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WOOD IDENTIFICATION OF CHARCOAL WITH CONFOCAL LASER SCANNING MICROSCOPY

IDENTIFIKACIJA LESA OGLJA S POMOČJO KONFOKALNE LASERSKE VRSTIČNE MIKROSKOPIJE

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Abstract / Izvleček

Abstract: *Wood identification of barbecue charcoal from commercial packages of three retailers (B1, B2, B3) in Slovenia and Croatia was performed with help of Confocal Laser Scanning Microscopy (CLSM). CLSM enabled us to image key identification features of charcoal wood that were compared with light micrographs of wood from the reference collection. Product B1 contained charcoal made exclusively of beech wood* (Fagus sylvatica) *and the declaration indicated the address of the producer, in Serbia which allowed traceability of the wood. The selection of wood species in product B2, consisted of red oak* (Quercus cerris *or* Q. rubra)*, black locust* (Robinia pseudoacacia)*, and cherry* (Prunus avium)*, which could originate from Serbia, and it did not contain tropical wood as stated on the package. Product B3 contained wood from at least four (sub)topical species which could not be exactly identified to species/genus level. The declaration on the product did not allow traceability of wood. As the risks of illegal logging are high for wood of (sub)tropical origin, our results support the initiative that the monitoring of the charcoal trade should be covered by the EUTR - European Timber Regulations.*

Keywords*: charcoal, confocal laser scanning microscopy (CLSM), wood anatomy, wood identification, domestic wood species, tropical wood species, illegal logging*

Izvleček*: Identifikacijo lesa oglja iz treh trgovin v Sloveniji in na Hrvaškem smo opravili s pomočjo konfokalne laserske vrstične mikroskopije (CLSM). Predstavili smo slike in opise ključnih znakov za identifikacijo lesa oglja in lesa iz referenčne zbirke. Izdelek B1 je vseboval oglje iz lesa bukve* (Fagus sylvatica) *in deklaracijo z natančnim naslovom proizvajalca iz Srbije, ki omogoča sledenje izvora lesa. Izdelek B2 je vseboval oglje iz lesa enega od rdečih hrastov* (Q. cerris *ali* Q. rubra)*, robinije* (Robinia pseudoacacia) *in češnje* (Prunus avium)*. To potrjuje, da pošiljka ne vsebuje tropskega lesa, kot je navedeno na deklaraciji in da verjetno prihaja iz Srbije. Izdelek B3 je vseboval les iz vsaj štirih (sub)tropskih lesnih vrst, ki jih ni bilo mogoče natančno določiti. Ker so tropske vrste za oglje pogosto nezakonito posekane, se pridružujemo pobudi, da bi v Evropi nadzor trgovine z ogljem morali vključiti v Uredbo EU o lesu - EUTR.*

Ključne besede*: oglje, konfokalna laserska vrstična mikroskopija (CLSM), anatomija lesa, identifikacija lesa, domače lesne vrste, tropske lesne vrste, nezakonita sečnja*

1 INTRODUCTION
1 UVOD

Charcoal is mainly used as a solid fuel for heating. It is made through the process of carbonisation, where wood is burned in a low oxygen environment and volatile components are removed through pyrolysis. The lower the percentage of volatile components in the charcoal is, the higher its quality because in this way charcoal burns cleaner, with little or no smoke (Oyedun et al., 2012). Wood charcoal as a source of energy has played an important role in human history. In palaeobotany it is therefore an important macrobotanical remain providing information on the past environment, plant communities, and exploitation of resources (Chabal, 1994; Asouti & Austin, 2005). In archaeology it provides information related to the use of wood by past societies, in domestic and ritual contexts (Scheel-Ybert et al., 2010) and indicators of human mobility in the landscape (Byrne et al., 2013). Charcoal from archaeological sites (Begin & Marguerie, 2002) and in natural soils is studied to

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understand the economics of the fuel, which describes the relationship between humans and the forest (Marguerie & Hunot, 2006). A clear example are the studies conducted in Pompei, where the charcoal analysis was particularly focused to firewood (Coubray, 2013; Veal, 2014).

In Europe, especially large amounts of charcoal were used in pre-industrial times, before it was replaced by the massive use of coal and other sources of energy. From the worldwide perspective it still remains a crucial source of energy in many regions. According to the Food and Agriculture Organization of the United Nations (FAO), half the wood extracted from forests worldwide is used to produce energy and about 17% is converted into charcoal (FAO 2017; Haag et al., 2020; Zemke et al., 2020). In Europe, charcoal is currently mainly used for barbeques, and Germany has the biggest consumption, with over 230.000 tons in 2018. Slovenia is currently not among the big producers or consumers of charcoal, but in ancient times great amounts of beechwood charcoal were produced and used in metallurgy (Brus & Jarni, 2012; Cimperšek, 2012).

Charcoal is often sold without traceability and the country of origin is not indicated. As its trade is not yet covered by the European Timber Regulations, EUTR, (EU) No. 995/2010, it is suspected that considerable amounts of illegally cut timber from all around the world may be sold in form of charcoal. The recent study of Haag et al. (2017; 2020) examined several European countries and showed that charcoal on the market, especially products without FSC or PEFC certificates (Hansen et al., 1997), often contains timber from subtropical or tropical origin, and the declarations on the packaging were often incorrect and/or incomplete, as also confirmed by the German consumer association (Stiftung Warentest, 2019). Charcoal from illegal logging contributes to forest degradation, deforestation, desertification and soil erosion.

During charcoal production high temperatures and carbonisation modify the structure of wood (Leme et al., 2007; Zemke et al., 2020). Despite this, charcoal may preserve crucial anatomical features for wood identification, especially in case of well-known species with clear diagnostic features (Prior & Alvin, 1983, 1986; Prior & Gasson, 1993; Kwon et al., 2009; Leme et al., 2010; Gonçalves et al., 2012; Hubau et al., 2013; Haag et al., 2020; Zemke et al., 2020). However, good knowledge of wood anatomy combined with reference collections and databases is needed for the taxonomic identification of charcoal wood. As reported in the literature, the analysis and identification of charcoal based on wood anatomy are widely applied (e.g. Scheel-Ybert, 2000; Théry-Parisot et al., 2010; Dotte-Sarout et al., 2015; Andrič et al., 2016). When wood anatomical features are not sufficiently preserved, charcoal collections are especially important source of reference material to improve identification (Zemke et al., 2020).

The identification of charcoal wood based on anatomical characteristics is thus an important way to track its origin and prevent illegal logging and black market sales. This can be of great importance for ecosystem conservation and can make an effective contribution not only to the environment but also socioeconomics, by increasing the sustainability of the charcoal production chain (Gonçalves & Scheel-Ybert, 2012).

Although charcoal identification is relatively feasible for temperate and boreal wood species, the identification of tropical and subtropical species is more complicated due to the great biodiversity and number of taxa (Haag et al., 2020).

Identification of anatomical traits in charcoal is generally made on fragments not bigger than a few centimetres (Moser et al., 2018). Reflected light microscopy is often used to the taxon. The charcoals are classically identified based on anatomical features observed under the reflected light on prepared surfaces (i.e., transverse, radial, tangential). To obtain thin sections for observation under the transmitted light, time-consuming embedding methods and advanced equipment such as sledge-, rotation- or cryo-microtome are needed (Ludemann & Nelle, 2002; Di Pasquale, 2009; Schweingruber, 2012; Beeckman et al., 2020). Scanning electron microscopy with adequate sample preparation is also used to visualise detailed anatomical traits (e.g., Boutain et al., 2010; Hubau et al., 2013). Recently, modern methods like 3D-reflected light microscopy (Zemke et al., 2020) and Confocal Laser Scanning Microscopy (CLSM) (Balzano et al., 2019; Čufar at al., 2019) have proved to be useful for obtaining three-dimensional information on the anatomical structure of wood, allowing species identification.

The main advantage of CLSM is the possibility to directly observe the surface of interest without time-consuming sample preparation, and at the same time, thanks to the 3D image acquisition, to obtain an adequately high resolution to recognise wood anatomical features.

A recent study of the quality of barbeque charcoal (Stare & Prislan, 2020) showed that 10 out of 11 products on the Slovenian market did not meet the standard for charcoal quality SIST EN 1860-2 (2005). However, Stare and Prislan (2020) do not report on wood identification.

In this article, we collected and analysed barbeque charcoal sold by three retailers in Slovenia and Croatia. We used CLSM to identify the charcoal wood and show the potential of the method for identification on fracture plains without specific sample preparation. Furthermore, we aimed to identify which wood species can be found in selected barbeque charcoal products, if the wood is of European origin or also imported from (sub)topical areas, and finally, if the selection of wood species agrees with the declarations on the commercial products.

2 MATERIALS AND METHODS
2 MATERIAL IN METODE

We randomly collected charcoal from three consignments from local retailers. We inspected the content of three bags, B1, B2 and B3, and their declaration labels (Table 1). The first product B1 was bought in a small shop for gardening products in Croatia, known for high quality products from trustworthy sources; the label reported the country of origin and exact address of the producer in Serbia. The bag B2 was bought in a small shop for chemical products in Slovenia. Its label reported the country of origin – Serbia, and declared that the product did not contain tropical wood. The third bag B3 was bought in a supermarket in Slovenia, which belongs to an international discount chain that operates thousands of stores across Europe and the United States. Its label only reported that the charcoal was made for the given chain.

We checked the content of each bag and macroscopically pre-identified wood species with help of a magnification lens and grouped the charcoal pieces which presumably belonged to the same species. Finally, larger samples, possibly containing mature wood of the stem (avoiding twigs and juvenile wood), were selected for detailed inspection.

The samples were split with a cutter, to obtain sub-samples of typical wood anatomical surfaces (transverse, tangential and radial) (Scheel-Ybert, 2004). The sub-samples of the same charcoal piece were labelled.

The samples were put in a petri dish with table salt, and placed on the stage of the Confocal Laser Scanning Microscope (CLSM) Olympus LEXT OLS5000 (Olympus Corporation Tokyo 163-0914, Japan) (Figure 1) to observe anatomical features with the following objectives: MPLFLN5x (numerical aperture 0.15, working distance 20 mm), MPLFLN10xLEXT (numerical aperture 0.3, working distance 10.4 mm), and LMPLFLN20XLEXT (numerical aperture 0.45, working distance 6.5mm). By moving the stage in real-time, we first used the optical system to get a panoramic overview to inspect the entire surface. Then, once we detected the key identification features, high resolution laser confocal images were acquired at different focus positions using the stitching function. Measurements of the anatomical traits were performed with the OLS5000 image analysis software.

Wood identification was performed with the available identification keys and wood anatomical literature, dichotomous microscopic keys and illustrated descriptions of the species (e.g., Grosser, 1977; Schweingruber, 1978, 1990; Wagenführ, 1996, 1999; Schoch et al., 2004; Čufar & Zupančič, 2009; Signorini et al., 2014). Wood identification software such as DELTA/ INTKEY for microscopic and macroscopic identification of commercial hardwoods and CITES species (Richter & Trockenbrodt, 1995; Richter & Dallwitz, 2000; Richter et al., 2002, 2005), or programmes available online such as InsideWood (IAWA committee, 1989; InsideWood, 2004 onwards; Wheeler, 2011), and an atlas and macroscopic wood identification software package for Italian timber species (Ruffinatto et al., 2017, 2019; Ruffinato & Crivellaro, 2019) were used. Wood samples and anatomical sections of the collection of the Department of Wood Science and Technology were used as a reference material. Reference slides were observed with a Nikon Eclipse E 800 light microscope, and the micrographs were taken with a Nikon DS-fi1 digital camera and NIS Elements Br 3.0 software.

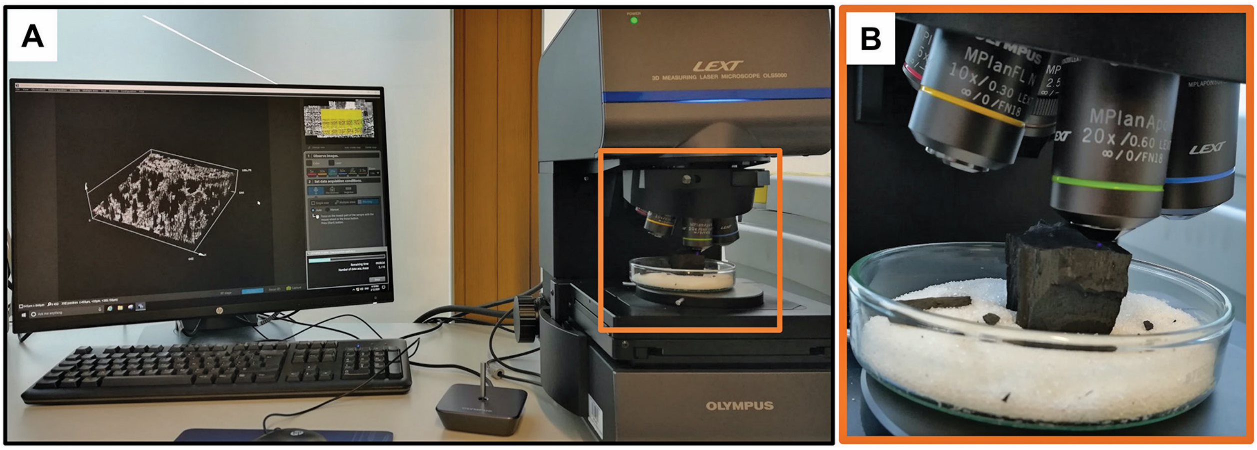

Figure 1. Charcoal observation: (A) Confocal Laser Scanning Microscope (CLSM) Olympus LEXT OLS5000 (Olympus Corporation Tokyo 163-0914, Japan), and (B) a detailed view of charcoal sample in a petri dish with table salt placed on the stage, under the objective of 20x magnification.
Slika 1. Opazovanje oglja: (A) konfokalni laserski vrstični mikroskop (CLSM) Olympus LEXT OLS5000 (Olympus Corporation Tokyo 163-0914, Japonska) in (B) vzorec oglja v petrijevki s kuhinjsko soljo, na mizici mikroskopa pod objektivom 20-kratne povečave (podroben pogled).

3 RESULTS AND DISCUSSION
3 REZULTATI IN RAZPRAVA

CLSM enabled us to observe the main wood anatomical features for precise identification of the charcoal samples. Sample preparation only took few minutes to split the charcoal and get an oriented fracture plain. It was the most challenging to obtain a clear cross-section. The 3D system of the microscope allowed us to capture high resolution and sharp images, even if the surfaces of the samples were not completely flat. In this way we could find key features for the identification of domestic species from temperate Europe. We recorded also four (sub)tropical taxa where exact wood identification to the level of species or genus was not possible (Table 1).

Table 1. Wood identification of the investigated barbecue charcoal
Preglednica 1. Identifikacija lesnih vrst raziskanega oglja za žar

Consignment / Pošiljka	Declaration label / Navedba na embalaži	Taxon / Takson
B1 small shop for gardening products, Croatia / manjša trgovina za vrtne potrebščine, Hrvaška	produced in Serbia, producer's address known / proizvedeno v Srbiji, naslov proizvajalca znan	*Fagus sylvatica*
B2 small shop for cleaning products, Slovenia / manjša trgovina s čistili, Slovenija	distributer's address in Slovenia known, made in Serbia, no tropical wood / distributerjev naslov v Sloveniji znan, narejeno v Srbiji, ni tropski les	*Prunus avium*
		Quercus sp.
		Robinia pseudoacacia
B3 supermarket of an international discount chain, Slovenia / veleblagovnica mednarodne diskontne verige, Slovenija	made for the chain / proizvedeno za prodajno verigo	T1 (sub)tropical species / (sub)tropska vrsta
		T2 (sub)tropical species / (sub)tropska vrsta
		T3 (sub)tropical species / (sub)tropska vrsta
		T4 (sub)tropical species / (sub)tropska vrsta

In the following we present the wood anatomy of the investigated charcoal as observed with CLMS and images of the reference material from the collection observed with a light microscope.

3.1 EUROPEAN BEECH, *Fagus sylvatica*, FAGACEAE

3.1 NAVADNA BUKEV, *Fagus sylvatica*, FAGACEAE

The cross-section of the selected sample contains distinct growth rings and growth ring boundaries demarcated by thicker-walled fibres, rays of two distinct sizes, and larger rays nodded at the growth ring boundaries. The distribution of vessels is diffuse-porous to semi-ring-porous. Average tangential vessel diameter is up to 80 µm. Axial parenchyma is apotracheal, diffuse and diffuse-in-aggregates (Figure 2 A, B).

On the tangential section we can observe rays of two distinct sizes and vessels with simple perforation plates (Figure 2 C, D). Small rays are more frequent than the multiseriate ones (> 10 cells wide and commonly >1000 µm high). The radial section shows heterogenous rays (with body ray cells procumbent and one row of square marginal cells), and vessels with simple and scalariform perforation plates (Figure 2 E, F).

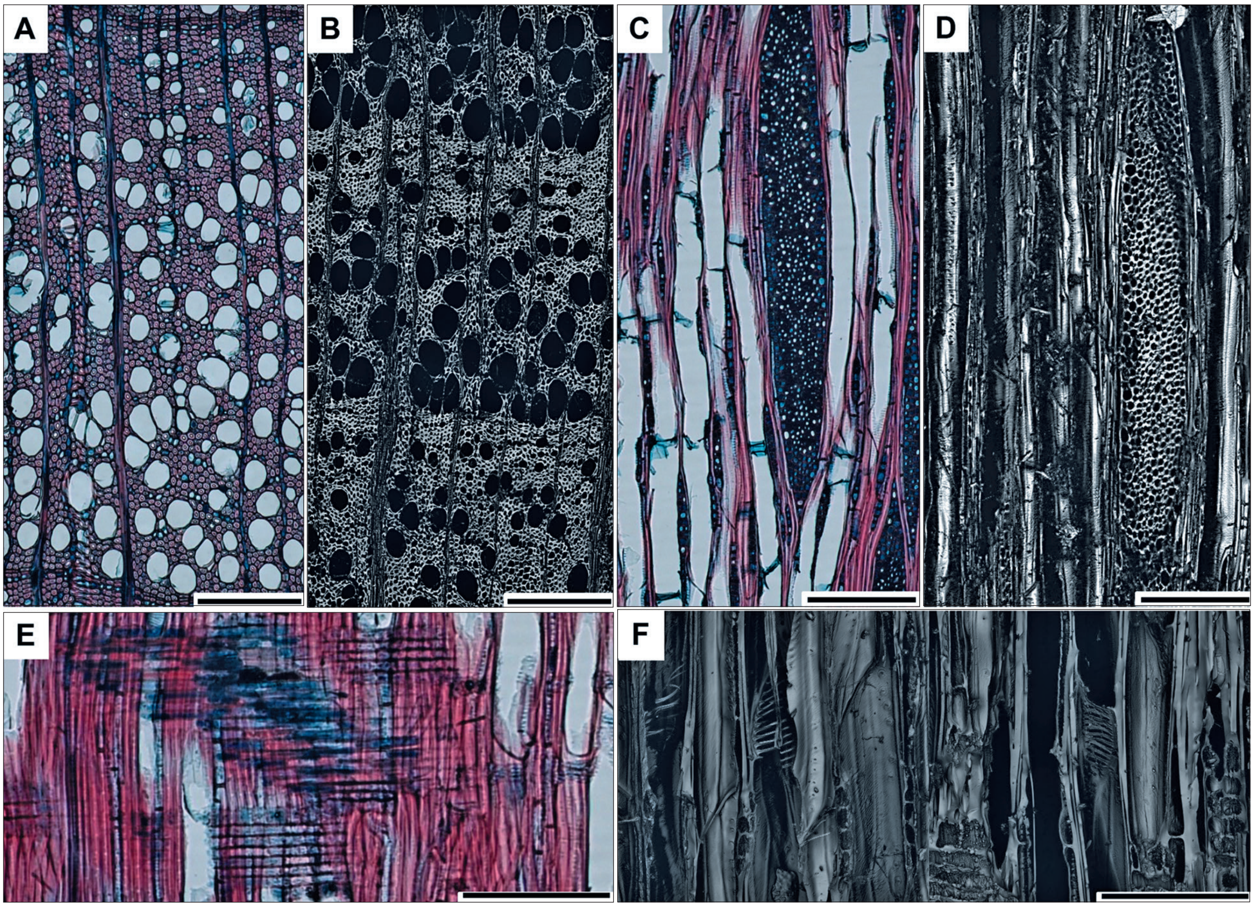

Figure 2. European beech Fagus sylvatica *(A, C, E) wood from the collection of the Department of Wood Science and Technology, light microscopy and (B, D, F) charcoal, CLSM. (A, B) Cross-sections with diffuse-porous to semi-ring-porous distribution of vessels; (C, D) tangential section with rays of two sizes; (E, F) radial section with heterogenous rays (body ray cells procumbent with generally one row of square marginal cells) and vessels with simple perforation plates. Scale bars = 250 µm.*

Slika 2. Les navadne bukve Fagus sylvatica *(A, C, E) iz zbirke Oddelka za lesarstvo; svetlobna mikroskopija in (B, D, F) oglje, tehnika CLSM. (A, B) prečna prereza, difuzen do polvenčast razpored trahej; (C, D) tangencialni prerez, dve velikosti trakov; (E, F) radialni prerez s heterogenimi trakovi (celice trakov ležeče, ob robu običajno ena vrsta kvadratnih robnih celic) in traheje z enostavnimi in lestvičastimi perforacijami. Merilne daljice = 250 µm.*

3.2 CHERRY, *Prunus avium*, ROSACEAE
3.2 ČEŠNJA, *Prunus avium*, ROSACEAE

The charcoal sample presents a cross-section with distinct growth ring boundaries and semi-ring-porous vessel distribution. Average tangential diameter of earlywood vessels is up to 70 µm. Fibre cell walls are of medium wall thickness. Axial parenchyma is absent or extremely rare (Figure 3 A, B). On the tangential section we can see mainly up to 4-seriate and 15-30 cells high rays (Figure 3 C, D). The radial section contains vessels with simple perforation plates and distinct spiral thickenings throughout the entire bodies of the vessel elements. Rays are mainly heterogenous with square cells mainly restricted to one marginal row (Figure 3 E, F). The described anatomy and good agreement with the reference material indicates that the wood belongs to cherry (*Prunus avium*) from the family Rosaceae.

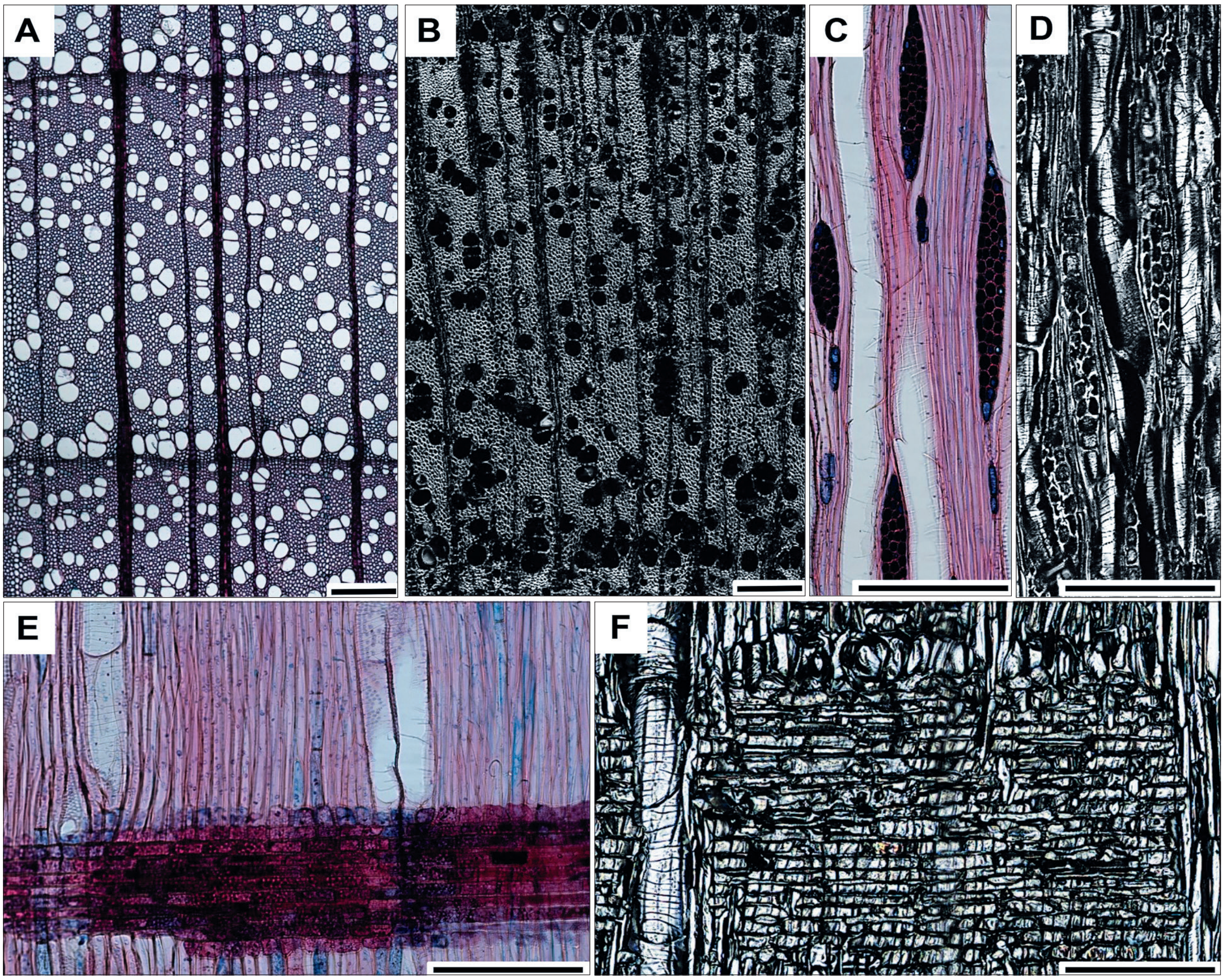

Figure 3. Cherry, Prunus avium *(A, C, E) wood from the collection of the Department of Wood Science and Technology, light microscopy and (B, D, F) charcoal, CLSM. (A, B) Cross-section of semi-ring-porous wood, vessels solitary or in small clusters; (C, D) tangential section with mainly 3-seriate rays; (E, F) radial sections with heterogeneous rays containing one marginal row of square cells, vessels with simple perforation plates and distinct spiral thickenings. Scale bars = 250 µm.*

Slika 3. Les češnje Prunus avium *(A, C, E) iz zbirke Oddelka za lesarstvo; svetlobna mikroskopija in (B, D, F) oglje, tehnika CLSM. (A, B) prečna prereza polvenčasto poroznega lesa, traheje posamezne ali v manjših skupinah; (C, D) tangencialni prerez s pretežno 3-rednimi trakovi; (E, F) radialni prerezi s heterogenimi trakovi, ki vsebujejo po eno robno vrsto kvadratastih celic, traheje z enostavnimi perforacijami in spiralnimi odebelitvami. Merilne daljice = 250 µm.*

3.3 RED OAK, *Quercus* sp., FAGACEAE
3.3 RDEČI HRAST, *Quercus* sp., FAGACEAE

The sample is ring-porous wood, vessels are exclusively solitary and in latewood arranged in diagonal and/or radial pattern. Latewood vessels are round and thick-walled. Earlywood vessels occasionally contains thin walled tyloses. Ground tissue consists of vasicentric tracheids (around the vessels) and libriform fibres of medium cell wall thickness. Axial parenchyma is predominantly apotracheal, diffuse and diffuse-in-aggregates (Figure 4 A, B). On the tangential section we see homogenous rays of two distinct sizes. They are uniseriate and multiseriate; the large rays are >10 cells wide and > 1mm high (Figure 3 C, D). Vessels have simple perforations, rays are homogeneous (Figure 3 C, D).

The described features are typical for red oaks, which are in our region (Slovenia and surrounding countries) represented by the native Turkey oak (*Q. cerris*) or by artificially introduced American red oak (*Q. rubra*). The two red oaks can be distinguished from the white oaks in the region mainly represented by the pedunculate (Q. *robur*) and sessile oak (Q. *petraea*) oak (Richter & Dallwitz, 2000; Merela & Čufar, 2013).

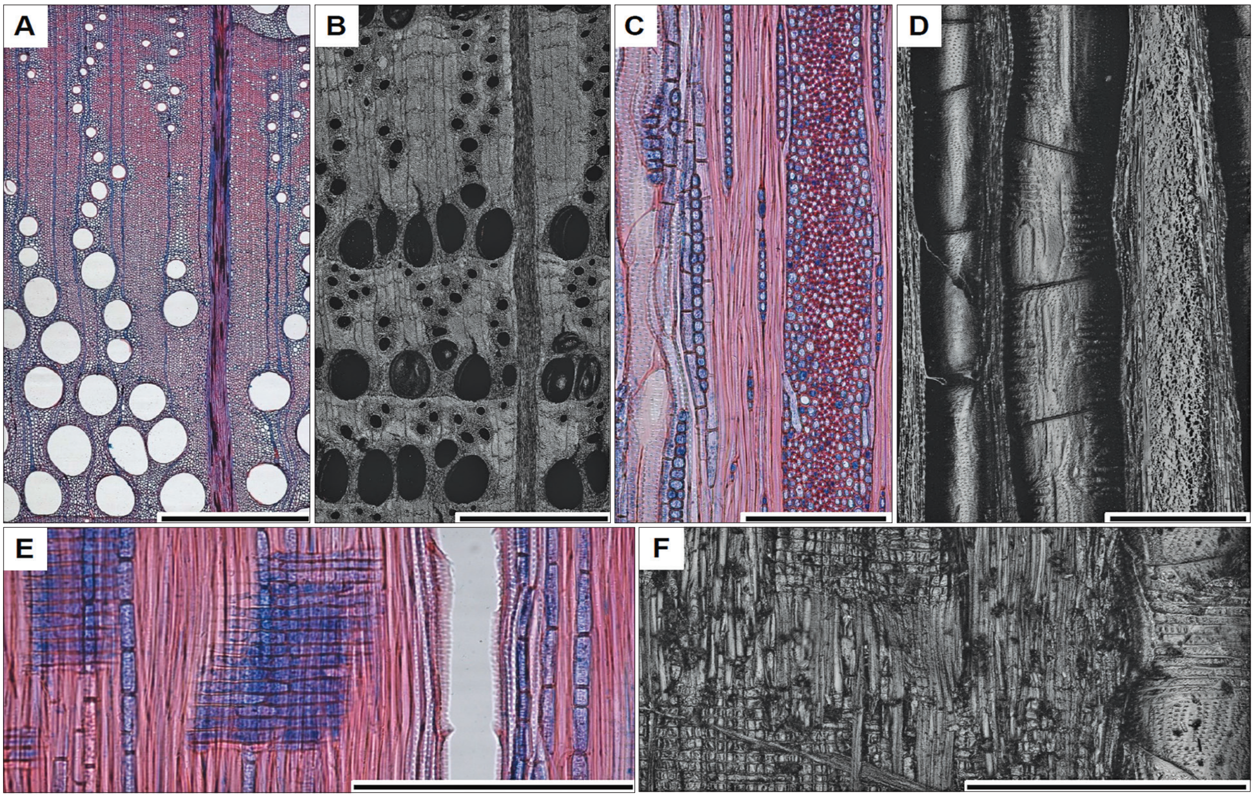

Figure 4. Red oak, Turkey oak Quercus cerris *or American red oak* Q. rubra*. (A, C, E) wood from the collection of the Department of Wood Science and Technology, light microscopy and (B, D, F) charcoal, CLSM. (A, B) cross-sections of ring porous wood with exclusively solitary vessels. In the latewood vessels are round, thick walled, and arranged in diagonal and/or radial pattern. Earlywood vessels contain few tyloses (B), ground tissue consists of tracheids (around the vessels) and fibres. Apotracheal axial parenchyma is diffuse and diffuse-in-aggregates; (C, D) tangential section with uniseriate to very large multiseriate rays; (E, F) radial section with homogeneous rays and vessels with simple perforation plates. Scale bars = 250 µm.*

Slika 4. Rdeči hrast, cer Quercus cerris *ali ameriški rdeči hrast* Q. rubra*. (A, C, E) les iz zbirke Oddelka za lesarstvo; svetlobna mikroskopija in (B, D, F) oglje, tehnika CLSM. (A, B) prečna prereza venčasto poroznega lesa z izključno posameznimi trahejami. V kasnem lesu so traheje okrogle, debelostene in diagonalno in / ali radialno razporejene. Traheje ranega lesa vsebujejo malo til (B), osnovno tkivo je sestavljeno iz traheid (okoli trahej) in vlaken. Apotrahealni aksialni parenhim je difuzen in difuzen v agregatih; (C, D) tangencialni prerez z enorednimi in velikimi trakovi; (E, F) radialni prerez s homogenimi trakovi in traheje z enostavnimi perforacijami. Merilne daljice = 250 µm.*

3.4 BLACK LOCUST, *Robinia pseudoacacia*, FABACEAE

3.4 ROBINIJA, *Robinia pseudoacacia*, FABACEAE

The wood is ring-porous with early- and especially latewood vessels arranged in clusters. Tyloses are particularly abundant in earlywood vessels. Fibres have medium thick cell walls. Axial parenchyma is paratracheal, scanty, vasicentric, and aliform (Figure 5 A, B, C). On the tangential section we can observe 3- or 4-cell wide rays, axial parenchyma and vessel elements are storied (Figure 5 D, E, F). On the radial section we can see vessels with simple perforation plates filled with tyloses and homogeneous rays (Figure 5 G, H, I). These features helped us to identify black locust, *Robinia pseudoacacia* from the family Fabaceae-Faboideae.

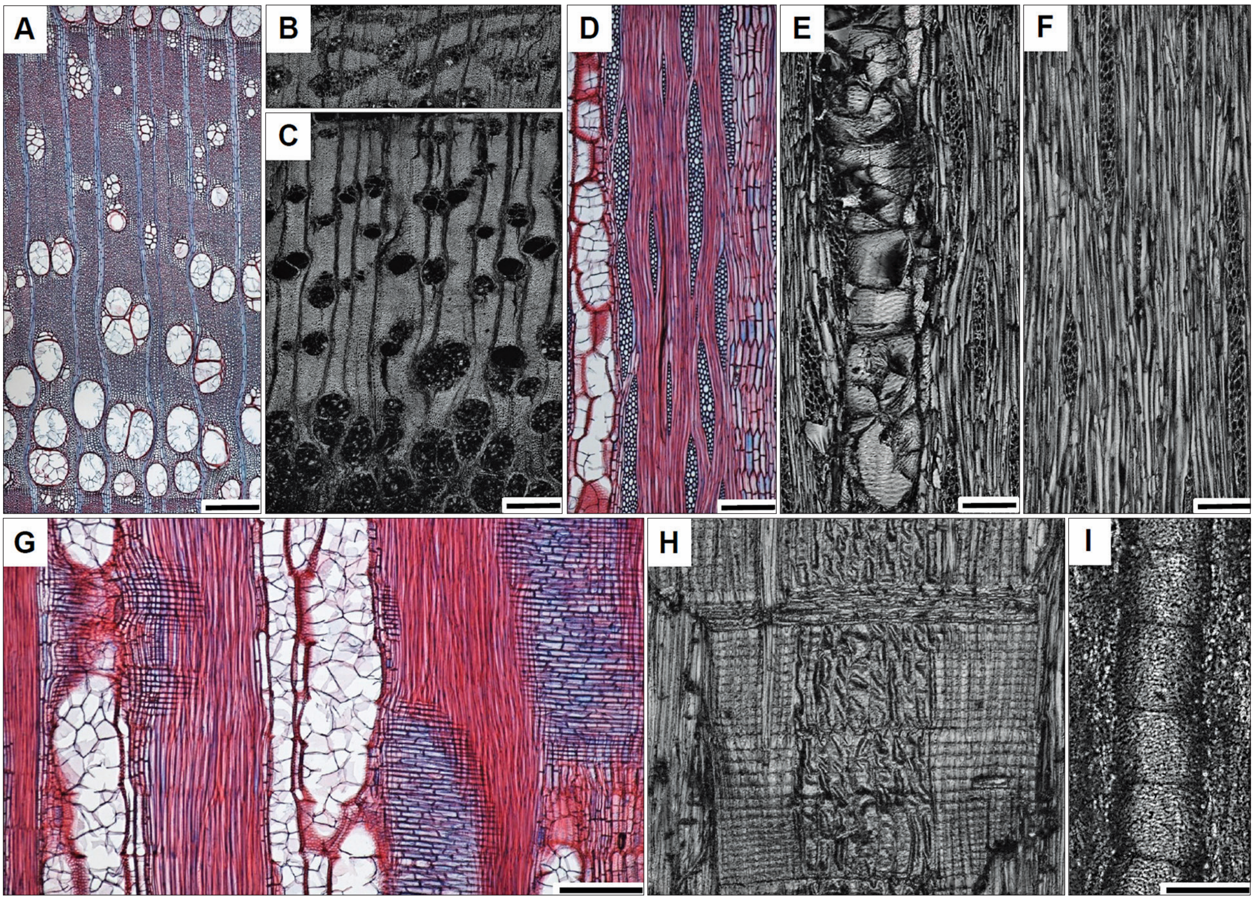

Figure 5. Black locust, Robinia pseudoacacia *(A, D, G) wood from the collection of the Department of Wood Science of Technology, light microscopy and (B, C, E, F, H, I) charcoal, CLSM. (A, B, C) Cross-section with ring porous wood, vessels arranged in clusters, earlywood vessels contain tyloses, axial parenchyma paratracheal vasicentric and aliform; (D, E, F) tangential sections with 3 or 4 seriate rays and storied axial parenchyma (D); (G, H, I) radial sections, vessels with simple perforation plates filled with tyloses and homogeneous rays. Scale bars = 250 µm.*

Slika 5. Les robinije, Robinia pseudoacacia *(A, D, G) iz zbirke Oddelka za lesarstvo; svetlobna mikroskopija in (B, C, E, F, H, I) oglje, tehnika CLSM. (A, B, C) prečna prereza venčasto poroznega lesa, traheje razporejene v gnezdih, traheje ranega lesa močno otiljene, aksialni parenhim paratrahealen, vasicentričen in krilast; (D, E, F) tangencialni prerezi s 3 - 4-rednimi trakovi in etažnim aksialnim parenhimom (D); (G, H, I) radialni prerezi, traheje z enostavnimi perforacijami, zapolnjene s tilami, trakovi homogeni. Merilne daljice = 250 µm*

3.5 CHARCOAL MADE OF WOOD OF TROPICAL OR SUBTROPICAL WOOD ORIGIN

3.5 OGLJE IZ LESA TROPSKEGA ALI SUBTROPSKEGA IZVORA

The B3 package of barbeque charcoal mainly contained charcoal made of wood species of (sub) tropical origin. We could recognise four different taxa (T1, T2, T3, T4) with several diagnostic features which do not appear in species of temperate or boreal forests. However, due to the great number and anatomical variety of subtropical or tropical species and lack of reference material, the observed fragments could not be assigned definitively to either a subtropical or tropical region. As exact identification to level of genus or species was not possible, and we only present the wood structure of four typical samples (Figures 6, 7).

In T1 and T2 we could observe all three an-

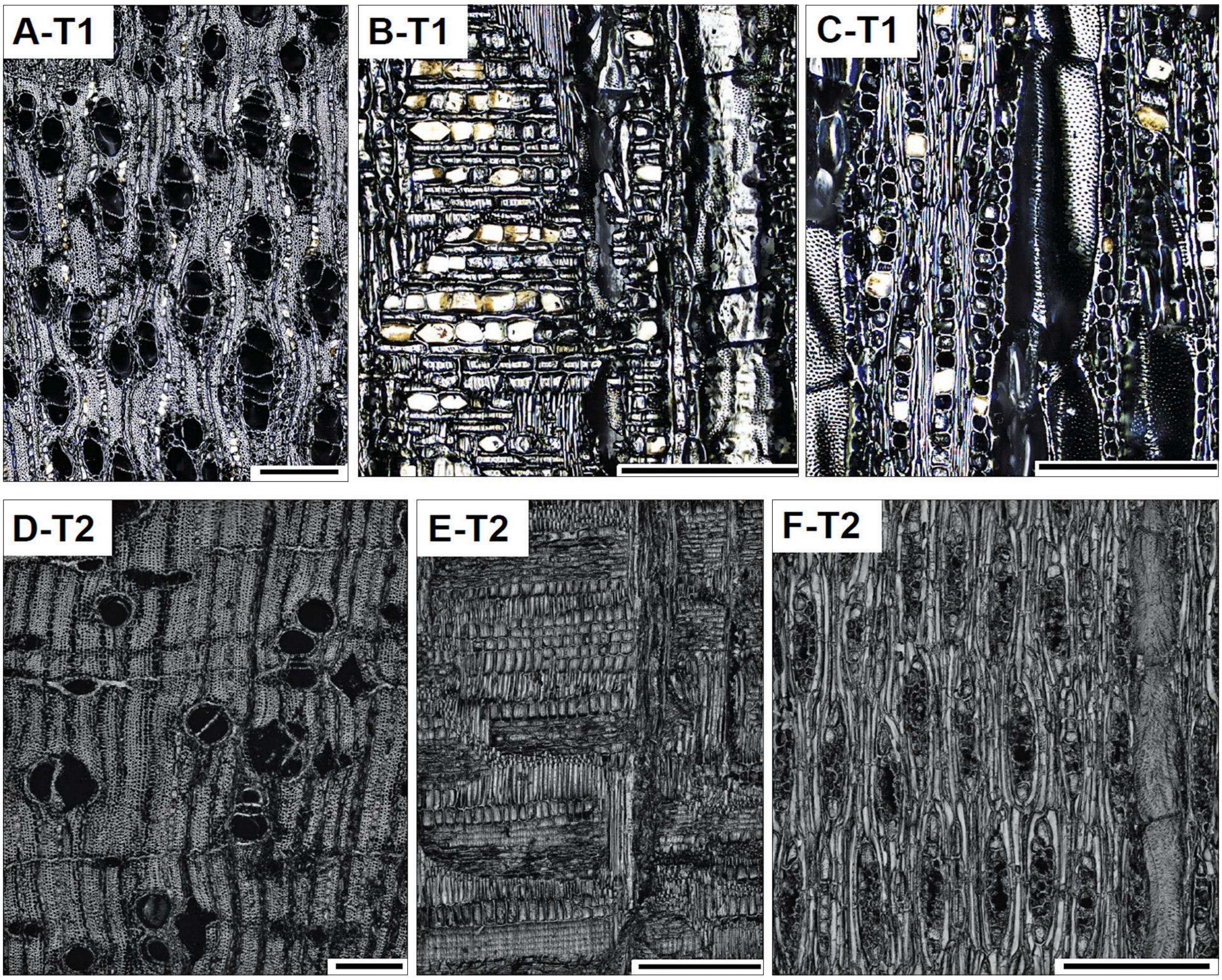

Figure 6. Diffuse porous species T1 and T2 of sub-tropical or tropical origin with diagnostically important anatomical features: (A) large vessels in radial multiples, axial parenchyma aliform, thick-walled fibres, (B) heterogenous ray with numerous crystals and vessels with simple perforations, (C) uniseriate rays, intervessel pits alternate to opposite, (D) large vessels, axial parenchyma aliform and banded, thick-walled fibres, (E) rays heterogenous, (F) rays and axial parenchyma storied. Scale bars = 250 µm.

Slika 6. Difuzno porozni (sub)tropski vrsti T1 in T2 z diagnostično pomembnimi anatomskimi znaki: (A) velike traheje v radialnih skupinah, aksialni parenhim krilast, vlakna z debelimi stenami, (B) heterogeni trak s številnimi romboidnimi kristali in stiloidi, traheje z enostavnimi perforacijami, (C) enoredni trakovi, intervaskularne piknje izmenične in nasprotne, (D) velike traheje, aksialni parenhim je krilast in v pasovih, vlakna z debelimi stenami, (E) heterogeni trakovi, (F) trakovi in aksialni parenhim v etažah. Merilne daljice = 250 µm

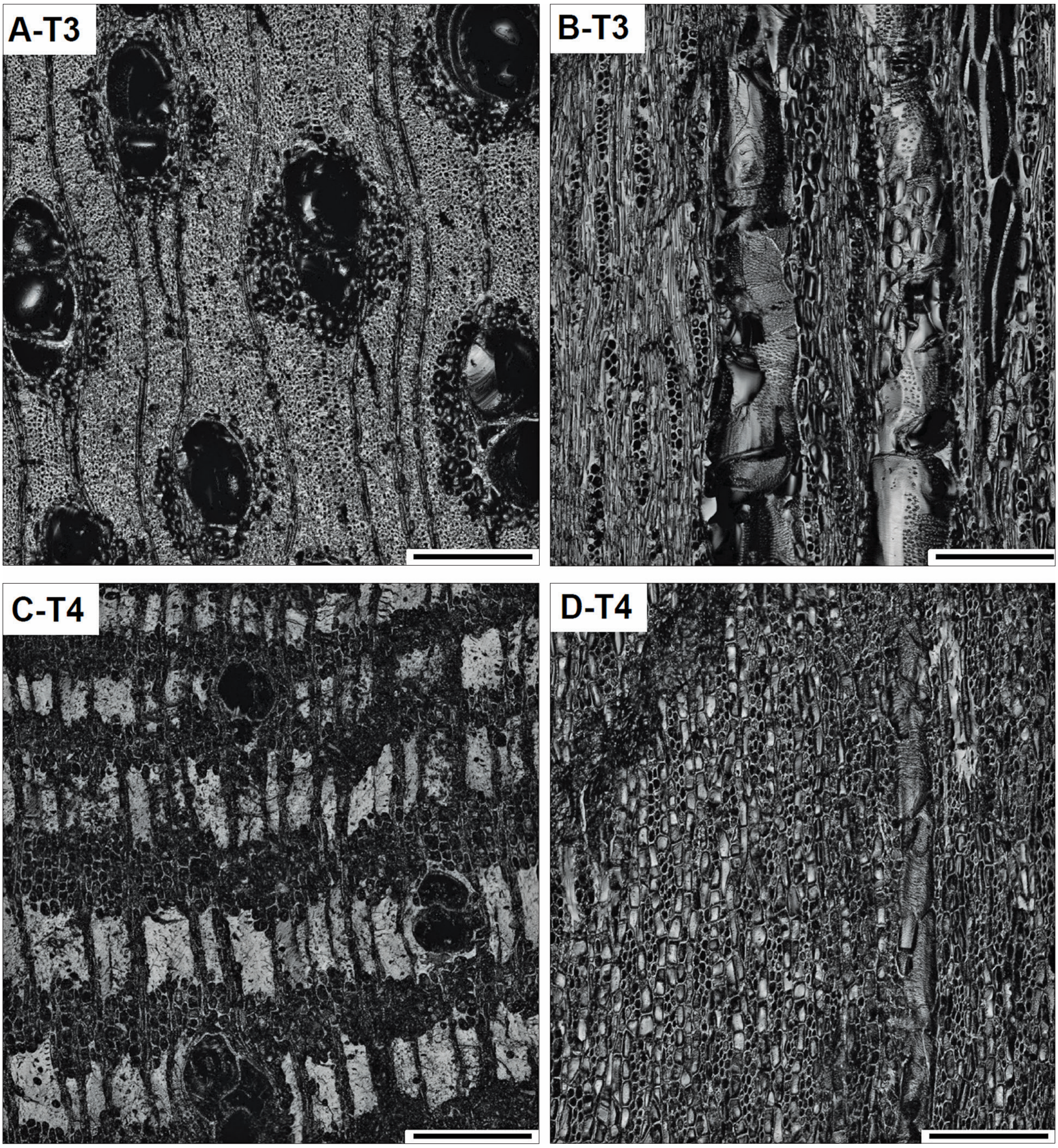

Figure 7. Species of sub-tropical or tropical origin T3 and T4 with diagnostically important anatomical features: (A) large vessels filled with tyloses, axial parenchyma aliform - lozenge, thick-walled fibres, (B) rays homogenous mainly 2 cells wide, vessels with simple perforations and intervessel pits alternate, (C) large vessels, axial parenchyma banded, bands more than 3 cells wide, fibres very thick walled, (D) rays mainly 2 or 3 cells wide. Scale bar= 250 µm.

Slika 7. Difuzno porozni (sub)tropski vrsti T3 in T4 (sub)tropskega ali tropskega izvora z diagnostično pomembnimi anatomskimi znaki: (A) velike traheje zapolnjene s tilami, aksialni parenhim krilast – romboidne oblike, vlakna z debelimi stenami, (B) homogeni trakovi široki pretežno 2 celici, traheje z enostavnimi perforacijami in intervaskularne piknje izmenične, (C) velike traheje, aksialni parenhim v pasovih, širokih več kot 3 celice, vlakna debelostena, (D) trakovi v glavnem široki 2-3 celice. Merilne daljice = 250 µm atomical sections. T1 (Figure 6 A, B, C) has large vessels in radial multiples, paratracheal aliform axial parenchyma, thick-walled fibres, heterogenous rays with numerous prismatic and elongated styloid crystals and vessels with simple perforations. Rays are uniseriate, intervessel pits are alternate to opposite.

T2 also has large vessels, axial parenchyma is paratracheal aliform and banded, fibres are thick walled, rays are heterogenous and 2 or 3 cells wide. Rays and axial parenchyma are storied (Figure 6 D, E, F). According to the recognised features the wood resembles *Dicorynia guianensis* (Angélique, basralocus), Fabaceae-Caesalpinioideae family with geographical distribution in tropical South America.

On samples T3 and T4 we could only observe transverse and tangential surfaces. Both species are diffuse porous with large vessels and other features indicating sub-tropical or tropical origin (Figure 7). T3 has large vessels filled with tyloses, axial parenchyma aliform - lozenge, thick-walled fibres, homogenous rays are mainly 2 cells wide, vessels have simple perforations and intervessel pits alternate (Figure 7 A, B). The structure can be described as *Afzelia*-like wood type. *Afzelia* spp. (Afzelia, doussie), Fabaceae-Ceasilpinoidae, from tropical Africa.

T4 has large vessels and banded axial parenchyma with bands more than 3 cells wide and thick-walled fibres (Figure 7 C, D). Rays are mainly 2 or 3 cells wide. The wood could be described as *Lophira alata* like type. *Lophira alata* (azobe, bongossi), Ochnaceae, grows in tropical Africa. Haag et al. (2020) described wood with similar vessel and axial parenchyma characteristics as *Milletia type* wood, however in their case the wood also had storied rays which were not found in our case.

The attribution of four hardwoods to (sub) tropical origin is assumed on a combination of typical wood anatomical features which do not appear in temperate or boreal species (e.g., diffuse distribution of very large vessels, no clear growth rings, no early- and late- wood, abundant paratracheal axial parenchyma, storied structures). Lack of features like colour, sapwood/heartwood differentiation, density, odour and potential deformation or loss of minute structures hamper more precise identification.

4 DISCUSSION AND CONCLUSIONS
4 RAZPRAVA IN ZAKLJUČKI

As previously demonstrated, CLSM enables the recognition of features crucial for wood identification on various objects (Balzano et al., 2019; Čufar et al., 2019), and this study confirms that the method is also useful for identification of charcoal wood without special sample preparation.

Regarding the origin of wood species and the issue of traceability, our study shows that the charcoal from two retailers, products B1 and B2, presumably originated in Serbia. Product B1 proved to be exclusively made of beechwood, as declared by the label on the package, which also enabled traceability. Product B2 contained red oak (*Q. cerris* or *Q. rubra*), black locust (*Robinia pseudoacacia*) and cherry (*Prunus avium*). The species grow in Serbia and are not tropical, therefore the statement on the label “made in Serbia” and “no tropical wood” can be confirmed. However, the declaration did not enable traceability of wood source.

The declaration on product B3 from the big international discount supermarket chain only reported that charcoal was produced for the given chain. However, the content of at least four tropical species revealed that we were dealing with a product of the global market, where the country of wood origin is not known and the possibility of illegal logging cannot be excluded.

Charcoal is generally considered of high quality if it is dry, solid, with pieces of uniform size, does not crumble excessively, emits heat evenly and burns (or smoulders) with a small amount of smoke (Stare & Prislan, 2020). The European standard SIST EN 1860-2: 2005 defines precise criteria for the quality of charcoal for grilling and contains five key quality indicators: (1) charcoal humidity (as low as possible or ≤ 8%), (2) quantity of ash, (3) volatile compound content (related to the amount of smoke,) (4) the amount of bound carbon (proportional to the amount of heat energy emitted per unit weight of coal and longer and cleaner combustion), and (5) the particle size of the charcoal.

Stare and Prislan (2020) tested charcoal from 11 distributers in Slovenia and found that only the Slovenian product from the “oglarska dežela”, or “charcoal country”, had adequate quality. This product is probably comparable to our B1 charcoal made of beechwood. Our product B2 contained a lot of dust, especially because oak (due to its wood structure) typically disintegrated into lamella like particles. Our B3 was a low-price product from a big supermarket chain; another product from the same chain that was tested for charcoal quality was found not to meet the standard EN 1860-2 (2005) (Stare & Prislan, 2020).

The identification of four European and four (sub)tropical wood species and incomplete information on wood origin shown in this study are in line with the situation observed in other European countries. Therefore, the general public and professionals should be notified that wood from commercial products that is sold without proper declarations cannot be traced and may originate from illegal logging in distant areas. As such, we support the initiative that charcoal trading should be controlled and included in the corresponding annex of the EU Timber Regulation (EUTR).

5 SUMMARY

5 POVZETEK

Z uporabo laserske konfokalne vrstične mikroskopije (Confocal Laser Scanning Microscopy – CLSM) smo raziskali lesno oglje za žar. Preverili smo uporabnost metode za prepoznavanje anatomskih znakov lesnih vrst, iz katerih je oglje narejeno. Želeli smo ugotoviti, katere lesne vrste vsebuje oglje v prodaji, če je les lokalnega izvora ali uvožen in če izvira tudi iz gozdov (sub)tropskega pasu. Zanimalo nas je tudi, kakšne podatke vsebujejo navedbe (deklaracije) na proizvodih. Preverili smo pomen identifikacije lesa oglja za ugotavljanje izvora lesa in ocenili, če je tudi v Sloveniji morda naprodaj oglje iz ilegalno posekanega tropskega lesa.

Raziskali smo 3 vreče oglja iz treh trgovin. Prvi izdelek (B1) je bil kupljen v majhni trgovini z vrtnarskimi potrebščinami na Hrvaškem. Na embalaži je bil naveden točen naslov proizvajalca iz Srbije, kar omogoča sledljivost izvora lesa. Izdelek B2 smo kupili v manjši trgovini v Sloveniji. Na embalaži je bilo navedeno, da izdelek ne vsebuje tropskega lesa in da je bil narejen v Srbiji. Vreča B3 je bila kupljena v Sloveniji, v diskontni trgovini velike mednarodne verige, ki upravlja trgovine po Evropi in ZDA. Napis na embalaži je navajal samo, da je izdelek narejen za omenjeno verigo.

Oglje vsake posamezne vreče smo pregledali pod lupo. Za podrobnejši pregled smo izbrali najprimernejše kose, ki so po možnosti vsebovali zrel (adulten) les debla in jih je bilo mogoče pripraviti za opazovanje zgradbe lesa na vseh treh anatomskih ravninah. Izogibali smo se lesu drobnih vej in juvenilnemu lesu. Izbrane vzorce smo razlomili ali razcepili z nožem, da smo lahko opazovali prečno, radialno in tangencialno lomno površino.

Razlomljeno oglje smo postavili v petrijevko s kuhinjsko soljo in ga pregledali s konfokalnim vrstičnim mikroskopom (Confocal Laser Scanning Microscope, CLSM) Olympus LEXT OLS5000 (Olympus Corporation Tokyo 163-0914, Japonska) z objektivi: MPLFLN5x (numerična odprtina 0,15, delovna razdalja 20 mm), MPLFLN10xLEXT (numerična odprtina 0,3, delovna razdalja 10,4 mm) in LMPLFLN20XLEXT (numerična odprtina 0,45, delovna razdalja 6,5 mm) (slika 1). S premikanjem mizice smo z optičnim sistemom najprej pridobili panoramsko sliko za pregled celotne površine. Poiskali smo ključne lesnoanatomske identifikacijske znake in s pomočjo funkcije sestavljanja slik zajeli laserske konfokalne slike visoke ločljivosti z veliko globinsko ostrino. Meritve dimenzij anatomskih znakov smo izvedli s programom za analizo slike OLS5000.

Identifikacijo lesa smo opravili s pomočjo različnih identifikacijskih ključev in specialne lesno anatomske literature (npr. Grosser, 1977; Schweingruber, 1978, 1990; Wagenführ, 1996, 1999; Schoch et al., 2004; Čufar & Zupančič, 2009; Signorini et al., 2014). Za mikroskopsko in makroskopsko identifikacijo lesa listavcev in lesnih vrst s seznama CITES smo uporabili program DELTA / INTKEY (Richter & Trockenbrodt, 1995; Richter & Dallwitz; Richter et al., 2002, 2005) in spletni računalniški ključ InsideWood (InsideWood, 2004; Wheeler, 2011), ki temelji na naboru mikroskopskih znakov za identifikacijo lesa listavcev (IAWA committee, 1989). V pomoč nam je bil tudi programski paket za makroskopsko identifikacijo lesa na italijanskem trgu (Ruffinatto et al., 2017, 2019) in atlas za makroskopsko identifikacijo lesa (Ruffinato & Crivellaro, 2019). Kot referenčni material smo uporabili les in mikroskopske preparate iz zbirke na Oddelku za lesarstvo. Referenčne preparate smo opazovali s svetlobnim mikroskopom Nikon Eclipse E 800, mikrofotografije pa smo posneli z digitalno kamero Nikon DS-fi1 in programom za analizo slike NIS Elements Br 3.0.

Predstavljena študija je ena prvih raziskav oglja z novo mikroskopsko metodo CLSM, ki se je izkazala za primerno, čeprav se zgradba lesa zaradi visokih temperatur in karbonizacije med izdelavo oglja spremeni. Domače vrste smo lahko natančno identificirali, tudi zato, ker jih dobro poznamo in imamo zanje na razpolago referenčni material. V oglju so bile zastopane tudi vrste, ki rastejo v subtropskem in tropskem pasu.

Oglje dobavitelja B1 je bilo narejeno iz lesa bukve (*Fagus sylvatica*) (slika 2, preglednica 1). Prodajalna na Hrvaškem, kjer smo ga kupili, že leta ponuja oglje istega manjšega proizvajalca iz Srbije, ki slovi po visoki kakovosti. Na embalaži je bil natančen naslov proizvajalca, kar omogoča sledljivost. Bukev je tradicionalna in najbolj cenjena vrsta za oglje v Sloveniji in širši regiji (Brus & Jarni, 2012; Cimperšek, 2012).

V vreči B2 smo identificirali les enega od rdečih hrastov, cera ali ameriškega rdečega hrasta (Q. *cerris* ali Q. *rubra*), robinije (*Robinia pseudoacacia*) in češnje (*Prunus avium*). Vse vrste, ki jih podrobno predstavljamo (slike 3, 4, 5), uspevajo tudi v Srbiji, kar je v skladu z napisom na embalaži „narejeno v Srbiji". Napis na embalaži tudi navaja, da izdelek ne vsebuje tropskega lesa, kar smo potrdili. Na embalaži ni podatkov o proizvajalcu.

V vreči B3 iz trgovine velike mednarodne diskontne verige je bilo oglje vsaj štirih tropskih vrst neznanega izvora, na embalaži pa je bilo navedeno samo, da je izdelek narejen za omenjeno trgovsko verigo. Da gre za les (sub)tropskih vrst (slika 6, 7) smo ugotovili na osnovi znakov, ki jih naše domače vrste iz zmerno celinskega ali hladnega podnebnega pasu nimajo. Preiskane (sub)tropske vrste so difuzno porozni listavci z zelo velikimi trahejami in obilnim paratrahealnim krilastim ali trakastim aksialnim parenhimom. Pri nobeni nismo zaznali jasno vidnih prirastnih plasti (branik in letnic) ter ranega ali kasnega lesa. Dve vrsti sta imeli aksialne elemente razporejene v etažah. Ugotovljeni znaki niso zadoščali, da bi natančno določili vrsto, rod ali regijo izvora, saj je nabor (sub)tropskih vrst izredno velik, lesnoanatomske razlike med vrstami in rodovi pa so pogosto majhne. Pri identifikaciji smo pogrešali znake kot so barva, razlika med beljavo in jedrovino, vonj in gostota, ki so se izgubili pri predelavi lesa v oglje. Glede na navedeno smo lahko samo navedli znane tropske vrste, ki imajo podobno zgradbo kot raziskani les.

Raziskana vrsta T1 (slika 6 A, B, C) ima diagnostično pomembne znake: velike traheje v radialnih skupinah, aksialni parenhim krilast, vlakna z debelimi stenami, heterogeni trakovi s številnimi romboidnimi kristali in stiloidi, traheje z enostavnimi perforacijami, enoredni trakovi, intervaskularne piknje izmenične in nasprotne.

Vrsta T2 je po naboru znakov podobna lesu vrste *Dicorynia guianensis* (Angélique, basralocus) iz družine Fabaceae-Caesalpinioideae, ki uspeva v tropski južni Ameriki. T3 ima podobno zgradbo kot vrste iz rodu *Afzelia* sp. (afzelia, doussie), Fabaceae-Ceasilpinoidae iz tropske Afrike. T4 pa je podoben vrsti *Lophira alata* (azobe, bongossi), Ochnaceae iz tropske Afrike.

Pri ugotovljenem naboru lesnih vrst in popolnem pomanjkanju navedb o izvoru lesa, je B3 tipičen izdelek globalne trgovine, kjer sledljivost ni mogoča. Tovrstni izdelki so pogosto tudi slabše kakovosti, kar sta pokazala Stare in Prislan (2020), ki sta preiskala izdelek iz iste verige kot je B3 in njegova kakovost ni bila skladna s standardom EN 1860-2 (2005).

V glavnem velja, da je kakovostno oglje suho, trdno, s kosi enakomerne velikosti in se pretirano ne drobi, toploto oddaja enakomerno in gori (oziroma tli) z zelo malo dima (Stare & Prislan, 2020). Pri tem Evropski standard SIST EN 1860-2:2005 opredeljuje natančna merila za kakovost oglja za žar in navaja pet ključnih kazalnikov z vidika: (1) vlažnosti oglja (čim nižja ali ≤ 8 %), (2) količine pepela, (3) vsebnosti hlapnih snovi (povezane s količino dima), (4) količine vezanega ogljika (sorazmerno s količino oddane toplotne energije na enoto teže oglja ter daljšim in čistejšim izgorevanjem) in (5) velikosti delcev oglja. Stare in Prislan (2020) ugotavljata, da je imelo kakovost skladno s standardom samo slovensko oglje iz oglarske dežele, ki je po naši oceni primerljivo z našim izdelkom B1. Čeprav vrsta lesa za oglje ni vključena v kriterije standarda, se v Sloveniji pričakuje, da je najboljše oglje iz bukovine. Ugotovljeni širok nabor domačih in tropskih vrst v preiskanih izdelkih s polic trgovin zato nakazuje, da bi bilo treba splošno in strokovno javnost vključno z nadzornimi službami v Sloveniji in širši regiji ozavestiti, da tudi v primeru oglja pomanjkanje sledljivosti povečuje nevarnost nedovoljene trgovine z lesom. Na osnovi ugotovitev raziskave se pridružujemo pozivom obsežnejših projektov v Evropi, da bi bilo tudi trgovino z ogljem treba nadzorovati v skladu z Uredbo EU o lesu (EUTR).

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