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 BURSAPEHELCHUS XYLOPHILUS

L. Fonseca1, C. Vieira dos Santos1, S. Costa2, L. Abrantes1 and M.S. Santos1

1Instituto do Ambiente e Vida, Departamento de Zoologia, Universidade de Coimbra, 3004-517 Coimbra, Portugal
2Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ & Lancaster University, Lancaster, LA1 4YW, UK
nemz@iav.uc.pt

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 micro, respectively. Our morphological studies showed a wide variation in the female tail, from round to micro 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 Iwahori, 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

Z.A. Handoo1, A.P. Nyczepir2, D. Esmenjaud2, J.G. van der Beek4, P. Castagnone-Sereno3, L.K. Carta2 and A.M. Skantar1

1Nematology Laboratory, USDA, ARS, Henry A. Wallace Beltsville Agriculture Research Center, Beltsville, MD 20705, USA
2USDA, ARS, Southeastern Fruit and Tree Nut Research Laboratory, 21 Dunbar Road, Byron, GA, USA
3UMR IPMSV, INRA, BP 167, 06903 Sophia Antipolis Cedex, France
4Plant Research International P.O. Box 16, NL-6700 AA Wageningen, The Netherlands
handooz@ba.ars.usda.gov

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 meliotic species M. hapla, M. fallax and M. chitwoodi. The new species reproduces by meliotic 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

N. Kanzaki1 and K. Futai2

1Laboratory of Forest Protection, Faculty of Agriculture, Kagoshima University, Korimoto, Kagoshima, 890-0065, Japan
2Laboratory of Environmental Mycossence, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto, 606-8502, Japan

kanzaki@kais.kyoto-u.ac.jp

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 apherelench (code: A1) and diplogradist (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 phototactic vectors. The D2 was identified as belonging to a new genus, which is morphologically intermediate between two families, Cylindrocorporidae and Diplodactyloidae. 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.

M. Oli1, M. Choudhary1, W. Ahmad1 and M.S. Jairajpuri2

1Nematology Res. Lab., Dept. of Crop Science, Shahrekord University, Shahrekord, Iran
2Section of Nematology, Dept. of Zoology, Aligarh Muslim University, Aligarh 202 002, India

olia100@yahoo.com

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 Bachtiar 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 Anatonicus 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. Oesophage-intestinal junction is prominently tuberculate; gonads are amphidiphic; 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 rainitalensis are recorded for the first time in Iran.

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

A.C. Penas, A. Cruz and M.A. Bravo

Departamento de Proteccio das Plantas - Estacao Agronomica Nacional (EAN) INIA, Quinta do Marques, 2784-505 Oeiras, Portugal
acpenas@hotmail.com

Spicule shape and size has been often considered an important character for differentiation among many nematode groups such as members of Rhabditida, Apherelenchida and a few genera of Tylenchida. Within each group, at specific level, spicule morphology assumes major importance in some apherelenchids (e.g. Bursapelenchus spp.) and cephalobids. Studies on Bursapelenchus species has shown that shape and spicule measurements are nearly sufficient for species identification. Bursapelenchus 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 Bursapelenchus 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 micro pipette; after coating with gold, the spicules were viewed and photographed using Jeol 33 SEM. Observations of excised spicules ob-