‘*Candidatus* Phytoplasma ulmi’ affecting *Ulmus laevis* in Germany

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Phytoplasmas are wall-less obligate parasites of the plant phloem and associated with diseases in many important crops and trees worldwide. Elm yellows phytoplasma (EY) belongs to the ribosomal group 16SrV subgroup A and is assigned as ‘*Candidatus* Phytoplasma ulmi’. It is known to be associated with elm phloem necrosis, leaf yellowing, stunting, witches broom and decline in various elm species. In 2013, European white elms (*Ulmus leavis* PALL.) were investigated for EY infection in Berlin (*N*42), in the palace Caputh (*N*4) and in the riparian forest Spreewald (*N*12) (Brandenburg; Fig. 1 & 2).

Fig. 1: *Ulmus laevis* in Germany.
A: elms in the riparian forest Spreewald; **B:** leaf exhibiting mild yellowing symptoms (indicated by arrow); **C:** asymptomatic leaves.

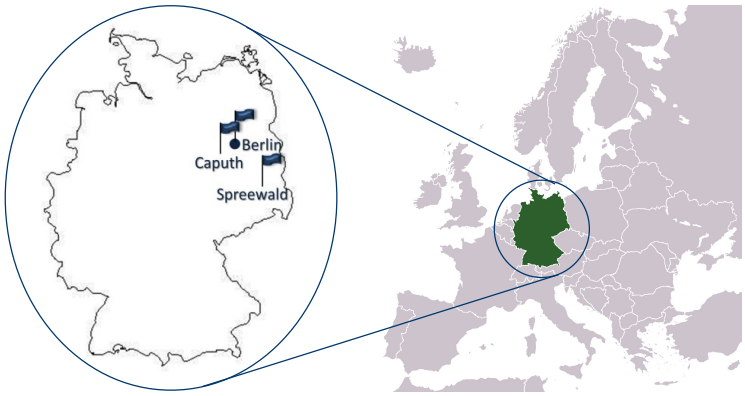

Fig. 2: Location of *U. laevis* trees analyzed in Germany.

MATERIAL & METHODS

- DNA extraction by CTAB approach (Ahrens & Seemüller 1992, modified)
- diagnostic direct & nested PCR for amplification of partial 16S rRNA by applying primer pairs P1/P7 (Smart et al. 1996) and R16F2n/R16R2 (Gunderson & Lee 1996)
- sequence determination and alignment to reference sequence EY1^T (Lee et al. 2004)

RESULTS & CONCLUSIONS

- 30/58 *U. laevis* trees (Fig. 3) were tested positive for phytoplasma infection in Germany
- sequence analysis allowed assignment to 16SrV-A
- this study strengthens the results of Serbian EY isolates obtained from *U. laevis* (Jović et al. 2011, Tab. 1)
- **first report of ‘*Ca*. P. ulmi’ infecting *U. laevis* in Germany**

RESULTS

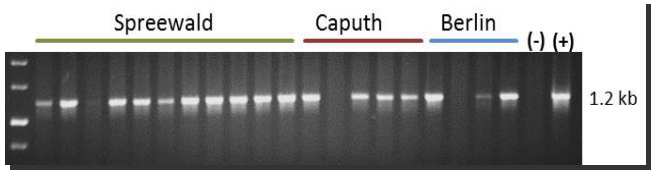

Fig. 3: Nested PCR of phytoplasma detection by partial 16S rRNA amplification.
(-): water control; (+): ‘*Ca.* P. asteris‘ strain AY1 from Vinca; M: 1kb Marker.

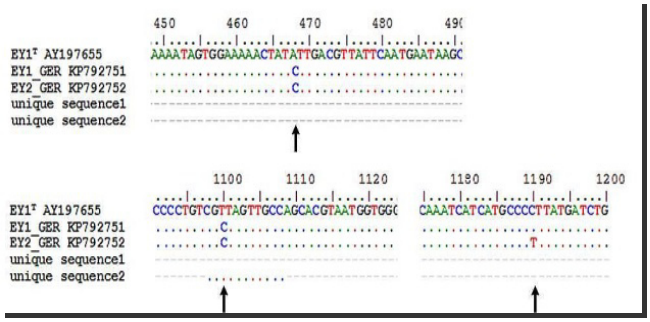

Fig. 4: Alignment of EY isolates.
Sequence variations are indicated by arrows. Substitution at nt 1100 is located within a conserved (unique) sequence for EY 16S-*rRNA* gene (Lee et al. 2004).

EY isolate	Acc.no.	host	bp position 468	bp position 1100	bp position 1190
EY1^T	AY197655	*Ulmus americana*	A	T	C
EY627	AY197658	*U. minor*	C	T	C
EY10_SRB	HM038457	*U. laevis*	C	C	C
EY18_SRB	HM038458	*U. laevis*	C	C	C
EY20_SRB	HM038459	*U. laevis*	C	C	C
EY1_GER	KP792751	*U. laevis*	C	C	C
EY2_GER	KP792752	*U. laevis*	C	C	T

Unique sequence to ‘*Ca*. P. ulmi‘ in 16S rRNA:

5‘-CGT TAG TTG CC-3‘

Tab. 1: Variations of the partial 16S rRNA sequence.
Sequences of EY isolates from Germany show two and respectively three nt substitutions.

REFERENCES
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Gunderson & Lee 1996, Phytopathol Mediterr 35, 144 – 151
Jović, Cvrković, Mitrović et al. 2011, Plant Pathol 60, 356-368
Lee, Martini, Marcone et al. 2004, Evol Microb 48, 1153-1169
Smart, Schneider, Blomquist et al. 1996, Appl Environ Microb 62, 2988-2993