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## IS *HYALESTHES OBSOLETUS* A SPECIES COMPLEX UNDERGOING CRYPTIC SPECIATION? MORE EVIDENCE OF HOST-ASSOCIATED GENETIC DIFFERENTIATION IN SOUTHEAST EUROPE

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Research on integrative taxonomy, geographical center of origin and diversity, host-shift patterns and genetic variation in insect pests are of essential importance to perceive their invasive capacity and adaptability to new habitats. These can be clarified by combining population genetic methods with morphological, ecological and biogeography data (GRAPPUTO et al., 2005). In the case of cixid planthoppers (Cixiidae), which establish close relationships with their host plants and affect numerous crops by vectoring phytoplasmas (NICKEL, 2003), this combined approach is of particular importance because it can help to trace distinct genetic lineages that potentially have different modes of behaviour and consequently epidemiological cycles.

*Hyalesthes obsoletus* Signoret, 1865 (Hemiptera: Cixiidae) is the main vector of 'Candidatus Phytoplasma solani' (CPs) and of the Bois Noir disease of grapevine. It is the type species of the genus and a member of the *Hyalesthes obsoletus* species group (HOCH and REMANE, 1985); i.e. a species complex comprising at least six closely related and by outer morphology very similar species. *H. obsoletus sensu stricto* is the most widely distributed of the species within the complex, which has a Mediterranean diversity centre (HOCH and REMANE, 1985; NICKEL, 2003). It co-occurs in sympatry with all the five other species in the complex: *H. lacotei* in south France, *H. thracicus* in Greece, *H. yozgaticus* and

*H. hani* in Turkey and Lebanon, and *H. flavovarius* in Uzbekistan. Although *H. obsoletus* is generally treated as a polyphagous species, so far only few plant species are considered as wild-host plants in central and southeastern Europe: *Convolvulus arvensis*, *Urtica dioica* and *Vitex agnus-castus* (MAIXNER et al., 1995; SHARON et al., 2005; KESSLER et al., 2011). Recent findings of genetic differentiation in *H. obsoletus* populations associated with different host plants revealed segregation of two host races on the north-western edge of the distribution range that were associated with *C. arvensis* and *U. dioica*, respectively (JOHANNESSEN et al., 2008; IMO et al., 2013), while preliminary findings from Southeast Europe have revealed the existence of a genetically divergent lineage associated with *Crepis foetida* (KOSOVAC et al., 2013). In addition, *V. agnus-castus* is now experimentally confirmed as a pathogen source plant in Bois noir epidemiology in the Mediterranean (KOSOVAC et al., 2016). This new link imposes a new element in the host-plant affiliations of *H. obsoletus* at the population level.

In order to understand ecological specialisation and cryptic speciation potential in *H. obsoletus*, seven nuclear markers (microsatellites) (IMO et al., 2013) were analysed to determine the level of differentiation among 16 populations associated with four host plants in Southeast Europe: *C. arvensis*, *U. dioica*, *C. foetida* and *V. agnus-castus*. Multilocus genotypes of 280 specimens were analyzed using Structure

2.3.4 Bayesian-based clustering to determine the most likely number of genetic clusters. The analyzed populations were unambiguously separated into three genetic entities with more than 92% membership assignment per population. The first cluster comprised populations associated with *C. arvensis* and *U. dioca*, while members of the second and third cluster were *H. obsoletus* populations affiliated to *V. agnus-castus* and *C. foetida*, respectively. Molecular variance analysis, performed with Arlequin 3.5.2, estimated that 82% of the total genetic variance was explained by genetic divergence among the three clusters (host-plant groups) ( $p < 0.001$ ). Phylogenetic analysis based on three mitochondrial gene regions showed separation into three closely related haplotype groups that corresponded to host plant affiliation and clusters obtained from nuclear markers. Sympatric and often syntopic co-occurrence of genetically divergent *H. obsoletus* populations associated with different host plants throughout Southeast Europe implicate ecological specialisation leading to cryptic speciation. It questions the presumed polyphagy of this cixiid and indicates much greater species diversity than is currently recognized within the *Hyalesthes obsoletus* species complex. Considering that the four plant species studied here represent dual host plants for pathogen and vector, i.e. inoculum sources, cryptic species should be of concern in all future epidemiological studies of CPs induced diseases.

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