



## Detection

# Use of mitochondrial divergence in plant-specialized populations of *Hyalesthes obsoletus* for identification of 'Candidatus Phytoplasma solani' epidemiology

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## Abstract

*Hyalesthes obsoletus*, a major vector of 'Candidatus Phytoplasma solani' is considered as a polyphagous species associated with diverse wild host-plants. A recent study performed in southeastern Europe documented genetic differentiation among host-plant associations of *H. obsoletus*, while numerous data are pointing to specialized plant-associated epidemiological cycle of 'Ca. P. solani'. Hence, based on the mitochondrial *COI* gene sequences, a method for routine identification of plant-specialized vector populations and epidemiological routes of pathogen transmission were developed and verified.

**Keywords:** *COI* gene, epidemiology, genetic segregation, host-plant, insect vector, molecular identification

## Introduction

The cixiid planthopper *Hyalesthes obsoletus* (Hemiptera: Cixiidae) is a principal vector of 'Candidatus Phytoplasma solani' (16SrXII-A ribosomal subgroup), with a major role in pathogen spread, transmission routes and disease epidemiology (Jovic *et al.*, 2019). Generally, *H. obsoletus* is considered as a polyphagous species associated with diverse wild host-plants which, often simultaneously, serve as pathogen reservoirs and consequently are the source of disease transmission. However, recent research performed in southeastern Europe, the distribution centre of the planthopper and the area of many 'Ca. P. solani'-associated plant diseases, indicated specific host-plant associations of the vector and specific vector-based routes of transmission (Kosovac *et al.*, 2018). Genetic differentiation and host-driven segregation (mitochondrial and nuclear) among three morphologically inseparable host-plant associations of *H. obsoletus* (Figure 1) was evidenced for populations affiliated to: i) *Urtica dioica* and *Convolvulus*, ii) *Vitex agnus-castus*, and iii) *Crepis foetida*.

In different parts of Europe, several studies have also documented the presence of a specialized plant-associated 'Ca. P. solani' epidemiological cycle and life cycles of *H. obsoletus* (Langer and Maixner, 2004; Johannesen *et al.*, 2008; Imo *et al.*, 2013; Sharon *et al.*, 2015; Kosovac *et al.*, 2016, 2019). The two principal transmission routes of 'Ca. P. solani' in the continental Europe are based on vector association and pathogen specialization towards *U. dioica* and *C. arvensis*,

while each plant is hosting a specific pathogen strain according to the elongation factor *Tu* gene, tuf-type a and tuf-type b, respectively (Langer and Maixner, 2004). In the Mediterranean coastal zone, along with the aforementioned transmission routes, *V. agnus-castus* is a preferred host-plant of *H. obsoletus* (Sharon *et al.*, 2015) and an independent source of pathogen transmission and of tuf-type b strain (Kosovac *et al.*, 2016). Finally, an additional, independent and recently evidenced, epidemiological cycle of tuf-type b 'Ca. P. solani' strain in the Balkan region is sourced by *C. foetida* and transmitted by its associated *H. obsoletus* population (Kosovac *et al.*, 2019). The three tuf-type b epidemiological cycles vectored by plant-specialized populations of *H. obsoletus* can be differentiated either by insect vector genetic divergence (Kosovac *et al.*, 2018, 2019) or pathogen divergence using variable, epidemiologically informative marker genes (Cimerman *et al.*, 2009; Fabre *et al.*, 2011) or with a combination of both (Kosovac *et al.*, 2019). The aim of this study was to point to possibilities of using mitochondrial divergence in plant-specialized populations of insect vector for a routine identification of epidemiological routes of 'Ca. P. solani' strain transmission.

## Materials and Methods

The material used in this study is in detail described in Kosovac *et al.* (2018). DNA of selected *H. obsoletus* individuals representing each of the formerly identified AB, EC, ZN, YM and JH mtDNA haplotypes were used for

analysis of nearly full-length of cytochrome oxidase subunit I (*COI*) mitochondrial gene. Obtained sequences were compared and analyzed using MEGA 7. Nucleotide diversity and variability was assessed within and between host-specialized mt*COI* haplogroups. These data were used for designing host-plant-specific multiplex PCR method.



**Figure 1.** *Hyalesthes obsoletus* on *Convolvulus arvensis* (left) and on *Crepis foetida* (right).

## Results

The 1476-bp long mt*COI* gene sequences were obtained for 54 *H. obsoletus* specimens affiliated with one of the three host-plant associations: *U. dioica*-*C. arvensis*, *V. agnus-castus* or *C. foetida*. Nucleotide analyses confirmed the genetic segregation according to host-plant specialization in all the 18 identified haplotypes originating from a wide geographic area (6 *U. dioica*-*C. arvensis*, 2 *V. agnus-castus* Greece, 4 *V. agnus-castus* Montenegro and 6 *C. foetida* haplotypes). Mean genetic distance within host-specialized haplogroups was 0.2% for *U. dioica*-*C. arvensis* and *C. foetida*, and 0.6% for *V. agnus-castus*, while between haplogroups the mean distance was 1.7-2.1%. The sequence comparison of all the haplotypes identified within the *H. obsoletus sensu lato* revealed a genetic variability represented by 57 variable, 11 singleton and 46 parsimony-informative sites. Out of these, 26 sites could be used as informative for the discrimination among *U. dioica*-*C. arvensis*, *V. agnus-castus* and *C. foetida* host-specialized *H. obsoletus*. Based on mt*COI* sequence variability three forwards primers were designed with binding positions in different regions of the gene, according to variable nucleotide sites specific for each host-specialized haplogroups. In addition, a single reverse primer was designed at the 3'-end of the gene containing nucleotide sequence corresponding to all three host-associated types. All four primers were used as a set, and methodology of differentiation of the three plant-specialized *H. obsoletus* populations was successfully tested on numerous specimens (Kosovac *et al.*, 2018).

## Discussion

Development of tools for molecular identification and separation of cryptic species or ecological host races is of great importance in insect vector-borne plant diseases of economic relevance. The presented methodology for plant-specialized *H. obsoletus* discrimination enables not only a reliable identification of the vector population (when

collected on cultivated plants), but at the same time provides information on the pathogen source plant and disease epidemiology. Although more data are needed regarding 'Ca. *P. solani*' differentiation in association with each plant-specialized vector population, available data are supportive of this conclusion (Kosovac *et al.*, 2016; 2019).

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## References

- Cimerman A, Pacifico D, Salar P, Marzachi C and Foissac X 2009. Striking diversity of *vmp1*, a variable gene encoding a putative membrane protein of the "stolbur" phytoplasma. *Applied and Environmental Microbiology*, 75 (9): 2951-2957.
- Fabre A, Balakishiyeva G, Ember I, Omar A, Acs Z, Kölber M, Kauzner L, Della Bartola M, Danet J-L and Foissac X 2011. StAMP encoding the antigenic membrane protein of "stolbur" phytoplasma is useful for molecular epidemiology. *Bulletin of Insectology*, 64(Supplement): S129-S130.
- Imo M, Maixner M and Johannesen J 2013. Sympatric diversification vs. immigration: deciphering host-plant specialization in a polyphagous insect, the "stolbur" phytoplasma vector *Hyalesthes obsoletus* (Cixiidae). *Molecular Ecology*, 22: 2188-2203.
- Johannesen J, Lux B, Michel K, Seitz A and Maixner M 2008. Invasion biology and host specificity of the grapevine yellows disease vector *Hyalesthes obsoletus* in Europe. *Entomologia Experimentalis et Applicata*, 126: 217-227.
- Jovic J, Riedle-Bauer M and Chuche J 2019. Vector role of cixiids and other planthopper species. In: *Phytoplasmas: Plant Pathogenic Bacteria-II: Transmission and Management of Phytoplasma Associated Diseases*, pp 79-113. Eds A Bertaccini, PG Weintraub, GP Rao and N Mori, Springer, Singapore.
- Kosovac A, Radonjic S, Hrcnic S, Krstic O, Tosevski I and Jovic J 2016. Molecular tracing of the transmission routes of "bois noir" in Mediterranean vineyards of Montenegro and experimental evidence for the epidemiological role of *Vitex agnus-castus* (Lamiaceae) and associated *Hyalesthes obsoletus* (Cixiidae). *Plant Pathology*, 65: 285-298.
- Kosovac A, Johannesen J, Krstic O, Mitrovic M, Cvrkovic T, Tosevski I and Jovic J. 2018. Widespread plant specialization in the polyphagous planthopper *Hyalesthes obsoletus* (Cixiidae), a major vector of "stolbur" phytoplasma: evidence of cryptic speciation. *Plos One*, 13(5): e0196969.
- Kosovac A, Jakovljevic M, Krstic O, Cvrkovic T, Mitrovic M, Tosevski I and Jovic J. 2019. Role of plant-specialized *Hyalesthes obsoletus* associated with *Convolvulus arvensis* and *Crepis foetida* in the transmission of 'Candidatus Phytoplasma solani'-inflicted "bois noir" disease of grapevine in Serbia. *European Journal of Plant Pathology*, 153: 183-195.
- Langer M and Maixner M 2004. Molecular characterisation of grapevine yellows associated phytoplasmas of the "stolbur" group based on RFLP analysis of non-ribosomal DNA. *Vitis*, 43: 191-199.
- Sharon R, Harari AR, Zahavi I, Raz R, Dafny Yelin M, Tomer M, Sofer-Arad C, Weintraub PG and Naor V 2015. A yellows disease system with differing principal host plants for the obligatory pathogen and its vector. *Plant Pathology*, 64: 785-791.