Genetic diversity of ITS sequences of
*Bursaphelenchus xylophilus*

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**ABSTRACT.** The sequence variation of internal transcribed spacer (ITS) regions of ribosomal DNA has been routinely used for species identification and species-level phylogeny of the pinewood nematode, *Bursaphelenchus xylophilus*. In this study, the intraspecies ITS genetic diversity of *B. xylophilus* was evaluated. Three pinewood nematode isolates from the United States, Japan, and Portugal were used for polymerase chain reaction (PCR) ITS region amplification and sequencing. Multiple peaks were observed in sequencing chromatograms from ITS regions of American and Japanese isolates, suggesting the presence of more than one ribosomal sequence for each isolate. PCR products were further cloned and 10 clones of each isolate were subsequently sequenced. Additionally, the ITS regions of individual nematodes from each isolate were amplified, cloned and sequenced. Among the 3 *B. xylophilus* isolates analyzed, an intraspecific and intra-isolate molecular variability was found. The intra-isolate ITS molecular diversity in the American isolate was higher than that in the Japanese and Portuguese isolates. However, the level of sequence variation observed within isolates was about the same as that described among ITS repeats within individuals.

**Key words:** ITS diversity; Pine wilt disease; Pinewood nematode; Ribosomal DNA