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First record of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in Costa Rica

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Abstract

A survey of entomopathogenic nematodes was conducted in the north Pacific (Guanacaste Conservation Area) and southeast Caribbean (Gandoca-Manzanillo Natural Refuge) regions of Costa Rica. Out of a total of 41 soil samples, 5 were positive for entomopathogenic nematodes (20.5%), with 3 (12.3%) containing *Steinernema* and 2 (8.2%) *Heterorhabditis* isolates. Morphological and molecular studies were undertaken to characterize these isolates. The *Heterorhabditis* isolates were identified as *Heterorhabditis indica* and the three *Steinernema* isolates were identified as two new undescribed species. *H. indica* was recovered from a coastal dry forest. *Steinernema* n. sp. 1 was isolated from a rainforest valley, between volcanoes. *Steinernema* sp. n. 2 was isolated from sand dunes in the Caribbean Coast (Punta Uva) near the rainforest strip along the coast. Although limited to two geographic regions, this study suggests entomopathogenic nematodes may be diverse and perhaps widely distributed in Costa Rica. A more intensive survey, covering all geographic regions is currently undergoing.

Keywords: Costa Rica; Diversity; Entomopathogenic nematodes; *Steinernema*; *Heterorhabditis*

1. Introduction

Entomopathogenic nematodes (EPN) of the genera *Steinernema* Travassos and *Heterorhabditis* Poinar are obligate pathogens that infect a wide range of soil insects (Kaya and Gaugler, 1993). These nematodes are mutualistically associated with bacteria in the genus *Photorhabdus* Boemare, Akhurst, and Mourant for *Heterorhabditis* or *Xenorhabdus* Thomas and Poinar for *Steinernema* (Boemare et al., 1997). The life cycle of all known entomopathogenic nematode/bacterium complexes is similar. The only stage that survives outside of the host is the non-feeding, third-stage infective juvenile (IJ). The IJ carries cells of the bacterial symbionts in its intestine. When the IJ finds a susceptible host, it invades and penetrates into the host's hemocoel through natural openings (i.e., anus, mouth, or spiracles). The IJ then releases the symbiotic bacterium that kills the host within 48 h by septicemia. The bacterium produces antibiotics that prevent other microorganisms from colonizing the cadaver. In addition to serving as a food source for the nematode, the bacterium digests the host tissues, thereby providing suitable nutrients for nematode growth and development.

In the broadest geographic sense, EPN are widespread. The only continent where they have not been found is Antarctica (Griffin et al., 1991). However, soil surveys conducted in different areas of the world have demonstrated variability in abundance across seasons, habitats, and geographic regions. Factors such as soil texture, moisture content, temperature, and host availability are thought to be important in determining distri-

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bution of entomopathogenic nematodes (Griffin et al., 1991; Hominick and Briscoe, 1990; Hara et al., 1991; Hominick et al., 1996; Steiner, 1996; Stock et al., 1999).

In contrast to human modified areas, natural habitats are likely uncontaminated by introduced nematodes and offer increased opportunities for finding native nematode species (Stock et al., 1999, 2003). In this respect, and with particular reference to entomopathogenic nematodes, the isolation of native species and/or populations provides a valuable resource not only from a biodiversity perspective but also from a more applicable standpoint (Stock et al., 1999). Indigenous EPN may result more suitable for inundative release against local insect pests because of adaptation to local climate and other population regulators (Bedding, 1990). In addition, many countries are concerned about the introduction of exotic entomopathogenic nematodes because they may have negative impact on non-target organisms (Bathon, 1996).

At present, only a few studies have focused on entomopathogenic nematodes in Central America but considering only the Caribbean islands of Puerto Rico, Cuba, Guadeloupe, and the Dominican Republic (Figueroa et al., 1993; Fischer Le Saux et al., 1998; Mráček et al., 1994; Román and Figueroa, 1994). Although, these studies have been restricted in geographical scope, at least two new *Steinernema*, *S. cubanum* and *S. puertoricense*, were recovered and described (Mráček et al., 1994; Román and Figueroa, 1994).

In Costa Rica, information on the diversity of entomopathogenic nematodes and their symbiotic bacteria is currently non-existent. Until now, no systematic survey has been conducted to document the presence of these nematodes in this country.

Costa Rica, a country of 50,900 km^2, is the nexus of two continents and two oceans. It is located in the Central American isthmus, bordered by Panama to the south and Nicaragua to the north, with coastline on the Pacific and Atlantic oceans. This confluence of land and water makes the region one of nature's great and unique biodiversity bottlenecks (Carvajal-Alvarado, 1995). It has been estimated that 4% of the world's biodiversity is present in this country. Surprisingly, close to 84% of this biodiversity remains unknown to science (Janzen, 1983), and the unknown percentage exceeds 90% for groups such as insects, fungi, bacteria, viruses, and nematodes. These observations suggest that the diversity of nematodes associated to insects and other invertebrates may be vast. Therefore, and as a preliminary approach to help design a more extensive EPN diversity inventory, two of the six Costa Rican geographic regions, the Caribbean and the North Pacific were considered in this survey. Nematodes recovered during this study were identified using a combination of morphological and molecular methods. Results of this survey are herein presented.

2. Materials and methods

2.1. Geography and collection of soil samples

Costa Rica is divided into six geographic regions: Caribbean, Central Valley, Northern Plains, Central Pacific, Northern Pacific, and South Pacific. Each of these regions has unique geographic, ecological, and climatic characteristics (Carvajal-Alvarado, 1995). In this study, two the Northern Pacific and Caribbean regions were selected for sampling (see Fig. 1).

The Area de Conservación Guanacaste (ACG) located in the North Pacific region, comprises 110,000 ha and is located in the Northern Pacific region. Because of its rich biological diversity it is considered one of Central America's "hotspot" (Myers et al., 2000). It comprises the Guanacaste Cordillera and surrounding flatlands and coastal areas. The ACG includes a series of volcanoes, the most notable being Rincon de la Vieja, which has three craters and one lagoon. Volcanic rocks of high calcium carbonate content underlie the western region; sedimentary sandstones occur on the coastal flank and salt flats or "salinas" are found along littoral lowlands. The marine/coastal area includes various islands and islets, open ocean marine zones, beaches, and rocky coasts. The average annual precipitation is 1528 mm in Santa Rosa (ACG) with considerable variation in the

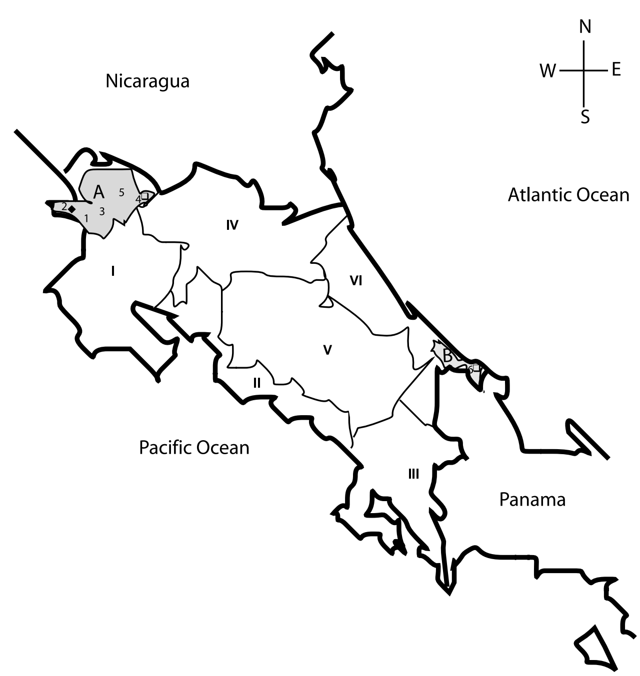

Fig. 1. Map of Costa Rica showing geographic regions and sampling sites. I. Northern Pacific; II. Central Pacific; III. Southern Pacific; IV. Northern Plains, V. Central Valley; and VI. Caribbean. (A) Area de Conservación Guanacaste (ACG), (B) Gandoca-Manzanillo Wildlife Refuge. 1. Playa Naranjo, 2. Santa Elena península, 3. Santa Rosa, 4. Volcano Valley, 5. Estación Pitilla, and 6. Punta Uva. □, Steinernematids; ◆, Heterorhabditids.