ACTA ENTOMOLOGICA SLOVENICA

LJUBLJANA, DECEMBER 2012 Vol. 20, št. 2: 147–152

TETRANEURA (*TETRANEURELLA*) *NIGRIABDOMINALIS* (SASAKI), GALL-FORMING APHID FOUND ON MAIZE ROOTS IN SLOVENIA

Špela MODIC, Jaka RAZINGER, Gregor UREK

Agricultural Institute of Slovenia, Plant Protection Department
Hacquetova ulica 17, SI-1000 Ljubljana, email: spela.modic@kis.si

Abstract - The root aphid *Tetraneura* (*Tetraneurella) nigriabdominalis* (Sasaki), (Pemphiginae: Eriosomatini) was recorded on corn roots (*Zea mays* L.) in August 2011 in northeast Slovenia in the village Benica. The species was classified morphologically and by molecular methods.

KEY WORDS: Pemphiginae, root aphids, fauna, Slovenia, DNA barcoding.

Izvleček – *TETRANEURA* (*TETRANEURELLA*) *NIGRIABDOMINALIS* (SASAKI), KORENINSKA UŠ NAJDENA NA KORENINAH KORUZE V SLOVENIJI

Koreninsko uš *Tetraneura* (*Tetraneurella) nigriabdominalis* (Sasaki), (Pemphiginae: Eriosomatini) smo ugotovili na koreninah koruze (*Zea mays* L.) avgusta 2011 v severovzhodni Sloveniji, v vasici Benica. Vrsto smo določili morfološko in na podlagi molekularno biološke analize.

KLJUČNE BESEDE: Pemphiginae, koreninske uši, favna, Slovenija, črtne kode DNA.

Introduction

The root aphid *Tetraneura nigriabdominalis* (Sasaki) has host alternation between leaf galls on trees from family Ulmaceae and roots of numerous species of Graminae (Poaceae) from the genera *Agropyron*, *Cenchrus*, *Chloris*, *Cynodon*, *Digitaria*, *Echinochloa*, *Eleusine*, *Eragrostis*, *Oryza*, *Panicum*, *Paspalum*, *Saccharum*, *Setaria*, and others. Species is distributed in Africa, India, Nepal, Bangladesh, Pakistan, Sri Lanka, Japan, China, Korea, Indonesia, Malaysia, the Philippines, Australia, New Zealand, Fiji, Tonga, Central America, Caribbean, the U.S.A. (Blackman and Eastop, 1984 & 1994 & 2006). It is also presented in Fauna Europaea (2011). Species is considered as a major pest of upland rice plants (Pathak and Khan, 1994). *Ulmus* spp. trees are its primary host where it forms galls

on upper sides of leaves. Galls are stalked, hairy, elongate; pouch or spindle shaped, usually with a pointed apex and differ from galls formed by *T. ulmi* L. In South and Eastern Europe *T. nigriabdominalis* is heteroecious holocyclic with *Ulmus* spp. as primary host; and probably anholocyclic where *Ulmus* is unavailable (Blackman & Eastop, 2006). Alate leave the gall through lateral slits in May and July to establish colonies on roots of Gramineae (Tanaka, 1961). Apterous are greenish or brownish white, plump, body length 1.5-2.5 mm. Alate have a shiny black head and thoracic lobes and a brown abdomen, body length 1.5-2.3 mm. Its presence on the roots of some hosts is indicated by a reddish-purple discoloration of the leaves (Blackmann & Eastop, 1984).

T. nigriabdominalis is often found in association with ants. The ants transport them and construct refuges in the roots. The ants attend them for honeydew and their presence confirms the root aphid attack (Galli and Bonvicini-Pagliai, 1998). The optimal temperature for population growth of *T. nigriabdominalis* is 30° C (Kuo et al., 2006).

The above mentioned morphological characteristics were duly observed during morphological classification of the species. Such an approach to classification can be termed 'classical' taxonomy. Classical taxonomy is based on detailed morphological analysis of morphometric characteristics of biological specimens. However, as DNA sequencing has become increasingly reliable and affordable more DNA sequences have become available online. This data can be used to classify an unknown specimen in a process designated DNA barcoding. The objective of DNA barcoding is to use large-scale screening of one or a few reference genes in order to, a), assign unknown individuals to species, and b), enhance discovery of new species (Moritz and Cicero, 2004). DNA barcoding is based on the premise that a short standardized sequence can distinguish individuals of a species because genetic variation between species exceeds that within species (Hajibabaei et al., 2007).

The purpose of this research was a), to confirm the occurrence of *T. nigriabdominalis* on secondary host in Slovenia and b), to provide the research community with additional DNA barcodes for this species.

Systematics – based on Remaudiere and Remaudiere, 1997

The species *Tetraneura nigriabdominalis* (Sasaki, 1899) has several synonyms: *Dryopeia hirsuta* Baker, 1921, *Tetraneura argrimoniae* Shinji, 1924, *Tetraneura* oryzae van der Goot ex van Herun, 1923, *Tetraneura akinire* Sasaki, 1904.

Materials and methods

Specimen collection

The aphid specimens were collected on August 11th 2011 from the secondary host – the aphids were discovered on roots of *Zea mays* in northeastern part of Slovenia, near the village Benica in Prekmurje. The exact location is longitude 16.5023 latitude

46.5135. Aphid samples were preserved in 75% ethanol on 4 to 5 °C in refrigerator for identification at a later day. Several insects were saved as vouchers at -80°C in entomological collection of the Agricultural Institute of Slovenia.

Classical taxonomical identification **-** based on Blackman and Eastop, 2006.

Apterae of the genus *Tetraneura* have very short terminal process of the last antennal segment. Siphunculus have slightly elevated cones and broadly rounded caudae. Legs of females have only one tarsal segment. Alate females have forewings with unbranched medial veins. Wax glands are usually present.

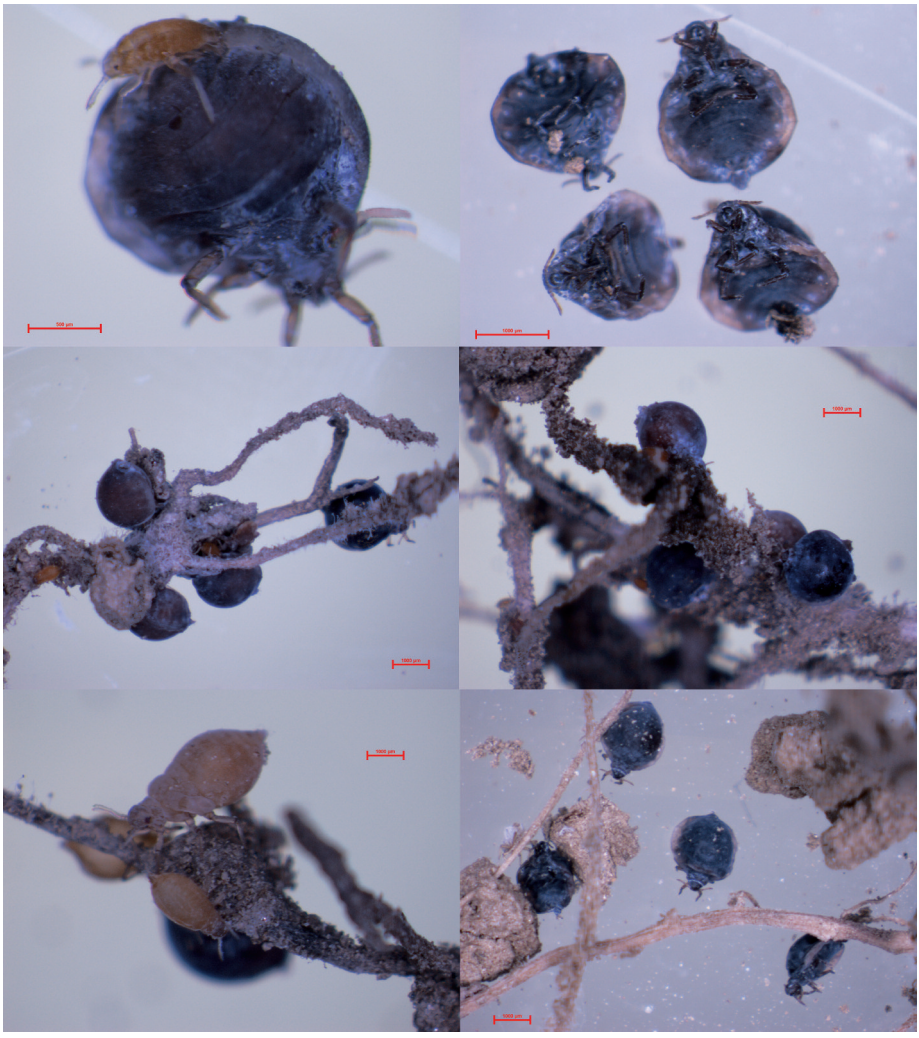

Fig. 1: Apterae clustered on roots of *Zea mays*.

Molecular classification

DNA purification. The specimens were preserved at -80°C until DNA extraction. Entire aphid was used for genomic DNA purification using the NucleoMag extraction kit (Macherey-Nagel, Germany) and MagMAX Express Magnetic Particle Processor (Applied Biosystems). The volumes of reagents used were smaller than those used in the NucleoMag kit's instruction manual. The tissue was homogenized manually in a 1.5 ml Eppendorf tube using micro-pestle. The lysis buffer consisted of 10 µl of pre-mixed proteinase K solution and 50 µl of T1 buffer. The lysate was transferred to the first well of the MagMAX cartridge, where additionally 10 µl of magnetic particles and 110 µl of MB2 buffer were added. 150 µl of MB3, 150 µl MB4 and 200 µl of MB5 were added to wells 2, 3 and 4, respectively. In the 5th well, 50 µl of MB6 (elution buffer) was added.

DNA barcoding. Partial cytochrome oxidase subunit I (COI) was amplified using forward primer LCO (5 -GGTCAACAAATCATAAAGATATTGG-3) and reverse primer HCO (5 -TAAACTTCAGGCTGACCAAAAAATCA-3) according to Folmer *et al*. (1994), with slight modifications. PCR was performed in Veriti thermocycler (Applied Biosystems). The 25 µl reactions contained 2.5 µl 10X PCR Buffer, 3.0 µl $MgCl_2$ (25 mM), 1.0 µl of each primer (10 µM), 0.4 µl dNTPs (10 mM), 0.2 µl Taq DNA polymerase (Fermentas), and 1.0 µl of DNA template. Thermocycling conditions were 95°C for 6.5 min, followed by 40 cycles of 40 s at 95°C, 40 s at 40°C, and 1 min at 72°C, with a final extension of 72°C for 7 min. The resulting PCR amplicon was checked on a 1.7% agarose gel, stained with ethidium bromide and visualized under UV light in Genegenius (Syngene). The amplicon was sequenced in Macrogen inc., Netherlands. The obtained sequence was deposited in Barcode of Life Database (www.boldsystems.org).

Results & Discussion

The species was previously confirmed in former Yugoslavia. It was discovered on an elm tree *Ulmus campestris* in Belgrade. It was classified as *Byrsocrypta hirsuta* Backer (N. Tanasijevič, 1965). Until now the species has expanded considerably on *Ulmus* trees in Slovenia (G. Seljak, pers. comm.). Their secondary host plants with a high potential economic value, *Zea mays* L. and *Sorghum bicolor* (L.) Moench, do not appear to be threatened.

This study shows benefits of combined morphological and molecular classification approach. Although molecular barcoding is a useful aid for taxonomic workflow in identifying specimens to a species level it is not meant as a replacement for classical morphological taxonomic analysis. For example, when an unknown specimen does not return a close match to existing records in the barcode library, the barcode sequence does not qualify the unknown specimen for designation as a new species. Instead, such specimens are marked for thorough morphological analysis (Hajibabaei et al., 2007).

This was also the case in this investigation. The aphid's sequence was obtained and analyzed by bioinformatics software. When it was discovered that it does not closely match any known sequences, the aphid was thoroughly morphologically analyzed. After our own morphological classification and confirmation by an independent foreign laboratory, we deposited the obtained sequence in the Barcode of Life Database (www.boldsystems.org). Thus the research community profited by one more specific genetic fingerprint of an aphid species.

Acknowledgement

We thank Dr. Thomas Thieme (BTL, Sagerheid, Germany) for confirmation of our taxonomic determination.

References

Blackman R.L., Eastop V.F., 2006. Aphids on the World's Herbaceous Plants and Shrubs. Vol. 2 The Aphids. John Wiley & Sons. ISBN-10 0-471-48973-5. 1439 pp.

Blackman, R. L. , Eastop V.F., 1984. Aphids on the World's Crops: An identification guide. John Wiley & Sons: Chichester, New York. ISBN 0 471 90426 0, 466 pp.

Blackman, R. L., Eastop V.F., 1994. Aphids on the world's trees. An identification and information guide. Cab International, London, 987 pp.+ 16 plates.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol*. 3: 294-299.

Galli E., Bonvicini-Pagliai A.M., 1988. Field and laboratory research on myrmecophily of *Tetraneura akinire*. Pp. 183-188 in Nieto Nafria J.M. and Dixon, A.F.G. (eds) Aphids in natural and managed ecosystem. University of Leon, Leon Spain.

Hajibabaei M, Singer GA, Hebert PD, Hickey DA., 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends Genet*. 23:167-172.

Kuo M., Lu W., Chiu M., Kuo, Y., Hwang S. 2006. Temperature dependent development and population growth of *Tetraneura nigriabdominalis* (Homoptera: Pemphigidae) on three host plant. *Journal of economic entomology*, 2006, 99, 4:1209-2113.

Moritz C., Cicero C., 2004. DNA barcoding: Promise and pitfalls. *PLoS Biol* 2(10): e354.

Pathak M.D., Khan Z.R., 1994. Insect pests of rice. International rice research institute. International centre of insect physology and ecology. Los Baños, Laguna. 89 pp.

Remaudiere G., Remaudiere M., 1997. Catalogue des Aphididae du monde = Catalogue of the world's Aphididae. INRA, Paris. ISBN 2-7380-0714-7. 473 pp.

Tanaka T., 1961. The rice root aphid, their ecology and control. *Spec. Bull. Coll. Agric. Utsunomiya* 10: 1-83.

Tanasijevič N. 1965. Fauna afida na žitima u Jugoslaviji. Agrohemija, Beograd, 1965, 12: 685-690.

Received / *Prejeto*: 2. 10. 2012