Chapter 6

Phylogeny and phylogeography of Heterorhabditis and Steinernema

Sergei E. SPIRIDONOV 1 and Sergei A. SUBBOTIN 1, 2

1 Center of Parasitology, A.N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences, Leninskii prospect 33, Moscow 119071, Russia
2 Plant Pest Diagnostic Center, California Department of Food and Agriculture, 3294 Meadowview Road, Sacramento, CA 95832, USA

Origin of Steinernema and Heterorhabditis

The evolutionary relationships of EPN were outlined for the first time by Poinar (1981), when he speculated that Steinernematidae and Heterorhabdititidae arose as two separate, independent monophyletic lineages. Poinar (1993) suggested that heterorhabditids evolved from a free-living Pellioditis-like ancestor in an arenicolous marine environment, whereas Steinernema probably evolved from a proto-Rhabditonema ancestor in a terrestrial environment. In his later publication, Poinar (2011) proposed a Permian origin (230-252 million years ago) for heterorhabditids and steinernematids.

The similarity of Steinernema to Strongyloides and affinity to free-living Panagrolaimoidea, as well as that of Heterorhabditis with Pellioditis and Rhabditidae, was proved by several analyses of 18S rRNA gene sequences (Blaxter et al., 1998; Holterman et al., 2006; van Megen et al., 2009; Zhang et al., 2012). Holterman et al. (2006) and van Megen et al. (2009) placed Steinernema in Clade 10 and Heterorhabditis in Clade 9, where bacterial-feeding nematode families dominated and only a few animal-parasitic nematodes are present. However, other analyses of 18S rRNA gene sequences and mitochondrial gene sequences studies have found the position of Steinernema to be unresolved (Smythe et al., 2006; Park et al., 2011).
Phylogeny and phylogeography of the genus *Heterorhabditis*

The molecular phylogeny of the genus *Heterorhabditis* has been reconstructed based on the analyses of partial sequences from the 28S rRNA gene (Curran & Driver, 1994; Nguyen et al., 2004, 2008; Edgington et al., 2011; Li et al., 2012; Malan et al., 2014), partial 18S of rRNA gene (Liu et al., 1997), ITS1 of rRNA gene (Adams et al., 1998; Phan et al., 2003), ITS1-5.8S-ITS2 of rRNA gene (Malan et al., 2008, 2014; Nguyen et al., 2008; Edgington et al., 2011; Li et al., 2012), partial *nd*4 gene (Liu et al., 1999) and partial *coxI* mtDNA gene (Kuwata et al., 2007). Whereas the analysis of the partial 18S rRNA gene showed that this fragment is too conservative and not useful for phylogenetic study of this genus, the analyses of other regions, especially ITS and D2-D3 of 28S rRNA gene, revealed resolved relationships between a number of species. The phylogenetic estimations based on the *nd*4 gene (Liu et al., 1999; Saeb & Grewal, 2014), however, differed in positions of some species, but it cannot be excluded that these differences occurred due to some misidentification of nematode samples.

The first comprehensive phylogenetic analysis of the genus *Heterorhabditis* was conducted by Adams et al. (1998). Nguyen et al. (2008) was the first proposed division of the genus into three clades (groups): ‘*Indica*’, ‘*Bacteriophora*’ and ‘*Megidis*’, which are presently recognised by the majority of authors. The species of the *Indica*-clade formed a highly supported clade in the phylogenetic tree (Fig. 6.1). This clade might be subdivided into two subclades: i) ‘*Indica*’ with *H. indica* (= *H. brevicaudis, H. gerrardi, H. hawaiiensis*) and *H. noenieputensis*; and ii) ‘*Baujardi*’ with *H. amazonensis, H. baujardi* (= *H. somsookae*), *H. floridensis, H. taysearae* (= *H. sonorensis*) and *H. mexicana*. The proposed synonymisation of *H. gerrardi* with *H. indica* and *H. sonorensis* with *H. taysearae* (see Chapter 2) is based on almost identical ITS rRNA gene sequences in these two pairs of heterorhabditids. All species of the *Indica*-clade are found in the tropics and subtropics or in the warm temperature zone. *Heterorhabditis indica* is the only species of the group, widely distributed in Asia, Africa, Australia, North and South America and the Caribbean region. *Heterorhabditis baujardi* is found in southeast Asia and Africa, whilst *H. taysearae* is known from western Asia, Africa and Mexico. Four other species are each from a certain area only: *H. noenieputensis* –