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CASE REPORT

Establishing a minimum postmortem interval of human remains in an advanced state of skeletonization using the growth rate of bryophytes and plant roots

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Abstract This paper illustrates the usefulness and efficiency of botanical evidence in establishing a minimum postmortem interval (PMI). The case under analysis refers to the remains of an adult male in an advanced state of skeletonization recovered from a wooded area in northern Portugal. The skeleton showed several taphonomical changes, which included the presence of green algae, bryophytes, and growing shrub roots in, around, and through the remains. By determining the age of both the bryophytes and shrub roots, it was concluded that the minimum amount of time elapsed since death was 3 years, to which several months or a few years have to be added to account for the complete decomposition of the remains. The disappearance of the presumptive individual had occurred 6 years before and is fully consistent with the estimate of the PMI. This report illustrates a novel use of bryophytes in a forensic setting.

Keywords Time since death · Forensic botany · Taphonomy · Skeletonization

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Introduction

The estimation of the postmortem interval (PMI) is a matter of crucial importance in forensic investigations. Determining the time between death and discovery of a corpse has critical value for providing a time line which allows for the narrowing of possible candidates for identification from lists of missing persons and exclude individuals. It can also provide a temporal reference point against which a suspect's alibi can be compared. Various physical [1], chemical [2], and biological [3, 4] methods provide a reasonable time frame for death in the early postmortem period. However, when the cadaver is in an advanced state of decomposition or completely skeletonized, the enormous variability in the rate of decomposition and postmortem change complicates this estimate. In addition, most indicators underlying those methods, such as insect life cycles or species succession, may simply not be useful at this stage [5]. Both experimentation with animal cadavers [6–8] and analysis of documented cases [9, 10] or personal experience with casework [6] have revealed useful information on the decomposition process, but they can only provide broad guidelines for PMI estimation. These guidelines not only provide wide intervals for estimation but also the longer the postmortem period, the broader the estimated time range. In

addition, PMI determination is done on an individual and empirical basis, drawing from the personal casework experience and subjective comparison of each case with others of similar characteristics [10]. To complicate matters further, there are climatic, regional, and topographic differences between locations that make descriptions and guidelines established in one region inapplicable to other regions. Therefore, there is often a great chance for error in PMI estimation.

Bodies left to decompose on the ground surface of wooded areas will slowly become integrated into the surrounding environment. Personal belongings, such as clothing, will provide a substrate for growth of sessile organisms and, once skeletonization is reached, so will bones. In particular, green algae, mosses, lichens, and fungi may grow on bone depending on various environmental circumstances. In addition, shrub and tree roots will grow and incorporate both bones and clothing if sufficient time elapses. Plant material is a perfect indicator of time because plants are permanently attached to a substrate (sessile) and the pattern and rate of plant growth can be easily identified [11, 12]. The growth period can then be converted into an estimate of the lower (minimum) limit for the PMI [13]. Only a minimum can be achieved because there is no certain manner to determine precisely when plant colonization was initiated relative to death or skeletonization. Although establishing the minimum postmortem period may only provide incomplete information, it can offer crucial data for the elimination or inclusion of possible candidates for identification.

The discovery of a fully clothed skeletonized human body in a forested area of northern Portugal provided the opportunity to evaluate the efficiency of estimating a minimum PMI from the growth rate of mosses and shrub roots. As soft tissue decomposed, the skeleton became slowly engulfed by the roots of a nearby rock rose and colonized by different species of bryophytes. Botanic evidence has been used to establish time of death of skeletonized remains by analysis of the growth of plant roots [11, 12, 14] but bryophytes, on the other hand, have not been previously utilized in this manner. The bryophytes, with more than 20,000 living species worldwide, are the most diverse group of land plants except for the flowering plants [15].These organisms are an important element of the communities' structure of the woodlands and other habitats. Bryophytes species have different ecologies that occur in association with a variety of substrata or microhabitats responding to a large spectrum of environmental factors. These features have made them important plants to provide botanical evidence for other areas of forensic practice [16, 17]. This case report describes how the growth analysis of bryophytes and of shrub roots retrieved was used to provide a minimum PMI, which represented additional evidence to support the circumstantial identification available.

Case history

In February 2008, skeletonized human remains were found in a wooded area of northern Portugal. The local and criminal police were called to the site and the remains were photographed, documented, and collected. They were sent to the pathology department of the northern division of the National Institute of Legal Medicine in Porto, Portugal where the remains were analyzed by a forensic anthropologist. Police enquiries revealed that only one person had been reported missing in the area where the remains had been found. Although the remains were fully skeletonized and no personal belongings were found, the remnants of the clothing were compatible with what had been described as the missing individual's clothes around the time of the disappearance and the location where the remains were found was consistent with the known whereabouts of the individual. The possible match refers to a 61-year-old indigent man of 156 cm of recorded height (national citizenship card) who went missing 6 years prior. There are no known relatives and no available antemortem DNA profiles for comparison. There were also no clinical or dental records available for this individual. The police was only able to recover an overall description of the individual from local inhabitants acquainted with him.

Since a putative positive identification of the decedent was suggested, verification through postmortem analysis of the remains was carried out by the forensic anthropologist. The analysis of the remains revealed a fully skeletonized body belonging to one single individual. All of the parameters of the biological profile were compatible with the possible match (sex, age, and stature). The osteological analysis also revealed distinguishing skeletal features which were consistent with information provided by the police report, namely, the fact that this individual walked with a limp. Since no antemortem records or DNA profiles were available to confirm identity, estimation of the PMI could provide additional circumstantial evidence for inclusion or exclusion of this individual.

Estimation of the postmortem interval

An estimate of the time elapsed since death was obtained from the taphonomic analysis of the skeletal remains and from an examination of the botanical material recovered from the remains and from the clothing remnants. Due to the advanced state of skeletonization of the remains, the works of Behrensmeyer [18], Galloway et al. [19], Haglund [20],

Fig. 1 Ribcage engulfed in a root mass (note lack of distinguishing traits due to high density of roots)

and Komar [9] were used to provide guidance in the estimation of the PMI at the later stages of decomposition of ground surface findings from taphonomic changes. Given the circumstances, these works were considered the most relevant and adequate for PMI estimation, as there is no available data for the Portuguese territory. Only the study by Prieto et al. [10] provides overall guidelines for the rate of body decomposition in various environmental conditions in the neighboring Spanish territory.

The remains were found to be almost completely skeletonized and relatively complete. There was only some soft tissue mummification in one foot, which was partially recovered from the remnants of a sock and boot. Most of the missing skeletal elements referred to the entire upper limbs, face, and mandible. The human remains were highly disarticulated and the body had probably been lying next to or on top of a shrub from which several roots subsequently grew through the remains. The ribcage had remained in an approximate anatomical position within the clothes and was bounded by a dense mass of roots (Fig. 1), as were several other skeletal elements. Green algae and mosses covered some of the bones and portions of the clothing and no insect material was retrieved. Several bone surfaces were sun bleached and most bones have also been stained with a brown color from soil and foliage cover suggesting that the decomposition occurred both in the shade and in the sun. Weathering was also common in exposed areas, mostly at the bone extremities. Table 1 summarizes the results of the taphonomic analysis and provides an estimate of the postmortem period. Given that the taphonomical analysis only permits a very wide and relatively uncertain PMI, the botanical evidence should prove more useful and effective when estimating a minimum PMI.

The estimate of the PMI from the botanical evidence relies on the assessment of the growth period of the plant samples obtained. As these plant parts grew through and on the bones and clothing, the PMI estimation is based on their direct association with the remains. Two types of botanical material were present: bryophytes (mosses) and vascular plants (shrubs). A sample of bryophyte was collected from the outer and inner lining (Fig. 2) of the clothes, as well as a sample collected from the proximal epiphysis of the right tibia (Fig. 3). The growth period of bryophyte species can be determined from annual segments in shoots and there are two growth forms [21, 22]. Sympodial growth occurs where the apical meristem ceases activity annually and growth is continued by a lateral bud, and monopodial growth occurs when the apex continues growth over a second growing season [22]. Species identified in this report are monopodial and some are ubiquitous, such as *Bryum capillare* Hedw. and *Hypnum cupressiforme* Hedw., making them particularly useful for estimating a minimum PMI of skeletonized remains. The age of the specimens was estimated by counting the number of annual segments along the stems [23] (Fig. 4) and provided the minimum PMI.

Table 1 Taphonomic analysis and PMI estimate

Taphonomic change	Description/degree	Postmortem period
Soft tissue	Only desiccated soft tissue remaining	4 months–3.5 years[a]
Greasiness and odor	No greasy substances; dry bones and no odor	>6 months[b]
Disarticulation	Total disarticulation; removal of upper extremities	5–52 months[c]
Bleaching	Part of cranium, innominates, sacrum, vertebra, and most of lower limbs	>6 months[b]
Exfoliation and bone loss	Exfoliation of iliac blade; cancellous bone exposure of tibial epiphyses and vertebra; destruction of facial bones	1–5.5 years[b] 2–6 years[d]
Estimated PMI		5 months–6 years+

[a] Komar [9]

[b] Galloway et al. [19]

[c] Haglund [20]

[d] Behrensmeyer [18]

Fig. 2 Bryophyte (*H. cupressiforme* Hedw.) in the inner lining of clothes

The sample of vascular plants referred to the fragment of shrub root with the greatest diameter obtained from the thoracic area. Plant age estimated by growth root rings is a valuable technique broadly applied independently of the plant species concerned [24]. However, roots are plant organs that may suffer partial necrosis and mortality during the plant's life. Furthermore, the distinctness of the growth rings generally decreases in the distal parts of the roots, a pattern which is also known for tree roots [25]. When assessing and collecting scattered or broken root fragments, such as in this case, the whole root organization may be lost, invalidating the use of growth ring counting to accurately determine plant age. Therefore, root age was qualitatively judged by visualizing branch diameter, root architecture, and topology according to Fitter and Stickland [26]. The maximum age of the root is the minimum PMI. Table 2 summarizes the results from the analysis of the botanical material.

Fig. 3 Bryophyte (*Bryum* sp.) growing in the proximal epiphysis of the right tibia

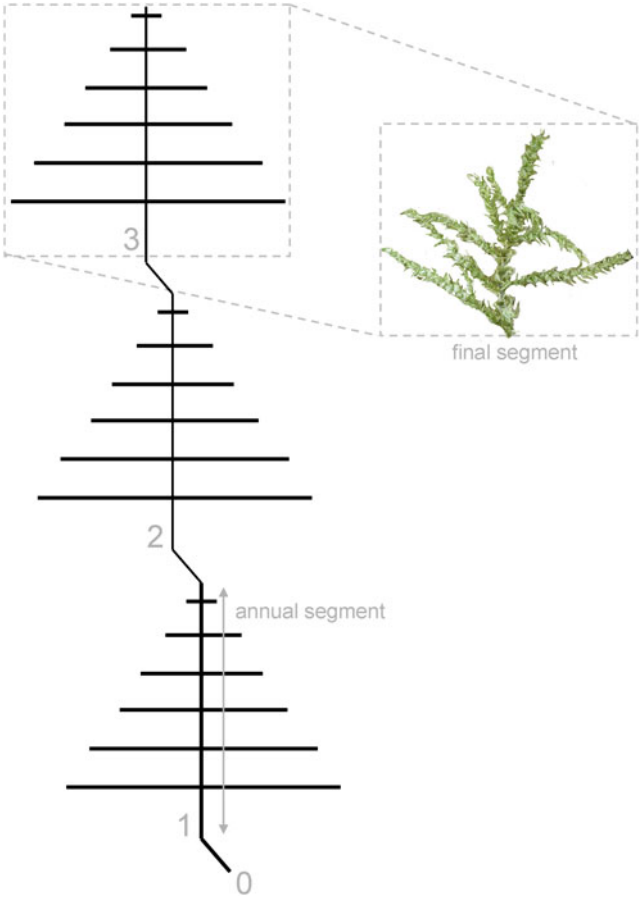

Fig. 4 Diagram of the main stem of a moss illustrating the growth process (monopodial growth). *0* represents the newest bud and years *1*, *2*, and *3* represent the annual segments (adapted from [22])

Discussion and conclusion

Forensic casework decisions must weigh all available evidence and translate that information in a manner that can be evaluated by the judicial authorities. In some circumstances, positive identification can be reached by dental methods or by simple exclusion, whereas in others, it

Table 2 Description of botanical samples and analysis of the specimens' ages

Location sampled	Species	Tissue	Age (years)
Bone (tibia)	*Bryum* sp.	Shoot	3
Clothes (inner lining)	*Bryum capillare* Hedw.	Shoot	1
	Hypnum cupressiforme Hedw.	Shoot	1
	Campylopus flexuosus (Hedw.) Brid. or related species	Shoot	1
Clothes (outer lining)	*Campylopus introflexus* (Hedw.) Brid.	Shoot	3
Thorax (ribcage)	Undet. (*Cistaceae* Juss)	Root	>3
Minimum PMI			3

may need to be founded on case-specific information, such as the location where the remains were found, clothing, cultural factors, PMI, etc. [27]. Due to the absence of any kind of antemortem records (dental or medical radiographs), only a strong circumstantial identification was achieved in this case, based on the biological profile, location of the remains, and clothes. However, an accurate PMI offers a temporal window for the elimination or inclusion of possible candidates, thus increasing the changes of a more certain identification.

The estimation of the PMI using taphonomic analysis of the skeletal remains can be considered uncertain based on published descriptions because of geographical, latitudinal, and overall environmental differences between the source samples and this case study. Latitude and local conditions may delay or advance the rate of decay, depending on factors such as temperature or rain. On the other hand, the information provided by the growth of the plant specimens provides a much more accurate minimum PMI estimation of 3 years. However, a certain amount of error may still be involved in estimating the age of bryophyte species. The main potential sources of error derive from major impacts on habitats, such as atmospheric pollution, and infrequent environmental conditions, like ultrabasic rock substrate or unusual temperature and precipitation. These factors may influence growth, density, biomass, and ultimately, the survival of these organisms. In the absence of such extreme environmental circumstances, monopodial species show a well-defined pattern of annual innovations which are good indicators of plant age [22]. Therefore, although the annual growth rate is dependent on environmental variables, especially temperature and precipitation, it is usually straightforward to assess and relatively constant in species of mosses with well-individualized annual growth increments, particularly species from areas with a well-defined dry season, such as this case. The absence of entomological evidence may reflect the natural decomposition of insect material (such as empty pupal cases and beetle exuviae) over such a long PMI and highlights the need for evidence like bryophytes because insects do not provide information on a multiannual scenario.

Considering that the bryophytes found on bone will only grow in the absence of organic material, we must assume skeletonization before plant colonization. If we wish to extend our postmortem period and estimate a maximum time since death, several months or a few years have to be added to the minimum PMI to account for the complete decomposition of the remains. This is not incompatible with the postmortem period obtained from the taphonomical analysis and additional evidence suggests that a maximum limit for the PMI of 6 years may in fact be reasonable. According to Sledzik [28], complete skeletonization occurs between 4 months and 3 years and observational data of known cases in Spain [10] also suggests that complete skeletonization occurs within 3 years. Despite the uncertainty with the estimate obtained from taphonomical change, the 3 years of moss and shrub root growth plus 3 years for complete skeletonization, is consistent with the presumptive identity's missing period.

Although only a strong circumstantial identification was obtained, this case illustrates the importance of other case-specific evidence for a decision. In particular, the analysis presented here provides an example of the usefulness and efficiency of botanical evidence, namely, bryophytes, in establishing a minimum PMI where the remains have been found completely or almost completely skeletonized. If the presumptive individual had disappeared between a few months and 3 years prior, then botanical evidence would have ruled him out as a possible candidate for identification.

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References

1. Nelson E (2000) Estimation of short-term postmortem interval utilizing core body temperature: a new algorithm. Forensic Sci Int 109:31–38
2. Bocaz-Beneventi G, Tagliaro F, Bortolotti F, Manetto G, Havel J (2002) Capillary zone electrophoresis and artificial neural networks for estimation of the post-mortem interval (PMI) using electrolytes measurements in human vitreous humour. Int J Legal Med 116:5–11
3. Arnaldos MI, Romera E, Presa JJ, Luna A, García MD (2004) Studies on seasonal arthropod succession on carrion in the southeastern Iberian Peninsula. Int J Legal Med 118:197–205
4. Byrd JH, Castner JL (2001) Forensic entomology: the utility of arthropods in legal investigations. CRC, Boca Raton
5. Ubelaker D (1997) Taphonomic applications in forensic anthropology. In: Haglund WD, Sorg MH (eds) Forensic taphonomy: the postmortem fate of human remains. CRC, Boca Raton, pp 77–90
6. Mann RW, Bass WM, Meadows L (1990) Time since death and decomposition of the human body: variables and observations in case and experimental field studies. J Forensic Sci 35:103–111
7. Weitzel MA (2005) A report of decomposition rates of a special burial type in Edmonton, Alberta from an experimental field study. J Forensic Sci 50:641–647
8. Janjua MA, Rogers TL (2008) Bone weathering patterns of metatarsal v. femur and the post-mortem interval in southern Ontario. Forensic Sci Int 178:16–23
9. Komar DA (1998) Decay rates in a cold climate region: a review of cases involving advanced decomposition from the medical examiner's office in Edmonton, Alberta. J Forensic Sci 43:57–61
10. Prieto JL, Magaña C, Ubelaker DH (2004) Interpretation of postmortem change in cadavers in Spain. J Forensic Sci 49:918–923
11. Willey P, Heilman A (1987) Estimating time since death using plant roots and stems. J Forensic Sci 32:1264–1270
12. Courtin GM, Fairgrieve SI (2004) Estimation of postmortem interval (PMI) as revealed through the analysis of annual growth in woody tissue. J Forensic Sci 49:781–783

13. Coyle HM, Lee C-L, Lin W-Y, Lee HC, Palmbach TM (2005) Forensic botany: using plant evidence to aid in forensic death investigation. Croat Med J 46:606–612
14. Quatrehomme G, Lacoste A, Bailet P, Grevin G, Ollier A (1997) Contribution of microscopic plant anatomy to post-mortem bone dating. J Forensic Sci 42:140–143
15. Mishler BD (2001) The biology of bryophytes—bryophytes aren't just small tracheophytes. Am J Bot 88:2129–2131
16. Korpelainen H, Virtanen VJ (2003) DNA fingerprinting of mosses. J Forensic Sci 48:804–807
17. Virtanen V, Korpelainen H, Kostamo K (2007) Forensic botany: usability of bryophyte material in forensic studies. Forensic Sci Int 172:161–163
18. Behrensmeyer AK (1978) Taphonomic and ecologic information from bone weathering. Paleobiology 4:150–162
19. Galloway A, Birkby WH, Jones AM, Henry TE, Parks BO (1989) Decay rates of human remains in an arid environment. J Forensic Sci 34:607–616
20. Haglund WD (1997) Dogs and coyotes: postmortem involvement with human remains. In: Haglund WD, Sorg MH (eds) Forensic taphonomy: the postmortem fate of human remains. CRC, Boca Raton, pp 367–382
21. During HJ (1992) Ecological classifications of bryophytes and lichens. In: Bates JW, Farmer AM (eds) Bryophytes and lichens in a changing environment. Clarendon, Oxford, pp 1–31
22. Ross SE, Callaghan TV, Ennos AR, Sheffield E (1998) Mechanics and growth form of the moss *Hylocomium splendens*. Ann Bot 82:787–793
23. Glime JM (2007) Bryophyte ecology, vol. 1. Physiological ecology. Ebook sponsored by Michigan Technological University and the International Association of Bryologists
24. Dietz H, Ullmann I (1997) Age-determination of dicotyledonous herbaceous perennials by means of annual rings: exception or rule? Ann Bot 80:377–379
25. Fritts HC (1976) Tree rings and climate. Academic, London
26. Fitter AH, Stickland TR (1992) Fractal characterization of root system architecture. Funct Ecol 6:632–635
27. Anderson BE (2007) Comment on: statistical basis for positive identification in forensic anthropology. Am J Phys Anthropol 133:741
28. Sledzik PS (1998) Forensic taphonomy: postmortem decomposition and decay. In: Reichs KJ (ed) Forensic osteology: advances in identification of human remains. Charles C. Thomas, Springfield, pp 109–119