

## Research Note

### CROSS HYBRIDIZATION STUDIES WITH THE ENTOMOPATHOGENIC NEMATODES *STEINERNEMA PUERTORICENSIS* AND *S. CUBANA*<sup>1,2</sup>

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In 1994 two new species of the entomopathogenic nematode *Steinernema* Travassos were described from the Caribbean islands: *S. cubana* Mracek, Arteaga-Hernández and Boemare from Cuba (Mracek et al., 1994) and *S. puertoricensis* Román and Figueroa from Puerto Rico (Román and Figueroa, 1994). Both species are related to the *S. glaseri* Steiner and *S. anomali* Kozodai group, whose infective larvae are longer than 1,000 µm. In spite of the fact that *S. cubana* and *S. puertoricensis* differ in certain morphological and morphometrical characters, their close relationship and geographical proximity raised the question of similarity between the two species. This paper reports research findings that establish their biological distinctness.

*S. cubana* and *S. puertoricensis*, maintained in stock cultures in the laboratory, were cross hybridized by using the following procedure:

*Galleria mellonella* L. larvae, washed in 0.02% formalin and rinsed in sterile distilled water, were slightly wounded in the posterior end with sterile dissecting scissors. Two drops of the hemolymph were placed on the bottom of each of 100 disposable polystyrene dishes (35 × 10 mm), covered and then transferred to dishes of the same kind (150 × 15 mm). Each dish had a filter paper saturated with water. Third-stage infective juveniles, washed in the same manner as the *Galleria* larvae, were hand picked with dental pulp canal files under a dissecting microscope after 24 hours. Specimens of each species were placed singly in each of 50 hemolymph drops. Two to three days after adult development, males and females of the same and different species were separated. They were placed with the corresponding opposite sex in the original hemolymph drops and fresh hemolymph was added. Dishes were wrapped in plastic bags and maintained in the laboratory at 26-28°C. Four trials were conducted with the following crossing treatments: a) *S. puertoricensis* ♂ × *S. cubana* ♀; b) *S. cubana* ♂ × *S. puertoricensis* ♀; c) *S. puertoricensis* ♂ and ♀; d) *S. cubana* ♂ and ♀. The number of nematodes and replications per treatment varied according to the number of active adults available. Daily observations were made for a period of eight to ten days. All trials were carried out in a laminar flow cabinet.

*Steinernema puertoricensis* did not hybridize with *S. cubana* in any of the four trials. In control dishes, males and females of either *S. puertoricensis* or of *S. cubana* mated and

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produced fertile progenies which developed to the second generation. Males of *S. puertoricensis* and of *S. cubana* were observed coiled around and moving throughout the entire female body of the same species and finally mating. A brief but similar type of courtship without mating was observed in males and females of the different species. One male and two females was the least number of specimens able to produce progeny.

Cabanillas et al. (1994) did not find any progeny after cross hybridizing *S. riobravis* Cabanillas, Poinar and Raulston with *S. carpocapsae* Weiser (All strain), *S. feltiae* Filipjev (SN strain), *S. glaseri* and *S. intermedia* Poinar. Similarly, Mracek et al. (1994) were unable to mate *S. cubana* n. sp. with *S. glaseri* (NC strain), *S. anomali* (Russia) and *S. kraussei* Steiner (Nomen dubium, Germany). Román and Figueroa (1994) reported negative hybridization with *S. puertoricensis* and *S. glaseri*. The aforementioned research, in addition to that reported herein, demonstrated that the three new species *S. riobravis*, *S. cubana* and *S. puertoricensis*, are different species which differ biologically from those most closely related.

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