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Evolution and taxonomy of nematode-associated entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus*: an overview

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Abstract
Entomopathogenic bacteria from the genera *Photorhabdus* and *Xenorhabdus* are closely related Gram-negative bacilli from the family *Enterobacteriaceae* (γ-*Proteobacteria*). They establish obligate mutualistic associations with soil nematodes from the genera *Steinernema* and *Heterorhabditis* to facilitate insect pathogenesis. The research of these two bacterial genera is focused mainly on their unique interactions with two different animal hosts, i.e. nematodes and insects. So far, studies of the mutualistic bacteria of nematodes collected from around the world have contributed to an increase in the number of the described *Xenorhabdus* and *Photorhabdus* species. Recently, the classification system of entomopatogenic nematode microsymbionts has undergone profound revision and now 26 species of the genus *Xenorhabdus* and 19 species of the genus *Photorhabdus* have been identified. Despite their similar life style and close phylogenetic origin, *Photorhabdus* and *Xenorhabdus* bacterial species differ significantly in e.g. the nematode host range, symbiotic strategies for parasite success, and arrays of released antibiotics and insecticidal toxins. As the knowledge of the diversity of entomopathogenic nematode microsymbionts helps to enable the use thereof, assessment of the phylogenetic relationships of these astounding bacterial genera is now a major challenge for researchers. The present article summarizes the main information on the taxonomy and evolutionary history of *Xenorhabdus* and *Photorhabdus*, entomopathogenic nematode symbionts.

Keywords *Xenorhabdus* · *Photorhabdus* · Entomopathogenic bacteria · Nematode symbionts · Evolution · Taxonomy

1 Introduction

Enthomopathogenic bacteria are widespread in nature and include mainly members of the genera *Bacillus, Peanibacillus*, *Brevibacillus, Serratia*, *Pseudomonas*, *Xenorhabdus,* and *Photorhabdus*. As obligate or facultative insect pathogens, entomopathogenic bacteria display different host ranges and mechanisms of infection; however, all of them have similar abilities to produce a huge repertoire of virulence factors to overcome insect immune responses and host microbiota (Boemare and Tailliez 2009; Glare et al. 2017). *Photorhabdus* and *Xenorhabdus* bacteria exhibit another essential feature: as obligate symbionts of infective juveniles (IJs) of entomopathogenic nematodes (EPNs) from the genera *Steinernema* and *Heterorhabditis*, respectively, they spend some part of their lives inside nematodes using them as a vector for efficient infection of insects (Goodrich-Blair and Clarke 2007; Koppenhöfer and Gaugler 2009; Stock 2015). Nematodes from the genus *Sternainem*a carry symbiotic bacteria in a specialized vesicle called the receptacle, which is placed in the anterior part of the gut. In turn, *Heterorhabditis* nematodes, which do not have such a specialized structure, use their intestinal lumen to harbor bacteria (Fig. 1) (Bird and Akhurst 1983; Boemare 2002; Snyder et al. 2007). The life cycle of all EPN microsymbionts is similar and can be divided into three phases: phoretic in the nematode host, pathogenic in the insect body, and saprophytic in the insect cadaver (Ciche et al. 2006; Herbert and Goodrich-Blair 2007; Stock and

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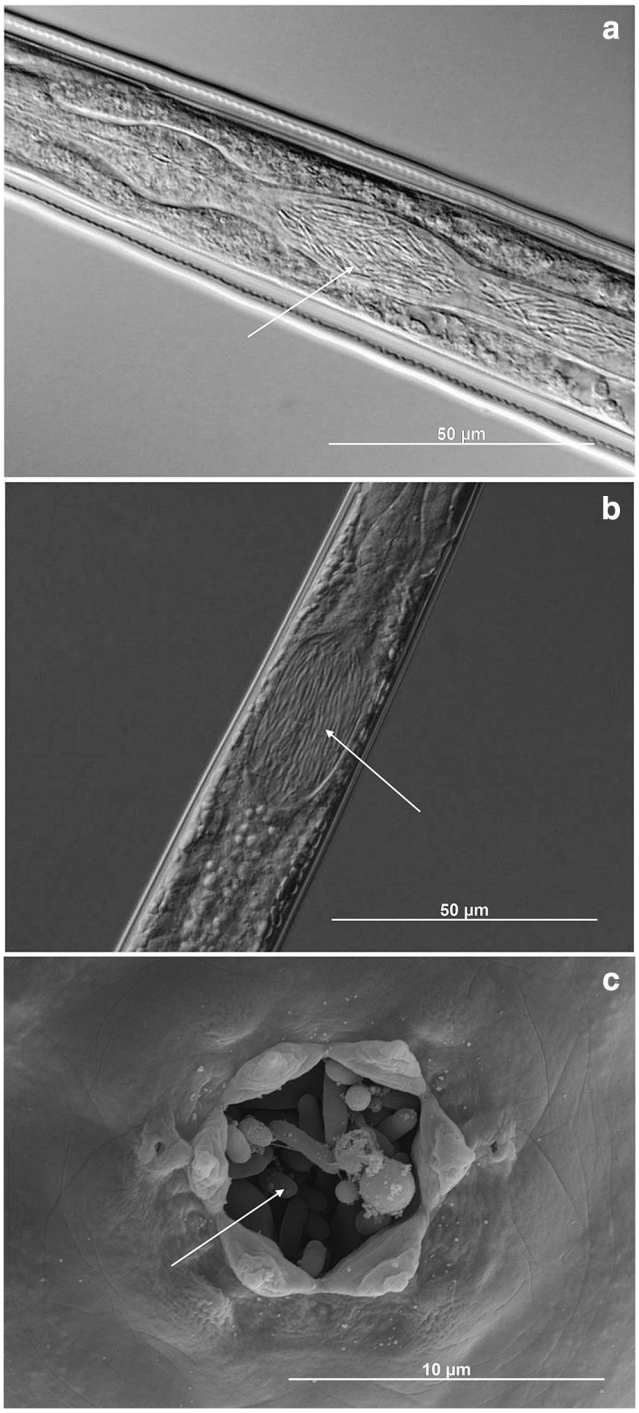

Fig. 1 **a** Anterior end of a *H. megidis* infective juvenile. *Photorhabdus temperata* rods visible (arrow) inside the whole lumen of the intestine. **b** Anterior end of a *S. intermedium* infective juvenile. *Xenorhabdus bovienii* rods visible (arrow) inside a bacterial pouch. **c** 1st generation female of *H. zealandica*. *Photorhabdus temperata* rods visible (arrow) inside the nematode mouth. Scale bars: as on images. The images were obtained using differential interference contrast microscope (**a**, **b**) and scanning electron microscope (**c**)

Goodrich-Blair 2008; Koppenhöfer and Gaugler 2009). In addition, some *Photorhabdus* bacteria have been identified as opportunistic pathogens of humans; however, the mechanism of infection is not known yet (see chapter 7 for details). There is also a report on a bacterial strain classified as *Photorhabdus luminescens*, which causes rare neonatal bacteremia and cutaneous lesions (Dutta et al. 2018).

The *Xenorhabdus* and *Photorhabdus* bacteria are easy to cultivate in the laboratory; isolated from infected insects or from the intestinal lumen of IJs of nematodes as their natural habitats, they grow fast in Luria-Bertani medium, with a doubling time of approx. 2 h. In fact, EPN symbiotic bacteria have never been found living freely in the soil; however, they have been detected in the bacterial biota of the insect larvae in metagenomic studies (Osimani et al. 2018).

EPNs together with their bacterial symbionts are well known for their long-term use in biological and integrated pest management since they display insecticidal activity against a wide range of soil-dwelling insect and other arthropods (Poinar and Grewal 2012; Hiltpold 2015). Furthermore, studies have advanced these organisms as a relevant biological model in fields of soil ecology, symbiotic relationships, and evolutionary biology (Stock 2005, 2015; Campos-Herrera et al. 2012). Recently, investigations of the virulence mechanisms and secondary metabolites of the *Xenorhabdus* and *Photorhabdus* bacteria have been aimed at their potential to be used for management of agricultural pests (Hinchliffe et al. 2010; Zhang et al. 2012; Kumari et al. 2015; Stock et al. 2017), as mosquito control repellents and feeding-deterrents (Yooyangket et al. 2018; Kajla et al. 2019), and in medical applications as a response to the need for novel antibiotics (Tobias et al. 2018; Xue et al. 2018).

2 General characteristic of the *Xenorhabdus* and *Photorhabdus* bacteria

The *Xenorhabdus* and *Photorhabdus* bacteria belong to the family *Enterobacteriaceae* within *Gammaproteobacteria* (Imhoff 2005; Koppenhöfer 2007). They are not the only γ-*Proteobacteria* that establish mutualistic symbiosis with nematodes. Research data indicate that *Moraxella osloensis* (family *Pseudomonadaceae*) can enter symbiosis with a slug-parasitizing nematode *Phasmarhabditis hermaphrodita,* whereas entomopathogenic *Serratia* sp. (family *Enterobacteriaceae*) have been found to associate with *Oscheius* and *Caenorhabditis* nematodes enabling their success as insect pathogens. Although these symbiotic associations seem not to be obligate, they suggest that the ability to engage in symbiosis with nematodes may have arisen several times during the evolution of γ-*Proteobacteria* (Husnik et al. 2011; Dillman et al. 2012).

Studies have established that the *Photorhabdus* and *Xenorhabdus* bacteria are phylogenetically close, in contrast to their EPN hosts *Steinernema* and *Heterorhabditis*,

which represent distinct clades (Liu et al. 1997; Koppenhöfer 2007; Tailliez et al. 2010; Stock 2015). As demonstrated by phylogenetic trees based on housekeeping genes, the *Xenorhabdus* and *Photorhabdus* bacteria form tight sister groups, while the genus *Proteus* is their nearest neighbor (Fig. 2). Current evidence suggests that a common ancestor of these bacteria lived possibly about 200–500 million years ago and it was able to associate with both *Steinernema* and *Heterorhabditis* nematode hosts. However, under the selective pressure of maintenance of long-term mutualistic interactions with the nematodes host, two separate genera of bacteria displaying host specific associations have evolved (Boemare 2002; Chaston et al. 2011). Studies have also revealed that a general trend in the EPN bacterial phylogeny is the increasing virulence associated with evolutionary trade-off between virulence and bacteriocin production abilities (Blackburn et al. 2016; Meli and Bashey 2018; Bhattacharya et al. 2019).

Phenotypically, the *Xenorhabdus* and *Photorhabdus* bacteria are defined as Gram-negative, facultatively anaerobic, non-spore forming rods. They are considered to be distinctive from other members of the family *Enterobacteriacae* in the same traits, including their inability to reduce nitrate to nitrite, which is the major positive feature of this family (Boemare 2002; Imhoff 2005). Another unique feature of the *Xenorhabdus* and *Photorhabdus* bacteria is their phenotypic variation, i.e. the existence of the primary and secondary form; however, the environmental stimuli and the role of the switch between two cell types in the life cycle of EPN symbionts are unclear (Boemare and Akhurst 1988). Recently, new information about differences in the transcriptome level between the two cell forms has contributed to formulation of a hypothesis that primary cells, which are not able to reassociate with nematodes, can live freely in the rhizosphere (Eckstein et al. 2019). Only bacteria of the genus *Photorhabdus* are positive for catalase and bioluminescence, thus they can be easily differentiated from bacteria of the genus *Xenorhabdus* by these two traits. The bioluminescence ability is the most curious phenotypic characteristic of the *Photorhabdus* bacteria. In fact, *Photorhabdus* are the only known bioluminescent terrestrial bacteria that are able to produce light; however, the function of this trait in *Photorhabdus* is unclear. Peat et al. (2010) showed a decline in bioluminescence intensity throughout the evolution of the *Photorhabdus* bacteria, supporting the hypothesis that this feature was acquired by their ancestor living in an aquatic environment but now is gradually being lost under terrestrial selection pressure. This agrees with the idea of bioluminescence in *Photorhabdus* as a non-functional evolutionary remnant that has not had enough time to be lost, as proposed earlier by Peat and Adams (2008).

3 Molecular diversity among EPN symbionts

To assess the genetic diversity of the *Xenorhabdus* and *Photorhabdus* bacteria, comparison of molecular typing profiles and gene sequences has been frequently used (e.g. Maiden et al. 1998; Tailliez et al. 2006; Adams et al. 2006). Recently, whole-genome analyses increasingly support studies on the diversity, biology, and evolutionary relationships of EPN bacteria (Murfin et al. 2012). Indeed, consideration of full genome sequences of different EPN microsymbionts has uncovered the large genomic divergence among the *Xenorhabdus* and *Photorhabdus* bacteria (Wilkinson et al. 2009; Chaston et al. 2011; Murfin et al. 2015a). It is now clear that the genomes of EPN bacteria encode diverse antibiotics, adhesins, hemolysins, proteases, and lipases, which are crucial for successful host invasion and bioconversion of the insect cadaver (Bode 2009). In fact, analysis of the *P. luminescens* TT01 genome revealed that nearly 6% of the genome sequences encode secondary metabolites, highlighting the potential of these bacteria for discovery of new drugs (Duchaud et al. 2003). Studies have established that many virulence genes of the *Xenorhabdus* and *Photorhabdus* bacteria are located within pathogenicity islands, probably acquired by extensive horizontal transfer (Waterfield et al. 2002; Duchaud et al. 2003; Chaston et al. 2011). Genes coding for insecticidal toxins and host-induced stress protection are regarded to be conserved, in contrast to genes involved in production of antimicrobial molecules limiting the growth of competitors, which appear to be highly diverse among EPN bacteria, even within a single population (An et al. 2009; Meli and Bashey 2018). Similarly, genes involved in mutualistic associations of the bacteria with nematodes exhibit considerable variation (Chaston et al. 2011). The studies reviewed above suggest that, despite the close phylogenetic relationships and similar lifestyle, the *Xenorhabdus* and *Photorhabdus* bacteria under different evolutionary pressures exerted by their two animal hosts as well as the environment (especially competitors) have developed a great number of different ways of interacting with nematodes, which is an example evolutionary convergence.

Xenorhabdus bovienii seems to be the most diverse species among EPN bacteria; nevertheless, genomic studies confirmed its species status according to the current taxonomic criteria (Tailliez et al. 2010; Murfin et al. 2015b). Unlike other EPN bacteria spp., *X. bovienii* is a mutualist of several *Steinernema* spp. belonging to two distant phylogenetic clades (Table 1) (Spiridonov et al. 2004; Nadler et al. 2006). Studies have revealed that *X. bovienii* strains differ significantly in the genome content and fitness strategy; however, this is related to the diversity of bioactive molecules and their regulation rather than the use of different mechanisms for mutualistic relationships with the nematodes (Murfin et al. 2015a, b; Bisch et al. 2016; McMullen et al. 2017a). Moreover, analysis of the multiple genomes of

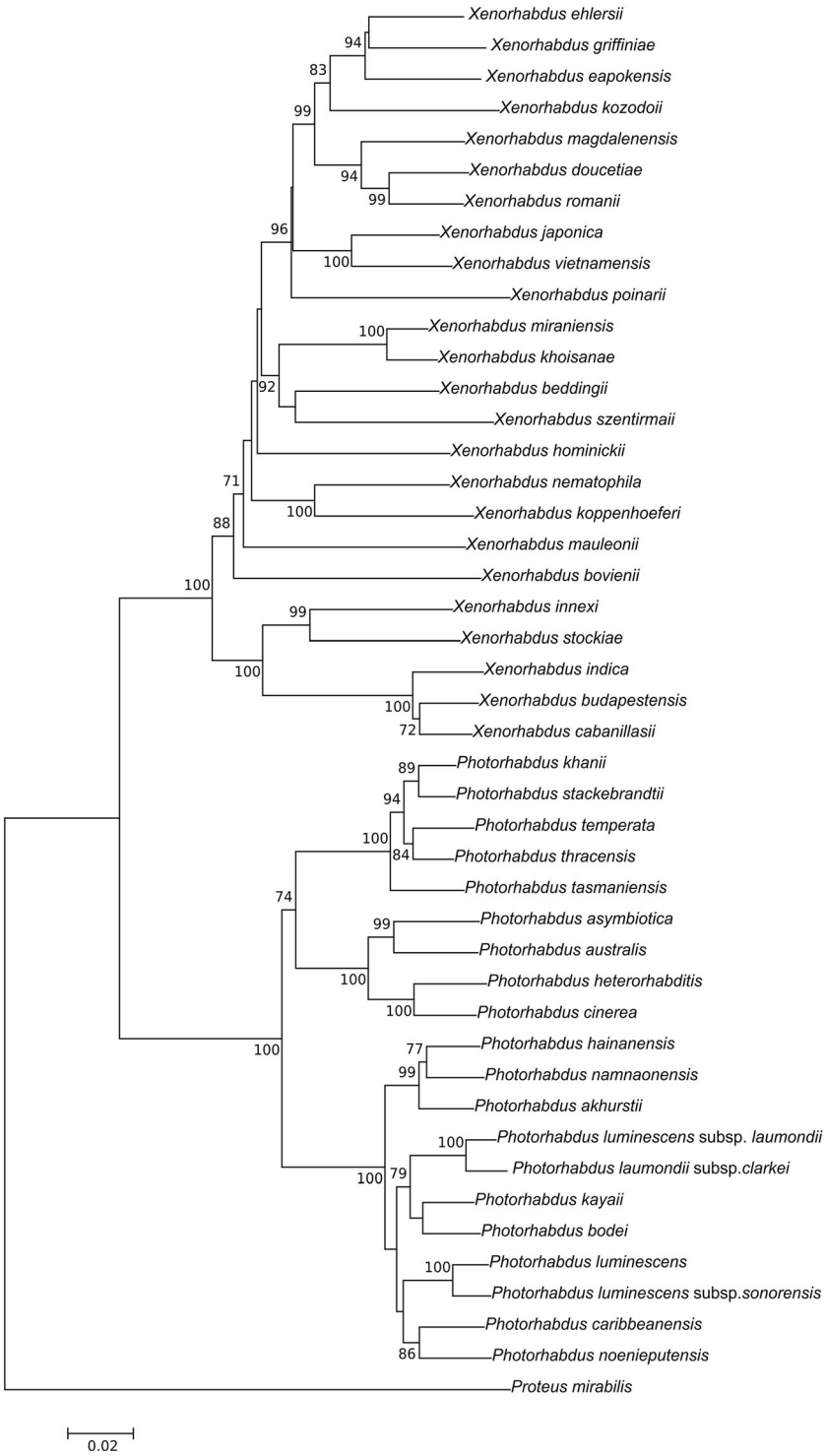

Fig. 2 Maximum-likelihood phylogenetic tree of the type strains of *Xenorhabdus* and *Photorhabdus* species reconstructed from concatenated nucleotide sequences of four protein-coding genes *recA, dnaN, gltX*, and *gyrB* (2823 bp). The numbers at the nodes show bootstrap values higher than 70% based on 1000 replications. The tree was rooted using *Proteus mirabilis* as an outgroup. Scale bar: 0,02 nucleotide substitutions per sequence position. The tree was inferred using MEGA6

X. bovienii as well as other EPN microsymbionts has supported their ecotype model of speciation. The ecotype, recognizing specific relationships between genes and the environment, is generally defined as an evolutionarily distinct group of strains within bacterial species that play a discrete ecological role (Koeppel et al. 2008). Since *Xenorhabdus* and *Photorhabdus* strains display specialization in producing particular bioactive compounds to interact with specific nematodes, despite being members of a single species, it is suggested that it is the ecotype definition that should be used to describe the fundamental units of their diversity (Chaston et al. 2011; Murfin et al. 2015a).

Table 1 List of currently described species in the genus *Xenorabdus* (Thomas and Poinar 1979) and their nematode symbionts

Xenorhabdus species	References	*Steinernema* species being a symbiont
X. beddingii	Akhurst and Boemare 1988	undescribed species
X. bovienii	Akhurst and Boemare 1988	*S. feltiae, S. kraussei, S. affinae, S. intermedium, S. weiseri, S. silvaticum, S. sichuanense, S. nguyeni, S. poinari, S. tbilisiensis, S. jollieti, S. puntauvense, S. oregeonense, S. litorale*
X. budapestensis	Lengyel et al. 2005	*S. bicornutum, S. ceratophorum*
X. cabanillasii	Tailliez et al. 2006	*S. riobrave*
X. doucetiae	Tailliez et al. 2006	*S. diaprepesi*
X. ehlersii	Lengyel et al. 2005	*S. longicaudum (S. serratum*[a]*)*
X. eapokensis	Kämpfer et al. 2017	*S. eapokensis*
X. griffiniae	Tailliez et al. 2006 Dreyer et al. 2017	*S. hermaphroditum,* undescribed species
X. hominickii	Tailliez et al. 2006	*S. karii, S. monticolum*
X. indica	Somvanshi et al. 2006	*S. thermophilum*[a]*, S. abbasi*
X. innexi	Lengyel et al. 2005	*S. scapterisci*
X. ishibashii	Kuwata et al. 2013	*S. aciari*
X. japonica	Nishimura et al. 1994	*S. kushidai*
X. khoisanae	Ferreira et al. 2013	*S. khoisanae, S. jeffreyense, S. saccharii*
X. koppenhoeferi	Tailliez et al. 2006	*S. scarabaei*
X. kozodoii	Tailliez et al. 2006	*S. arenarium*
X. magdalenensis	Tailliez et al. 2012	*S. australe*
X. mauleonii	Tailliez et al. 2006	undescribed species
X. miraniensis	Tailliez et al. 2006	undescribed species
X. nematophila	Poinar and Thomas 1965	*S. carpocapsae*
X. poinarii	Akhurst 1983	*S. glaseri, S. cubanum*
X. romanii	Tailliez et al. 2006	*S. puertoricense*
X. stockiae	Tailliez et al. 2006	*S. siamkayai*
X. szentirmaii	Lengyel et al. 2005	*S. rarum*
X. thuongxuanensis	Kämpfer et al. 2017	*S. sangi*
X. vietnamensis	Tailliez et al. 2010	*S. sangi*

[a] *nomina nuda*

4 Specificity of symbiotic relationships and coevolution between bacterial symbionts and their nematode host

In the symbiotic interactions between nematodes and bacteria, the strong specificity favoring symbionts with the most beneficial traits facilitates successful transmission of such a bacteria-nematode pair from one insect host to another (Adams et al. 2006). It is assumed that each species of the genus *Steinernema* establishes symbiosis with only one *Xenorhabdus s*pecies; in turn, many *Xenorhabdus* species can be associated with several nematode species. In contrast, the symbiotic *Heterorhabditis-Photorhabdus* relationships are more flexible: many species, both bacteria and nematodes, are able to engage in symbiotic associations with multiple species of symbiotic partners (Koppenhöfer 2007; Koppenhöfer and Gaugler 2009). While plenty of data support this specificity pattern, the mechanisms underlying it remain to be elucidated (Herbert and Goodrich-Blair 2007; Hillman and Goodrich-Blair 2016).

Laboratory studies on the specificity of symbiotic associations are usually based on removal of native microsymbionts followed by re-association of nematode-bacterium pairs with non-native bacteria. Such experiments demonstrated strong

partner preference, however with the potential for horizontal transfer of symbionts between different species of nematodes, depending on the bacterium-nematode pair used (e. g. Sicard et al. 2004; Sicard et al. 2005; Chapuis et al. 2009; Murfin et al. 2015b; McMullen et al. 2017b; Kazimierczak et al. 2017; Sajnaga et al. 2018). In fact, the bacterial host-switching between nematode species, even those representing different clades, seems to be rare but possible, especially that associations of nematodes with new symbiotic partners may enable colonization of new niches or extend one by conferring significant fitness benefits (Henry et al. 2013; Maher et al. 2017). Nevertheless, the microsymbiont switching has most often a detrimental effect on the nematode host because association with non-cognate symbionts results in a decline in their reproduction fitness, symbiont carriage, and virulence. As expected, nematode-bacterium pairs with bacteria closely related to its native microsymbiont are more efficient in terms of reproductive fitness and virulence than those with a distantly related microsymbiont. The reproductive success of the nematode associated with non-cognate bacteria is also strongly inversely correlated with the phylogenetic distance between nematodes being a donor and a recipient of microsymbionts, confirming that the nematode host diversity strongly influences coadaptation between symbiotic partners (Murfin et al. 2015b; McMullen et al. 2017b).

It is known that associations between obligate mutualists, involving fulfillment of their needs, often result in co-speciation or a larger phenomenon - coevolution, where symbionts share adaptive changes and their evolutionary history is congruent. To date, few studies based on comparisons of the gene phylogenies of the nematode-bacterium partners have been conducted to assess co-speciation or co-evolution events. Using a single-gene approach, Maneesakorn et al. (2011) provided evidence for a co-evolutionary pattern of relationships between a majority of the *Photorhabdus* and *Heterorhabditis* symbiotic associations tested; however, processes such us host switching and host duplication of bacteria, which can create incongruence between phylogenies of symbiotic partners, were detected as well. In contrast, using a multigene approach, Lee and Stock (2010b) found little evidence for the coevolution between *Xenorhabdus* bacteria and *Steinernema* nematodes. Furthermore, this analysis suggested that the host switching of the microsymbiont occurred rampantly in the evolutionary history of *Xenorhabdus*. Then, several studies have revealed cospeciation events as well as the ability of microsymbionts to transfer between distantly related nematodes in the mutualistic *Xenorhabdus/Steinernema* associations (Lee and Stock 2010b; Murfin et al. 2015b; Dreyer et al. 2017; Bhat et al. 2018). There are several reasons why the processes of host switching, could culminate in the evolutionary history of the *Xenorhabdus* and *Photorhabdus* bacteria. EPNs are cosmopolitan animals often coexisting in the same ecological niche (Grewal et al. 1994; Půža and Mráček 2010). As they also have a wide insect host range, coinfections of the same insect by more than one EPN species is likely to occur in nature (Koppenhöfer and Kaya 1996; Peters 1996; Grewal et al. 1997). Additionally, within the insect host, symbiotic bacteria are physically separated from the nematode, which is not in common with most of the other mutualistic symbioses, where partners do not disassociate naturally. In fact, in the case of co-infection of an insect by different nematodes, their microsymbionts are free to associate with other nematodes (Adams et al. 2006). In this respect, it is suggested that host switching is an evolutionary phenomenon responsible for the spread of bacterial symbionts to different nematode hosts, enabling dissemination of phylogenetically conservative traits, which is also called ecological fitting (Maneesakorn et al. 2011; Stock 2015).

5 Approaches used for taxonomy and identification of EPN symbiotic bacteria

Initially, the symbiotic properties and some phenotypic features were the main criteria used to differentiate among *Xenorhabdus* and *Photorhabdus* bacteria and classify them into two separate groups (Thomas and Poinar 1979). Subsequently, a polyphasic approach was introduced to classify prokaryotes based on integration of different kinds of phenotypic and genotypic data. It was followed by 16S rRNA gene sequence analysis and DNA/DNA hybridization (DDH), which have become "the gold standard" in the taxonomy of bacteria (Rosselló-Mora and Amann 2001; Stackebrandt et al. 2002; Stackebrandt and Ebers 2006). To accomplish the new species concept, the 16S rDNA sequence similarity threshold 97%, later changed into 98.7%, was applied in recognizing new species of EPN symbionts (Farmer et al. 1989; Boemare et al. 1993; Liu et al. 1997; Fischer-Le Saux et al. 1999). Additionally, the 70% DDH threshold introduced by the ad hoc Committee on Reconciliation of Approaches of Bacterial Systematics (Wayne et al. 1987) and the 80% DDH threshold proposed by Vandamme et al. (1996) effectively complemented the minimal standards in the taxonomy of *Xenorhabdus* and *Photorhabdus,* respectively.

Meanwhile, the rapid development of the gene sequencing techniques changed the approach to the definition of bacterial species. It became apparent that using solely ribosomal subunit sequences for taxonomic purposes was not suitable due to their low variation and lateral gene transfer (LTG) among different bacterial species (Akhurst et al. 2004; Tailliez et al. 2006; Lee and Stock 2010a; Tailliez et al. 2010). To overcome this limitation, a more robust phylogenetic relationship framework for the *Xenorhabdus* and *Photorhabdus* bacteria was developed by comparing the sequences of genes coding for proteins with conserved function (Sergeant et al. 2006; An and Grewal 2010; Lee and Stock 2010a). Finally, multilocus

sequence analysis (MLSA) of genes coding for proteins with conserved functions, called housekeeping genes, was widely adopted for inference of the phylogenetic relationships of nematode microsymbionts and gradually replaced the single-gene analysis and time-consuming DDH in identification and classification of these bacteria (Maiden et al. 1998; Stackebrandt et al. 2002; Glaeser and Kämpfer 2015). Nevertheless, the 16S rDNA sequence analysis is still a common approach for preliminary investigations of the genetic diversity of bacterial collections and for establishment of the genus status of newly isolated EPN bacterial strains (e.g. Kazimierczak et al. 2016, 2017; Godjo et al. 2018; Sajnaga et al. 2018). In 2010, Tailliez et al. (2010), by using concatenated sequences of four protein coding gene fragments: *recA* (recombinase A), *gyrB* (gyrase B), *dnaN* (DNA polymerase III subunit), and *gltX* (glutamyl-tRNA synthetase) for a thorough analysis of the EPN symbiont phylogeny, introduced the scheme of MLSA to identify EPN bacteria. Hence, the *Xenorhbdus* species was outlined as a collection of strains whose concatenated sequences of the housekeeping genes mentioned above (3396 bp) showed 97% identity, whereas the same threshold delimitated the subspecies boundary for *Photorhabdus* strains to fit the more strict taxonomic system of this genus (Tailliez et al. 2010). To improve the robustness of the phylogenetic study of EPN bacteria, the fifth *infB* gene sequence was soon added to the MLSA scheme (Tailliez et al. 2012). Such comparative sequence analysis has been widely used for the last years for identification of many new bacterial isolates and has led to delineation of several new species and subspecies of EPN bacteria (e.g. Tailliez et al. 2010; Ferreira et al. 2013a, b; Kuwata et al. 2013; Orozco et al. 2013; Glaeser et al. 2017).

Currently, advancement of the DNA sequencing technology allows the use full genome sequences for taxonomic purposes (Auch et al. 2010; Lee et al. 2016). In fact, to delineate new bacterial species the replacement of DDH with pairwise genome sequence-derived similarity has been proposed by several authors (Chun et al. 2018). The power of genomotaxonomy has recently been demonstrated by Machado et al. (2018), who presented a high-resolution taxonomy of the genus *Photorhabdus*. In their study, consideration of two whole genome-based phylogenetic methods, i.e. orthologous average nucleotide identity (OrthoAni) and *in silico* DNA-DNA hybridization (isDDH), supported by traditional methods, such as MLSA, MALDI-TOF, and phenotypic tests, deeply revisited the genus *Photorhabdus* phylogeny.

6 Changes in the taxonomy of *Xenorhabdus* and *Photorhabdus* - historical overview

Symbiotic bacteria isolated from the nematode *Steinernema carpocapse* were described for the first time in 1965 (Poinar and Thomas 1965; Poinar and Thomas 1966). They were named *Achromobacter nematophilus*, later transferred to a newly created genus *Xenorhbdus* and renamed as *Xenorhabdus nematophilus,* and finally renamed as *Xenorhabdus nematophila* to comply with the bacteriological nomenclature (Thomas and Poinar 1979; Euzéby and Boemare 2000). Meanwhile, symbiotic glowing bacteria isolated from *Heterorhabditis bacteriophora* were included into the genus *Xenorhabdus* as *Xenorhabdus luminescensens* (Thomas and Poinar 1979). Therefore, up to 1993, there were only two bacterial species in the genus *Xenorhabdus*, i.e. *Xenorhabdus nematophila* (type species) and *Xenorhabdus luminesces*, which comprised symbionts of *Steinernema* and *Heterorhabditis* nematodes, respectively (Akhurst 1983; Akhurst and Boemare 1988; Boemare and Akhurst 1988; Farmer et al. 1989). However, the significant differences in the phenotypic and molecular traits between these two species resulted in the transfer of all *Heterorhabditis* nematode symbionts into a new genus *Photorhabdus* as *Photorhabus luminescens* (Boemare et al. 1993).

Although the genus *Xenorhabdus* was found to be more homogenous than *Photorhabdus*, it quickly became relatively rich in terms of the number of species (Akhurst et al. 1996). At first, using a polyphasic approach, 3 new species were described by elevating the *X. nematophila* subspecies to the species level, i.e. *Xenorhabdus beddingi, Xenorhabdus poinarii*, and *Xenorhabdus bovienii* (Akhurst 1983; Akhurst and Boemare 1988). The delineation of new species of EPN symbionts based on sequence data of single gene sequences continued in the next years, and in 2007 Koppenhöfer reported a list of 20 *Xenorhabdus* spp. The recognition of the last few *Xenorhabdus* species was linked with the application of MLSA and whole genome sequence data to EPN symbionts. Currently, 26 species representing the genus *Xenorhabdus* have been described (Table 1).

After establishment of the genus *Photorhabdus* in 1993, bacteria of the only species *P. luminescens* were divided into two groups, i.e. *Heterorhabditis* nematode symbionts and human clinical isolates (Akhurst et al. 1996). However, based on phylogenetic analysis of 16S rRNA gene sequences, another two species in the genus *Photorhabdus* were delineated in 1997, i.e. *Photorhabdus temperata* and *Photorhabdus asymbiotica* for some nematode symbionts and all bacteria isolated from human specimens, respectively (Lie et al. 1997; Fischer-Le Saux et al. 1999). Meanwhile, the stricter taxonomic system for the genus *Photorhabdus* bacteria contributed to creation of many new taxa with a status of subspecies (e.g. Fischer-Le Saux et al. 1999; Hazir et al. 2004; Tóth and Lakatos 2008; An and Grewal 2010, 2011). In 2010, after a deep revision of the genus *Photorhabdus* phylogeny based on MLSA, there were only 3 species in this genus: *P. luminescens, P. temperata,* and *P. asymbiotica,* but they comprised 9, 6, and 2 subspecies, respectively (Tailliez et al. 2010). The fourth species in the genus *Photorhabdus,*

Photorhabdus heterorhabditis, was described by Ferreira et al. (2014) for bacterial symbionts of the nematode *Heterorhabditis zealandica*. Recently, based on whole-genome sequence data, Machado et al. (2018) proposed the elevation of most *Photorhabdus* subspecies to the species level and described one novel species - *Photorhabdus bodei*. At the time of writing, the genus *Photorhabdus* contained 19 species, 3 of which, i.e. *Photorhabdus laumondii, P. luminescens,* and *Photorhabdus khani*, include one or two subspecies (Table 2).

Table 2 List of currently described species in the genus *Photorhabdus* (Boemare et al. 1993) and their nematode symbionts

Photorhabduss species	References	*Heterorhabditis* species being a symbiont
P. akhurstii	Fischer-Le Saux et al. 1999, Machado et al. 2018	*H. indica*
P. asymbiotica	Fischer-Le Saux et al. 1999, Akhurst et al. 2004	undescribed species
P. australis	Akhurst et al. 2004, Machado et al. 2018	*H. gerrardi, H. indica*
P. bodei	Machado et al. 2018	*H. beicherriana*
P. caribbeanensis	Tailliez et al. 2010, Machado et al. 2018	*H. bacteriophora*
P. cinerea	Tóth and Lakatos 2008, Machado et al. 2018	*H. downesi, H. megidis, H. bacteriophora*
P. hainanensis	Tailliez et al. 2010, Machado et al. 2018	undescribed species
P. heterorhabditis	Ferreira et al. 2014	*H. zealandica*
P. kayaii	Hazir et al. 2004, Machado et al. 2018	*H. bacteriophora*
P. khanii	Tailliez et al. 2010, Machado et al. 2018	*H. bacteriophora*
subsp. *guanajuatensis*	Machado et al. 2019	*H. atacamensis*
P. kleinii	An and Grewal 2011, Machado et al. 2018	*H. georgiana, H. bacteriophora,*
P. laumondii	Fischer-Le Saux et al. 1999, Machado et al. 2018	*H. bacteriophora*
subsp. *clarkei*		*H. bacteriophora*
subsp. *laumondii*	Machado et al. 2018	*H. bacteriophora*
	Fischer-Le Saux et al. 1999, Machado et al. 2018	
P. luminescens	Thomas and Poinar 1979, Boemare et al. 1993	*H. bacteriophora, H. indica*
subsp. *sonorensis*		*H. sonorensis*
subsp. *mexicana*	Orozco et al. 2013	*H. mexicana*
	Machado et al. 2019	
P. namnaonensis	Glaser et al. 2017, Machado et al. 2018	*H. baujardi*
P. noenieputensis	Ferreira et al. 2013, Machado et al. 2018	*H. indica, Heterorhabditis* sp.
P. stackebrandtii	An and Grewal 2010, Machado et al. 2018	*H. bacteriophora, H. georgiana*
P. tasmanensis	Tailliez et al. 2010, Machado et al. 2018	*H. zealandica, H. marelatus*
P. temperata	Fischer-Le Saux et al. 1999, Machado et al. 2018	*H. megidis, H. downesi, H. zealandica*
P. thracensis	Hazir et al. 2004, Tailliez et al. 2010,	*H. bacteriophora*
	Machado et al. 2018	

7 *Photorhabdus* bacteria with ability to infect both insects and humans

A distinctive feature of some *Photorhabdus* bacteria is their pathogenicity to humans (Gerrard et al. 2004; Hapeshi and Waterfield 2017). Over a dozen human cases of *Photorhabdus* soft tissue infections and disseminated bacteremia have been documented so far, but many more are probably misdiagnosed (Gerrard et al. 2011; Gerrard and Stevens 2017). The first human pathogen from the genus *Photorhabdus* was isolated from a patient with a leg ulcer in the USA in 1989 (Farmer et al. 1989). This was followed by isolation of other *Photorhabdus* bacteria from human specimens in the USA and Australia (Gerrard et al. 2003). Since it was initially believed that these bacteria were not able to establish symbiosis with nematodes, the new species created for them was named *P. asymbiotica* to emphasize this feature (Fischer-Le Saux et al. 1999). However, in 2006, symbionts of the clinical *Photorhabdus* strain, i.e. heterorhabditid nematodes, were described and classified as *Heterorhabditis gerrardi* (Gerrard et al. 2004; Gerrard et al. 2006; Plichta et al. 2009). Thus, it is now accepted that human pathogenic *Photorhabdus* bacteria are able to form a mutualistic relationships with *Heterorhabditis* nematodes to infect insects efficiently, like other *Photorhabdus* nematode symbionts. Currently, two species: *P. asymbiotica* and *P. australis* comprise both clinical and entomopathogenic *Photorhabdus* strains derived from various locations (Kuwata et al. 2008; Thanwisai et al. 2012).

To address the particular life style of *Photorhabdus* human pathogens, their diversity and evolutionary relationships have been intensively explored by implementing whole genome sequencing and transcriptomic analysis. It was found that *P. asymbiotica* and *P. australis* bacteria have a relatively small genome, compared to that of *P. luminesces* (the closest relative), and display lower diversity of insecticidal toxins. The existence of many supplementary plasmids and pathogenicity islands in the genome has also been shown (Wilkinson et al. 2009, 2010). Current evidence suggests that the switch of these bacteria from nematodes to humans was facilitated by virulence genes (necessary for insect host infection) already present in the genome, as well as additional virulence factors (appropriate for infection of mammalian cells) acquired by horizontal transfer from other human pathogens (Hapeshi and Waterfield 2017). Unlike other species of the genus *Photorhabdus*, *P. asymbiotica* and *P. australis* can grow at 37 °C, undergoing the metabolic shift to adapt their metabolisms to the mammalian host (Mulley et al. 2015). Despite the considerable effort, the mechanisms of transmission of *Photorhabdus* bacteria to the human body are still unclear. The most probable scenario is that the nematode vector may penetrate the human skin and transmit microsymbionts, thereby causing infections (Hapeshi and Waterfield 2017).

8 Conclusion

The symbiotic bacteria of entomopathogenic nematodes emerged as a relevant model to study microorganism-host interactions. Although much interest in the *Xenorhabdus* and *Photorhabdus* bacteria has been historically driven by their symbiotic associations with nematodes, it is now clear that the uncovered richness of their secondary metabolites will accelerate investigations of these bacilli. Based on the recent advances in molecular phylogeny associated with the increasing number of available genes and genome sequences, the taxonomy of the symbiotic bacteria of entomopathogenic nematodes has been reassessed, with elevation of many subspecies to the species level and creation of new taxa. However, the current knowledge of the diversity, genealogy, and specificity of symbiotic relationships of *Photorhabdus* and *Xenorhabdus* bacteria with nematodes remains limited. In fact, despite the gradual identification of new EPN microsymbionts, mutualists of many existing nematode species have not been described yet. Therefore, substantial efforts should be made to improve the knowledge of the distribution of *Xenorhabdus* and *Photorhabdus* species linked to the host and geographic origin. It seems that implementation of whole genome-based phylogenetic methods can particularly provide ample information about the basic features and origin of these bacterial genera.

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