Thuja occidentalis L. IS COMMONLY A HOST FOR CYPRESS JEWEL BEETLE (*Ovalisia festiva* L.) IN SLOVENIA

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ABSTRACT

A significant drying and dying of white cedars (*Thuja occidentalis* L.) in an older hedge was observed in autumn 2010 in Ljubljana (Vič). When the branches were carefully examined, many were found to be hollow and full of channels filled with sawdust. In the channels pupae were discovered, from which blue-green beetles with a metallic sheen emerged after five days at 20°C. Beetles were morphologically analyzed and classified as Cypress Jewel Beetle (*Ovalisia festival* L.). In April and May 2012 similar damage was observed in several gardens in Ljubljana (Trnovo, Bežigrad), Posavje (Žadovinek) and Prekmurje (Lendava). We conclude that the native beetle that normally feeds on junipers found a niche in the white cedars growing in permanent sites or in nurseries. This suggests a need for regular and professional monitoring of the pest. A possible control measure to slow the insect's spread is a selection of ornamental plants, well adapted to their growth conditions.

Keywords: DNA barcoding, Coleoptera, insect pests, hedges, *Thuja* sp.

IZVLEČEK

AMERIŠKI KLEK V ŽIVIH MEJAH JE POGOSTO GOSTITELJ JUŽNEGA BRINOVEGA KRASNIKA (*Ovalisia festiva* L.) V SLOVENIJI

Spomladi 2011 smo v Ljubljani pregledali Ameriške kleke (*Thuja occidentalis* L.) v starejši živi meji, ki so se sušili že nekaj let. Pri razrezu polsuhih vej smo opazili v lesu rove z žagovino. V izdolbinah smo našli bube, iz katerih so se pri temperaturi 20°C v laboratoriju po petih dneh izlegli zelenomodri hrošči s kovinskim leskom in temnimi pikami na pokrovkah, dolgi približno 10 mm. Hrošče smo morfološko klasificirali in ugotovili, da je izvrtine povzročil južni brinov krasnik (JBK) (*Ovalisia* (*Palmar*) *festiva* L.). Pridobljeno sekvenco smo objavili na medmrežju (www.boldsystems.org). V letu 2012 smo v aprilu in maju opazili starejše kleke sorte Smaragd z enakimi poškodbami in ličinkami v lesu na več vrtovih v Ljubljani (Trnovo, Bežigrad), v Posavju (Žadovinek) in v Prekmurju (Lendava). Sklepamo, da JBK ogroža kleke na stalnem rastišču in v drevesnicah. Močno napadeni kleki se posušijo v celoti ali pa jih je zaradi slabega videza potrebno odstraniti. Možen ukrep za zmanjšanje škode je izbor okrasnih rastlin primernih za določeno rastišče.

Ključne besede: DNA črtno kodiranje, hrošči, škodljivci, *Thuja* sp., žive meje

1 INTRODUCTION

Thuja occidentalis L. has spread as ornamental plant for hedges in all parts of Slovenia regardless to climate and soil conditions. The prevailing cultivar is Smaragd. An older *Thuja*

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occidentalis hedge with the history of slow dying was inspected in Ljubljana in spring 2011. The tunnels with sawdust were observed in the wood of wilted branches and the chambers with pupae were found. The green- blue beetles with metallic shine, app. 10 mm long, with black spots on elytra, appeared after 5 days in laboratory at 20°C. By morphological classification the beetles which caused the injury of plants were identified as Cypress Jewel Beetle (CJB) *Ovalisia (Palmar) festiva* L.
Cypress jewel beetle is a native species which feeds mainly on Juniperus plants. *Thuja occidentalis*, probably weakened by environmental stress factors is an alternative host as it is reported also from some other parts of Europe. Plants which were heavily attacked were dying or had to be removed due to bad visual appearance. Neither the life cycle of Cypress Jewel beetle in our climate nor the control measures are known. Additionally we did not find a molecular barcode in the internet databases, to assist us with '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 properly identify a potentially harmful pest of hedges and b), to provide the research community with DNA barcode sequence of this species.

2 MATERIALS AND METHODS

2.1 Pest monitoring and insect collection

After the first finding of CJB in 2011 more Thuja hedges with damaged plants were inspected in 2012. Branches with symptoms of wilting and browning were dissected and observed for tunnels, sawdust, exit holes, larvae or pupae. The pest was sampled in several locations in Ljubljana, Žadovinek pri Krškem and Lendava, Slovenia.
Several insects were deposited as voucher specimens in entomological collection of the Agricultural Institute of Slovenia and several were stored in -80°C freezer for subsequent DNA analyses.

2.2 Classical taxonomy

The insects were morphologically classified using guides by Harde *et al*. (2000) and White (1983). Additionally, two Coleoptera experts were consulted for their opinion regarding the classification.

2.3 DNA barcoding

DNA purification. The specimens were preserved at -80°C until DNA extraction. Beetle's left hind leg was removed for genomic DNA extraction 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 premixed 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.
PCR conditions and sequencing. 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). 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).

3 RESULTS, PRESENTATION OF THE PEST AND DISCUSSION

Different stages of injuries were usually observed in individual hedges and also on individual plants. Some plants were already dead. On dead branches or branches almost entirely brown tunnels with sawdust and exit openings originating from infestations in previous years were observed. In contrast, on live, but wilting and somewhat chlorotic branches, active larvae were found under the bark. Older symptomatic Thuja *sp*. cv. Smaragd plants with larvae in the wood were found in central Slovenia, Posavje and Prekmurje. The attack was also confirmed on four year old Thuja occidentalis plants for planting in a nursery in Ljubljana where larvae were found at the base of trunks.

Table 1: Sites with confirmed Cypress Jewel Beetle attack

Location	Date of inspection and sampling	Host plant	Estimated age of host plant
Ljubljana, Vič; private garden	26 May, 2011	*Thuja occidentalis* L., cv. Smaragd	>10 years
Ljubljana, Trnovo; private garden	6 April, 2012	*Thuja occidentalis* L., cv. Smaragd	>10 years
Ljubljana, Bežigrad; private garden	3 April, 2012	*Thuja occidentalis* L., cv. Smaragd	8-10 years
Žadovinek pri Krškem; private garden	16 May, 2012	*Thuja occidentalis* L., cv. Smaragd	>10 years
Lendava; public green aerea	25 July , 2012	*Thuja occidentalis* L.,	>10 years
Ljubljana; nursery	16 April, 2012	*Thuja occidentalis* L., cv. Smaragd	4 years

3.1 Presentation of the pest

The adult Cypress Jewel Beetle has an ovoid-shaped body, 6-12 mm long. The head is partly located under the prothorax. The eyes are rather large. Wingspan is about 8 to 12 mm. The short antennae have 11 articles. The general colour is metallic green with dark patches. The pronotum shows two patches located in small shallow lateral depressions. The elytrae also show dark patches from the base to the apex. They are longitudinally striated (Harde *et al*., 2000). Larvae are 15-20 mm long, with a pronounced head which is wider than the abdominal segments. The head ends with strong maxillae. Larvae drill holes in branches and stems. They can attack healthy plants. Pupae are of similar size as imagos, ca. 8-12 cm in length and up to 6 mm in width (
Figure 1).

3.2 Systematics

Ovalisia festiva (Linnaeus, 1767) beetle is commonly known as Cypress Jewel Beetle. Other scientific names are *Lamprodila festiva, Lampra festiva, Scintillatrix festiva, Palmar festiva* (Harde *et al*., 2000) and *Poecilonota festiva* (Hellrigl, 2010). It belongs into order Coleoptera, family Buprestidae (White, 1983).

Figure 1: Larva, pupae and imagos of *Ovalisia festiva*.

3.3 Geographical distribution

The genus *Ovalisia* is spread in the Southern and Central Europe. North findings are known up to Württemberg, Gemany. It can also be found in North Africa and is known to extend its range northwards (White, 1983). Hellrigl (2010) reports findings in south Tirol in junipers (*Juniperus communis*) dating already from 1863. Findings of the beetle are reported also from Switzerland and Italy (Sistiana gulf near Trieste; Hellrigl, 1972). It is hypothesized that the beetle extends its range northward due to global warming (Wermelinger, 2011).

3.4 Biology

The Cypress Jewel Beetles naturally occur in forests of junipers or cypresses but can also be found in artificial habitats such as hedgerows of *Thuja* sp. Adults can be observed from May to September, whereas larvae and pupae all year round. They breed in stressed, injured and dying trees, freshly fallen branches, and in stumps and trash after logging (AgForests Queensland, 2008). Adults are active during the hottest hours of the day. Just after mating, females lay their eggs in crevices in the bark of trunks or branches. The beetles’ flight occurs from May to July (Harde *et al*., 2000). The larvae first drill flat tunnels, filled with sawdust, between bark and sapwood. After overwintering, the larvae drill further, making bigger tunnels and galleries causing branches to die, or even causing the whole plant to die. Usually the larvae overwinter twice. In the second spring the larvae dig out a gallery just bellow or in bark where it pupates. The adult beetles chew their way out using strong maxillae and make an oval exit hole of 2-4 mm (Figure 2). The whole life cycle takes from one to two years (Hellrigl, 1972) or even three years (Wermelinger, 2011).

3.5 Host plants and attack symptoms

Jewel beetle *Ovalisia (Palmar) festiva* (L., 1767) attacks mainly *Juniperus communis* L. (common juniper) in the Alps and the pre-Alpine regions. In southern Europe and in northern Africa it attacks *Juniperus oxycedrus* L., *Juniperus phoenicea* L., as well as *Juniperus thurifera africana*. It can also attack other species from the family Cupressaceae, like *Thuja occidentalis* L. (Figure 3), *Cupressus* sp., and *Chamaecyparis lawsoniana* (Murr.) Parl. (Hellrigl, 1972; Wermelinger, 2011).

Figure 2: Holes produced by emerging beetles.

Figure 3: Severly infested *Thuja* sp. hedge in Lendava, Prekmurje, Slovenia 25th Julij, 2012.

Damage to trees and shrubs is caused by larvae feeding in the inner bark and outer sapwood. A few larvae cause minimal damage. When there are many larvae they cause extensive damage to the sapwood and the tree can ultimately be ring-barked. External signs of infestation can include: cracking and lifting bark; frass (powder) on the trunk or ground and in badly infested trees, crown dieback due to the beetle damage ringbarking the tree (AgForests Queensland, 2008). The larvae prefer smaller branches or stems with a diameter of up to 2-10 cm (Wermelinger, 2011).

The beetles' damage to the xylem reduces the mechanical integrity of the trunk and/or branches. Simultaneously the attacked plant's water conductance is disturbed and the leaf water potential decreases (Ueda and Shibata, 2005). The first symptoms of damage are chlorosis and leaf drying. When a branch or stem is severely infested with larvae it can dry completely and die off. 2-4 mm exit holes can be observed on infested branches (Wermelinger, 2011).

3.6 Natural enemies

In our literature survey we did not find references of natural enemies of *O. festiva*, although there are some reports of Hymenopterans parasitizing beetles from f. Buprestidae. *Oobius agrili* was reported to parasitize emerald ash borer *Agrilus planipennis* (Duan *et al*., 2011). *Sclerodermus harmandi* is a more non-specialized parasite of several families of beetls. It was reported to also parasitize the genus *Ovalisia* (Lim *et al*., 2006).

3.7 Potential economic importance of *Ovalisia festiva* in Slovenia with conclusions

The predominant economic damage is foreseen on *Thuja* sp., a common conifer in Slovenia. Primarily *Thuja occidentalis* L., American Arborvitae, which is used for the planting of hedges and in cemeteries. *Thuja orientalis* L., Chinese Arborvitae, an invasive species in the western part of Slovenia, can also be attacked.

The climate conditions in Slovenia are very suitable for reproduction and development, especially hot and dry summers with little precipitation. Given the fact that we found no reports on effective natural enemies and there is an abundance of *Thuja* sp. hedges in Slovenia, we expect that the pest could greatly expand in the next few years and cause significant economic damage. Similar problems were reported for a weevil, *Phyllobius intrusus*, which was introduced to USA and later Norway through distribution of infected *Thuja* sp. hedge plants (Ødegaard and Berggren, 2010). Therefore, the authors of this paper believe that more emphasis should be put on proper selection of the hedge-plants.

Molecular barcoding in identifying specimens to a species level is a useful aid for taxonomic workflow however, 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 beetle’s sequence was obtained and analyzed by bioinformatics software. When it was discovered that it does not closely match any known sequences, the beetle was thoroughly morphologically analyzed. After our own morphological classification and confirmation by independent experts, we deposited the obtained sequence in the Barcode of Life Database (www.boldsystems.org). Thus the research community benefitted by having another species genetically barcoded.

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5 REFERENCES

AgForests Queensland, 2008. Management & Forest Products Guide; White Cypress Native Forests & Woodlands, pp. 1-21.

Duan, J.J., Bauer, L.S., Ulyshen, M.D., Gould, J.R., Van Driesche, R., 2011. Development of methods for the field evaluation of *Oobius agrili* (Hymenoptera: Encyrtidae) in North America, a newly introduced egg parasitoid of the emerald ash borer (Coleoptera: Buprestidae). Biological Control, 56: 170-174.

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, 5: 294-299.

Hajibabaei, M., Singer, G.A., Hebert, P.D., Hickey, D.A., 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. Trends Genet., 23: 167-172.

Harde, K.W., Severa, F., Möhn, E., 2000. Der Kosmos Käferführer: Die mitteleuropäischen Käfer. Franckh-Kosmos Verlags-GmbH & Co KG, Stuttgart, ISBN 3-440-06959-1.

Hellrigl, K.G., 1972. Revision der westpaläarktischen Arten der Prachtkäfergattung *Lampra* LAC, (Col., Buprestidae). Ann. Naturhistor. Mus. Wien, 76: 649-708.

Hellrigl, K.G., 2010. Faunistik der Prachtkäfer von Südtirol (Coleoptera: Buprestidae). Forest Observer, 5: 153–206.

Lim, J., Lyu, D., Gwang-Sik-Choi, Jeong, Y.-J., Shin, S.-C., Lee, S., 2006. A Taxonomic Note on *Sclerodermas harmandi*, Ectoparasite of Stem and Wood Boring Insect Larvae (Hymenoptera: Chrysidoidea: Bethylidae) in South Korea. Journal of Asia-Pacific Entomology, 9:115-119.

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

Ødegaard, F., Berggren, K. 2010. The first European records of the arborvitae weevil *Phyllobius intrusus* Kono, 1948 (Coleoptera, Curculionidae) in Norway. Norwegian Journal of Entomology, 57: 162–165.

Ueda, M., Shibata, E., 2005. Water status of hinoki cypress, *Chamaecyparis obtusa*, attacked by secondary woodboring insects after typhoon strike. J For Res, 10: 243–246.

Wermelinger, B., 2011. Der Grüne Wacholder-Prachtkäfer. g'plus die Gärtner-Fachzeitschrift, 3:30.

White, R.E., 1983. A field guide to the beetles of North America. Houghton Mifflin Company, Boston. ISBN 0-395-31808-7: 368 str.