trichodorid species occurring in Portugal and six non-indigenous trichodorid species, based on 18S rDNA gene sequences. A comprehensive survey for trichodorids was carried out in continental Portugal and ten species (five *Trichodorus* and five *Paratrichodorus*) were identified using classical taxonomy. Representative specimens from each species were selected for molecular studies. DNA was extracted from individual nematodes, a minimum of two per population, and using appropriate primer sets the 18S rDNA gene was isolated and subsequently sequenced. The 18S rDNA gene from six non-indigenous trichodorid species was also sequenced. A multiple sequence alignment was produced and used as a basis of a Maximum Likelihood phylogenetic analysis. With one exception, the resultant phylogenetic tree clearly separated both genera and species into groups that agree with currently accepted taxonomy of the Trichodoridae. However, populations of *P. minor* appeared more closely associated with *Trichodorus* species than other *Paratrichodorus* species.

223. CHARACTERIZATION OF MUCRONATE FORMS OF *BURSAPHELENCHUS XYLOPHILUS*

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Since the discovery of the pinewood nematode, *Bursaphelenchus xylophilus*, in Portugal, a survey has been undertaken to detect its presence and distribution in the country. We have analysed many samples of wood chips in our laboratory. In several samples, the accurate identification based on morphobiometry was difficult because of character variability. Most populations of *B. xylophilus* have been distinguished from *B. mucronatus*, a closely related but non-pathogenic species, by the shape of the female tail terminus, which is either rounded or has a distinct mucro, respectively. Our morphological studies showed a wide variation in the female tail, from round to mucro type. A rapid and reliable identification was possible using the so-called "single worm PCR" technique developed by Abad and collaborators in Antibes, France. Reference cultures of *B. xylophilus* and *B. mucronatus* were obtained from Institut National de la Recherche Agronomique, Antibes, France. Three of the mucronate populations of *B. xylophilus* were isolated and cultured on *Botrytis cinerea* on malt extract agar and characterized also by an ITS-RFLP technique slightly modified from that described by Iwahory, Kanzaki and Futai. The mucronate nematodes can be successfully identified by the two techniques we used. However, it is suggested that further investigations on their pathogenicity should be undertaken.

224. A NEW ROOT-KNOT NEMATODE (*MELOIDOGYNE* SP.) PARASITIZING PEACH (*PRUNUS PERSICA*) IN FLORIDA, UNITED STATES, WITH OBSERVATIONS ON ITS MORPHOLOGICAL, MOLECULAR AND DIFFERENTIAL-HOST CHARACTERIZATION

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A new root-knot nematode, (*Meloidogyne* sp.), was found parasitic on Nemaguard peach roots (*Prunus persica*) at Gainesville, Florida, United States. This new species resembles *M. incognita*, *M. christiei*, *M. graminicola* and *M. hispanica*, but LM and SEM observations indicate that it differs from these species either by the following: body length, shape of head, tail and tail terminus of second-stage juveniles; body length and shape of spicules in males; and distinctive female perineal pattern. This pattern has a high to narrowly rounded arch with coarsely broken and network-like striae in and around the anal area, faint lateral lines interrupting transverse striae, a sunken vulva and anus, and large, distinct phasmids. Second-stage juveniles possess a broad to bluntly rounded terminus. Males include both short and long forms. Molecular data from ribosomal IGS illustrate that *Meloidogyne* sp. is different from the mitotic species *M. arenaria*, *M. incognita* and *M. javanica*. Data from RAPDs confirm this and suggest that the new species lies in an intermediate phylogenetic position between the previous species and the meiotic species *M. hapla*, *M. fallax* and *M. chitwoodi*. The new species reproduces by meiotic parthenogenesis and/or amphimixis, with haploid chromosome number of $n=18$, maybe sometimes 19 or 20. Differential host tests based on annual crops and on *Prunus* accessions are reported. Considering the morphological, molecular and differential host-characteristics, we consider this root-knot nematode on peach as unique among all other species

of root-knot nematodes previously described. Additional information regarding the distribution of this nematode within the region and its economic importance in peach and other cultivated crops is under investigation.

225. NEMATODE ASSOCIATES OF THE JAPANESE OAK WILT DISEASE

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The Japanese oak wilt disease, the mass mortality of Japanese oak trees, *Quercus mongolica* var. *grosseserrata* and *Q. serrata*, is caused by a kind of ambrosia fungus, *Raffaelea quercivora*, vectored by the oak borer, *Platypus quercivorus*, which makes a mass attack on its host oak tree. The nematode associates with the oak borer and those of dead oak trees were surveyed in the present study. Two species of nematodes, unidentified aphelenchid (code: A1) and diplogasterid (code: D2) species, were isolated from the backside of the elytra of oak borer, and more than 10 species (including the above mentioned two species) were isolated from the dead wood. As for A1, the propagative fourth stage juveniles and adults were isolated from the beetle body. However, the nematode did not propagate on several species of the fungi examined. Thus re-isolation from the beetles and/or from dead woods is necessary for identification of A1 and for further study of its life cycle. While the D2 was isolated from the beetles at its dauer juvenile stage, and propagated on NGM and Asparagine-Mannitol agar feeding on bacteria. Thus the nematode may utilize the beetles only as phoretic vectors. The D2 was identified as belonging to a new genus, which is morphologically intermediate between two families, *Cylindrocorporidae* and *Diplogasteroididae*. Further morphological and molecular studies are also needed to determine the systematic affiliation of the new genus.

226. STUDY OF THE NEMATODES OF THE ORDER MONONCHIDA FROM IRAN WITH THE DESCRIPTION OF *ANATONCHUS* SP.N.

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Very little is known about the predatory nematodes of the Order Mononchida Jairajpuri, 1969 of Iran. During our studies on soil-inhabiting nematodes in Chaharmahal va Bakhtyari province in central Iran, specimens of the mononchs were collected. In this presentation, some known species of mononchs of the genera *Mononchus, Clarkus* and *Mylonchulus* are reported and illustrated and a new species of the genus *Anatonchus* is described in details. The new species has 2.3-2.7 mm long body; lip region 52-53 μm wide, 20-21 μm high; amphids cup-shaped; buccal cavity 43-48 μm long and 31-33 μm wide. Oesophago-intestinal junction is prominently tuberculate; gonads are amphidelphic; vulva transverse with three cuticularized pieces at vulva-vagina junction; vulval papillae absent and tail is elongate conoid, 5-5.5 anal body width long; caudal glands with a terminal opening. The genus *Mononchus* and *Mylonchulus nainitalensis* are recorded for the first time in Iran.

227. SPICULE: THE MOST IMPORTANT MORPHOLOGICAL CHARACTER FOR *BURSAPHELENCHUS* SPECIES DIFFERENTIATION

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Spicule shape and size has been often considered an important character for differentiation among many nematode groups such as members of Rhabditida, Aphelenchida and a few genera of Tylenchida. Within each group, at specific level, spicule morphology assumes major importance in some aphelenchids (e.g. *Bursaphelenchus* spp.) and cephalobids. Studies on *Bursaphelenchus* species has shown that shape and spicule measurements are nearly sufficient for species identification. *Bursaphelenchus* spicules are paired, usually separated, arcuate, with a capitulum with two characteristic projections, rostrum and apex, and in some species the distal end forms a swelling or a disc-like structure named cucullus. Several *Bursaphelenchus* spicules were excised and observed by scanning electron microscope (SEM) to clarify its three-dimensional structure. Males were transferred live to a drop of a mixture of lactic acid (45%) + acetic acid (45%) + Rotring® Brilliant Ultramarine Blue ink (120:4:0.1) and heated briefly over an alcohol lamp; spicules were cleaned of attached tissues using a cactus thorn and transferred to a 2% formalin drop placed on a coverslip; the formalin was removed with a fine micropipette; after coating with gold, the spicules were viewed and photographed using Jeol 35 SEM. Observations of excised spicules ob-