Identification and phylogenetic relationships within the stem nematode *Ditylenchus dipsaci* species complex (Tylenchida: Anguinidae) as inferred from analyses of the ITS-rDNA sequences

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Fig. 1. *Ditylenchus dipsaci*

The stem nematode *Ditylenchus dipsaci* has economic importance as a parasite of agricultural and horticultural crops (Fig. 1). It is distributed world-wide, infects over 500 plant species, and causes stunting and swelling of various plant organs. According to the present understanding, *D. dipsaci* should be considered as a species complex. *D. dipsaci* consists of many races and populations that differ mainly in host range and appear to be at different stages of speciation.

Analysis of coding and non-coding regions of ribosomal DNA has become a popular tool for identification and study of phylogenetic relationships of many nematode groups. Goals of our study were: (i) to revealed phylogenetic relationships between populations and races based on analysis of the ITS-rDNA, (ii) to estimate the taxonomic boundary of stem nematode *D. dipsaci* and (iii) to present RFLP-ITS-rDNA profiles and develop PCR with species specific primer for diagnostics of agricultural important races of the stem nematode.

The ITS region of rDNA from 23 populations of the *D. dipsaci* species complex (Fig. 2) were amplified using TW81 and AB28 primers and sequenced. The sequences were aligned with known seven ditylenchid sequences. Length for the entire ITS region including 5.8S gene ranged from 671 to 673 bp. The pairwise divergence of the sequences varied from 0 to 7.6% (0 - 50 nucleotides) within the studied populations. Maximum parsimony analyses of the ITS1-5.8S-ITS2 and the ITS2 using PAUP* generated several trees with seven distinct clades (Fig. 3): (1) *D. dipsaci sensu stricto* including all populations collected from cultivated plants (red clover, onion, alfalfa, oat, red beet, corn, garlic, strawberry and others), (2) "giant race" or *D. dipsaci sensu lato* "sp.B" from *Vicia faba,* (3-6) four clades with *Ditylenchus* populations parasitising plants from the Asteraceae and (7) a clade with populations from *Plantago maritima*. The molecular data support suggestions by several authors that differences in karyotype can indicate that biological races presently grouped under the nominal species *D. dipsaci* (2n = 24 for *D. dipsaci s. l.*), such as the giant race from *Vicia faba* (2n = 48-60) and populations from *Cirsium setosum* (2n = 52), *Plantago maritima* (2n =48-54), *Pilosella officinarum* (2n = 46) or *Crepis praemorsa* have deserve species status. The molecular analyses allow to justify the previous synonymisation of *D. allii, D. trifolii* and *D. phloxidis* with *D. dipsaci* and reconsider the taxonomic status for *D. sonchophilus* (2n = 52-56). Our study supports the distinction of at least six new and undescribed species, which were known as *D. dipsaci*.

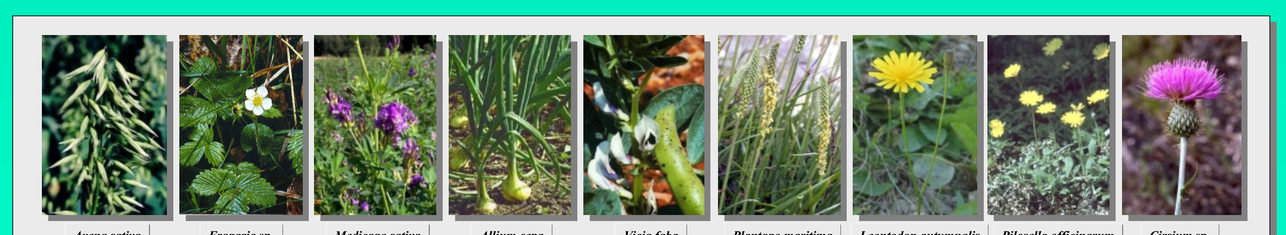

Fig. 2. Host–plants for the *Ditylenchus dipsaci* complex

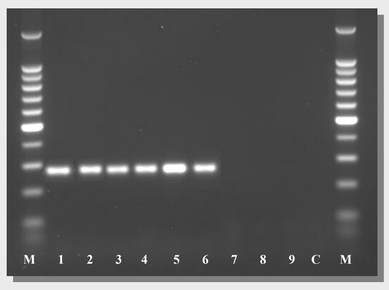

Fig. 5. PCR with species specific primer for *D. dipsaci sensu stricto*. M: 100 kb DNA marker, Promega, 1-6: D. *dipsaci s. st*. populations, 1: alfalfa, 2: oat, 3: garlic, 4: clover, 5: corn, 6: *Lusmachia*, 7-8: "Giant race", or *D. dipsaci s. l.* "sp.B" from *Vicia faba*, England and Syria, 9: *D. dipsaci s. l.* "sp.H" from *Plantago maritima,* C: Control without DNA.

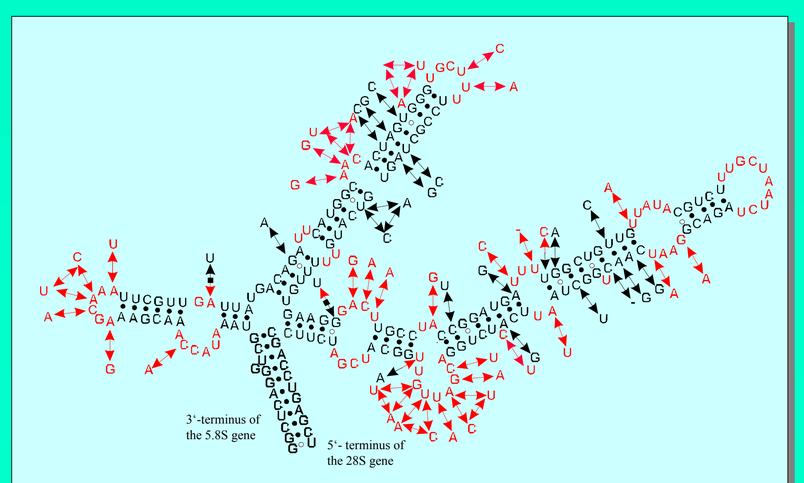

Fig. 6. Putative secondary structure of the ITS2 in the 5.8S-28S premature RNA for *Ditylenchus dipsaci sensu lato.*

Diagnostic RFLP profiles generated by eleven enzymes for *D. dipsaci s. st*. are presented in Fig. 4. PCR-RFLP and sequence data can not allow to distinguish most populations and races of *D. dipsaci s. st*. from each others. However, it needs to test more samples from each race, especially, from strawberry to support this statement. PCR-RFLPs is suited to identify a pure nematode culture and this approach does not allow mixed species populations to be identified. To overcome this limitation in the *D. dipsaci* detection, PCR with species specific primer was developed. Our method detected specimens from all *D. dipsaci s. st*. populations. No positive reactions were observed with other species of the *D. dipsaci* complex and other nematodes (Fig. 5). This method is of important relevance to regulatory services where information on species identity is limited.

Although the biological role of the ITS spacers is not well understood, the utilization of the yeast model has definitely shown their importance for production of the mature rRNAs. Using the energy minimization approach and comparative mutation analysis we found that the secondary structure of ditylenchid ITS2 was organized in three main domains emerging from a preserved central core (Fig. 6). The knowledge of RNA structure is becoming increasingly important in assisting accurate sequence alignment, weighting of nucleotides within stem and loops for phylogenetic analysis and may also serve as valuable proof-reading method for newly obtained sequences.

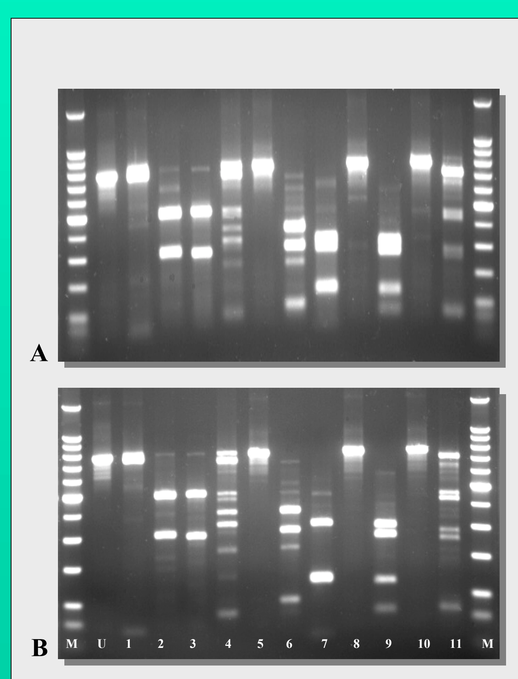

Fig. 4. RFLPs of the ITS region for the onion (*Allium cepa*, Germany) (A) and oat (*Avena sativa*, Germany) (B) populations of *D. dipsaci sensu sticto.* M: 100 kb DNA marker, Promega, U: unrestricted PCR, 1: *Alu*I, 2: *Bsh*126I, 3: *Cfo*I, 4: *Hae*III; 5: *Hin*dIII, 6: *Hinf*I, 7: *Hpa*II, 8: *Pvu*II, 9: *Rsa*I, 10: *Ssp*I, 11: *Mva*I.

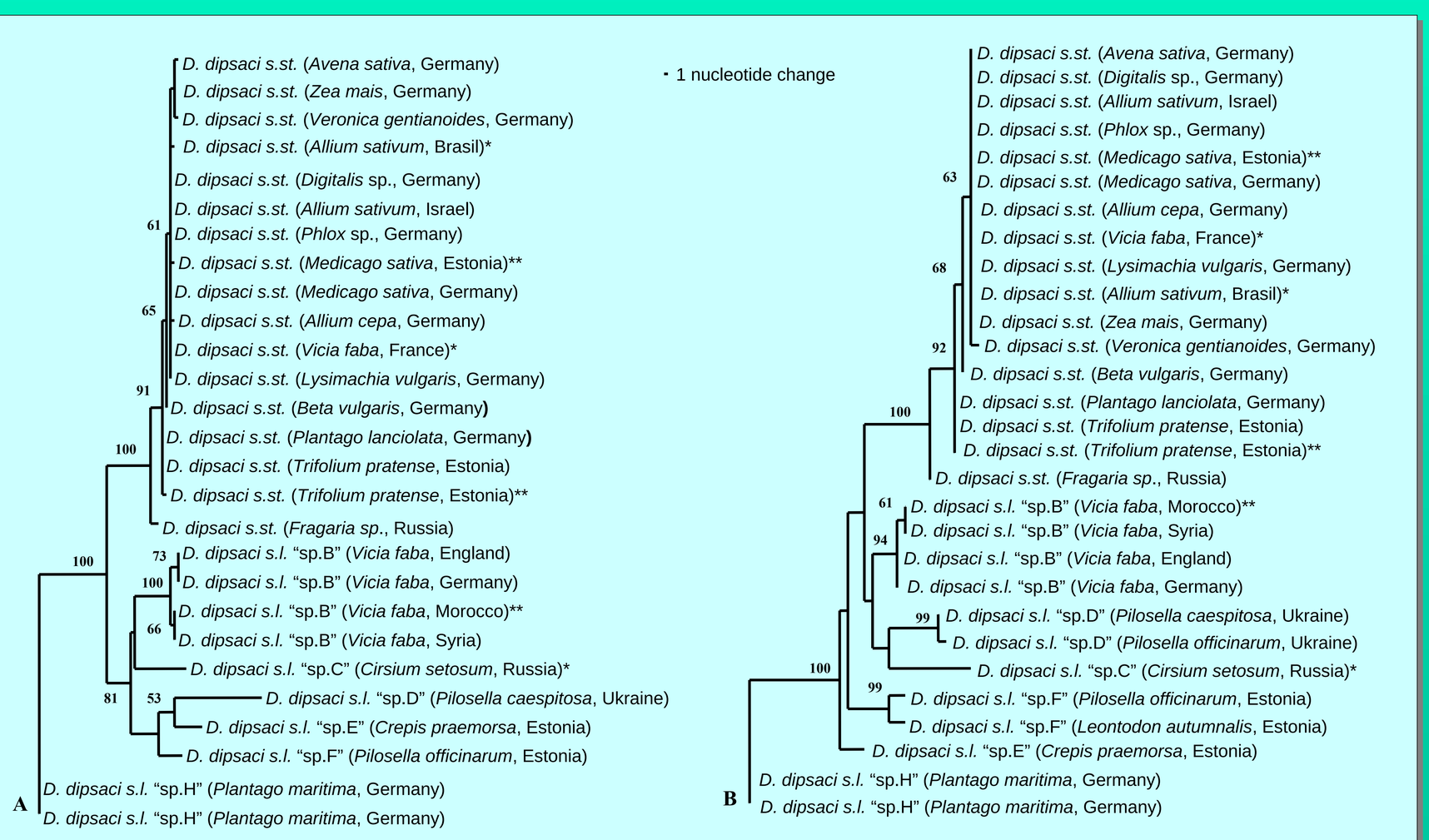

Fig. 3. Unrooted maximum parsimonious trees obtained from the analyses of the IIS1-5.8S-ITS2 (**A**) and ITS2 (**B**) sequences of *D. dipsaci sensu lato*. Bootstrap supports more 50% are given for appropriate clade.

* Sequence data from Leal-Bertioli *et al*., 2000. *Nematologia Brasileira*, 24: 83-85; ** Sequence data from Subbotin *et al.,* 2003. *Molecular Phylogenetic and Evolution*, (in press).