

WOOD IDENTIFICATION USING NON-DESTRUCTIVE CONFOCAL LASER SCANNING MICROSCOPY

IDENTIFIKACIJA LESA S POMOČJO NEDESTRUKTIVNE KONFOKALNE LASERSKE MIKROSKOPIJE

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Abstract / Izvleček

Abstract: Exact wood identification is usually based on observation of wood features under the microscope. For this, we have to take a sample of the wood from the object and cut thin slides, possibly of all three anatomical sections. Such destructive sampling is often not possible on valuable historical objects, and therefore there is a need for non-destructive approaches. The objective of the study is to present the potential of Confocal Laser Scanning Microscopy (CLSM) using an Olympus LEXT OLS5000 for the identification of wood. We present work on an example of a gothic sculpture, "St. George Defeating the Dragon". Conventional sampling and microscopical wood identification showed that St. George is made of Norway spruce (*Picea abies*), and the dragon of poplar (*Populus sp.*) or willow (*Salix sp.*). We present crucial features needed for the identification of these species and the limitations with identification if the samples are too small. Finally, we demonstrate the possibility of wood identification of the abovementioned species using CLSM on wood samples without special preparation of the surfaces. CLSM enabled us to observe all the features needed for wood identification.

Keywords: confocal laser scanning microscopy (CLSM), wood anatomy, wood identification, *Picea abies*, *Populus sp.*, *Salix sp.*

Izvleček: Natančna identifikacija lesa običajno temelji na opazovanju anatomskih znakov pod mikroskopom. Za to moramo odvzeti vzorec lesa in narezati tanke preparate, po možnosti vseh treh anatomskih prerezov. Takšno destruktivno vzorčenje na dragocenih zgodovinskih predmetih pogosto ni sprejemljivo, zato iščemo nedestruktivne pristope. Cilj te študije je predstaviti možnosti identifikacije lesa s pomočjo konfokalnega laserskega mikroskopa (CLSM) Olympus LEXT OLS5000. Identifikacijo predstavljamo na primeru gotskega kipa svetega Jurija z zmajem. Konvencionalno vzorčenje in mikroskopska identifikacija lesa sta pokazala, da je sveti Jurij narejen iz lesa navadne smreke (*Picea abies*), zmaj pa iz lesa topola (*Populus sp.*) ali vrbe (*Salix sp.*). Predstavljamo ključne značilnosti, potrebne za identifikacijo naštetih vrst, in omejitve identifikacije, če so vzorci premajhni. Na koncu pokažemo možnost identifikacije lesa zgoraj omenjenih vrst s CLSM na vzorcih lesa brez posebne priprave površine. CLSM nam je omogočil opazovanje vseh anatomskih znakov, potrebnih za identifikacijo lesa.

Ključne besede: konfokalna laserska vrstična mikroskopija (CLSM), anatomija lesa, identifikacija lesa, *Picea abies*, *Populus sp.*, *Salix sp.*

1 INTRODUCTION

1 UVOD

Art objects made of wood represent an important part of our cultural heritage. For their optimal conservation, restoration, and valuation, it is important to know from which wood and how they were made. For wood identification, we can apply traditional macroscopic and microscopic methods (Čufar & Zupančič, 2000).

Macroscopic identification is more or less non-destructive and is mainly based on features which can be observed with the naked eye or simple magnification lens (e.g., Wagenführ, 1999; Richter et al., 2002; Ruffinatto et al., 2019). Such a methodology has its limitations, however, as the number of wood characteristics which can be recorded is relatively small, and some of them, like colour and odour, change or even disappear with ageing. The use of macroscopic identification keys may sometimes lead to subjective decisions or even wrong identification (Haag et al., 2018).

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Microscopic wood identification is more reliable, especially in the case of aged or painted wooden objects. However, this method is not non-destructive, as we need to collect an oriented piece of wood from the object and cut thin cross, radial and tangential sections. When the sections are made, identification is based on observation of wood features under the microscope.

For identification of softwoods and hardwoods, we dispose with numerous identification keys. If we know the origin of the wood, for instance Europe, we can use 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; Ruffinatto et al., 2017).

More universal are multiple entry keys that enable identification based on searching for the presence or absence of wood features. The International Association of Wood Anatomists (IAWA) organised committees to define the lists of microscopic features for hardwood (IAWA committee, 1989) and softwood (IAWA committee, 2004) identification. The lists enabled the development of widely used computer added identification tools like the publicly available InsideWood (InsideWood, 2004 onwards) which is also an indispensable resource of information on hardwood anatomy (Wheeler, 2011). There are also identification keys supported by the DELTA/INTKEY program package (Richter & Trockenbrodt, 1995) for microscopic identification of commercial hardwoods (Richter & Dallwitz, 2000), microscopic identification of softwoods (Richter, personal communication) and CITESwoodID for identification of CITES protected trade timbers (Richter et al., 2005).

Exact wood identification is therefore based on observation of wood features on microscopic sections under the microscope. The sections are cut from samples taken from the objects. With regard to valuable historical objects, such destructive sampling is usually not possible, and thus there is a need for less destructive or if possible non-destructive approaches.

The recent development of non-destructive methods has helped to study valuable (historical) objects, where destructive sampling of wood specimens is not possible. Such methods have been, for example, used to study the wood of historical musical instruments (Fioravanti et al., 2016; Haag et al., 2018), bows of historical stringed instruments

(Fioravanti et al., 2017) and wood species of historic gala berlines (Giulio, et. al., 2019). Different pieces of equipment have been used for this, like the portable digital microscopes Dino-lite pro AD413T and AM4113ZT4 (Fioravanti et al., 2016), Dino-lite and synchrotron light X-ray CT in phase-contrast mode (Fioravanti et al., 2017), or digitised image microscope analysis systems Cell[^]F[®], Olympus and KEYENCE[®] VHX-5000 (Haag et al., 2018).

In the present study, we present the possibility to use a Confocal Laser Scanning Microscope (CLSM) Olympus LEXT OLS5000 for wood identification on the example of a gothic statue of St. George from Ptuj, Slovenia. We compared wood identification based on light microscopy of sections obtained from tiny wood samples taken from the statue, on conventional (large) sections from the slide collection, and wood identification using CLSM on the samples of the same wood species without special surface preparation.

2 MATERIALS AND METHODS

2 MATERIAL IN METODE

2.1 MATERIAL

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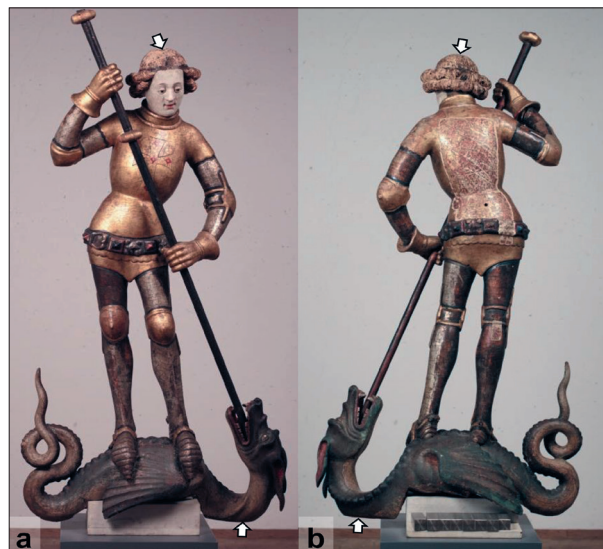


Figure 1. Gothic statue of St. George defeating the dragon. Arrows show the locations where small samples of wood were taken for microscopic wood identification.

Slika 1. Gotski kip svetega Jurija z zmajem. Puščice prikazujejo mesto na glavi svetnika in mesto na spodnji strani zmaja, kjer sta bila odvzeta majhna vzorca lesa za mikroskopsko identifikacijo lesa.

The main church in Ptuj holds the statue of the patron saint St. George defeating a dragon (Figure 1), presumably made between 1370-1380 AD (Vnuk, 2013, 2019).

The statue was presented at the exhibition “A knight, a lady and a dragon – the heritage of medieval warriors” in the National Museum of Slovenia in Ljubljana from December 2012 until February 2014 (Vnuk, 2013). After the exhibition it was possible to study the statue to identify the wood it is made of.

2.2 SAMPLE PREPARATION, LIGHT MICROSCOPY, AND WOOD IDENTIFICATION

2.2 PRIPRAVA VZORCEV, SVETLOBNA MIKROSKOPIJA IN IDENTIFIKACIJA LESA

We examined the statue in the restoration workshop. Together with the restorers, we defined the sites where the tiny samples of the wood could be taken for research. We took two samples of minimal dimensions, one from the head of St. George and one from the dragon (Figure 1).

The sample taken from the head of the statue of St. George was an oriented splinter (ca. 3 × 3 × 7 mm) taken from a location where the head was previously damaged. The splinter was further oriented and fixed into a holder of the Leica SM

2000 R slide microtome (Figure 2a), where we cut 20 µm thick slices of cross and tangential sections, as the amount of wood collected was not sufficient to obtain the radial section as well. The slices were stained with safranin and astra blue, dehydrated and embedded in Euparal (Čufar & Zupančič, 2000; Prislan et al., 2009). Thus, permanent slides (Figure 2b) were prepared.

From the dragon, we cut three shavings using a scalpel and obtained thin slices of approximate size 7 × 9 × 0.3 mm, which were immediately put on a glass slide and embedded into glycerol without staining. The slices only contained the cross-section of the wood.

All slides were observed with a Nikon Eclipse E 800 light microscope. Micrography was performed with a Nikon DS-fi1 digital camera and NIS Elements Br 3.0 software. Microscopic identification of wood was performed with keys for the microscopic identification of softwoods and hardwoods (Grosser, 1977; Richter & Dallwitz, 2000; Schoch et al., 2004).

For wood identification, we also observed the reference material, the slides of the same species from the slide collection of the Department of Wood Science and Technology, for comparison.

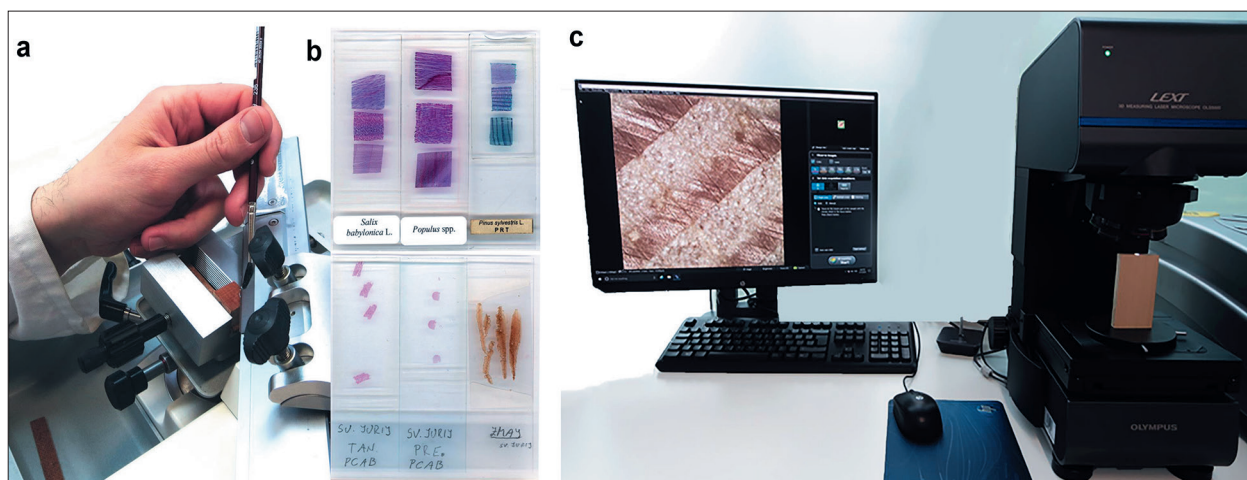


Figure 2. Laboratory work: (a) cutting of thin sections with a sliding microtome, (b) slides from the statue and from the collection prepared for light microscopy, (c) confocal laser scanning microscope LEXT OLS5000 3D (Olympus Corporation Tokyo 163-0914, Japan) equipped with an optical system for colour observation with an oriented sample of wood from the collection.

Slika 2. Delo v laboratoriju: (a) rezanje tankih preparatov z drsnim mikrotomom, (b) preparati iz kipa in iz zbirke, pripravljeni za opazovanje pod svetlobnim mikroskopom in (c) konfokalni laserski mikroskop LEXT OLS5000 3D (Olympus Corporation Tokyo 163-0914, Japonska), opremljen z optičnim sistemom za opazovanje barvne slike z orientiranim vzorcem lesa iz zbirke.

2.3 CONFOCAL LASER SCANNING MICROSCOPY 2.3 KONFOKALNA LASERSKA MIKROSKOPIJA

We took samples from the wood collection of the Department of Wood Science and Technology to check if the features crucial for the identification of wood from the statue could be observed with Confocal Laser Scanning Microscope (CLSM) (Figure 2c). The wood was observed with a CLSM Olympus LEXT OLS5000 (Olympus Corporation Tokyo 163-0914, Japan) with the following objectives: MPLFLN5x (numerical aperture 0.15, working distance 20 mm), MPLFLN10xLEXT (numerical aperture 0.3, working distance 10.4 mm), and MPLAPON20xLEXT (numerical aperture 0.6, working distance 1 mm).

We used the optical system for colour observation. The system acquired microscope colour images by illuminating the sample with the white light-emitting diode (LED) light source and imaging the reflected light with the complementary metal-oxide semiconductor (CMOS) image sensor.

The wood objects without special surface preparation were placed on the stage. Moving the stage, we selected the position for imaging. As the focal depth of the colour images was relatively deep, we could enlarge the colour images to observe the detailed wood anatomy features or to define the positions for detailed observations using the polarised light.

3 RESULTS AND DISCUSSION 3 REZULTATI IN RAZPRAVA

Due to the small dimensions of the wood sample taken from the head of the statue, the obtained cross-section only contained part of one tree-ring with earlywood and some tracheids indicating a gradual transition to latewood (Figure 3a). The section contained no axial resin canals. However, the tangential section contained radial resin canals with thick-walled epithelial cells (Figure 3b). Assuming that the wood of the sculpture possibly originated from the local forests (Slovenia and its surroundings), the features lead us to the conclusion that the wood is Norway spruce (*Picea abies*).

In the optimal case the structure of Norway spruce should be observed on all three anatomical sections (transversal, tangential, radial) to observe the key features of *Picea abies* including the gradual transition from early to latewood, axial and radial resin canals with thick-walled epithelial cells, heterocellular rays with radial tracheids and ray parenchyma cells containing piceoid cross-field pits, as well as the presence of multiseriate rays with radial resin canals and the predominating uniseriate rays (Figure 4).

Observation with CLSM on a parallel sample of *Picea abies* wood showed that the abovementioned key features can be observed on the wood surface without time-consuming sample preparation (Figure 5).

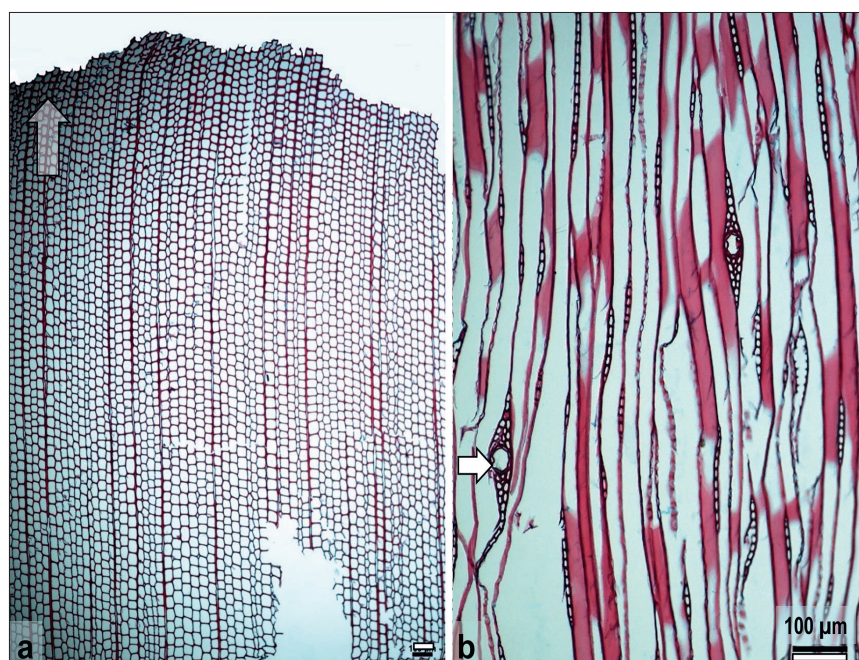


Figure 3. Sections obtained from a wooden splinter of the St. George statue with the (a) cross-section and (b) tangential section indicating the wood of Norway spruce (*Picea abies*). Light microscopy – bright field, safranin, and astra blue staining. Scale bars – 100 μm .

Slika 3. Prerezi lesa vzorca iz glave kipa svetega Jurija s (a) prečnim prerezom in (b) tangencialnim prerezom, ki nakazuje les navadne smreke (*Picea abies*). Svetlobni mikroskop - svetlo polje, obarvanje safranin in astra modro. Merilne daljice - 100 μm .

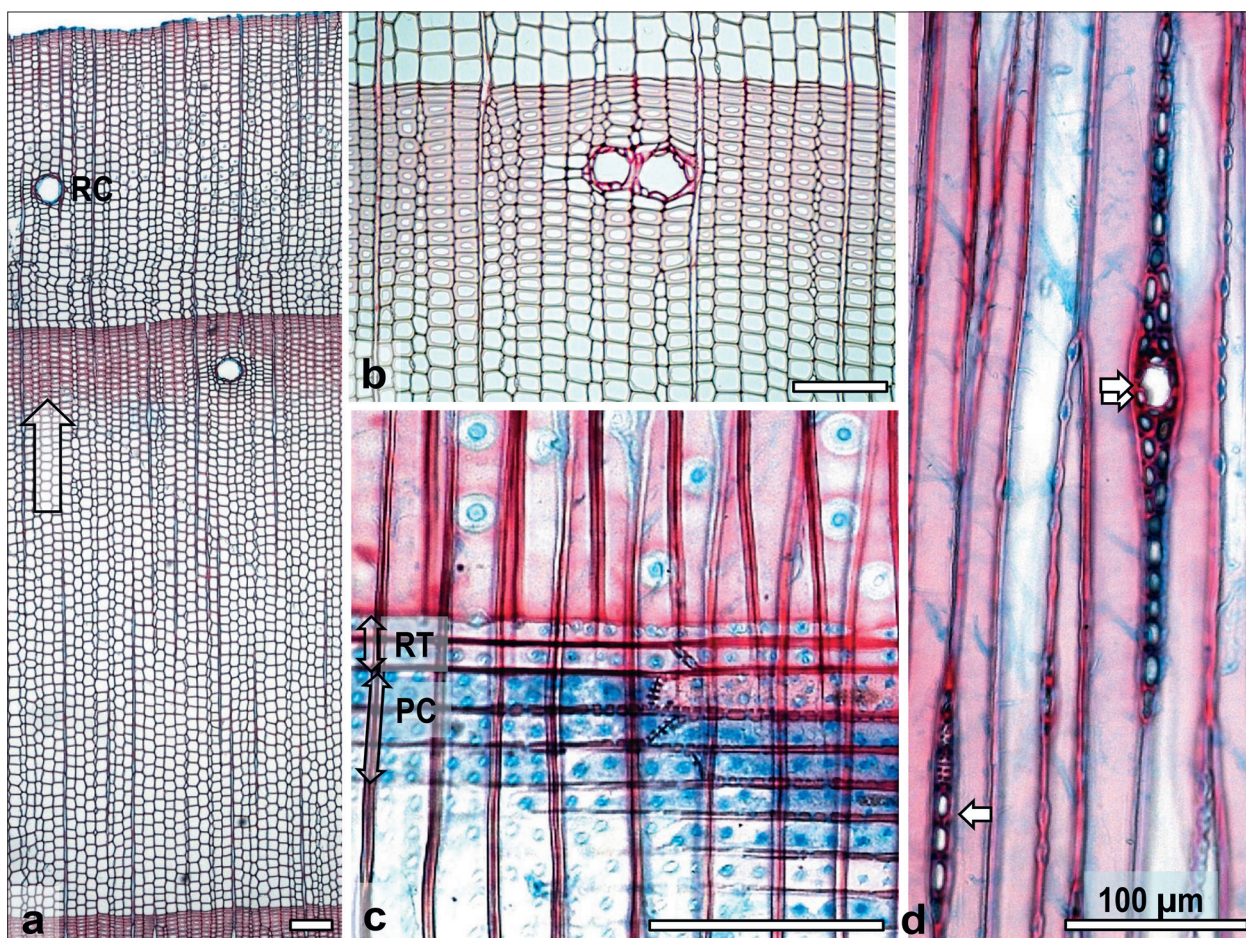


Figure 4. Norway spruce (*Picea abies*) wood from the collection of the Department of Wood Science and Technology, light microscopy – bright field, safranin and astra blue staining: (a) cross-section with gradual transition from early to latewood (arrow) and axial resin canals (RC), (b) cross-section with axial resin canals with thick-walled epithelial cells, (c) radial section with heterocellular ray composed of radial tracheids (RT) and parenchyma cells (PC) with piceoid cross-field pits, (d) tangential section with a multiseriate ray containing radial resin canal RC (double arrow) and an uniseriate ray (arrow). All scale bars – 100 μm .

Slika 4. Prerezi lesa navadne smreke (*Picea abies*) iz zbirke preparatov Oddelka za lesarstvo. Svetlobni mikroskop - svetlo polje, obarvanje safranin in astra modro: a) prečni prerez, branika s postopnim prehodom iz ranega v kasni les (puščica) in aksialnimi smolnimi kanali (RC), (b) prečni prerez z aksialnimi smolnimi kanali z debelostenimi epitelnimi celicami, (c) radialni prerez s heterocelularnim trakom, ki vsebuje radialne traheide (RT) in trakovne parenhimske celice (PC) s piceoidnimi piknjami v križnih poljih, (d) tangencialni prerez z večrednim trakom, ki vsebuje radialni smolni kanal RC (dvojna puščica) in enoredni trak (puščica). Vse merilne daljice - 100 μm .

The shavings from the dragon only contained cross-sections. We could observe that the wood is a diffuse-porous hardwood with small diameter vessels and uniseriate rays (Figure 6). These features are typical of willows (*Salix* sp.) (Figure 7) and poplars (*Populus* sp.) (Figure 9). The two species have similar wood anatomy and can be differentiated based on the type of the rays visible on the radial section, which we did not have. The rays in *Salix* are

heterogeneous (Figure 7 b, c) whereas in *Populus* they are homogenous (Figure 9 b, c).

CLSM images of *Populus* and *Salix* structure on parallel samples enabled us to see the anatomical features of both species (Figures 8 and 9), especially the upright cells defining heterocellular rays seen on the radial surface (Figure 8 b, c). They are crucial for the identification of *Salix* and its differentiation from *Populus*.

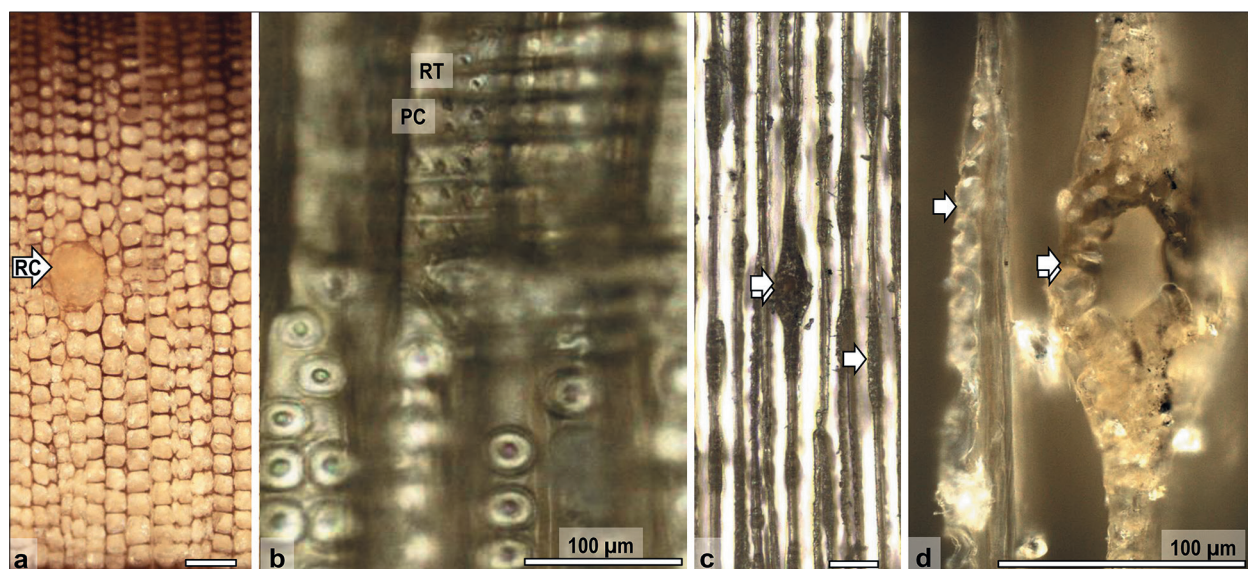


Figure 5. Norway spruce (*Picea abies*), sample of wood from the collection, Confocal Laser Scanning Microscopy: (a) cross-section with gradual transition from early to latewood and axial resin canal (RC), (b) radial section with heterocellular ray with radial tracheids (RT) and parenchyma cells (PC) containing piceoid cross-field pits, (c, d) tangential section with a multiseriate ray containing radial resin canal RC (double arrow) and an uniseriate ray (arrow). All scale bars – 100 µm.

Slika 5. Les navadne smreke (*Picea abies*) iz zbirke Oddelka za lesarstvo. Konfokalni laserski mikroskop: (a) prečni prerez, branika s postopnim prehodom iz ranega v kasni les in aksialnim smolnim kanalom (RC), (b) radialni prerez s hetero celularnim trakom, ki vsebuje radialne traheide (RT) in trakovne parenhimske celice (PC) s piceoidnimi piknjami v križnih poljih, (c, d) tangencialni prerez z večrednim trakom, ki vsebuje radialni smolni kanal RC (dvojna puščica) in enoredni trak (puščica). Vse merilne daljice - 100 µm.

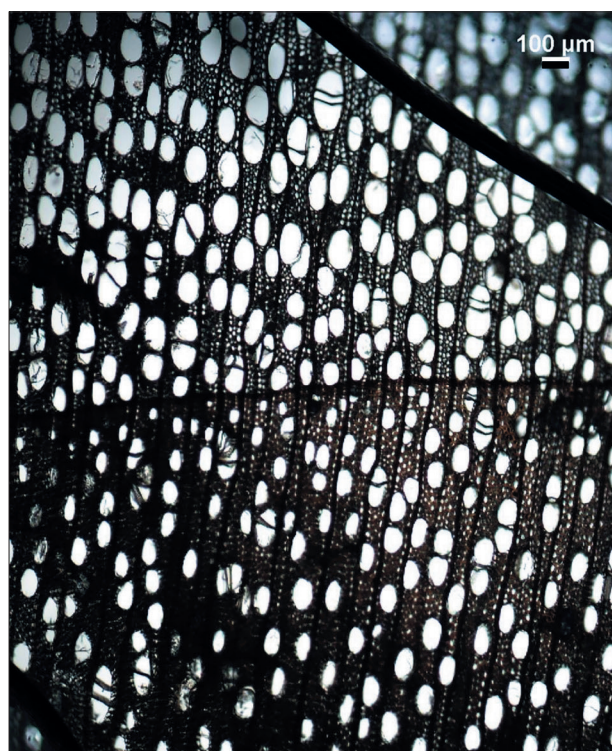


Figure 6. Shaving taken from the dragon – unstained cross-section under a light microscope. Scale bar – 100 µm.

Slika 6. Ostružek s spodnjega dela zmaja – neobarvan prečni prerez pod svetlobnim mikroskopom. Merilna daljica - 100 µm.

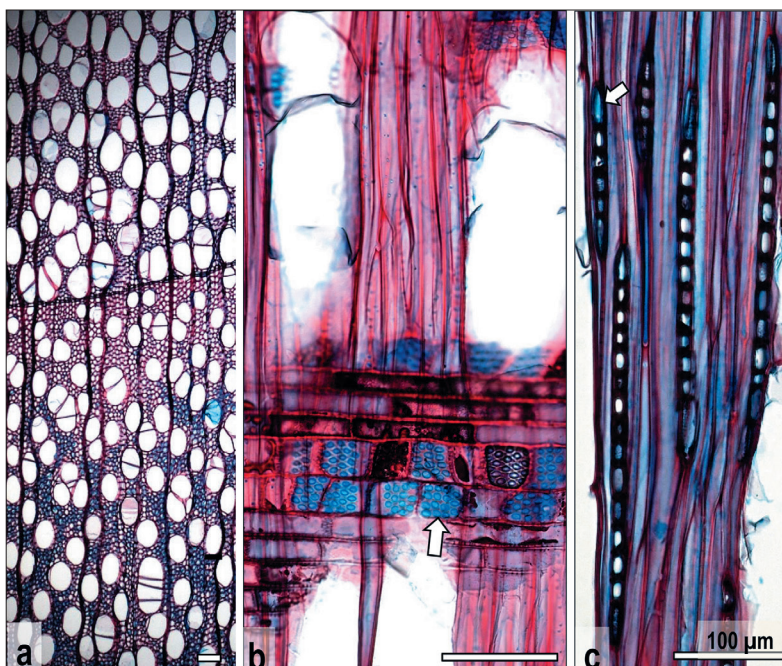


Figure 7. Willow (*Salix alba*) wood from the slide collection, light microscopy – bright field, safranin and astra blue staining: (a) cross-section – diffuse-porous wood with uniseriate rays, (b) radial section – heterogenous ray consisting of procumbent and upright cells (arrow), (c) tangential section – uniseriate rays with procumbent and upright cells (arrow). All scale bars – 100 μm .

Slika 7. Les bele vrbe (*Salix alba*) iz zbirke preparatov, svetlobna mikroskopija – svetlo polje, obarvanje, safranin in astra modro: (a) prečni prerez – difuzno porozen les z enorednimi trakovi, (b) radialni prerez – heterogeni trak, sestavljen iz ležečih in pokončnih celic (puščica), (c) tangencialni prerez – enoredni trakovi sestavljeni iz ležečih in pokončnih celic (puščica). Vse merilne daljice – 100 μm .

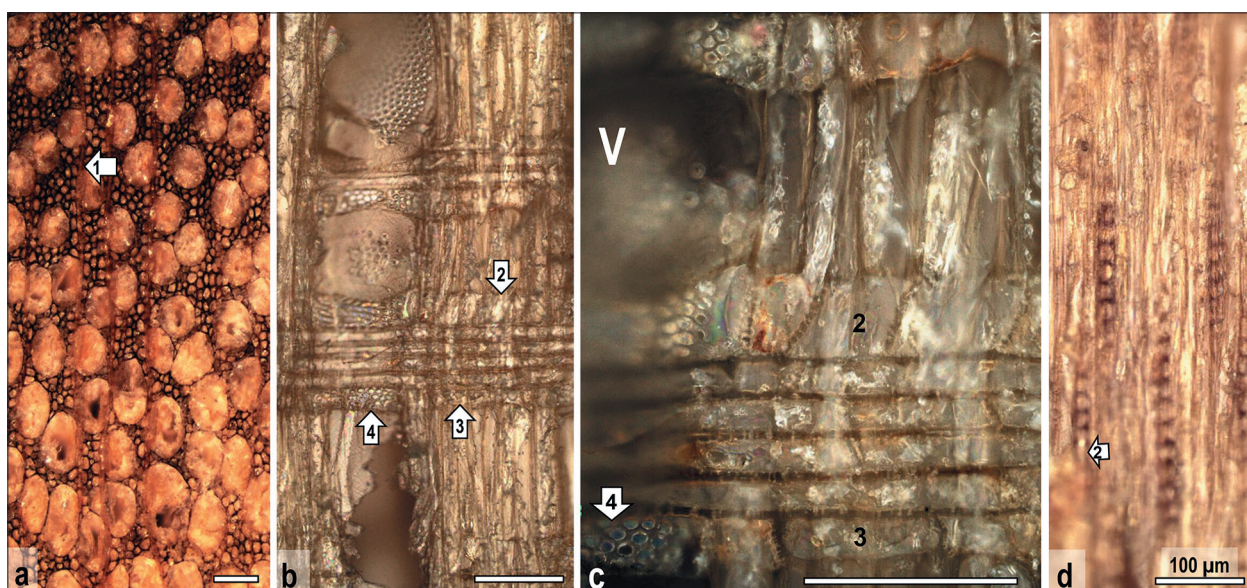


Figure 8. Willow (*Salix sp.*), confocal laser scanning microscope: (a) cross-section – diffuse-porous wood with uniseriate rays (arrow), (b, c) radial section – heterogenous ray with procumbent (3) and upright cells (2), vessel (V), and large vessel-ray pits (4), (d) tangential section – uniseriate ray, with upright marginal cells (arrow) indicate the heterogeneous ray. All scale bars – 100 μm .

Slika 8. Les vrbe (*Salix sp.*) iz zbirke lesa, konfokalni laserski mikroskop: (a) prečni prerez - difuzno porozen les z enorednimi trakovi, (b, c) radialni prerez - heterogeni trak, sestavljen iz ležečih (3) in pokončnih celic (2) in traheje (V), ter velike piknje med trakom in trahejo (4) (c) tangencialni prerez – enoredni trakovi sestavljeni iz ležečih in pokončnih celic (puščica) nakazujejo heterogeno trakovno tkivo. Vse merilne daljice – 100 μm .

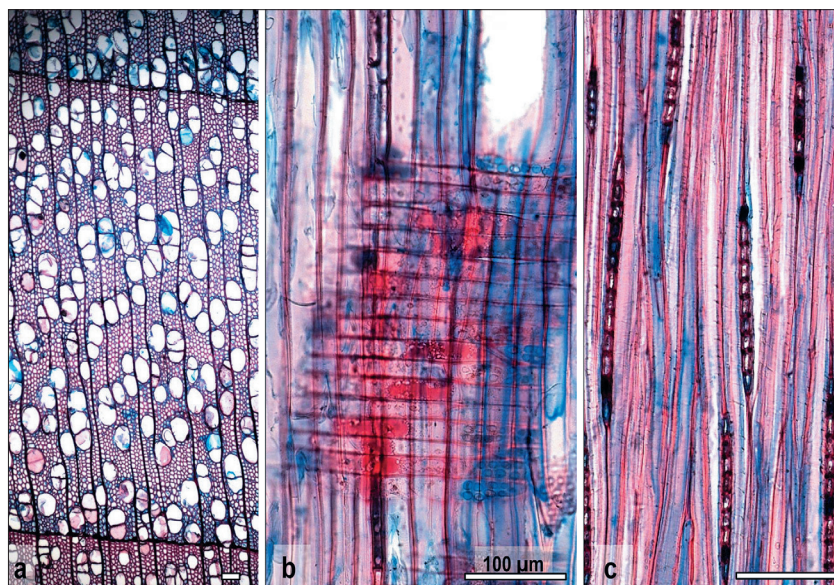


Figure 9. Poplar (*Populus nigra*) wood from the slide collection, light microscopy – bright field, safranin and astra blue staining: (a) cross-section – diffuse-porous wood with uniseriate rays, (b) radial section – homogenous ray consisting of procumbent cells, (c) tangential section – uniseriate homogenous rays. All scale bars – 100 μm .

Slika 9. Les črnega topola (*Populus nigra*) iz zbirke preparatov, svetlobna mikroskopija – svetlo polje, obarvanje, safranin in astra modro: (a) prečni prerez - difuzno porozen les z enorednimi trakovi, (b) radialni prerez – homogeni trak, sestavljen iz ležečih celic, (c) tangencialni prerez – enoredni homogeni trakovi. Vse merilne daljice – 100 μm .

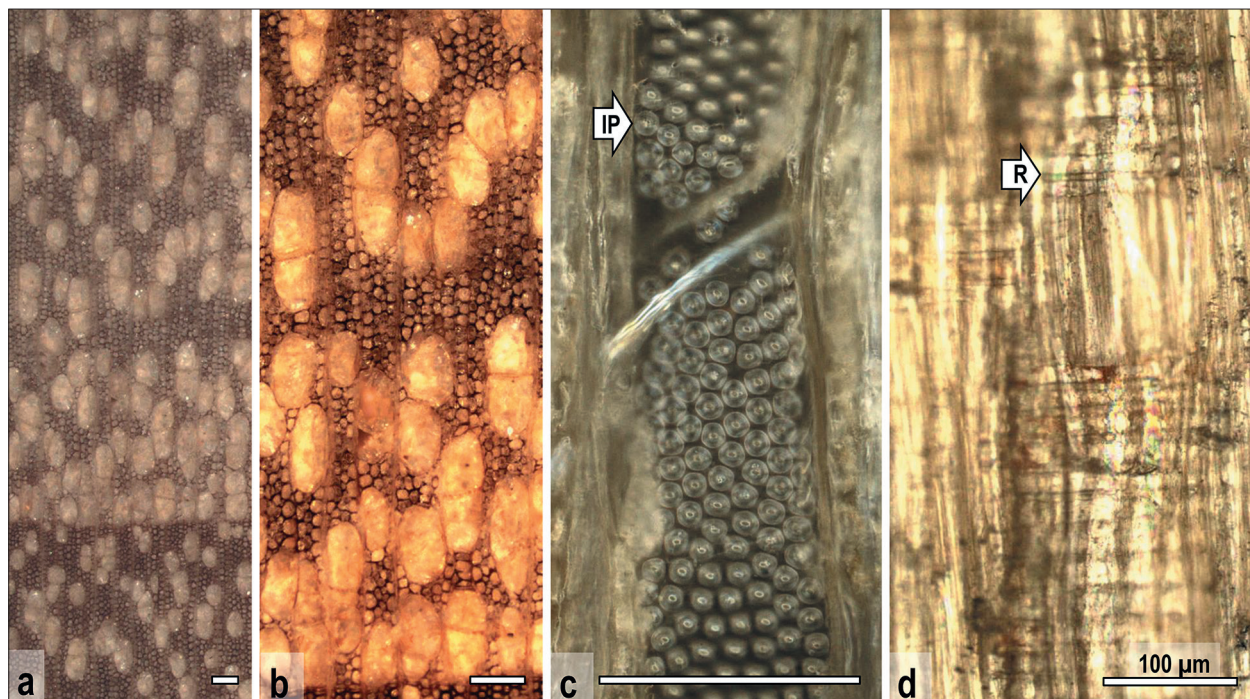


Figure 10. Poplar (*Populus sp.*), confocal laser scanning microscope: (a, b) cross-sections – diffuse-porous wood with uniseriate rays, (c) radial section – vessel elements with typical alternate intervacular pits (IP), (d) radial section – homogenous rays (R) consisting of procumbent cells. All scale bars – 100 μm .

Slika 10. Les topola (*Populus sp.*) iz zbirke lesa, konfokalni laserski mikroskop: (a, b) prečni prerez – difuzno porozen les z enorednimi trakovi, (c) radialni prerez – traheja z izmeničnimi intervaskularnimi piknjami (IP), (d) radialni prerez – homogeni trakovi, sestavljeni iz ležečih celic. Vse merilne daljice – 100 μm .

4 CONCLUSIONS

4 ZAKLJUČKI

The use of a confocal laser scanning microscope made it possible to identify the species *Picea abies*, *Populus nigra* and *Salix alba* based on microscopic wood anatomy features. The key features necessary to identify the mentioned central European species could be observed on oriented wood samples without special preparation of the surfaces. The CLSM methodology would possibly be applicable to identify the wood of the statue of St. George defeating the dragon in a non-destructive way if the working distance of the microscope would allow observing the features directly on the object.

5 SUMMARY

5 POVZETEK

Leseni umetniški predmeti so pomemben del naše kulturne dediščine. Za njihovo optimalno konserviranje, restavriranje in vrednotenje je pomembno vedeti, iz katerega lesa in kako so bili izdelani. Za prepoznavanje vrste lesa lahko uporabimo tradicionalne makroskopske in mikroskopske metode (Čufar & Zupančič, 2000; Čufar, 2006; Čufar & Merela, 2014).

Makroskopska identifikacija lesa je bolj ali manj nedestruktivna, saj temelji predvsem na lastnostih posameznih vrst, ki jih je mogoče videti s prostim očesom ali z lupo (npr. Wagenführ, 1999; Richter et al., 2002; Ruffinatto et al., 2019). Ta metodologija ima svoje omejitve, saj je število znakov za makroskopsko identifikacijo lesa razmeroma majhno, nekateri znaki, kot sta na primer barva in vonj, pa se s starostjo spremenijo ali celo izginejo. Zato lahko uporaba makroskopskih identifikacijskih ključev privede do subjektivnih odločitev ali celo napačne identifikacije (Haag et al., 2018).

Mikroskopska identifikacija lesa je bolj zanesljiva, zlasti pri lesenih predmetih kulturne dediščine. Ta metoda je žal destruktivna, saj zahteva odvzem usmerjenega kosa lesa iz predmeta, iz katerega narežemo tanke prečne, radialne in tangencialne rezine za opazovanje pod mikroskopom.

Za prepoznavanje lesa iglavcev in listavcev so na voljo številni identifikacijski ključi. Če les izvira iz srednje Evrope, lahko uporabimo dihonomne mikroskopske ključe in s slikami opremljene opise les-

nih vrst (npr. Grosser, 1977; Schweingruber, 1978, 1990; Wagenführ, 1996, 1999; Schoch et al., 2004; Signorini et al., 2014; Ruffinatto et al., 2017).

Bolj vsestranski so večvhodni ključi, ki temeljijo na prepoznavanju anatomskih znakov v lesu. Mednarodno združenje lesnih anatomov (IAWA) je organiziralo odbore za pripravo seznamov mikroskopskih znakov listavcev (IAWA Committee, 1989) in iglavcev (IAWA Committee, 2004). Seznami so omogočili razvoj računalniško podprtih orodij za identifikacijo lesa kot na primer javno dostopni portal InsideWood (InsideWood, 2004 naprej), ki je tudi nepogrešljiv vir za identifikacijo in zbirko podatkov o anatomiji listavcev (Wheeler, 2011).

Veliko se uporabljajo tudi računalniški ključi za identifikacijo lesa, ki jih podpira programski paket DELTA / INTKEY (Richter & Trockenbrodt, 1995). V uporabi so ključi za mikroskopsko identifikacijo listavcev (Richter & Dallwitz, 2000), mikroskopsko identifikacijo iglavcev (Richter, osebna komunikacija) in CITESwoodID za identifikacijo lesa zaščitene vrst s seznama CITES (Richter et al., 2005).

Omenjeno destruktivno vzorčenje lesa na dragocenih zgodovinskih predmetih običajno ni mogoče, zato laboratoriji razvijajo uporabo nedestruktivnih metod, ki so jih na primer uporabili za proučevanje lesa zgodovinskih glasbenih instrumentov (Fioravanti et al., 2016; Haag et al., 2018), lokov godal (Fioravanti et al., 2017) in razkošnih kočij (Giulio et al., 2019). Za to so uporabili različno opremo, kot so prenosni digitalni mikroskopi Dino-lite pro AD413T in AM4113ZT4 (Fioravanti et al., 2016), Dino-lite in sinhrotronski rentgenski CT (Fioravanti et al., 2017) ali sisteme za zajem in analizo slike Cell[^]F[®], Olympus in KEYENCE[®] VHX-5000 (Haag et al., 2018).

V tej študiji predstavljamo možnost uporabe konfokalnega laserskega skenirnega mikroskopa Olympus LEXT OLS5000 (Olympus Corporation Tokyo 163-0914, Japonska) (Balzano et al., 2019) za identifikacijo lesa na primeru gotskega kipa svetege Jurija s Ptuja v Sloveniji (slika 1). Primerjali smo identifikacijo lesa na podlagi svetlobne mikroskopije rezin iz vzorcev, odvzetih iz kipa, nato na običajnih preparatih iz zbirke Oddelka za lesarstvo in nato še identifikacijo lesa z uporabo CLSM na vzorcih lesnih vrst, iz katerih je izdelan kip (slike 5, 8 in 10).

Natančna identifikacija lesa običajno temelji na opazovanju anatomskih znakov s pomočjo mikrosko-

pa. Za tak način moramo odvzeti vzorec lesa in narezati tanke preparate, po možnosti vseh treh anatomskih prerezov, prečnega, radialnega in tangencialnega. Sledi pregled s svetlobnim mikroskopom, določitev anatomskih znakov in identifikacija lesa.

Po pregledu kipa in posvetu z restavratorji smo sklepali, da sta sveti Jurij in zmaj verjetno izdelana iz različnih lesnih vrst. Skupaj z restavratorji smo poiskali mesta, kjer bi bilo mogoče les natančno pregledati ali po potrebi na čim manj invaziven način odvzeti vzorce lesa za raziskave. Ker zaradi polihromacije nismo mogli videti lesno anatomskih znakov, potrebnih za določitev lesne vrste, smo odvezli dva vzorca minimalnih dimenzij, enega na glavi svetega Jurija, enega pa na zčaju (slika 1).

Iz glave smo na mestu manjše predhodne poškodbe odvzeti vzorec v obliki orientirane trske (dimenzije 3 x 3 x 7 mm), ki smo ga v laboratoriju dodatno orientirali, poravnali in vpeli v drsni mikrotom Leica SM 2000 R (slika 2a) ter odrezali rezine debeline 20 µm prečnega in tangencialnega prereza. Rezine smo obarvali z barvili safranin in astra modro, jih dehidrirali in vklopili v vklopni medij (Euparal 3C-129; Chroma) (Čufar & Zupančič, 2000).

Iz zčaja smo v restavratorski delavnici s skalpelom odrezali ostružke oz. tanke rezine približne velikosti 7 x 9 x 0,3 mm, ki smo jih takoj po odvzemu neobarvane položili na objektno steklo in vklopili v glicerol. Odvezti material oz. rezine so zajemale samo prečni prerez lesa.

Tako smo pripravili preparate (slika 2b) za opazovanje s svetlobnim mikroskopom Nikon Eclipse E 800. Mikrografija je bila opravljena z digitalno kamero Nikon DS-fi1 in programsko opremo NIS Elements Br 3.0. Mikroskopsko identifikacijo lesa smo opravili s pomočjo standardnih identifikacijskih ključev (Čufar, 2006; Čufar & Merela, 2014; Schoch et al., 2004; Signorini et al., 2014).

Trska lesa iz glave kipa svetega Jurija je bila dovolj velika, da smo izdelali prečni in tangencialni prerez lesa (slika 3). Prečni prerez je zajel del široke branike, ki je vsebovala samo rani les s tipičnim postopnim prehodom iz ranega v kasni les (slika 3a), na tangencialnem prerezu pa so bili jasno vidni radialni smolni kanali z debelostenimi epitelnimi celicami (slika 3b).

Kljub temu, da na prečnem prerezu nismo videli aksialnih smolnih kanalov in lesa ni bilo do-

volj, da bi odrezali radialni prerez ter potrdili prisotnost heterogenega trakovnega tkiva in majhnih piceoidnih pikenj v križnem polju, smo na podlagi radialnih smolnih kanalov lahko ugotovili, da je kip svetega Jurija narejen iz lesa navadne smreke (*Picea abies*).

Vse ključne mikroskopske znake lesa smo poiskali in prikazali na preparatih lesa smreke iz zbirke preparatov Oddelka za lesarstvo (slika 4). V naslednjem koraku smo vse ključne znake za identifikacijo te lesne vrste poiskali na orientiranih vzorcih lesa smreke iz zbirke lesa Oddelka za lesarstvo in jih preiskali s konfokalnim laserskim mikroskopom. Posneli smo znake kot so: postopni prehod iz ranega v kasni les, aksialne in radialne smolne kanale z debelostenimi epitelnimi celicami, radialni prerez s heterocelularnim trakom, ki vsebuje radialne traheide in trakovne parenhimske celice s piceoidnimi pikenjami v križnih poljih (slika 5).

Ostružek lesa, ki smo ga odvzeli iz zčaja, je vseboval samo prečni prerez, na katerem smo lahko videli, da je les difuzno porozen listavec z majhnimi trahejami in da ima enoredne trakove (slika 6). Našteti znaki so značilni za les vrbe (*Salix* sp.) (slika 7) ali topola (*Populus* sp.) (slika 9). Obe vrsti imata podobno anatomijo lesa in ju je mogoče razlikovati po trakovih, ki so pri vrbi heterogeni (slika 7 b, c), pri topolu pa so homogeni (slika 9 b, c). Omenjenega znaka na prečnem prerezu ni mogoče videti.

Na CLSM posnetkih lesa vrbe in topola iz zbirke Oddelka za lesarstvo smo lahko videli vse znake, potrebne za določitev topola in vrbe in za razlikovanje vrbe in topola (sliki 8 in 10). Pri tem sta bila ključna radialna prereza. Pri vrbi smo na radialnem prerezu lahko videli heterogeni trak (slika 8 b, c), sestavljen iz ležečih in pokončnih celic, pri topolu pa homogeni trak, sestavljen samo iz ležečih celic (slika 10 d).

Konfokalni laserski vrstični mikroskop je omogočil prepoznavanje vseh ključnih znakov za mikroskopsko identifikacijo lesa vrst *Picea abies*, *Populus nigra* in *Salix alba*. Znake smo lahko posneli na orientiranih vzorcih lesa brez posebne priprave površin. Metodologija CLSM bi bila najverjetneje uporabna tudi za identifikacijo lesa direktno na kipu, če bi delovna razdalja mikroskopa omogočala, da bi kip postavili pod mikroskop.

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