НАУЧНОМ ВЕЋУ
ИНСТИТУТА ЗА ФИЗИКУ
БЕОГРАД

Предмет: Молба за покретање поступка за стицање звања истраживач сарадник

МОЛБА

С обзиром да испуњавам критеријуме прописане од Министарства просвете, науке и технолошког развоја за стицање звања истраживач сарадник, молим Научно веће Института за физику у Београду да покрене поступак за мој избор у наведено звање.

У прилогу достављам:

1. Мишљење руководиоца пројекта са предлогом комисије за избор у звање
2. Стручну биографију
3. Списак објављених радова и других публикација
4. Фотокопије објављених радова и других публикација
5. Потврду о уписаним докторским студијама
6. Потврду о просеку на основним и мастер студијама
7. Кратак преглед научне активности
8. Потврду о пријављеној и прихваћеној теми докторске дисертације

У Београду,

5.5.2017.

С поштовањем,

Даница Павловић

Научно веће
Институт за физику
Београд

Датум:
Београд, 5.5.2017.

Предмет:
Мишљење руководиоца пројекта за избор Данице Павловић у звање истраживач сарадник

Даница Павловић, запослена у Центру за фотонику Института за физику у Београду, ангажована је на пројекту интегралних интердисциплинарних истраживања Министарства просвете, науке и технолошког развоја ИИИ45016 под називом „Генерисање и карактеризација нанофотонских функционалних структура у биомедицини и информатици“.

С обзиром да колегиница испуњава све критеријуме прописане Правилником за изборе у научна звања Министраства просвете, науке и технолошког развоја, као руководилац наведеног пројекта, сагласан сам са покретањем поступка за избор Данице Павловић у звање истраживач сарадник.

За састав комисије за избор Данице Павловић у звање истраживач сарадник предлажем:

1. др Бранислав Јеленковић, научни саветник, Институт за физику, Београд
2. др Дејан Пантелић, научни саветник, Институт за физику, Београд
3. др Срећко Ћурчић, ванредни професор, Биолошки факултет, Београд

Руководилац пројекта

др Бранислав Јеленковић,
Научни саветник,
Институт за физику, Београд

Биографија Данице Павловић

Даница Павловић је рођена 10. 4. 1990. године у Београду. Основну школу „Филип Кљајић- Фића“ у Београду завршила је као носилац Вукове дипломе а школовање је потом наставила као ђак XIII београдске гимназије. У току школовања била је награђивана више пута за резултате постигуте у области биологије. Освојила је друго место на државном такмичењу из биологије 2004. године. Добитник је Повеље београдски анђели за област науке. Освојила је прво место на републичком такмичењу из биологије 2009. године, где је представила и своја прва истраживања радећи под менторством биолога са Института за биолошка истраживања „Синиша Станковић“ у Београду. Награђена је од стране општине Чукарица за постигнуте резултате у школовању, и ослобођена полагања пријемног испита на свом будућем факултету, због остварених изузетних резултата на такмичењу.

Основне академске студије уписује 2009/2010 године на Биолошком факултету у Београду и 2013. године стиче академски назив „дипломирани биолог“. Мастер академске студије уписује 2013/2014 на истом факултету на модулу Екологија, подмодул Заштита животне средине. Студије завршава у року, 2014. године са просечном оценом 9.41, чиме стиче академски назив „ мастер еколог“.

По завршетку студија искуство је стекла радећи две праксе: Универзитетска радна пракса у ЈКП „Србијагас“ на позицији стручни сарадник на пословима заштите животне средине и БГ праксу у Секретаријату за заштиту животне средине, бавећи се проблемима сузбијања штеточина (инсеката, глодара, корова) и Секретаријату за инспекцијске послове, одељења за заштиту жив. средине, где се бавила инспекцијско-правним пословима заштите природних добара, вода, земљишта, ваздуха, заштити од буке, итд. Од октобра 2014. до јануара 2015. године волонтира на Институту за физику у Београду.

Докторске академске студије уписује 2014/2015 године на Биолошком факултету у Београду, модул Биологија, подмодул Ентомологија. Од јануара 2015. године је запослена на Институту за физику у Београду као истраживач-приправник. Тренутно је у звању истраживач-приправник ангажована на пројекту ИИИ45016, под називом „Генерисање и карактеризација нанофотонских функционалних структура у биомедицини и информатици“. Ради на истраживачким проблемима у области биофизике, са посебним интересовањем у области биофотонике, биомиметике и магнетобиологије.

Кратак преглед научне активности кандидата

Кандидат Даница Павловић се успешно бави научно-истраживачким радом на терену и у лабораторијама Центра за фотонику, Института за физику у Београду. У оквиру групе за биофизику, овладала је савременим методама и техникама нелинеарне и SEM (Scanning electron microscopy) микроскопије. Блиску сарадњу остварује са Институтом за зоологију, Биолошког факултета у Београду и Институтом за биолошка истраживања „Синиша Санковић".

Област научног истраживања у којем је Даница Павловић ангажована је биофизика, и то области: Биофотоника - где се бави нано-фотонским структурама код инсеката; Биомиметика где испитује потенцијалну функционализацију/имитацију природних нанофотонских структура и њихову примену; и Магнетобиологија- где се бави испитивањем утицаја магнетних поља на различите нивое биолошке организације.

Има прихваћену тему докторске дисертације под насловом „Фотоничка карактеризација кутикуларних структура одабраних врста Coleoptera и Lepidoptera". Предмет кандидаткињиног истраживања и будуће докторске дисертације је испитивање фотонских и морфолошких карактеристика кутикуларних структура одабраних врста из два највећа реда инсеката – Coleoptera (тврдокрилци) и Lepidoptera (лептири и мољци). Први део тезе биће посвећен испитивању структурне обојености одабраних врста инсеката, која није последица присуства пигмената, већ настаје као резултат интеракције микронских и субмикронских структура на њиховом телу и упадног зрачења. Испитиваће се спектрална својства рефлектованог и трансмитованог зрачења, као и њихова зависност од угла осветљавања и посматрања, како читавих кутикуларних зона, тако и њихових компонената (нпр. крилних љуспица Lepidoptera). Истраживаће се начин формирања фотонских структура на телима инсеката и њихов начин интераговања са светлошћу. Други део тезе ће се базирати на испитивању морфологије наведених фотонских кутикуларних структура помоћу различитих микроскопских и оптичких метода, као и утврђивању везе између грађе и функције самих структура, што је веома значајно за расветљавање биологије и екологије испитиваних врста инсеката. Биће развијени и одговарајући модели који квантитативно објашњавају експерименталне резултате. Трећи део тезе биће посвећен коришћењу добијених резултата за различите технолошке апликације. Испитиване фотонске кутикуларне структуре инсеката ће бити третиране као извор инспирације за унапређење постојећих технологија или дизајнирање нових иновативних оптичких уређаја.

Даница Павловић је коаутор три рада на SCI листи (2 рада M21 и један рад M23) и учесник више међународних и домаћих научних конференција. Коаутора је три поднесене међународне патентне пријаве, које представљају иновацију у области заштите докумената коришћењем и манипулацијом неорганских биолошких структура. Члан је Ентомолошког друштва Србије и Оптичког друштва Србије.

Списак објављених радова и других публикација

Радови и конгресна саопштења из уже научне области:

Б1. Радови у часописима међународног значаја

Радови у врхунском међународном часопису (М 21)

1. Pavlović, D., Petković, B., Ćurčić, S., Todorović, D., Vesović, N., Pantelić, D. & Perić-Mataruga, V. (2016). Increased motor activity of the beetle Laemostenus punctatus caused by a static magnetic field of 110 mT. Entomologia Experimentalis et Applicata, 160 (2), 188-194.

2. Pantelić, D., Savić-Šević, S., Stojanović, D., Ćurčić, S., Krmpot, A., Rabasović, M., Pavlović, D., Lazović, M. & Milošević, V. (2017). Scattering-enhanced absorption and interference produce a golden wing color of the burnished brass moth, Diachrysia chrysitis. Physical Review E, 95 (3), 032405.

Радови у међународном часопису (М 23)

1. Duletić-Laušević, S., Alimpić, A., Pavlović, D., Marin, P. D. & Lakušić, D. (2016). Salvia officinalis of different origin – antioxidant activity, phenolic and flavonoid content of extracts. Agro FOOD Industry Hi-Tech, 27 (1), 52-55.

Б2. Поглавља у монографијама националног значаја

Поглавље у књизи М 41 или рад у истакнутом тематском зборнику водећег националног значаја (М 44)

1. Pantelić, D., Krmpot, A., Stojanović, D. V., Rabasović, M. D., Ćurčić, S., Savić-Šević, S., Lazović, V. & Pavlović, D. (2016). Svetlost na krilu leptira. In: Popović, Z. V. & Jelenković, B. (Eds.): Svetlost u razvoju društva. Prošlost, sadašnjost i budućnost. Srpska akademija nauka i umetnosti, Beograd, pp. 45-53.

Б3. Конгресна саопштења на скуповима међународног значаја

Радови саопштени на скупу међународног значаја штампани у целини (М 33)

1. Dikić, G., Pavlović, D., Vasiljević, D., Tomić, Lj. & Pantelić, D. (2016). The thermographic analysis of photonic characteristics of Rosalia alpina surfaces. 3rd International Conference on Electrical, Electronic and Computing Engineering IcETRAN 2016. Zlatibor, Serbia, 13-16 June 2016. Proceedings, MOI1.2.1-5.

2. Kostić, I., Pavlović, D., Lazović, V., Vasiljević, D., Stojanović, D., Knežević, D., Tomić, Lj., Dikić, G. & Pantelić, D. (2016). Thermal and camouflage properties of Rosalia alpina longhorn beetle with

structural coloration. 7th International Scientific Conference on Defensive Technologies OTEH 2016. Belgrade, Serbia, 06-07 October 2016. Proceedings, 525-529.

Радови саопштени на скупу међународног значаја штампани у изводу (М 34)

1. Alimpić, A., Pavlović, D., Lakušić, D., Marin, P. D. & Duletić-Laušević, S. (2015). Seasonal variation of flavonoid content and antioxidant activity of Salvia officinalis of different origin. 2nd International Conference on Plant Biology, 21st Symposium of the Serbian Plant Physiology Society, COST ACTION FA1106 QUALITYFRUIT Workshop. Petnica, Serbia, 17-20 June 2015. Book of Abstracts, 70, Belgrade.

2. Vesović, N., Ćurčić, S., Vujisić, Lj., Nenadić, M., Krstić, G., Perić-Mataruga, V., Milosavljević, S., Antić, D., Mandić, B., Petković, M., Vučković, I., Marković, Đ., Vrbica, M., Pavlović, D., Ćurčić, B. & Makarov, S. (2015). Does life in caves reduce the diversity of chemicals produced by the pygidial glands of carabids? 17th European Carabidologists Meeting 2015. Primošten, Croatia, 20-25 September 2015. Book of Abstracts, 108, Primošten.

Б4. Конгресна саопштења на скуповима домаћег значаја

Радови саопштени на скупу националног значаја штампани у изводу (М 64)

1. Pavlović, D., Petković, B., Ćurčić, S., Todorović, D., Vesović, N., Pantelić, D. & Perić-Mataruga, V. (2015). Hipermotorno ponašanje vrste Laemostenus punctatus (Dejean, 1828) (Coleoptera: Carabidae) izazvano statičkim magnetnim poljem. X Symposium of Entomologists of Serbia 2015. Kladovo, Serbia, 23-27 September 2015. Abstracts, 17, Belgrade.

2. Pantelić, D., Krmpot, A., Rabasović, M., Pavlović, D. & Lazović, V. (2016). Structures of biological origin as optical security elements. IX radionica fotonike. Kopaonik, Srbija, 02-08. mart 2016. Book of Abstracts, 11, Belgrade.

Б5. Пријаве међународних патената (М 86)

1. Pantelic, D., Rabasovic, M., Krmpot, A., Lazovic, V. & Pavlovic, D. (2015). Security device individualized with biological particles. PCT/EP2015/081398.

2. Pantelic, D., Rabasovic, M., Krmpot, A., Lazovic, V. & Pavlovic, D. (2015). Security tag containing a pattern of biological particles. PCT/EPO2015/081400.

3. Pantelic, D., Rabasovic, M., Krmpot, A., Lazovic, V. & Pavlovic, D. (2015). Security tag with laser-cut particles of biological origin. PCT/EP2015/081407.

УНИВЕРЗИТЕТ У БЕОГРАДУ
БИОЛОШКИ ФАКУЛТЕТ

Студентски трг 16
11000 БЕОГРАД
Република СРБИЈА
Тел: +381 11 2186 635
Факс: +381 11 2638 500
Е-пошта: dekanat@bio.bg.ac.rs

Број: **sl**

Београд, 5. мај 2017.

Број индекса: **Б3002/2014**

ИБ: **1493972381120**

Универзитет у Београду, Биолошки факултет, на захтев који је поднела студенткиња **Даница Павловић** издаје следећу

П О Т В Р Д У

Студенткиња **Даница Павловић**, број индекса **Б3002/2014**, уписала је школску **2016/2017**. годину на Универзитету у Београду, Биолошком факултету, студијски програм **Биологија - докторске академске студије**, као **самофинансирајући студент**.

Потврда се издаје на лични захтев ради **сваке законске употребе**.

ОВЛАШЋЕНО ЛИЦЕ СТУДЕНТСКЕ СЛУЖБЕ БИОЛОШКОГ ФАКУЛТЕТА

Петар Мршовић

РЕПУБЛИКА СРБИЈА УНИВЕРЗИТЕТ У БЕОГРАДУ - БИОЛОШКИ ФАКУЛТЕТ "СЛУЖБА ЗА СТУДЕНТСКА ПИТАЊА" БЕОГРАД

УНИВЕРЗИТЕТ У БЕОГРАДУ
БИОЛОШКИ ФАКУЛТЕТ

Студентски трг 16
11000 БЕОГРАД
Република СРБИЈА
Тел: +381 11 2186 635
Факс: +381 11 2638 500
E-пошта: dekanat@bio.bg.ac.rs

Број: **5429213**

Београд, 28. октобар 2013.

На основу члана 161. Закона о општем управном поступку („Службени лист" 33/97, 31/01 и „Службени гласник РС" 30/10) и члана 1. Правилника о листи стручних, академских и научних назива („Службени гласник РС" 30/07, 112/08, 72/09, 81/10, 39/11, 54/11), по захтеву који је поднела Даница Павловић, издаје се следеће

У В Е Р Е Њ Е

ПАВЛОВИЋ Зоран ДАНИЦА, рођена 10. априла 1990, општина Савски венац, Србија, уписала је школске 2011/2012. године основне академске студије, студијски програм

Биологија

и завршила студије 9. јула 2013, са просечном оценом 9,30 (девет и 30/100) и оствареним укупним бројем ЕСП бодова 247 (двеста четрдесет седам).

Испунила је обавезе предвиђене наставним планом и програмом наведеног студијског програма на Биолошком факултету Универзитета у Београду. Тиме је стекла стручно звање

Дипломирани биолог

Уверење се издаје на лични захтев, а служи као доказ да су завршене основне академске студије до издавања дипломе.

ДЕКАН БИОЛОШКОГ ФАКУЛТЕТА

проф. др Јелена Кнежевић-Вукчевић

УНИВЕРЗИТЕТ У БЕОГРАДУ
БИОЛОШКИ ФАКУЛТЕТ

Студентски трг 16
11000 БЕОГРАД
Република СРБИЈА
Тел: +381 11 2186 635
Факс: +381 11 2638 500
Е-пошта: dekanat@bio.bg.ac.rs

Број: **4696714**
Београд, 15. октобар 2014.

На основу члана 161. Закона о општем управном поступку („Службени лист" 33/97, 31/01 и „Службени гласник РС" 30/10) и члана 1. Правилника о листи стручних, академских и научних назива („Службени гласник РС" 30/07, 112/08, 72/09, 81/10, 39/11, 54/11), по захтеву који је поднела Даница Павловић, издаје се следеће

У В Е Р Е Њ Е

ПАВЛОВИЋ Зоран ДАНИЦА, рођена 10. априла 1990, општина Савски венац, Србија, уписала је школске 2013/2014. године мастер академске студије, студијски програм

Екологија

и завршила студије 29. септембра 2014, са просечном оценом 9,90 (девет и 90/100) и оствареним укупним бројем ЕСП бодова 60 (шездесет).

Испунила је обавезе предвиђене наставним планом и програмом наведеног студијског програма на Биолошком факултету Универзитета у Београду. Тиме је стекла академско звање

Мастер еколог

Уверење се издаје на лични захтев, а служи као доказ да су завршене мастер академске студије до издавања дипломе.

ДЕКАН БИОЛОШКОГ ФАКУЛТЕТА

проф. др Јелена Кнежевић-Вукчевић

УНИВЕРЗИТЕТ У БЕОГРАДУ

Адреса: Студентски трг 1, 11000 Београд, Република Србија
Тел.: 011 3207400; Факс: 011 2638818; E-mail: officebu@rect.bg.ac.rs

ВЕЋЕ НАУЧНИХ ОБЛАСТИ
ПРИРОДНИХ НАУКА

Београд, 27.04.2017.
02-07 Број: 61206-1636/2-17
МЦ

На основу члана члана 47. став 5. тачка. 3. Статута Универзитета у Београду ("Гласник Универзитета у Београду", број 186/15-пречишћени текст и 189/16) и чл. 14. – 21. Правилника о већима научних области на Универзитету у Београду ("Гласник Универзитета у Београду", број 134/07, 150/09, 158/11, 164/11 и 165/11), а на захтев Биолошког факултета, број: 33/86-1 од 13.04.2017. године, Веће научних области природних наука, на седници одржаној 27.04.2017. године, донело је

О Д Л У К У

ДАЈЕ СЕ САГЛАСНОСТ на предлог теме докторске дисертације ДАНИЦЕ ПАВЛОВИЋ.
Усвојени наслов: „Фотоничка карактеризација кутикуларних структура одабраних врста Coleoptera и Lepidoptera";
Предложени наслов: „Фотонске и морфолошке карактеристике кутикуларних структура одабраних врста Coleoptera и Lepidoptera":

ПРЕДСЕДНИК ВЕЋА

Проф. др Душан Сладић

Доставити:
- Факултету
- архиви Универзитета

УНИВЕРЗИТЕТ У БЕОГРАДУ
БИОЛОШКИ ФАКУЛТЕТ

Студентски трг 16
11000 БЕОГРАД
Република СРБИЈА
Тел: +381 11 2186 635
Факс: +381 11 2638 500
Е-пошта: dekanat@bio.bg.ac.rs

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О Д Л У К У

Прихвата се Извештај Комисије за оцену испуњености услова и научне заснованости теме докторске дисертације кандидата:

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„Фотонске и морфолошке карактеристике кутикуларних структура одабраних врста Coleoptera и Lepidoptera“

За менторе се именују:

1. др Срећко Ћурчић, ванредни професор, Универзитет у Београду-Биолошки факултет
2. др Дејан Пантелић, научни саветник, Универзитет у Београду-Институт за физику

Декан Биолошког факултета

Проф. др Жељко Томановић

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Increased motor activity of the beetle *Laemostenus punctatus* caused by a static magnetic field of 110 mT

Danica Pavlović[1], Branka Petković[2]*, Srećko Ćurčić[3], Dajana Todorović[2], Nikola Vesović[3], Dejan Pantelić[1] & Vesna Perić-Mataruga[2]

[1]*Institute of Physics, University of Belgrade, Pregrevica 118, 11080 Zemun, Belgrade, Serbia,* [2]*Institute for Biological Research, University of Belgrade, Despota Stefana Blvd. 142, 11060 Belgrade, Serbia, and* [3]*Institute of Zoology, Faculty of Biology, University of Belgrade, Studentski Trg 16, 11000 Belgrade, Serbia*

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Abstract

The aim of this study was to investigate the effects of a static magnetic field on six behavioral parameters (travel distance, average speed while in motion, travel distance of the head, body rotations, time in movement, and immobility time) of the ground beetle *Laemostenus (Pristonychus) punctatus* (Dejean) (Coleoptera: Carabidae). Adults of this troglophilic and guanophilic beetle were randomly divided into two groups, the first exposed to a static magnetic field of 110 mT for 5 h, and the second a control group. Beetle behavior after these 5 h was monitored in an open-field test for 12 min and analyzed using ANY-maze software. Exposure to a static magnetic field of 110 mT increased motor activity (travel distance and average speed while in motion) in the first 4 min. After that there were no significant differences. We conclude that the applied static magnetic field affects motor activity of adult specimens of *L. (P.) punctatus*, and we discuss the mechanism, possibly through acting on the control centers responsible for orientation and movement.

Introduction

Living beings are exposed to the Earth's magnetic field, to which they have been adapted over millions of years of natural selection. Many organisms use this magnetic field for orientation in space and time (Wiltschko & Wiltschko, 2005). In the previous century, there was a rapid increase in the use of electrical appliances emitting additional electromagnetic fields. It has been shown that various types of these manmade magnetic fields, which are very often more than 1 000× stronger than the Earth's magnetic field, also affect organisms (Kavaliers & Ossenkopp, 1994). Magnetobiology is a relatively young scientific discipline that investigates the effects of magnetic fields on organisms. It encompasses the principles of several sciences unified around biophysics. Many things such as the theoretical foundation and general physical concepts of magnetobiology, as well as mechanisms underlying the biological effects of magnetic fields, need to be elucidated and explained. However, magnetobiology lacks predictive theoretical models (Binhi & Savin, 2003).

Magnetic fields are important ecological factors that can influence biological processes at any level of organization (Blank, 1995; Balcavage et al., 1996; Binhi & Savin, 2003; Rosen, 2003). Besides the Earth's magnetic field, there are manmade static magnetic fields (SMFs) generated, for example, in households, traffic, and industrial processes. Many studies have examined the possible effects of SMFs on animals. The most relevant data on their impact on human health are those obtained in studies examining the acute effects of field strengths considerably higher than that of the Earth's magnetic field and equivalent to ones used in industrial processes or in magnetic resonance imaging (WHO, 2006).

A magnetite-based magnetoreception sensor is one of the proposed mechanisms of SMF perception (Kirschvink, 1989; Kirschvink & Kirschvink, 1991). It has been shown that SMFs can influence several classes of organic chemical reactions as a result of effects on the electronic spin states of reaction intermediates (Grissom, 1995; Timmel et al., 2001). Some studies also indicate light-sensitive detection of magnetic fields in the retina (Ritz et al., 2002). Deutschlander et al. (1999a,b) have shown that

*Correspondence: Branka Petković, Department of Neurophysiology, Institute for Biological Research, University of Belgrade, Despota Stefana Blvd. 142, 11060 Belgrade, Serbia.
E-mail: janac@ibiss.bg.ac.rs

wavelength-specific effects occur in avian and reptile magnetic orientation. In the case of salamanders, Phillips et al. (2001) proposed a model where two separate and antagonistic magnetic field-sensitive systems are activated by short- and long-wavelength light. Recent research on magnetoreception has dealt with the question of cryptochromes as photopigment receptors (Thompson & Sancar, 2004; Gegear et al., 2008; Wiltschko & Wiltschko, 2014).

There is growing evidence that insects are able to detect and react to SMFs. These fields can have an impact on their daily activities, behavior, and spatial orientation (Klotz & Jander, 2003; Vácha, 2006; Vácha et al., 2008). In addition, the neuroendocrine system of insects plays an important role in controlling their motor behavior, which can be altered by SMFs (Osborne, 1996). Published data of SMF effects on beetles are scarce, especially for carabid beetles (Prolić & Nenadović, 1994, 1995; Vácha et al., 2008; Todorović et al., 2013; Spasić et al., 2015). Thus, the aim of this study was to investigate effects of an SMF (110 mT) on six behavioral parameters (travel distance, average speed while in motion, travel distance of the head, body rotations, time in movement, and immobility time) in adults of *Laemostenus* (*Pristonychus*) *punctatus* (Dejean) (Coleoptera: Carabidae).

Material and methods

Insects

Laemostenus (*P.*) *punctatus* is a troglophilic and guanophilic beetle. The adults have an average body size of about 13–17 mm (Casale, 1988). Our experiment used adults of *L.* (*P.*) *punctatus* from a field population. Specimens were collected during late March 2015 in the Ogorelička Pećina cave (43°20′52.29″N, 22°5′38.41″E), Svrljiške Planine Mountains, village of Sićevo, near the city of Niš, southeastern Serbia. At the time of collection, average air temperature in the cave was 12.6 °C and relative humidity 68.7%. After collecting beetles and until the end of experiments, insects were stored in a 1.5-l container at 11 ± 2 °C. Once a day they were fed earthworms (*Lumbricus terrestris* L.).

Static magnetic field

Insects were exposed to an SMF of 110 mT generated by a permanent magnet of the type described in Prolić & Nenadović (1995). Briefly, a double U-shaped magnet was used (model 6002; Raytheon, Waltham, MA, USA) made of two symmetric halves: the upper half of the magnet has two north (N) poles, at the terminal end of the magnet, and a centrally positioned south (S) pole. Inversely, the lower half of the magnet has two S poles (terminal end) and one N pole, centrally positioned (Figure 1). A relatively homogenous magnetic field was created between the poles. Maximal magnetic induction at the poles was ca. 320 mT as measured by a GM05 gaussmeter with a PT 2837 probe (Hirst Magnetic Instruments, Falmouth, UK). During the experiments, values of the local geomagnetic field (448380N, 208460E) were in the range of 47 761–47 763 nT for the total intensity.

Experimental procedure

The adult beetles used in the experiment were randomly divided into two groups: control (n = 9) and exposed to an SMF of 110 mT (n = 8). Each beetle was placed in an empty separate plastic Petri dish (r = 85 mm). The treated beetles were exposed to an SMF (110 mT) for 5 h, while the controls were kept under the same conditions (23 ± 1 °C, 65 ± 10% r.h.), but outside the reach of the magnetic field. Afterwards, the beetles were placed in another Petri dish (r = 85 mm), where their behavior was recorded for 12 min (open-field test).

Behavior monitoring

The behavior of all 17 individual insects was videotaped by a web-camera (Microsoft LifeCam VX-6000; Microsoft, Redmond, WA, USA) positioned above the Petri dish. Recordings and all experiments were performed under laboratory conditions (23 ± 1 °C, 65 ± 10% r.h.). ANY-maze software v.4.96 (Stoelting, Wood Dale, IL, USA) was used to analyze six behavioral parameters of the beetles during tests: (1) travel distance (m), (2) average speed while in motion (m s^{-1}), (3) travel distance of the head (m), (4) number of times the body completed a full 360° rotation, (5) time in movement (s), and (6) immobility time (s) (beetles had to remain immobile for at least 10 s

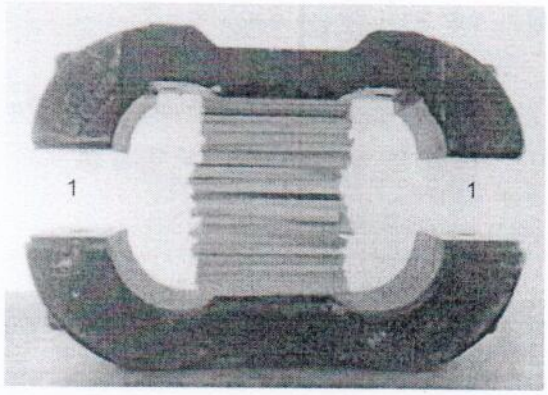

Figure 1 Setup for exposure of *Laemostenus* (*Pristonychus*) *punctatus* beetles to a relatively homogenous magnetic field with average magnetic induction of 110 mT (zones indicated with '1').

to be considered immobile). Behavior of the beetles was analyzed in the whole open-field arena, as well as in the arena arbitrarily divided into peripheral and central zones (about 70 and 30% of total area, respectively).

Statistical analysis

The Kolmogorov–Smirnov test was used to check for normality of the data. Because the data were not distributed normally, they were analyzed with the Mann–Whitney U test ($\alpha = 0.05$). All analyses were performed with STATISTICA v.7.0 software (StatSoft, Tulsa, OK, USA).

Results

Significant effects of an SMF of 110 mT on motor activity of adult *L. (P.) punctatus* beetles were found during the first 4 min of observation for travel distance (U = 14.0, P = 0.034) and average speed while in motion (U = 12.5, P = 0.024) (Figure 2). After 4 min, there were no significant differences.

For behavior in peripheral and central zones results are presented as the percent of motor activity in the given zones in relation to the whole open-field arena. In both

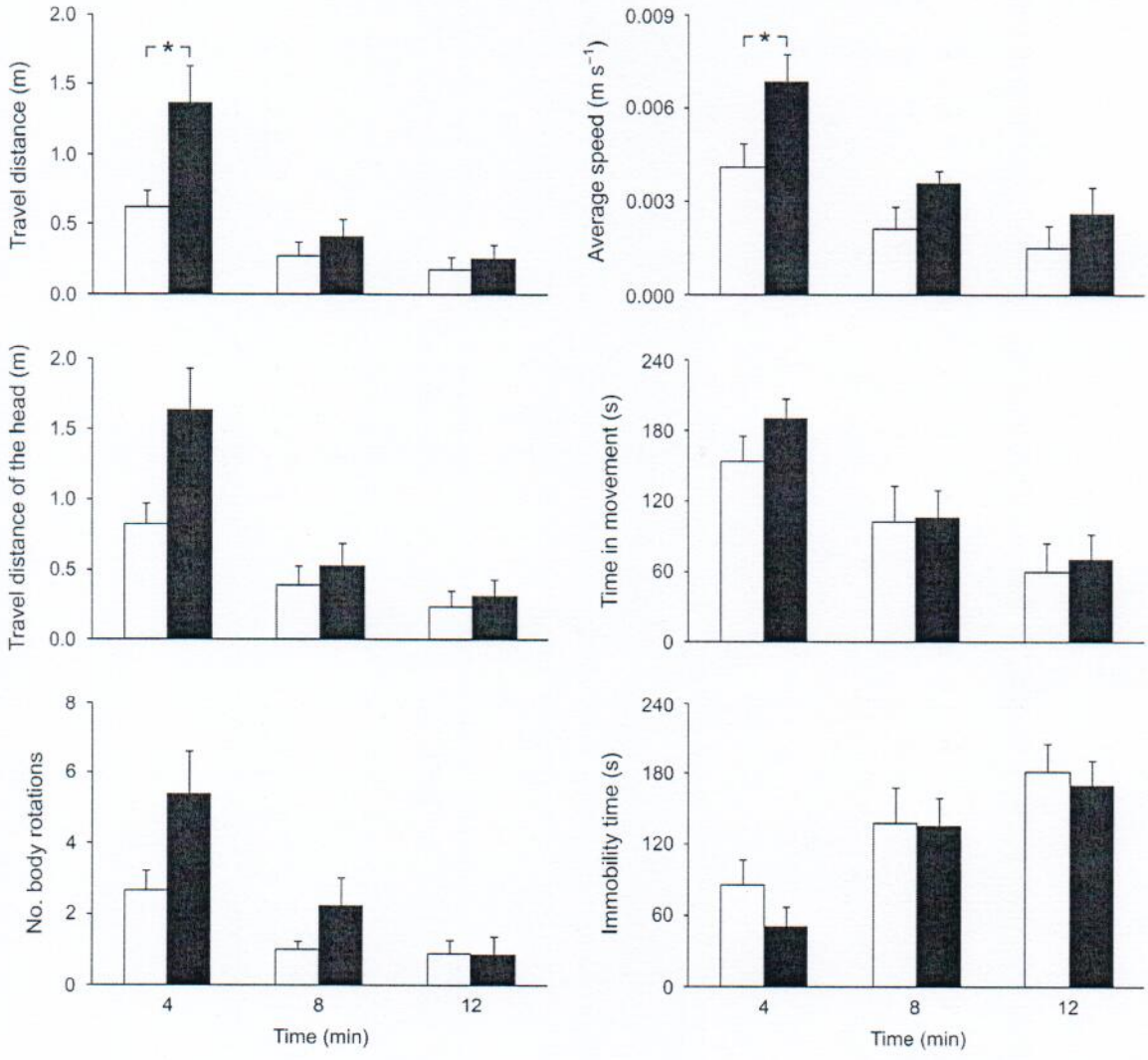

Figure 2 Effect of a static magnetic field (SMF) (110 mT) on six behavioral parameters of *Laemostenus (Pristonychus) punctatus* adults in the whole open-field arena: mean (+ SEM; control, white bars: n = 9; exposed to SMF, black bars: n = 8) travel distance (m), average speed while in motion (m s^{-1}), travel distance of the head (m), time in movement (s), number of body rotations, and immobility time (s). Means are calculated for three 4-min sequences within the 12-min total period of observation. Asterisks indicate significant differences between treatment and control (Mann–Whitney U test: $P<0.05$).

groups, control and SMF, adult *L.* (*P.*) *punctatus* beetles were mostly in the peripheral rather than in the central zone of the open-field arena (Figure 3). Differences between peripheral vs. central zone were significant for almost all combinations of the three 4-min intervals, the four variables (travel distance, travel distance of the head, time in movement, and immobility time), and the two groups (control and SMF) (U range: 0.0 to 16.0, P range: <0.001 to 0.031). Only for travel distance, travel distance of the head, and time in movement in the control group, the differences in the third 4-min interval were not significant.

Discussion

There is a considerable body of literature about the influence of magnetic fields of different characteristics on various species of insects. Effects of SMFs on behavior have been documented in most detail in honeybees (*Apis mellifera* L.) and homing pigeons (Towne & Gould, 1985). Honeybees are able to detect and respond to extremely small changes in intensity of the Earth's magnetic field (Walker & Bitterman, 1985, 1989). These authors also showed that movement is essential for this ability, with stationary honeybees being unable to distinguish changes of magnetic field intensity (Walker et al., 1989). As an explanation for this phenomenon, the authors proposed involvement of a ferromagnetic transduction mechanism that makes use of magnetite. Kirschvink (1989) and Kirschvink & Kirschvink (1991) showed a single-domain and superparamagnetic material to be present in honeybees, and that magnetite is a possible translator of magnetic field changes in neuronal activity. However, Gould et al. (1980) discovered that demagnetized bees can still orient to a magnetic field, indicating that magnetite is probably not the only molecule responsible for detection of the Earth's magnetic field. Static magnetic fields have been shown to influence the behavior and orientation of several other species of insects, including a number of Diptera (Becker, 1965; Picton, 1966), some Lepidoptera

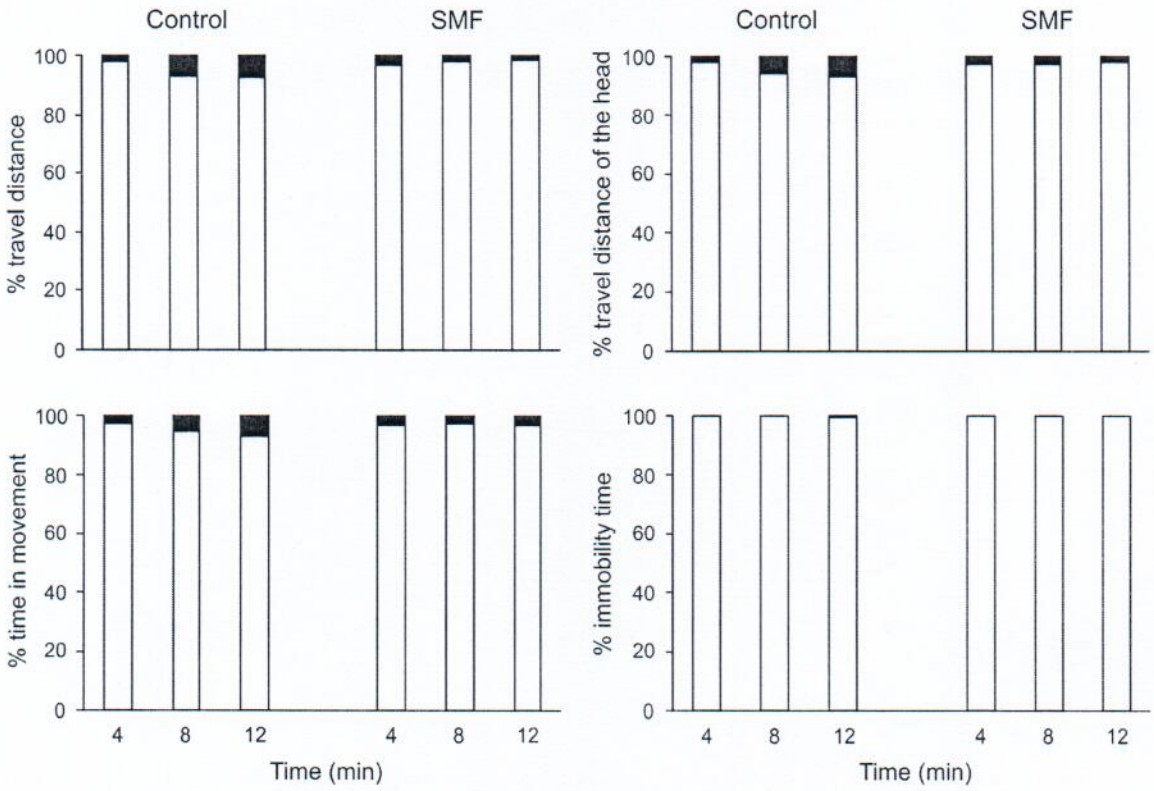

Figure 3 Effect of a static magnetic field (SMF) (110 mT) on distribution (%) of four behavioral parameters – travel distance, travel distance of the head, time in movement, and immobility time – of *Laemostenus* (*Pristonychus*) *punctatus* adults over the peripheral zone (white) vs. central zone (black) of the open-field arena (control: n = 9; exposed to SMF: n = 8). Percentages are calculated for three 4-min sequences within the 12-min total period of observation.

[monarch butterflies (MacFadden & Jones, 1985), the heart and dart moth (Baker, 1987)], Coleoptera (*Tenebrio* spp.) (Todorović et al., 2013), and others. There also have been studies of the influence of SMFs on insect viability (Rauš et al., 2009), development (Ramírez et al., 1983; Prolić & Nenadović, 1994; Todorović et al., 2013), and genetic material (Takashima et al., 2004). Static magnetic fields stimulated pupal metamorphosis in *A. mellifera*, *Drosophila melanogaster* Meigen, and *Tenebrio molitor* L., respectively (Prolić & Nenadović, 1994, 1995; Prolić et al., 2001).

Effects of SMFs on Coleoptera species have been poorly investigated to date, especially for the Carabidae family. Todorović et al. (2013) studied the effect of a 50 mT SMF on development and behavior of *Tenebrio* spp. They found that this field did not affect pupa-to-adult development of two *Tenebrio* spp., but did modulate their adult motor behavior. In *T. molitor* adults, the SMF modulated travel distance, average speed, and immobility time. Todorović et al. (2007) examined the influence of an SMF (2 mT) on neuronal population activity in adults of a longhorn beetle, *Morimus funereus* Mulsant. They found that 5-min exposure produced both excitatory and inhibitory effects on neuronal activity of the antennal lobe of *M. funereus*. Their results also indicated that the induced effects were mostly irreversible for both the population and the nearest neuron.

In our study, exposure of *L. (P.) punctatus* adults to an SMF (110 mT, 5 h) changed their motor activity, namely significantly increased their travel distance and average speed while in motion in the first 4 min of observation. This could mean that these beetles, compared to control ones, did not walk for longer, but when they were walking, they were going faster and therefore they walked a greater distance. Absence of significant changes after 4 min could be explained by the disappearance of SMF effects, or any alterations induced by an SMF are not large enough to be expressed on behavioral level. One more observation is that the beetles – control and exposed to an SMF – were mostly in the peripheral rather than central zones of the open-field arena during the whole period of observation. This is in accordance with earlier findings indicating that insects often avoid central zones and stay in the periphery, close to the walls of open-field arenas. This phenomenon is known as centrophobism/thigmotaxis (Besson & Martin, 2005).

How an SMF influences the nervous system is still undetermined. Studies on motor behavior are therefore important because they indirectly provide more information about magnetic field effects on integrity of the nervous system (Thomas et al., 2001). The neuroendocrine system controls insect development and behavior. Static magnetic fields could cause alterations in these vital processes. It has been proven that some biogenic amines (dopamine, norepinephrine, serotonin, octopamine, and tyramine) govern motor behavior (Saraswati et al., 2004; Dernovici et al., 2007; Socha et al., 2008). A possible explanation for changes in the motor activity of *L. (P.) punctatus* adults is that the employed SMF induces alterations in metabolism or transmission of the above-mentioned biogenic amines. Static magnetic field effects can also be mediated by induced changes in calcium ion concentration (Teodori et al., 2002).

Another question concerns the possible connection between cryptochromes and magnetoreception in *L. (P.) punctatus*. It has been shown that cryptochromes in the photoreceptor neurons of bird eyes have an impact on magnetic orientation during migration (Heyers et al., 2007). Cryptochrome could be involved in various tasks in the life of insects. It may help to determine the position of the geomagnetic north and aid orientation in space. *Drosophila* species appear to have cryptochrome-dependent magnetic orientation. A photosensitive molecule of cryptochrome is essential for their light-dependent ability to sense magnetic fields (Gegear et al., 2008). Bazalova et al. (2016) demonstrated a Cry-dependent sensitivity to the direction of geomagnetic field in two cockroach species – American cockroach, *Periplaneta americana* (L.), and German cockroach, *Blattella germanica* (L.) – and the importance of the eye for the directional geomagnetic field response.

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Scattering-enhanced absorption and interference produce a golden wing color of the burnished brass moth, *Diachrysia chrysitis*

Dejan Pantelić,[1,*] Svetlana Savić-Šević,[1] Dejan V. Stojanović,[2] Srećko Ćurčić,[3] Aleksandar J. Krmpot,[1] Mihailo Rabasović,[1] Danica Pavlović,[1] Vladimir Lazović,[1] and Vojislav Milošević[1]

[1]*Institute of Physics, University of Belgrade, Pregrevica 118, 11080 Zemun, Belgrade, Serbia*

[2]*Institute of Lowland Forestry and Environment, University of Novi Sad, Antona Čehova 13, 21000 Novi Sad, Serbia*

[3]*Institute of Zoology, University of Belgrade—Faculty of Biology, Studentski Trg 16, 11000 Belgrade, Serbia*

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Here we report how interference and scattering-enhanced absorption act together to produce the golden wing patches of the burnished brass moth. The key mechanism is scattering on rough internal surfaces of the wing scales, accompanied by a large increase of absorption in the UV-blue spectral range. Unscattered light interferes and efficiently reflects from the multilayer composed of the scales and the wing membranes. The resulting spectrum is remarkably similar to the spectrum of metallic gold. Subwavelength morphology and spectral and absorptive properties of the wings are described. Theories of subwavelength surface scattering and local intensity enhancement are used to quantitatively explain the observed reflectance spectrum.

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I. INTRODUCTION

Fascinating "inventions" of evolution have been discovered in a large number of recent studies dealing with the biophysics of living creatures. In that respect, insects are an excellent research subject due to their diversity and abundance.

Biophotonics of insects is a particularly active subject which aims to explain function and imitate intricate micro- and nanostructures on their bodies. Several studies present a comprehensive overview of the current research [1–3]. Surprising results are still being published, such as the photonic system of a Saharan silver ant, enabling radiative dissipation of heat, directly through the IR atmospheric window [4]. This and a number of other studies suggest that nature has developed many technologies which can be used to solve everyday problems, if successfully imitated [5,6].

Optical photonic structures in the living world are diverse and have been classified by Land [7], based on their biological function, including tapeta (light-path doubling or image forming), camouflage, display, optical filters (e.g., corneal nipples of insect compound eyes), and anatomical accidents (features whose optical properties have no obvious biological function, e.g., mother-of-pearl in some mollusks).

More specifically, the biophotonics of Lepidoptera draws much attention, mostly due to the attractiveness of butterflies. Much less is known about moths (suborder Heterocera), which represent a group of Lepidoptera, characterized by the wings mostly having drab colors (gray or brown), and feathery or saw-edged antennae (vs club-shaped in butterflies). While moths are more numerous than butterflies, the number of structural coloration studies is significantly smaller. Only attractive and conspicuous moth species were explored, such as the Madagascan sunset moth [8]. The lack of interest is, possibly, due to the simpler wing-scale structure of moths, compared to really complex features present on the scales of day flying butterflies (e.g., Bragg gratings or photonic crystals).

Golden wing patches are prominent features of some noctuid moths. The patches might be just small marks as in *Autographa jota* (Linnaeus, 1758) and *A. bractea* (Denis & Schiffermüller, 1775), or large areas, as in *Diachrysia balluca* Geyer, 1832. The physics behind the golden moth color was previously analyzed using the diffraction theory of Stratton-Silver-Chu [9] in the case of *Thysanoplusia orichalcea* (Fabricius, 1775) (previously included in the genus *Trichoplusia* McDunnough, 1944) (Noctuidae family). However, a correspondence between theory and experimentally recorded spectra was qualitative, probably due to approximations of the mathematical formalism.

Other insects with a golden cuticle do exist, such as *Chrysina aurigans* (Rothschild & Jordan, 1894) (Coleoptera: Scarabaeidae) [10], whose broadband metallic reflection is due to a chirped Bragg mirror within the cuticle. Some species have a tunable color, which depends on atmospheric humidity [11] or stressful events [12], enabling the insects to change the color from red to golden.

Rothschild *et al.* [13] found that carotenoid pigments may also contribute to golden metallic areas of Danainae butterfly pupae. Similar results were obtained by Taylor [14] and Neville [15]. In contrast, Steinbrecht *et al.* [16] proved that golden reflections of *Euploea core* (Cramer, 1780) (Lepidoptera: Nymphalidae) pupae have an entirely physical nature. They showed that reflectance spectra of the cuticle (possessing multiple endocuticular thin alternating layers) and metallic gold are very similar, with a characteristic edge at 450–550 nm. The authors also emphasize that the carotenoids in the epidermis cannot contribute to the color effects because the cuticle practically does not transmit yellow light at all.

Scattering from an irregular surface is a secondary mechanism of structural coloration—interference and diffraction being dominant ones. For example, lycaenid butterflies (in particular, subfamily Polyommatinae) scatter light from the internal, pepper-pot-like, Bragg layers (having holes of 100 nm average diameter). The wing-scale laminae are almost hollow and permit the blue radiation to escape [17]. Pieridae can also be mentioned due to the nanobeads (pigment granules), which fill the space between the laminae [18], where the scattering

*pantelic@ipb.ac.rs

and fluorescence extend the reflection spectrum [19,20]. Scattering in the living world is sometimes accompanied by light trapping, as in diatoms [21]. This seems to be a significant mechanism for efficient light harvesting in photosynthetic organisms.

Here we describe how several optical mechanisms interact to produce the golden wing color of the burnished brass moth, *Diachrysia chrysitis* (Linnaeus, 1758). We have studied spectral properties of the golden wing patches, as well as the internal and external ultrastructure of the wing scales and wing membranes. It was found that the scattering from irregular internal surfaces of each scale suppresses the UV-blue reflection, while interference efficiently reflects the red-infrared spectral range. A theoretical model is proposed, which combines interference and scattering from the scale laminae. The finite element method (FEM) is used to confirm the trapping and local intensity enhancement of light inside the laminae, while the modified transfer matrix method is used to calculate the reflection spectrum.

II. OPTICAL PROPERTIES AND STRUCTURE OF *D. CHRYSITIS* WING

The burnished brass moth (*D. chrysitis*; shown in Fig. 1) is a common species of the Noctuidae family (Insecta: Lepidoptera). It inhabits temperate climates in the Palearctic region [22]. *D. chrysitis* is a remarkable moth with big, golden (sometimes brassy-green) areas on each forewing. The wingspan is 28–35 mm, while the length of each forewing is 16–18 mm [23]. The burnished brass moth is usually found in marshy areas or in slightly moist forb communities. The larvae feed on plants such as *Urtica* spp., *Lamium* spp., or *Cirsium* spp. [24]. The moth flies from May to October depending on the location. It flies regularly in dusk, and was seen visiting flowers of various plants. Sometimes it can be noticed during the day, even sucking nectar. The species is widespread in Serbia [25].

It is supposed that irregular golden patches in the forewings of *D. chrysitis* are an example of disruptive coloring, as an excellent way of hiding oneself by breaking up the body contours [26]. Additionally, it was also postulated that specular reflection of the sun's rays may imitate the glittering of dew droplets. Even more, it seems that the wing color is optimized to efficiently reflect the yellowish light of the sun. As a dusk species, the moth may emit a signal in the IR spectral range from the golden patches, which might be used as a signal for intraspecific recognition. Anatomical accidents seem unlikely because metallic areas on *D. chrysitis* forewings form species-specific patterns [27].

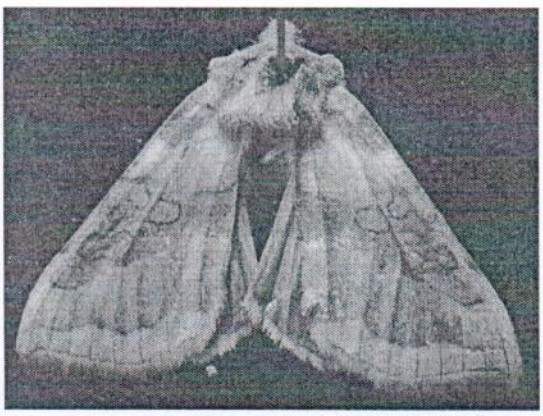

FIG. 1. Burnished brass moth (*D. chrysitis*) with golden areas on its forewings.

From the optical point of view, the most prominent features of the studied moth are golden-color wing patches, as seen in Fig. 1. The corresponding wing spectra were recorded in reflection using a fiber optic spectrometer (manufactured by Ocean Optics, HR2000CG-UV-NIR), with a 400-μm core diameter fiber. A halogen lamp was used as a light source, and spectra were referenced to a standard white surface. The light collection angle was limited by the numerical aperture of the fiber (NA = 0.22, which is equivalent to an angular range of $\pm 12.7°$), positioned such that an approximately 40-mm^2 area is observed. This means that the spectra of individual scales are integrated both angularly and across the wing surface. This fact was accounted for in the numerical simulations.

The spectrum of *D. chrysitis* is broad, with a cutoff wavelength at approximately 500 nm. Its exact shape slightly depends on the angle between light source, wing, and detector. There is a close similarity with the spectrum of metallic gold, as shown in Fig. 2. The specular reflectance spectrum of gold is taken tabulated from Ref. [28], where it is treated as a reference standard.

Optical reflection microscopy of the *D. chrysitis* forewing reveals an almost uniform, intense golden sheen as shown in Fig. 3(a). In contrast, reflection from individual scales is yellowish, with occasional red and green bands, presented in Fig. 3(b). Overlapped scales show increased reflection and color bands, as can be observed in the same image. If observed in transmission, an individual scale in air [see Fig. 3(c)] is quite transparent, with a slight residual absorption. By immersing

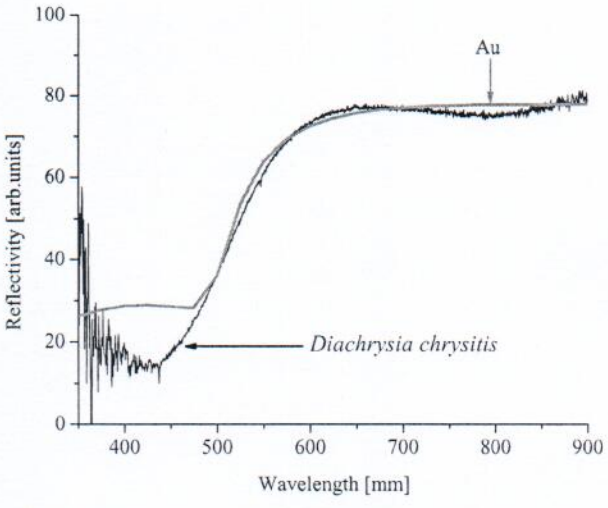

FIG. 2. Spectral reflectance of metallic gold (red curve) and golden wing patch of *D. chrysitis* forewings (black curve). Reflectance of *D. chrysitis* is scaled to emphasize the similarity with the gold.

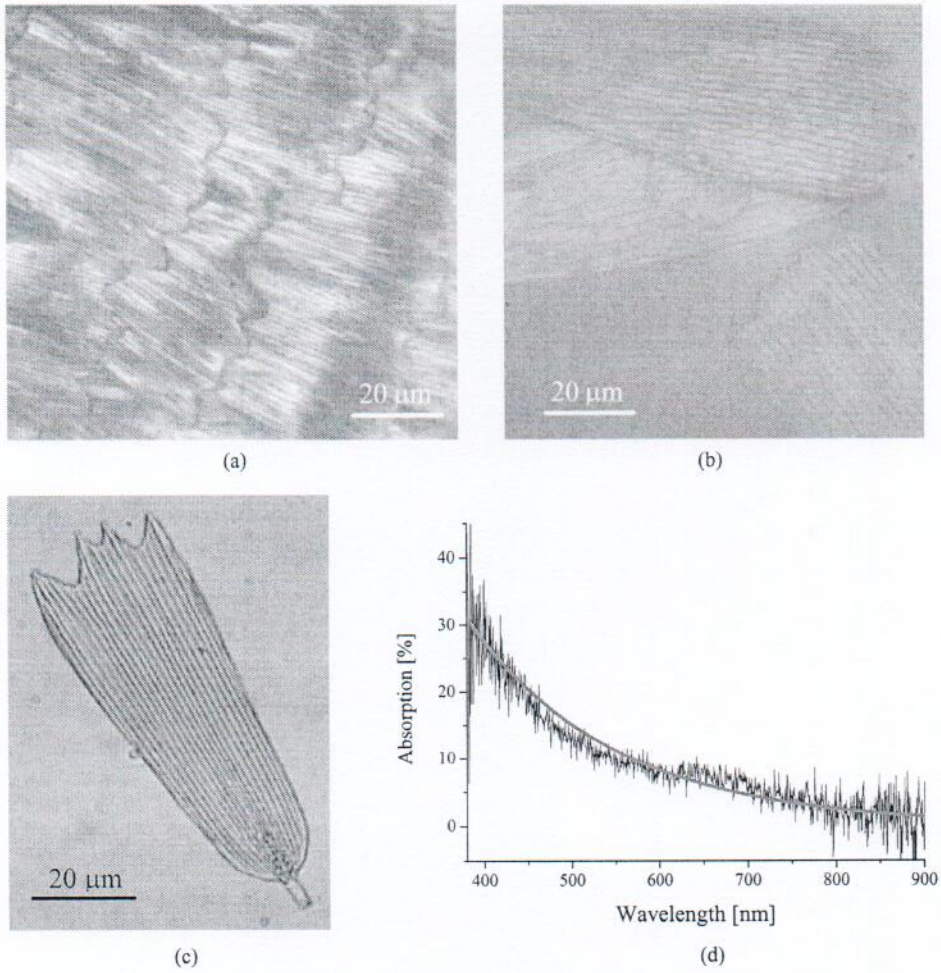

FIG. 3. Optical microscope images of *D. chrysitis*: (a) Scales on the forewing showing the uniformly golden reflection. (b) A microscope reflection image of two individual, overlapped scales. (c) A transmission image of an individual scale in air. (d) Absorption spectrum of a single scale placed in an immersion oil (black line) and the corresponding exponential fit (red line).

the scale in an index matching liquid (manufactured by Cargile, series A, with the certified refractive index 1.5700 ± 0.0002) Fresnel reflection was suppressed. We measured the absorption spectrum [Fig. 3(d)], which is very similar to that of melanin [29], showing exponential decrease from the UV to the IR part of the spectrum. We were able to estimate the value of the absorption coefficient α (or the imaginary part of the complex index of refraction $k = \alpha\lambda/4\pi$), and use it in further calculations. We have found that k ranges between 0.081 (at 380 nm) and 0.0013 (at 800 nm).

A field-emission gun scanning electron microscope (FEGSEM) was used to study the fine anatomy of the moth scales. The *D. chrysitis* forewing possesses a number of overlapping scales [as in Fig. 4(a)], but we were not able to see a difference between cover and ground scales. At higher magnification, as in Fig. 4(b), we can see that the upper lamina is ornamented with very thin lamellar ridges (separated by approximately 1.8 μm). They are connected with herringbone shaped cross ribs, which constitute a subwavelength diffraction grating with the period of roughly 150 nm. A dual wing membrane seems to be an important optical component, too. Its thickness is of the order of 500 nm and contains a number of 300-nm diameter, randomly dispersed, hemispherical protuberances [see inset in Fig. 4(a)].

Individual scales were prepared for scanning electron microscopy by the double transfer method, which was begun by detaching a scale with a low-surface-energy adhesive (adhesive layer of "Post-it" sticky note), followed by the transfer to a high-surface-energy tape (conductive carbon). By that means, the original scale orientation was preserved. Figure 5 shows one of the partially destroyed scales and its internal and external laminae structures. We see that the external side of the upper lamina (the one facing outwards) is strongly patterned, as explained above, while its internal surface is very irregular, with linear grooves directly beneath the ridges. The external side of the lower lamina (the one facing the wing membrane) is smooth, while its internal surface is completely irregular, similar to nanometer-sized "pebbles," with a diameter less than 60 nm.

We have observed a strong autofluorescence of scales, which is enough for nonlinear (NL) fluorescence microscopy. We used a nonlinear microscope for laser processing and

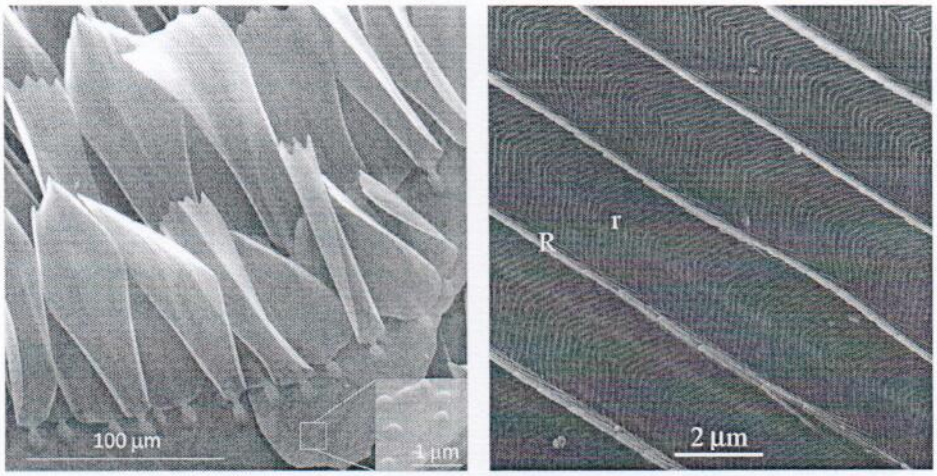

FIG. 4. (a) Scales of *D. chrysitis* in their natural position on the wing. The inset shows the enlarged part of the wing membrane with 300-nm-diameter protuberances. (b) Enlarged image of a single scale, showing lamellar ridges (R) and herringbone shaped cross ribs (r).

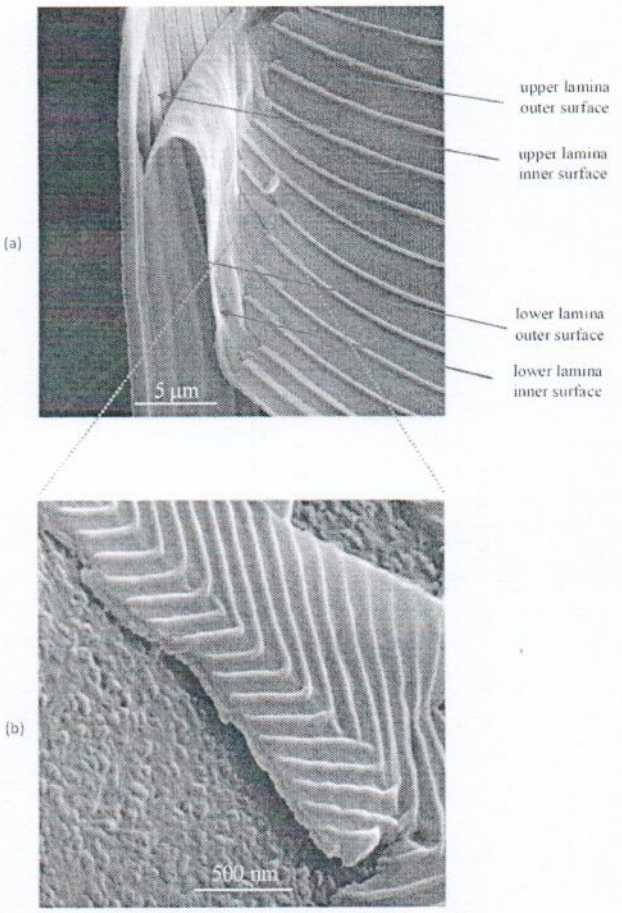

FIG. 5. FEGSEM images of a single *D. chrysitis* scale at two different magnifications. (a) This image reveals the internal and external structure of a single scale. (b) Enlarged zone of a scale showing rough internal surfaces.

cutting, too, which turned out to be a good tool for exposing otherwise hidden features. The beam power was increased above the threshold level and software was modified to enable drawing arbitrary shapes using vector images. At 840 nm and ~100 fs pulse length we were cutting moth chitinous structures with as low as a few milliwatts of laser power. However, continuous wave (cw) radiation at the same wavelength required an order of magnitude higher power. It was interesting that the laser-cut lines were rather irregular in the case of *D. chrysitis*, in contrast to scales of other lepidopteran species, which produced clear, well defined, lines.

To further reveal the cross-sectional geometry of *D. chrysitis* moth scales, we cut them as explained above. A SEM image of a laser-cut scale is shown in Fig. 6. The internal space of the scale is not visible due to the welding of the upper and lower laminae, but we were able to estimate the thickness of the scale at 300 nm, and the height of the ridge at 400 nm. Based on the scanning electron microscope images we are able to draw a general scheme of an individual scale as presented in Fig. 7.

We emphasize that the external features of the wing scale (such as the distance between the ridges) can be measured accurately from FEGSEM images, because they are recorded at normal incidence. Other characteristics, such as laminae thickness, are more complicated to quantify due to difficulty in determining the exact relative position of the scale and the scanning electron microscope optics (see Fig. 6, where the scale is partially lifted from the substrate). In such cases, measurements were performed using external features as a reference—e.g., lamina thickness was determined at approximately 75 nm by observing that it is approximately one half of the distance between the cross ribs (150 nm). Anyway, such measurements served just as a starting point for a wing-scale model.

Variability of the moth scales is another source of uncertainty. We recorded a number of SEM images, measured relevant features at several positions, and were able to find that they vary between 15% and 20% (depending on the measured characteristics).

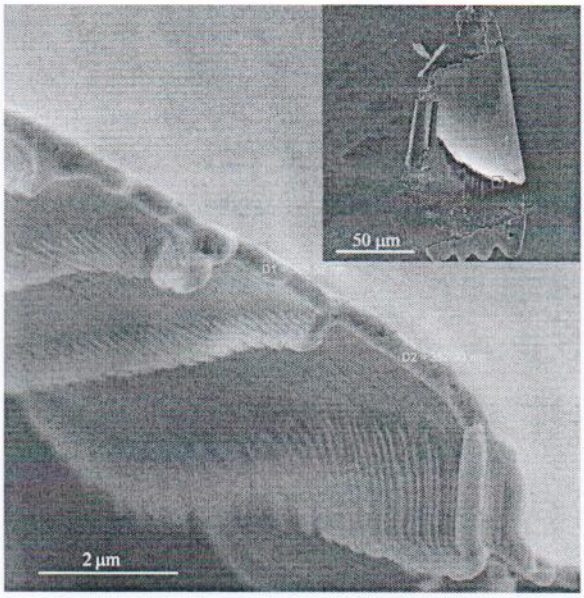

FIG. 6. A cross-sectional image of a femtosecond-laser-cut *D. chrysitis* wing scale. The image of the whole, laser-cut scale is shown in the inset.

III. OPTICAL MODELING OF THE *D. CHRYSITIS* WING SCALES

The transparency of *D. chrysitis* scales and the apparent simplicity of their internal and external structure pose a problem in explaining the golden wing color. We show that all the wing components (a double layer of scales and a wing membrane, possibly also the pigmented scales on the wing underside) work together to produce the final effect. Several features operate synergistically: slight absorbance of each scale, scattering on internal scale surfaces, interference of light within the scale, reflection of light from the wing membrane, and diffraction on the upper lamina grating.

We first demonstrate that the scattering on the internal scale surfaces leads to significant dispersal of incident light. As shown in Fig. 5, the internal scale surfaces are highly irregular, with the root mean squared (RMS) roughness estimated between 10 and 30 nm. The wavelength of the incident visible light (inside material) is much larger than the roughness and

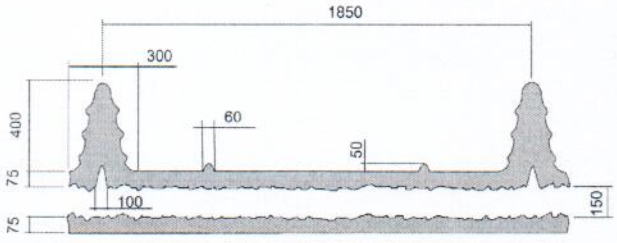

FIG. 7. A dimensional scheme of a *D. chrysitis* scale cross section. All dimensions (expressed in nanometers) are estimated from SEM images and vary across the scales. Due to the variability of features in the living world, uncertainty of all the dimensions is between 15% and 20%.

the widely used scalar surface-scattering theory [30,31] is applicable. Under these circumstances, the light is split into two parts: one, regular, propagating as if the surface is perfectly flat, and the other diffusely scattered (haze).

Quantitatively, both components are described relative to transmittance T_0 and reflectance $R_0 = 1 - T_0$ of an ideally flat surface, when Fresnel equations hold. Accordingly, the haze transmittance $T_H(\lambda)$ and reflectance $R_H(\lambda)$ of a rough surface are described by [31]

$$T_H(\lambda) = T_0\left(1 - \exp\left\{-\left[\frac{2\pi\sigma}{\lambda}(n_i\cos\phi_i - n_t\cos\phi_t)\right]^2\right\}\right), \tag{1}$$

$$R_H(\lambda) = R_0\left\{1 - \exp\left[-\left(\frac{4\pi\sigma}{\lambda}n_i\cos\phi_i\right)^2\right]\right\}, \tag{2}$$

where T_0 and R_0 are the transmittance and the reflectance of a perfectly flat surface, respectively; λ is the wavelength in vacuum; ϕ_i and ϕ_t are the angles of incidence and refraction; n_i and n_t are corresponding refractive indices; σ is the surface RMS roughness.

A simple calculation, based on Eqs. (1) and (2), shows that between 1% and 3% of incident radiation is scattered at each interface, depending on the wavelength and assuming normal angle of incidence ($\phi_I = 0$), RMS roughness $\sigma = 20$ nm, and the refractive index of chitin $n_i = 1.57$. As expected, short wavelengths are scattered more than long ones. The scattered light has a tendency to be trapped inside chitin layers, in a manner similar to textured solar cells [32]. It was shown in [33] that the local light intensity is increased by $2n^2$, and absorption by $4n^2$, where n is the refractive index. This was verified for the *D. chrysitis* moth by using the finite element method (FEM) with periodic boundary conditions, applied to the model simulating a double layer of scales, as shown in Fig. 8(a). The corresponding electromagnetic field distributions can be seen in Fig. 8(b) showing the strong electromagnetic field enhancement.

Local field enhancement due to scattering is accompanied by increased absorption as predicted by the model described in [33]. We made slight modifications to correctly describe the scales of *D. chrysitis*.

The change of the beam cross section is ignored due to the thinness of the scales. This is justified by the following arguments: Assume that the angle of divergence is $\theta = 40°$ and scale laminae thickness is $D = 75$ nm; then the beam spread is defined by $2Dtg(\theta/2) = 55$ nm. This is insignificant for a beam width of approximately 7 mm, as used in our spectral measurement. The surface absorption was disregarded, too, because the residual melanin is expected to be distributed inside the laminae.

Under these assumptions, the absorption A_{int} inside the planar layer can be described by

$$A_{\text{int}} = \frac{4n^2T_{\text{inc}}}{T_{\text{esc}} + 4n^2\alpha l}, \tag{3}$$

where α is absorption coefficient, n is the refractive index, I_{inc} is incident light intensity, l is the layer thickness, and T_{inc} is a fraction of light transmitted through the interface. T_{esc} is an

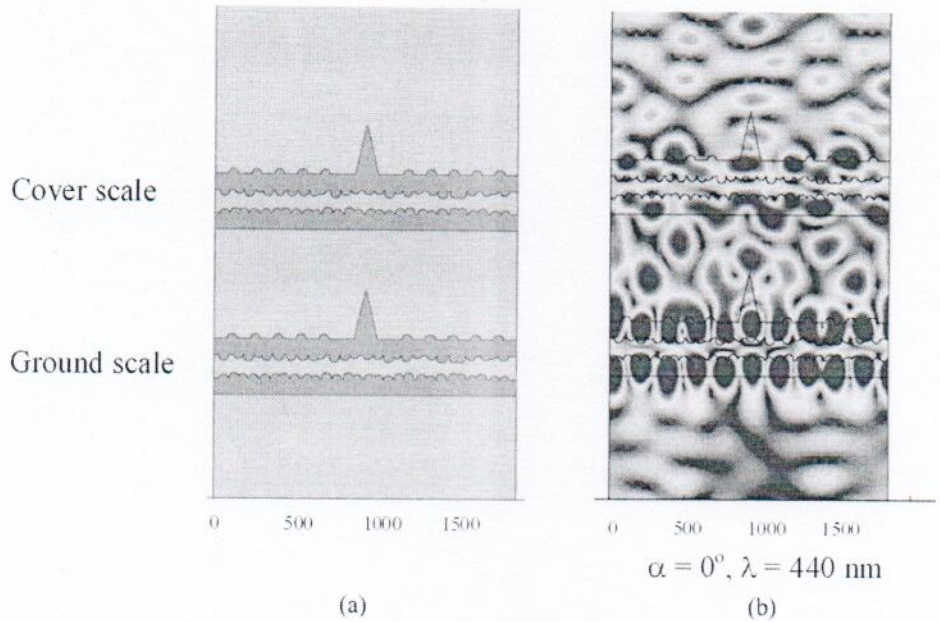

FIG. 8. (a) Geometry of the FEM model. (b) Intensity enhancement at 440 nm wavelength. The electromagnetic field is enhanced both in the ground and cover scales. Calculations were performed assuming that the angle of incidence is $\alpha = 0$ and there is no absorption.

average transmission factor of the escaping radiation, due to partial (Fresnel) transmission at the interface. According to the same model, fraction of the incident radiation escaping the layer is described by

$$F_{\mathrm{esc}} = \frac{T_{\mathrm{inc}} T_{\mathrm{esc}}}{T_{\mathrm{esc}} + 4n^2 \alpha l}. \tag{4}$$

Now we have tools to treat the problem of the golden coloration of the burnished brass moth. Its geometry includes two layers of scales and two wing membranes as shown in Fig. 9(a). It is assumed that the outside surfaces of the scales are flat, which is strictly true only for the lower lamina. The upper lamina is structured with two gratings. The coarse one will produce diffraction orders which will be treated similarly during the propagation through the scales, the only difference being the angle of incidence. The dense grating is incapable of generating any propagating modes and will not enter the calculations. The inside scale surfaces are rough with RMS roughness of 10–30 nm, and the wing membranes are treated as flat. Fresnel reflection and transmission will be taken into account at flat surfaces, while rough surfaces will also include haze in reflection and transmission as schematically shown in Fig. 9(b). In the latter case, haze $R_H(\lambda)$ and $T_H(\lambda)$ diminish Fresnel coefficients R_0 and T_0 by the amounts $R_0 R_H(\lambda)$ and $T_0 T_H(\lambda)$. The resulting transmission and reflection coefficients are described by $R_0 - R_0 R_H(\lambda)$ and $T_0 - T_0 T_H(\lambda)$.

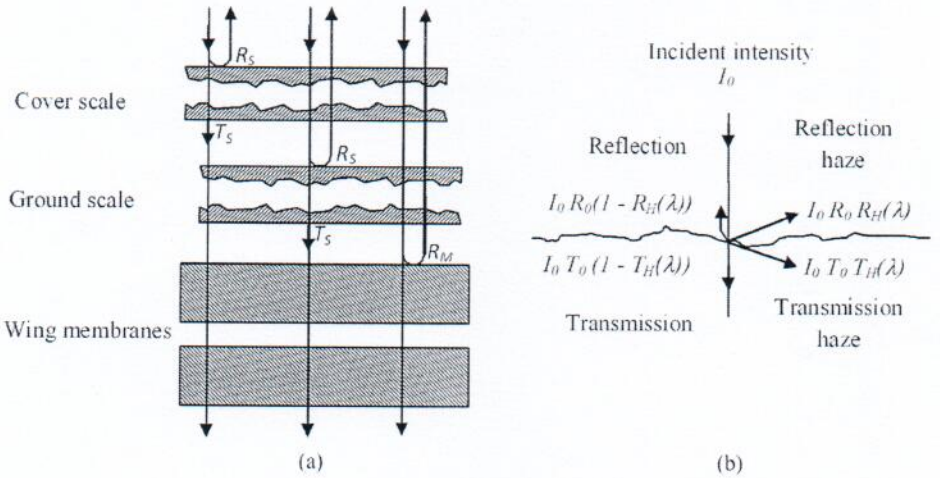

FIG. 9. (a) Geometry of the model used to simulate the wing of *D. chrysitis*. R_S and R_M are reflectance of a scale and a wing membrane, respectively. T_S is a transmittance of a single scale. (b) Reflection and transmission through the interface, as treated in a model. Incident intensity I_0 is split into four components: Fresnel reflectance (R_0), Fresnel transmittance (T_0), reflection haze (R_H), and transmission haze (T_H).

TABLE I. Numerical values of the parameters used for modeling optical reflection from *D. chrysitis* moth scales.

Parameter	Meaning	Value
N	Refractive index of chitin	1.57
α_0	Melanin absorption parameter, Eq. (5)	0.23 (1/nm)
A	Melanin absorption parameter, Eq. (5)	90 (nm)
λ_0	Melanin absorption parameter, Eq. (5)	380 (nm)
σ	RMS surface roughness	30 (nm)

The interference problem will be solved for an individual wing scale, as well as for the wing membranes, but not for the wing as a whole. This is a reasonable assumption, because the relative distances between the scales and the wing membrane are highly variable and the resulting effect is averaged across the wing surface. As a consequence, the resulting reflection spectral intensities of scales and membranes will be incoherently added.

Optical parameters of the model were estimated from the measurements performed on the scale embedded in an immersion liquid ($n = 1.57$), as described in the previous section. Therefore, the refractive index was taken to be 1.57 (consistent with the results published in [34]). According to the same study [34], the refractive index dispersion is less than 4% within the wavelength range of interest (380–900 nm) and the resulting effects were found to be insignificant. The coefficient of absorption α was modeled with an exponential function (assuming that the residual pigment is most probably melanin):

$$\alpha = \alpha_0 \exp\left(-\frac{\lambda - \lambda_0}{A}\right). \tag{5}$$

All parameters of the model are summarized in Table I.

Finding the exact solution to the multilayer interference is a problem requiring numerical tools. Here we adopt the transfer matrix method, described and used in [35] to analyze light scattering and trapping in silicon thin film solar cells. We divide incident light into two components: a scattered one, which is mostly absorbed and diffused, and an unscattered one, which interferes in wing scales and wing membranes. For the unscattered component we apply a transfer matrix method, where scattering from subwavelength rough surfaces is treated as a wavelength-dependent correction for Fresnel coefficients.

Reflection and transmission from a single scale are treated coherently using the transfer matrix method. As a result, the spectral reflectance R_S and transmittance T_S were found. Similarly, the reflection from the double wing membrane was treated coherently (with the resulting reflectance R_M). Scattering from the wing membrane was not included in calculations due to the sparsity and large dimensions of scattering inclusions (as illustrated in Fig. 4). The resulting spectrum of the wing as a whole is composed of three components: one which is reflected from the cover scales, the other reflected from the ground scales, and the final one due to the wing membranes. We combine them incoherently, because the mutual position between scales and membranes is stochastic and highly variable. The final reflected spectral distribution of the whole wing is thus

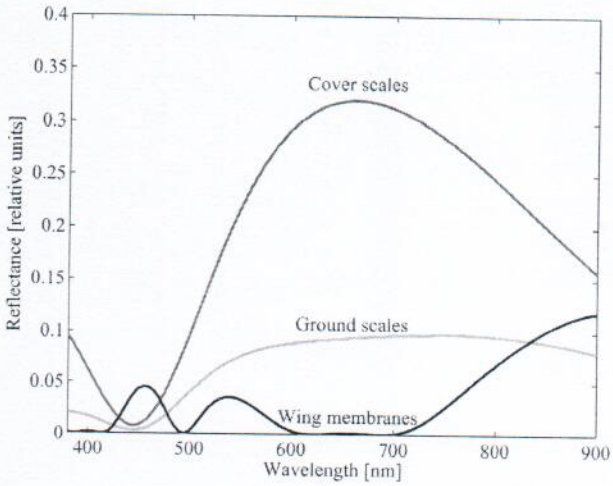

FIG. 10. Contributions of wing membranes, cover scales, and ground scales to the resulting wing spectrum. They are calculated using the transfer matrix method and normalized to the intensity of the incident light before being transmitted through the layers.

$$R = R_S + T_S R_S T_S + T_S^2 R_M T_S^2. \tag{6}$$

The first term corresponds to the reflection from the cover scale, the second to the transmission through the cover scale, followed by the reflection from the ground scale and return path through the cover scale. The third term describes transmission through the cover and ground scales, followed by the reflection from the wing membrane, and return path through both layers of the scales. The calculated contribution of each term to the final spectrum is shown in Fig. 10 (normalized to the intensity of light before being transmitted through the layers).

Spectral contributions of wing components significantly depend on their geometry, i.e., scale laminae and wing membrane thicknesses. For some combination of dimensional parameters, even a single scale can quite faithfully reproduce an experimentally recorded spectrum (as in Fig. 11). However, there are slight modulations within the whole spectral range, due to thin film interference effects. They disappear when spatial and angular averaging is included, as further explained. As explained above, losses are much higher inside the blue-UV spectral range, as can be seen in Fig. 11. According to Eqs. (3) and (4), most of the light energy is absorbed, and the rest of the radiation is scattered. It is interesting to note that a significant amount of radiation is transmitted through the wing membranes (green curve in Fig. 11). However, the UV component of transmitted radiation is efficiently absorbed by the dark, pigmented scales, on the wing underside.

There is an important word of caution. In order to get a consistently golden wing color, the dimensional and optical parameters of each individual scale should be kept within quite tight tolerances—a task completely impossible in the living world. It is more realistic to expect significant variability of all the parameters. Thus they were varied in our simulations (according to the normal distribution) within ±15% of the values producing fit in Fig. 11. The angle of incidence was also

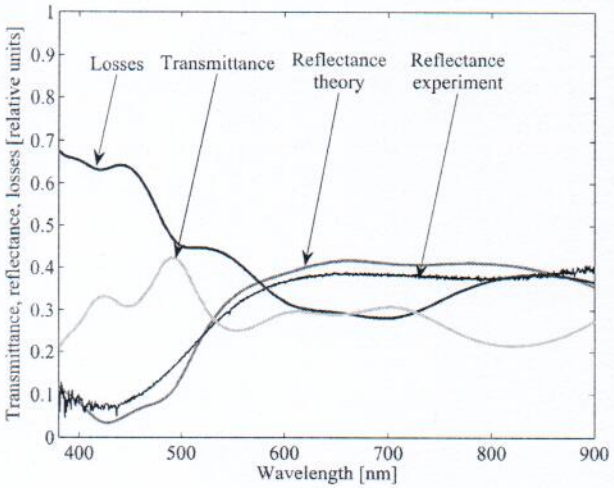

FIG. 11. A spectral reflectivity (red curve) of a *D. chrysitis* moth wing, calculated by the transfer matrix method is shown. The corresponding experimental curve (black curve) is added for reference. Losses (due to scattering and absorption, blue curve) and the wing transmittance (green curve) are displayed, too.

allowed to fluctuate within ±20°, which imitates variability of scale orientations.

As a result, 100 different spectra were calculated (for clarity, only 25 of them are displayed in Fig. 12 as light blue curves). They were consequently averaged, in agreement with our experimental procedure where the light is collected from the wing area and within an angular range (the resulting curve is shown in blue). By comparing the calculated spectrum with the experimentally recorded one (red curve in the same figure), agreement appears remarkably good, except for the radiation above 800 nm.

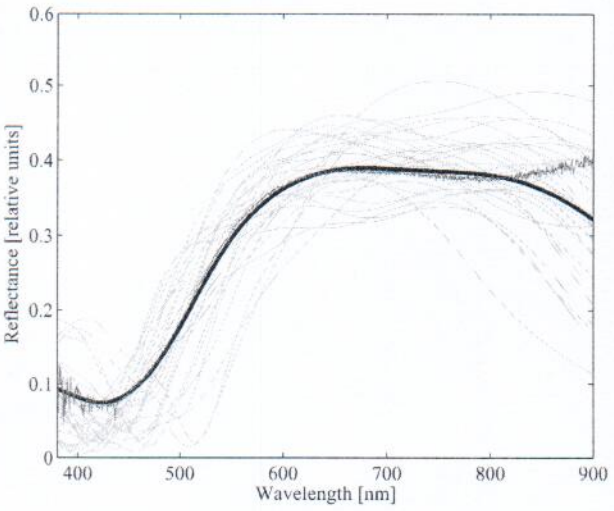

FIG. 12. Spectral averaging of light reflected from *D. chrysitis* wing, calculated by the transfer matrix method which includes scattering and local field enhancement. Light blue curves are individual spectra, blue curve is the averaged spectrum, and the red curve is the experimentally recorded spectrum.

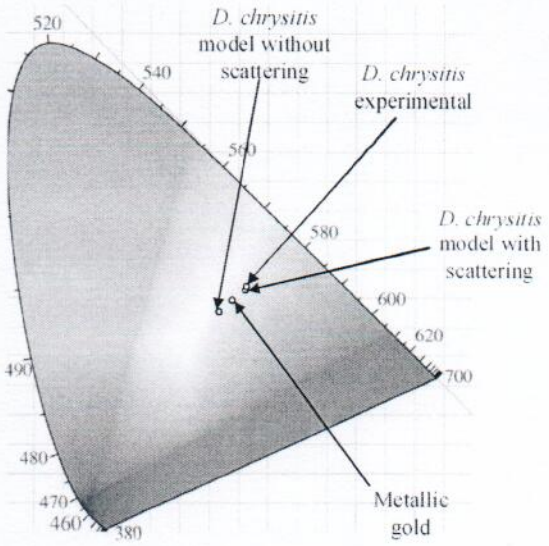

FIG. 13. CIE color coordinates of metallic gold and *D. chrysitis* forewing golden patches, with and without scattering.

The final shape of the spectrum is influenced by the layer thicknesses and the RMS surface roughness. They collectively influence the final calculated spectrum, but in general terms we have observed that increased thickness shifts the spectrum towards the red, while increased roughness depletes the blue part of the spectrum and decreases overall reflectivity.

All the computations are based on a transfer-matrix code, developed in [36] (declared free to use and made public at the URL provided therein). The program was modified by including scattering and local field enhancement effects [as defined by Eqs. (1)–(4)].

IV. DISCUSSION AND CONCLUSIONS

In contrast to most other lepidopteran species, each and every wing structure of *D. chrysitis* plays a certain role in iridescent color production. The same goes for the optical mechanisms—interference, diffraction, absorption, and surface scattering. They seem to be intricately intertwined in a synergistic manner—by omitting any of them, a significant change of the spectral profile would result. To demonstrate the fact, we calculated the resulting spectrum without taking into account scattering-enhanced absorption. As expected, reflection in the UV-blue spectral range is not attenuated. The corresponding CIE color coordinate testifies that the resulting color is whitish, as presented on a CIE 1931 diagram (Fig. 13). For reference, we have also displayed points corresponding to metallic gold, experimentally recorded spectrum, and full simulation of *D. chrysitis* wing. It is obvious that without scattering, the color coordinate would approach the achromatic center of the diagram—i.e., the wing will have only a slight coloration. It is interesting to note that the moth wing looks "yellower" than the gold.

As noted before, scattering stimulates confinement of light and increases the intensity by the $2n^2$ factor. It seems that the ground scale layer further amplifies the confinement [see Fig. 8(b)]. One possible explanation is that the cover scales

diffuse the incoming light, while the ground scales additionally disperse it and make it amenable for wave guiding within the layer.

Angular variability of *D. chrysitis* wing coloration is noticeable, and the golden color is observable within the $\pm 20°$ from the specular direction. Beyond that, the color abruptly changes from golden to brown-gray. However, within the specular range, dependence of the reflected spectrum is slight for several reasons. On one hand, it results from irregular internal surfaces of the scale laminae and irregular mutual position of scales in ground and cover layers. The other reason is that the reflectivity at air-chitin interfaces is almost constant between 0° and 30°, as predicted by Fresnel equations. Therefore, the overall shape of the spectrum is not altered, but slightly shifted with the angle of incidence. Coarse diffraction grating on the upper lamina additionally diminishes angular dependence. From whichever direction light enters the scale, at least one of the diffraction orders is being reflected. This is what gives the notable stability of the optical effect with respect to the illumination direction (within the stated angular range).

We have not observed polarization sensitivity of the described reflection spectra, even though it certainly exists at the single wing-scale level. If observed macroscopically, across the whole wing, polarization effects cancel out due to strong variability of individual scale orientations with respect to incident radiation.

It is also interesting to note that the scales from brown-gray and golden areas of the wing have very similar morphology if observed under the scanning electron microscope. The most important difference is optical: while the golden area scales are almost transparent, the others contain a significant amount of absorbing pigment which leads to suppression of the specular component in the brown wing regions. Also, the brown scales are flatter, while the golden ones are slightly curled.

Calculations, according to Eqs. (3) and (4), have shown that approximately 70% of incoming radiation at 380 nm is scattered. Forty percent of the scattered component is absorbed, while the rest is uniformly dispersed all over the full solid angle. At the other end of the spectrum (above 800 nm), only 20% of light is scattered, without being absorbed to any significant extent. As a whole, the spectrum of unabsorbed light is quite flat from UV to IR. Its contribution to the wing reflection is really small, because it is dispersed over the 4π solid angle, while the reflected light is concentrated around the specular direction.

A dual wing membrane is densely covered with nanosized spherical inclusions, conveniently situated, just beneath the scales. It seems that this could be an additional mechanism to scatter light back through the layer of scales. Transmission of the membranes is high and light further propagates to underside scales. They are gray, most probably due to melanin, which will further absorb the UV-blue part of the spectrum. In the red-infrared range, melanin absorbance is insignificant, and scales are again capable of reflecting light back through all the previous layers. This might account for the increased reflectivity in the infrared, which is not predicted by the theory described in the previous section.

Insects with golden body parts are rare and interesting from the biological point of view. The roles of the golden color may be diverse and are related mostly to possible defense mechanisms. We suppose that in *D. chrysitis* the golden forewing patches may appear to predators as warning and/or they can facilitate the conspecific recognition [15].

To summarize: All the structures—cover and ground scales, wing membranes, and underside wing scales—contribute to the golden wing color of the burnished brass moth. Interference, scattering, and absorption enhancement are optical mechanisms responsible for the effect. In short, interference on the scales produces a broad reflection spectrum with a peak in the green part of the spectrum. The blue part of the spectrum is absorbed due to scattering-enhanced absorption on a residual pigment. The red part of the spectrum is transmitted to the wing membrane, where it reflects, goes back through the scales, and combines with the reflection from the scales. The resulting spectrum is strongly attenuated below 520 nm, being almost flat up to 800 nm. The forewings of the *D. chrysitis* moth seem to be a remarkable, finely tuned, optical filter.

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Administrative & Account
Alba Aprea - alba@teknoscienze.com

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SONJA DULETIĆ-LAUŠEVIĆ[1], ANA ALIMPIĆ[1]*, DANICA PAVLOVIĆ[2], PETAR D. MARIN[1], DMITAR LAKUŠIĆ[1]

*Corresponding author

1. Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade, Takovska 43, Belgrade, Serbia

2. Institute of Physics, University of Belgrade, Pregrevica 118, 11080 Zemun, Belgrade, Serbia

Ana Alimpić

Salvia officinalis of different origins

Antioxidant activity, phenolic and flavonoid content of extracts

KEYWORDS: *Salvia officinalis*, sage, antioxidant activity, phenolics, flavonoids.

Abstract *Salvia* officinalis L. plants originated from continental (Mt. Pleš, Serbia) and Mediterranean part of Central Balkans (Luštica peninsula, Montenegro) grew under the same conditions in Belgrade. Various extracts of plant material, collected during summer and winter season, were analyzed for the antioxidant activity and phenolic and flavonoid contents. DPPH, ABTS, and FRAP assays for antioxidant activity, as well as total phenol and flavonoid content, were measured spectrophotometrically. Phenolic and flavonoid content and antioxidant activity of the extracts mostly depended on extraction solvent and harvesting season. The extracts of plants originated from Serbia showed stronger antioxidant activity. Generally, plants collected in summer season performed higher activity. Among tested extracts, ethanol extract showed better antioxidant activities compared to other analyzed extracts.

INTRODUCTION

Salvia (sage), one of the largest and the most important aromatic and medicinal genera of the Lamiaceae family, comprises about 1000 worldwide distributed species (1). *Salvia* species are reported to have antioxidant, antibacterial, antifungal, antiviral, cytotoxic, neuroprotective, antiinflammatory and other biological activities (2-8). *Salvia officinalis*, known as Dalmatian sage, common sage or garden sage, is a perennial subshrub native to the northern coastal region of the Mediterranean, but widely cultivated in many countries (9) due to its culinary and medicinal significance. It is used for food preservation, as a spice for flavouring, and for treatment of many diseases (2).

Free radicals and reactive oxygen species (ROS) are well known inducers of cellular and tissue pathogenesis leading to several human diseases such as cancer, inflammatory disorders, atherosclerosis and cardiovascular diseases (10). *S. officinalis* is proven to be biologically active, and promising as antioxidant agent of natural origin (5, 11-18).

The aim of this study was to determine and compare the antioxidant potential, phenolic and flavonoid contents of different extracts obtained from plant material of *Salvia officinalis* L. originated from continental part of Eastern Serbia and Mediterranean part of Montenegro, cultivated under the same conditions in Belgrade and collected during summer and winter season.

MATERIAL AND METHODS

Plant material

Salvia officinalis plants from their natural habitats at Mt. Pleš (Eastern Serbia) and Luštica peninsula (Montenegro) were transplanted in Belgrade and cultivated under the same conditions. Plants from Pleš were transplanted in December 2008, and plants from Luštica in August 2009. After five seasons living under the same environmental conditions, aerial parts were harvested in vegetative stage during winter (December 2013) and flowering stage in summer (June 2014).

Preparation of plant extracts

Whole aerial plant parts (10 g) were grounded in small pieces (2-6 mm) in the cylindrical crusher. The extraction was performed successively, in a series of consecutive extraction and filtration of plant material, by increasing polarity solvents (100 ml of dichloromethane (DCM), chloroform (CHL), ethyl acetate (ETAC) and ethanol (ETOH)). The plant-solvent mixture was exposed to the ultrasound 1h before and after 24h-maceration at 30°C. The liquid extracts were filtered and evaporated under reduced pressure by the rotary evaporator (Buchi rotavapor R-114). The obtained crude extracts were stored in the fridge at +4°C for further experiments.

Experimental measurements

Crude extracts of *S. officinalis* were dissolved by methanol to obtain stock extract solution at concentration 500 µg/mL (w/v). As standard antioxidants, BHA (2(3)-t-Butyl-4-hydroxyanisole) and BHT (3,5-di-tert-butyl-4 hydroxytoluene), were dissolved in methanol in concentrations of 100 µg// mL and tested for antioxidant activity. All of applied spectrophotometric measurements were performed using JENWAY 6305UV/Vis spectrophotometer.

Determination of total phenolic content

The total phenolic content (TPC) was measured using specrophotometric procedure (19). 0.2 mL of extract solution and 1 mL of 10% Folin–Ciocalteu reagent were mixed and after

six minutes was added 0.8 mL of 7.5% Na_2CO_3. Absorbance was recorded at 740 nm after two hours incubation. The same procedure was repeated for standard gallic acid in order to construct calibration curve. Phenolic content of samples was calculated from standard curve equation and expressed as gallic acid equivalents (mg GAE/g dry extract).

Determination of total flavonoid content

Total flavonoid content (TFC) was measured using specrophotometric procedure (20). The reaction mixture was prepared to contain 1 mL of extract solution, 4.1 mL of 80% ethanol, 0.1 mL of 10% $Al(NO_3)_3 * 9 H_2O$, and 0.1 mL 1M solution of CH_3COOK. After 40 min of incubation at room temperature, absorbance was measured at 415 nm. The same procedure was repeated for standard (flavonol quercetin) in order to construct calibration curve. Concentration of flavonoids in samples was calculated from standard curve equation and expressed as quercetin equivalents (mg QE/g dry extract).

DPPH assay

DPPH (2,2-dyphenyl-1-picrylhydrazyl) free radical scavenging method (21) with slight modifications was performed. Stock solutions of extracts were mixed with methanolic solution of DPPH (40 µg/mL) to adjust concentrations of 10-100 µg/mL (v/v) of reaction mixture (2000 µL). Methanol was used as a blank, while methanol with DPPH solution was used as a control. Absorbance of the reaction mixture was measured after 30 minutes in the dark at room temperature at 517 nm. The decrease of absorption of DPPH radical at 517 nm was calculated using equation:

$$\text{Inhibition of DPPH radical (\%)} = [(A_C - A_S)/A_C] * 100\%$$

where A_C-absorbance of control and A_S-absorbance of the test samples at different concentrations. IC_{50} values (µg/mL) were calculated from DPPH absorption curve at 517 nm.

ABTS assay

ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline–6-sulfonic acid) assay was performed (22) with some modifications. Fresh $ABTS^+$ solution was prepared 12-16 hours before use by dissolving ABTS in the potassium-persulfate solution (2.46 mM). The $ABTS^+$ solution was dissolved by distilled water to obtain an absorbance of working solution 0.700 ± 0.020 at 734 nm. 50 µL of test samples and/or standard antioxidants were mixed with 2 mL of working $ABTS^+$ solution and after incubation of 30 min at 30°C, absorbance was recorded at 734 nm. Distilled water was used as blank. ABTS activity was calculated from ascorbic acid calibration curve (0-2 mg/L) and expressed as ascorbic acid equivalents per gram of dry extract (mg AAE/g).

Ferric-reducing ability of plasma (FRAP) assay

The FRAP assay was performed (23) with slight modifications. FRAP reagent, prepared freshly to contain sodium acetate buffer (300 mmol/L, pH 3.6), 10 mmol/L TPTZ in 40 mmol/L HCl and $FeCl_3 * 6H_2O$ solution (20 mmol/L) in proportion 10:1:1 (v/v/v), respectively, was warmed to 37°C prior to use. 100 µL of test sample were added to 3 mL of FRAP reagent and absorbance was recorded at 593 nm after 4 minutes. Blank was prepared to contain distilled water instead of extract. The same procedure was repeated for standard solution of $FeSO_4 * 7H_2O$ (0.2-1.6 mmol/L) in order to construct calibration curve. FRAP values of sample were calculated from standard curve equation and expressed as µmol $FeSO_4 * 7H_2O$ /g dry extract).

Statistical analysis

All experimental measurements were carried out in triplicate and are expressed as average of three measurements ± standard deviation. Pearson's correlation coefficients, calculated between TPC, TFC and antioxidant assays, were interpreted according to Taylor (24). Calculations and constructing of the charts were performed using the MS Office Excel, 2007. Analysis of variance (ANOVA) was used in order to calculate critical value from F-test (F) and p-statistical significance (p) for analyzed characters. Statistical analyses were performed using the package Statistica 5.1 (25).

		Yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH[a] (IC_{50}, mg/ml)	ABTS[b] (mg AAE/g)	FRAP[c] (µmol Fe (II)/g)
Locality (N=16)	Pleš	2.50 ± 1.555	98.84 ± 38.048	31.75 ± 9.919	28.28 ± 10.241*	1.11 ± 0.509	613.81 ± 328.363
	Luštica	2.03 ± 0.617	96.36 ± 38.941	29.30 ± 8.530	48.62 ± 29.181*	0.99 ± 0.538	593.79 ± 410.621
Season (N=16)	Winter	2.24 ± 0.404	84.94 ± 46.067*	26.41 ± 2.230*	46.89 ± 29.707*	0.91 ± 0.644	521.90 ± 461.568
	Summer	2.29 ± 1.661	110.26 ± 22.598*	34.64 ± 11.580*	30.02 ± 12.152*	1.18 ± 0.327	662.22 ± 276.585
Solvent (N=16)	DCM	3.23 ± 1.875*	77.38 ± 20.864*	27.33 ± 6.185*	37.48 ± 8.652*	0.95 ± 0.313*	426.64 ± 147.153*
	CHL	2.47 ± 0.415*	80.07 ± 25.038*	29.74 ± 4.674*	57.35 ± 36.104*	0.79 ± 0.392*	412.18 ± 211.743*
	ETAC	1.47 ± 0.844*	81.15 ± 23.365*	37.74 ± 12.348*	42.06 ±16.825*	0.75 ± 0.274*	438.80 ± 126.640*
	ETOH	1.90 ± 0.344*	151.82 ± 17.659*	27.30 ± 8.483*	16.93 ± 3.156*	1.77 ± 0.371*	1157.72 ± 239.975*

Table 1. Yield of extracts, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities of S. officinalis extracts.

Results are expressed as mean ± standard deviation; N is the number of samples
* Values within column are significantly different ($p<0.05$)
[a] BHA (IC_{50} 13.37 µg/ml) and BHT (IC_{50} 17.94 µg/ml)
[b] BHA (2.82 mg AAE/g) and BHT (2.75 mg AAE/g)
[c] BHA (583.72 µmol Fe (II)/g) and BHT (445.34 µmol Fe (II)/g)

RESULTS AND DISCUSSION

Extract yields, total phenolic and flavonoid content

The yields of extracts were expressed in percentage (%) of the dry weight extract compared to the initial mass of dry plant material used for successive extraction (Table 1).

Yields of extracts varied depending on the origin, season and the solvent used for extraction. The yields of plant extracts originating from Pleš (2.50%) were higher than those originating from Luštica (2.03%). The yields in the summer and winter season were very similar (2.29 and 2.24%, respectively). The highest extract yield was obtained by dichloromethane extract (3.23%), while the ethyl acetate extract showed the lowest yield (1.47%). Extracts yields were mostly influenced by extraction solvent. Similar results were obtained before using successive extraction (6). Yield of methanolic extract of *Salvia* species varied depending on collection locality and harvesting time (7, 8).

Results of measurements of total phenolic content (TPC) and total flavonoid content (TFC) are presented in the Table 1. The total phenolic content was generally higher in the plant extracts originating from Pleš (98.84 mg GAE/g) comparing to results obtained for Luštica (96.36 mg GAE/g). Extracts of the summer season showed higher phenolic content (110.26 mg GAE/g). Type of extraction solvent affects the efficiency of extraction of phenols as indicated before (6). It was found that *S. officinalis* from different collection sites showed TPC ranging from 207.48 to 251.73 mg GAE/g (7), with the highest yield in the fruiting stage comparing to vegetative and flowering ones (8).

The values of total flavonoids content in the extracts were measured as 31.75 mg QE/g for Pleš locality and 29.30 mg QE/g for Luštica locality (Table 1). Higher flavonoid content was found in extracts from plants collected in summer (34.64 mg QE/g) than in winter (26.41 mg QE/g), which is consistent with previous reports (8). The highest content of total flavonoids showed the ethyl acetate extract (37.74 mg QE/g), while the lowest content of total flavonoids was obtained in ethanolic extracts (27.30 mg QE/g), which is consistent to previous reports on *Salvia* species successively obtained extracts (6). Several authors (3,6,7,18,26) concluded that differences in results of yield of total phenolics and flavonoids could be caused by genetic and environmental (climate, location, temperature, fertility, pests and diseases) factors, the plant part used for extraction, time of sampling, choice of the extraction solvent, extraction techniques, etc. Considering the plants in our study were cultivated under the same environmental conditions for five years, statistically significant differences in total phenolic and flavonoids contents of extracts should be attributed only to harvesting season and extraction solvent.

Evaluation of antioxidant activity

Antioxidant activity of extracts was evaluated using three parallel test assays (DPPH, ABTS, and FRAP assay) and results are presented in Table 1.

DPPH activity of *S. officinalis* extracts, originating from Pleš and Luštica, was measured as 28.28 µg/ml and 48.62 µg/ml, respectively. Summer season extracts were more successful against DPPH radical, compared to extracts of winter season (30.02 µg/ml and 46.89 µg/ml, respectively). Ethanol extracts showed the strongest activity (16.93 µg/ml) while chloroform extract was the weakest (57.35 µg/ml). It was reported that successively obtained ethanol extracts of fourteen *Salvia* species showed the strongest DPPH activity comparing to their dichloromethane and ethyl acetate extracts (6). The results obtained in this study are consistent with previous findings on *S. officinalis*, with high efficiency of ethanolic extract (16,27,28). The ethanol extracts showed very strong DPPH activity, close to synthetic antioxidants BHA and BHT (13.37 and 17.94 µg/ml, respectively).

The measured ABTS activity proved to be higher in the extracts originating from the locality Pleš than those from Luštica (1.11 mg AAE/g and 0.99 mg AAE/g, respectively). Stronger activity was obtained in the summer season (1.18 mg AAE/g), comparing to winter season (0.91 mg AAE/g). Among examined extracts, ethanol extracts performed the strongest ABTS activity (1.77 mg AAE/g). In the ABTS assay (15), IC_{50} values of antioxidant capacity showed a similar range of activities as well as in the DPPH assay (12-95 mg/ml and 8-94 mg/ml, respectively).

FRAP activities of *S. officinalis* plant extracts originating from the locality Pleš (613.81 µmol Fe(II)/g) was stronger than those obtained for Luštica (593.79 µmol Fe(II)/g). Extracts showed higher FRAP capacity in the summer season (662.22 µmol Fe(II)/g). Ethanol extracts of plants showed the strongest activity (1157.72 µmol Fe(II)/g). The results obtained in this study are consistent with results of other authors who have measured the FRAP activity of extracts of *S. officinalis* (29). It was reported on the strongest activity of ethanol extract in the analysis of the antioxidant activity of dichloromethane, ethyl acetate and ethanol extracts of the aerial parts and roots of 14 Turkish *Salvia* species, using a FRAP method (6). The extracts obtained using higher polarity solvents were more effective radical scavengers comparing to those obtained using less polar ones (16).

Previously reported data on antioxidant activity of *S. officinalis* methanol extract tested using DPPH, ABTS and FRAP assays showed that activity varied depending on collection site and harvesting time (7, 8). Comparing the antioxidant activity in the vegetative and fruiting stage of *S. officinalis* from two localities in northern Tunisia, DPPH and ABTS assays showed stronger activity in the vegetative, while FRAP assay showed stronger activity in the fruiting stage (8). In the present study, differences in the DPPH activities of extracts were caused by plant origin, harvesting season and extraction solvent, while those in the ABTS and FRAP assays could be attributed only to extraction solvent (Table 1). Similarly to phenolic and flavonoid contents, antioxidant activity of extracts varied depending on plant part, solvent and techniques chosen for extraction as was indicated for other *Salvia* species (6,12,18). Our results are in agreement with above-mentioned data.

The correlation between antioxidant activity and total phenolic and flavonoid contents

Based on the values of correlation coefficients (r) shown in Table 2, it can be concluded that the antioxidant capacities of the extracts (measured using DPPH, ABTS and FRAP assays) were strongly correlated to total phenol (r ranging from ±0.695 to ±0.975) and weakly to total flavonoid content (r from ± 0.065 to ± 0.255). The results indicated that the total phenols are more responsible for the antioxidant activity than total flavonoids as it was previously reported (12). The strong correlation between antioxidant activity and total phenolic content in plant extracts was obtained in previous studies (4, 5, 8, 16).

Antioxidant tests were strongly correlated between each other, especially FRAP and ABTS tests (r=0.962). According to previous reports, it was established a statistically significant strong correlation (r ≥0.669) between DPPH and ABTS antioxidant activity in all tested genera of Lamiaceae family, whether they are examined as a group or separately (16).

	DPPH	ABTS	FRAP
TPC	-0,695[c]	0,950[c]	0,975[c]
TFC	0,065[a]	-0,222[a]	-0,255[a]
DPPH vs. ABTS	-0,718[c]		
ABTS vs. FRAP	0,962[c]		
DPPH vs. FRAP	-0,683[c]		

Table 2. Linear correlation coefficients (r) between antioxidant activity and total phenolic and flavonoid contents of *S. officinalis* extracts.

According to literature data (24): [a]rs0.35 weak correlation; [b] 0.36<r<0.67 moderate correlation; c 0.68<r<1 strong correlation

CONCLUSIONS

Determined variations in phenolic contents and antioxidant capacity of *S. officinalis* extracts revealed the association of the plant origin, harvesting time and applied solvent. Comparing to plant origin, extraction solvent and harvesting season showed statistically higher impact on obtained differences in measured parameters. Strong correlation coefficients between total phenolics and the antioxidant tests were verified. Aerial parts extracts of *S. officinalis* are proved to be valuable as an effective and safe source of natural antioxidants, but observed variations in yield and antioxidant properties could serve as basis for the selection of plants with high level of polyphenolic compounds and good antioxidant properties for future commercial exploration in public health.

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ЦИКЛУС
ПРЕДАВАЊА

СВЕТЛОСТ У РАЗВОЈУ ДРУШТВА

СВЕТЛОСТ
У РАЗВОЈУ ДРУШТВА

ЦИКЛУС
ПРЕДАВАЊА

1841-2016

1. СВЕТЛОСТ У РАЗВОЈУ ДРУШТВА
2. ГЕНИ И ГЕНОМ

СВЕТЛОСТ У РАЗВОЈУ ДРУШТВА

Д. ПАНТЕЛИЋ[1], А. КРМПОТ[1], Д. В. СТОЈАНОВИЋ[2,4], М. Д. РАБАСОВИЋ[1],
С. ЋУРЧИЋ[3], С. САВИЋ-ШЕВИЋ[1], В. ЛАЗОВИЋ[1], Д. ПАВЛОВИЋ[1]

СВЕТЛОСТ НА КРИЛУ ЛЕПТИРА

А п с т р а к т . – Боје у живом свету су последица како пигмената, тако и физичких ефеката на нанометарским структурама. Овде ће бити речи о низу оптичких механизама који одређују боје припадника реда инсеката Lepidoptera (лептира и мољаца): интерференцији, дифракцији, расејању и поларизацији. Као пример ћемо навести три врсте које живе у Србији: *Apatura ilia* (Denis & Schiffermüller, 1775), *Diachrysia chrysitis* (Linnaeus, 1758) и *Jordanita globulariae* (Hübner, 1793).

УВОД

Многи софистицирани механизми, за које често сматрамо да су производ савремене цивилизације, постоје већ милионима година у живом свету: ултразвучни локатор код слепих мишева и његово активно ометање код неких ноћних Lepidoptera (мољаца), жироскоп код неких врста инсеката лепезара (Strepsiptera), хемијско оружје код буба бомбардера (Carabidae: Brachininae), детекција инфрацрвеног зрачења код пирофилних инсеката, а листу би било могуће наставити у недоглед. Поменућемо још само једну од најновијих студија у којој је утврђено постојање функционалног система од два спрегнута зупчаника код ларве инсекта *Issus coleoptratus* (Fabricius, 1781) [1].

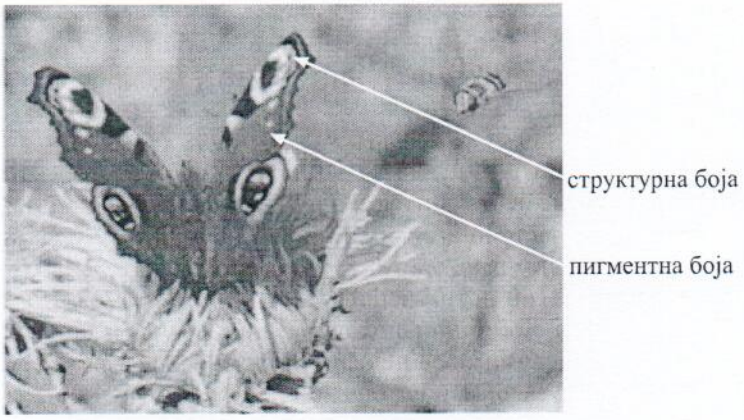
У овом тексту ћемо се детаљније задржати на оптичким феноменима у свету лептира и мољаца, који овим, изванредно прилагодљивим бићима омогућавају интра- и интерспецијску комуникацију. На телима инсеката могуће је наћи дифракционе решетке, поларизационе елементе, антирефлексионе

[1] Институт за физику, Универзитет у Београду
[2] Национални парк Фрушка гора
[3] Институт за зоологију, Универзитет у Београду – Биолошки факултет
[4] Универзитет у Новом Саду, Институт за низијско шумарство и животну средину

слојеве, фотонске кристале, селективне оптичке филтере – чуда модерне технологије, за која смо дуго сматрали да су искључиви производ човекове интелигенције [2]. За оне који се баве овом облашћу, „домишљатост" еволуције представља инспирацију за развој нових оптичких уређаја и техника [3].

СТРУКТУРНЕ БОЈЕ У СВЕТУ ЛЕПТИРА И МОЉАЦА (LEPIDOPTERA)

Богатство боја у живом свету, а посебно код инсеката, је последица њихове потребе да се сакрију и стопе са околином, пошаљу сигнал упозорења или сигнал привлачења јединкама супротног пола. Неке од боја су последица хемијске структуре пигмената који се налазе на површини њиховог тела (интегумента). Ипак, опсег боја које су у стању да хемијски синтетишу је ограничен. Зато је током еволуције искоришћен читав низ оптичких ефеката, попут интерференције и дифракције да се генеришу боје од љубичасте преко плаве, до зелене, па и даље, према инфрацрвеном делу спектра. Боје које се на овај начин добијају називају се структурним, јер су условљене микронским и субмикронским структурама на телу живих јединки. Код неких организама структурна обојеност може бити независна од угла осветљавања и посматрања. Код других, боја се, у зависности од упадног угла светлости, мења. Ову појаву називамо иридесценцијом.

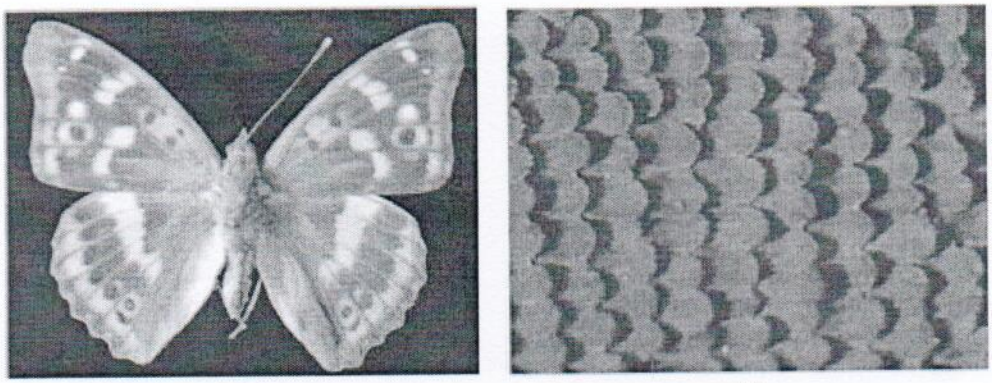


Слика 1. Црвена, жута и црна боја лептира дневног пауновца (*Aglais io*) (Linnaeus, 1758) су последица пигмената, док плава и бела боја настају због структурних ефеката

Опште је позната раскошна обојеност крила дневних лептира (нпр. пауновац – *Aglais io* (Linnaeus, 1758) – сл. 1). Занимљиво је да су често неки делови крила обојени структурно, док је боја других зона пигментна. Код дневног лептира *Apatura ilia* (Denis & Schiffermüller, 1775) (сл. 2а) лако се уочава иридесцентна природа љубичасте боје крила – дорзална страна левог крила има жуто-мрку, пигментну боју, док је дорзална страна десног крила љубичаста, због погодног угла осветљавања. У анатомском смислу, боју генеришу танке

микронске плочице (љуспице), које покривају читаву површину крила попут црепова на крову куће (сл. 2б). Само крило као основу има двоструку мембрану, у коју су љуспице усађене са обе стране у издигнуте дршке. На сл. 3 се види део крила са једном љуспицом, снимљен помоћу нелинеарне двофотонске флуоресцентне микроскопије. Ова техника даје могућност да се формира тродимензионална слика, те да се крило посматра под различитим угловима. Дата су два карактеристична погледа на којима се види сама двострука крилна мембрана, једна љуспица и неколико дршки из којих су љуспице испале.

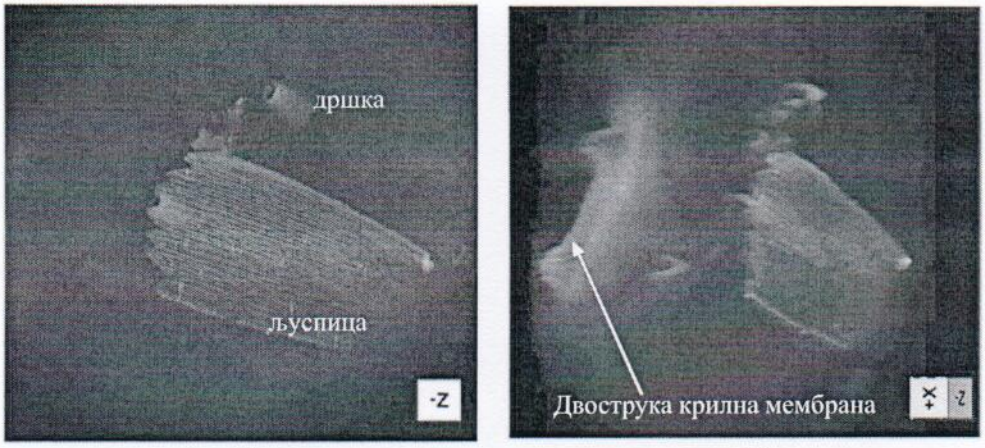
Једино детаљно посматрање под електронским микроскопом омогућава разумевање порекла структурних боја. На малом повећању (сл. 4) види се да свака љуспица има структуру дифракционе решетке. На већем увеличању (сл. 5а), види се да дифракциона решетка поседује ламеларну структуру, где се појединачни гребени (на међусобном растојању од око 1 μm) састоје од низа слојева. Попречни пресек (добијен помоћу трансмисионог електронског микроскопа – сл. 5б) показује да се слојеви налазе на међусобном растојању од око 150 nm. На истој слици је шематски приказана делимична рефлексија светлости на сваком од слојева. Услов за конструктивну интерференцију свих рефлектованих снопова одређује да ће светлост таласне дужине од око 380 nm бити одбијена, што заиста и одговара љубичастој боји крила [4].



а) б)

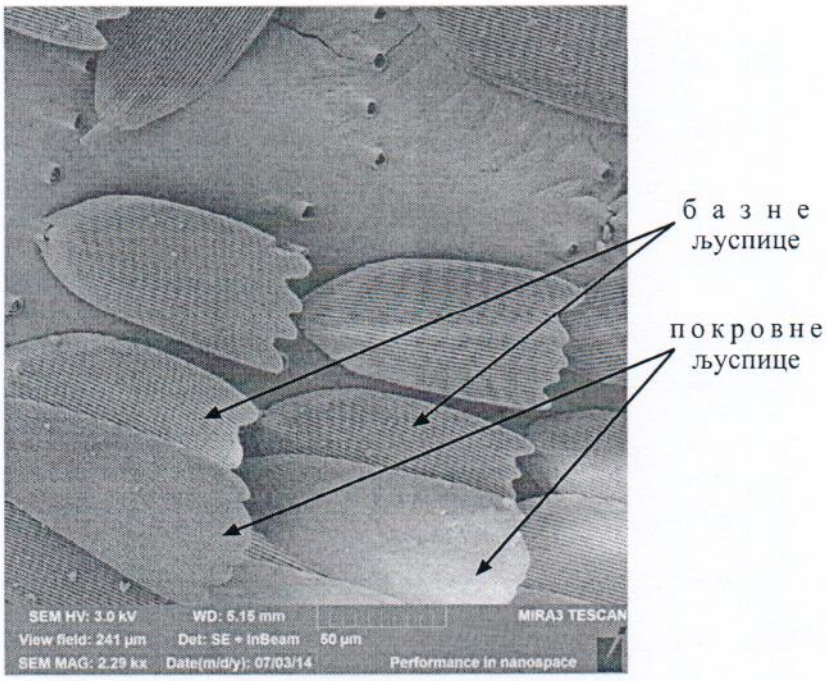
Слика 2. а) Љубичаста структурна боја крила код лептира преливача (*A. ilia*). б) Увеличани део крила који показује присуство иридесцентних љубичастих љуспица, које покривају крило лептира

И неки ноћни лептири (у народу звани мољци) имају структурну обојеност крила, иако је геометрија њихових љуспица значајно другачија. Ламеларна решетка постоји, али има велики период (неколико микрона) и мали број ламела, те су дифракциони ефекти слабо изражени. Зато је доминантна попречна решетка, која има периоду мању од таласне дужине светлости у видљивом делу спектра (између 380 и 760 nm). Овакве решетке не допуштају формирање виших дифракционих редова и функционишу у режиму нултог реда [5].

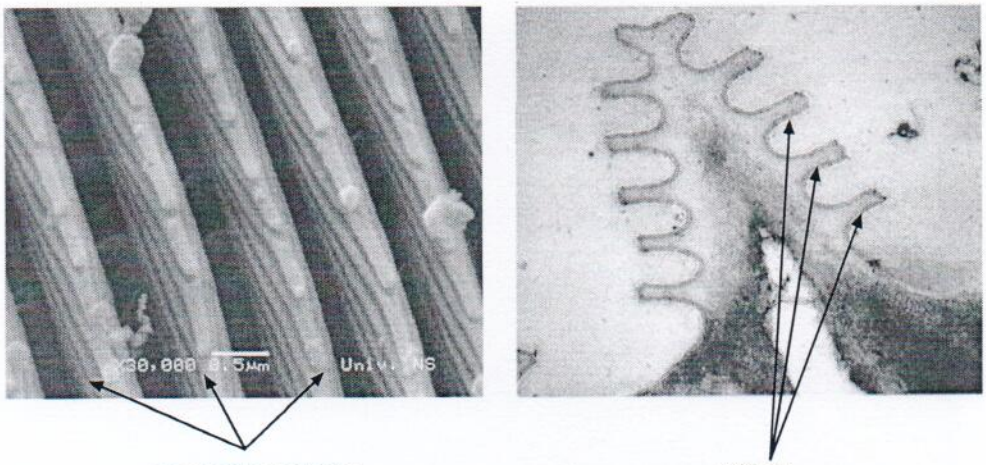


а) б)

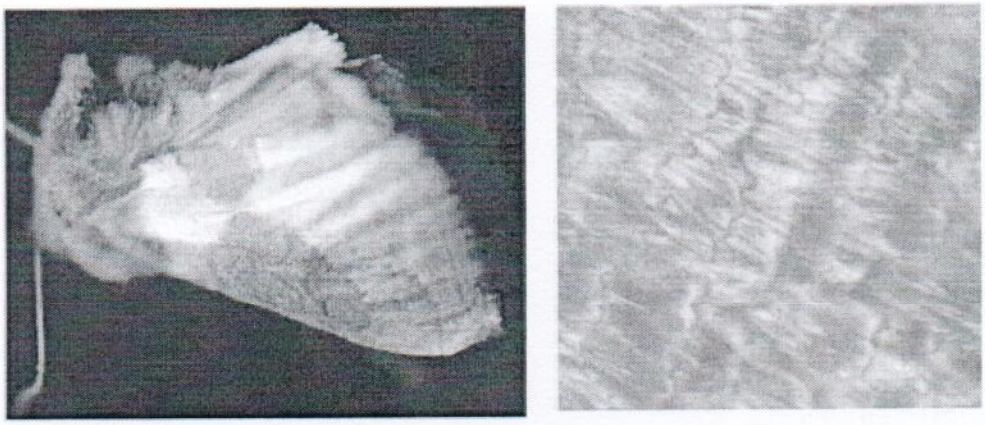
Слика 3. Положај љуспице на двострукој крилној мембрани лептира *A. ilia*, снимљен под два различита угла (а, б). Коришћена је нелинеарна дво-фотонска флуоресцентна микроскопија развијена у Центру за фотонику, Института за физику у Београду



Слика 4. Два типа љуспица лептира A. Ilia (покровне и базне) имају структуру дифракционе решетке



Слика 5. а) Слојевита структура љуспице дневног лептира *A. ilia*, посматрана под скенирајућим електронским микроскопом. б) Попречни пресек љуспице дневног лептира *A. ilia* посматран под трансмисионим електронским микроскопом

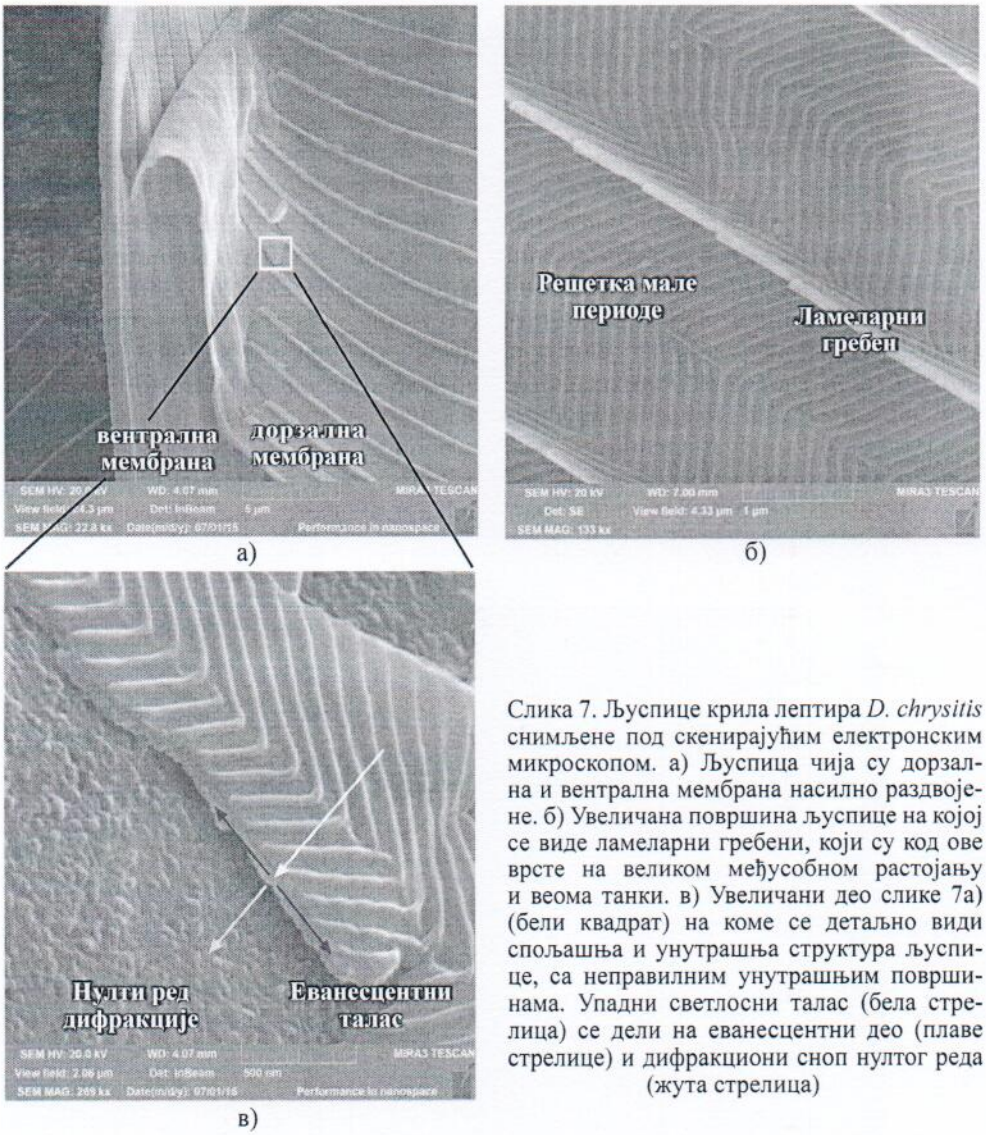


Слика 6. а) Мољац *Diachrysia chrysitis* (Linnaeus, 1758), чија крила имају један део површине златне боје. б) Увеличана слика златног дела крила *D. chrysitis*, снимљена оптичким рефлексионим микроскопом, на којој се виде појединачне љуспице

У том погледу, занимљив је пример ноћног лептира *Diachrysia chrysitis* (Linnaeus, 1758), који има лепу златну боју дорзалне површине предњих крила (сл. 6а и 6б). Слика 7а, снимљена под скенирајућим електронским микроскопом, приказује љуспицу крила *D. Chrysitis* која је преломљена, тако да се јасно види да постоје две мембране – вентрална, која нема значајних и добро организованих структура и дорзална, која је структурисана двема ортогоналним решеткама, једном ламеларном велике периоде (око 2.5–3 μm) и другом

веома густом (периоде 100–150 nm) профила рибље кости (сл. 7б). Запажа се да је унутрашњост и дорзалне и вентралне мембране веома неправилна (сл. 7в). Дебљина сваке од њих је око 150 nm и налазе се на међусобном растојању 150 nm, што чини читаву љуспицу изузетно танком (мање од 500 nm).

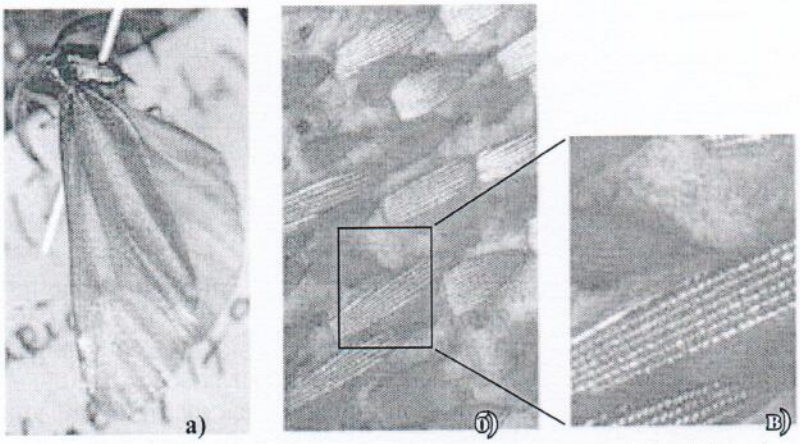
У оптичком погледу, трансверзална дифракциона решетка је толико густа да није могуће формирање других дифракционих редова осим нултог. Могуће је, међутим, да се део енергије користи за формирање еванесцентног или вођеног таласа [6], који се простире дуж мембрана љуспице (сл. 7в). Овај процес код Lepidoptera није до краја разјашњен, али спектар рефлектованог зрачења показује да се таласне дужине изнад 500 nm ефикасно рефлектују, што даје спектралну расподелу и визуелни изглед сличан злату.



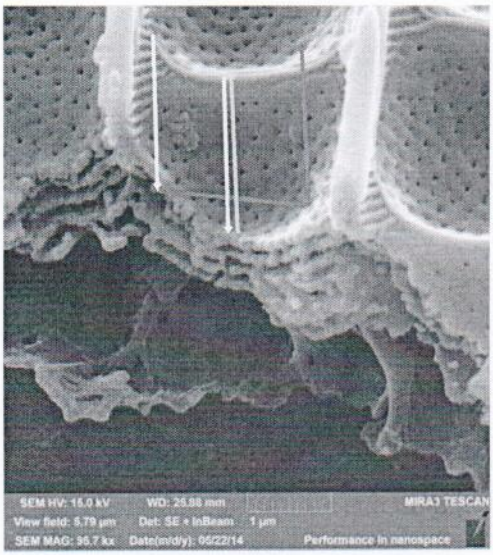
Слика 7. Љуспице крила лептира *D. chrysitis* снимљене под скенирајућим електронским микроскопом. а) Љуспица чија су дорзална и вентрална мембрана насилно раздвојене. б) Увеличана површина љуспице на којој се виде ламеларни гребени, који су код ове врсте на великом међусобном растојању и веома танки. в) Увеличани део слике 7а) (бели квадрат) на коме се детаљно види спољашња и унутрашња структура љуспице, са неправилним унутрашњим површинама. Упадни светлосни талас (бела стрелица) се дели на еванесцентни део (плаве стрелице) и дифракциони сноп нултог реда (жута стрелица)

У бескрајном низу могућих структура љуспица погледајмо још једну, којом се одликује мољац *Jordanita globulariae* (Hübner, 1793) (сл. 8а). Предња крила су покривена низом зелених, иридесцентних, као и браон, пигментних, љуспица које су ретко распоређене и не прекривају се. Известан број љуспица је иридесцентан, као што се види на сл. 8б, која је снимљена под оптичким микроскопом. Уколико се слика иридесцентне љуспице додатно повећа види се да на њој постоји низ дискретних тачака зеленог и жутог обојења (сл. 8в). Да бисмо објаснили њихово постојање неопходно је да завиримо у дубљи ниво, повећавајући преломљену љуспицу скоро 100.000 пута (сл. 9). На попречном пресеку је најуочљивија вишеслојна структура која се налази испод горње мембране и прати њен конкавни облик. Она функционише као Брагова решетка, што значи да представља селективни спектрални филтер. Из спектра беле светлости ће бити рефлектована само она боја светлости (таласна дужина) која је одређена растојањем између слојева и упадним углом светлости. Са сл. 9 видимо да се, за светлост која пада на центар удубљења, упадни угао на Брагову решетку разликује од светлости која пада на ивичне делове. Последица је да се боја рефлектоване светлости у ове две зоне разликује, као што потврђује сл. 8в.

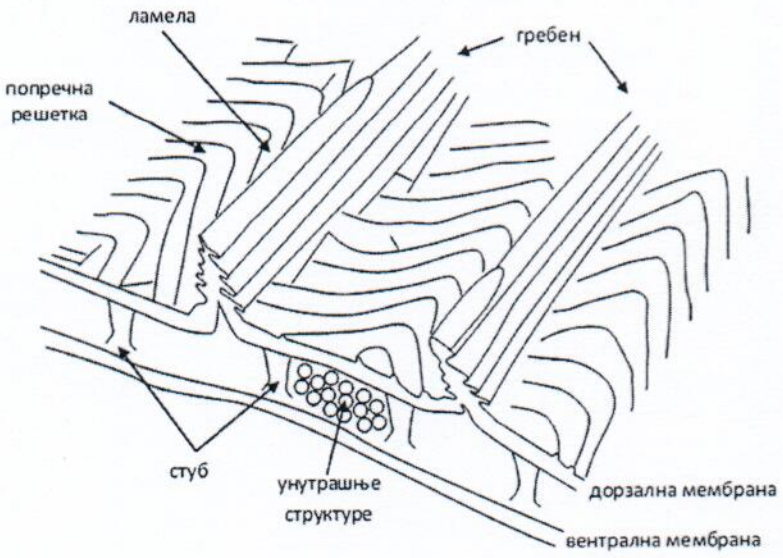
На основу свега што је речено, запажамо да је свака љуспица код Lepidoptera пљосната структура дебљине неколико микрона, ширине око 50–100 μm, а дужине од 150–300 μm. Она представља мртви остатак једне једине ћелије, састављен од хитина, пигмената и протеина. Уочавамо и општи принцип конструкције љуспице (слика 10). Она се састоји од две мембране – вентралне, која је скоро сасвим равна и дорзалне која је структурисана системом уздужних ламеларних гребена и попречних решетки. Две мембране повезују, ретко постављени, стубови између којих постоји простор, који може бити празан или испуњен додатним структурама попут гранула, запреминских решетака и фотонских кристала. Структуре могу бити веома сложене на нанометарском нивоу, а оптички ефекти који се на њима уочавају јесу комбинација интерференције, дифракције, расејања, флуоресценције и апсорпције.



Слика 8. а) Мољац *J. globulariae*. б) Увеличана слика крила *J. globulariae* под оптичким микроскопом на којој се истовремено виде иридесцентне и неиридесцентне љуспице. в) Увеличани део слике 8б) где се види да се иридесценција састоји од дискретних тачака зелене и жуте боје



Слика 9. Попречни пресек љуспице мољца *J. globulariae*. Испод дорзалне мембране уочава се низ конкавних слојева који функционишу као Брагова решетка. Беле стрелице означавају упадни сноп светлости, који се у централној зони директно рефлектује (жута стрелица), а у бочној трпи две рефлексије (зелене стрелице)



Слика 10. Општа шема структуре љуспице на крилима Lepidoptera

ЗАКЉУЧАК

Овај текст представља кратак приказ оптичких ефеката на крилима лептира и мољаца, који овим инсектима дају карактеристично обојење. Ради се о постојању структура микронских и субмикронских димензија на којима се одигравају процеси карактеристични за таласну природу светлости. Њихово проучавање даје увид у фасцинантне резултате еволутивног развоја живих бића, али и идеје како да се откривени ефекти практично искористе за нове типове козметичких препарата, иридесцентне боје, заштиту докумената али и детекцију зрачења и нове типове сензора.

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D. Pantelić, A. Krmpot, D. V. Stojanović, M. D. Rabasović,
S. Ćurčić, S. Savić-Šević, V. Lazović, D. Pavlović

LIGHT ON THE BUTTERFLY WING

Summary

Colors in the living world are either a consequence of pigments, or physical effects on nanometer-sized structures. Here we explain the mechanisms responsible for colors of Lepidoptera (butterflies and moths): interference, diffraction, scattering, polarization. Three species, living in Serbia, will be analyzed as an example of structural coloration: *Apatura ilia* (Denis & Schiffermüller, 1775), *Diachrysia chrysitis* (Linnaeus, 1758) and *Jordanita globulariae* (Hübner, 1793).

The Thermographic Analysis of Photonic Characteristics of *Rosalia alpina* Surfaces

Goran Dikić, Danica Pavlović, Ljubiša Tomić, Dejan Pantelić, Darko Vasiljević and Dejan Stojanović

Abstract—**Materials developed by nature during the evolution, have a significant impact in search for artificial materials having useful absorptive properties, especially for solar energy collection, thermal energy dissipation and camouflage. Four prominent black spots on the elytra of *Rosalia alpina* have attracted our attention. We have found that the light absorption is not the sole consequence of dark pigments, but that it is strongly influenced by the underlying structure. We decided to perform thermo-physical investigations of the optical mechanism responsible for the absorption of light in *Rosalia alpina*. This paper presents the results obtained using testing procedure applied in pulsed thermography.**

Index Terms—**Pulse thermography, photonic structure.**

I. Introduction

Excellent survival mechanisms have been developed in nature during evolution of species. Good example is color matching with intention to attract the attention of other members of the same specie or to be hidden using similarities to the environment. The heat energy exchange with environment is also well known example which can be based on use of colors. Thermoregulation can be improved using natural photonic processes. Considering specific structure of longhorn beetle antennas and wings we have been analyzing thermal images of an insect *Rosalia alpina* (Linnaeus, 1758), to these processes.

Rosalia alpina is a large longhorn beetle (family *Cerambycidae*) with flat, blue-gray elytra (hardened front wings) with large, dominating black spots. It is 15-38 mm long, with the long antennas and striking black tufts of hair on the central segments of the antenna [1]. Such coloration serves as a good camouflage with their preferred habitat, the European Beech [2].

Goran Dikić is with the Military Academy, University of Defence, Pavla Jurišića Šturma 33, 11000 Belgrade, Serbia (e-mail: goran.dikic@mod.gov.rs).

Danica Pavlović is with the Photonics Center Institute of Physics, University of Belgrade, Pregrevica 118, 11080 Belgrade, Serbia (e-mail: danica.pavlovic@ipb.ac.rs).

Ljubiša Tomić is with the Military Technical Institute, Ratka Resanovica 1, 11000 Belgrade, Serbia (e-mail: ljubisa.tomic@gmail.com).

Dejan Pantelić is with the Photonics Center Institute of Physics, University of Belgrade, Pregrevica 118, 11080 Belgrade, Serbia (e-mail: pantelic@ipb.ac.rs).

Darko Vasiljević is with the Photonics Center Institute of Physics, University of Belgrade, Pregrevica 118, 11080 Belgrade, Serbia (e-mail: darko@ipb.ac.rs).

Dejan Stojanović is with the Fruška Gora National Park, Zmajev Trg 1, 21208 Sremska Kamenica, Serbia (e-mail: dejanstojanovic021@yahoo.co.uk)

The largest part of the beetles' body is covered with a dense tomentum, consisting of very fine, light blue, blue gray and dark blue hairs. The black spots on the elytra and pronotum are also covered with dense black hairs which give the spots their velvety appearance [1] and [3]. Figure 1 shows the photography of *Rosalia alpina* (test sample).

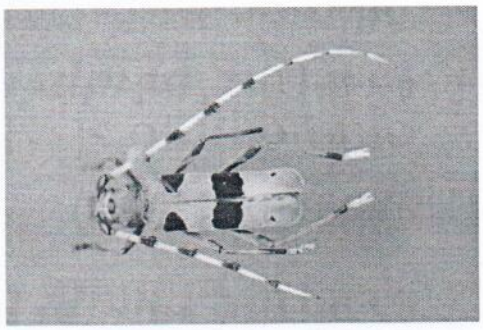

Fig. 1. The potography of *Rosalia alpine*.

Natural photonic structures have the main purpose of producing colors that would have been impossible to generate by the pigments alone. In the insect world this is particularly true for blue colors (generated by pepper-pot structures found in many Polyommatinae [4]) and green colors (produced by chiral photonic structures in *Calophrys rubi* (Linnaeus, 1758), [5]).

Sometimes photonic structure enhances the pigment color, particularly for generation of strongly absorbing black spots, as observed in some snakes [6] or butterflies [7]. Micro and nano-structures localize light, and increase the average path length within the structure causing the increased absorption. The biological purpose of such structures might be the camouflage or thermoregulation, as in Lycaenid butterflies [8].

More advanced structures have dual purpose as in Saharan silver ant (*Cataglyphis bombycina* (Roger, 1859) [9]): to reflect maximum amount of the visible light, and to simultaneously dissipate infrared radiation directly into the atmospheric window at mid-infrared. This enables insect to efficiently regulate its body temperature in very hostile desert environment.

Materials developed by nature during the evolution, have a significant impact on search for artificial materials having useful absorptive properties, especially for solar energy collection, thermal energy dissipation and camouflage.

Four prominent black spots on the elytra of *Rosalia alpina* have attracted our attention. We have found that the light

absorption is not the sole consequence of dark pigments (most probably melanin), but that it is strongly influenced by the underlying structure. Figure 2 shows hairs in this area. The typical tufts of hair at the ends of the individual antenna members are shown in Fig. 3.

Fig. 2. Hairs in the area of grey spots.

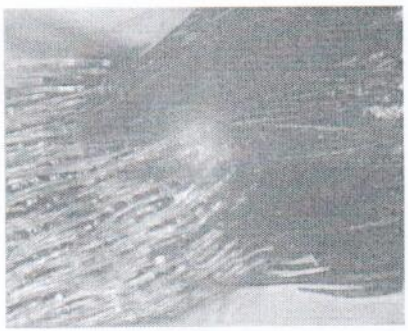

Fig. 3. The typical tufts of hair at the ends of the individual antenna members.

In related species, such as *Morimus funereus* (Mulsant, 1863), electron microscopy investigations have shown that the fine structure of the dark spots is characterized by tiny and densely packed hairs. Hairs are curved and as a whole represent an efficient light trap. Length of the hair is approximately 50 μm, its width is of the order of 10 μm, while its thickness is several micrometers. The distance between hairs is 15 μm. Optical microscopy of the single scale has revealed that it strongly absorbs light with the absorption coefficient estimated at 8000 cm^{-1}.

We decided to perform thermo-physical investigations of the optical mechanism responsible for the absorption of light in *Rosalia alpina*. This paper presents the results which are based on use of pulse thermography (PT) [10]. This method involves the analysis of images that have been registered by an infrared (IR) camera as a consequence of infrared irradiation of the test sample (in this case beetle).

II. Experimental Setup

The test equipment comprised a photographic flash a thermal camera and a personal computer (PC), which recorded digital data in real time. The surface of test sample (*Rosalia alpina*) was heated using the photographic flash (BOWENS BW-3955 Gemini R & Pro), positioned at a distance of 50 cm from the sample, as this distance enabled homogeneous heating.

The optimal position of the flash was when the light was normal relative to test sample. For better orientation of the luminous flux to the test sample, a reflector (having 65° divergence angle) with an aperture of 20 cm is used. The light source positioned as shown in Fig. 4 produces a strong heat impulse which homogeneously heats the beetle surface.

The maximum power of the light source BW-3955 BOWENS is 1500 Ws. The duration of the light pulse at full power is 0.7142 ms. The pulse duration must be much shorter relative to the frame duration. Considering the possible frame duration t_f (8.33 ms, 16.66 ms and 33.33 ms) this rquirement is fulfiled in case of any of three modes of operation.

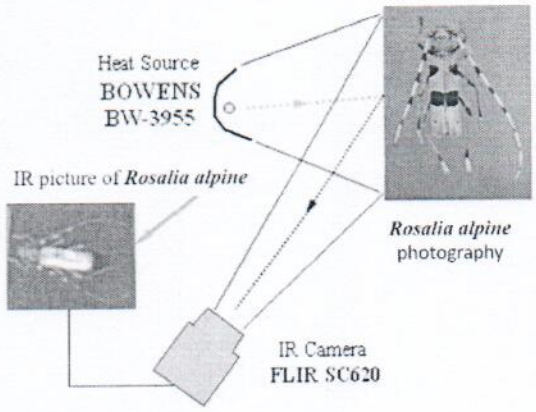

Fig. 4. Position of the IR camera relative to the test sample during the experiment.

Cooling of the test sample, previously heated by means of a short light pulse, was monitored using a commercial thermal camera "FLIR SC620", operating in the spectral range from 7.5 to 13 μm. This type of camera has a focal plane matrix of 640x480 consisting of semiconductor detector (Vanadium Oxide - VOx). Each detector measures the intensity of infrared radiation. These values can be represented on a monitor as a thermal image coded in shades of gray or in color and can be converted to temperature values using the appropriate table.

In order to obtain optimal experimental results zoom has been set manually. After that, the all other parameters (ambient temperature, emissivity, the distance of the object from the thermal imaging camera, humidity, etc) have been set. The temperature and emissivity of the test sample have been assessed by means of the thermal camera. Measurements, presented in this paper, have been carried out at short distances, (about 50 cm) so that the distance as a parameter does not have a major impact.

Thermal imaging camera allows conversion of spatially inhomogeneous distribution of radiation flux of scene, due to differences in the distribution of temperature and/or emissivity, in the visible image. The right choice of measuring geometry and correct interpretation of the results is based on

the knowledge of the spatial and temperature resolution (sensitivity) of thermal imaging cameras.

III. Experimental results

Analysis of IR reflection of irradiated object has been achieved by analyzing video sequences consisting of 2,232 frames. Filming has been carried out at the frame rate of 120 Hz. The dimensions of the frame are 120 pixels × 724 pixels. In order to optimize the number of calculations the pixels have been processed only in the area whose dimensions are 120 pixels × 140 pixels, that is marked as white rectangle in Fig. 5.

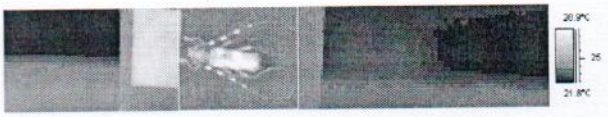

Fig. 5. The original form of 75th frame, taken at the frequency of 120 Hz.

In this way it is possible to extract the compact three-dimensional data matrix in which the coordinate information and temporal distribution of the intensity of each pixel in the immediate environment of the recorded object are stored. Fig. 6 provides an insight into the distribution of intensity in an area that is separated during processing of the seventy fifth frame. In accordance with the needs of processing, the intensity of each pixel may be converted to the corresponding temperature value. Figure 7 shows the time distribution of pixels' intensity, for pixel at coordinates (40, 40), as shown in Fig. 6, and has an index that determines gray level in the amount of 0.8784.

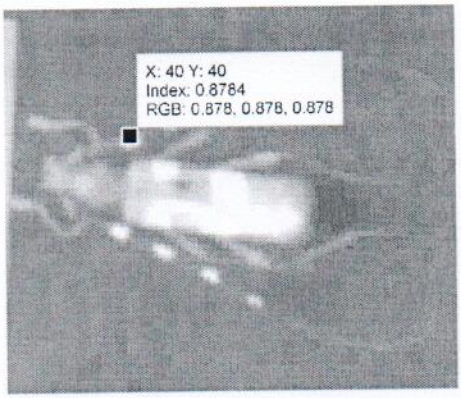

Fig. 6. IR reflection of the irradiated object and its immediate environment with the characteristics of highlighted pixel.

Figure 7 shows the situation which corresponds to the frames 73, 74, 75 and 76. Frame 73 is still unlit. Heating has been started just before the frame 74. Maximum (the pixel at coordinate 40, 40) has been reached in 75th frame. Note that the left wing is lighter in frame 76. This must be consequence of weaker contact of the wing with the body of the insect. Thus, the heating process has been faster at this side.

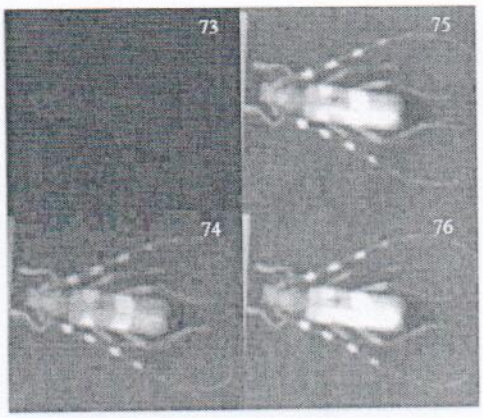

Fig. 7. The situation which corresponds to the frames 73, 74, 75 and 76.

Figure 8 shows the time distribution of gray level of this pixel within the first 500 frames. It is obvious that the irradiation of the subject achieved immediately before seventy fifth frame. The gray levels that correspond to the pixel at coordinates (40, 40) in frames 72, 73, 74, 75 and 76 are 0.1882, 0.1882, 0.6275 0.8784 and 0.8471. These data show that the test sample has been irradiated at the moment between frames 73 and 74.

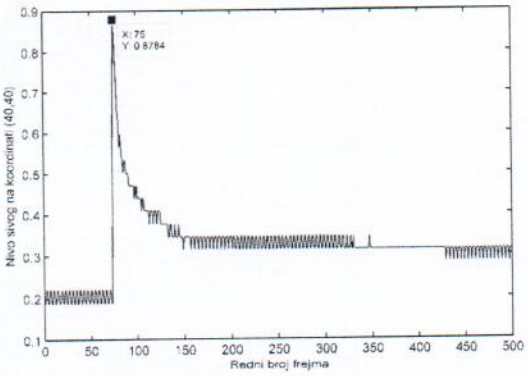

Figure 8. The time distribution of gray level for pixel at coordinates (40, 40).

Figure 9 shows the IR reflection of the irradiated object obtained within every tenth frame starting from 75th to 185th. The order of images is arranged by columns, from top to bottom, and from left to right. The cooling process, after irradiation of the recorded object, can be clearly observed.

The bright details, at the first shot (in the upper left corner), belong to the parts of the antenna that are covered by dense hairs.

We believe that the rapid heating of these areas relative to the other parts occurs, basically, for two reasons. The first reason is the better energy absorption that occurs because of the dark color. Another reason is very structure of these areas. Specific dimensions, shape and arrangement of hairs reduce the emissivity of these areas.

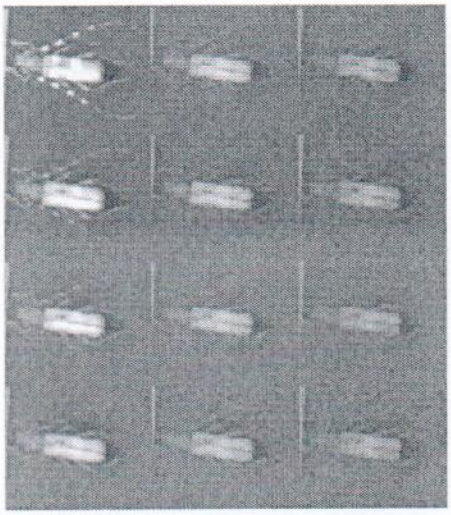

Fig. 9. IR reflection registered in the immediate vicinity of the irradiated object; (presented from top to bottom and from left to right).

In addition, hairs in the area of dark spots at the wings, decrease the emission of the energy that has been accumulated during heating process. That is the reason for increasing of the temperature that is visible as brighter details in region of dark spots at the wings.

The existence of individual surfaces (wings) is very visibly because of dark line existence in central part of body. During cooling both halves of the body, uniformly change the gray level that originate from the heat of dominant body mass.

Data processing in the frequency domain is based on the application of Fast Fourier Transformation (FFT). Processing of the thermal images has been achieved using two ways of selection of appropriate frames. In the first case, 128 succesive frames, starting with 75th, has been separated. Subsequently, the FFT processing has been applied on 128 consecutive values of the levels of gray, for each pixel, respectively.

The data are stored in the form of a matrix of values of each spectral component modules. After that, with intention to ensure better visibility of the results each matrix has been normalized. In this way, maximum values are the brightest (presented by the gray level corresponding to the value of 1), and the lowest values are black (presented by the gray level corresponding to the value of 0).

Figure 10 shows the results, in the ordering, from the top to bottom, and from the left to right. Thus, the details in the upper left corner of the picture present the modules that correspond to the first spectral component, i.e. the first harmonic of each pixel. In the same way, in the lower right corner the details correspond to the twelfth harmonic of the each pixel.

It can be seen that each harmonic brings additional information on the structure of the object to be analyzed based on its IR reflection. Comparing the first result (above left) and the last (below right), it is clear, there is a difference in the details corresponding to the insects' head. In addition, the visibility of bright parts of antennas in every segment of image is consequence of rapid change of the temperature of these parts.

This is consistent with the theory of signal processing. The intensive, short-term signal consists of more spectral components in frequency domain, comparing to the similar signal with higher duration. By the analogy, the insect fuselage is the most visible in the first three segments of the image. In accordance with the Fig. 9 the insects' body is visible much longer compared to the light areas of the antenna. Thus, the appropriate spectrum consists of fewer components.

In the second case, the processing has been achieved by having formed sequences composed of 128 frames, first selecting every successive frame, then selecting every second frame, every third frame and so on. The final sequence has been formed by selecting every twelfth frame. In all cases the 75th frame was adopted as the first in the sequence. Apparently, in this way, the variable sampling frequency of the frames has been provided. Processing of these sequences in the field of non-destructive interrogation of materials subsurface defects can detect at different levels. Considering this fact, it can be expected that identical treatment, in particular case, should also provide better insight into the structure of analyzed insect. The results shown in Fig. 11 just confirm this expectation.

The small details which belong to the antennas are clearly visible in upper-left segment of this figure. Reducing the sampling frequency, i.e. increasing the interval selection of frames, cause the worsening of visibility of antenna details, but the details of the body become more clear. The bright parts that correspond to the dark spots at the central part of wings become visible. These parts are barely visible at the first segment (top left).

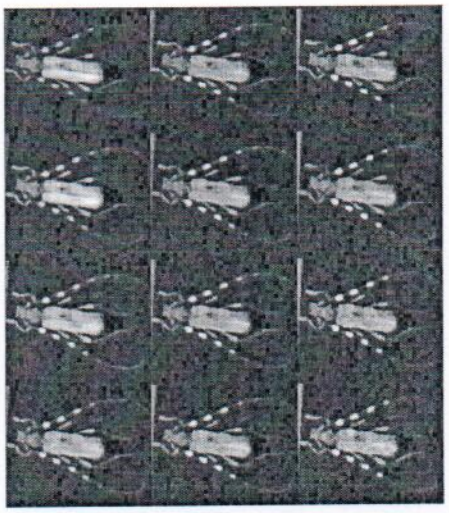

Fig. 10. Visual representation of the results obtained by FFT processing 128 successive frames starting from 75th frame.

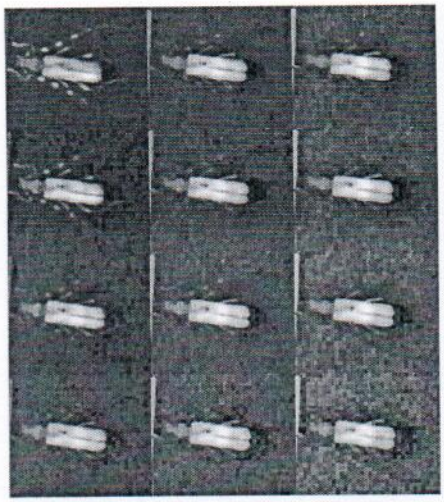

Fig. 11. Visual representation of the results obtained by FFT processing sequences with different sampling frequencies.

IV. Conclusion

Laboratory tests based on the application of thermography procedures were aimed to check our assumptions about the existence of photonic structures on surfaces within the areas of tentacles and wings of beetle known as *Rosalia alpina*. The existence of the hairs with specific dimensions, positioned in some areas of the antenna, and dark spots on the wings reduces the emissivity and increases the temperature in these areas. The increase of temperature is visible in form of brighter pixels in thermal images that were recorded during the cooling of test samples, after heating by the appropriate source of IR radiation. The existence of local differences in temperature of the test sample parts, such as antennae and wings, confirms our presumptions about the existence of natural photonic structures in these areas.

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THERMAL AND CAMOUFLAGE PROPERTIES OF *Rosalia alpina* LONGHORN BEETLE WITH STRUCTURAL COLORATION

IVANA KOSTIĆ
Technical Test Center, Belgrade, kostic.ici@gmail.com

DANICA PAVLOVIĆ
Photonics Center Institute of Physics, University of Belgrade, danica.pavlovic@ipb.ac.rs

VLADIMIR LAZOVIĆ
Photonics Center Institute of Physics, University of Belgrade, vladimir.lazovic@ipb.ac.rs

DARKO VASILJEVIĆ
Photonics Center Institute of Physics, University of Belgrade, darko@ ipb.ac.rs

DEJAN STOJANOVIĆ
Fruška Gora National Park, Sremska Kamenica, dejanstojanovic021@yahoo.co.uk

DRAGAN KNEŽEVIĆ
Military Technical Institute, Belgrade, dragankn@gmail.com

LJUBIŠA TOMIĆ
Military Technical Institute, Belgrade, ljubisa.tomic@gmail.com

GORAN DIKIĆ
Military Academy, University of Defence in Belgrade, goran.dikic@mod.gov.rs

DEJAN PANTELIĆ
Photonics Center Institute of Physics, University of Belgrade, pantelic@ipb.ac.rs

Abstract: *Rosalia alpina is a longhorn beetle possessing distinctive gray body with several black spots. They serve as a camouflage within its environment (beech forest) and we suppose that insect also uses them to control the body temperature. We have studied the optical properties of this particular insect, ranging from the visible to far infrared part of the spectrum. Optical analysis has shown strong absorption in the visible, while thermal camera (operating in the spectral range from 7.5 to 13 μm) has shown quite uniform emissivity of the whole body. Numerical ray tracing was used to explain the exact optical mechanism of strong absorption of black spots. Possible military applications of the natural camouflage and absorption mechanism are outlined.*

Keywords: *Infrared imaging, natural photonics, camouflage.*

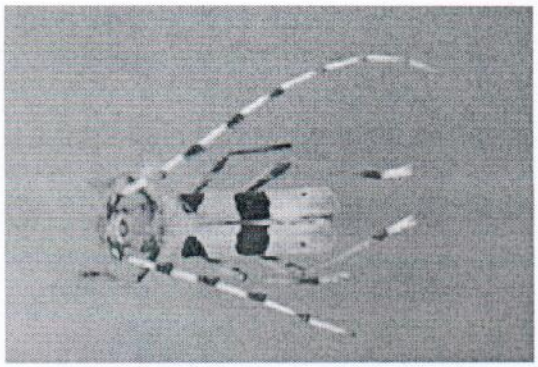
1. INTRODUCTION

Coloration has multiple purposes in the living world: to hide, attract or warn. It can be also used for heat energy exchange with the environment [1]. Thermoregulation can be improved using natural photonic processes. In that respect, we have analyzed antennas and elytra (modified forewings of Coleoptera, which are used as a hard shield for their body) of *Rosalia alpina* (Linnaeus, 1758) (see photograph in Picture 1).

This is a large longhorn beetle (family *Cerambycidae*) with flat, blue-gray elytra with large, dominating black spots. It is 15-38 mm long, with the long antennas and striking black tufts of hair on the central segments of the antenna [2]. Such coloration serves as a good camouflage with their preferred habitat, the European Beech [3].

The largest part of the beetles' body is covered with a dense tomentum, consisting of very fine, light blue, blue gray and dark blue hairs. The black spots on the elytra and pronotum are also covered with dense black hairs which give the spots their velvety appearance [2,4]. Picture 1 shows the photography of *Rosalia alpina* (test sample).

Natural photonic structures have the main purpose of producing colors that would have been impossible to generate by the pigments alone. In the insect world this is particularly true for blue colors (generated by pepper-pot structures found in many Polyommatinae [5]) and green colors (produced by chiral photonic structures in *Calophrys rubi* (Linnaeus, 1758), [6]).

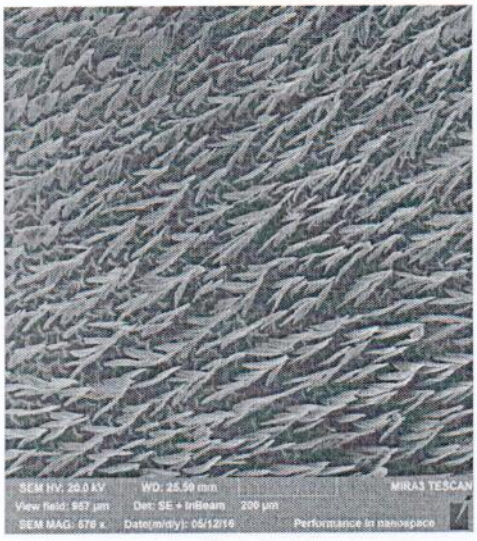


Picture 1. The potography of *Rosalia alpina.*

Sometimes photonic structure enhances the pigment color as observed in some snakes [7] or butterflies [8]. Micro and nano-structures localize light, and increase the average path length within the structure thus increasing absorption. The biological purpose of such structures might be the camouflage or thermoregulation, as in Lycaenid butterflies [9].

More advanced structures have dual purpose as in Saharan silver ant (*Cataglyphis bombycina* (Roger, 1859) [1]): to reflect maximum amount of the visible light, and to simultaneously dissipate infrared radiation directly into the atmospheric window at mid-infrared. This enables insect to efficiently regulate its body temperature in very hostile desert environment.

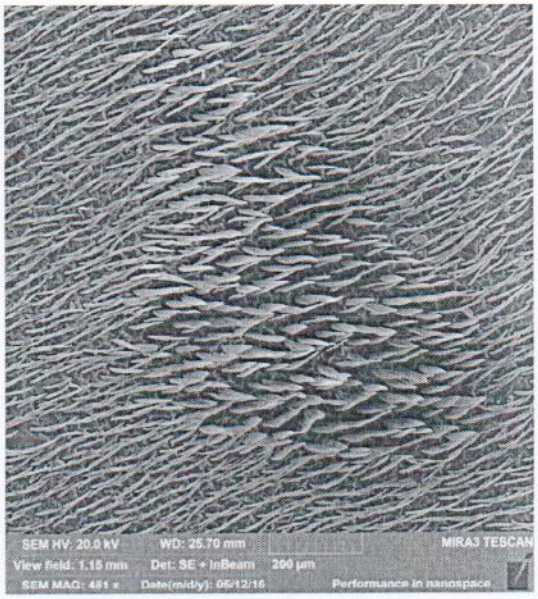
Four prominent black spots on the elytra of *Rosalia alpina* have attracted our attention. We have found, that the light absorption is not the sole consequence of dark pigments (most probably melanin), but that it is strongly influenced by the underlying structure [10]. We have been using Scanning Electron Microsope to examine the structure of *Rosalia alpina* hairs within the black spot (Picture 2). In adjacent grey zones hairs have completely different structure, as shown in Picture 3.



Picture 2. The hairs in black spots area of *Rosalia alpina.*

Both structures can be viewed, in the same scale, in picture 4. The different structure of hairs can be very easy notified in this picture.

Picture 3. The hairs in gray zones of *Rosalia alpina.*



Picture 4. The black spot area of *Rosalia alpina* surrounded by hairs with differenrt structure.

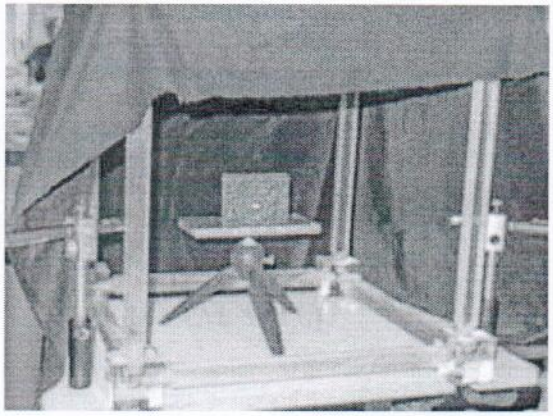
Materials developed by nature during the evolution, have a significant impact on search for artificial materials having useful absorptive properties, especially for solar energy collection, thermal energy dissipation and camouflage.

Bearing in mind possible military application we have been examining cooling process in the area of black spots of *Rosalia alpine.* The first results have been obtained using thermographic analysis based on pulse thermogaphy [10]. This method involves the analysis of images that have been recorded by an infrared (IR) camera irradiating the test sample with infrared radiation. We have found that the light absorption is not the sole consequence of dark pigments (most probably melanin), but that it is strongly influenced by the underlying structure.

Here we analyze spectral absorption of black spots using several laser wavelengths.

2. EXPERIMENTAL SETUP

The test equipment comprised a set of laser pointers, a thermal camera and a personal computer (PC), which recorded digital data in real time. The surface of test sample (*Rosalia alpina*) was heated using the red, green and blue laser pointers (650 nm, 532 nm and 405 nm), positioned at a distance of 50 cm from the sample. Picture 5 shows the experimental setup.



Picture 5. The experimental setup.

Cooling of the test sample, previously heated by means of a short laser pulse, was monitored using a commercial thermal camera "FLIR SC620", operating in the spectral range from 7.5 to 13 μm with FLIR T197189 macro lens. This type of camera has a focal plane matrix of 640x480 consisting of semiconductor detector (Vanadium Oxide - VOx). Each detector measures the intensity of infrared radiation. These values can be represented on a monitor as a thermal image coded in shades of gray or in color and can be converted to temperature values using the appropriate table.

In order to obtain optimal experimental results zoom has been set manually. After that, the all other parameters (ambient temperature, emissivity, the distance of the object from the thermal imaging camera, humidity, etc) have been found. Measurements, presented in this paper, have been carried out at short distances, (about 50 cm) and are not affected by atmospheric absorption.

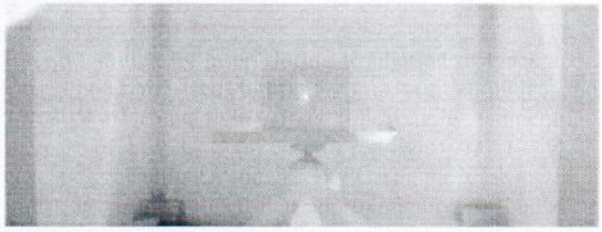
Thermal imaging camera allows conversion of spatially inhomogeneous distribution of radiation flux of scene, due to differences in the distribution of temperature and/or emissivity, in the visible image. The right choice of measuring geometry and correct interpretation of the results is based on the knowledge of the spatial and temperature resolution (sensitivity) of thermal imaging cameras.

3. EXPERIMENTAL RESULTS

Experiments have been organized in two ways. In the first case laser has been pointed to the black spot. In the second it has been used to irradiate the gray area near the black spot. During the short time (several seconds) laser pulse has been heating the irradiated place. Complete process has been recorded by IR camera and analyzed later using MATLAB software.

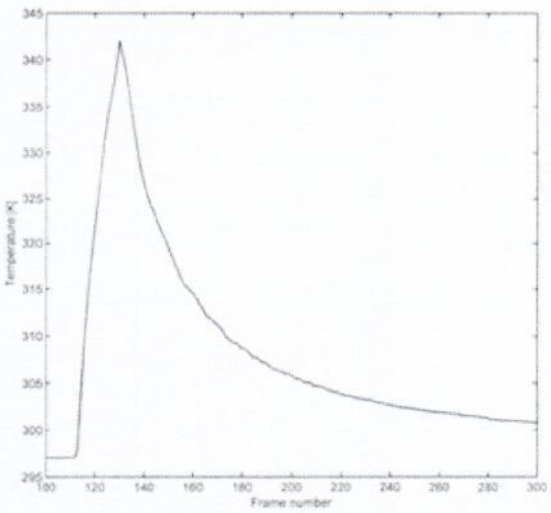
Energy emitted by laser, during irradiation of black spot, was 49.632 mJ (red light), 5.73 mJ (green light) and 176.305 mJ (blue light). It is followed by temperature increase of 44.226 °C (in case of red light), 23.512 °C (in case of green light) and 4.531 °C (in the case of blue light). It can be noticed that approximately 10 times less energy had been emitted in case of green light but temperature increase was only 2 times less than in case of red light. In addition, 3.5 times more energy had been emitted in case of blue light but temperature increase was nearly 10 times less than in case of red light.

Picture 6 shows a thermal image during the experiments with red laser in case of frame number 700. This frame is chosen because the temperature difference between maximal and minimal temperature is small enough that both, details of the target and the background can be visible.



Picture 6. Thermal immage recorded during the 700th frame in case when red laser has been pointed at black spot.

Picture 7 shows a typical temperature change during the experiments.



Picture 7. Temperature change in case when red laser had been pointed at black spot.

Here we try to establish the dominant cooling mechanism: convection, conduction or radiation. We have started with analysis based on the Newton's law of cooling.

This law states that the rate of heat loss of a body is proportional to the difference in temperatures between the body and its surroundings. This means that the heat transfer coefficient, which mediates between heat losses and temperature differences, is a constant. This condition

is true in thermal conduction, but approximately true in conditions of convective heat transfer.

Newton's law of colling is described by equation

$$\frac{dT}{dt} = k(T_t - T_a) \tag{1}$$

Whose solution is

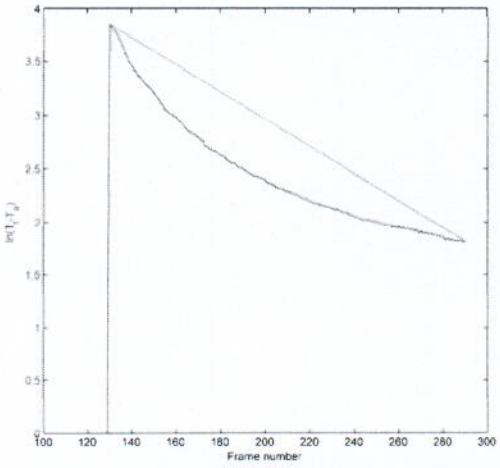
$$T_t = T_a + (T_0 - T_a)e^{-kt} \tag{2}$$

In these equation T_t is the temperature at time t and T_a is the ambient temperature, T_0 is the initial temperature of the body, and k is a constant.

From equation 2 it folows that

$$\ln\frac{(T_t - T_a)}{(T_0 - T_a)} = -kt \tag{3}$$

We can see that appropriate curve, in ideal case of cooling, should be the straight line. In picture 8 we can see slightly displacement of real process (blue line) comparing to the ideal case (red line)

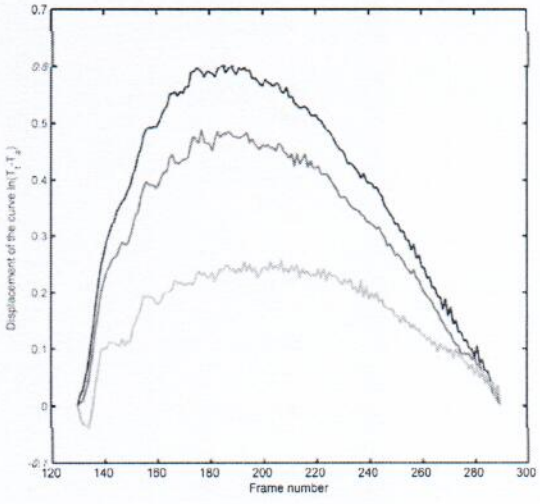


Picture 8. Natural logarithm of temperature difference in case of red laser; blue line real; red line in accordance with the Newton's law of cooling

Picture 9 shows the temperature difference in case when laser with red light has been pointed to the black spot. The blue line represents results obtained at the position of pixel with maximal temperature obtained during irradiation. The red line represents the results for the pixel in the same colon but one row below. Line marked by green colour represents the results in the same colon two row below. The similar results has been obtained in case of laser with the green and blue light.

Obviously the cooling process not coincide ideally with the Newton's law of cooling. There is a process of heat conduction in space that surround the irradiated point. The existence of negative values in case of green curve is clear evidence of that. Maximal temperature is achieved later than in case of red curve, after conducting of energy from the space with higher temperature.

During experiments the radiation time of laser has not controlled precisely. The next experiments will be organized with possibility for strictly control of radiation time. Also, with intention to have more comparable results, more attention will be paid to the positioning of the laser beam. During the last experiments irradiation of the object has been realized pointing laser from the hand. In addition, a couple of lasers with different wavelengths will be used for better covering of radiation spectra.



Picture 9. Displacement of real curve comparing to the curve obtained by the Newton's law of cooling; blue in position of maximal temperature; red in the same colon but one raw below, and green in the same colon but two rows bellow.

4. CONCLUSION

Our experiments are in progress. The results we have up to now show the existence of different photonic structure in the area of black spots. It is confirmed by the scanning electronic microscope but we want to confirm comparing the results of irradiation of the black spots and area outside of them.

In this paper we present the preliminary results of thermal analysis of *Rosalia alpina*. Insect was irradiated with laser radiation at three wavelengths, spanning the whole visible spectrum (405 nm, 532 nm and 650 nm). Significant departure from the Newton's law of cooling indicates that the thermal dissipation is regulated by radiation from the photonic structures. It seems that the structure is optimized such that it maximizes absorption in the visible part of the spectrum and simultaneously minimizes thermal losses due to radiation. More experimental and theoretical research is needed to better assess the thermal effects.

If proven true, the described effect could be used in construction of military personnel and arms clothes, which will diminish thermal dissipation through radiation. The effect could be important from the military point of view, because it can help the solder to survive in cold weather, while reducing its thermal trace.

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Serbian Plant Physiology Society

Institute for Biological Research „Siniša Stanković", University of Belgrade

2nd International Conference on Plant Biology

21th Symposium of the Serbian Plant Physiology Society

COST ACTION FA1106 QUALITYFRUIT Workshop

Petnica Science Center, June 17-20, 2015

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PROGRAMME

2nd International Conference on Plant Biology • 21th Symposium of the Serbian Plant Physiology Society • COST ACTION FA1106 QUALITYFRUIT Workshop

PETNICA SCIENCE CENTER 17-20 JUNE, 2015

Wednesday 17th June, 2015

09:00-14:00	*Registration*	
14:00-15:00	*Lunch*	
Section I:	**Plant Biotechnology**	
15:00-15:30	*Opening Ceremony*	
15:30-16:00	(Invited talk) **Alain Tissier**	Systems biology of a plant cell factory, the tomato glandular trichomes
16:00-16:20	(Invited talk) **Jules Beekwilder**	Biotechnological production of plant compounds
16:20-16:40	(Invited talk) **Milen Georgiev**	Metabolomics, lead, discovery and plant biotechnology: perfect holistic match?
16:40-17:00	(Invited talk) **Dragana Božić**	Exploring the secondary metabolism in trichomes of *Salvia fruticosa* and *Rosmarinus officinalis*: the case of carnosic acid
17:00-17:30	*Coffee break*	
17:30-17:45	(Selected talk) **Milica Bogdanović**	Problems in detecting activity of fluorescent reporter genes – case of DsRED and GFP
17:45-18:00	(Selected talk) **Stevan Jeknić**	Alteration of flower color in *Solanum lycopersicum* through ectopic expression of a gene for capsanthin-capsorubin synthase from *Lilium lancifolium*
18:00-18:15	(Selected talk) **Miloš Prokopijević**	Characterization of soybean hull peroxidase immobilized on glycidyl methacrylate copolymers
18:30-19:30	Poster session: *Plant Biotechnology*	
20:00-21:00	*Dinner*	
21:00-	Wine tasting	

Thursday 18th June, 2015

08:00-09:00	*Breakfast*	
Section II:	**Plant Growth, Development, Metabolism and Nutrition**	
09:00-09:30	(Invited talk) **James Giovannoni**	Harnessing genetic diversity to better understand regulation of tomato fruit ripening and nutritional quality
09:30-09:50	(Invited talk) **Christian Fankhasuer**	Photosensory receptor-mediated growth responses in Arabidopsis
09:50-10:10	(Invited talk) **David Honys**	Male germline development: lesson from the -omics
10:10-10:30	(Invited talk) **Dragan Vinterhalter**	Acid growth theory, auxin and potato phototropism
10:30-10:50	(Invited talk) **Bojana Banović**	How to avoid self-fertilization in plants- a buckwheat story
10:50-11:20	*Coffee break*	

POSTER PRESENTATIONS

Seasonal variation of flavonoid content and antioxidant activity of *Salvia officinalis* of different origin

PP3-1

Ana Alimpić, Danica Pavlović, Dmitar Lakušić, Petar D. Marin, Sonja Duletić-Laušević
(alimpic.ana@bio.bg.ac.rs)

University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac", Takovska 43, Belgrade, Serbia

Sage (*Salvia officinalis* L., Lamiaceae) is widely known as an important culinary and medicinal plant. This study was aimed to investigate the antioxidant activity and flavonoid content in ethanol extracts of four samples of this species. Plants from Pleš (Eastern Serbia) and Lustica (Montenegro), were transplanted in Belgrade, and cultivated under the same conditions. Aerial parts were harvested during summer and winter season, grounded and extracted by ethanol to obtain crude extracts. Antioxidant activity was evaluated using DPPH assay and results were expressed as IC50 values (μg mL^{-1}). Flavonoid contents (FC) were measured spectrophotometrically and data were presented as mg of quercetin equivalents per gram of dry extract (mg QE g-1). All of the extracts performed DPPH activity ranged from 13.12-20.05 μg mL^{-1}, which was evaluated as good comparing to values obtained for standards BHA (1.3.37 μg mL^{-1}) and BHT (17.94 μg mL^{-1}). Flavonoid content ranged from 20.08 to 40.72 mg QE g-1. Extracts of plants originated from Pleš showed stronger activity and higher FC than plants from Lustica. As expected, extracts of summer samples exhibited stronger DPPH activity and higher FC than the winter ones. Taking into account that uniform procedures have been applied for all of the plant samples, it could be concluded that flavonoid content and DPPH activity of the extracts depended on the locality of origin and season of the plant material collection.

Keywords: *Salvia officinalis*, ethanol extract, DPPH activity, flavonoid content

Hydrolysis of secoiridoid glycosides from *Centaurium erythraea* Rafn increases their antioxidative potential

PP3-2

Jelena Boljević, Suzana Živković, Jasmina Glamočlija, Dragana Božić, Neda Aničić, Branislav Šiler, Marina Soković, Danijela Mišić
(jelena.boljevic@ibiss.bg.ac.rs)

Institute for Biological Research "Siniša Stanković", University of Belgrade, Bulevar despota Stefana 142, 11060 Belgrade, Serbia

Dominant secondary metabolites of common centaury (*Centaurium erythraea* Rafn) are bitter taste secoiridoid glycosides; swertiamarin, gentiopicrin and sweroside, and their function is proposed to be in plant responses against pathogens and herbivores. It can be assumed that secoiridoid glycosides and hydrolytic enzymes (β-glucosidases) form a dual defense system in common centaury, where β-glucosidase plays an essential role in removing non-reducing terminal glucosyl residues from glycosylated compounds, leading to highly active but unstable aglycones. The present study was designed to evaluate antioxidative and antimicrobial activity of hydrolyzed and non-hydrolyzed methanol extracts of *Centaurium erythraea* aboveground parts and its main components sweroside, swertiamarin and gentiopicrin. To examine the concentrations of secoiridoid glycosides and their aglycones in methanol extracts, before and after hydrolysis, UHPLC/DAD/+HESI-MS/MS method was developed and evaluated. Hydrolysis was performed enzymatically using commercial β-glucosidase isolated from almond. Results of FRAP, ABTS and DPPH assays showed higher antioxidative activity of hydrolyzed *C. erythraea* methanol extract and pure compounds than non-hydrolyzed ones. Conversely, hydrolysis of *C. erythraea* methanol extracts led to lower antifungal activity and had weak or no influence on antibacterial activity. Based on this study it can be presumed that biosynthesis of secoiridoid glycosides, and their degradation mediated by β-glucosidases are regulated by various biotic factors, and are involved in defense system against herbivores and pathogens.

Keywords: secoiridoid glycosides, β-glucosidase, *Centaurium erythraea*

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (ON173024).

Antibacterial activity of Lady's Mantle

PP3-3

Tatjana Boroja[1], Vladimir Mihailović[1], Jelena Katanić[1], Milan Stanković[2], Nevena Stanković[1], Milan Mladenović[1]
(tatjanaboroja@gmail.com)

[1] Department of Chemistry, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac
[2] Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac

Lady's Mantle (*Alchemilla vulgaris* L.) belongs to the Rosaceae family. In traditional medicine, it was used as herbal treatment for menstrual disorders. Due to the high content of phenolic compounds, *Alchemilla* species were also shown to possess anti-inflammatory, antioxidant, antiinfluenza and anticarcinogenic activity. The purpose of this work was to evaluate the antibacterial properties of *A. vulgaris*. The methanolic extract of aerial parts of *A. vulgaris* prepared by maceration has been used to estimate the antibacterial activity against nine bacterial strains. The *in vitro* antibacterial activity was performed by microdilution method. Minimal inhibitory concentrations (MIC) were evaluated based on the color change of resazurin. The most sensitive bacterial strain was *Micrococcus lysodeikticus* (MIC 0.156 mg mL^{-1}). The methanolic extract of *A. vulgaris* also showed remarkable antibacterial potential against both ATCC and clinically isolated strains of *Enterococcus faecalis* (0.312 mg mL^{-1} and 0.156 mg mL^{-1}, respectively). *Pseudomonas aeruginosa* was the most resistant species with MIC values 20 mg mL^{-1}. MIC values for chloramphenicol, used as standard, were in the range of 2.5-10 mg mL^{-1}. The results of the present investigation suggest that *A. vulgaris* possesses strong antibacterial activity against tested bacterial species, with MIC values ranging from 0.156 mg mL^{-1} to 20 mg mL^{-1}. Based on these results, further chemical and pharmacological investigation, as well as isolation of bioactive compounds may be recommended.

Keywords: *Alchemilla vulgaris*, antibacterial activity, phenolic compounds

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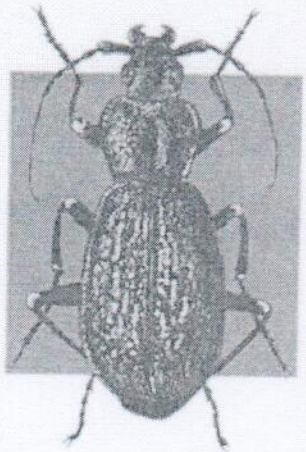
17th European Carabidologists Meeting

Learning about carabid habits and habitats – a continuous process in a continuously changing environment



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Learning about carabid habits and habitats – a continuous process in a continuously changing environment

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Drawing of *Carabus croaticus* Dejean, 1826 on front cover by Iva Čupić
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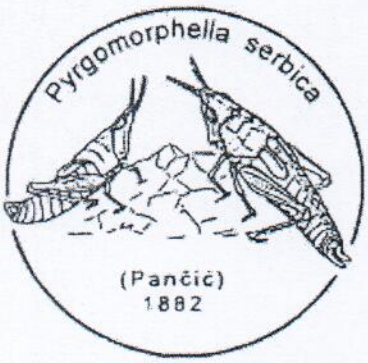
Does life in caves reduce the diversity of chemicals produced by the pygidial glands of carabids?

N. Vesović[1], S. Ćurčić[1], Lj. Vujisić[2], M. Nenadić[1], G. Krstić[2], V. Perić-Mataruga[3], S. Milosavljević[2], D. Antić[1], B. Mandić[2], M. Petković[1], I. Vučković[2], Đ. Marković[1], M. Vrbica[1], D. Pavlović[4], B. Ćurčić[1], S. Makarov[1]

[1]Institute of Zoology, University of Belgrade - Faculty of Biology, Studentski Trg 16, 11000 Belgrade, Serbia, nikola.vesovic@bio.bg.ac.rs
[2]University of Belgrade - Faculty of Chemistry, Studentski Trg 12-16, 11000 Belgrade, Serbia, ljubaw@yahoo.com
[3]Siniša Stanković Institute for Biological Research, University of Belgrade, Bulevar Despota Stefana 142, 11060 Belgrade, Serbia, vesper@ibiss.bg.ac.rs
[4]Institute of Physics, University of Belgrade, Pregrevica 118, 11080 Zemun, Serbia, danicap@ipb.ac.rs

Carabids have paired pygidial glands in the abdomen that produce a great diversity of chemicals. Adults of three cave-dwelling (both troglophilous and troglobite) ground beetles from southeastern Serbia were induced to discharge secretions of the pygidial glands into vials. Extraction with dichloromethane was used in order to obtain the secretions, and the compounds were identified by gas chromatography-mass spectrometry (GC-MS). The aims of the study were to identify the chemical contents of the released compounds, to check whether the underground way of life had influenced both composition of the secretions and the number of the pygidial chemicals, and to search for possible new compounds that have not previously been reported in Carabidae. Totally, 42 compounds were identified. *Pheggomisetes ninae* contained 32, *Laemostenus* (*Pristonychus*) *punctatus* 13, while *Duvalius* (*Paraduvalius*) *milutini* had nine glandular compounds. Caproic, oleic, palmitic and stearic acids were present in the samples of all analyzed species. Heptacosene and nonacosadienes were predominant in the pygidial extract in *P. ninae*. Undecane was the major component in the secretion of *L. punctatus*. The most abundant compound in *D. milutini* secretion was palmitic acid. The adaptation to underground life did not lead to a reduction or changes of chemical defense mechanism in all analyzed Platyninae and Trechinae taxa.

Keywords: Carabidae, Platyninae, Trechinae, cave-dwelling insects, gas chromatography-mass spectrometry (GC-MS).



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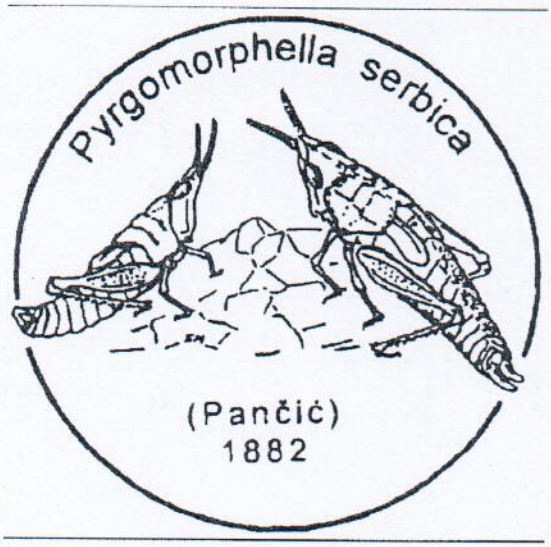

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ENTOMOLOGICAL SOCIETY OF SERBIA

HIPERMOTORNO PONAŠANJE VRSTE *LAEMOSTENUS PUNCTATUS* (DEJEAN, 1828) (COLEOPTERA: CARABIDAE) IZAZVANO STATIČKIM MAGNETNIM POLJEM

DANICA PAVLOVIĆ[1], BRANKA PETKOVIĆ[2], SREĆKO ĆURČIĆ[3], DAJANA TODOROVIĆ[2], NIKOLA VESOVIĆ[3], DEJAN PANTELIĆ[1], VESNA PERIĆ-MATARUGA[2]

[1]Institut za fiziku, Univerzitet u Beogradu, Pregrevica 118, 11080 Zemun, Beograd, Srbija
E-mail: danica.pavlovic@ipb.ac.rs, dejan.pantelic@ipb.ac.rs
[2]Institut za biološka istraživanja „Siniša Stanković", Univerzitet u Beogradu, Bulevar despota Stefana 142, 11060 Beograd, Srbija
E-mail: vesper@ibiss.bg.ac.rs, dajana@ibiss.bg.ac.rs, janac@ibiss.bg.ac.rs
[3]Institut za zoologiju, Univerzitet u Beogradu - Biološki fakultet, Studentski trg 16, 11000 Beograd, Srbija
E-mail: srecko@bio.bg.ac.rs, nikola.vesovic@bio.bg.ac.rs

Uticaj magnetnog polja na žive sisteme od velikog je značaja i predmet je intenzivnog ispitivanja, naročito poslednjih godina u eri sve većeg elektromagnetnog zagađenja. *Laemostenus punctatus* (Dejean, 1828) je vrsta troglofilnog tvrdokrilca iz porodice trčuljaka (fam. Carabidae). Cilj ove studije je bio da se ispita efekat statičkog magnetnog polja na različite parametre ponašanja (pređeni put, prosečna brzina, pokreti glave, rotacije tela, vreme kretanja i vreme mirovanja) jedinki *L. punctatus*. U eksperimentu su korišćene adultne jedinke (n = 17) ove vrste izlovljene u martu 2015. godine u Ogoreličkoj pećini, selo Sićevo, u blizini Niša, jugoistočna Srbija. Jedinke su bile podeljene u dve grupe: kontrolna (n = 9) i eksperimentalna (n = 8). Eksperimentalna grupa jedinki izlagana je statičkom magnetnom polju (110 mT) u trajanju od 5 sati. Ponašanje kontrolnih i tretiranih jedinki, neposredno nakon izlaganja, je praćeno u „open field" testu u trajanju od 12 minuta i automatski analizirano korišćenjem Any-maze programa. Rezultati su grafički prikazani u intervalima od po 4 minuta. Pokazano je da izlaganje statičkom magnetnom polju povećava motornu aktivnost (pređeni put, prosečna brzina, pokreti glave, rotacije tela i vreme kretanja) i stoga smanjuje vreme mirovanja jedinki *L. punctatus*. Najznačajnije promene za sve navedene parametre uočene su u prvih 4 minuta, pri čemu je statistička značajnost utvrđena za sledeća dva parametra: pređeni put i prosečna brzina ($p<0,05$; Mann-Whitney U test). Na osnovu dobijenih rezultata možemo zaključiti da statičko magnetno polje utiče na ponašanje adultnih jedinki vrste *L. punctatus*, odnosno da deluje na centre odgovorne za kontrolu motorne aktivnosti.

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др Александер Ковачевић, виши научни сарадник Института за физику (председник)

др Драган Лукић, виши научни сарадник Института за физику

др Александар Крмпот, научни сарадник Института за физику

др Дарко Васиљевић, виши научни сарадник Института за физику

др Бранислав Јеленковић, научни саветник Института за физику

Спонзори Конференције:

Министарство просвете, науке и технолошког развоја Републике Србије

Оптичко друштво Србије

„Сензор инфиз" д.о.о.

„Оптикс" ("Optix") д.о.о.

Structures of biological origin for optical document security

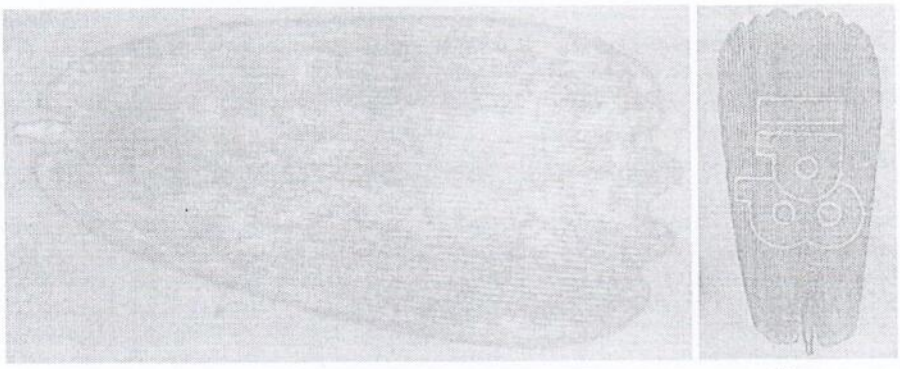
Dejan Pantelić, Aleksandar Krmpot, Mihailo Rabasović, Danica Pavlović, Vladimir Lazović

Institute of Physics, University of Belgrade, Pregrevica 118 Zemun, Belgrade, Serbia

Contact: D. Pantelić (pantelic@ipb.ac.rs)

Abstract. Optically variable devices (OVD) are a common protective element on various types of documents (e.g. identity cards, passports, visas, bank cards) [1]. Holograms and other diffractive elements are mainly used, because their protective value is based on complexity of micron and submicron structures. As a result, a huge number of identical OVDs is manufactured, thus facilitating counterfeiting.

Here we use a complexity and variability of biological structures to individualize documents. We show that butterfly wing-scales are good candidates for this way of document protection. Their internal structure is of micron and submicron complexity (as revealed by scanning electron microscopy), while the resulting optical effects are strongly variable and unpredictable (as found by optical microscopy) – see Fig. 1a. We use butterfly scales as physical one-way functions – i.e. as something that is easy to manufacture and use, yet prohibitively complicated to copy or counterfeit [2].



a) b)

Fig. 1: a) Iridescence of a butterfly scale, showing complex pattern of colored dots. b) Laser processing of a butterfly scale.

We have observed a number of local degrees of freedom of an individual butterfly scale: local spectra, polarization, iridescence, fluorescence, overall shape, to mention just a few of them. In order to further increase the protective capacity, we use laser processing to introduce artificial, covert and overt, features into the scale. We are able to write arbitrary pattern with microscopic features: letters, symbols, figures, shapes (as in Fig. 1b) [3].

REFERENCES

[1] *Optical Document Security*, ed. by R. L. Van Renesse, Artech House, (1998).

[2] R. Pappu, B. Recht, J. Taylor, N. Gershenfeld, "Physical One-Way Functions," *Science* **297** (2002), 2026–30

[3] D. Pantelić, A. Krmpot, M. Rabasović, V. Lazović, D. Pavlović, PCT patent applications PCT/EP2015/081407, PCT/EP2015/081400, PCT/EP2015/081398

УНИВЕРЗИТЕТ У БЕОГРАДУ
ИНСТИТУТ ЗА ФИЗИКУ БЕОГРАД
Прегревица 118, 11080 Земун - Београд, Србија
Телефон: +381 11 3713000, Факс: +381 11 3162190, www.ipb.ac.rs
ПИБ: 100105980, Матични број: 07018029, Текући рачун: 205-66984-23

Београд, 9.3.2016. година

ПОТВРДА О АУТОРСТВУ

Поштовани,

Овим дописом потврђујем да је Даница Павловић, истраживач приправник, ангажована на Институту за физику у Београду један од проналазача у следећим међународним патентним пријавама (*енг.* PST):

1) *"Security device individualized with biological particles"*,
Institute of Physics Belgrade,
PCT application number PCT/EP2015/081398;

2) *"Security tag with laser-cut particles of biological origin"* ,
Institute of Physics Belgrade,
PCT application number PCT/EP2015/081407;

3) *"Security tag containing a pattern of biological particles"*,
Institute of Physics Belgrade,
PCT application number PCT/EP2015/081400;

Потврда је издата на захтев аутора, а за потребе избора у звање истраживач сарадник. У случају било каквих додатних питања стојим Вам на располагању.

Саша Лазовић

др Саша Лазовић
Руководилац
Иновациони центар Института за физику

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

PCT

To:

Winkler, Andreas
Postfach 347013
28339 Bremen
ALLEMAGNE

NOTIFICATION OF THE INTERNATIONAL APPLICATION NUMBER AND OF THE INTERNATIONAL FILING DATE

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Date of mailing *(day/month/year)*	27-01-2016
Applicant's or agent's file reference AW-P0076WO	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2015/081407	30 December 2015 (30-12-2015)	

Applicant

INSTITUTE OF PHYSICS BELGRADE, UNIVERSITY OF...

Title of the invention

1. The applicant is hereby notified that the international application has been accorded the international application number and the international filing date indicated above.

2. The applicant is further notified that the record copy of the international application:

[X] was transmitted to the International Bureau on **see above date of mailing**

[ ] has not yet been transmitted to the International Bureau for the reason indicated below and a copy of this notification has been sent to the International Bureau*:

- [ ] because the necessary national security clearance has not yet been obtained
- [ ] because *(reason to be specified)*

* The International Bureau monitors the transmittal of the record copy by the receiving Office and will notify the applicant (with Form PCT/IB/301) of its receipt. Should the record copy not have been received by the expiration of 14 months from the priority date, the International Bureau will notify the applicant (Rule 22.1(c)).

Name and mailing address of the Receiving Office	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040 Fax: (+31-70) 340-3016	Masterson, Claire

Form PCT/RO/105 (April 2007)

PCT REQUEST

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0	**For receiving Office use only**	
0-1	International Application No.	PCT/EP2015/081407
0-2	International Filing Date	30 DEC 2015 (30.12.2015)
0-3	Name of receiving Office and "PCT International Application"	RO/EP

0-4	**Form PCT/RO/101 PCT Request**	
0-4-1	Prepared Using	PCT Online Filing Version 3.5.000.244e MT/FOP 20141031/0.20.5.20
0-5	**Petition** The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	European Patent Office (EPO) (RO/EP)
0-7	**Applicant's or agent's file reference**	AW-P0076WO
I	**Title of Invention**	Security tag with laser-cut particles of biological origin
II	**Applicant**	
II-1	This person is	Applicant only
II-2	Applicant for	All designated States
II-4	Name	Institute of Physics Belgrade, University of Belgrade
II-5	Address	Pregrevica 118 11080 Belgrade Republic of Serbia
*RO/EP		
II-6	State of nationality	*RS
II-7	State of residence	RS
II-11	Applicant's registration No. with the Office	0.0
III-1	**Applicant and/or Inventor**	
III-1-1	This person is	Inventor only
III-1-3	Inventor for	All designated States
III-1-4	Name (LAST, First)	PAVLOVIC, Danica
III-1-5	Address	Boška Živikovica 18c 11260 Belgrade Republic of Serbia

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III-2	**Applicant and/or inventor**	
III-2-1	This person is	Inventor only
III-2-3	Inventor for	All designated States
III-2-4	Name (LAST, First)	LAZOVIC, Vladimir
III-2-5	Address	Jurija Gagarina 127 11070 Belgrade Republic of Serbia
III-3	**Applicant and/or inventor**	
III-3-1	This person is	Inventor only
III-3-3	Inventor for	All designated States
III-3-4	Name (LAST, First)	KRMPOT, Aleksandar
III-3-5	Address	Jasenova 8/15 11030 Belgrade Republic of Serbia
III-4	**Applicant and/or inventor**	
III-4-1	This person is	Inventor only
III-4-3	Inventor for	All designated States
III-4-4	Name (LAST, First)	RABASOVIC, Mihailo
III-4-5	Address	Svetozara Papica 1/51 11080 Belgrade Republic of Serbia
III-5	**Applicant and/or inventor**	
III-5-1	This person is	Inventor only
III-5-3	Inventor for	All designated States
III-5-4	Name (LAST, First)	PANTELIC, Dejan
III-5-5	Address	Nemanjina 7 11080 Belgrade Republic of Serbia

PCT REQUEST

Print Out (Original in Electronic Form)

IV-1	**Agent or common representative; or address for correspondence**		
	The person identified below is hereby/ has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	Agent	
IV-1-1	Name (LAST, First)	WINKLER, Andreas	
IV-1-2	Address	P.O. Box 347013 28339 Bremen Germany	
IV-1-3	Telephone No.	00494212233912	
IV-1-5	e-mail	winkler@winkler-ip.de	
IV-1-5(a)	E-mail authorization The receiving Office, the International Searching Authority, the International Bureau and the International Preliminary Examining Authority are authorized to use this e-mail address, if the Office or Authority so wishes, to send notifications issued in respect of this international application:	as advance copies followed by paper notifications	
IV-1-6	Agent's registration No.	0.0	
V	**DESIGNATIONS**		
V-1	**The filing of this request constitutes under Rule 4.9(a), the designation of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.**		
VI-1	**Priority Claim**	NONE	
VII-1	**International Searching Authority Chosen**	European Patent Office (EPO) (ISA/EP)	
VIII	**Declarations**	Number of declarations	
VIII-1	Declaration as to the identity of the inventor	-	
VIII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	-	
VIII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	-	
VIII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	-	
VIII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	-	

PCT REQUEST

Print Out (Original in Electronic Form)

IX	Check list	Number of sheets	Electronic file(s) attached
IX-1	Request (including declaration sheets)	4	✓
IX-2	Description	13	✓
IX-3	Claims	3	✓
IX-4	Abstract	1	✓
IX-5	Drawings	10	✓
IX-7	TOTAL	31	
	Accompanying Items	Paper document(s) attached	Electronic file(s) attached
IX-8	Fee calculation sheet	--	✓
IX-20	**Figure of the drawings which should accompany the abstract**	1	
IX-21	**Language of filing of the international application**	English	
X-1	**Signature of applicant, agent or common representative**	(PKCS7 Digital Signature)	
X-1-1	Name	Institute of Physics Belgrade, University of Belgrade	
X-1-2	Name of signatory	Andreas Winkler 35778	
X-1-3	Capacity (if such capacity is not obvious from reading the request)	(Applicant)	

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10-1	**Date of actual receipt of the purported international application**	30 DEC 2015 (30.12.2015)
10-2	**Drawings:**	Received
10-2-1	Received	
10-2-2	Not received	
10-3	**Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application**	
10-4	**Date of timely receipt of the required corrections under PCT Article 11(2)**	
10-5	**International Searching Authority**	ISA/EP
10-6	**Transmittal of search copy delayed until search fee is paid**	

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NOTIFICATION OF THE INTERNATIONAL APPLICATION NUMBER AND OF THE INTERNATIONAL FILING DATE

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Date of mailing *(day/month/year)*	19-01-2016
Applicant's or agent's file reference AW-P0075WO	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2015/081400	30 December 2015 (30-12-2015)	

Applicant

INSTITUTE OF PHYSICS BELGRADE, UNIVERSITY OF...

Title of the invention

1. The applicant is hereby notified that the international application has been accorded the international application number and the international filing date indicated above.

2. The applicant is further notified that the record copy of the international application:

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Name and mailing address of the Receiving Office	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040 Fax: (+31-70) 340-3016	Pasche, Constantinus

Form PCT/RO/105 (April 2007)

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0-1	International Application No.	**PCT/EP2015/081400**
0-2	International Filing Date	**30 DEC 2015 (30.12.2015)**
0-3	Name of receiving Office and "PCT International Application"	**RO/EP**

0-4	**Form PCT/RO/101 PCT Request**	
0-4-1	Prepared Using	PCT Online Filing Version 3.5.000.244e MT/FOP 20141031/0.20.5.20
0-5	**Petition** The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	European Patent Office (EPO) (RO/EP)
0-7	**Applicant's or agent's file reference**	AW-P0075WO
I	**Title of Invention**	Security tag containing a pattern of biological particles
II	**Applicant**	
II-1	This person is	Applicant only
II-2	Applicant for	All designated States
II-4	Name	Institute of Physics Belgrade, University of Belgrade
II-5	Address	Pregrevica 118 11080 Belgrade Republic of Serbia
II-6	State of nationality	RS
II-7	State of residence	RS
II-11	Applicant's registration No. with the Office	0.0
III-1	**Applicant and/or inventor**	
III-1-1	This person is	Inventor only
III-1-3	Inventor for	All designated States
III-1-4	Name (LAST, First)	PANTELIC, Dejan
III-1-5	Address	Nemanjina 7 11080 Belgrade Republic of Serbia

RO/EP

III-2	**Applicant and/or inventor**	
III-2-1	This person is	Inventor only
III-2-3	Inventor for	All designated States
III-2-4	Name (LAST, First)	RABASOVIC, Mihailo
III-2-5	Address	Svetozara Papica 1/51 11080 Belgrade Republic of Serbia
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III-3-1	This person is	Inventor only
III-3-3	Inventor for	All designated States
III-3-4	Name (LAST, First)	KRMPOT, Aleksandar
III-3-5	Address	Jasenova 8/15 11030 Belgrade Republic of Serbia
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III-4-3	Inventor for	All designated States
III-4-4	Name (LAST, First)	LAZOVIC, Vladimir
III-4-5	Address	Jurija Gagarina 127 11070 Belgrade Republic of Serbia
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IV-1	**Agent or common representative; or address for correspondence**		
	The person identified below is hereby/ has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	Agent	
IV-1-1	Name (LAST, First)	WINKLER, Andreas	
IV-1-2	Address	P.O. Box 347013 28339 Bremen Germany	
IV-1-3	Telephone No.	00494212233912	
IV-1-5	e-mail	winkler@winkler-ip.de	
IV-1-5(a)	E-mail authorization The receiving Office, the International Searching Authority, the International Bureau and the International Preliminary Examining Authority are authorized to use this e-mail address, if the Office or Authority so wishes, to send notifications issued in respect of this international application:	as advance copies followed by paper notifications	
IV-1-6	Agent's registration No.	0.0	
V	**DESIGNATIONS**		
V-1	**The filing of this request constitutes under Rule 4.9(a), the designation of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.**		
VI-1	**Priority Claim**	NONE	
VII-1	**International Searching Authority Chosen**	European Patent Office (EPO) (ISA/EP)	
VIII	**Declarations**	Number of declarations	
VIII-1	Declaration as to the identity of the inventor	–	
VIII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	–	
VIII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	–	
VIII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	–	
VIII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	–	

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IX	Check list	Number of sheets	Electronic file(s) attached
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IX-3	Claims	2	✓
IX-4	Abstract	1	✓
IX-5	Drawings	13	✓
IX-7	TOTAL	33	
	Accompanying Items	Paper document(s) attached	Electronic file(s) attached
IX-8	Fee calculation sheet	–	✓
IX-20	**Figure of the drawings which should accompany the abstract**	1	
IX-21	**Language of filing of the international application**	English	
X-1	**Signature of applicant, agent or common representative**	(PKCS7 Digital Signature)	
X-1-1	Name	Institute of Physics Belgrade, University of Belgrade	
X-1-2	Name of signatory	Andreas Winkler 35778	
X-1-3	Capacity (if such capacity is not obvious from reading the request)	~~(Applicant)~~ XX Agent	RO/EP

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10-1	**Date of actual receipt of the purported international application**	30 DEC 2015 (30.12.2015)
10-2	**Drawings:**	
10-2-1	Received X	
10-2-2	Not received	
10-3	**Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application**	
10-4	**Date of timely receipt of the required corrections under PCT Article 11(2)**	
10-5	**International Searching Authority**	ISA/EP
10-6	**Transmittal of search copy delayed until search fee is paid**	

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NOTIFICATION OF THE INTERNATIONAL APPLICATION NUMBER AND OF THE INTERNATIONAL FILING DATE

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Date of mailing *(day/month/year)*	11-01-2016
Applicant's or agent's file reference AW-P0074WO	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2015/081398	30 December 2015 (30-12-2015)	

Applicant

INSTITUTE OF PHYSICS BELGRADE, UNIVERSITY OF...

Title of the invention

1. The applicant is hereby notified that the international application has been accorded the international application number and the international filing date indicated above.

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Name and mailing address of the Receiving Office	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040 Fax: (+31-70) 340-3016	Olsder, Florence +0031 70 340 3647 Rijswijk

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0-1	International Application No.	PCT/EP2015/081398
0-2	International Filing Date	30 DEC 2015 (30.12.2015)
0-3	Name of receiving Office and "PCT International Application"	RO/EP

0-4	**Form PCT/RO/101 PCT Request**	
0-4-1	Prepared Using	PCT Online Filing Version 3.5.000.244e MT/FOP 20141031/0.20.5.20
0-5	**Petition** The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	European Patent Office (EPO) (RO/EP)
0-7	**Applicant's or agent's file reference**	AW-P0074WO
I	**Title of Invention**	Security device individualized with biological particles
II	**Applicant**	
II-1	This person is	Applicant only
II-2	Applicant for	All designated States
II-4	Name	Institute of Physics Belgrade, University of Belgrade
II-5	Address	Pregrevica 118 11080 Belgrade Republic of Serbia
II-6	State of nationality	*RS *RO/EP
II-7	State of residence	RS
II-11	Applicant's registration No. with the Office	0.0
III-1	**Applicant and/or inventor**	
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III-4-5	Address	Jurija Gagarina 127 11070 Belgrade Republic of Serbia
III-5	**Applicant and/or inventor**	
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IV-1-3	Telephone No.	00494212233912	
IV-1-5	e-mail	winkler@winkler-ip.de	
IV-1-5(a)	E-mail authorization The receiving Office, the International Searching Authority, the International Bureau and the International Preliminary Examining Authority are authorized to use this e-mail address, if the Office or Authority so wishes, to send notifications issued in respect of this international application:	as advance copies followed by paper notifications	
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VII-1	**International Searching Authority Chosen**	European Patent Office (EPO) (ISA/EP)	
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VIII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	-	
VIII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	-	
VIII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	-	
VIII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	-	

PCT REQUEST

Print Out (Original in Electronic Form)

IX	Check list	Number of sheets	Electronic file(s) attached
IX-1	Request (including declaration sheets)	4	✓
IX-2	Description	12	✓
IX-3	Claims	3	✓
IX-4	Abstract	1	✓
IX-5	Drawings	10	✓
IX-7	TOTAL	30	
	Accompanying Items	Paper document(s) attached	Electronic file(s) attached
IX-8	Fee calculation sheet	–	✓
IX-20	**Figure of the drawings which should accompany the abstract**	1	
IX-21	**Language of filing of the international application**	English	
X-1	**Signature of applicant, agent or common representative**	(PKCS7 Digital Signature)	
X-1-1	Name (LAST, First)	Winkler, Andreas	
X-1-3	Capacity (if such capacity is not obvious from reading the request)	(Representative)	

FOR RECEIVING OFFICE USE ONLY

10-1	**Date of actual receipt of the purported international application**	30 DEC 2015 (30.12.2015)
10-2	**Drawings:**	
10-2-1	Received X	
10-2-2	Not received	
10-3	**Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application**	
10-4	**Date of timely receipt of the required corrections under PCT Article 11(2)**	
10-5	**International Searching Authority**	ISA/EP
10-6	**Transmittal of search copy delayed until search fee is paid**	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	**Date of receipt of the record copy by the international Bureau**	