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'*Candidatus* Phytoplasma ulmi' causing yellows in *Zelkova serrata* newly reported in Italy

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The Japanese elms are temperate broad-leaved tree species in the genus *Zelkova*. This genus includes species that are nowadays distributed in East Asia (three species), Western Asia (one species) and the Mediterranean (two species), while it is absent from North America (Denk & Grimm, 2005). Even though a member of the elm family, the Japanese elm had no disease or pest problems of significance, and is not susceptible to Dutch elm disease. Since June 2006, however, several plants of *Z. serrata* grown in Ancona, Marche region (central eastern Italy), have shown symptoms of chlorosis which involve the whole plant or some of the branches, foliar reddening on one or more branches, attenuation of apical dominance and proliferation of lateral shoots, witches' broom, reduced growth and stunting of the plant.

Leaf samples from plants with and without symptoms were collected and the DNA was extracted using the Plant DNeasy mini kit (Qiagen). A molecular diagnosis was carried out to detect phytoplasma by PCR with universal primers P1/P7 (Seemüller *et al*., 1998) followed by a nested PCR with specific group primers 16Sr(V)F1/R1 (Lee *et al*., 1994). All of the five plants with symptoms yielded a PCR product of the expected size (1100 bp), both during 2006 and 2007. This PCR product was purified from the 1% agarose gels using Spin Column Wizard SV Gel and the PCR Clean–Up System (Promega), and eluted with sterile ultrapure water, according to the manufacturer's instructions. Five-μl aliquots of the PCR product were digested with 3 U of restriction endonuclease *Bfa*I (New England BioLabs), over night at 37°C. The restriction pattern was characterized by two bands of 650 and 450 bp, identical to those in the reference strain of '*Candidatus* Phytoplasma ulmi' (EY, kindly provided by C. Marzachì, IVV-CNR, Torino, Italy) (Lee *et al*., 2004). The pathogen was not found in samples from symptomless plants. Those plants were inoculated by grafting with samples from plants with symptoms, and disease symptoms appeared in the entire plant. Leaf samples from artificially inoculated plants tested positive for the presence of the phytoplasma. The sequenced isolate from *Z. serrata* showed more than 99% identity with '*Ca.* Phytoplasma ulmi' strain EY1^T (GenBank Acc. No. AY197655) (Lee *et al*., 2004).

Several natural infections of '*Ca.* Phytoplasma ulmi' have been described on different species of the genus *Ulmus* (Lee *et al*., 2004), although it has not been reported on *Zelkova* spp. This thus appears to be the first report of '*Ca.* Phytoplasma ulmi' infection in affected *Z. serrata*, in Italy and worldwide.

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New strain of '*Candidatus* Phytoplasma ulmi' infecting *Ulmus minor* and *U. laevis* in Serbia

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Elm yellows (EY) phytoplasma ('*Candidatus* Phytoplasma ulmi') is the causal agent of a decline in American elms in North America, and in Eurasian elm species and hybrids in Europe (Lee *et al*., 2004). EY is known to infect different *Ulmus* species: *U. americana*, *U. minor*, *U. rubra*, *U. alata*, *U. serotina*, *U. crassifolia* and *U. chenmoui*, showing different symptoms such as stunting, witches' broom, yellowing and general decline of the plants (Marcone *et al*., 1997; Griffiths *et al*., 1999). In September 2007 leaves with petioles from eighteen elm trees showing symptoms of discrete leaf yellowing were collected from three different sites in northeast Serbia near the villages of Srednjevo, Ljubičevo and Šuvajić. From each site six samples were collected. At two sites (Srednjevo and Ljubičevo) the affected plants were of European field elm (*U. minor*), and at the third site they were of European white elm (*U. laevis*). Leaves of six symptomless young elm trees (*U. minor*) collected near Belgrade served as the controls.

Total nucleic acids were extracted from fresh leaf midribs and petioles using the CTAB method (Angelini *et al*., 2001). Phytoplasma identification was conducted using a nested PCR assay with P1/P7 and F2n/R2 primers on the 16S rRNA gene, followed by RFLP analysis with *Mse*I restriction enzyme. Positive results were obtained in nine affected *U. minor* samples and five *U. laevis* samples, with RFLP profiles indicating the presence of phytoplasmas of the 16SrV group. None of the symptomless plants were positive for the presence of phytoplasma. Further characterization was performed by amplifying the ribosomal protein genes *l22* and *s3* using primers rp(V)F1/rpR1 followed by rp(V)F1A/rp(V)R1A, finally by digestion with *Mse*I and *Tsp*509I (Lee *et al*., 2004). RFLP profiles with *Mse*I enzyme showed the presence of EY phytoplasmas of 16SrV-A group, but profiles obtained with *Tsp*509I enzyme were different from the EY control sample and were more similar to FD-C (16Sr V-C group). Subsequently two of these products, one from *U. minor* and one from *U. laevis*, were sequenced (GenBank Acc. No. EU592500, EU592501) and showed identical nucleotide sequence to each other. BLAST analyses showed 99% similarity of these isolates with reference strain EY1^T (AY197675). Nucleotide changes are located in two out of three unique regions of the *rpl22–rps3* genes reported by Lee *et al.* (2004) as being species specific for '*Candidatus* Phytoplasma ulmi'.

This is the first report of elm yellows phytoplasma belonging to rRNA group 16SrV-A infecting elm species in Serbia and of its association with *U. laevis*. It is also the first evidence of strain differences in '*Candidatus* Phytoplasma ulmi' detectable by RFLP analysis of ribosomal protein gene PCR products.

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