

SHORT COMMUNICATION:**MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF *HYALESTHES OBSOLETUS* SIGNORET (AUCHENORRHYNCHA: CIXIIDAE) IN HERZEGOVINA VINEYARDS**ZORICA ĐURIĆ¹, SNJEŽANA HRNČIĆ² AND DUŠKA DELIĆ¹¹ University of Banja Luka, Faculty of Agriculture
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During 2016 a survey for *Hyalesthes obsoletus* SIGNORET (Auchenorrhyncha: Cixiidae) was conducted in and around three vineyards in the Herzegovina district of Bosnia and Herzegovina (BiH). *H. obsoletus* were collected using sweep net and mouth aspirator from *Convolvulus arvensis* and *Vitex agnus-castus*. The identity of the caught adults was checked using morphological characters of adults and male genitalia and employing molecular methods such as PCR/RFLP on COI and ITS2 region and sequence analyses. All analyses confirmed the presence of *H. obsoletus* in the herbaceous flora of Herzegovina's vineyards for the first time.

Keywords: Cixiidae, *Convolvulus arvensis*, *Vitex agnus-castus*, morphological identification, PCR, sequence analyses

Morphologische und molekulare Identifizierung von *Hyalesthes obsoletus* SIGNORET (Auchenorrhyncha: Cixiidae) in Weinbergen Herzegowinas. Im Jahr 2016 wurde das Auftreten von *Hyalesthes obsoletus* SIGNORET (Auchenorrhyncha: Cixiidae) in und um drei Weinberge im Kanton Herzegowina von Bosnien und Herzegowina erfasst. *H. obsoletus* wurde mittels Kescher und Exhaustor von *Convolvulus arvensis* und *Vitex Agnus-Castus* gesammelt. Zur Identifizierung der gefangenen Adulten wurden ihre äußeren morphologischen Merkmale sowie die der männlichen Genitalien herangezogen., weiters wurden molekulare Methoden wie PCR/RFLP in der COI- und ITS2-Region und Sequenzanalysen eingesetzt. Alle Analysen bestätigten das Vorkommen von *H. obsoletus* in der Krautschicht der Weinberge Herzegowinas zum ersten Mal.

Schlagwörter: Cixiidae, *Convolvulus arvensis*, *Vitex agnus-castus*, morphologische Identifikation, PCR, Sequenzanalysen

Hyalesthes obsoletus Signoret (Auchenorrhyncha: Cixiidae) is a confirmed planthopper vector of 'Candidatus Phytoplasma solani' (genetic group 16Sr-XII-A) to grapevine and several horticultural crops in Euro-Mediterranean regions (QUAGLINO et al., 2013; MAIXNER, 1994). However, it has been observed that *H. obsoletus* prefers weeds such as *Convolvulus arvensis* L., *Urtica dioica* L. and, recently found, *Vitex agnus-castus* L., which simultaneously act as a host for the vector and a phytoplasma reservoir (BRESSAN et al., 2007; KOSOVAC et al., 2015). In Bosnia and Herzegovina (BiH) 'Ca. P. solani' has been identified in grapevine, maize, pepper and celery (DELIC et al., 2011 a, b; DELIC et al., 2016 b; KOVAČEVIĆ et al., 2014). Multigene characterisation on 'Ca. P. solani' isolates from grapevine, pepper and celery (DELIC et al., 2016 a, b) implied the role of *H. obsoletus* in the epidemiologic route of the phytoplasma in the studied hosts. On the other hand *H. obsoletus* has never been identified in the cixiid fauna of BiH agro-ecosystems. So the main aim of this work was to explore the presence of *H. obsoletus* on the main phytoplasma reservoir plants such as *C. arvensis* and *V. agnus-castus* surrounding vineyards in the Herzegovina region where previously the epidemiology of 'Ca. P. solani' was confirmed by DELIC et al. (2011 a, b). Furthermore, the focus was to confirm the identity of *H. obsoletus* adults using morphological and molecular characters.

MATERIAL AND METHODS

In July 2016, a small-scale survey was carried out in vineyards at three sites in the Herzegovina region (Trebinje, GPS: 42°40'37"N18°19'52"E; Popovo polje GPS:42°51'22"N18°27'52"E; and Ravno, GPS: 42°53'0"N17°99'20"E). Leafhoppers and planthoppers were collected using sweep net and mouth aspirator directly from *C. arvensis* and *V. agnus-castus* found inside and outside of the vineyards. All specimens were kept in 96 % ethanol until morphological identification and DNA extraction. Collected specimens were individually identified to the family or genus level, observing morphological features through a stereo microscope. Identification was confirmed according to the identification guides by HOLZINGER et al. (2003) and BIEDERMAN and NIEDRINGHAUS (2004).

The total DNA from three individual *H. obsoletus* speci-

mens (two collected from *V. agnus-castus* and one from *C. arvensis*) was isolated using the EXTRACTME DNA Tissue Kit (Blirt DNA-Gdansk, Gdansk, Poland), according to manufacturer's instructions.

For the PCR analyses primers amplifying the ITS2 region and mtCOI fragment were employed. The amplification of the ITS2 region was carried out with ITS2fw and reverse ITS2rv primers, respectively (COLLINS and PASKEWITZ, 1996). A fragment of COI mitochondrial gene was amplified using C1-J-2195 and L2-N-3014 primer pair (SIMON et al., 1994). The ITS2 and COI fragments amplified from *H. obsoletus* were digested with the *TaqI* restriction enzyme at 65 °C for two hours.

For additional identification, selected PCR products of ITS2 and COI fragments were sent to Macrogen (Amsterdam, Netherlands) for sequencing. BLAST analysis (Basic Local Alignment Search Tool; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used to compare the sequences obtained with those from the GenBank.

RESULTS AND DISCUSSION

In total twenty-five *H. obsoletus* adults were captured from the field (10 from *V. castus-agnus* and 15 from *C. arvensis*) from all surveyed vineyards. The species is easily recognized because of its whitish collar-like pronotum (Fig. 1). Still, the main characters for morphological identification are the shape of the aedeagus and the genital style (Fig. 2a, b, c). In addition to *H. obsoletus* the presence of *Reptalus cuspidatus*, *Philaenus spumarius*, *Aphrophora alni*, *Dictyophara europaea*, *Eupelix cuspidata* and unidentified species from the Cicadellidae family was revealed.

The amplification of the ITS2 region provided fragments with a size of 880 bp while fragments of 890 bp were amplified using a primer specific for the mitochondrial COI gene. The obtained RFLP profiles with *TaqI* of COI sequences provided species-specific digestion patterns for *H. obsoletus* (~210 bp, ~680 bp) and of ITS2 amplicons ~800 bp what is in line with specific RFLP patterns for *H. obsoletus* described in BERTIN et al. (2010). The ITS2 sequences from all the three *H. obsoletus* specimens are available in GenBank (accession numbers: KY320566-68), and the BLAST analyses revealed that they were 99 % identical with the *H. obsoletus* specimen collected from Romania (GenBank Acc.No.GU552996) (BERTIN et al., 2010).

Comparing the sequenced COI fragment, we found out that the obtained sequences of *H. obsoletus* GenBank accession numbers: KY320569-70 revealed a 98 % identity with *H. obsoletus* specimens collected from Russia (GenBank Acc.No.GU553002), Italy (GenBank Acc.No.FN179291) and Romania (GenBank Acc.No. GU553001) (BERTIN et.al, 2010).

This study provided evidence for the presence of *H. obsoletus* in the Herzegovina district of BiH using morphological and molecular tools. According to literature data

this is also the first report of *H. obsoletus* on BiH territory. In the survey, *H. obsoletus* adults were collected inside (*C. arvensis*) and outside (*V. castus-agnus*) of vineyards. Considering BLAST analyses of sequenced specimens, PCR/RFLP assays on ITS2 and COI regions showed to be suitable for correct assignment of the individuals to species in accordance with the previous morphological identification.



Fig. 1: *H. obsoletus* – male



Fig. 2: *H. obsoletus* – a) male anal segment; b) genital style; c) aedeagus

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