Botanical Journal of the Linnean Society, 2025, **00**, 1–10
https://doi.org/10.1093/botlinnean/boaf096

Original article

Adhesive metagenomics: unlocking information on the assembly of historic herbaria

Annabelle R. de Vries[1,2,3,*], Lochlan Chadwick[1], Mark Carine[2], Robin G. Allaby[1]

[1]School of Life Sciences, Gibbet Hill Campus, University of Warwick, Coventry, United Kingdom
[2]Department of Botany, The Natural History Museum, London SW7 5BD, United Kingdom
[3]Naturalis Biodiversity Center, Leiden 2333, The Netherlands

*Corresponding author. Naturalis Biodiversity Center, Darwinweg 2, Leiden 2333 CR, The Netherlands. E-mail: Annabelle.devries@naturalis.nl

ABSTRACT

Adhesives have been integral to the production of herbaria for paper making, securing plant material to paper, and, in the case of bound volumes, for bookbinding. The adhesives used may be of plant, animal, or synthetic origin. Here we investigated herbarium specimens from the Natural History Museum London (NHM), collected between 1698 and 1970, to determine whether information on the adhesives used in the preparation of herbarium specimens can be established using ancient DNA analysis of the mounted plant material. Ancient DNA was obtained from leaf tissue of 14 herbarium specimens of *Trochetiopsis* and sequenced using Illumina MiSeq. Non-*Trochetiopsis* DNA was identified using metagenome analysis software (MEGAN). Reads identified as animal were further analysed using the metagenomics pipeline Phylogenetic Intersection Analysis (PIA). Two specimens showed distinct animal reads. One specimen from 1698, which had glue residue observable on the leaf material, showed evidence for Pecora and Bovidae, specifically *Bos*, and with lower read counts also for both Leporidae and *Ovis*. The bones of cattle, rabbits, and sheep are all likely to have been used in the preparation of glue in this period, and consequently the animal DNA retrieved is probably from the glue used for mounting. The second sample was from 1970 and showed reads of Pecora, Bovidae, and *Bos*. Latex adhesives were used at the NHM during the 1970s with synthetic adhesives used thereafter. We infer that the animal DNA retrieved is probably from gelatine used for paper sizing. The results of this study demonstrate that the genetic analysis of plant material can also provide insights into the process of making herbaria.

Keywords: ancient DNA; herbariomics; adhesive; herbarium glue; specimen mounting

INTRODUCTION

It is unclear who made the first herbarium (Stefanaki *et al.* 2019) although Luca Ghini was certainly a pioneer in their development (Thijsse 2016). The earliest surviving herbaria date back to the mid-16th century, Bologna, Italy (Stefanaki *et al.* 2018, 2019, Thiers 2020). Such early herbaria were used by physicians and apothecaries, or were decorative presents (Thiers 2020). Today, herbaria have an extraordinarily diverse range of users and uses (Carine *et al.* 2018, Davis 2023, Swain and Chakraborty 2024).

Herbaria are a paper-based technology in which dried pressed plant material is attached to paper sheets for long-term preservation. A range of products derived from biological sources may be used in the production of paper used in herbaria. Fibres from plants such as flax and hemp, and from clothes and rags made from linen, cotton ,and wool may all have been used in the production of paper (Bloom 2017, Clark 1986). From the 19th century onwards, wood pulp was also used for paper production (Barrett *et al.* 2016). Today, paper may still be described as 'rag' to indicate the paper was made from cotton or linen, even though rags are rarely used themselves (Clark 1986). Sizing of the paper, to make it less permeable to water for ease of writing (Gimat *et al.* 2021), may also make use of biological products. Paper can be sized using a range of different products including starch, polyvinyl alcohol, and gelatine (Clark 1986, Gimat *et al.* 2021). Sizing with gelatine, which is thought to have originated in Italy around the end of the 13th century (Barrett and Mosier 1995, Gimat *et al.* 2021), is considered to have advantages over other sizing agents, improving the strength, resistance to abrasion, and protection against soiling of paper (Barrett and Mosier 1995).

Attaching plant specimens to paper can be done by using a range of techniques, for example stitching, or using adhesives in different ways, with paper strips, pre-coated paper, point adhesion,

Received: 9 April 2025; revised 5 September 2025; accepted 9 September 2025

or overall adhesion (Croat 1978, Clark 1986, Egenberg and Moe 1991, Berli and Belhadj 2020, Grenda-Kurmanow 2021, Davies *et al.* 2023, Yesilyurt et al 2025). The adhesives used may be synthetic but they may also be derived from plant or animal tissue (Bridson and Forman 1999, Grenda-Kurmanow 2021). Plant-based adhesives used in herbaria have included starch pastes, gums, and resins from a variety of plant species (Fay 2021, Grenda-Kurmanow 2021). Early descriptions of animal glue recipes for herbaria were provided by Van den Spiegel (1606) and De Tournefort (1694). Van den Spiegel wrote in *Isagoge in rem herbariam libri duo*: 'This glue is made of transparent hide glue (bovine glue), called the German glue ("colla Tedescha") in Padova. One ounce is macerated in six ounces of vinegar throughout the night and then heated slowly until it becomes liquid. Then allow the glue to cool down and add a drachma [1/8 of an ounce] of "hepatic" aloe extract, one scrupulus [1/24 of an ounce] of clove powder and mix.' (Grenda-Kurmanow 2021). De Tournefort (1694) suggested use of hide glue together with mercury chloride, warning that decoctions of 'Semen contra' [*Seriphidium cinum* (O. Berg & C.F. Schmidt) Poljakov, Asteraceae], 'Absinthe commune' (*Artemisia absintium* L., Asteraceae), aloe, or 'similar drugs' cause a discoloration of the plant material (Grenda-Kurmanow 2021).

A fish-based recipe using isinglass (collagen derived from the swim bladders of fish), from the early 18th century, in the hand of James Petiver (1663–1718) can be found in the Sloane Herbarium at the Natural History Museum London (NHM) (Jarvis 2021). Petiver does not state the type of fish used as the source of swim bladders but they were typically obtained from fish such as sturgeon (Ova 2000, Werrett 2025). Linnaeus (1751) also later published recipes for isinglass or fish-based adhesives that he used for mounting his own herbaria (Grenda-Kurmanow 2021). While fish-based glues are rarely used in herbaria today, gelatine glues are still in use (Bridson and Forman 1999, Schellmann 2007, Grenda-Kurmanow 2021).

The biological origin of adhesives in artworks and archival documents has been investigated using a range of techniques. Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) is a technique with which the presence of gelatine in paper can be detected (Rouchon *et al.* 2010). Gas chromatography–mass spectroscopy (GC/MS) has been used to determine the gelatine content in paper (Stephens *et al.* 2008). Other techniques used include pyrolysis-GC/MS (Py-GC/MS) (Chiavari *et al.* 2006) and thin layer isoelectric focusing, a method of protein separation, that has been used to characterize parchment and animal glue (Van Oosten 1989). DNA sequencing techniques, such as species-specific PCR, have also been used even though exposure of DNA to water and destructive pH levels during the glue making process (Schellmann 2007) could degrade DNA. Mitochondrial DNA analysis on glue from the Madonna of Citerna by Donatello showed that the adhesive was probably made from *Bos taurus* L. (Albertini *et al.* 2011).

Grenda-Kurmanow (2021) noted that there has, to date, been no study of DNA from adhesives in herbaria. Whole genome sequencing technologies are increasingly being used to examine plant diversity and evolution using herbarium specimens (Davis 2023). Herbarium material contains fragmented DNA of approximately 40–120 bp in length, so-called ancient DNA (Orlando *et al.* 2021). Often the focus of analysis is on the successful recovery of ancient endogenous DNA from the focal organism(s) (Gutaker and Burbano 2017, Bakker *et al.* 2020, Kistler *et al.* 2020, Davis 2023). Here we focus on the analysis of ancient exogenous DNA (Bieker *et al.* 2020, Grasso *et al.* 2025), i.e. ancient DNA that is not from the expected taxon and that is typically filtered out from the analysis. Ancient exogenous DNA may provide insights into the material used in the preparation of herbarium specimens.

In this project, we used a metagenomics pipeline to study the exogenous DNA which is a by-product of sequencing herbarium material of *Trochetiopsis* specimens and that is typically filtered out. Fourteen herbarium specimens from the NHM ranging in age from 1698 to 1970 were sequenced. We screened the ancient exogenous DNA for evidence of taxa consistent with the use of animal- or plant- based glues or paper making. We specifically investigated for reads from animal species used in isinglass (fish) glue, or in animal-based glue, notably those derived from the bones of butchered animals including rabbits, pigs, and other domesticated ungulates (Haupt *et al.* 1990, Schellmann 2007, Fay 2021, Grenda-Kurmanow 2021). We also investigated for reads consistent with the use of plant-based adhesives derived from maize, rice, potato, wheat, cassava, *Aloe vera*, rubber, gums, or resins (Fay 2021, Grenda-Kurmanow 2021). Other plant species such as flax, hemp, and cotton associated with paper making were also investigated. Our overall goal was to ascertain whether ancient exogenous DNA can give insights into the material history of herbarium specimens.

METHODS

Herbarium material sampling

Dried leaf samples were obtained from the herbarium of the NHM, London, UK. Samples of 0.5–1 cm^2 were taken from 14 specimens of *Trochetiopsis* (Malvaceae), a genus of three species endemic to St Helena recently considered to be part of *Melhania* Forssk (Dorr and Wurdack 2021).

The date of collection ranged from 1698 to 1970. The oldest specimens sampled (Table 1, samples HS92.7 and HS87.24) were stored in bound volumes originally assembled by Leonard Plukenet (1641–1706) and were subsequently integrated into the Herbarium of Hans Sloane (1660–1753) (Dandy 1958, Carine 2020, Scott *et al.* 2025). These samples were adhered to the paper and were released by slow and partial dissolving of the glue using 5% agarose gel blocks. Glue residues remaining on the leaves were subsequently removed using tweezers to carefully scratch the glue off. Figure 1 shows the leaf samples before and after glue removal. The remaining 12 herbarium specimens (Table 1) were sampled from the General Herbarium. While these specimens were also mounted using adhesives, no glue was observed on the leaf material sampled.

Ancient DNA extraction and next-generation sequencing of ancient DNA

Ancient DNA extractions were performed using a modified cetyltrimethylammonium bromide (CTAB) protocol (Rogers and Bendich 1985, Doyle and Doyle 1990). This was followed by the single-tube BEST method for library preparation (Mak *et al.* 2017, Carøe *et al.* 2018) with the modifications of Liu (2019). The PCR protocol followed Meyer and Kircher (2010) and the clean-up used Sera-Mag SPRI beads (Roland and Reich 2012). The volume

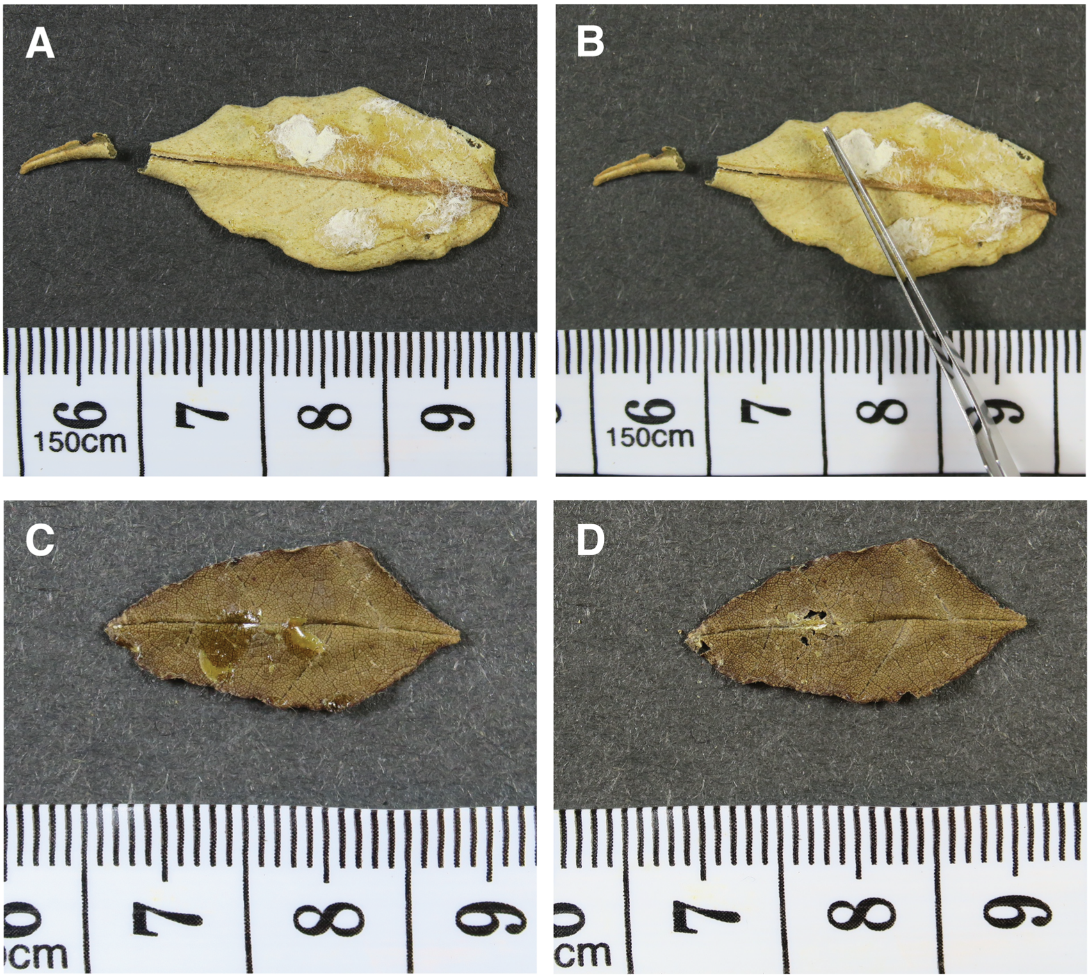


Figure 1. Leaf material of Sloane Herbarium samples HS87.24 (A and B) and HS92.7 (C and D). Before (A and C) and after (B and D) removing glue.

of SPRI beads was adjusted to ×3 (120 µL) (beads/DNA ratio 2.67:1). During the library preparation process, duplicates were made for six of the 14 specimens, where a low DNA yield was obtained on the first attempt (duplicate samples are: 080a and 080b, 25b and 25a, 118a-a and 118a-b, 79 and 79b, and 86a and 86b). All 20 libraries with negative controls were diluted to 4 nM using molecular-grade water and pooled for sequencing. DNA was indexed double ended and, to allow for covering more template molecules, single end sequencing was done (Roycroft *et al.* 2022). Sequencing of the libraries was performed on the MiSeq Illumina platform using reagent Kit v.3 (150 cycles) with a low-concentration PhiX spike-in of 1% as a control.

Bioinformatics pipeline

After demultiplexing DNA reads, FASTQC reports were made for quality control using FASTQC 0.11.6 (Andrews 2010) obtained via BioConda (Grüning *et al.,* 2018). AdapterRemoval v.2.2.2 (Schubert *et al.* 2016) was used to remove the adapter sequences.

BLASTn v.2.2.31+ (Altschul *et al.* 1990) was used (with parameters: -max_target_seqs 500 -outfmt '6 std staxids') to search the resulting sequences to determine whether the material being sequenced is (predominantly) *Trochetiopsis* using the National Center for Biotechnology Information (NCBI) nucleotide database downloaded on 3 May 2021 (Altschul *et al.* 1990). These results were visualized using MEtaGenome ANalyzer (MEGAN) v.6.23.4 (Huson *et al.* 2007). The data were filtered using MEGAN by extracting only Vertebrate or Viridiplantae data. The Phylogenetic Intersection Analysis (PIA) pipeline was followed for both Viridiplantae and Vertebrata data with the minimum taxonomic diversity score parameter changed from 0.1 to the less restrictive 0.04 (Cribdon *et al.* 2020). MetaDamage v.2.8 is sensitive for low copy numbers and was used to determine whether the DNA showed a damage signal (Everett and Cribdon 2023).

RESULTS

DNA extraction yields

DNA quantities are shown in Table 1. DNA quantity following extraction ranged from 1.50 to 11.53 ng/µL. Yields measured before sequencing ranged from 0.22 to 11.53 ng/µL. Samples 080a, 118a-a, 118b-b, 117a, and 117b exhibited DNA yields below 1 ng/µL. These low DNA yields were not found to be related to species or age of the specimens. Following sequencing, insufficient data were retrieved for analysis of samples 117a, 117b, and 118a-a [coloured in grey in Supporting Information Table S1 (Viridiplantae) and Table S2 (Vertebrata)]. No contamination was found in the negative controls (NC_a to NC_e coloured grey in Tables S1 and S2).

Table 1. List of herbarium specimens at the NHM that were sampled for the initial study with results of quantification of the ancient DNA extractions following the protocol of Doyle and Doyle (1990) with small modifications.

Sample number	*Trochetiopsis* species	Specimen registration number	Collector	Collector no.	Collection year	DNA quantity post-extraction (ng/μL)	DNA yield before sequencing (ng/μL)	Number of reads found, Viridiplantae	Number of reads found, Vertebrate
311	*T. ebenus*	BM001024311	S. Robson	s.n.	c. 1772	22.97	4.62	29781	10
19	*T. ebenus*	BM013862119	Roxburgh	s.n.	1814	1.50	1.63	85610	2
HS92.7	*T. erythroxylon*	HS92 f.7	sin coll.	s.n.	c. 1700	3.48	11.53	35671	4
81	*T. erythroxylon*	BM000630081	P. Miller	s.n.	1772–1774?	2.86	4.24	37847	2
21	*T. erythroxylon*	BM013862121	J. Banks & D. Solander	s.n.	1771	8.28	4.90	49357	4
080a	*T. erythroxylon*	BM001124080	J.R. & G. Forster	159	1775	11.70	0.87	22115	2
080b							2.23	16376	3
20	*T. erythroxylon*	BM013862120	W. Roxburgh	s.n.	1814a	4.27	5.10	23041	5
25b	*T. erythroxylon*	BM013862125	W. Roxburgh	s.n.	1814b	5.30	1.53	37882	2
25a							5.73	34780	6
118a-a	*T. erythroxylon*	BM013862118	J.C. Melliss	s.n.	c. 1860–1871	11.97	0.29	27	0
118a-b							0.68	14812	0
86a	*T. erythroxylon*	BM000810086	F. E. Grant	s.n.	1884	2.21	4.05	37490	9
86b							10.12	62614	5
117a	*T. erythroxylon*	BM013862117	A.B. Rendle	80	1929	14.77	0.22	0	0
117b							0.25	0	0
87	*T. erythroxylon*	BM000810087	N.R. Kerr & A.C. Hill	27	1970	6.78	4.55	41312	413
HS87.24	*T. melanoxylon*	HS87 f.24	Stonestreet	s.n.	1698	2.22	8.52	56983	2955
79	*T. melanoxylon*	BM001124079	J. Banks & D. Solander	s.n.	1771	12.60	5.91	41824	6
79b							3.67	58670	3

Table 2. PIA output for Viridiplantae total number of reads for all samples combined for the taxa associated with *Trochetiopsis*.

Taxon	Total read count per taxon for all samples combined (proportions)
Ingroup	686192
Rosids	68823 (0.100)
Rosids/Malvids	22932 (0.033)
Rosids/Malvids/Malvales	7626 (0.011)
Rosids/Malvids/Malvales/Malvaceae	181909 (0.265)
Rosids/Malvids/Malvales/Malvaceae/Malvoideae	19521 (0.028)
Rosids/Malvids/Malvales/Malvaceae/Dombeyoideae	2567 (0.004)
Rosids/Malvids/Malvales/Malvaceae/Dombeyoideae/*Trochetiopsis*	145 (<0.001)

PIA pipeline: what non-*Trochetiopsis* DNA of higher plants or animals is present?

Reads were compared using NCBI blast and the output sorted into Viridiplantae and Vertebrata groups using MEGAN (Huson *et al.* 2007). The Viridiplantae and Vertebrata groups were then subject to stringent phylogenetic assignations using PIA (Cribdon *et al.* 2020).

Results from the PIA focusing on the Viridiplantae showed that the total number of reads for the higher taxa Viridiplantae was 6195, for Mesangiospermae was 120629, and for Pentapetalae was 189129. Table 2 gives details of the higher taxa of *Trochetiopsis* and the total of numbers of reads found. Total numbers of reads found per sample for the higher taxa of *Trochetiopsis* are listed in Supporting Information Table S3. The majority of sequences were assigned to clades that include *Trochetiopsis* with most reads counted for Malvaceae, the family in which *Trochetiopsis* is placed (181909 reads). While for *Trochetiopsis* there is no genome and data from few loci are available on public databases, there were 145 reads that mapped to *Trochetiopsis* which are shared between sample 21 with 51 reads and sample 25b with 94 reads. Other samples did not show read counts for *Trochetiopsis* specifically, but 13 samples did show reads for Dombeyoideae (Table S1, Viridiplantae). All samples, excluding 117a, 117b, and 118a-a, had between 4846 and 20102 read counts for Malvaceae.

Total read counts of between 119 and 291 were found for the families Euphorbiaceae, Cucurbitaceae, Convolvulaceae, Solanaceae, Brassicaceae, and Poaceae. For *Brassica* and *Solanum*, a total read count of 159 and 225 was found, respectively, with each sample containing between four and 24 reads (Supporting Information Table S1, Viridiplantae). While the total read count for both these genera is higher than found for *Trochetiopsis*, the read count per sample is much lower.

For Asparagales, the order that includes *Aloe vera*, only five reads were found in total (Supporting Information Table S1, Viridiplantae). For Poaceae, which includes maize and wheat, both of which have been used in the production of adhesives, the total number of reads was 382. Of these, 233 reads belong to sample 20, and the other samples each have below 20 reads (Table S1). No reads were found specifically for wheat or maize.

Results from the PIA focusing on the Vertebrates showed five samples where there were more than five reads in total (Supporting Information Table S2, Vertebrata). These five samples are presented in Table 3. Samples 87 and HS87.24 exhibit total read counts for Vertebrata of 413 and 2955 respectively (Table 3). Sample 87 is a *Trochetiopsis erythroxylon* specimen collected in 1970 and curated in the General Herbarium at the NHM. Reads were found for *Bos* (32 reads), Bovidae (285), and Pecora (88; Table 3). Sample HS87.24 is a specimen of *Trochetiopsis melanoxylon* from the Sloane Herbarium. This sample had 66 reads found for *Bos*, 2032 reads for Bovidae, and 751 reads for Pecora with lower counts for Leporidae (39 reads) and *Ovis* (19 reads; Table 3). Samples 87 and HS87.24 were processed in separate batches during lab work and cross-contamination between these samples can therefore be ruled out. Large numbers of reads were not found for bony fish or specifically sturgeon.

Animal reads show DNA damage signals

To authenticate the animal reads as ancient, the *Bos* and Bovidae reads in both samples 087 and HS87.24 were tested for a damage signal using MetaDamage (Everett and Cribdon 2023). Sample HS87.24 shows elevated C to T (5′ end, red line) and G to A (3′ end, blue line) base misincorporations at fragment ends consistent with ancient DNA damage (Fig. 2). In contrast, sample 87 did not show a clear damage signal for *Bos* and Bovidae with no evidence of increasing substitutions at the 3′ and 5′ end.

DISCUSSION

The aim of this study was to determine if the use of a metagenomics pipeline to analyse ancient exogenous DNA in herbarium samples could provide insights into the way specimens were prepared.

The results showed a strong signal of endogenous DNA. As expected, within Viridiplantae, read counts were high for taxa within which *Trochetiopsis* is resolved. *Trochetiopsis* reads were recorded for two of the 20 samples (10%), reads for the subfamily Dombeyoideae were found in 13 of the 20 samples (65%), and all samples for which sequence data were generated showed relatively high read counts for Malvaceae (21.0–34.5%). A higher read count was observed for Malvoideae rather than Dombeyoideae (the subfamily to which *Trochetiopsis* belongs) and this is probably explained by the limited reference data in GenBank for Dombeyoideae relative to Malvoideae. When a taxon present in a sample is not included in the database used, the reads are often assigned to closely related taxa present in the database (Smith *et al.* 2015, Warinner *et al.* 2017, Cribdon *et al.* 2020). PIA has been shown to accrue fewer false positives than other standard approaches (Cribdon *et al.* 2020).

Among the ancient exogenous DNA recovered, distinct animal reads were found for two of the 14 *Trochetiopsis* herbarium specimens sequenced. Sample HS87.24 is stored in a bound volume that was assembled by Plukenet before his death in 1706. It showed the highest animal read counts and the most variation in animal taxa found. Not only are *Bos* (66 reads), Bovidae (2032 reads), and Pecora (751 reads) present in quantity, consistent with the use of cow products in glue, but there are also reads found for Leporidae (rabbit) and some reads of *Ovis* (sheep). MetaDamage

Table 3. The five samples with total read counts greater than five per sample for Vertebrata from the PIA with minimum diversity score of 0.04; bold text indicates read counts greater than 15.

Taxon	HS87.24 Sin. Coll. 1698	311 Robson c. 1772	25a Roxburgh 1814	86a Grant 1884	87 Kerr & Hill 1970
Total read count	2955	10	6	9	413
Cyprinoidei	0	1	0	0	0
Teleostei	0	0	0	1	0
Osteoglossocephalai	1	0	0	1	0
Clupeocephala	1	1	0	1	1
Acanthomorphata	1	0	0	0	0
Euacanthomorphacea	0	0	0	1	1
Percomorphaceae	2	2	1	1	0
Eupercaria	1	0	0	0	0
Leporidae	39	0	0	0	0
Ruminantia	3	0	0	0	0
Pecora	751	1	0	0	88
Bovidae	2032	0	0	0	285
Bos	66	0	0	0	32
Caprinae	5	0	0	0	0
Ovis	19	0	0	0	0
Euteleostomi	11	3	3	3	1
Tetrapoda	1	0	0	0	0
Amniota	15	1	0	0	3
Mammalia	1	0	0	0	0
Theria	4	0	1	0	1
Glires	1	0	0	0	0
Lagomorpha	1	0	0	0	0
Cricetidae	0	0	0	0	1
Caniformia	0	0	1	0	0
Galloanserae	0	0	0	1	0

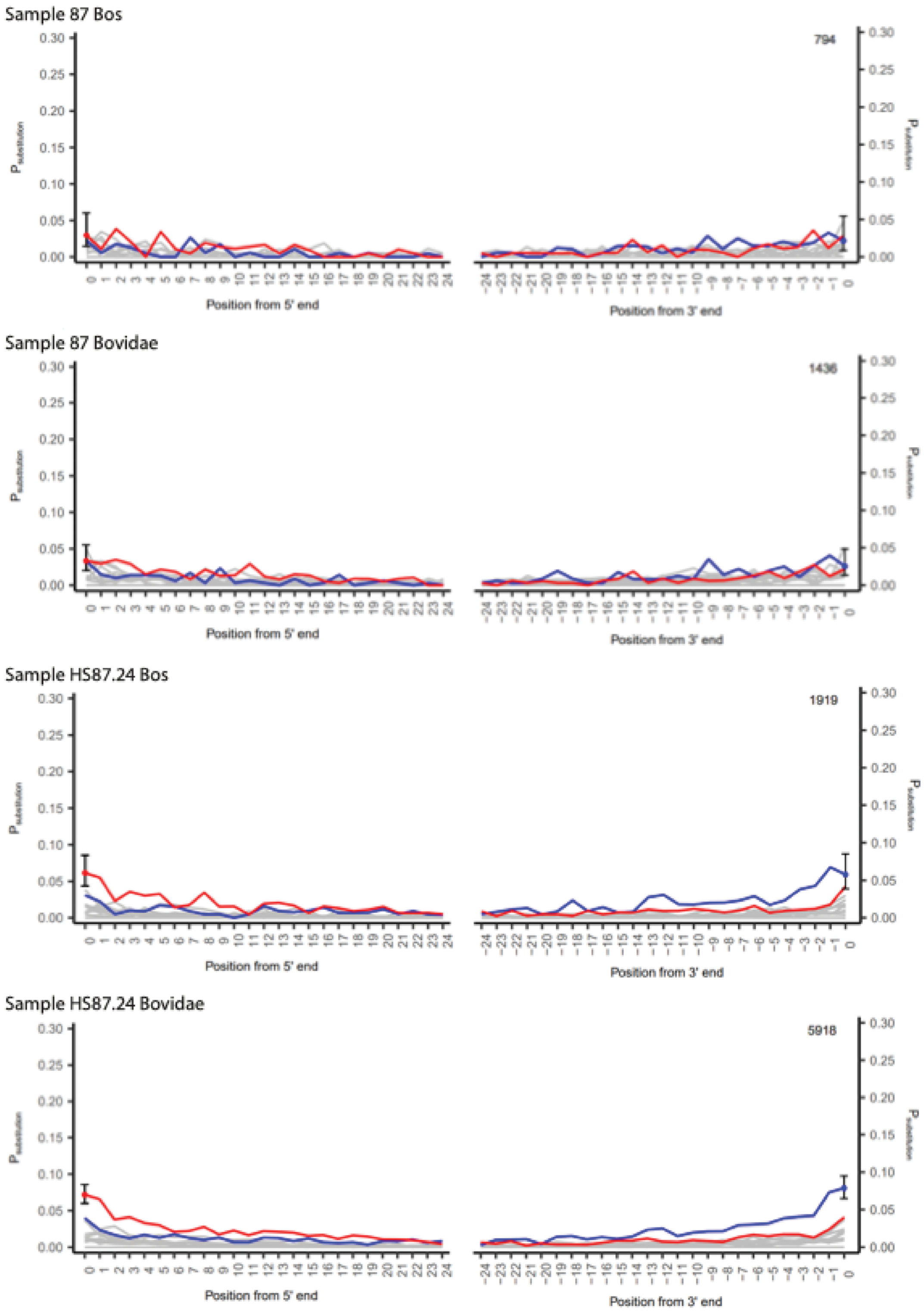
analysis for *Bos* and Bovidae sequences show a damage signal indicating that they are not recent contamination. A signal of deamination, evidence of DNA damage, is expected at the ends of the molecule because water has easier access to the bases. Therefore, an increase in deamination at the ends of the molecule is expected in the MetaDamage plots (Fig. 2) as is observed here. For Leporidae and *Ovis* the assigned reads were not sufficient for assessment of ancient DNA damage with MetaDamage.

There are no records of the glue recipe used by Plukenet to mount specimens but in the 18th century, bones from butchered animals were generally used for glue making, which could include cattle, pigs, sheep, and rabbits. The results of our study are consistent with the use of a mix of different animal bones in the glue used by Plukenet to mount his specimens. The sample did not show a high quantity of reads for bony fish, which we would have expected had Plukenet been using the recipe recorded in the Sloane Herbarium by Petiver or that published by Linnaeus (1751). It is evident that a range of different glue recipes were used to prepare herbarium specimens in the 17th/18th centuries.

It is notable that both specimens sampled from the Sloane Herbarium showed visible evidence of glue (Fig. 1) but reads associated with animals were only observed for specimen HS87.24 and not for sample HS92.7. In the preparation of the specimens for DNA extraction, the removal of glue was more successful on HS92.7 than on HS87.24, but the absence of animal reads in the former and, indeed, in most other samples included in this study could mean that there was insufficient glue present to be detected using this approach or that the DNA was too degraded by the glue preparation process.

Sample 87, a specimen of *Trochetiopsis erythroxylon* collected in 1970 was the other specimen to exhibit reads of animal DNA with reads matching *Bos* (32), Bovidae (285), and Pecora (88) (Table 3). MetaDamage plots for the *Bos taurus* reads (Fig. 2) show no clear damage signal but this could be due to insufficient reads, which could be clarified by deeper sequencing (Everett and Cribdon 2023). While herbaria today still use animal-based adhesives (Grenda-Kurmanow 2021), it is likely the specimen was mounted with latex adhesives which were used at the NHM from the 1970s due to their ease of use and price (R. Vickery, Dr R. Huxley, Prof. S. Blackmore, pers. comm.). Only later, around the 1980s, a shortage of latex arose due to the AIDS crisis and new adhesives were investigated, at which point the NHM switched to using polyvinyl acetate glue (PVA). We would not, therefore, expect to find evidence of cattle from the glue used. The herbarium paper used at the NHM still includes sizing with gelatine and it is plausible that the bovine reads in this sample are likely to come from the gelatine used for this process. Further investigation using near-infrared (NIR) spectroscopy or GC/MS for identification and estimation of the gelatine content in historical papers could be carried out to test this hypothesis (Stephens *et al.* 2008, Ormsby Barrett and Lang 2011, Barrett *et al.* 2016).

To investigate plant material that was used in the adhesives, we focused on the families and genera for maize (*Zea*; Poaceae), potato (*Solanum*; Solanaceae), wheat (*Triticum*; Poaceae), cassava (*Manihot*; Euphorbiaceae), *Aloe vera* (Asphodelaceae), and


Sample 87 Bos

Sample 87 Bovidae

Sample HS87.24 Bos

Sample HS87.24 Bovidae

Figure 2. MetaDamage graphs for samples 087 and HS87.24 with reference genome *Bos* for the reads of *Bos* and Bovidae. For each, two graphs are presented that show the 5′ and 3′ ends of the DNA molecule primer. The *x*-axis shows the position of the base along the molecule. The fraction of bases that differ from the reference base for C to T (red), G to A (blue), and other substitutions (grey) are shown on the y-axis.

rubber (*Hevea*; Euphorbiaceae), and plants associated with gums or resins (Fay 2021, Grenda-Kurmanow 2021).

Reads were found for Cucurbitaceae, Convolvulaceae, Euphorbiaceae, Solanaceae, Brassicaceae, and Poaceae. At a genus level, *Solanum* and *Brassica* occurred in every sample. Sample contamination during processing in the lab can be excluded since there were no reads assigned to Viridiplantae or Vertebrata in the negative controls (NC_a to NC_e in Supporting Information Table S1, Viridiplantae, and Table S2, Vertebrata). Both *Solanum* and *Brassica* have a much lower read count per sample than found for *Trochetiopsis*. The genus *Solanum* includes *S. tuberosum*, the potato, which could be an indicator of a starch-based glue. However, read counts per sample for *Solanum* and *Brassica* were low and it is likely that the recovery of these sequences reflects the high number of sequences available in GenBank for these economically important taxa. We found no reads for *Manihot*, *Zea*, *Triticum*, or *Aloe*. For Asparagales only five reads were found in total. There was therefore no evidence for the use of plant-based adhesives in the preparation of the samples examined.

We also consider plants used to make the paper. Flax or hemp could be used in paper making, but we did not observe reads assigned to either Linaceae or Cannabaceae. Another plant species that could be expected in paper making, including the herbarium paper currently used at NHM, is cotton (*Gossypium*). This species is a member of Malvaceae, which is the same family as the target taxon *Trochetiopsis.* It is notable, however, that no peak in reads was found for *Gossypium*, despite the abundance of sequence data available for this economically important taxon. In a future study of herbarium material of species outside of Malvaceae, the potential to detect sequences from the paper on which the sample is mounted may be more easily investigated.

The sequencing data were originally produced for the study of *Trochetiopsis*. The data were not specifically produced for the metagenomic analysis performed in the present article. During sample preparation visible adhesives were avoided and removed as much as possible. The generation of sequence data was therefore not focused on the products used in the production of paper and adhesives. Nevertheless, we were able to use the metagenomics pipeline PIA to detect reads in the ancient exogenous DNA from materials that were used in the preparation of herbarium specimens. Reads of ancient exogenous DNA from samples 087 and HS87.24 allowed us to identify animal products to the genus level and provide insights into the glue used in a 17th century herbarium and the process of paper sizing in the late 20th century. We found no evidence for plant-based glues or a signal from the material used to make paper.

For many samples, we were unable to detect reads for the curatorial products used. Increased sequencing depth might enable further detection of herbarium materials. A focus on the adhesives specifically could improve the success of genetic analysis of the materials used (Albertini *et al.* 2011, Fortes and Paijmans 2015). Other techniques such as GC/MS and FTIR spectroscopy, thin layer isoelectric focusing, or Py-GC/MS (Chiavari *et al.* 2006, Stephens *et al.* 2008, Albertini *et al.* 2011) may also be used to investigate herbarium adhesives further. Nevertheless, this metagenomic study suggests that insights into the history of mounting specimens can be obtained by using ancient exogenous DNA. While not all sequences may yield information, it is plausible that information on the history of herbarium practices could also be obtained from the discarded ancient exogenous DNA of other herbariomics studies.

ACKNOWLEDGEMENTS

Thanks are extended to Prof. Stephen Blackmore, Dr Robert Huxley, and Roy Vickery for their comments on adhesives being used at the NHM around the 1970s, and to Victoria Pickering for help with the transcription of Petiver's glue recipe. Special thanks to paper conservator Konstantina Konstantinidou for her help to release the glue on the plant material from the Sloane Herbarium. In addition to this, the authors would like to thank the referees for their valuable input on this paper.

AUTHOR CONTRIBUTIONS

Annabelle R. de Vries (Conceptualization, Methodology, Software, Formal analysis, Data curation, Writing—Original draft, Visualization, Investigation), Lochlan Chadwick (Methodology, Formal analysis), Robin G. Allaby (Conceptualization, Methodology, Software, Writing—Reviewing and Editing, Supervision, Funding acquisition, Resources), and Mark Carine (Conceptualization, Methodology, Writing—Reviewing and Editing, Supervision, Funding acquisition, Resources).

SUPPLEMENTARY DATA

Supplementary data are available at *Botanical Journal of the Linnean Society* online.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

This project was part of the PhD of A.R.d.V., who was supported by an Central England NERC Training Alliance 2 grant NE/S007350/1.

DATA AVAILABILITY STATEMENT

Raw sequence data have been deposited to the NCBI Sequence Read Archive (SRA): BioProject PRJNA1367884.

REFERENCES

Albertini E, Raggi L, Vagnini M *et al.* Tracing the biological origin of animal glues used in paintings through mitochondrial DNA analysis. *Analytical and Bioanalytical Chemistry* 2011;**399**:2987–95. https://doi.org/10.1007/s00216-010-4287-2

Altschul SF, Gish W, Miller W *et al.* Basic local alignment search tool. *Journal of Molecular Biology* 1990;**215**:403–10. https://doi.org/10.1016/S0022-2836(05)80360-2

Andrews S. *FastQC-A Quality Control Application for FastQ Files*. Babraham, UK: Babraham Bioinformatics, 2010.

Bakker FT, Bieker VC, Martin MD. Herbarium collection-based plant evolutionary genetics and genomics. *Frontiers in Ecology and Evolution* 2020;**8**:603948.

Barrett TD, Mosier C. The role of gelatin in paper permanence. *Journal of the American Institute for Conservation* 1995;**34**:173–86.

Barrett T, Ormsby M, Lang JB. Non-destructive analysis of 14th-19th century European handmade papers. *Restaurator* 2016;**37**:93–135. https://doi.org/10.1515/res-2015-0017

Berli J, Belhadj O. Consolidating herbarium specimens using two-sided hydroxylpropylcellulose pre-coated paper. *Journal of Paper Conservation* 2020;**21**:31–4. https://doi.org/10.1080/18680860.2020.1835112

Bieker VC, Sánchez Barreiro F, Rasmussen JA *et al.* Metagenomic analysis of historical herbarium specimens reveals a postmortem microbial community. *Molecular Ecology Resources* 2020;**20**:1206–19.

Bloom JM. Papermaking: the historical diffusion of an ancient technique. In: *Mobilities of Knowledge*. Cham: Springer International Publishing, 2017, 51–66. https://doi.org/10.1007/978-3-319-44654-7_3

Bridson D, Forman L. *The Herbarium Handbook* (3rd ed.). England: Royal Botanic Gardens Kew, 1999.

Carine MA, Cesar EA, Ellis L *et al.* Examining the spectra of herbarium uses and users. *Botany Letters* 2018;**165**:328–36.

Carine M (ed.). *The Collectors: creating Hans Sloane's Extraordinary Herbarium. Georg Handisyd*. London: Museum of Natural History, 2020, 192–7.

Carøe C, Gopalakrishnan S, Vinner L *et al.* Single-tube library preparation for degraded DNA. *Methods in Ecology and Evolution* 2018;**9**:410–9. https://doi.org/10.1111/2041-210X.12871

Chiavari G, Fabbri D, Galletti GC *et al.* Use of pyrolysis-gas chromatography/mass spectrometry to characterise binding media and protectives from a Coronelli's terrestrial globe. *Journal of Cultural Heritage* 2006;**7**:67–70.

Clark SH. Preservation of herbarium specimens: an archive conservator's approach. *Taxon* 1986;**35**:675–82.

Cribdon B, Ware R, Smith O *et al.* PIA: more accurate taxonomic assignment of metagenomic data demonstrated on sedaDNA from the North Sea. *Frontiers in Ecology and Evolution* 2020;**8**:1–12. https://doi.org/10.3389/fevo.2020.00084

Croat TB. Survey of herbarium problems. *TAXON* 1978;**27**:203–18.

Dandy JE. *List of British vascular plants*, 1958.

Davies, N. M., Drinkell, C., Utteridge, T. M. (eds.). (2023). *The Herbarium Handbook: Sharing Best Practice from Across the Globe*. Kew: Kew Publishing.

Davis CC. The herbarium of the future. *Trends in Ecology & Evolution* 2023;**38**:412–23.

De Tournefort JP. *Élémens de botanique: ou Méthode pour connoître les plantes*, vol. 1. Paris: de l'Imprimerie Royale, 1694. https://bibdigital.rjb.csic.es/idurl/1/13697

Dorr LJ, Wurdack KJ. Indo-Asian Eriolaena expanded to include two Malagasy genera, and other generic realignments based on molecular phylogenetics of Dombeyoideae (Malvaceae). *Taxon* 2021;**70**:99–126.

Doyle J, Doyle J. Isolation of plant DNA from fresh tissue. *Focus* 1990;**12**:13–5.

Egenberg IM, Moe D. A "stop-press " announcement. Damage caused by a widely used herbarium mounting technique. *Taxon* 1991;**40**:601–4.

Everett R, Cribdon B. MetaDamage tool: Examining post-mortem damage in sedaDNA on a metagenomic scale. *Frontiers in Ecology and Evolution* 2023;**10**:888421.

Fay PA. A history of adhesive bonding. In *Adhesive Bonding: Science, Technology and Applications* (2nd ed.). Amsterdam: Elsevier Ltd, 2021.

Fortes GG, Paijmans JLA. Analysis of whole mitogenomes from ancient samples. *Whole Genome Amplification: Methods and Protocols* 2015;**1**:179–95. https://doi.org/10.1007/978-1-4939-2990-0_13

Gimat A, Michelin A, Belhadj O *et al.* Paper sizing with gelatine: from the macro- to the nano-scale. *Cellulose* 2021;**28**:2419–32. https://doi.org/10.1007/s10570-020-03655-z

Grasso G, Debruyne R, Adamo M *et al.* Ancient microbiomes as mirrored by DNA extracted from century-old herbarium plants and associated soil. *Molecular Ecology Resources* 2025;**25**:e14122.

Grenda-Kurmanow M. Adhesives used in herbaria: Current practice with regard to what we know from written sources on mounting herbarium specimens and conservation. *Taxon* 2021;**70**:1–15. https://doi.org/10.1002/tax.12413

Grüning B, Dale R, Sjödin A; Bioconda Team *et al.* Bioconda: sustainable and comprehensive software distribution for the life sciences. *Nature Methods* 2018;**15**:475–6.

Gutaker RM, Burbano HA. Reinforcing plant evolutionary genomics using ancient DNA. *Current Opinion in Plant Biology* 2017;**36**:38–45.

Haupt M, Dyer D, Hanlan J. An investigation into three animal glues. *The Conservator* 1990;**14**:10–6. https://doi.org/10.1080/01410096.1990.9995051

Huson DH, Auch AF, Qi J *et al.* MEGAN analysis of metagenomic data. *Genome Research* 2007;**17**:377–86. https://doi.org/10.1101/gr.5969107

Jarvis CE. An annotated bibliography of the printed works of James Petiver (c. 1663–1718). *Archives of Natural History* 2021;**48**:346–67.

Kistler L, Bieker VC, Martin MD *et al.* Ancient plant genomics in archaeology, herbaria, and the environment. *Annual Review of Plant Biology* 2020;**71**:605–29.

Linnaeus C. *Philosophia botanica*. Stockholmiae [Stockholm]: apud Godofr. Kiesewetter, 1751. https://doi.org/10.5962/bhl.title.37652. http://linnean-online.org/120027/

Liu H. Grape evolution in the human environment. PhD thesis, University of Warwick, 2019.

Mak SST, Gopalakrishnan S, Carøe C *et al.* Comparative performance of the BGISEQ-500 vs Illumina HiSeq2500 sequencing platforms for palaeogenomic sequencing. *GigaScience* 2017;**6**:1–13. https://doi.org/10.1093/gigascience/gix049

Meyer M, Kircher M. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harbor Protocols* 2010;**2010**:pdb.prot5448. https://doi.org/10.1101/pdb.prot5448

Orlando L, Allaby R, Skoglund P *et al.* Ancient DNA analysis. *Nature Reviews Methods Primers* 2021;**1**:14.

Ormsby M, Barrett T, Lang J. *et al.* Estimation of gelatin content in historical papers using NIR spectroscopy. *Iowa Research Online*, 2011. https://doi.org/10.17077/pbxf-a1p0

Ova TYN. A history of fish glue as an artist's material: applications in paper and parchment artifacts. *The Book and Paper Group Annual* 2000;**19**. https://cool.culturalheritage.org/coolaic/sg/bpg/annual/v19/bp19-29.html

Rogers SO, Bendich AJ. Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. *Plant Molecular Biology* 1985;**5**:69–76. https://doi.org/10.1007/BF00020088

Roland & Reich. Genome Research: SPRI beads, 2012. https://genome.cshlp.org/content/suppl/2012/01/20/gr.128124.111.DC1/Supplements_1_18_2012.pdf

Rouchon V, Pellizzi E, Janssens K. FTIR techniques applied to the detection of gelatine in paper artifacts: from macroscopic to microscopic approach. *Applied Physics A* 2010;**100**:663–9.

Roycroft E, Moritz C, Rowe KC *et al.* Sequence capture from historical museum specimens: maximizing value for population and phylogenomic studies. *Frontiers in Ecology and Evolution* 2022;**10**:931644.

Schellmann NC. Animal glues: a review of their key properties relevant to conservation. *Studies in Conservation* 2007;**52**:55–66. https://doi.org/10.1179/sic.2007.52.supplement-1.55

Schubert M, Lindgreen S, Orlando L. AdapterRemoval v2: Rapid adapter trimming, identification, and read merging. *BMC Research Notes* 2016;**9**:88–7. https://doi.org/10.1186/s13104-016-1900-2

Scott B, Pickering V, Coulton R *et al.* Collecting and cataloguing the world: the botanical collections of Hans Sloane (1660–1753). *Systematics and Biodiversity* 2025;**23**:2455440. https://doi.org/10.1080/14772000.2025.2455440

Smith O, Momber G, Bates R *et al.* Sedimentary DNA from a submerged site reveals wheat in the British Isles 8000 years ago. *Science (New York, N.Y.)* 2015;**347**:998–1001.

Stefanaki A, Thijsse G, Van Uffelen GA *et al.* The En Tibi herbarium, a 16th century Italian treasure. *Botanical Journal of the Linnean Society* 2018;**187**:397–427. https://doi.org/10.1093/botlinnean/boy024

Stefanaki A, Porck H, Grimaldi IM *et al.* Breaking the silence of the 500-year-old smiling garden of everlasting flowers: The En Tibi book herbarium. *PloS One* 2019;**14**:e0217779.

Stephens CH, Barrett T, Whitmore PM *et al.* Composition and condition of naturally aged papers. *Journal of the American Institute for Conservation* 2008;**47**:201–15.

Thiers BM. *Herbarium: The Quest to Preserve and Classify the World's Plants*. Portland: Timber Press, 2020.

Thijsse G. Tusschen pampier geleyt: Ontstaan, verspreiding en gebruik van de vroegste herbaria. *De groene Middeleeuwen. Duizend jaar gebruik van planten (600–1600)*. Eindhoven: Lecturis BV, 2016, 64–93.

Swain H, Chakraborty K. Science behind herbarium and its importance in recent years. *Nordic Journal of Botany* 2024;**2024**:e04499.

Van den Spiegel A. Isagoge in rem herbariam libri duo. Patavii [Padua]: apud Paulum Meiettum, 1606. https://archive.org/details/BIUSante_pharma_res012063

Van Oosten TB. Characterisation of parchments and animal glues from different kinds of animals by thin layer isoelectric focusing. *Leather Conservation News* 1989;**5**:1–4.

Warinner C, Herbig A, Mann A *et al.* A robust framework for microbial archaeology. *Annual Review of Genomics and Human Genetics* 2017;**18**:321–56.

Werrett S. The marvellous bond: Animal glue and isinglass as vital waste in England, 1600–1800. *Journal of Material Culture* 2025;**30**:76–90.

Yesilyurt J, Dominguez-Santana F, Carine MA. Herbarium specimens: is there a best approach to mount dried plant specimens?*Journal of Natural Science Collections* 2025;**13**:52–63.