that phylogeny based on the *gyr*B gene provided a more robust tree for determining intrageneric relationships among *Serratia* spp., whereas the 16S rDNA gene was effective for determining phylogenetic relationships among more distantly related enteric bacteria. To resolve the intrageneric relationships for both *Xenorhabdus* and *Photorhabdus*, more robust trees need to be developed by comparing sequences from additional strains for carefully selected protein-coding gene sequences.

3.4. Gnotobiology

Taxonomic studies have established that each entomopathogenic nematode is associated with a specific bacterial symbiont. In nature, the nematode develops and reproduces in a monoxenic environment that is established by its symbiont within an insect cadaver. Gnotobiological techniques have been employed to investigate the level of specificity between the nematode host and its symbiont. Accordingly, nematodes are freed from their symbiont and then associated with a bacterium of interest, either the natural symbiont or another potential partner. With this approach it is possible to rear *Steinernema* axenically, but an artificial diet has not yet been developed for *Heterorhabditis* (Boemare et al., 1997). However, bacteria-free infective juveniles of *Heterorhabditis bacteriophora* Poinar and *Heterorhabditis indica* Poinar, Karunakar and David can be produced by culturing the nematodes on *P. temperata* and the symbiont of *H. bacteriophora* H06, respectively (Han and Ehlers, 1998). Otherwise, surface-sterilized eggs harvested from *Heterorhabditis* sp. are compatible only with their symbiont (Boemare et al., 1997). Due to the specificity of the host–symbiont relationship, heteroxenic associations are difficult to establish (Boemare et al., 1997; Gerritsen and Smits, 1993). Specificity is determined on three levels: recovery of the IJ, nutritive properties to support nematode reproduction and development, and retention of the bacteria in the intestinal tract of the IJ (Grewal et al., 1997; Han and Ehlers, 1998).

Grewal et al. (1997) reported that a food signal was secreted in the cell-free filtrate of the *Steinernema scapterisci* Nguyen and Smart symbiont. Although *S. scapterisci* is able to use and retain symbionts of other steinernematids, their recovery was delayed but improved upon supplementation

with the cell-free filtrate of its natural symbiont (Grewal et al., 1997), indicating a high degree of specificity between the nematode and its symbiont for optimal IJ recovery.

Strauch and Ehlers (1998) reported that an unknown food signal is required for recovery of *Heterorhabditis* and is secreted in the medium by the bacterial symbiont. The food signal appears to be a common signal recognizable by *H. bacteriophora*, since in most cases it is able to recover in response to both *P. luminescens akhurstii* and *P. temperata* (Han and Ehlers, 1998). Han and Ehlers (1998) established that the signals responsible for nematode recovery are not the same compounds necessary for completion of the life cycle. *P. luminescens* bacteria isolated from *H. indica* are nutritionally incompatible for *H. bacteriophora* H06, and the nematodes are unable to develop (Han and Ehlers, 1998). However, Gerritsen et al. (1998) produced a successful monoxenic combination of *Heterorhabditis megidis* Poinar, Jackson and Klein with *P. luminescens*. The nematodes could use an alternative symbiont, but virulence was reduced (Gerritsen et al., 1998). The best nutritional condition for nematode growth is not necessarily established by its indigenous symbiont, but nematode growth appears to be unrelated to nematode virulence (Han et al., 1991).

Some combinations of nematodes with nonsymbionts support nematode development, but then fail to maintain long-term associations (Akhurst and Boemare, 1990; Ehlers et al., 1990; Han and Ehlers, 1998). Other bacteria may produce conditional associations with a nematode, but the symbiont always appears to be the most efficient partner for pathogenicity, reproduction, and development of the nematode (Bonifassi et al., 1999). At present, our understanding of the nematode–bacterium symbiosis is insufficient, and requires further study to explain the provisions given by the symbiont and the durability of the association over many generations (Boemare, 2002; Bonifassi et al., 1999).

3.5. Associated microbial species

Associations between entomopathogenic nematodes and their bacterial symbionts are summarized in Table 2. Each steinernematid nematode shares a mutualistic relationship with a single bacterial species, yet some *Xenorhabdus* spp. are associated with more than one nematode species. These multiple host relationships include *X. beddingii* Akhurst, the natural symbiont of *Steinernema longicaudum* Shen and Wang, and an undetermined species of *Steinernema*; *X. bovienii*, the symbiont of *Steinernema affine* (Bovien), *Steinernema feltiae* (Filipjev) *Steinernema intermedium* (Poinar), and *Steinernema kraussei* (Steiner); and *X. poinarii* Akhurst and Boemare, which is associated with both *Steinernema glaseri* (Steiner) and *Steinernema cubanum* (Mrácek, Hernandez and Boemare) (Boemare, 2002). On the contrary, *H. bacteriophora* strains NC1, Brecon, and HP88 are associated with two different species and two different subspecies of *Photorhabdus*, which are *P. temperata, P. luminescens* ssp. *luminescens*, and *P. luminescens* ssp. *laumondii*, respectively. Most of the symbiotic bacteria of described nematode species have yet to be characterized and identified.

The specificity between the nematode and its bacterial resident is a result of the exclusion of bacterial competitors and a specific recruitment of its symbiont upon exiting the insect. Both *Xenorhabdus* and *Photorhabdus* produce bacteriocins, which are proteins that have antimicrobial activity against closely related strains or species of bacteria. The occurrence of bacteriocins is useful for symbiosis so that the natural symbiont can outcompete closely related bacteria (Boemare et al., 1997). Xenorhabdicin is a bacteriocin isolated from *X. nematophila* that has antibiotic activity against other *Xenorhabdus* spp., *P. luminescens*, and species of *Proteus* (Thaler et al., 1995). Bacteriocins called lumicins have been isolated from *P. luminescens* (Sharma et al., 2002). Lumicins have proven to be active against other *Photorhabdus* spp. as

Table 2
Taxonomic correspondence of symbiotic bacterium to host nematode(s)

Bacterium	Host nematode(s)
Xenorhabdus/Steinernema	
X. nematophila (Poinar and Thomas, 1965) Thomas and Poinar, 1979	*S. carpocapsae*
X. bovienii (Akhurst, 1983) Akhurst and Boemare (1993)	*S. affine*, *S. feltiae*, *S. intermedium*, *S. kraussei*
X. poinarii (Akhurst, 1983) Akhurst and Boemare (1993)	*S. glaseri*, *S. cubanum*
X. beddingii (Akhurst, 1986) Akhurst and Boemare (1993)	*S. longicaudum*
X. japonica Nishimura et al., 1994	*S. kushidai*
X. budapestensis Lengyel et al., 2005	*S. bicornutum*
X. ehlersii Lengyel et al., 2005	*S. serratum*
X. innexi Lengyel et al., 2005	*S. scapterisci*
X. szentirmaii Lengyel et al., 2005	*S. rarum* (Cordoba, Argentina)
Photorhabdus/Heterorhabditis	
P. luminescens (Thomas and Poinar, 1979) Boemare et al., 1993; subsp. *luminescens* Fischer-Le Saux et al., 1999	*H. bacteriophora* Brecon
P. luminescens subsp. *akhurstii* Fischer-Le Saux et al., 1999	*H. indica*
P. luminescens subsp. *laumondii* Fischer-Le Saux et al., 1999	*H. bacteriophora* HP88
P. temperata Fischer-Le Saux et al., 1999	*H. zealandica*, *H. bacteriophora* NC1, *H. megidis* (Nearctic strains)
P. temperata subsp. *temperata* Fischer-Le Saux et al., 1999	*H. megidis* (Palearctic strains)
P. luminescens subsp. *kayaii* Hazir et al., 2004	*H. bacteriophora* (grassland or clover fields, Turkey)
P. luminescens subsp. *thracensis* Hazir et al., 2004	*H. bacteriophora* (sunflower field, fallow field or pine forest, Turkey)

well as distantly related bacteria such as *E. coli* (Migula), suggesting that these bacteriocins may be involved in clearing the insect gut microflora and are not just active against competing symbionts (Sharma et al., 2002).

The primary form of *X. nematophila* produces mannose-resistant fimbrae, resembling the fimbrae of *Proteus mirabilis* Hauser (Forst and Clarke, 2002; Moureaux et al., 1995). Fimbrae are surface appendages that are thought to be involved in host-specific colonization. The secondary form of *X. nematophila* does not produce fimbrae, which may account for its reduced ability to colonize the nematode (Forst and Clarke, 2002). Recently, the entire genome of *P. luminescens* TT01 was sequenced, and it was found to have a large repertoire of fimbrial genes (Duchaud et al., 2003). Among the fimbrial genes were two gene clusters coding for proteins similar to the mannose-resistant fimbrae as well as pili similar to *E. coli* and *Salmonella enterica* (Gaertner) which may help the bacteria colonize the nematode gut and invade insect tissues (Duchaud et al., 2003). Genes have been identified in *X. nematophila* that are required for mutualistic interactions between the host and bacterium. The transcription factor, σ^s, which controls regulons that can mediate stress resistance, survival or host interactions, is required for mutualism; for when this gene was disrupted, bacteria were unable to colonize the vesicle (Vivas and Goodrich-Blair, 2001). The gene product may be involved in regulating other colonization functions (Heungens et al., 2002). Several other genes encoding proteins that inhibit the symbiotic interactions have been identified as well. Among these are genes that encode regulatory proteins, biosynthetic proteins, an outer membrane protein, and novel proteins that may have a specific role in allowing the bacterium to colonize the nematode (Heungens et al., 2002).

Despite the fact that the entomopathogenic nematodes share an exclusive mutualistic relationship with their respective bacteria, it should be noted that occasionally other bacteria are isolated from the nematode or the host insect post infection. These bacteria include *Pseudomonas aeruginosa* (Schroeter), *Ochrobactrum* spp., *Acinetobacter* spp., and *Providencia rettgeri* Hadley and are generally associated with the cuticle of the nematode (Aguillera et al., 1993; Jackson et al., 1995; Lysenko and Weiser, 1974; Walsh and Webster, 2003). *Ochrobactrum* spp. were found to naturally associate with *Photorhabdus luminescens* subsp. akhurstii (Babic et al., 2002). Although *Xenorhabdus* spp. produce antimicrobial compounds, *Acinetobacter* was found to be resistant and could proliferate in the host insect (Walsh and Webster, 2003). Therefore, it may be possible for the nematodes to transport other nonsymbiotic bacteria, and these unnatural associations may be detrimental to the symbiotic relationship (Bonifassi et al., 1999; Walsh and Webster, 2003). A recently described species, *Paenibacillus nematophilus* Enright, establishes an enduring relationship with *Heterorhabditis* spp. (Enright and Griffin, 2004, 2005; Enright et al., 2003). This species of *Paenibacillus* (and probably others yet to be described) adheres to the surface of the nematode and is capable of overcoming the antibiotics produced by the endogenous *Photorhabdus* symbiont, reproducing in sympatry inside the host cadaver.

4. Systematics, diversity, and biogeography of entomopathogenic nematodes

4.1. Diversity and phylogenetic position of the Nematoda

The Nematoda are tremendously diverse and abundant (Wall and Virginia, 2000; Wall et al., 2001). Estimates of the number of species in the phylum range from 400,000–10,000,000 (Hammond et al., 1995) to as high as 100,000,000 (Lambshead, 1993), on par with some of the higher insect estimates (Erwin, 1991).

The phylogenetic position of the Nematoda relative to other metazoans, and among metazoans in general, is currently controversial and hotly contested. Challenging old ideas such as the Vermes, erected by Linnaeus in 1758, and later the Aschelminthes of Grobben (Claus and Grobben, 1910) is the idea that nematodes belong in a clade of molting animals, the Ecdysozoa (Aguinaldo et al., 1997). Thus, nematodes have been hypothesized to share a most recent common ancestor with arthropods, kinorhynchs, nematomorphs, onychophorans, priapulids, and tardigrades [but see De Ley and Blaxter (2002) for discussion of early classifications which also advocated relationships between nematodes and other Ecdysozoan phyla]. Although recognition of the Ecdysozoa comes primarily from analyses of molecular data, considerable effort is underway to identify and scrutinize morphological and developmental synapomorphies (Copley et al., 2004; Nielsen, 2003; Schmidt-Rhaesa, 2003; Telford, 2004). Questions concerning the validity of the Ecdysozoa have emerged from analyses that have emphasized character sampling over taxon sampling and analytical rigor (Hedges, 2002; Wolf et al., 2004). But as more thoughtful analyses have emerged, the placement of nematodes within the Ecdysozoa appears to stand up as the best-supported hypothesis (Copley et al., 2004; Giribet and Wheeler, 1999; Giribet, 2003; Mallatt et al., 2004; Mallatt and Winchell, 2002).

4.2. Entomopathogenic nematode taxonomy

Recent years have seen an increased interest in studying entomopathogenic nematodes (EPN) not only because of their biological control potential but also to answer other research questions in the fields of ecology, biodiversity, evolution, biochemistry, symbiosis, and molecular genetics (Burnell and Stock, 2000). More than half of the currently recognized EPN species have been described since 1995 (Figs. 4 and 5).

At the First International EPN Conference in Asilomar, California in 1990, confusion and frustration with nomenclatural changes and nematode variability contributed to the consideration of the taxonomy of entomopathogenic nematodes as “in a state of flux” (Akhurst, 1995). Five years later, at the Second International EPN Conference held in Honolulu, Hawaii, when molecular characters and cross-fertiliza-

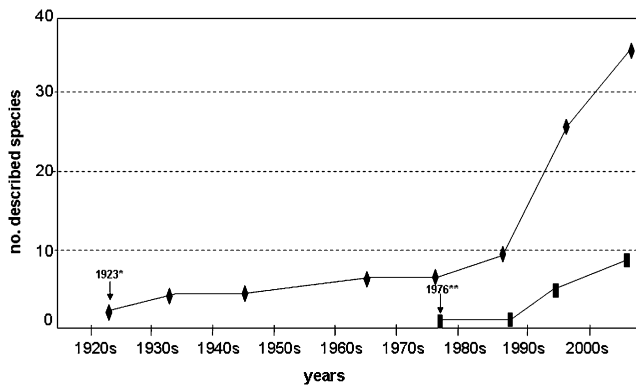

Fig. 4. Historical growth in the description of entomopathogenic nematode taxa. (1) First International Nematode Conference (Asilomar, CA). (2) Second International Nematode Conference (Honolulu, HW). (3) Third International Nematode Conference (Wooster, OH); ◆, steinernematids; ■, heterorhabditids; *, description of first steinernematid; **, description of first heterorhabditid.

tion methods had just started being incorporated into species descriptions, EPN taxonomy was described as "in a state of transition" (Powers et al., 1995). Now, almost a decade after the Hawaii meeting, numerous events have led to much improved taxonomic statements, including: (1) a standardization of criteria for species descriptions (Hominick et al., 1997); (2) proposal of name emendations (Adams and Nguyen, 2002; Hominick et al., 1997); (3) an interpretation of phylogenetic relationships in the phylum Nematoda based on molecular evidence (Blaxter et al., 1998); (4) a theoretical and applied concept of species (Adams, 1998, 2001); and an updated classification for Nematoda (De Ley and Blaxter, 2002). Contributions such as these have placed EPN systematics in a phase of stability and growth.

Perhaps the greatest contributions to systematic stability arose from the inclusion of phylogenetic hypotheses in taxonomic statements. Blaxter et al.'s (1998) molecular phylogenetic framework for the Nematoda depicted the Heterorhabditidae as being most closely related to the Strongylida, a group of parasites of vertebrates that shares a most recent common ancestor with *Pellioditis*, a free-living bacterivore. The same hypothesis depicted the Steinernematidae as being most closely related to the Panagrolaimoidea (free-living and insect associates) and Strongyloididae (vertebrate parasites), and as a member of a larger clade that includes free-living, fungal-feeding, and plant parasitic taxa. This phylogenetic study supports Poinar's (1993) hypothesis that the heterorhabditids probably arose from a free-living bacterivorous ancestor, while for steinernematids, reconstruction of the trophic habits of the ancestors remains ambiguous (Blaxter et al., 1998).

This molecular framework and some of the later work that builds on it served as the basis for the most recent classification of Nematoda by De Ley and Blaxter (2002). This new classification scheme places the Steinernematidae within the suborder Tylenchina, which also includes insect parasitic allantonematids and neotylenchids. The Heterorhabditidae were positioned within the suborder Rhabditina which includes, among others, free-living Rhabditidae and animal parasitic Strongylidae.

The most recent taxonomic account for the Steinernematidae recognizes *Steinernema* as the type genus with 40 recognized species. The second genus, *Neosteinernema* contains only one species, *N. longicurvicauda* (Table 3).

The Heterorhabditidae contain a single genus, *Heterorhabditis*, with 10 currently recognized species. However, two of these taxa, *H. brevicaudis* and *H. poinari* are species *inquirendae* based on their incomplete morphological descriptions and lack of molecular and cross-breeding supporting data (Adams and Nguyen, 2002; Stock and Hunt, 2005) (Table 4).

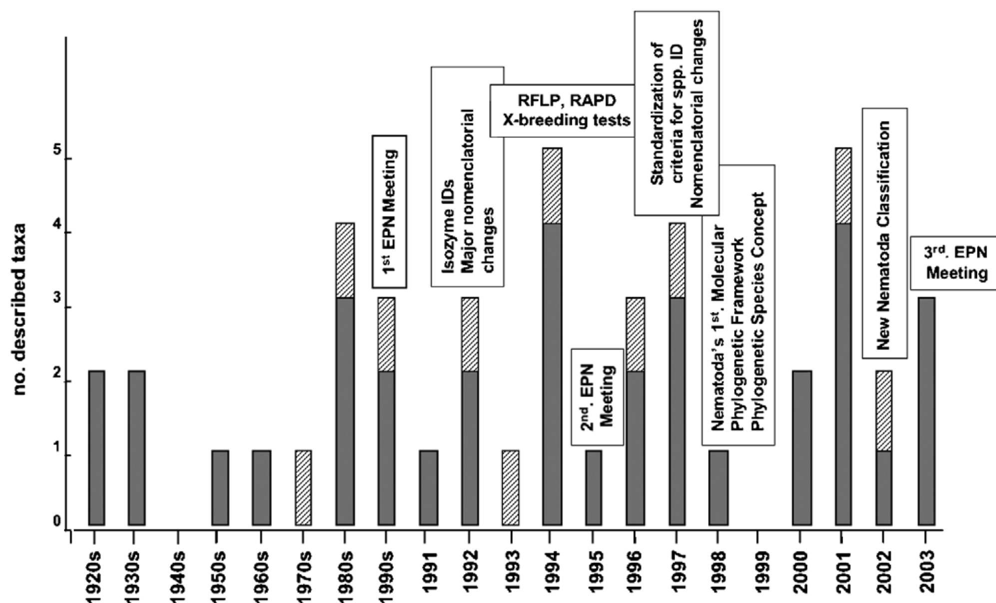

Fig. 5. Major events in entomopathogenic nematode systematics. History of events in entomopathogenic nematode systematics. ▭, steinernematids; ▨, heterorhabditids.

Table 3
Taxonomic summary of the family Steinernematidae and their authorities

Family: Steinernematidae Chitwood and Chitwood, 1937
Type genus: *Steinernema* Travassos, 1927
Type species: *S. kraussei* (Steiner, 1923) Travassos, 1927
Syn. *Aplectana krausseei* Steiner, 1923

Other species:
- *S. abbasi* Elawad et al., 1997
- *S. aciari* Qiu et al., 2005
- *S. affine* (Bovien, 1937) Wouts et al., 1982
- *S. anatoliense* Hazir et al., 2003
- *S. arenarium* (Artyukhovsky, 1967) Wouts et al., 1982
- *S. asiaticum* Anis et al., 2002
- *S. apuliae* Triggiani et al., 2004
- *S. bicornutum* Tallosi et al., 1995
- *S. carpocapsae* (Weiser, 1955) Wouts et al., 1982
- *S. caudatum* Xu et al., 1991
- *S. ceratophorum* Jian et al., 1997
- *S. cubanum* Mrácek et al., 1994
- *S. diaprepesi* Nguyen and Duncan, 2002
- *S. feltiae* (Filipjev, 1934) Wouts et al., 1982
- *S. glaseri* (Steiner, 1929) Wouts et al., 1982
- *S. guangdongense* Qiu et al., 2004
- *S. intermedium* (Poinar, 1985) Mamiya, 1988
- *S. hermaphroditum* Stock, Griffin and Chaenari, 2004
- *S. jollieti* Spiridonov et al., 2004
- *S. karii* Waturu et al., 1997
- *S. kushidai* Mamiya, 1988
- *S. loci* Phan et al., 2001a
- *S. longicaudum* Shen and Wang, 1991
  - Syn. *S. serratum* Liu, 1992
- *S. monticolum* Stock et al., 1997
- *S. neocurtillae* Nguyen and Smart, 1992
- *S. oregonense* Liu and Berry, 1996b
- *S. pakistanense* Shahina et al., 2001
- *S. puertoricense* Román and Figueroa, 1994
- *S. rarum* (de Doucet, 1986) Mamiya, 1988
- *S. riobrave* Cabanillas et al., 1994
- *S. ritteri* de Doucet and de Doucet, 1990
- *S. robustispiculum* Phan et al., 2005
- *S. sangi* Phan et al., 2001b
- *S. scapterisci* Nguyen and Smart, 1990
- *S. scarabaei* Stock and Koppenhöfer, 2003
- *S. siamkayai* Stock et al., 1998
- *S. tami* Van Luc et al., 2000
- *S. thanhi* Phan et al., 2001a
- *S. thermophilum* Ganguly and Singh, 2000
- *S. websteri* Cutler and Stock, 2003
- *S. weiseri* Mrácek et al., 2003

Genus: *Neosteinernema* Nguyen and Smart, 1994
Type and only species: *Neosteinernema longicurvicauda* Nguyen and Smart, 1994

Table 4
Taxonomic summary of described *Heterorhabditis* species

Type and only genus: *Heterorhabditis* Poinar, 1976
Syn. *Chromonema* Khan et al., 1976
Type species: *H. bacteriophora* Poinar, 1976
Syn. *Chromonema heliothidis* Khan et al., 1976
H. heliothidis (Khan et al., 1976) Poinar et al., 1977
H. argentinensis Stock, 1993

Other species:
- *H. baujardi* Phan et al., 2003
- *H. brevicaudis* Liu, 1994[a]
- *H. downesi* Stock et al., 2002
- *H. indica* Poinar et al., 1992
  - Syn. *H. hawaiiensis* Gardner et al., 1994
- *H. marelatus* Liu and Berry, 1996a,b,c
  - Syn. *H. hepialius* Stock et al., 1996
- *H. megidis* Poinar et al., 1987
- *H. mexicana* Nguyen et al., 2004
- *H. poinari* Kakulia and Mikaia, 1997[a]
- *H. taysearae* Shamseldean et al., 1996
- *H. zealandica* Poinar, 1990

[a] Species *inquirenda*.

4.3. Methodology: Species identification and diagnosis

With the increasing number of described species, traditional approaches such as comparative morphology have become of limited utility for EPN taxonomy and/or diagnosis. Morphological limitations arise from: (1) the lack of morphological variation, particularly for taxa that are recently diverged or closely related (i.e., *Heterorhabditis* species), and (2) most of the characters used for species identification are only good for diagnostic purposes and lack phylogenetic information (they represent either plesiomorphic states or are highly homoplasious) (Stock, 2002; Stock and Reid, 2003). Applying the biological species concept via cross-hybridization methods has also been questioned, mainly because it is a labor and time intensive task, and because the outcomes may have little evolutionary meaning (Adams, 1998). Additionally, the discovery of hermaphroditism in steinernematids by Griffin et al. (2001) has set a "caution signal" for the consideration of hybridization assays to test the validity of biological species in this group.

To overcome these difficulties, a number of molecular methods have been investigated as potential substitutes or complements to traditional morphological approaches. Techniques such as protein electrophoresis have been considered good at the species level, particularly in the identification of sibling species, but have little use above the level of genus or for separating organisms at or below the level of subspecies (Akhurst, 1987). Restriction fragment length polymorphisms (RFLPs) have shown to be good diagnostic tools for discriminating *Steinernema* species (Hominick et al., 1997; Pamjav et al., 1999; Reid, 1994; Reid and Hominick, 1992; Stock et al., 1998; Triga et al., 1999). They have widely been applied as a complementary approach to morphological descriptions of uncharacterized *Steinernema* spp. and also to estimate phylogenetic relationships among species in this genus.

Random amplified polymorphic DNA (RAPD) methods have also been considered for diagnoses and to assess phylogenetic relationships of EPN (Gardner et al., 1994; Liu and Berry, 1996c). Moreover, they have also been considered to measure genetic variability among *Heterorhabditis* and *Steinernema* isolates and species (Hashmi et al., 1996). However, RAPDs are not widely used, mainly because the reproducibility of results can be affected by many factors, such as the quality and concentration of DNA and PCR cycling conditions (including type of PCR machine used).

More recently, nucleotide sequence analysis has proven to be a good tool not only for diagnostics at different taxonomic levels, but also for providing valuable data for phylogenetic studies (Adams et al., 1998; Nguyen et al., 2001, 2004; Stock et al., 2001). Microsatellite DNA sequences have been proposed as diagnostic tools for identifying *Heterorhabditis* and *Steinernema* at the population level, but they have yet to be employed successfully (Grenier et al., 1997).

Although molecular techniques have provided a tremendous amount of objective data towards EPN systematics, they too can produce spurious results, even when care is taken to use and analyze them appropriately. It would be a mistake to replace classical morphological approaches with molecular methods. Research programs in systematic biology have shown convincingly that consideration of both morphological and molecular approaches lend the greatest explanatory power to investigators (Kluge, 1998, 2004). Together, morphological and molecular data will continue to provide a more comprehensive view of EPN evolution, and more robust taxonomic statements (Stock and Reid, 2003).

4.4. Phylogenetic systematics

Several approaches have been used to assess the evolutionary relationships of EPN. Early studies of phylogenetic relationships of EPN included PCR RFLP analyses of the ITS repeat unit of rDNA region, first considering both EPN families (Reid, 1994), and later focusing only on Steinernematidae (Reid et al., 1997). RAPD and morphological data have also been considered for assessment of evolutionary relationships in Steinernematidae (Liu and Berry, 1996c). However, these methods are of limited utility for reconstructing evolutionary histories of EPN due to several factors, including insufficient number of phylogenetically informative characters, tenuous information content and homology inference of the characters, or analytical methods that perform poorly at inferring evolutionary history (Stock et al., 2001). Moreover, taxon sampling has also been problematic, as less than half of the described *Steinernema* spp. were considered in early studies.

Recently, nucleotide sequence analyses of different nuclear (18S, ITS, and 28S) and mitochondrial genes (ND4) have been employed to assess evolutionary relationships of EPN at different taxonomic levels (Adams et al., 1998; Liu et al., 1999; Nguyen et al., 2001, 2004; Stock et al., 2001; Szalanski et al., 2000). Within the Steinernematidae, the study conducted by Stock et al. (2001), based on 28S rDNA sequences and morphological characters illustrates how the combined use molecules and morphology can be applied to address several aspects of EPN systematics. This study not only developed a framework for interpreting evolutionary relationships among *Steinernema* spp., but also considered the analysis of character evolution patterns for diagnostic morphological traits. Moreover, all terminal taxa considered in this study were delimited as entities with individual evolutionary histories (each of them with unique autapomorphies) based on the phylogenetic species concept.

The internal transcribed spacer region of the rDNA cistron (ITS, including the 5.8S gene) has also been used to assess phylogenetic relationships and delimit species of several *Steinernema* spp. (Nguyen et al., 2001). The ITS region might only be useful for resolving relationships among closely related *Steinernema* species, and is perhaps too variable to reliably infer relationships among all species in this genus (Nguyen et al., 2001; Stock et al., 2001). However, more extensive taxon and geographic sampling needs to be conducted to test the utility of this marker to adequately address the nature of variability within and among individuals and populations of *Steinernema* (Stock and Reid, 2003).

4.5. Biodiversity and biogeography

4.5.1. Patterns of geographic distribution

At a global scale, both families of EPN are nearly ubiquitous, as they have been found on all continents except Antarctica (Hominick, 2002; Hominick et al., 1996). Table 5 lists the continental distributions of nominal taxa. Note that the distributions as presented in Table 5 ignore historical relationships among areas and are most likely highly correlated with the effort, trained scientist-hours, and expenditure invested in searching for species.

4.5.2. Patterns of habitat distribution

Before 1995, most published surveys had insufficient data to test for correlations between habitat variables and EPN populations (Hominick et al., 1996). However, as more surveys occur, providing larger sample sizes and accurate identification, habitat associations of certain species have become more apparent. In all cases it is important to keep in mind that factors such as soil type, availability of suitable hosts, and physiological and behavioral adaptations are key factors affecting the distribution of different taxa.

In a broad sense, heterorhabditids have been isolated primarily from sandy coastal soils. Some taxa have greater presence in more calcareous soils (i.e., *H. indica* in Guadaloupe), or more acidic soils (*H. bacteriophora* and *Heterorhabditis marelatus*), whereas other species range beyond coastal regions (*H. bacteriophora*) and are broadly distributed in turf and weedy habitats (*H. megidis*) (Constant et al., 1998; Stock et al., 1996; Stuart and Gaugler, 1994) and tropical forests (Phan et al., 2003).

Prevalence of steinernematids seems to be highest in woodlands (Hominick et al., 1996). Recent extensive and intensive surveys conducted in Europe and the USA have revealed habitat associations for several steinernematids (Hominick et al., 1995; Stock et al., 1999; Sturhan, 1999; Sturhan and Liskova, 1999). *S. feltiae* is common in grass-

Table 5
Entomopathogenic nematode species diversity by continent based on published accounts of described species and their distributions

Species	Continent	% Diversity[a]
S. bicornutum, *S. carpocapsae*, *S. cubanum*, *S. diaprepesi*, *S. feltiae*, *S. glaseri*, *S. intermedium*, *S. kraussei*, *S. longicaudum*, *S. neocurtillae*, *S. puertoricense*, *S. oregonense*, *S. rarum*, *S. riobrave*, *S. scarabaei*	North and Central America	40.5
Neosteinernema longicurvicauda		100
H. bacteriophora, *H. indica*, *H. marelatus*, *H. megidis*		40
S. carpocapsae, *S. feltiae*, *S. glaseri*, *S. rarum*, *S. ritteri*, *S. scapterisci*	South America	16.2
H. bacteriophora		10
S. abbasi, *S. anatoliense*, *S. carpocapsae*, *S. caudatum*, *S. feltiae*[b], *S. glaseri*, *S. hermaphroditum*, *S. kushidai*, *S. longicaudum*, *S. loci*, *S. monticolum*, *S. pakistanense*, *S. sanghi*, *S. thanhi*, *S. thermophilum*, *S. siamkayai*, *S. websteri*	Asia	43.2
H. brevicaudis, *H. baujardi*, *H. indica*, *H. megidis*		40
S. affine, *S. arenarium*, *S. bicornutum*, *S. carpocapsae*, *S. feltiae*, *S. glaseri*, *S. intermedium*, *S. kraussei*, *S. weiseri*	Europe	24.3
H. bacteriophora, *H. downesi*, *H. megidis* (NW European type)		20
S. carpocapsae, *S. feltiae*, *S. longicaudum*	Australia	8.1
H. bacteriophora, *H. indica*, *H. zealandica*		30
S. karii	Africa	2.7
H. bacteriophora, *H. taysearae*		20

S., *Steinernema*; *H.*, *Heterorhabditis*.
[a] Percentage diversity is based on current number of described species.
[b] Species identity needs further confirmation.

lands and woodlands (Hominick, 2002). This species and *S. affine* are the only steinernematids so far recorded from arable soils in Germany (Sturhan, 1999). *S. kraussei* and *S. intermedium* are mainly forest/woodland species. *S. kraussei* has mainly been found in coniferous forests in Europe and North America (USA and Canada) on both the east and west coasts (Sturhan, 1999; Sturhan and Liskova, 1999).

5. Conclusions

The global biodiversity of bacteria and nematodes is tremendous, yet only a tiny fraction of this diversity consists of described species, and an even smaller subset of taxa exists for which phylogenetic hypotheses have been generated. Viewed in this light it would appear that the phylogenetic systematics of EPNs and their bacterial symbionts is complete, with little more to do than add a few more taxa every so many years. This statement is betrayed by the fact that the rate at which new EPNs and their bacterial endosymbionts are described shows no sign of slowing, and that only an extremely small portion of the globe has been intensively sampled for them. Advances in the concepts of nematode and bacterial species and the exploration of multiple sources of molecular genetic and phenotypic data will contribute to an increasingly stable systematic framework. Such a framework can in turn be utilized by many other disciplines that require an accurate representation of history and diversity to make logical scientific inference (i.e., ecology, behavior, biogeography, ecological genomics, transgenics, and selective-breeding programs, host range, to name only a few). As more robust phylogenies continue to emerge for these taxa, their utility as model systems for research in evolution, molecular genetics, and cell biology is increasingly evident. For example, how do free-living prokaryotes and eukaryotes enter into symbioses? How do nematode and bacterial cells signal and negotiate? Acquiring genome sequences of nematode hosts that complement their endosymbiotic counterparts will empower numerous research programs in making progress towards questions pertinent to improving the performance of EPNs as biological control organisms, and as models for the study of numerous other biological phenomena.

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