

Identification of some cyst forming nematodes based on PCR-RFLP, sequence of rDNA and specific primers



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More than 50 populations of cyst-forming nematodes collected in Italy and Belgium were analysed using PCR-RFLP, ITS-rDNA sequences, and PCR with species-specific primers. Restriction of PCR products by eight restriction enzymes *AluI*, *AvaI*, *Bsh1236I*, *CfoI*, *HaeIII*, *MvaI*, *PstI* and *RsaI* allowed to identifying several agricultural important cyst nematode species and separating from their sibling species. RFLP profiles for some European populations of *Heterodera carotae*, *H. fici*, *H. filipjevi*, *H. goettingiana*, *H. hordecalis*, *H. humuli*, *H. mediterranea*, *H. riparia* (Fig. 1), *H. schachtii*, *Globodera pallida*, *G. rostochiensis* (Figs. 2 & 3) are given. *Bsh1236I* digestion of the PCR product separated both *Globodera* species from each other and from *G. tabacum* (Fig. 3). The species-specific primer developed by Bulman & Marshall (1997) clearly allowed to discriminate *G. pallida* from *G. rostochiensis* (Fig. 4). Comparison of obtained sequences with those deposited in GenBank showed high similarities (99.8-100%) (Fig. 5). Relative high level of sequence divergence between populations of *H. hordecalis* (1.5%) suggests the presence of two biological species presently grouped under this taxon (Fig. 6).

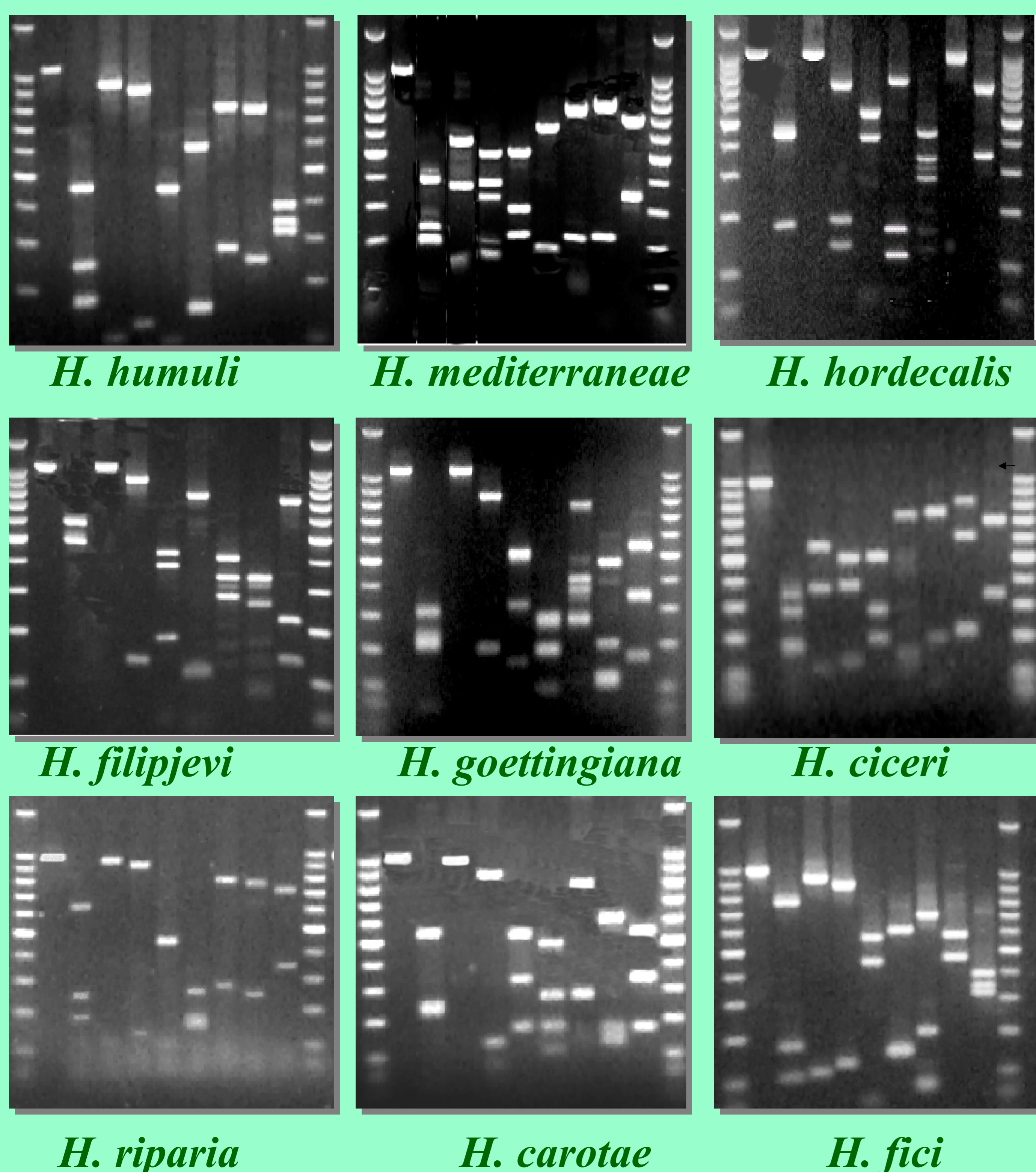


Fig. 1. RFLPs of the ITS region for several cyst nematode species. (100 kb DNA marker, Promega and *AluI*, *AvaI*, *Bsh1236I*, *HaeIII*, *CfoI*, *MvaI*, *RsaI* and *PstI* restriction enzymes)

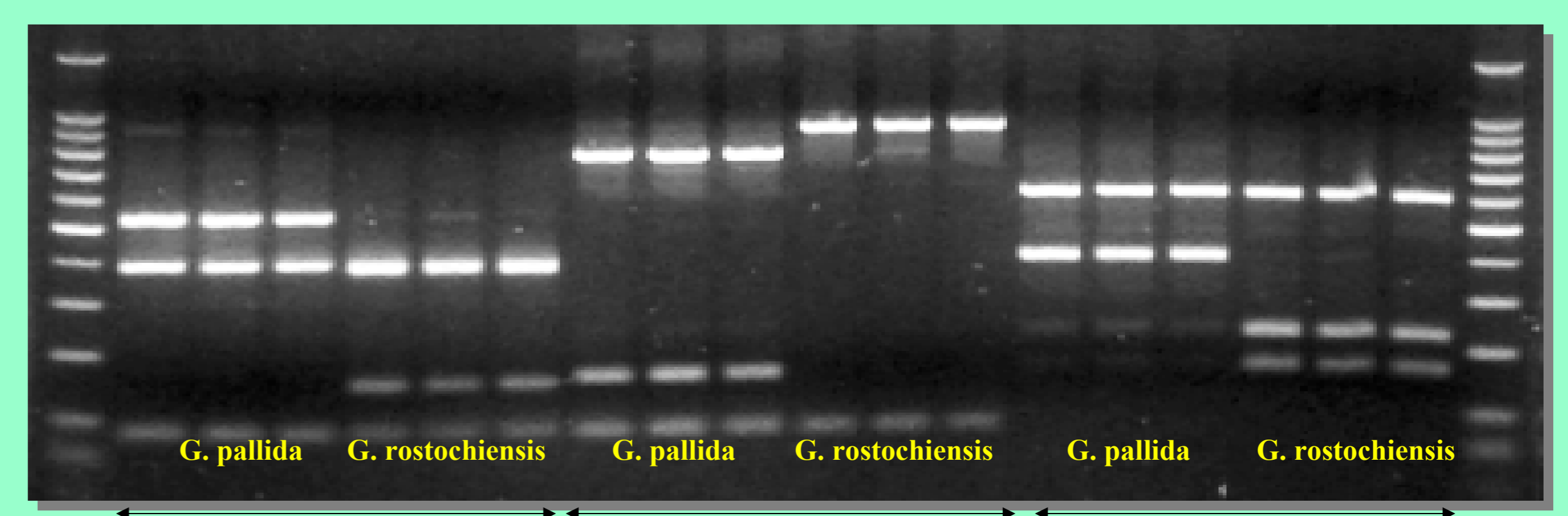


Fig. 2. RFLPs of the ITS region for two *Globodera* species generated by three enzymes.

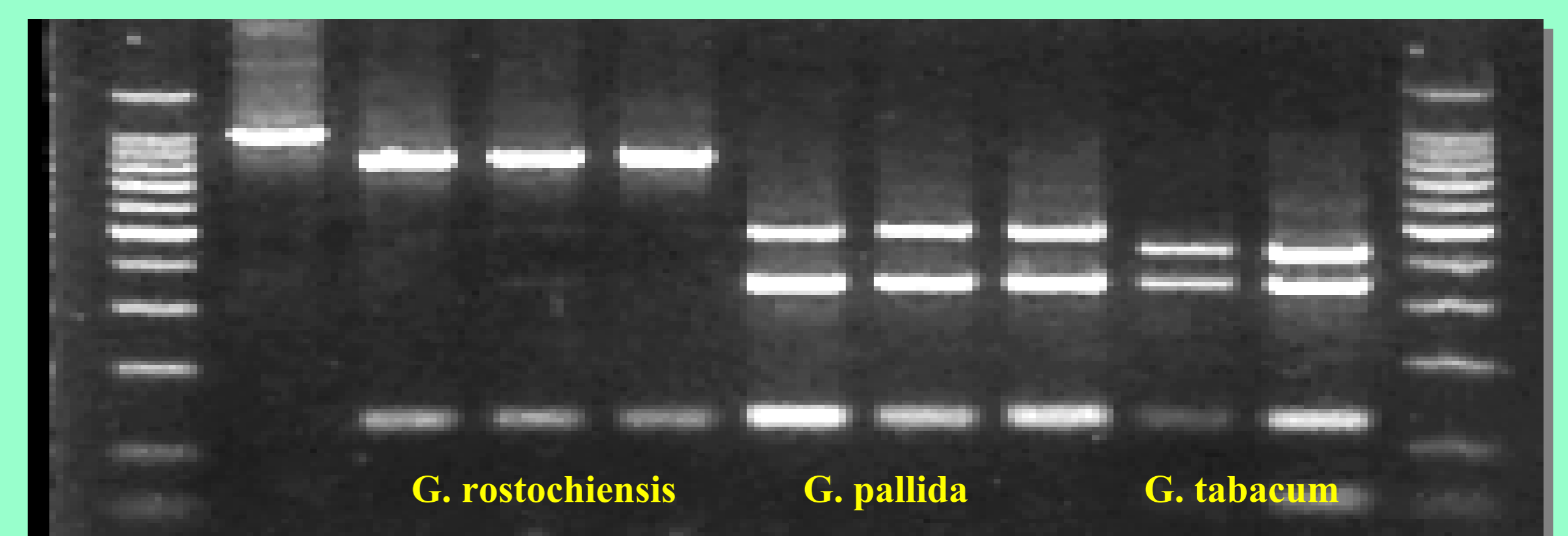


Fig. 3. RFLPs of the ITS region for three *Globodera* species generated by *Bsh1236I*.

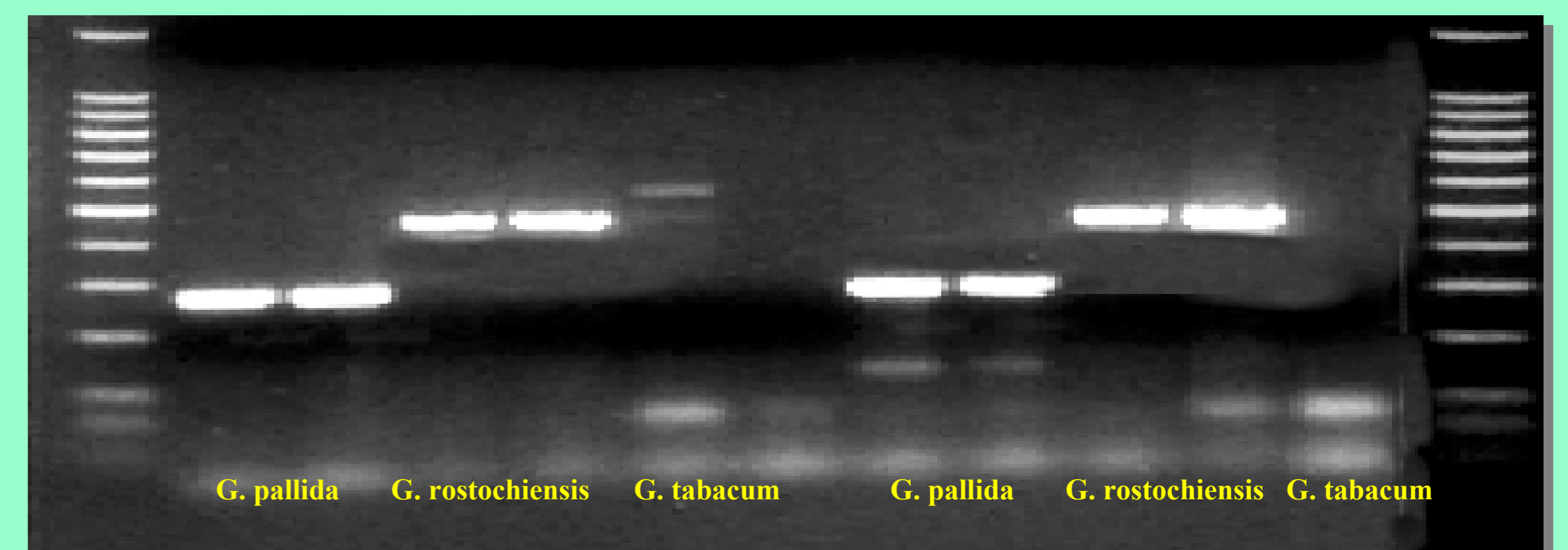


Fig. 4. Multiplex PCR with species-specific primer.

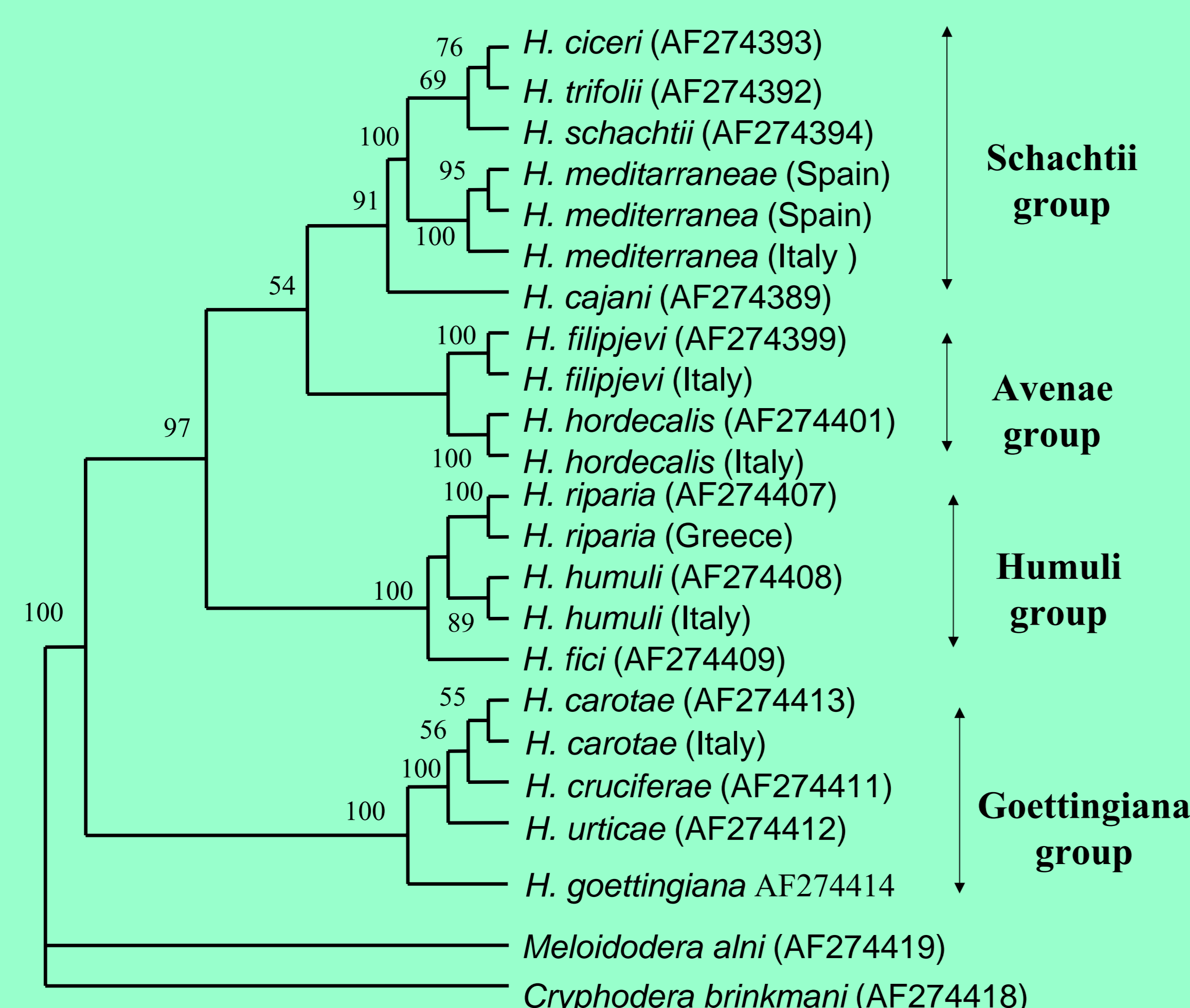


Fig. 5. Maximum parsimonious tree obtained in the result of analyses the ITS sequences of cyst-forming nematodes.

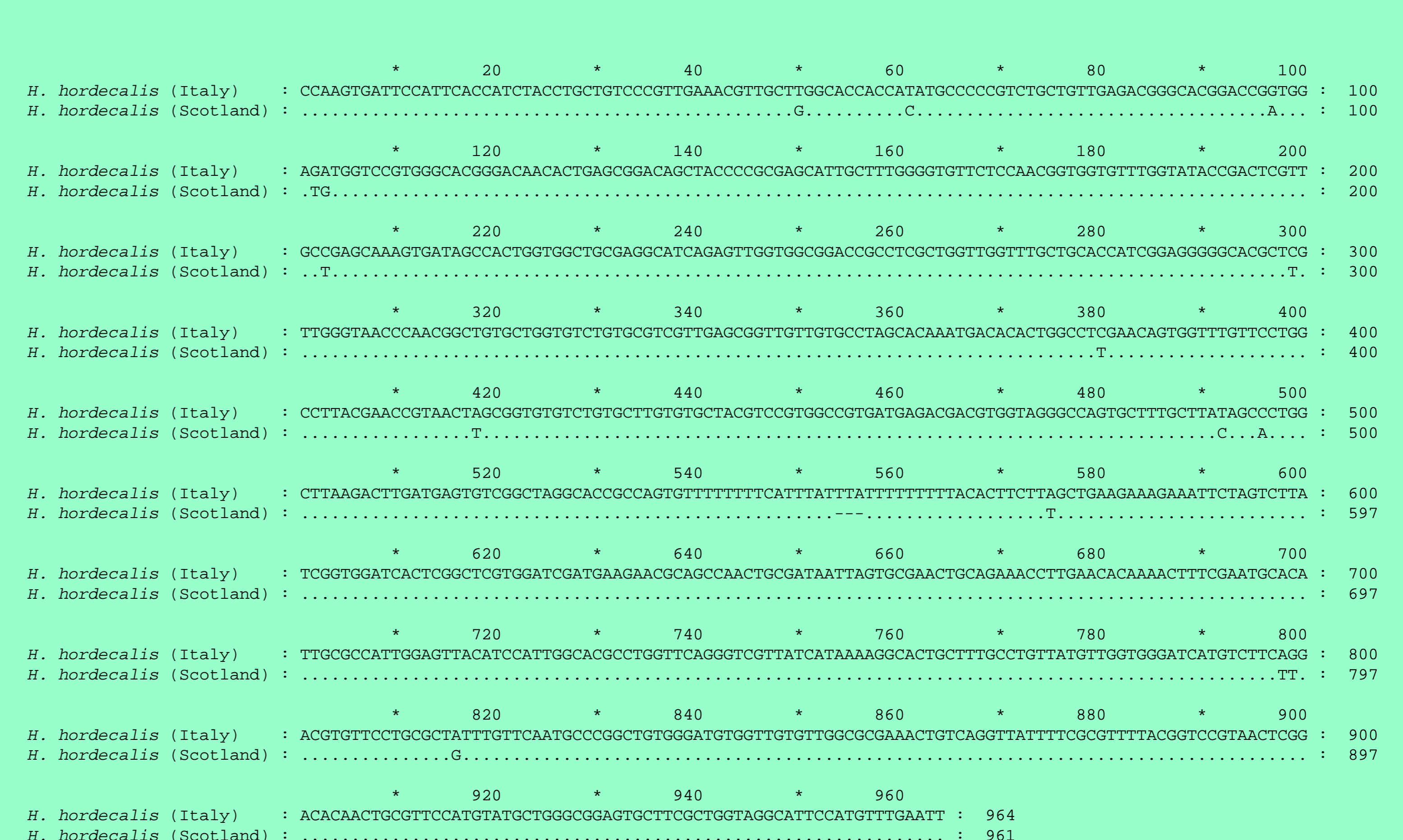


Fig. 6. Sequences of the ITS1+5.8S gene+ITS2 region for two populations of *Heterodera hordecalis*.