above 71%. Isolate 2B clusters with *S. affine* and *S. intermedium*, sharing 97.5% of identity with *S. affine* and 40.5% of identity with *S. intermdium* (Fig. 2). By maximum parsimony analysis of ITS rDNA, isolate 20F branches with species from the *kraussei/feltiae*-group (*S. feltiae, S. oregonense* Liu *et* Berry and *S. kraussei*) with 99% bootstrap support. Isolate 20F groups with *S. kraussei* (Fig. 2) with an identity of 92.2%. MP analysis of ITS rDNA groups isolates 59F and 15G together, sharing an identity of 85.5% (Fig. 2). These isolates group with species from the *glaseri*-group (*S. cubanum* Mráček, Hernández *et* Boemare, S. *diaprepsi* Nguyen *et* Duncan, *S. glaseri* and *S. longicaudum* Shen *et* Wang, etc.) suggesting that these two isolates could represent a new species.

28S rDNA region, domain D2 and D3. For isolates 15G and 59F, sequences from the 28S domain D2D3 rDNA were not obtained. For this reason, the phylogenetic analysis of this region does not include these isolates. MP analysis of the 28S domain D2D3 rDNA dataset was constructed based upon 959 positions involving 21 nucleotide sequences (Fig. 3). Isolate 2B also groups with the clade comprising *S. intermedium* and *S. affine*, as its ITS MP analysis, supported by strong bootstrap values (100%) (Fig. 3), shares an identity of 91.8% with *S. affine* and of 90% with *S. intermedium*. 28S rDNA sequence of isolate 20F groups with *S. kraussei*, sharing with it an identity of 80.7% (Fig. 3).

Cytochrome c oxidase subunit I (COI). MP analysis of cytochrome c oxidase subunit 1 (*COI*) of the mtDNA dataset was constructed based upon 889 positions involving 21 nucleotide sequences. *COI* sequence shows the same results already found with ITS and D2D3, identifying isolate 2B as *S. intermedium*, isolate 20F as *S. kraussei* and isolates 15G and 59F as *Steinernema* sp. belonging to the *glaseri*-group (Fig. 4).

DISCUSSION

Accurate identification of entomopathogenic nematodes has important implications in systematics and population genetics and is of major importance for selection of species for future use in biological control. The combination of molecular and morphological methods is necessary to solve a variety of issues in EPN taxonomy. A recent study of phylogenetic relationships among *Steinernema* species combined morphological and molecular methods and showed that most morphological features are not phylogenetically informative (Spiridonov *et al.*, 2004). Still, it is possible to obtain some areas of agreement between morphological and molecular results. For example, taxa with morphological differences, such as *S. affine* and *S. intermedium*, are closely related in molecular data (Nguyen *et al.*, 2007). At present, *S. intermedium sensu lato* includes a number

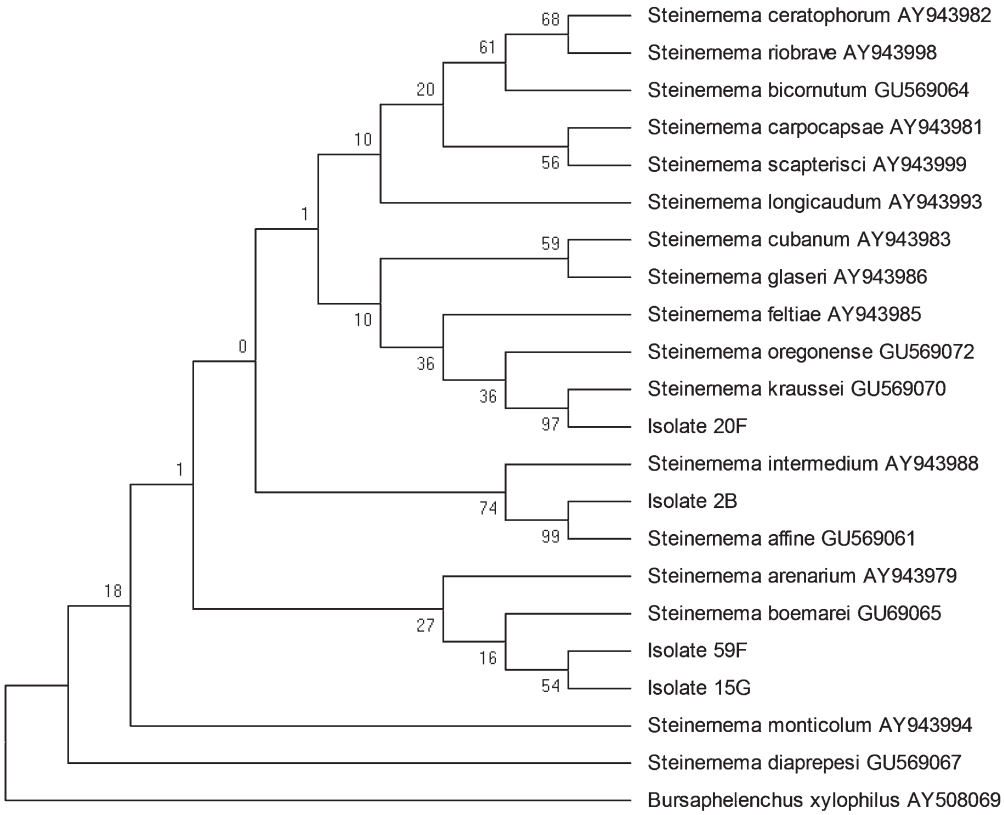

Fig. 4. Phylogenetic relationships based on Maximum Parsimony between 17 Steinernema species and isolates 2B, 20F, 59F and 15G with bootstrap analysis of *COI* regions.