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Abstract: The ubiquitous presence of plant species makes forensic botany useful for many criminal cases. Particularly, bryophytes are useful for forensic investigations because many of them are clonal and largely distributed. Bryophyte shoots can easily become attached to shoes and clothes and it is possible to be found on footwear, providing links between crime scene and individuals. We report a case of suicide of a young girl happened in Siena, Tuscany, Italia. The cause of traumatic injuries could be ascribed to suicide, to homicide, or to accident. In absence of eyewitnesses who could testify the dynamics of the event, the crime scene investigation was fundamental to clarify the accident. During the scene analysis, some fragments of *Tortula muralis* Hedw. and *Bryum capillare* Hedw were found. The fragments were analyzed by a bryologists in order to compare them with the moss present on the stairs that the victim used immediately before the death. The analysis of these bryophytes found at the crime scene allowed to reconstruct the accident. Even if this evidence, of course, is circumstantial, it can be useful in forensic cases, together with the other evidences, to reconstruct the dynamics of events.

Perugia, 21/1/2015

Dear Editor,

please find enclosed a copy of our manuscript: "Forensic botany as a useful tool in the crime scene: report of a case" to be considered for publication in Journal of Forensic and Legal Medicine.

All the authors have made substantial contributions to the analysis and interpretation of data.

I revised the article critically for important intellectual content.

The authors have read and approved submission of the manuscript in its final form. The manuscript has not been published nor it is being considered for publication elsewhere in whole or in part, in any language.

We hope that the manuscript will meet the requirements for publication in your prestigious journal, and look forward to receiving from you.

We thank you for your kind attention and please do not hesitate to contact me for any further information.

Sincerely Yours

Dr. Gabriele Margiotta

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Forensic botany as a useful tool in the crime scene: report of a case.

Dear Editor,

Thank you very much for the opportunity to revise our Manuscript. We would also thank the two Referees for their interesting and useful comments. Attached for your consideration is the revised version of our paper. Please find embedded a detailed reply to the reviewers

#Reviewer 1

- 1) Question: This study seems very interesting. I suggest to include more information in the abstract about case and methods

Answer: Following the referee's advice, we extended the abstract by including direct references to the case study

- 2) Question: I suggest to add some references that I insert below: 1. Aquila I, Ausania F, Di Nunzio C, Serra A, Boca S, Capelli A, Magni P, Ricci P. J Forensic Sci. 2014 May; 59(3):820-4. The role of forensic botany in crime scene investigation: case report and review of literature. 2. The Green Revolution: botanical contributions to forensics and drug enforcement. Miller Coyle H, Ladd C, Palmbach T, Lee HC. Croat Med J. 2001 Jun;42(3):340-5. 3. The forensic entomologist in the context of the forensic pathologist's role. Forensic Sci Int. 2001. Campobasso CP1, Introna F. Forensic Sci Int. 2001 Aug 15;120(1-2):132-9.

Answer: We would like to thank the referee for the suggested references. After reading these papers, we modify the text according to the new information and furthermore, we included these paper in the reference list.

#Reviewer 2

- 3) Question: This article starts as a good case report and then somewhat abruptly shifts to a superficial and incomplete review of the field of forensic botany. First and foremost for the authors is to improve the case report involving the mosses. To do this, they need to better explain certain aspects of moss biology since one of their underlying premises is that mosses are particularly useful. But what makes them useful? The authors ambiguously reference their clonal growth and diffuse occurrence, but it is not clear to a general reader what these mean. Why is clonal growth a useful attribute? What do the authors mean by "diffuse"? Where are investigators more likely to come across mosses generally and why?

Answer: In this new MS version, we deeply described moss biology and we also include some reference to those features that could be used as diagnostic characters in forensic botany field.

- 4) Question: Additionally, the authors should clarify the facts of the case at hand: 1) was moss present on the terrace floor? They imply no but they should be explicit. 2) Did the stairs/latter leading to the terrace show foot prints and areas of damage that could plausibly be linked to the suicide victim? 3) Did the video on the lower level actually show the woman climbing onto the stairs/latter? It is not clear. 4) Be explicit for the readers as to why moss on the terrace wall suggests suicide. I figured this out, but the authors should not leave it to the reader to interpret the evidence.

Answer: In order to give as much information as possible on the environmental features in which the suicide happened, and in agreement with the referee's suggestion, we largely improved the

text in the Case Study section. In its current shapes, the new text addresses all the referee's posed questions.

- 5) Question: Rather, the authors should provide clear interpretation in every aspect for the reader. They should control the message. Additionally, I recommend that the authors provide scale bars on the photos and then also introduce a third figure diagramming the relationship of the terrace to the stairs to the street where the victim was found. Making this case report the best it can be is of primary importance.

Answer: We believe the referee is right! For this reason, we improved the whole graphical part of the MS: 4 new photos are now presented, with an increased resolution, detailing mosses parts. A scale bar was added to each picture. A composite image was realized to clearly display the street, the terrace and the whole commercial area where the described case study take place.

- 6) Question: Lastly, I'm not sure it is appropriate for the authors to provide an overview of the field of forensic botany. If this is what the journal wants (a review article), then it would have to be more complete and more extensive with regards to references. For example, the authors' discussion of DNA evidence suggests in parts that DNA evidence is a thing of the future, but it is in fact a thing of the present. The books by Coyle and the more recent Forensic Botany book by David Hall have entire chapters on this. Additionally, their discussion of DNA evidence is limited to the use of DNA barcodes or similar approaches to identify plants at the species-level. However, using fingerprinting techniques such as microsatellites or AFLPs can and have been used to link suspects to a particular crime scene by identifying the very population (not just species) from which botanical trace evidence has come. Hardy and Steinhart (2013; attached) discuss the usefulness and limitations of DNA evidence in their case report involving destruction of a maize crop in the eastern United States.

Answer: We reduce the overview about forensic botany and we corrected the discussion about DNA evidence.

- 7) Question: Indeed, the Discussion section of a paper usually warrants expansion of some sort, but perhaps it could be a bit more focused on things that are relevant to the case report, such as moss biology, why morphology alone (rather than DNA) was sufficient in this case and why it will often be the most cost effective (i.e., DNA work, especially detailed population level studies take time to develop). What about other case studies involving mosses, or other cases where plant material linked suspects to a scene or other similar case reports?

Answer: We corrected the discussion taking into consideration comments of the reviewer.

Conflict of Interest Statement

I declare that there isn't conflict of interest for the article: "Forensic botany as a useful tool in the crime scene: report of a case." .

Gabriele Margiotta.

*Highlights (for review)

- 1) Forensic botany is a powerful tool for crime scene investigation.
- 2) Botanic materials may connect a suspect with a crime scene.
- 3) Botanic materials may reveal if a death is due to suicide, accident or homicide.
- 4) Even a small piece of plant may reveal important information for the court.
- 5) Careful collection of botanical evidence is critical for its evaluation.

INTRODUCTION

The study of plants and of their role in criminal investigations is referred as “Forensic botany”¹. During the past century the use of forensic botany was very limited in criminal or civil cases, because a little number of specialists are trained for this purpose. Furthermore, academic or specialized centers able to train specialist in this field are almost absent². Nevertheless the value of botanical trace evidence in criminal cases has been demonstrated and it is accepted as suitable scientific evidence by the courts of many countries because the ubiquitous presence of plant species makes forensic botany useful for many criminal cases³. Forensic Botany includes many subdisciplines, for example Palynology (the study of pollen and spores), Dendrochronology (the study of growth rings of trees stems and roots), Lichenology (the study of lichen communities), Mycology (the analysis and the identification of Fungi), Bryology (the study of Bryophytes). Particularly, bryophytes are useful for forensic investigations because many of them occur in every type of environment, even those unable to host vascular plants and other organisms. Furthermore, some studies demonstrate that fragments of bryophytes can easily remain attached to shoes and clothes, and even if the plant has been fragmented, their DNA can be analyzed⁴. Traditionally, “bryophytes” include the mosses, liverworts, and hornworts. Together, these groups comprise some 15 000 – 20 000 species and, if combined, are more diverse than the nonflowering vascular plants. The three bryophyte groups share a similar life cycle in which the gametophyte is perennial and dominant about size and longevity and the sporophyte is unbranched, monosporangiate and completes its entire development remaining attached to the maternal gametophyte. The sporophyte generation begins as a fertilized egg and eventually produces spores via meiosis in a terminal sporangium. In the last phase, the sporangium stops to be photosynthetic and so it dries and senesces. The sporophytes of many moss species mature in about a year but do not continue to grow after spore production and they are for the most part essentially annual⁵. The growth period of bryophyte species can be determined by examining annual segments on the stems, which have two different growth forms: the sympodial growth occurs when the apical meristems die at the end of the growing season and growth is continued by lateral buds, which in the next season initiate a new segment with lateral branches. Conversely, the monopodial growth occurs when the apical meristems continue

growing for the whole life of the plant, resuming growth from the previous year. This kind of growth is more regular than sympodial one, and the annual segments are easier to identify because they are situated in an area where side slings change their dimension and positioning. Typically bryophytes (height 0,1-10cm) grow close and form mats patches on ground, rock, wood and many other substrate, in all environments but not in the sea. Bryophytes are rootless and they have single- to multi-branched stems with leaves. The stems is fragile, so, fragments of the plants can easily breaks and the fragmentation allows the vegetative reproduction. Furthermore, mosses (and to a lesser extent, liverworts and hornworts) are unique among land plants in that both the gametophyte and sporophyte generation have sufficient morphological variability and complexity to be phylogenetically informative. Nevertheless, they are hardy in a wide microclimatic range. For these reasons, they provide useful evidence for PMI determination, especially when it is not possible to use morphological-based and/or entomological-based methods⁶. That is why some authors^{7,8} used mosses to establish the PMI of human skeletal remains from the growth rate of mosses. With regard to their usefulness in the field of forensic botany, it should be stressed that there are some published cases where, for instance, the study of moss, in particular the use of monopodial growth bryophytes, is extremely useful when the complete skeletonization of a human individual took place in a shorter period than the expected because of peculiar biological or environmental conditions. It should be considered that environmental conditions do not affect the development of annual growth units, even though they can influence the leaves' density and the final length of the plants or of their secondary branches. In this way, mosses can be used to disregard the microenvironmental conditions in these particular cases. Moreover, as demonstrated in some experiments⁴, bryophyte shoots can easily become attached to shoes and clothes and, because of the common occurrence of bryophytes in many areas, it is possible to find them on footwear, providing links between crime scene and individuals.

The present paper instead deals with a case in which the study of bryophytes represents a link between crime scene and individual. Due to the fact that forensic botany is a discipline that is still poorly understood by nonbotanists, sharing the details of this case would be instructive to the community of law enforcement or forensic operators who may be involved in similar cases in the future⁹: in fact, prosecutors are frequently

unaware of the potential of botanical evidence to provide linkages between crime scenes and individuals. The forensic pathologist has a pivotal role in this development, because he has the legal authority to take charge of the dead body at the death scene: the primary functions of the forensic pathologist are the exterior and interior examination of the corpse and the recovery of the physical evidence¹⁰. Because an adequate death investigations requires the combined efforts and cooperation of experts in different disciplines, forensic scientist have the responsibility to encourage all local jurisdictions to spend some resources for adopting, in death investigations, different professionals or, at least, training crime-scene technicians to recognize and properly preserve botanical evidences.

CASE REPORT

Scene Investigation: We report the case of S.B. , a Caucasian, 22 year old girl, student at the Faculty of Medicine of the University of Siena, Tuscany, Italy. The girl was being treated for a few months at the Department of Psychiatry of the Hospital of Siena for previous episodes of major depression. The girl was in drug therapy and psychotherapy. She was regularly subjected to visits and colloquies at the Department of Psychiatry of the Hospital of Siena, and during all the last interviews, she specifically denied to have suicidal intentions, as it was possible to verify during the subsequent analysis of the medical records. Despite therapy, the girl had a depressed mood. After a few months of therapy, the girl committed suicide by jumping from the terrace of a shopping mall in the center of Siena. The Institute of Forensic Medicine of the University of Siena was immediately informed. Two medical examiners, the prosecutor and several police officers were brought on site to begin the investigation of the case. The crime scene was the terrace of a four-storey building housing a shopping center, the bus station of the city and the University for Foreigners of the University of Siena. The terrace was not accessible by the users of the building: it was necessary to cross a walkway to reach the service stairs, that are reserved only to maintenance of the building. First of all, the medical examiners proceeded to the investigation of the places that the girl had gone through to reach the terrace, in order to obtain useful information: in fact, there were no eyewitnesses who could testify the dynamics of the event. So, it was necessary to use other elements to clarify the accident. Obviously, the investigations were addressed immediately to the surveillance cameras, but the ones present

on the terrace were out of order, and the last images of the girl were those obtained by surveillance cameras on the lower floor. Inspection of the surveillance videos from the camera on the lower floor showed the girl, alone, crossing the walkway to reach the service stairs for the terrace. It was necessary to reconstruct what happened as accurately as possible, so the medical examiners proceeded to a careful analysis of the crime scene, including the walkway and the service stairs. During the inspection, traces of removed mosses were found on the masonry parapet of the terrace and under the soles of the shoes of the victim. The medical examiners detected the absence of moss on the floor of the terrace. The analysis of the walkway and of the service stairs leading to the terrace, instead, allowed to detect the presence of moss. In particular, some areas between the walkway and the service scale showed areas of damage compatible with the trampling. A sample of moss was removed from these areas. Collected specimen was preserved into sterile test tubes. All the traces of moss on the masonry parapet of the terrace and under the shoes of the victim were carefully taken and stored in a test tube for following analysis. The hands and the nails of the victim were carefully analyzed searching for foreign matter. Autopsy was performed 48 hours later. The external examination of the victim showed traumatic injuries to be attributed to a fall. All clothes and external injuries were photographed, described, and measured. The autopsy revealed that the cause of death were bone lesions and skull-encephalic lesions, but the cause of traumatic injuries could be ascribed to suicide, to homicide, or to accident. The reconstruction of the clinical history and the absence of injury by other person suggested the suicide as cause of death, but other elements were essentials to clarify the dynamics of the events.

Botanical Surveys: In order to identify the species and compare the samples, bryologists and plant ecologists of University of Trieste, Department of Life Sciences, were consulted. Botanical material was sampled, mounted on a slide, and observed with a stereomicroscope. Dichotomous identification keys were used to identify sampled materials: two species were determined, *Tortula muralis* Hedw. and *Bryum capillare* Hedw.¹¹. These species are ubiquitous mosses and they are very common in urban bryofloras, growing preferentially in humid environments. More in details, *Tortula muralis* is an acrocarpous moss species that can be frequently found on walls, house enclosures and stony soils in urban areas in Central and North Italy.

Some studies pointed out that *Tortula muralis* is suitable for monitoring air quality based on the accumulation of pollutants in its tissues. *Bryum capillare*, a moss of worldwide distribution, is ubiquitous to cities where it is commonly found growing within the cracks of sidewalks. Because of its occurrence in the harsh and depauperate urban environment, it was hypothesized that this moss is resistant to the deleterious effects of pollutants, such as acid rain. The presence of removed bryophytes on the shoes of the girl and on the masonry parapet of the terrace and the presence of fresh bryophytes on the stairs allowed to reconstruct the accident: most likely the girl had walked up to the service stairs alone, dirtying her shoes of mosses, she climbed on the parapet of the terrace, and threw herself down. Instead, it was less likely that someone could push her down during a fight (in the latter case we would not have found traces of moss on the masonry parapet, because if someone had thrown the girl by pushing her, she could not put her foot on the parapet, leaving the trace of removed moss. There was not moss on the masonry parapet or at the floor of the terrace). This evidence, of course, is circumstantial and would on its own not convict anyone of an offense. However, with other evidences, such as the video of surveillance cameras and the absence of injury by other person, it provided enough direct evidence.

DISCUSSION

Biodiversity, i.e. the variety and complementarity of living organisms occurring at any organization level of the biological world, provides an ideal scenario for forensic botany. For example, most locations are characterized by unique combinations of pollen, resulting in the possibility of using it as a tool to associate individuals with a geographical region or a crime scene, especially for the regions where extensive published pollen records exist^{12,13}. Despite all, botanical evidence in forensics are under-utilized because of the lack of botanical knowledge among evidence collection teams and prosecutors¹⁴.

In most of the world, the samples are collected by generalist crime scene examiners¹⁵. There are many publications¹⁶⁻¹⁷⁻¹⁸⁻¹⁹⁻²⁰ that explain how to collect and store comparator (control) and evidential samples, but often the control collection techniques used at one scene would not be appropriate at another scene. The experience of the collector and the characteristic of the place where the event occurred determine the

best sampling method. For these reasons, scene samples will be taken by an experienced forensic palynologist. To avoid potential contamination or inadvertent destruction of the scene, to stop continued biological activity (microbial destruction) in the samples, and to avoid continual transfer of evidential and control materials, the forensic palynologist should be called to the crime scene immediately. If this is not possible, the samples must be collected with clean implements, placed into sterile containers, and sampling implements must be cleaned after each sample is taken or preferably use new ones each time. Sterile gloves should be worn and changed after each sample is gathered¹⁵.

Plant identification can provide links between crime scene and individuals, determine a sample's geographic origin, ascertain the possession in forbidden species, and more³. His major limit is inability to routinely identify degraded specimens by morphology²¹. In such cases, molecular methods can be used: if botanical trace evidence does not contain the necessary morphological or histological features to identify a plant at the genus or species level, forensic botanists can use DNA technology, especially for fragmented and deteriorated plant material²¹. DNA profiling methods can discern genetic differences among individuals of the same species and they are now widely employed in human criminal and civil cases⁹. Molecular biology analyses should support the traditional forensic botany in the identification of the plant species evidence, but the use of these techniques in the court of law is still rare⁹. The costs are the major limitations to the use of these methods, instead, traditional botany uses simple, inexpensive methods for the identification of a plant species. In our case, it was not necessary to perform the genetic investigation because morphological and histological features were sufficient for the identification of the species.

CONCLUSIONS

Forensic botany is a powerful tool for crime scene investigation. Botanic materials may connect a suspect with a crime scene, or reveal whether a death is due to suicide, accident or homicide¹⁴. Even a small piece of plant may reveal important information for the court. For example, pollen samples can be useful to distinguish one year from another²², the growth rate of some species of moss is a valid method in estimating the minimum PMI²². But only if an investigator is aware of the potential existence of that

evidence any efforts will be made to search for it. It must be highlighted that a botanical evidence can be present in a crime scene at the microscopic level (such as grains of pollen)¹⁴ and that careful collection, documentation, and preservation of botanical evidence are critical to the evaluation of plant evidence. Unfortunately, often samples have been collected by untrained personnel¹⁵. Guidelines should dictate that plant materials should be collected along with control samples. Plant material may be examined using simple microscopy or more sophisticated plant DNA testing¹. Forensic botany first requires the identification of the plant species by morphological characteristics, microscopy, or molecular biology²³⁻²⁴⁻²⁵⁻²⁶, and, after the species has been identified, an attempt to individualize the sample is made. If a plant is rare in the area of the sampling, source attribution may not be difficult, however, the effectiveness of source attribution will depend on how rare the species into the geographic area is²⁷.

Forensic botany should be considered in every case of crime, especially, but not exclusively, when it happens in an open area²⁸. Botanic proof is circumstantial and alone is a poor evidence, however, with the other finds, it can provide enough direct and circumstantial evidence for the courts²⁹. For many regions forensic botany is not yet accepted, even if it is now clear that it can play an important role in many forensic cases¹⁴.

Acknowledgments

We are grateful to Dr. Elena Pittao for her kind assistance in getting moss microscopy images.

Figure Legend:

Fig.1: This picture shows the terrace, the walkway and the street where the victim was found. The arrows show the point of throwing and the point of impact of the victim.

Fig.2: Traces of removed mosses on the masonry parapet of the terrace (up) and under the shoes of the victim (down)

Fig.3: Specimen of *Tortula muralis* Hedw found on the crime scene. Magnification 10x with focal magnification 0,5x.

Fig.4: Specimen of *Tortula muralis* Hedw found on the crime scene. Magnification 6.3x.

Fig.5: Specimen of *Bryum capillare* Hedw found on the crime scene. Magnification 10x with focal magnification 0,5x.

Fig.6: Specimen of *Bryum capillare* Hedw found on the crime scene. Magnification 6,3x.

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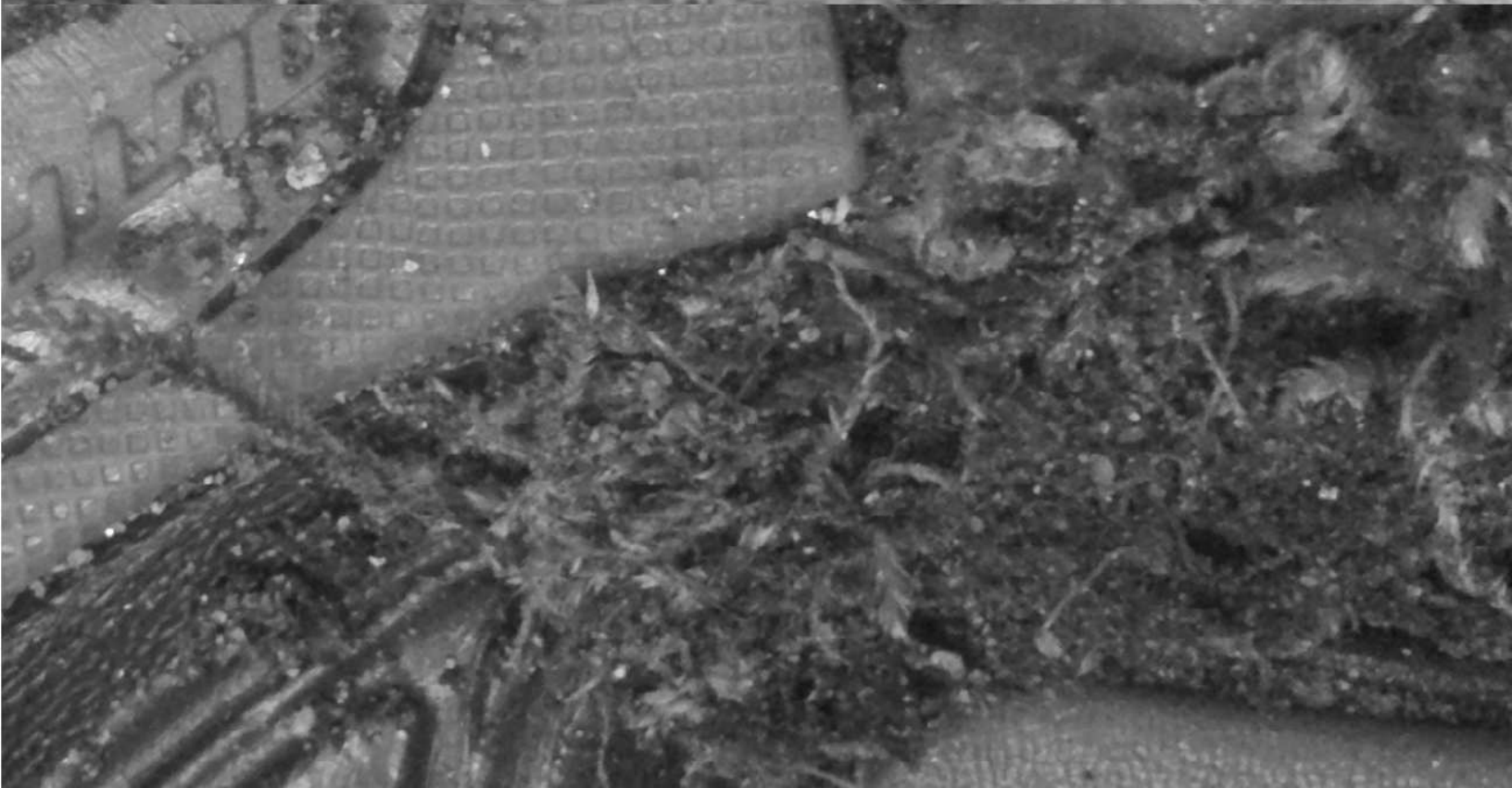
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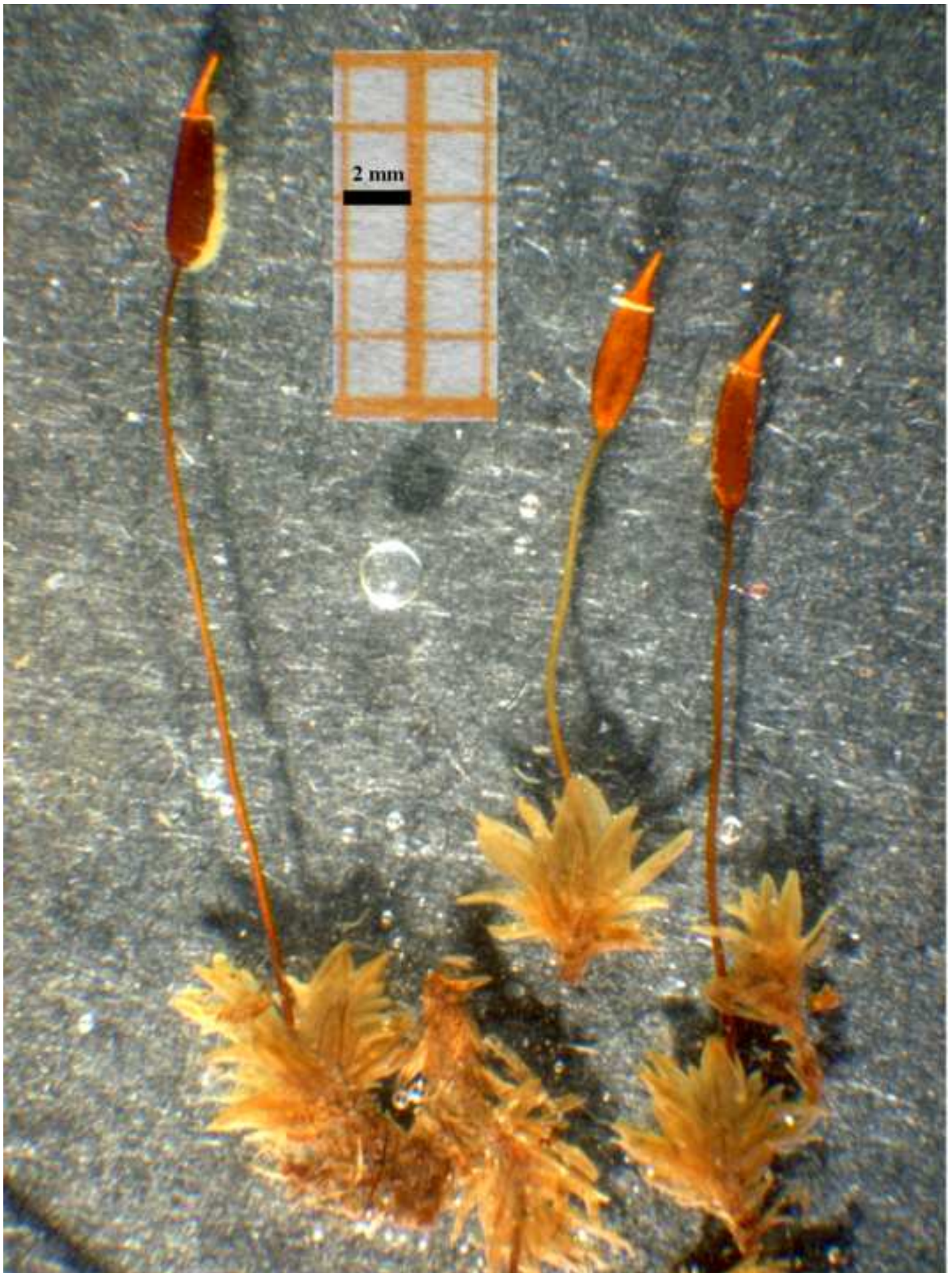
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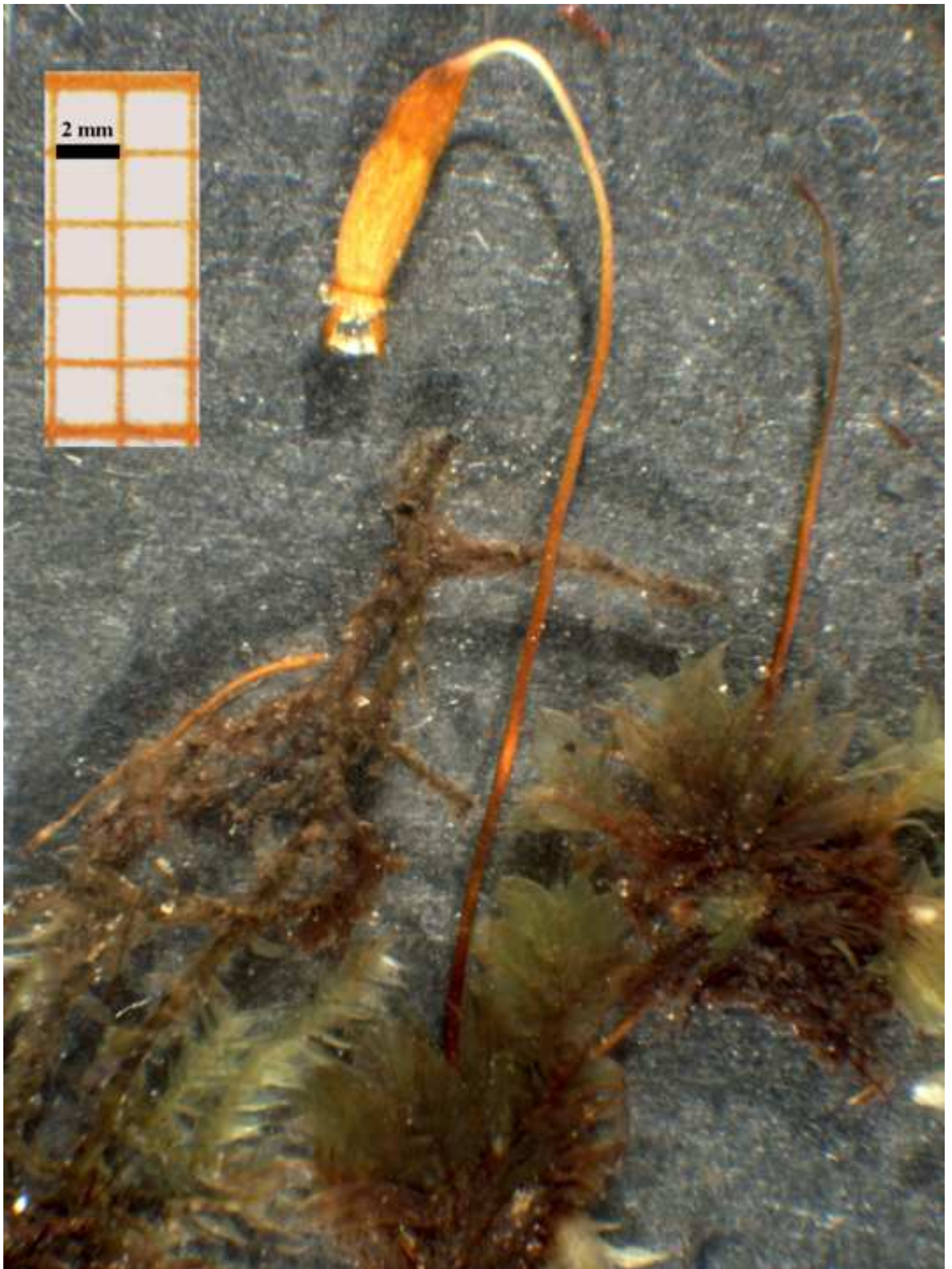
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