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**UTVRĐIVANJE ASOCIJACIJA CIKSINA
(HEMIPTERA: AUCHENORRHYNCHA) SA BILJKAMA DOMAĆINIMA
MOLEKULARNOM IDENTIFIKACIJOM LARVI**

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Cikade podfamilije *Cixiinae* Spinola, 1839 su floemski polifazi koji biljke domaćine, pored ishrane koriste i kao pogodnu nišu za parenje, ovipoziciju i larvalni razvoj, kao i zaštitu od predatora. Prezimljavaju endogejski, u stadijumu larve u trajanju od nekoliko nedelja do nekoliko godina. Broj generacija različitih vrsta ciklina varira u zavisnosti od nadmorske visine, ekspozicije terena i klimatskih karakteristika podneblja, dok su u umerenim regionima uglavnom jednogeneracijske. Nimfe se hrane na korenju, podzemnim izdancima ili gljivicama i uglavnom se zadržavaju u rizosferi biljaka u čijoj blizini su se razvile iz jaja. Krug biljaka domaćina larvi može biti znatno uži u odnosu na imaga jer korenov sistem mnogih biljaka koje adulti koriste u ishrani nije dostupan tokom cele godine (osim u slučajevima višegodišnjih biljaka). Biljke domaćini ciklina su slabo poznate, izuzev sporadičnih podataka koji se prihvataju uz oprez jer je larvene stadijume različitih vrsta, pa čak i rodova, teško morfološki razlikovati. Problematika identifikacije larvi ciklina i njihovih asocijacija sa biljkama domaćinima je posebno značajna zbog njihove uloge vektora fitoplazmi (Mollicutes: '*Candidatus* Phytoplasma') čije biljke domaćini predstavljaju izvore inokuluma patogena. Metod molekularne identifikacije larvalnih stupnjeva insekata analizom mtDNK regiona COI gena (citohrom oksidaza c subjedinic 1) pruža precizniji uvid u identitet vrsta (Jinbo et al., 2011) i stoga je ovo metod izbora u determinaciji larvi ciklina u cilju utvrđivanja asocijacija sa biljkama domaćinima.

Nimfe ciklina su tokom maja 2012 godine sakupljene ekshaustorom na mezofilnoj livadi na teritoriji Negotina, neposredno uz korenov sistem pojedinačnih biljaka do 20cm dubine zemlje. Uzorkovanje je sprovedeno neselektivnim odabirom prisutnih biljnih vrsta, dok je materijal primarno grupisan na osnovu larvalnog stupnja (L1-L5). Molekularna identifikacija larvi izvršena je umnožavanjem 3' kraja regiona COI gena približno dužine 850 bp. Poređenjem sa nukleotidnim sekvencama iz NCBI baze korišćenjem blastn algoritma, detektovane su 3 vrste ciklina: *Hyalesthes phylesakis* (L4), *Reptalus melanochetus* (L3-L5) i *R. quinquecostatus* (L4 i L5). Jasnu asocijaciju sa određenom biljnom vrstom su pokazali *H. phylesakis* i *R. quinquecostatus*, čije su nimfe sakupljene sa korena *Sanguisorba minor*, odnosno *Koeleria macrantha*, dok je *R. melanochetus* pored *K. macrantha*, prisutan i na korenu vrsta *Hordeum murinum* i *Nonea pulla*. Navedene asocijacije predstavljaju nove nalaze a posledica su nasumičnog uzorkovanja i odabira biljaka.

Usled kompleksnog odnosa sa biljkama domaćinima, istraživanje distribucije određenih biljnih vrsta može biti adekvatan mehanizam praćenja potencijalnih staništa ciklina. Jedan od načina utvrđivanja životnih strategija ovih insekata su laboratorijska gajenja koja mogu biti uspešna i na biljkama sa kojima se date vrste nikada ne susreću u prirodi, što je često praćeno variranjem stope preživljavanja, reprodukcije i larvalnog razvoja. Do sada nije definisan uniforman metodološki pristup u istraživanjima ekoloških osobnosti ciklina, ali uz opservaciju ponašanja, laboratorijsko praćenje razvoja i postavljanje eksperimenta različitih limitirajućih faktora, molekularna identifikacija vrsta na nivou larvalnog stadijuma daje najkompletniji uvid u životni ciklus vrsta i definisanje kruga biljaka domaćina.

Provisional translation:

X SIMPOZIJUM ENTOMOLOGA SRBIJE 2015 – X SYMPOSIUM OF ENTOMOLOGISTS OF SERBIA 2015
KLADOVO, 23-27 IX 2015

Determining the host plant associations of the Cixiinae (HEMIPTERA: AUCHENORRHYNCHA) by molecular identification of the nymphs

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Members of the subfamily Cixiinae Spinola, 1839 are phloem phytophages that use their host plants as nutrition source, as an adequate niche for mating, oviposition and protection from predators. Nymphs of all five instars (N1-N5) are overwintering endogeic and underground development can last from five weeks up to few years. Number of generations of different Cixiinae species varies depending on altitude, exposition of the terrain and climatic characteristics of the area, while in moderate regions they are mainly univoltine. Nymphs feed on the root, underground shoots or fungi and prefer to stay in the rhizosphere of the plants on whose roots they have hatched from the eggs. Nymphal host plant range can be much smaller comparing to the adult plant preferences because root systems of many suitable plants species that they use as nutrition sources, are not available during whole year (except in the case of perennial plants). Host plants of the Cixiinae are poorly known, except for occasional data that should be considered carefully because of difficult morphological distinguishing of different species or even genera in the nymphal stage. Identification issue of the Cixiinae nymphs and their host plant associations is of special importance knowing their vector ability of the fastidious phytoplasmas (Mollicutes: 'Candidatus Phytoplasma') because insects host plants are also inoculum sources for the pathogen. Methodology of the molecular identification of insects based on analysis of mtDNA COI gene (cytochrome oxidase C subunit I) in the nymphal stage gives the better insight into the species identity (Jinbo et al., 2011) and that is why this approach was chosen for revealing host plant association of the Cixiinae nymphs.

Nymphs were collected during May 2012 on the mesophil meadow in east Serbia near city of Negotin by nonselective sampling individuals directly from the rootlets using mouth aspirators up to 20cm deep in the ground. Primary grouping of the samples was done only into the instars groups using key for distinguishing immature stages of *Hyalesthes obsoletus* provided by Sforza and associates (1999). Molecular identification of the nymphs is conducted by amplifying 3' end of the COI gene (forward C1-J-2195, reverse TL2-N-3014; 487 bp).

Comparing nucleotide sequences with those available in NCBI database we have detected three species of the Cixiinae group: *Hyalesthes phylesakis* (N4), *Reptalus melanochetus* (N3-N5) i *R. quinquecostatus* (N4 i N5). Clear association with specific plant species has been shown for *H. phylesakis* and *R. quinquecostatus* whose nymphs were collected from the rhizosphere of the *Sanguisorba minor* and *Koeleria macrantha*, respectively, while *R. melanochetus* nymphs were beside *K. macrantha* also present in the

rhizosphere of *Hordeum murinum* and *Nonea pulla*. All recorded associations are new findings and are consequence of the random sampling and plant selection.

Because of complex relations with host plants, analyzing the distribution of detected/confirmed host plants can be an adequate method for tracking potential habitats of the Cixiinae. One way to comprehend life strategies of this group of insects is laboratory rearing that can be successful using plants which some Cixiinae species are never going to encounter in the nature, and it is usually followed by varying survival, reproduction and nymphal development. Until now an uniform methodological approach is not defined in the research of the ecological specificities of the Cixiinae, but the observation of the behavior, laboratory controlled development and setting up experiments with limiting factors combined with molecular identification of the species in the nymphal stage gives the most complete insight into the lifecycle of the species and defining the host plants.