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Contribution of plant anatomy to forensic investigation: tree bark morphology

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Abstract:	Highlights • Tree bark is a common botanical element in a crime scene • Bark could be a powerful tool to link a body back to a primary crime scene • Identification keys were prepared for 16 lowland tree species from Northern Italy • Is an identification at the species level from small bark fragments possible? Abstract Plant science has been more and more utilized in forensic investigation, although its full potential is still to be reached. Plant macroremains are a powerful tool to link a body or other evidence back to a primary crime scene as they can provide detailed information about its previous ecological and geographic location. However, plant macroremains are often poorly preserved and difficult to identify, as diagnostic elements are seldom present within the assemblage occurring on the scene. Plant fragments most likely to be found are those exposed to the environment and resistant to degradation. The bark of woody plants meets these requirements but the possibility of its identification at species level from small fragments is not known. Starting from a real homicide case, where bark splinters were found on the victim, we aimed to assess the forensic potential of bark identification from small fragments like those likely occurring on a crime scene. Two identification keys were prepared for 16 common lowland tree species from Northern Italy; one key used all the available anatomical traits, the second only those from the outer bark. The second key was not able to discriminate unambiguously some couples of species, but could identify the bark fragments of the homicide as Robinia pseudoacacia , as confirmed from direct comparison with a reference sample. Bark fragments deserve to be included into the macroremains to be analyzed during an investigation, but small samples could easily lack diagnostic traits, and the building of a reference collection should be encouraged.

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Contribution of plant anatomy to forensic investigation: tree bark morphology

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Highlights

- Tree bark is a common botanical element in a crime scene
- Bark could be a powerful tool to link a body back to a primary crime scene
- Identification keys were prepared for 16 lowland tree species from Northern Italy
- Is an identification at the species level from small bark fragments possible?

Contribution of plant anatomy to forensic investigation: tree bark morphology

Abstract

Plant science has been more and more utilized in forensic investigation, although its full potential is still to be reached. Plant macroremains are a powerful tool to link a body or other evidence back to a primary crime scene as they can provide detailed information about its previous ecological and geographic location. However, plant macroremains are often poorly preserved and difficult to identify, as diagnostic elements are seldom present within the assemblage occurring on the scene. Plant fragments most likely to be found are those exposed to the environment and resistant to degradation. The bark of woody plants meets these requirements but the possibility of its identification at species level from small fragments is not known.

Starting from a real homicide case, where bark splinters were found on the victim, we aimed to assess the forensic potential of bark identification from small fragments like those likely to occur on a crime scene. Two identification keys were prepared for 16 common lowland tree species from Northern Italy; one key used all the available anatomical traits, the second only those from the outer bark. The second key was not able to discriminate some couples of species unambiguously, but could identify the bark fragments of the homicide as *Robinia pseudoacacia*, as confirmed from direct comparison with a reference sample. Bark fragments deserve to be included into the macroremains to be analyzed during an investigation, but small samples could easily lack diagnostic traits, and the building of a reference collection should be encouraged.

Key words: Forensic botany, identification key, macroremains

Introduction

The role of plant science in forensic investigation has proved more and more useful in the recent decades [1-4]; many botanical disciplines have been involved such as palynology [5], micro- , macroremain and living plant analysis [4,6,7,8], dendrochronology [9,10], study of vegetation dynamics [11], thus addressing different questions such as post-mortem interval (PMI), identification of clandestine burial, previous location of remains, etc.

Plant macroremains (seeds, fruits, flowers, leaves and other vegetative parts and/or their fragments) are a powerful tool to link a body or other evidence back to a primary crime scene, as they can provide detailed information about the ecological and geographic location where the body has been exposed; their primary importance has been outlined in forensic-like investigations carried out in the archeological context [12]. In comparison with pollen analysis, plant macroscopic remains are easier to collect and process, less prone to contamination, and cheaper to examine. On the other hand, their identification is often problematic even for experienced taxonomists, particularly when only small and/or non significant parts occur and their conservation status is poor. Amongst plant parts, reproductive structures usually provide the maximum amount of taxonomic information; however, other structures can be more frequently found, particularly the most resistant ones and those directly exposed to the environment. The outer layer of ligneous seed plants (trees and shrubs), commonly known as "bark", meets these conditions. Its direct contact with the external environment and its resistance to decomposition make its occurrence very likely each time a contact with a woody plant takes place. Unfortunately, little is known about specific identification tools for the anatomical features of bark, or at least about the individuation of specific marks which can be used for the direct comparison with samples of known provenance, even if reference texts are increasingly available [13,14].

What is commonly known as bark consists of many layers of different origin which may or not be present in different species [15,16]. It results from the activity of a living meristem (phellogen) which originates two distinct tissues, phelloderm inwards and phellem ("cork") outwards. These tissues together form the periderm. During the life of the tree, many periderms may originate: each one isolates the outer tissues, causing their death and the formation of the rhytidome, or outer bark. Depending on the position of the periderm, rhytidome may include dead cells from different tissues such as phloem or parenchyma: sieve cells, fibers, phellem could thus be found, more or less modified. Little is known about the different occurrence and appearance of such elements in the different tree and shrub species, and whether they could be used as identification tools [17-20]; many bark features have been recently outlined by Schweingruber et al. [13] but mainly concerning the phloem and the cortex and not the outer bark. In archeological contexts, however, bark used to create artifacts, identified at species level, has given important environmental information such as, for example, about the Neolithic Iceman discovered on the Alps [21].

The present work originates from a real homicide case which occurred in Northern Italy, where bark fragments were found on the victim and were analyzed to help the reconstruction of the dynamics of the homicide. The aim of the research was to investigate the macroscopic anatomical features of bark useful for species identification, the evaluation of its use in forensic investigations, and the preliminary construction of an identification key for bark fragments of some common tree species, representative of different bark structures.

Materials and methods

Case report

In August 2010 the body of a 20-year-old man was found in a woodland, in Northern Italy, next to a large tree, with several gunshot injuries to the head and thorax. The victim was the son of a known leader of a mafia clan. He had been abducted in the evening by two men disguised as policemen who summoned him to follow them in a car. He was then seen entering the car which drove off. The body was found the following morning next to a tree, with many abrasions on the face, wrists and forearms. As a witness later told authorities that the victim had been handcuffed to a tree, shot, and the body left near the tree, the prosecutor requested to verify if the man had been handcuffed around a tree, shot, and then the handcuffs removed (they hadn’t been found on the body). This was particularly important in order to verify the reliability of the specific witness who claimed to have been present and later decided to collaborate with the Police. The wrists indeed had some linear abrasions and bruises, findings which could be consistent with handcuffing. However, what seemed more important to the pathologist at the scene and upon autopsy was a series of small splinters of botanical origin inserted within the skin of the ventral surface of the forearms, in the context of abrasions. Once these splinters were removed from the skin, they were analyzed with the intention of verifying whether they could come from the bark of the tree adjacent to the body. The hypothesis was that such splinters could indicate the consequence of the forearms being tightly bound (handcuffed) around the tree; this position would have caused rubbing and friction of the skin against the bark and the insertion of small fragments.

Sample collection and analysis

Bark splinters collected from the body were glued on a wood support in vertical position (i.e. with fibers and vessels placed vertically as in living position) and finely sanded on the cross section.

Samples for the identification keys were taken from 16 tree species common in Northern Italy lowlands and representative of the main macroscopic bark shapes [22]. Samples were extracted from living trees with a Pressler increment borer, air-dried and finely sanded (Fig. 1). The pressler borer, used for dendrochronological analysis, allows the extraction of small samples (c. 4 mm wide) which are not optimal for the complete observation of bark features, but are representative of the small fragments that could detach following occasional contact or impact with tree stem or branches, as in the homicide case under investigation. Stem cross sections of the investigated species were also examined for a complete overview of bark organization.

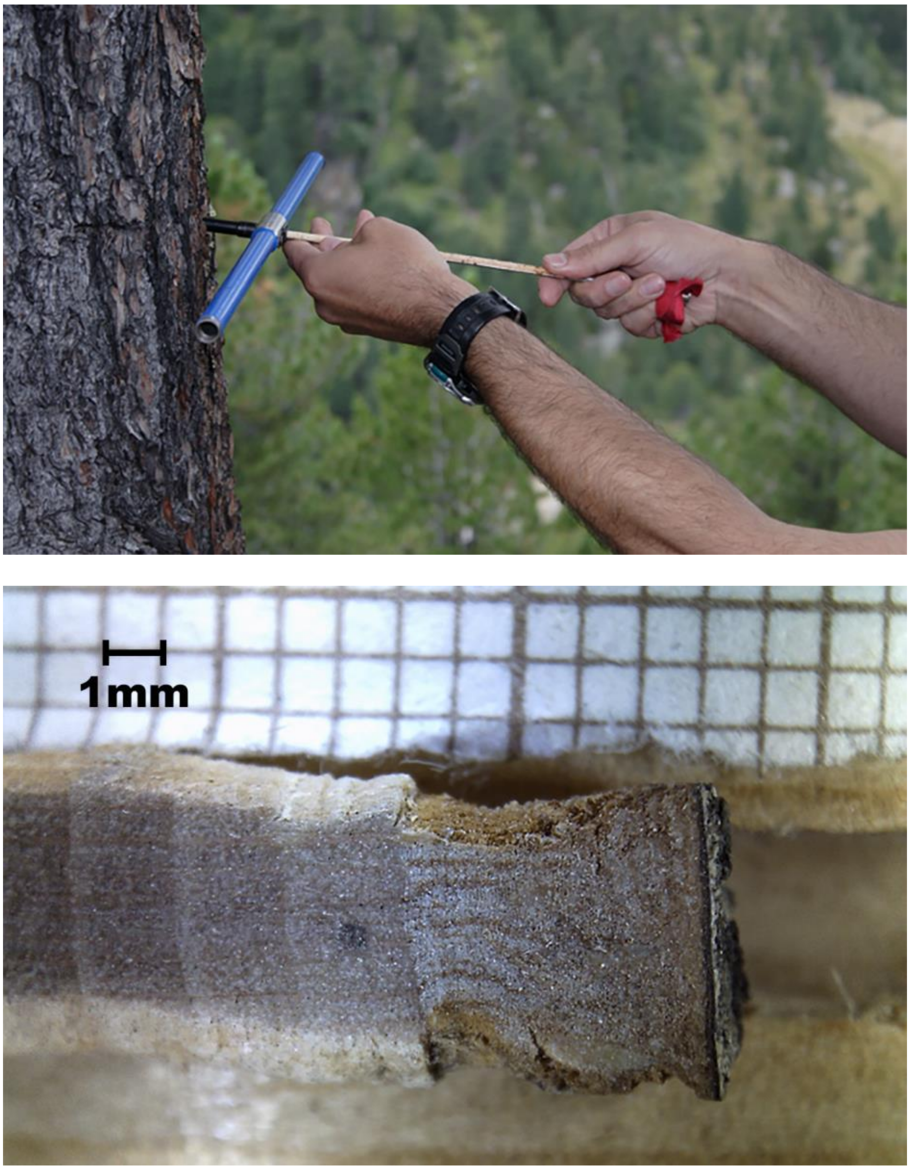

Fig. 1. Top: Extraction of a sample with the Pressler increment borer. Bottom: sample (*Fagus sylvatica*) glued on a wooden support and sanded.

Examined species were *Acer campestre* L., *Alnus glutinosa* (L.) Gaertn., *Alnus incana* (L.) Moench., *Betula pendula* Roth, *Castanea sativa* Mill., *Quercus robur* L., *Fagus sylvatica* L., *Fraxinus excelsior* L., *Pinus sylvestris* L., *Populus nigra* L., *Sambucus nigra* L., native in lowland/montane forests and frequently planted as ornamental species; *Prunus serotina* Ehrh, *Robinia pseudoacacia* L., invasive species very frequent in lowland forests and, the latter, the dominant species in the site of the homicide; *Ginkgo biloba* L., *Juglans nigra* L., *Juglans regia* L., frequently cultivated as ornamental species and/or fruit tree (*J. regia*, walnut) in parks and gardens.

All the samples were observed with a stereomicroscope (Leica zoom 2000) at 7x-30x. The main macroscopic characteristics of the outer part of the stem (from secondary phloem to the surface) were examined. The identification keys were obtained primarily basing on the features that could be observed on the samples obtained with the increment borer, integrated where necessary with those visible on the cross sections. A first identification key was prepared on the basis of all the anatomical traits observable through a stereomicroscope. A second identification key was then prepared considering only the features of the outer bark, which represents the portion more likely to detach from a trunk and occur in the examined real case.

The identification of the bark splinters collected from the body was performed both through the identification keys and through visual comparison with outer bark samples collected from the nearby trees (*Robinia pseudoacacia* L.).

Results

Description of the bark fragments found on the body

The fragments found on the body were very small (between 2 and 8 mm; Fig. 2), and the description of the whole bark could not be performed; only part of the outer bark (rhytidome) was present. Within the rhytidome, dead phloem tissue could be observed, with lighter rectangular-shaped fibers.

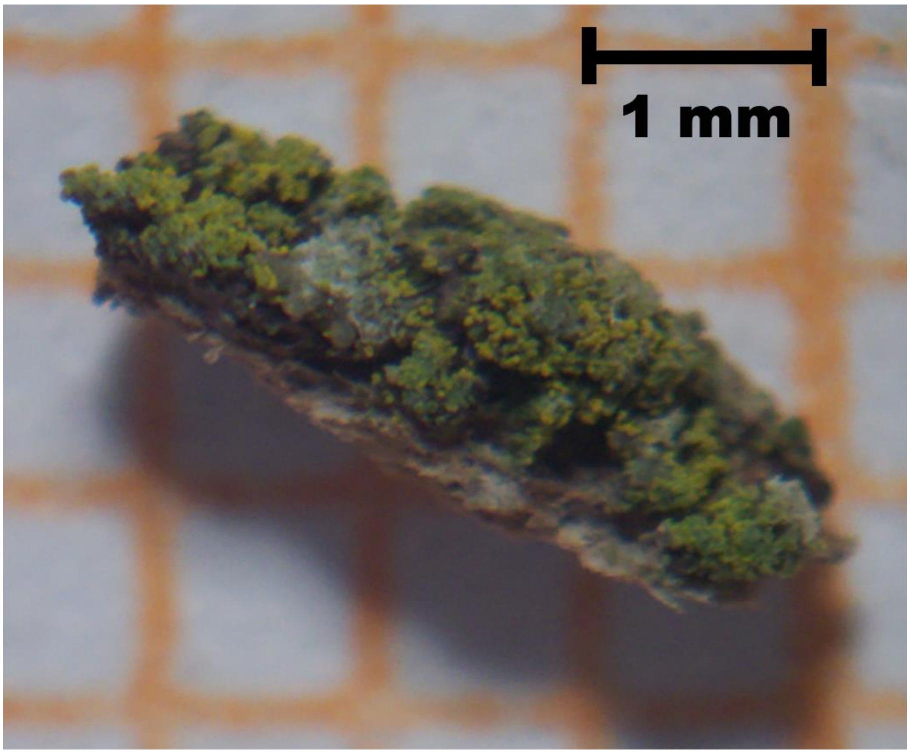

Fig. 2. Top: bark fragment collected at autopsy on the inner surface of the left forearm of the victim in the investigated case. Bottom: magnified view of the bark fragment.

Bark appearance in the selected species and identification key

The sampled species showed very variable bark structures (Fig. 3). The main features differentiating the samples were the occurrence and number of periderms, their appearance (i.e., color, thickness, disposition, occurrence of dead secondary phloem and of its fibers), the morphology of the secondary phloem in the inner bark (color, occurrence of growth rings, of fibers), the occurrence and distribution of expansion tissue, the arrangement of fibers and of expansion tissue elements in sclereids, the occurrence of specific traits (rays, resin ducts). A certain degree of variability could be observed within a species or an individual, particularly in terms of thickness and appearance of the outer bark. The first identification key (table 1) allowed the discrimination of all the collected species; only *Juglans nigra* and *J. regia*, although visually different, could not be distinguished on the basis of unambiguous traits.

Fig. 3. Scheme of the different bark structure observed in the investigated species

The second identification key (table 2) was not able to discriminate unambiguously some couples of species (*Quercus robur/Alnus glutinosa; Sambucus nigra/Gingko biloba; Fagus sylvatica/Alnus incana*).

Identification of the bark fragments found on the body

A comparison with the bark of *Robinia pseudoacacia,* the dominant tree species on the crime scene, showed almost identical features of its outer bark (Fig. 4), characterized by secondary phloem with radially-arranged fibers, which are the dominant feature also in the lighter inner bark, alternating with interposed periderms which did not occur in the splinters found on the body.

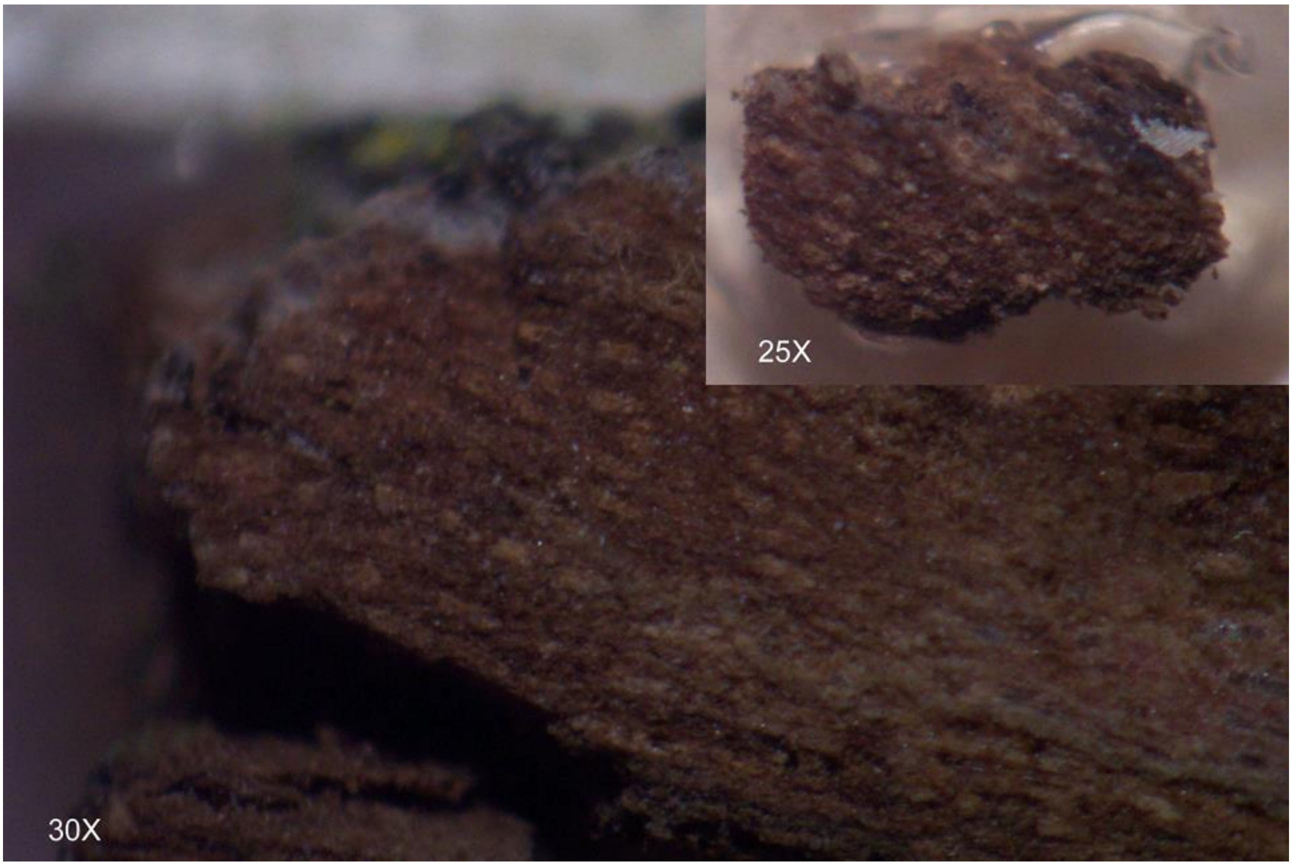

Fig. 4. Comparison between the transverse section of the outer bark of *Robinia pseudoacacia* collected on the crime scene and that of one of the splinters found on the body (inset).

The identification of the fragments with the complete key of table 1 was not possible, as dichotomy n. 8 could not be resolved because of the absence of expansion tissue. On the contrary, the simplified key reported in table 2 allowed the correct identification of the samples.

Discussion

Species identification from small bark fragments

Our data confirm the occurrence of specific, distinctive traits in the layers forming tree bark. Their use as an identification tool is promising, and the increase in the number of species could greatly improve the effectiveness of the identification key. However, it should be noticed that the dimension of the samples obtained through the increment borer is seldom sufficient to properly observe the diagnostic features of the bark such as the occurrence of localized expansion tissue or of rays in the secondary phloem. For example, outer bark fragments of *Quercus* and of *Alnus glutinosa* may result almost undistinguishable if wedge-shaped expansion tissue and prominent rays typical of *Quercus* do not occur in the sample. The number of rhytidome layers in the outer bark can also be variable, even within the same tree individual.

Occasional severance of bark portions usually involves only the outermost layers, which may be very variable in number, while secondary phloem and inner bark are unlikely to be detached from a living trunk. For this reason, a simplified key like that reported in table 2, even if less able to discriminate species visually very different from each other, could result more effective, as it proved to be in the reported case.

However, the lack of the inner bark makes the interpretation of the diagnostic traits of the outer bark more difficult, as cells and tissues can be observed only in their degraded phase without any information about their original status. For this reason, sclerified cells of different origin like those deriving from phloem fibers, expansion tissue and other parenchyma are often not distinguishable from each other when occurring in the outer bark. A diagnostic key developed for forensic purpose should take these caveats into account and include statements somewhat incorrect from the botanical point of view, merging elements of different nature (see point 4 of the identification key in table 1).

Forensic potential of bark identification

Bark fragments can frequently occur following a contact with tree trunk or branches; furthermore, they show a variable appearance as a consequence of the species of origin; many features could be observed and recognized with simple macroscopic observation with lens or stereomicroscope; finally, bark tissues can last a long time without deterioration and can be easily handled. For these reasons, bark fragments deserve to be included into the macroremains to be collected and analyzed during an investigation. On the other hand, some critical points should be taken into account, particularly due to the small size of the samples that could likely occur and their lack of completeness, as discussed in the previous paragraph.

However, the use of diagnostic keys like those obtained in the present paper could at least focus on few genera or species and the diagrams of bark sections can help identify the main tissue and structures occurring in tree bark; both tools would be particularly effective when used together with a good reference collection or with reference material sampled ad hoc for direct comparison of the samples. In this case, even small and partial samples like those of the case report could be identified rather easily. Unfortunately, to our knowledge, bark reference collections are quite uncommon, while for forensic purposes it would be important to have easy access to a wide range of reference collections covering both native and exotic taxa [5]. Our results represent a starting point, to be further developed within different biogeographic contexts, and to develop a tool to be used together with the direct comparison with reference material or alone when such comparison is not possible.

Our work emphasized the usefulness of a complete survey of botanical remains associated with forensic samples, including those apparently lacking the traditional identification tools, and the need to establish reference collections including plant parts more likely preserved from decomposition. Methods and knowledge derived from archaeological or palaeoecological studies (e.g. Heiss and Oeggl [12]) may be useful for achieving the full potential of such techniques, which is still to be reached and recognized.

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1.Phellem absent or very thin; bark generally smooth

2. Outer bark made of numerous tightly packed periderms (phellem) easily esfoliating. Secondary phloem with numerous stone cells (scereids).. *Betula pendula*

2. Outer bark absent or very thin (1-2mm) with one phellem layer

3. Outer bark generally absent. Expansion tissue continuous at surface, inwards continuing in wide rays within secondary phloem.. *Fagus sylvatica*

3. Outer bark formed by a discontinuous thin phellem layer. Phloem reddish, with scattered stone cells sometimes forming thin rays.. *Alnus incana*

1. Phellem present, one or different thick periderm layers form the outer bark

4.Expansion tissue, phloem fibers or sclereids absent

5. Periderms not parallel to cambium, delimiting phellem layers of irregular width.................. *Sambucus nigra*

5. Periderms parallel to cambium

6. Periderms generally thick; resin ducts present... *Pinus sylvestris*

6. Periderms c. 2 mm thick isolating regularly stratified secondary phloem............... *Acer campestre*

4.Expansion tissue, phloem fibers or sclereids present

7. Phellem c 1 mm thick; expansion tissue diffuse in secondary phloem; bark smooth............................ *Prunus serotina*

7. Phellem thicker, often arranged in numerous layers

8. Expansion tissue absent (but phloem fiber may become conspicuous in the older portion of phloem)

9. Phloem fibers confluent in tangential layers, penetrating also the periderms and becoming larger in the older phloem (to 0.5 mm) and in the outer bark; rays uniseriate. Outer bark thick (to 6-7 mm) with many rhytidome layers.. *Castanea sativa*

9. Periderms often anastomosed isolating portions of secondary phloem; phloem fibers small and homogeneous.. *Ginkgo biloba*

8. Expansion tissue present

10. Expansion tissue localized, arranged in rows or wedges

11. Expansion tissue organized in wedges (cuneiform)

12. Expansion tissue homogeneous with small (<0.5 mm) sclerified cells compactly packed; secondary phloem regularly arranged in light and dark bands... *Juglans nigra, J. regia*

12. Expansion tissue composed of parenchyma cells, some of which become sclerified and form large, circular stone cells. *Quercus robur*

Phloem interrupted by large rays; outer bark thick, composed of layers 1-2 mm thick..	
11. Expansion tissue mainly organized in rows perpendicular to vascular cambium.	
13. Expansion tissue in light radial rows within darker phloem; phloem fibers homogeneous and rectangular. Phloem alternated with periderm layers. Outer bark thick with still visible phloem fibers	*Robinia pseudoacacia*
13.Expansion tissue in very thin radial rows, often indistinct. Phloem fibers and sclereids heterogeneous, in tangential rows becoming less regular in the outer bark; periderms few; outer bark thin (2-4 mm)...	*Fraxinus excelsior*
10.Expansion tissue diffuse, not arranged in rows or wedges	
14. Sclereids in the outer bark conspicuous (up to 1 mm)..............	*Alnus glutinosa*
14. Sclereids small (less than 0.5 mm) and sparse...........................	*Populus nigra*

Table 1. Identification key for common lowland tree taxa in Northern Italy based on features of the whole bark

1.Outer bark thin (< 4 mm)and generally smooth

2. Outer bark 1-4mm thick, with one or more phellem layers

3. Outer bark made of numerous tightly packed periderms (phellem) easily esfoliating.................. *Betula pendula*

3. Outer bark c not as above

4. Outer bark c 1-2 mm thick; periderm one or more, separated by dark lines without conspicuous sclereids... *Prunus serotina*

4. Outer bark 2-4 mm thick with conspicuous, heterogeneous phloem fibers and sclereids ; periderms few;... *Fraxinus excelsior*

2. Outer bark absent or very thin (1-2mm) with one phellem layer.. *Fagus sylvatica, Alnus incana*

1. Phellem present and thick; one to many thick periderm layers form the outer bark

5. resin ducts present... *Pinus sylvestris*

5. resin ducts absent

6.Sclereids present

7. Sclereids large (0.5-1 mm).. *Quercus robur, Alnus glutinosa*

7. Sclereids small (<0.5 mm)

8. Sclereids uniformly distributed.. *Populus nigra*

8. Sclereids arranged in wedges alternated with tightly-packed secondary phloem... *Juglans regia, J. nigra*

6. Sclereids absent (but phloem fiber sometimes conspicuous)

9. Phloem fibers conspicuous

10. Phloem fibers in tangential layers, bigger and less regularly arranged with increasing age of bark... *Castanea sativa*

10. Phloem fibers homogeneous and rectangular, radially arranged..................... *Robinia pseudoacacia*

9. Phloem fibers not evident

11. Regularly stratified secondary phloem visible in the outer bark..................... *Acer campestre*

11. Secondary phloem and periderms homogeneous... *Ginkgo biloba, Sambucus nigra*

Table 2. Identification key for common lowland tree taxa in Northern Italy based on features of the outer bark

Dear Editor

We have made the requested amendments. In fact the text has been considerably shortened as the part on lichens has been removed. Below are our rebuttals. We trust the article can now be considered adequate for publication.

Reviewer #1: This work would be worthy of a technical note in some journal, but is of insufficient calibre for publication in FSI.

English is obviously not the native language of the authors and there are many errors in syntax, some in spelling, and there is considerable ambiguity in some areas of the text.

We apologise. We fixed the errors and tried to improve the clarity of the text.

There is some value in providing diagrams of bark sections as the authors have done but most forensic scientists would probably obtain reference material on an ad hoc basis where necessary.

In the discussion section we tried to better emphasize the role of our key and diagrams beyond our specific case and geographic location.

In reality, although tree bark is present in woodland sites where body disposal occurs, it is not frequently collected as trace evidence at crime scenes or from victims or exhibits.

That's exactly the point that we tried to address with our research. Although bark fragments are usually overlooked, they could provide useful information in many investigations occurring in outdoor (not only woodland) environments.

The authors have ignored much work that has already been reported on plant macro-remains in criminal cases, and seem to be unfamiliar with the field generally.
It could have been useful to know which papers the referee actually refers to. Anyway, we added some references to papers that emphasize the contribution of macroremain analysis to criminal investigation. We would be happy and grateful to know if we overlooked important and pertinent literature.

The drawings of bark sections are valuable and this kind of approach is to be encouraged. However, the photographs of bark sections and the lichen were out of focus and of insufficient calibre for publication.

We removed the photograph of the lichens (see point below) and added two new photographs to improve the quality of the pictures

The paper is too long and insufficiently succinct although the authors have made a valuable point in suggesting that more attention should be given to bark anatomy.
In order to shorten the paper and make it more focused on the main topic (bark anatomy), we decided to remove the part dedicated to lichen identification. This topic deserves to be treated separately and more completely, as we discussed it only with respect of our specific case study, without a broader contextualization. We hope that this will made the paper more effective and clear.

Reviewer #2: This article represents an interesting contribution to the fields of forensic botany and forensic science.

The methods and sciences of the article are valid.

The novelty of the method of bark identification appears clear and scientifically based.

This article is in my opinion important because it focuses on an important discipline such as forensic botany. It also provides important guidelines such as the retrieval and analysis of microremains and includes among them the cortex providing important information for proper analysis and identification.

However, the authors also emphasize the limitations of the proposed method.

In my opinion it is really important to describe real cases in which this subject could be applied.

The manuscript is clearly structured and well written. I would suggest this document for publication with this small suggestion for authors:

could you add a figure or chart for sampling collection and fixation of bark?

We thank the referee for the positive comments. We added a picture showing sample collection and a bark sample fixed and prepared for observation.

Credit Author Statement

Marco Caccianiga: Conceptualization., methodology, resources, investigation, writing-original draft, writing-review & editing supervision

Chiara Compostella: resources, data curation, visualization

Giulia Caccia: writing-review & editing, visualization

Cristina Cattaneo: Conceptualization., methodology, resources, investigation, supervision, writing-review & editing