Journal of Natural Science Collections

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NatSCA
Natural Sciences Collections Association

The Natural Sciences Collections Association

The Natural Sciences Collections Association (NatSCA) is a UK based membership organisation and charity which is run by volunteers elected from the membership.

NatSCA's mission is to promote and support natural science collections, the institutions that house them and the people that work with them, in order to improve collections care, understanding, accessibility and enjoyment for all.

More information about NatSCA can be found online at: **natsca.org**

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Journal of Natural Science Collections

Aims and scope
The *Journal of Natural Science Collections* is a place for those working with these collections to share projects and ways of working that will benefit the museum community. The Journal represents all areas of work with natural science collections, and includes articles about best practice and latest research across disciplines, including conservation, curatorial methods, learning, exhibitions, and outreach. Articles in the Journal should be relevant and accessible to all of our diverse membership. Submissions are peer reviewed, resulting in high quality articles.

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Editorial Board

Front cover image: Examples of ferns and climbers (Image: J. Jackson, Natural History Museum, Photo Unit, 2024). See pp.42-53 of this volume.

Editorial

Glenn Roadley

Welcome to Volume 13 of the *Journal of Natural Science Collections*, and my first volume as the Editor for NatSCA. NatSCA has been a huge influence and support for me since I first began my career in museums back in 2014. Starting out as a Curatorial Trainee at Leeds Museums, I found NatSCA's conferences, training events and publications to be invaluable, providing a range of knowledge and insight that can far exceed that available within a single institution. As my career took me to exciting new roles and new cities, the network of amazing people I'd met though NatSCA remained a comforting constant, always happy to provide advice and support.

Being part of the NatSCA Committee has been incredibly rewarding and I'm genuinely happy to be able to give back to a community which has provided me with so much. Since taking on the role of Editor at the 2024 AGM, I've had the privilege of working with expert museum professionals to bring you this volume, which offers research on subjects spanning decolonisation, pest management, conservation and curation of collections.

We begin Volume 13 with an article from **Schlunke and Schwarz**, examining how 19th century attitudes to a thylacine displayed in Germany portrayed the species as 'primitive' and destined for extinction. We then pivot to IPM, as **Holloway and Querner** report the occurrence of *Trogoderma glabrum* in Austrian museums and provide a guide to identification. An awkward side-step keeps us on the subject of taxonomic identification as **Roberts *et al*** give us a demonstration of a piece of software designed to spot potentially mis-identified specimens by comparing collection and occurrence data. While no doubt useful for any biological collections, this paper makes use of bryophyte specimens as a case study, and thus links nicely to the next three papers, each on the subject of botany curation.

Yesilyurt *et al* provide a practical test of different herbarium mounting methods by seeing which specimens best survive the hardships of international post, while **Prakash *et al*** give us an overview of the methods used in a project to re-curate over 3,000 specimens in the Natural History Museum's fluid-stored seed plant collection. **Granget *et al*** have also been busy with botanical wet collections to bring us the results of a study into the effect of different preservative recipes on colour retention.

Smith and Callaghan round off our trio of wet collection papers with a study of the use of Steedman's preservative, including an assessment of the condition of Cole Museum of Zoology specimens and a survey of other museums. And finally we have **Castelain's** study showing how different materials and treatments can be differentiated by their fluorescence under ultra-violet light - and yes, it has all the glowing specimen photos you could hope for.

As you can see, we have another bumper volume this year, thanks to the hard work and patience of each of these brilliant authors. Thank you also to all of the anonymous reviewers who generously volunteered their time to read and provide feedback on the articles - *you know who you are*.

Our fab Editorial Board, comprised of Paolo Viscardi, Bethany Palumbo, Verity Burke, Lisa Winters, Emilie Pearson and Jan Freedman also deserve a huge thanks for all of their support in getting each article through the peer review process.

And finally my gratitude to Jan Freedman, our out-going Editor, who has steered the helm of the *Journal of Natural Science Collections* to where it is today. In addition to staying on as part of the Editorial Board, Jan has provided support, advice, notes and templates which ensured a painless transition to my tenure as Editor.

View from the Chair

Co-Chairs Jen Gallichan and Isla Gladstone

There have been many positives for NatSCA in 2024. We have sustained a thriving programme of events and publications, and are helping advocate for future opportunities for UK natural sciences collections in the DiSSCo UK initiative. In an environment of continued financial and workplace pressures for the sector, we have been able to support the community through opportunities for connection and knowledge sharing using our platforms, and for financial support through subsidised costs, bursaries and small grant funding.

NatSCA's 2024 conference 'Trials and Triumphs' aimed to celebrate triumphs and amplify success in museums, but also share pitfalls and lessons learned. The conference was generously hosted by the Oxford University Museum of Natural History, with their team providing a fantastic welcome and tours. Positive feedback showed the conference highly met attendee expectations, with comments such as *"It was very well organised and felt like a positive and supportive environment".* Our conference sub-group are now working on our 2025 conference which will be hosted by Manchester Museum.

Our training programme this year has included our regular informal monthly lunchtime chats, as well as fully booked online events 'An Introduction to Natural Sciences Collections Legislation', delivered by specialists from across the sector, and 'An Introduction to Natural Sciences Collection Georeferencing' delivered by the team working at the Natural History Museum London on the DiSSCo project. The topics covered were informed by a survey sent out to members, which has also created a long-list of priorities for future training delivery.

We will be looking to improve how we communicate across all NatSCA social media platforms in the future. Work has already started on the development of a new NatSCA website including updates to the sector job vacancies, committee member profiles and pages for committee nominations, membership and bursaries. New pages have been created pulling together resources and recordings from the 2023 and 2024 NatSCA conferences and there is a new page highlighting the DiSSCo project.

The NatSCA blog is consistently now getting around 2000 views per month, a trend we have been building on for the last 2 years. It feels that our members appreciate the flexibility of options available for publishing, rather than just a journal paper which was not always appropriate. We now have regular contributors as well as unprompted submissions from both the NatSCA community and beyond. It is so good seeing engagement with the blog grow and to be able to showcase the work of so many colleagues and institutions.

After reviewing the NatSCA reserves policy in 2023, the committee has continued to deliver a financial model that sustainably increases spending for the benefit of our users and mission, to bring reserves down to the recommended level. This includes reducing upfront conference costs for attendees, increasing the number of bursaries, and raising funds available for the Bill Pettit award. Moving forward, we are planning discrete projects that advocate for natural history collections, as well as production of specialist resources to further support the sector.

We would like to finish by thanking the committee and everyone who has contributed to NatSCA this year. Special thanks to Glenn Roadley for the successful publication of this journal, the first issue under his new role as Editor. Glenn has expertly navigated the hand-over, working closely with the editorial board to ensure that work on the journal continues to be an open and collaborative process.

The First Beutelwolf: How Berliners were taught to see the thylacine

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Abstract

This article examines the ways in which visitors came to see the Beutelwolf (thylacine) that is in the collection of the Museum für Naturkunde (MfN) in Berlin. We analyse nineteenth-century zoo-related materials, key popular German natural history writings, and historical museum guides to show how the emphasis on 'seeing' specimens, combined with the production of images inside and outside the museum, created a particular view of the species as 'primitive' and destined for extinction due to its inability to adapt to the modern world. We conclude with some suggestions for how contemporary representations of extinction in the MfN might need to be reconsidered in the light of these findings.

Keywords: Beutelwolf, Tasmanian Tiger, thylacine, Berlin Museum für Naturkunde (MfN), visuality, extinction

Introduction: the Wall of Life?

We have come to Berlin's Museum für Naturkunde (MfN) to see the thylacine. Or as it has also been called: coorinna, loarinna, laoonana, lagunta, or Tasmanian tiger. Here, it is called Beutelwolf ("pouched wolf"), a name that curiously combines notions of the fearsome Eurasian wolf looming large in the German imagination with the soft pouch characteristic of marsupials. A mythical animal in more ways than one.

Up the building's stately staircase and through the Dinosaur Hall, we turn left to look for the animal on display in the MfN's Evolution in Action Hall. But the first thing that catches our eye, at twelve metres wide and stretching across almost the entire hall's entrance, is the Biodiversity Wall, one of the museum's centrepieces (Fig. 1). The Wall is a visual delight. No taxonomical principles – Linnaean, ecological, genetic or otherwise – appear to organise its presentation of 3,000 animal specimens against a neutral background. Instead, museum visitors are invited to be overwhelmed by the mass of animal bodies alone, their abundant beauty and dazzling diversity (Toepfer, 2019; te Heesen, 2017). Popular in natural history museums around the world, such as the American Museum of Natural History, biodiversity displays are a particularly apt example of what Pollock and Zemans describes as the specific visuality of museums, where "knowing, seeing, visually mastering leaves the viewer centered and disembodied in a perfect fantasy" (2007, p. 13).

Thus aestheticised, the animal bodies displayed in Berlin's Biodiversity Wall are regularly recontextualised in the political sphere and read as symbols of humanity's threat to nature. As Johannes Vogel, the museum's director likes to stress, "even [former] German Chancellor Angela Merkel gives her political speeches on biodiversity and climate protection in front of the Berlin

Fig. 1. Biodiversity Wall at the Museum für Naturkunde Berlin (DE-MUS-813712). © Carola Radke, MfN.

Biodiversity Wall" (Vogel, 2016, p. 237). Both museumgoers and politicians, then, value the wall for the intense affective responses it evokes. And for many of them, the pleasure of this visual feast is mixed with the knowledge and sense of grief that so many of the ecologies that these creatures are part of are under threat and that many of the animals are endangered (Pike, 2017; Massol de Rebetz, 2020). Building on these responses, the wall acts a prominent symbol of the museum's proven and sustained commitment to act across a range of platforms as a lobbyist on behalf of the Earth's biodiversity in the face of the unfolding Holocene extinction ("Strengthening Engagement").

What the "hyperbolic optics" (Bezan, 2019, p. 222) of the wall does not invite us to see, however, is the role of natural history in colonial practices that ultimately contributed to species extinction, nor does it encourage conversations about how natural history museums have historically naturalised and depoliticised the "impact of anthropogenic change upon nonhuman life" through their exhibition practices (Bezan, 2019, p. 222; see also pp. 214, 224 and Westergaard, 2023, p.10). This past, we will argue, continues in the MfN's current practice of exhibiting endangered animals, particularly the Beutelwolf. In that sense, the MfN is very much like the Humboldt Forum: the institution that exhibits Berlin's most prized ethnological collections, acquired over the long nineteenth century from peoples whose cultures were thought to be threatened by an encroaching Western modernity, and which today styles itself as a "site of world culture" (Parzinger, 2011, p. 6). The MfN also 'worlds': The pedagogy of its exhibitions allows us to see the minerals, plants and animals according to the universalising ordering principles and narratives – evolution, ecosystems, climate change or species extinction – of the natural sciences. And like the ethnological collections on display in the Humboldt Forum, the MfN has only recently begun to acknowledge imperial expansion and colonial violence as the underlying logic that brought many of these riches to Berlin. But while the Humboldt Forum, whose controversial collections are currently at the centre of an intense reckoning with Germany's colonial past, is reluctantly becoming the 'forum' of public discourse that its name suggests, the MfN, although like other natural museums increasingly committed to researching its colonial past ("Colonial Contexts"; Das and Lowe, 2018; Ashby and Machin, 2021), is not imagined in this way. Or perhaps not yet.

Seeing and Unseeing Double Death

Just fifteen metres from the Biodiversity Wall there is a Beutelwolf mount (thylacine; known in popular English as the Tasmanian Tiger) near the very end of the Evolution in Action Hall, opened in 2007 (Fig. 2). The mount on display is just one item from the museum's larger collection of skins, bones, mounts and organs of the species, whose last known living individual died in 1936. It's role in

Fig. 2. Beutelwolf in the "Extinction through Human Activity" cabinet in the Evolution in Action Hall at the Museum für Naturkunde Berlin (DE-MUS-813712). © Katrina Schlunke

the exhibition is carefully scripted. From their encounter with the Biodiversity Wall, visitors are invited to journey from diversity to extinction to reflection, through the introduction of key ideas such as evolution, mutation, variability, convergent evolution and displayed busts of major contributors to evolutionary thought, such as Carl Linnaeus, "the man who systematised life" and Charles Darwin, who demonstrated diversity and adaptation through "Darwin's finches". There is also a panel dedicated to Amalie Dietrich, an "unusual woman," botanist and researcher who collected in Australia and Tonga between 1863 and 1873. What the panel chooses not to mention is Dietrich's involvement in the looting and trade of human remains from colonial Queensland (Turnbull, 2020).

Like other animals shown in the hall, the Beutelwolf is displayed in a large original glass cabinet from 1889, used to emphasize the role of the "extensive scientific collections of the Museum, compiled over several hundred years" (Damaschun, Faber and Steiner, 2019, p. 75). It sits between an extant but endangered Siberian tiger and the extinct quagga while above all three, perch two extinct huia wattlebirds. The cabinet is titled "Extinction through Human Activity", and the interpretive stand in front of it offers more information on "When the Natural Habitat Shrinks". Alongside the now iconic film footage of one of the last thylacines in captivity, walking around its cage at the Hobart Zoo in 1933, we are told that "in many cases human activity has directly caused the extinction of species". The example given is of the huia wattlebird, which was hunted and traded mainly for its feathers with the last official sighting occurring in 1907 (Boyle, 2019, p. 223). The text goes on: "Often the destruction of habitats occurred so quickly that organisms had no time to develop survival strategies. The South African quagga, and the Beutelwolf are examples of animals that were unable to withstand the new environmental conditions that humans made".

The sentence is curiously unspecific in its reference to human-made "new environmental conditions". It glosses over the specific geopolitical processes of European settler colonialism that resulted in this destruction of habitats over the past two hundred years. After all, quagga, huia and Beutelwolf had coexisted with particular groups of humans for millennia without either species becoming extinct. The text also fails to mention that the thylacine, like the quagga, was hunted, and that a bounty was put on its head by early colonists. Thylacines were also traded to menageries, museums, circuses and zoos. Dead thylacines were actively sought for museum displays and collections and as their numbers dropped, the value of thylacines in the global network of museums went up (Möller 1997, pp. 133–137; Maynard and Gordon, 2014, p. 28; on a similar shift in value of Galápagos tortoises, see Bezan, 2019, p. 232). Instead, the panel's projection of a universal human responsibility for species extinction ties in well with a series of topical questions printed on the back of the thylacine's display case. The questions: "What is a human being?" and "What does nature mean to us?" (all translations from German are our own) invoke a shared positionality of all humans in relation to nature. They leave no room, for example, for the articulation of the specific cultural relations that Tasmania's Indigenous palawa people have maintained with this particular animal, regardless of its extinction. Furthermore, the display text seems to suggest that it was a deficiency in the thylacine itself that left this particular species "unable to withstand the new environmental conditions" in which others thrived. Ashby has warned that such a view of Australian marsupials as "inevitably doomed to be outcompeted by a superior evolutionary force from the north" has real implications for conservation efforts today (2021, p. 43). He argues that species deemed inferior are unlikely to receive the same protection, and museum displays may be "accidentally complicit" in perpetuating this view (Ashby, 2021, p. 36).

The MfN's Beutelwolf display thus invites us into a particular process of seeing that encourages the viewer to know (through seeing) this Beutelwolf as yet another extinct animal, aesthetically contained within a procession of ordered mounts. Indeed, processes of visibilisation have been

identified as crucially structuring extinction exhibits across different contemporary natural history museums (Guasco, 2020; O'Key, 2021). In a discussion of the Pinta Island tortoise Lonesome George, exhibited in the Hall of Hope at the Charles Darwin Research Centre (Santa Cruz, Galápagos), Bezan argues that visitors' understanding of species extinction is shaped by a number of factors, including, crucially, exhibition technology and biological discourse, "which together sketch the parameters of what we can see – *and consequently of what we also fail to see* – of the anthropogenic processes that contribute to the loss of species" (2019, p. 214; emphasis in the original). Such exhibits, Bezan argues, channel visitors' responses to "the macrohistorical processes of extinction that, due to their scale and complexity, evade full comprehension" (ibid).

Like Lonesome George, visitors to MfN's Beutelwolf display are invited to see the animal as doubly dead in Deborah Bird Rose's sense of the term. Placed next to footage of one of the last known living thylacines at Hobart Zoo, they are encouraged to understand it not as an individual but as an endling: an animal that simultaneously embodies "the irreparable loss not only of the living but of the […] capacity of evolutionary processes to regenerate life" (Rose, 2012, p. 128; see also Jørgensen, 2017, p. 134; *The Endling exhibition*).

Our article juxtaposes this reliance on all-too-familiar footage of 'the last' and the effect it has of silencing histories of colonization and anthropogenic biodiversity loss with what we can learn about the singular life and afterlife of Berlin's first Beutelwolf. By using the definite article in the paper's title, we want to insist on the singularity of this animal, which Berliners, as well as a wider German-speaking public, came to know first in the zoo, then in zoological publications, and later in the museum. And we seek to trace the visual and cultural regimes that shaped what was seen in real life and through a range of different media from 1864 to the present day. We will call this individual animal Beutelwolf, while using the term thylacine to refer to the species as a whole. In the course of the telling of this story, however, the function of the definite article will repeatedly shift from that of a marker of singularity to that of something else: in accounts of its zoo life, as a museum exhibit, and in the printed depiction of its mounted skeleton, Berlin's first Beutelwolf became THE Beutelwolf – that is, representative of the entire thylacine species. Through its mounted display and its portrayal in the well-known zoological reference book *Brehm's Thierleben*, it achieved a unique but supra-individual status in the public imagination, close to that of a type specimen, or indeed the 'endling' in the video in the present exhibition, representing not an individual animal but the thylacine species as a whole.

Turned into a type, the animal has since performed symbolic work for the dissemination of the grand narratives of natural history to a wider German-speaking public. While in the current exhibition it serves to illustrate the devastating effects of habitat destruction, historically it has been used to support speculation about the workings of evolution and to prove the supposed superiority of placental mammals over marsupials. Throughout this article, we will insist on the singularity of Berlin's first Beutelwolf in order to interrogate those grand narratives. There are certainly limits to this approach – not least the projection of a modern Eurocentric notion of (human) individuality onto the being of another species and from another place. For now, however, we will follow it in the hope that it will allow us to attend to the ongoing "coloniality of knowledge" (Quijano 1997) that has structured and continues to structure the various lives of Berlin's first Beutelwolf, and which works to prohibit the recognition of other ways of relating to the animal and its extinction.

"Such an animal […] belongs in a museum": Zoo life

The bare facts: Berlin's first Beutelwolf arrived at the city's zoo on 5 July 1864. His arrival was reported by several newspapers, including the *Berlinische Nachrichten* and the Leipzig-based *Illustrirte Zeitung* (also: *Erheiterungen*; *Morgenblatt*). Captured in colonised lutrawita (Tasmania), he had been shipped to London in 1856, where he lived eight years in captivity at the London Zoo, only to be transported to another imperial city, Berlin. Here he would die some three months later, becoming the longest-lived captive thylacine.

When he arrived in Berlin, this Beutelwolf was only the third of its kind to be shown in a zoo, and the first in mainland Europe. Only fourteen years earlier, arriving in 1850, the first thylacine exhibit had been a three-year wonder at London Zoo. As William Allen Drew remarked at the time: "Amongst the first, I noticed Lions and Lionesses, Jaguars, Pumas, Chans and the Tasmanian Wolf or Dog-headed Oppossum, of which no other living example has ever been seen in civilized life" (Drew, 1852, p. 312). The Berlin Zoo was in desperate need of such remarkable animals to

attract the crowds. According to Wilhem Peters, the zoo's director, despite its "favourable conditions" and state support, the zoo "lagged behind all other [zoos] in its achievements, in the condition of its animals and in its scientific results" (cited in Bruce, 2017, p. 42).

So, what did visitors see when they visited the zoo's latest attraction? Some may have tried to recognise in the Beutelwolf the real-life animal they knew from an old children's book *Bilderbuch für Kinder*, a lavishly illustrated natural history series. An illustration of the "Hundsköpfige Beutelthiere [dog-headed pouch animal]" appears in volume 10 of the 1821 edition, where it shares the page with other "Strange Marsupials" in the ever-expanding "Miscellaneous" section of the publication project (Fig. 3). Their depiction is preceded by an entry on "The Interior of the Great Temple of Ybsambul" and followed by a discussion of "Strange Amphibians". Lacking any serious attempt at categorisation, the *Bilderbuch*'s publisher J. F. Bertuch defended the series' "most lively and colourful mixture of objects" by pointing out that he "only wanted to amuse" (Bertuch, 1790, p. 7). The brief description accompanying the picture, however, attempted to impart "Beutelthier" knowledge and invited readers to consider the animal's similarity with dogs "especially its head" while stressing that its "internal structure" was consistent with marsupials. It also commented on the animal's "particularly wild, vicious appearance", albeit acknowledging that "on the whole, little is known [about the animal], as only two specimens have been caught, and both males" (Bertuch, 1821, p. 21; for context see Freeman, 2014).

Fig. 3."Das hundsköpfige Beutelthier [dog-headed pouch animal]" in Bertuch's Bilderbuch für Kinder (1821). The illustration is an adaptation of the image published in Harris 1808.

The grouping of the thylacine with "wild, vicious" animals and the comparative gaze that this description invites, appear to have been modes of looking with which visitors some 40 years later also approached the zoo's "Käfig für reißende Thiere [Cage for ferocious animals]", a construction of five adjoining wooden enclosures with iron bars measuring approximately 3x3x2 metres. Here, Berlin's Beutelwolf was placed next to a leopard, a jaguar, a striped hyena and a placental wolf. The zoo's guidebook for 1864 foregrounded the animal's novelty ("It has not yet been brought to Europe alive") and invited viewers to compare him with his European namesake and visual relatives, stressing the similarity of physiology and behaviour to wolves and dogs: "The stature and size are like those of a young wolf or hunting dog, the head also resembles that of a dog, only the mouth is more widely divided" (Zoologischer Garten Berlin, 1864, p. 43). In fact, the dog-like appearance of the animal's head, in particular, seems to have been something everyone could agree on. Remarked upon by Bertuch and in the zoo guide, it was also emphasised in a drawing by animal illustrator Heinrich Leutemann, who portrayed the Beutelwolf's head from life during a visit to the zoo, as part of a one-page tableau of "animal types" for an 1867 issue of the *Illustrirte Zeitung* (Fig. 4).

But 1864 was not 1821, and instead of happily placing the Beutelwolf in a "most lively and colourful mixture of objects", people were now trying to sort out its position within rapidly changing ideas of taxonomy. The zoo's placement of the Beutelwolf with "ferocious animals" was reflective of such sorting, as was the guidebook's related assertion that "its way of life differs little from that of its relatives, the predators" (Zoologischer Garten Berlin, 1864, p.43). It is difficult for us today to grasp exactly how viewers would have understood this claim of the animal's affinity with "the predators". Was the zoo guide suggesting a direct biological link? Or was it hinting at ideas of convergent evolution?

If so, then for Alfred Brehm, eminent zoologist and founding director of the Hamburg Zoo, this affinity was no more than "implied", masking an underlying fundamental difference in evolutionary status (Brehm, 1867, p. 423). In an essay accompanying Leutemann's drawing, he reasoned that marsupials were nothing but Creation's imperfect first, primitive, "attempt" at producing

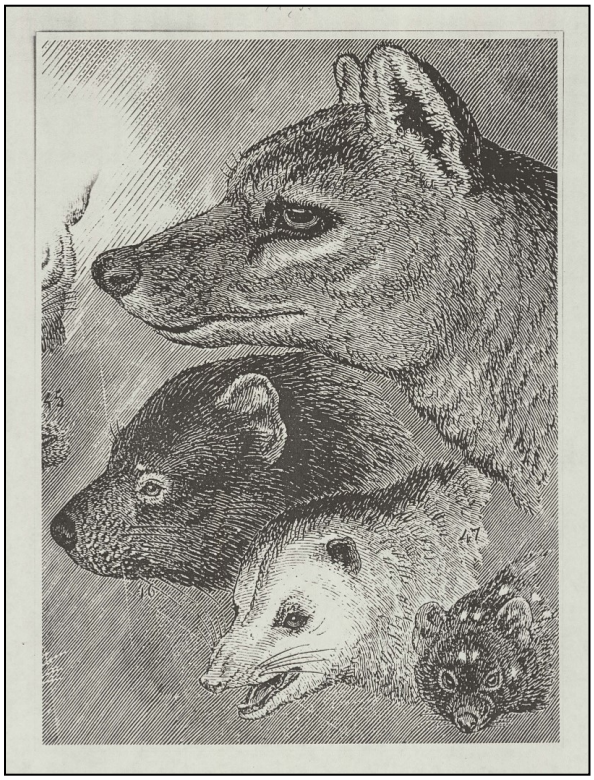

Fig. 4. Head of Berlin's first Beutelwolf (top), drawn from life by Heinrich Leutemann for the Illustrirte Zeitung, 7 December 1867, p. 389.

animals. Compared to placental mammals, he argued,

> the marsupial always appears as an unfinished, imperfect creature, which is far surpassed by the animals as whose predecessors we regard them. And this, as has been remarked, by no means refers to the outer form alone, but also to the way of life, to the spiritual being. Among the marsupials there is not a single one which in cleverness equals other superior mammals, and several of them might be regarded as paragons of stupidity (Brehm, 1867, p. 423).

Brehm's comments form part of what Ashby (2021, 2023a) has identified as a dominant way of thinking about Australian animals. Already in 1834, Richard Owen, for example, first superintendent of the British Museum (Natural History) in London, had described marsupials as "characterized by a low degree of intelligence" (cited in Ashby, 2023a, p. 14). For a German audience, Brehm built on these insights in his 1867 essay, and more extensively in the second edition of his monumental *Illustrirtes Thierleben* (1877). Here, in a veritable diatribe against the subclass of marsupials as a whole, he described them as "a group whose heyday is to be sought in the days of the clumsy amphibians of the land, the flying lizards of the air, the sea dragons of the oceans [...] [as] descendants of past stages of creation, as the earliest mammals, forerunners of more highly developed forms, the attempt of creative nature to form a mammal for the first time" (p. 539). Brehm's verdict culminated in the insight that "the marsupial is in every way inferior in form, development and perfection to the carnivorous or rodent animals" that it resembles (p. 541).

Although never well-known in Britain or North America, Brehm's *Illustrirtes Thierleben* was the most important zoological encyclopaedia ever published in German. Translated into French, Russian, Hungarian and Swedish, Brehm's writing "had an enormous impact on how Europeans of his generation […] observed the animal kingdom" (Reichenbach 2010, p. 186). His descriptions of marsupials such as the thylacine therefore carried weight and promoted a particular view of the animal. Like other nineteenth-century authors, Brehm introduced a temporal dimension to the classical notion of a *scala naturae*. In line with this older view, he regarded marsupials as physically and intellectually inferior, as if they occupied different rungs of the 'ladder of life' (Baum, 2008). At the same time, however, he saw marsupials as remnants of an earlier stage of evolution, as anachronistic precursors of their superior 'modern' successors, the placental mammals. Disregarding the severe pressures on the species from habitat loss and settler violence, he blamed deficiencies in thylacine biology for the species' decline, which he ultimately saw as incompatible with modernity (see Ashby, 2023b, p. 250; Ashby 2023a, p. 288–289). It is this view of marsupials that we identified earlier as still haunting the language of the MfN's current thylacince exhibit.

And yet: While Brehm's writing is primarily concerned with the supposed inferiority of the thylacine species, it also allows us glimpses into the lives and deaths of individual Beutelwolfs in Berlin. In the first edition of *Thierleben*, we find the intriguing comment that thylacines are "difficult to keep alive" (Brehm 1864, p. 6), which could have been written with the Berlin animal in mind. The 1877 edition then devoted an entire paragraph to thylacine life in captivity, evidently based on observations of live animals. By this time, the Berlin Zoo had also housed the city's second Beutelwolf from 1871 to 1873 (Campbell, 2024), so we must assume that Brehm's comments were written with both animals in mind. Echoing his

earlier disparaging remarks, Brehm describes the species as "stupid and mindless":

> Newly captured Beutelwölfe are said to behave very defiantly and unruly in the beginning, climbing around in their cage or in the roof of a house with cat agility and performing movements of 2-3m height. In long captivity, the wild nature in the presence of a human being subsides; […] they run around in their cage for hours without paying much attention to the outside world, or lie resting and sleeping just as apathetically in one and the same place. Their clear, dark brown eyes stare blankly at the observer and completely lack the expression of a real predator's eye (1877, p. 547).

What is new in Brehm's 1877 description is the expressed lack of interest on both sides of the iron bars. Berlin's Beutelwolf 1 and 2 were not interested in Berliners (or, as Brehm seems to speculate, lacked the mental capacity for curiosity), while they could only arouse fleeting interest in zoo visitors as well. Or were the Beutelwolfs simply hard to watch for those who recognised in the animals' trancelike pacing, apathy and blank stares the telltale signs of stressed animals in captivity?

We get a better idea of what zoo visitors might have seen from illustrator Leutemann, who a few years earlier had laconically commented on this mutual lack of recognition in a satirical essay on the deplorable state of the Berlin Zoo:

> The Berlin institution, in its conscious self-sufficiency, had hardly bothered with acquiring new, unprecedented animals, and so, year after year, a certain number of, as it were, immortal animals formed a venerable foundation, the members of which seemed to wrathfully ask any newcomer, who had come here almost in error, how he could dare to disturb the tranquillity of their contemplation. Such arrivals usually soon lost their desire to stay alive. Once when a really rare animal, a Beutelwolf, was in the garden, I heard the words from influential people: Such an animal is not for the public, it must be dead and belongs in a museum. And behold, the Beutelwolf was so attentive that he soon followed this recommendation: it took hardly any time at all before he was dead (Leutemann, 1871, p. 37).

According to Leutemann's verdict, the mere fact of being an "unprecedented animal" seems to have provoked other animals' desire to see the Beutelwolf gone. But why would "influential people" have been prompted to wish for its demise?

Of Bones, Teeth and Pouch: Skeleton Afterlife

Death, however, is far from the end of the first Beutelwolf's story. Zoo director Wilhelm Peters has been identified as the person behind Leutemann's "influential people" cipher (Möller, 1997, p. 145). He ensured that the animal would become valuable source material for natural history in general and the status of Peters and the Zoo in particular. After its demise on 14 November 1864, the Beutelwolf's body was quickly transported to Humboldt University's Zoological Museum, whose entry catalogue records the animal's arrival on the same day (MfN Cat, ZMB-Mam-2986). Here he was dissected and divided into two separate Beutelwolfs, one as a mounted skeleton, henceforth part of the University's Anatomical-Zootomical Museum, and the other as a taxidermy mount. There is also a record of a brain preserved in alcohol, which was later transferred to the Zoological Museum of Kiel University in the 1960s, but which is no longer identifiable in the Kiel collection. The inclusion of a drawing of his skeleton (Fig. 5) in Brehm's popular *Thierleben* from 1877 with the caption "Skeleton of the Beutelwolf (from the Berlin anatomical museum)" suggests that at least the skeleton was used for research and teaching purposes at the time (Brehm 1877, p. 545).

Any attempt to see Berlin's first Beutelwolf today, therefore makes it necessary to visit the exhibition as well as the MfN's research collection. It is, effectively, an act of piecing together those violently separated parts of the animal's body to see a poignant whole. But in 1864 it was the skinning of fur, the bottling of organs and the scraping of bone that gave value to an animal that had come to be considered disappointing in life. By the time Peters consigned the Beutelwolf to the MfN, the 'currency' of the dead thylacine had already risen through scientific attention. London's first thylacine after its death in 1853, had quickly become the subject of Edward Crisp's paper "On some points relating to the Anatomy of the Tasmanian Wolf" (Crisp, 1855). And Peters himself is described by Gary Bruce in his history of the Zoo as "interested in a 'thick description' of the animal and its place in the pantheon of species," which is only possible by studying it from the inside out, "rather than in animal behavior or preservation" (Bruce, 2017, p. 41).

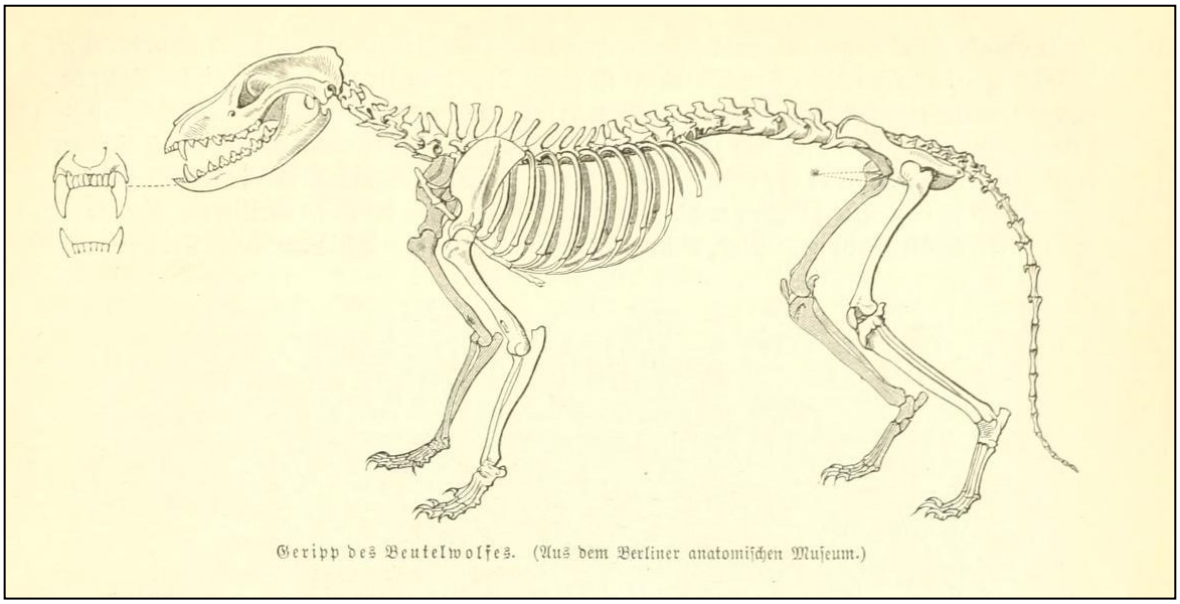

Fig. 5. "Gerippe des Beutelwolfes (aus dem Berliner anatomischen Museum) [Skeleton of the Beutelwolf (from the Berlin anatomical museum)]" in A.E. Brehm (1877), Brehms Thierleben, p. 545.

In adopting this approach, Peters and Brehm were learning from Georges Cuvier, one of the founders of comparative anatomy, who had argued that the study of skeletons – through vivisection and drawing – would reveal the particular anatomical organisation unique to a species and provide the basis for comparing it with others. In his own skeletal study of the thylacine, Cuvier had found that it shared features with much smaller, omnivorous marsupials (Cuvier, 1863, p. 205). The inclusion of the skeleton in Brehm's *Thierleben* must be seen in the light of this development. Its particular focus on certain dental details and the dotted lines indicating epipubic bones, thought at the time to support the pouch, became key to understanding the nature of the animal. Still labelled as an illustration of a specific animal "from the Berlin Anatomical Museum", the drawing in fact functioned as a tool for seeing the thylacine species as a whole.

In this act of seeing, the pouch was crucial in explaining the special ability of marsupials to care for their altricial young and the teeth were key to understanding the thylacine as a marsupial rather than a placental mammal. Thylacine teeth had already been described by Tomes in 1849 (p. 409) and again by Flower in 1868 (p. 636), and it seems reasonable to assume that Brehm's illustrator would have appreciated their scientific importance. Emphasised by a zoomed-in, front-on view, the drawing clearly shows eight upper incisors as a point of differentiation from both the wolf and the dog, which have only six. At the same time, Brehm, in his comments was keen to describe thylacine teeth as 'primitive': "incomplete and backward […] always more imperfectly arranged [than in corresponding placental mammals], either more irregularly set or blunter, even less beautiful in colouring, less white and pure than those of the more perfect predator of later times" (Brehm, 1877, p. 541).

And yet, on the whole, their teeth – much like their bodies when seen whole and moving about in a zoo – were strangely and even confusingly 'almost' dog-like to human observers. Four years before Brehm's anatomical sketch F.H. Balkwill had written about this "Difficulty for Darwinists":

> Mr. Darwin lays it down that the controlling forces which direct the path of variation in a species are the other species with which it has to struggle; and if these forces were sufficiently definite and restricted in their action to produce two such similar dental types as those of the thylacine and dog, independently of each other, it strikes me that classification of mammals would no longer be possible; should we not have dogs, cats, rodents and ruminants arising from independent sources all over the world? (Balkwill, 1873, p. 3698).

Seeing the material reality, even of the skeleton, was not enough, Balkwill argued. Observation alone would only lead to the position that this marsupial, this already decidedly more primitive organism could be seen as dog-like. The solution was not simply 'inside' – the truth of skeleton and

teeth – but classification. As Balkwill went on to quote:

> Darwin himself says […] 'I believe that something more is included; and that propinquity of descent, the only known cause of the similarity of organic beings, is the bond, hidden as it is by various degrees of modification, which is partially revealed to us by our classifications' (Darwin cited in Balkwill, 1873, p. 3698).

It was therefore only in death, and through the mental operation of classification that it facilitated, that the link of Berlin's first Beutelwolf to its permanent, 'real' taxonomic family could be firmly established. In its emphasis on an abstracted pouch and teeth, and their spectral removal from the whole skeleton, Brehm's diagram teaches us that these are the two key things to 'really' see about the thylacine, and that to see them is to understand the animal both inside and out, and in both cases dead. It was only in death that the teeth and bones could be examined in detail and recorded in drawing. Only in death was the animal still enough to be properly 'seen'. And only through that classification could one tell what was a dog or a vicious predator. Or what was a fearsome wolf and what was an apathetic marsupial.

On Display: The Taxidermy Revenant

Although possibly familiar from the skeletal diagram in Brehm's *Illustrirtes Thierleben*, it was not until 1889, when the university collections were amalgamated into the Museum für Naturkunde, that Berliners were invited to see the Beutelwolf in what Leutemann had foreseen as the animal's more appropriate museum setting. Or rather: some of it. A mount of the animal's skin – previously housed in the university's zoological museum and restricted to a scientific audience – was put on display and has been the object of museumgoers' gazes ever since. However, the meanings ascribed to the animal body underwent several shifts during this period, which can be traced by a cursory reading of the various museum guides published by the MfN between 1899 and the 1930s. These allow us to extend our analysis of how Berliner's saw the Beutelwolf beyond the zoo animal's death and its discussion in contemporary zoological literature to show how these representations continued to inform the presentation of the animal body right up to the current exhibition.

Museum guidebooks are a particularly pertinent example of the processes of visibilisation described by Bezan, Guaso and O'Key, as they prescribe a tour of the collection and offer instructions to museumgoers on how to see and make sense of the exhibits (Bezan 2019; Guasco, 2020; O'Key, 2021). A guide from 1932 states in this regard: "The guide is intended to be, in a sense, the detailed text to the demonstration material housed in the cabinets of the hall, and this material forms the illustration to the text" (Zimmer, 1932, p. 3). Printed in large numbers for visitors to purchase at low cost, they also served as souvenirs and mini-biology textbooks whose impact on popular perceptions of biology went far beyond their immediate use.

The first evidence of the mounted animal on display at the MfN comes from a guide published in 1899 by the museum's founding director Karl Möbius. By this time, the status of Berlin's zoological collections had changed dramatically (Schwarz, 2024). With the founding of the German nation-state in 1871, the museum had become a national project meant to reflect, not least, the country's newfound status as a colonial power since 1884. The Bundesrat resolutions of 1889 and 1891 had cemented this status by centralising the processing of ethnographic and natural history specimens collected by colonial troops and the scientists who travelled with them in Berlin's imperial institutions ("Colonial Contexts"). This shift also saw the introduction of evolutionary displays alongside the older taxonomic principles that had organised the University's Zoological Museum grouping – for example, the great apes together with human skeletons and skulls from all orders of mammals. In this display, a prominent place was given to marsupials. Their description focused on the altricial nature of their newborns and the physical characteristics of the animals that enabled mothers to feed their young: "Marsupials are born undeveloped," Möbius informed his readers, "in the pouch, a skin cavity on the abdomen, in which the milk warts lie, they receive the food for further development. In front of their pelvis there is one marsupial bone on each side." The Beutelwolf formed part of this display and was described as a predator with many pointed teeth in a list evidently intended to illustrate the diversity of the marsupial species (Möbius, 1899, pp. 16-17).

Eight years later, in 1907, the Beutelwolf was no longer explicitly mentioned in the revised museum guide, and the overall space devoted to marsupials in the publication was reduced to make room for

a greater number of animals, particularly from the German colonies on the African continent. This decline in interest was accompanied by a change in status, reminiscent of the scala naturae we have already discussed in relation to Brehm's writings on the life of the Beutelwolf in the Berlin Zoo. Published under the new directorship of Prof Dr August Brauer, marsupials were now described as the "lowest mammals" and featured in a potentially shocking display: "In the last rows of the cabinet are the lowest mammals, the marsupials and the monotremes. The former get their name from the fact that the young develop in a pouch that surrounds the teats. One specimen shows a pouch cut open and the young hanging from the teats inside" (Brauer,1907, p. 17).

When Brauer published a new guide only three years later (1910), the tone of the publication had shifted significantly towards a glorification of the German colonial enterprise. The mammal hall, in particular, appears to have been radically altered, with taxonomic principles disregarded in favour of colonial heroes with whom the animals were associated. Among those singled out in the guide were a "rare okapi, which His Highness Duke Adolf Friedrich zu Mecklenburg brought back from his great inner-African expedition" and a chimpanzee, who "lived for several years at the biological station in Amani in German East Africa and was given to the museum by Privy Councillor Prof. Dr. Stuhlmann" (Brauer, 1910, pp.14-18). In this context, it is striking that the text for the marsupials underwent little change apart from being shortened once again: animals not associated with German colonial heroes were apparently not considered to be of equal educational value to museum visitors.

The 1918 and 1921 editions retain this tone, still referring to "our colony of German East Africa" even in 1921, years after Germany had lost its colonies to the Versailles Allies (Kükenthal, 1921, p. 19). By 1931, however, these colonial references have disappeared. Here marsupials and monotremes were once again described as "especially remarkable". What made them remarkable now was their juxtaposition as "primitive forms", displayed on one side of the room, with the placental mammals on the other side: "On the left, the primitive forms: the Australian monotremes – the only egg-laying mammals – furthermore marsupials […]; on the right, on the other hand, the highest mammals, the human-like apes, including a huge gorilla with a skeleton" (Museum für Naturkunde, 1931, p. 5).

Taken together, these guides suggest that the taxidermy mount of Berlin's first Beutelwolf has been on display continuously ever since it moved to the Museum für Naturkunde in 1889. While he would never generate the excitement of animals associated with German colonial heroes, he was consistently shown with other marsupials, a group initially singled out for their curious peculiarities and later denigrated as "lowest" and "primitive" mammals. This subordinate placement of the thylacine on an imaginary evolutionary ladder in 1931 echoes Brehm's earlier description of the animal as "in every way inferior" to placental mammals; a status indicated, among other things, by its "imperfect" teeth. It reminds us to ask how the concepts, 'primitive' and 'extinct' have been used to enable each other long before the Beutelwolf was singled out from the group of marsupials to become the charismatic endling that we are invited to see today. The repercussions of that long-held view of "primitive" continues to reverberate in the current "Extinction through Human Activity" cabinet.

Seeing is believing

Throughout this article we have had an awkward relationship with individuality. On the one hand, our focus on the life, death and afterlife of an individual animal has allowed us to trace how Berlin's first Beutelwolf was subjected to a succession of generalising ways of seeing and understanding, first in the Berlin Zoo, then in zoological publications, and later in the museum. These rendered it emblematic of larger discourses about the workings of evolution, the place of marsupials within it, and the effects of habitat destruction. By focusing on this singular animal, we were also able to address some of the links between settler colonialism, natural history and species extinction; links that have largely remained outside the scope of the MfN's demonstrated commitment to biodiversity advocacy today.

At the same time, however, we remain wary of an individualising strategy that ascribes to non-human animals the attributes of historical actors. This not least, because it further removes the coorinna of lutrawita (Tasmania) from what his relations were and might still be to Country and Indigenous life (Araluen, 2022); relations, human and non-human, that potentially also encompass thylacines who lived across mainland Australia and appear in Pilbara and Kakadu rock art, song and ceremony (Vasseleu, 2022). We do not suggest that the MfN can resolve this tension by simply incorporating Indigenous ways of knowing the thylacine into the exhibition space (Schlunke, 2024). And certainly

not without a proper reckoning with the implications of how our contemporary ways of knowing and representing extinction follow a long tradition of seeing certain animals, such as the thylacine, as 'primitive'. After all, the mutually legitimising notions of 'primitive' and 'destined for extinction' were also used to justify the attempted genocide of the Indigenous palawa people of lutrawita with bounties placed on both the palawa and the coorinna (Ashby, 2023b). In this context, the MfN exhibition's silence on Amalie Dietrich's looting of human remains in colonial Queensland, just a few metres from the thylacine exhibit, is telling. Rather than treating Dietrich's actions as an isolated incident, irrelevant to the practices of nineteenth-century natural history, natural history museums need to acknowledge such violence as structurally embedded in their institutional and disciplinary history (Das and Lowe, 2018; Ashby and Machin, 2021). How might such pasts be adequately addressed while at the same time making the MfN and other colonial institutions appropriate keeping places for coorinna and culturally safe for Indigenous staff and visitors? And how much might a German museum audience, potentially well-educated in the effects of scientific racism when enacted in a human world, appreciate that this way of seeing had mutually reinforcing repercussions for all living beings?

Through our own reflections on Berlin's first Beutelwolf, we have come to understand that such steps towards change would need to move away from the focus on death that is entrenched in the thinking and practice, past and present, around thylacines, that we have outlined in this paper. Whether museum taxidermy, nineteenth-century evolutionary thought, or present-day concerns about habitat loss, they all rely on what we have described as the animal's double-death, individually and as a species, and they cut Berlin's Beutelwolf off from his living relations with kin and Country. Australia's First Peoples have consistently emphasised the importance of Country and their relations with animals, extant and extinct, and Australian natural history museums are beginning to reflect this in their exhibition spaces and museum pedagogy ("Starting where you are"; "Debunking"). To follow their lead would be to make connection the true organising principle of both biodiversity and extinction exhibits. Such museum spaces would acknowledge living relations, even with extinct species, and take responsibility for the multiple and often violent disruptions of those relations which colonial natural history thought and practice contributed.

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Recognition of *Trogoderma glabrum* (Coleoptera; Dermestidae) and differentiation from *Anthrenocerus australis*

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Abstract

As climate change progresses, the distributions and activities of many insect species are changing, in particular those that live out of doors. *Trogoderma glabrum* occurs widely across continental Europe Here we report on the occurrence of *T. glabrum* in Austrian museums and consider how to identify the species. It is compared with *Anthrenocerus australis*, a common pest in some historic houses and museums, and a species that resembles *T. glabrum* both in terms of size and colouration.

Keywords: IPM, pest management, museum, historic houses, identification

Introduction

One of the cornerstones of integrated pest management (IPM) in museums is the correct identification of pest species (Pinniger, 2015; Querner, 2015). Different species could utilize different food sources, e.g., α-keratins (mammalian hair and skin), β-keratins (feathers), or plant-based material (Querner, 2015), and what an insect feeds on could influence where in the museum it is likely to be found or how it enters the building, so correct identification is crucial. Several Coleoptera species are well known to IPM managers in museums and historic houses, for example *Anthrenus verbasci* (Linnaeus, 1767), *A. sarnicus* Mroczkowski, 1963, *Attagenus smirnovi* Zhantiev, 1973, *Stegobium paniceum* (Linnaeus, 1758), and *Lasioderma serricorne* (Fabricius, 1792). In Europe, especially NW Europe, some of these species are almost entirely found in buildings (e.g., *A. sarnicus* and *At. smirnovi*) as self-sustaining populations, whilst others occur out of doors, e.g., *A. verbasci* and *S. paniceum*, and probably enter buildings on an annual basis. It is possible that distributions and activities of species found naturally out of doors are influenced by climate change, so the current communities of beetles found in museums and historic houses could change as new species enter the fray (Pinniger, 2013; Querner *et al*. 2022).

The genus *Trogoderma* Dejean, 1821 contains a number of species that are difficult to identify and to differentiate from each other (Peacock, 1993). One such species is *T. glabrum* (Herbst, 1783). *Trogoderma glabrum* is spread widely across Europe, Russia, USA (Peacock, 1993), and Australia (Rees, 2004). It can be found out of doors under bark feeding on insect remains (Mulsant and Rey, 1868), sap (Mroczkowski, 1962), and in Hymenoptera nests (Hämäläinen and Mannerkoski, 1984). From the little information available, it does not appear to be very common. It is the least common of the *Trogoderma* species in California (Peacock, 1993), rare in Finland (Hämäläinen and Mannerkoski, 1984), and it wasn't found at all in a survey of *Trogoderma*

Fig. 1. Trogoderma glabrum Herbst, 1783 A: male habitus (scale = 1 mm), B: male antenna (scale = 100 µm), C: female antenna (scale = 100 µm)

species in Spanish mills (Castañé *et al*. 2020). In the UK, it has only been found on imports (Peacock, 1993). *Trogoderma glabrum* is a minor pest of stored products in North America (Peacock, 1993), but since it is able to feed on a variety of commodities, both plant and animal based, it is possible that their status as pests of natural science collections could develop.

The purpose of the current study is to raise awareness of *T. glabrum* to museum IPM managers following the discovery of specimens in Vienna and southern Austria . In addition, *Trogoderma glabrum* is compared with *Anthrenocerus australis* Hope, 1843, the most likely species to be found in museums with which it can be confused.

Methods

Sticky traps set in museums and historic houses across Austria in 2022 were examined for Coleopteran pests. Along with the usual species, a number of *T. glabrum* were found. Beetles were lifted from the sticky trap glue using ethyl acetate, which makes the glue fluid, and specimens were then dropped into dry cleaning fluid (K2r ®) to remove any remaining surface glue. Insects were mounted on card and the antennae were teased out for imaging.

Habitus images were captured at ×20 magnification using a Canon EOS 2000D camera mounted on a BMSL microscope. Images of antennae were captured at ×100 magnification using a Canon EOS 1300D camera mounted on a Brunel monocular SP28 microscope. All images were fed through Helicon Focus Pro version 8.2.2 focus-stacking software. All figure scales were made using DsCap.Ink software version 3.90.

Results

Trogoderma glabrum

Figure 1 shows an image of male *T. glabrum* (Fig. 1A). Identification was confirmed using culture specimens from the Pest Infestation Laboratory, York, UK, held by Oxford Natural History Museum (ONHM). The sexes are similar in appearance, both broad bodied, but not very convex. The pronotum is black and the elytra are blackish basally, progressively becoming a reddish brown towards the elytral apices. The elytra are sparsely covered in white and yellow hairs, but the white hairs are focussed in three horizontal bands across the elytra, and the yellow hairs are most notable around the shoulders of the elytra. Body length 2 – 4.2 mm (Herrmann, 2023). The male antennal segments (Fig. 1B) progressively expand from the base rather than forming a well-defined club. The basal four antennomeres are yellowish, the rest are brown apart from the apical half of the terminal segment which is yellow. The male antenna shown in Fig. 1B is long, >0.6 mm. The

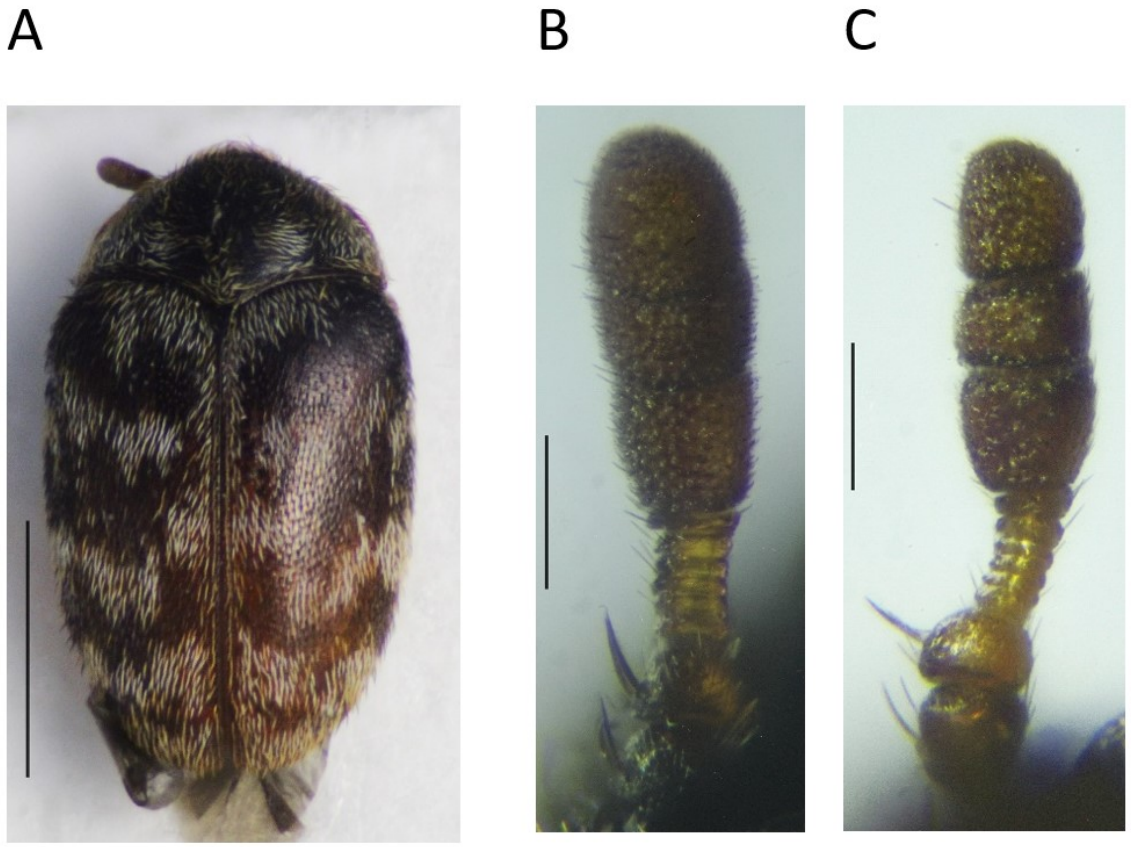

Fig. 2. Anthrenocerus australis Hope, 1843 A: male habitus (scale = 1 mm), B: male antenna (scale = 100 µm), C: female antenna (scale = 100 µm)

female antenna (Fig. 1C) is shorter (0.4 – 0.45 mm) with the terminal five segments forming a more well-defined club than the male. All antennal segments are brown apart from the yellowish terminal segment.

Anthrenocerus australis

Fig. 2A shows an image of male *An. australis*. The sexes are similar in appearance, small and convex. The pronotum is dark brown and the elytra are dark brown basally, usually a lighter reddish brown in the apical half. The elytra have three bands of white hairs, sub-basal, medial, and sub-apical. The bands curve posteriad, particularly the sub-basal and medial bands. There are also patches of white hairs on the elytral base and apex, the outer corners of the pronotum and on the pronotum anterior to the scutellum. Body length 2 – 3.4 mm (Herrmann, 2023). The male antennae (Fig. 2B) have a well-defined, brown cylindrical club consisting of the terminal three segments that contrasts with the yellow of the basal eight segments. The antenna is about .45 mm long with the club accounting for about 0.25 mm of the total length. The female antenna is in many respect similar to the male, except that the antennal club segments are more accentuated, and is approximately the same length as the male antenna.

Discussion

Many species are altering their distributions in response to climate change, although for the majority of insect species we do not have enough information to be able to predict accurately how their distributions might be changing. *Trogoderma glabrum* is found naturally out of doors in Europe and consequently subject to pressures from climate change, so the distribution of this species might be changing. *Trogoderma glabrum* appears on the checklist of beetles of the British Isles (Duff, 2018), but it has not been noted as a self-sustaining population and as such it should not appear on the British Isles list (Peacock, 1993; Holloway, 2020; 2023). It has been recorded on stored product imports (Peacock, 1993). *Trogoderma glabrum* has the capacity to feed on a wide variety of food types, both plant and animal based, and should be a species of concern for IPM managers in museums and historic houses. At a

glance, *T. glabrum* could be confused easily with *An. australis*; they are dark, about the same size and shape with white hairs on the elytra. A more detailed examination should reveal the structure of the antennae and *An. australis* has more extensive white hairs across the elytra.

IPM managers are familiar with the possibility of new species becoming established in the UK and developing into pests of collections and historic artefacts. For example, *An. australis* was first noted in the UK fewer than 100 years ago (Hinton, 1945), and *A. sarnicus* fewer than 60 years ago (Woodroffe, 1967). *Anthrenus sarnicus* has become significant pests of collections and *A. australis* has remained present in many historic houses since then (Pinniger, 2010; Pinniger, 2015; Pinniger and Lauder, 2022; Holloway and Pinniger, 2024). *Anthrenus sarnicus* is a major pest in some establishments in the UK (e.g., Natural History Museum, London), but has no pest status beyond the UK (Holloway and Pinniger, 2024). *Anthrenus museorum* is a pest in collections in many parts of the world, but not in the UK (Holloway and Pinniger, 2020). Other species, such as *A. flavipes*, can devastate collections in warmer parts of Europe (Holloway and Bakaloudis, 2021), but is of less significance in cooler northern latitudes. It is not possible to predict how a new species will react to new conditions, so we do not know whether *T. glabrum*, should it become established in the UK, could develop into a major pest, remain a minor pest as it currently is across continental Europe, or develop no pest status at all. The first line of defence against this uncertainty is for IPM managers to monitor which insects are entering their establishments, and to be aware that new species are always a possibility.

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Suspicious specimens: a new tool to find potentially misidentified and misnamed specimens in biological data using a case study of bryophytes

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Abstract

Natural history collections contain a vast quantity of biological data that provide information on past populations, the impact of invasive species or diseases, evolutionary changes, as well as the effects of climate change. Specimens which are misidentified or misnamed will produce problems for researchers, however checking identifications in large datasets is time-consuming. The new tool described here can be used to screen collection data using three analyses to generate a list of specimens that are likely to be misidentified or misnamed – termed 'suspicious specimens', flagging them for curation. The package identifies outlying biological specimens whose metadata indicates a higher risk of misidentification as well as comparing the collection dataset with a reference dataset and flagging up discrepancies. It is free to use and can be adapted for any collection of biological data. This study uses data from bryophyte specimens in National Museum Wales (NMW) and British Bryological Society (BBSUK) herbaria as a case study to demonstrate the functionality of the package. Of the 10 most suspicious species produced by the analysis and examined in this case study, 70% of the species required redeterminations, showing the effectiveness of this tool in improving the accuracy of collection records.

Keywords: botanical specimens, collections, data, mis-identification, curation, tool

Introduction

Natural history collections are an important source of information. The specimens contained vary across broad temporal and geographic ranges and often include rare and extinct species. This wealth of information has been used in a wide variety of ways by researchers to model past populations and evolutionary changes, and show responses to climate change (Andrew *et al*., 2019; Lang *et al*., 2019), past epidemics (Bieker and Martin, 2018), analysis of invasive species (Ivison *et al*., 2023) and changes in biodiversity of habitats (Mannino *et al*., 2020). These collections have also been used to detect when a new species has been introduced to an area as well as to predict species distributions (Mannino *et al*., 2020). These analyses are dependent on the accurate identification of specimens.

However, several studies have highlighted that misidentified or misnamed specimens are a consistent presence in herbarium collections. Older specimens may have information missing or be incorrectly transcribed (Mannino *et al*., 2020) as well as being named using old or contradictory

taxonomic concepts (Xu *et al*., 2015). Furthermore, some misidentified specimens have been found to be unidentified species (Olds *et al*., 2023) whilst other misidentifications have been found at the genus level (Bradshaw *et al*., 2022). Misidentification also extends to voucher specimens (Łuczaj, 2010). This is particularly important as voucher specimens are used as a verifiable record of a species cited directly in scientific studies and can help resolve taxonomic issues (Bieker and Martin, 2018), thus misidentified voucher specimens are likely to propagate misidentifications in future specimen records. In a study on 4,500 specimens of African gingers (Goodwin *et al*., 2015), it was found that 58% of the specimens were misnamed. Misidentifications do not need to only be tracked once in museum collections but in the field observations as well where plant specimens are misidentified at both species and genus level (5.9% and 1.9% respectively; Scott and Hallam, 2003). Other misidentifications in the field can for example lead to invasive species such as the algae *Lophocladia lallemandii* (Montagne) F.Schmitz in the Mediterranean Sea being mismanaged, with impacts on the native ecosystem (Golo *et al*., 2023). Bias from collectors in areas where there is little interest can also produce taxonomic errors (Isaac and Pocock, 2015), and in some cases misleading and false species information can even be recorded (Pearman and Walker, 2004). Such misidentifications in the field can find their way into museum collections but could be caught beforehand.

Bryophytes are an understudied group of plants (Smith, 2020) that can be difficult to identify with some species requiring microscopy to distinguish them from others and yet they have a great abundance in the UK with about two thirds of European species existing here (Atherton *et al*., 2010). They are used as an example for this study as it is likely that the bryophyte specimens reviewed will include misidentifications and such errors are detailed here as an example case study. In this paper, an analysis of around 100,000 bryophyte specimen records from England, Wales, Scotland, and the Isle of Man – consisting of the databased portion of the NMW and the BBSUK herbaria for these regions (Thiers, accessed 2023) – has been conducted using a newly developed R Package created for this study by the authors (Roberts, 2023). Both NMW (National Museum Wales) and BBSUK (British Bryological Society UK) herbaria are held at National Museum Cardiff by Amgueddfa Cymru-Museum Wales.

An R Package is a piece of software created using the statistical coding language R and can be easily downloaded and used by anyone. These specimens are those which use the Watsonian vice county numbers 1 – 112 (Watson, 1847). Vice counties are a convenient way to section areas of Britain and Ireland for comparative analysis including historical and modern material and is still used by the BBS recording system driven by local as well as taxonomic expertise. Northern Ireland and Ireland were not included in this study due to the different vice county system requiring additional coding. The package identifies outlying biological specimens whose metadata indicates a higher risk of misidentification as well as comparing the collection dataset with a reference dataset and flagging up discrepancies. This new tool is a free and time saving method for cleaning data that can work alongside a variety of Collection Management Systems, providing the curator with an accessible method for verifying collection data with different historic data entry practices.

Analysing the distribution of locations, collections, and taxonomic species, produced 61 museum specimens that may require data verification as well as taxonomic reassessment and shows that the published distributions of some species differ substantially from the narrative offered by museum collections.

Materials and Methods

1. The NMW and BBSUK herbaria

National Museum Cardiff is part of Amgueddfa Cymru – Museum Wales and was founded in 1905 with art, geology, zoology, and botany collections currently in the museum. The total botanical collection has around 750,000 specimens including the bryophyte collection consisting of around 308,000 specimens with collections dating back to the 18th century (K. Slade, pers. comm.).

The British Bryological Society (BBS) was inaugurated in 1923, replacing the Moss Exchange Club formed in 1896 (Foster, 1979). Many of the private collections formed during this time are still part of the BBSUK herbarium which has been held at National Museum Cardiff since 1971 (Harrison, 1980). In 2001, the ownership and copyright of the BBSUK herbarium transferred to Amgueddfa Cymru (Cleal *et al*., 2022). The society compiles reliable records of bryophytes and their distributions published in census catalogues, the most recent from 2021 (Blockeel *et al*., 2021b), with an interim census released online in 2023 (Pilkington and Hodgetts, 2023) and the *Atlas of British and Irish Bryophytes* (Blockeel *et al*., 2014).

Being a voucher specimen collection, the greater accuracy of identifications allows for active research with additional voucher specimens being added frequently. The current collection houses around 47,000 specimens.

2. Analyses

The R package created as a tool for identifying outlying biological specimens conducts three separate analyses to determine specimens or observations with a high risk of misidentification due to inaccurate data verification and validation (Roberts, 2023).

2.a. Species Distributions

The first of the three analyses uses species distribution (including varieties and subspecies) from Watsonian vice county (Watson, 1847) data and compares that to published species distribution records. The biological census data for bryophytes – the British Bryological Society Census Catalogue (Blockeel *et al*., 2021b) – uses vice county records and can be used to show the known distributions of species.

Other mapping tools that use distribution datasets utilise specimen coordinates such as ModestR (García-Roselló *et al*., 2013) and DIVA-GIS (Hijmans *et al*., 2001). However, coordinates are not always available especially for older specimens and cannot be reliably retrospectively assigned. Whilst difficulties exist in assigning a vice county to specimens, particularly for specimens found on borders or for those labelled with old place names, it is nevertheless viable and has been generally carried out as standard curatorial procedure at Amgueddfa Cymru when adding specimens to the botany collection.

The R package displays the species distribution from biological specimen data onto Watsonian vice county boundaries GIS layers from the Biological Records Centre (Biological Records Centre, 2019). Specimens from Northern Ireland and Ireland, whilst available and databased, were not included in the analysis due to using a different system for vice counties (Praeger, 1896) requiring additional coding.

In this analysis, a threshold of the number of specimens in a vice county for a species is set. For example, in a smaller dataset, only one specimen found in a vice county could be suspicious as it is an anomalous result compared to the rest of the dataset. For larger datasets, the user may wish to set a higher threshold. The package produces maps for both the species distribution created from the specimen data, and census data distribution. Another tool in this analysis produces a list of specimens where the vice county it is found in is different to that of the census data.

2.b. Collectors

For the second of the three analyses, the number of collectors for a species was analysed to find any potential bias in the collection data. This analysis uses all collectors for every specimen to produce a list of collectors for each species. A threshold is set for the number of collectors that equates as being potentially suspicious. For example, if the threshold is set at one, then a list of species across all specimens with only one collector is produced. A low number of collectors is more likely to show collector bias and potential species misidentification.

It is also important to note that some taxonomic groups may only have a small number of collectors or recorders across the world. When interpreting the results of this analysis it is essential to be aware of the popularity and recording effort going into a group.

2.c. Orphan Species and Specimens

Finally, the program considered orphan species and specimens. An orphan species is one where there is only one species in the database for a given genus. Similarly, an orphan specimen is one where there is only one specimen in the database for a given species. This analysis identified genera or species with the specified number of orphan species or specimens. For example, if the threshold has been set at one, then a list of either genera with one species or species with one specimen will be produced. This method is useful for finding records of rare or under collected/ observed species as well as taxa that have been subject to excessive taxonomic splitting. Where data contains orphan species and specimens, different systems of classification could have been used. Such confusion of classification can lead to problems with identification (Christenhusz and Chase, 2018).

These methods in combination will flag up species that have either suspect distributions, biased collectors, or lack of specimen information. Suspect specimens after analysis can then be checked for their correct identification and then if relevant, sent for further verification to be recorded as new vice county records for a species. The R package can be utilised with any list

of specimens that utilises Watsonian vice counties (numbered 1 – 112) for location data and can be compared with any corresponding census data. Thus, the package can be a useful tool in reviewing a broad range of biological datasets.

3. Data verification and taxonomic reassessment

After conducting the three analyses, the results were combined to produce a list of the species which have specimens most likely to be misidentified or misnamed. From this, ten species named as the most suspicious species were selected and the corresponding specimens reviewed and inspected microscopically. For each specimen, the herbarium labels were inspected for original identifications and further information about the specimen. For specimens that required taxonomic reassessment, small sections of the specimen were removed and observed microscopically using *The Moss Flora of Britain and Ireland* (Smith, 2004) and *The Liverwort Flora of the British Isles* (Paton, 1999) for species identification.

Results

NMW and BBSUK Herbaria

From each analysis, suspicious specimens were produced using set thresholds. For the NMW and BBSUK dataset the thresholds for vice county distributions were species which for any vice county had one specimen. When comparing the distribution maps, the species that differed significantly from the census data (i.e., specimens not found in vice counties adjacent to those in the census data, see Figure 2) were considered suspicious. Species with one collector were also considered suspicious and either species with one specimen or genera with one species were also deemed suspicious.

Once all three analyses had been run, the list of species was filtered to only show species that had specimens that qualified as suspicious for all three analyses. For example, having a distribution different to that of the census data, having one collector and being an orphan specimen. This produced a list of the most suspicious species having specimens potentially misidentified. The filtering was then run again for species that only qualified for two of the analyses and so on to produce a ranked list of specimens by suspiciousness (Table 1). Note that while taxonomic names on the database are currently being manually updated using Blockeel *et al.* (2021), Tropicos.org (accessed, 2023) and the United Kingdom Species Inventory (Raper 2014, last updated 12/02/2021), they may differ from currently accepted names (Katherine Slade pers.comm.).

The bryophyte specimens deemed most suspicious are listed subsequently (with accession numbers in brackets). *Neckera pennata* Hedw. (NMW C96.7.333) had a vice county vastly different to that of the census data and being an orphan specimen. *Pseudocampylium radicale* (P.Beauv.) Vanderpoorten (NMW C.2010.030.8020), *Aongstroemia longipes* (Sommerf.) Bruch & Schimp. (NMW C96.16.259), *Heterocladiella dimorpha* (Brid.) Ignatov & Fedosov (NMW C.2000.002.528), *Homomallium incurvatum* (Brid.) Loeske (NMW C96.18.127) and *Paraleucobryum longifolium* (Hedw.) Loeske (NMW C97.12.161) had a vice county vastly different to that of the census data and being an orphan species. *Philonotis tomentella* Molendo (NMW 13.68.49, 15.54.1, 20.7.m.10, 20.7.m.11, 20.7.m.12, 22.187d.977, 23.92.685, 24.457.44, 24.457.45, 25.152.4046, 25.152.4047, 25.152.4050, 25.152.4068, 40.443.46, 42.13.4, 44.265.8, 48.29.48, 64.97.488, 66.230.104, 71.1B.122) and *Riccia crystallina* L. emend Raddi (NMW C96.15.130, C96.15.2959, C96.15.2961, C96.15.2962, C96.15.2963, C96.15.2964, C96.15.2965, C96.15.2966, C96.15.2967, C96.15.2971, C96.15.2972, C96.15.2973, C96.15.2974, C96.15.2975, C96.15.2976, C96.15.2977, C96.15.2978, C97.3.1682, C97.3.1687, C97.3.1688, C.1999.028.3603, C.1999.028.3616, C.1999.028.3619, C.1999.028.3942, C.1999.028.3943, C.1999.028.3944, C.1999.028.3945, C.1999.028.3946, C.1999.028.3947, C.2000.008.186) and (BBSUK C.2001.020.8617, C.2001.020.8618, C.2001.020.8619) which had many different vice counties that were different to the census, ranking it highly as there were many specimens for this species that were found in unexpected locations. *Cirriphyllum cirrosum* (Schwaegr.) Grout (NMW C.2000.002.641) had a vice county vastly different from the census. *Plagiothecium platyphyllum* Moenk. (NMW C.2000.020.28) had a vice county different to that of the census. Of these specimens, most were from the NMW herbarium (58 specimens) and only three specimens were from the BBSUK herbarium (C.2001.020.8617, C.2001.020.8618, C.2001.020.8619), reflecting their respective levels of verification.

These top species flagged for curation were then checked against the literature and analysed microscopically to confirm if species required taxonomic reassessment.

Table 1: The ranking of bryophyte species based on the suspiciousness of specimens after running the three analyses. Species ranked from most suspicious to least suspicious based on outcome of analyses. Species names are those listed in Amgueddfa Cymru-Museum Wales Botany Collections Management System database in June 2023. The reason column dictates which analyses produced suspicious results.

Ranking	Species	Reason
Most suspicious	*Neckera pennata* Hedw.	Vice county vastly different to census Orphan specimen
	Pseudocampylium radicale (P. Beauv.) Vanderpoorten	Vice county vastly different to census Orphan species
	Philonotis tomentella Molendo *Riccia crystallina* L. emend Raddi	Many vice counties different to census
	Cirriphyllum cirrosum (Schwaegr.) Grout	Vice county vastly different to census
	Aongstroemia longipes (Sommerf.) Bruch & Schimp. *Heterocladiella dimorpha* (Brid.) Ignatov & Fedosov *Homomallium incurvatum* (Brid.) Loeske *Paraleucobryum longifolium* (Hedw.) Loeske	Vice county different to census Orphan species
Least suspicious	*Plagiothecium platyphyllum* Moenk.	Vice county different to census

1. *Neckera pennata*

(NMW C96.7.333)

The most suspicious of the moss species was *Neckera pennata* (NMW C96.7.333), which has only one specimen in the collection found in a vice county different to that of the census (Figure 1). *Neckera pennata* is a circumpolar boreal-montane species which has only been recorded once in Scotland in 1823 (Blockeel *et al*., 2014). The flagged specimen was found in VC 9 (Dorset).

When this specimen was observed under the microscope, the leaves were noted to be distinctly smooth rather than undulate (Figure 2). Undulated leaves are a feature in *N. pennata* and other *Neckera* species but not in *Neckera complanata* (Hedw.) Huebener. The specimen showed broad oblong leaves with obtuse apiculate apex and did not have a nerve present (Figure 2) This leaf shape is not like that of *N. pennata* whose leaf gradually tapers to an apex (Smith, 2004). The elongated mid-leaf cells were around 3 – 4 times as long as wide (Figure 2) whereas in *N. pennata* they are 4 – 8 times as long as wide (Smith, 2004). These characteristics, in particular the lack of undulations, points towards this specimen being *Neckera complanata*, the distribution of which includes VC 9, where this specimen was found (Figure 3; Blockeel *et al*., 2014).

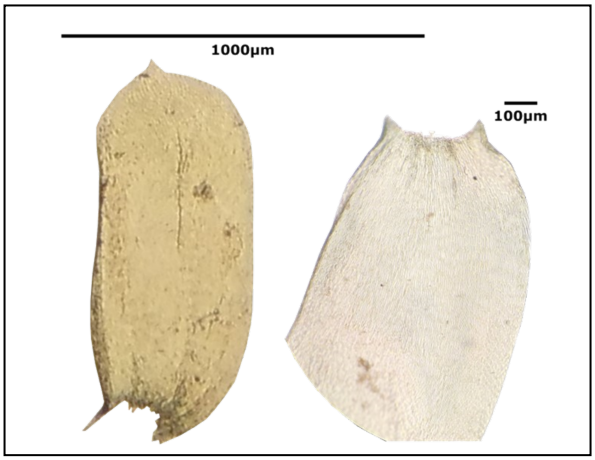


Figure 2. Microscope image of (NMW C96.7.333). Image on the left shows the whole leaf missing undulations and nerve with obtuse apiculate apex. Image on the right shows the elongated mid-leaf cells that are 3 – 4 times long as wide. This description is closer to that of Neckera complanata.

Figure 1. The vice county data for Neckera pennata *NMW C96.7.333. The image on the left shows the distribution from the herbarium data: VC 9. The image on the right shows the British Bryological Society 2021 Census Catalogue (Blockeel* et al.*, 2021b) distribution: VC 90.*

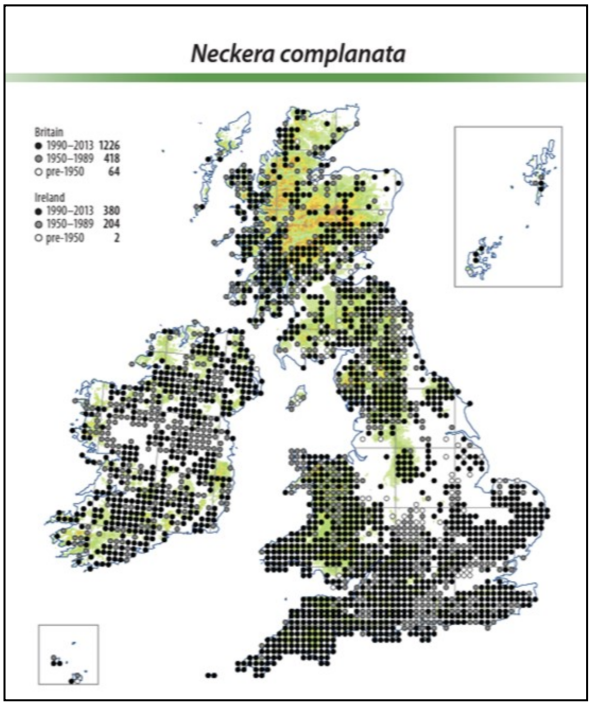


Figure 3. The distribution of Neckera complanata *from the Atlas of British Bryophytes (Blockeel* et al.*, 2014). This species has a large distribution and includes Dorset where the specimen NMW C96.7.333 was found.*

2. *Pseudocampylium radicale*

(NMW C.2010.030.8020)

This specimen was found in VC 13 (West Sussex), deviating from the census data which shows a more westerly distribution (Figure 4). This specimen is from a historical book of pressed bryophytes dating from the 1850s and so was only observed *in situ* under a stereo microscope so as not to damage the specimen. The original identification for this specimen was *Hypnum radicale* P. Beauv (now *Pseudocampylium radicale*). From inspection, the leaf of this specimen has a distinctive bend in the nerve which extends into the apex of the leaf like that of *Hygroamblystegium varium* (Hedw.) Mönk. (Figure 5) which can be found in West Sussex (Figure 6). The leaves of *Hygroamblystegium varium* are ovate with long acumen and stem leaves are 1.0 – 1.4 mm long (Smith, 2004). These characteristics can be seen in Figure 5.

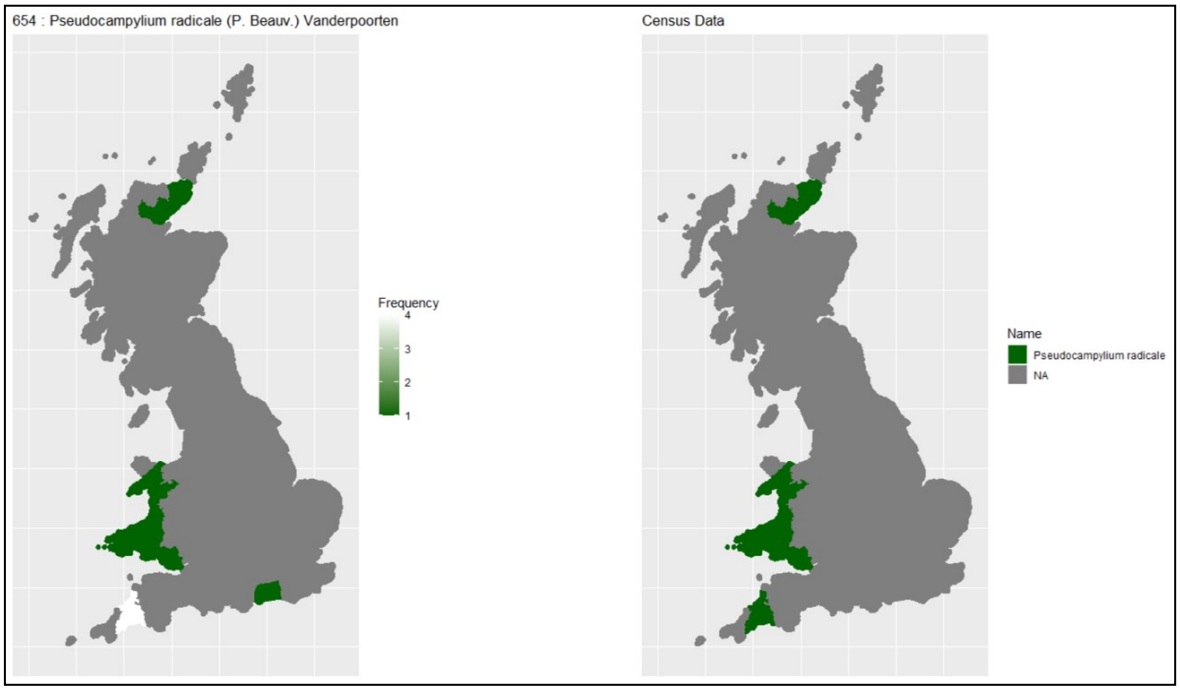


Figure 4. The vice county data for Pseudocampylium radicale *(NMW C.2010.030.8020). The image on the left shows the distribution from the herbarium data: vice counties 2, 13, 41, 44 – 46, 48, 49, 107, 109. The image on the right shows the British Bryological Society 2021 Census Catalogue (Blockeel* et al., *2021b) distribution: vice counties 2, 41, 44 – 46, 48, 49, 107, 109.*

Figure 5. Microscope image of (NMW C.2010.030.8020). Image shows the distinct bend in the nerve of the leaf like that of Hygroamblystegium varium.

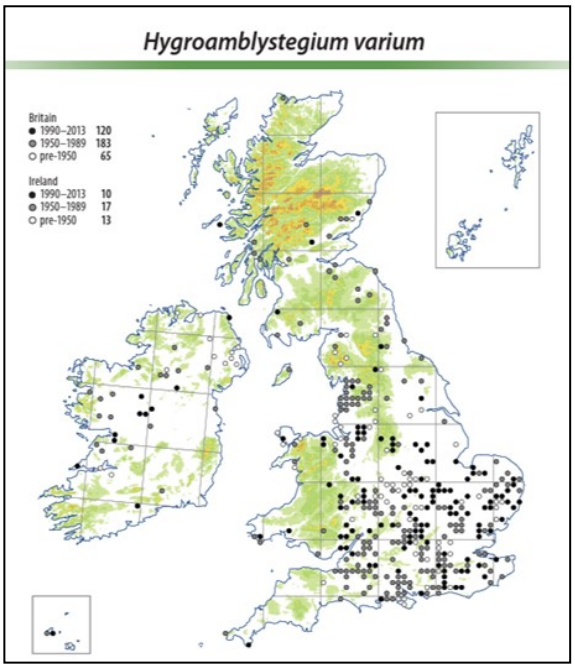


Figure 6. The distribution of Hygroamblystegium varium *from the Atlas of British Bryophytes (Blockeel* et al., *2014). This species has a wide distribution across England and includes West Sussex where the specimen NMW C.2010.030.8020 was found.*

3. *Philonotis tomentella*

(NMW 13.68.49, 15.54.1, 20.7.m.10 - 12, 22.187d.977, 23.92.685, 24.457.44 - 45, 25.152.4046 - 47, 25.152.4050, 25.152.4068, 40.443.46, 42.13.4, 44.265.8, 48.29.48, 64.97.488, 66.230.104, 71.1B.122)

Philonotis tomentella specimens in the collection had many vice county records that were not found in the census data (Figure 7). The vice counties recorded for *P. tomentella* not in the census data are: 5, 35, 47, 40 – 42, 46, 48, 58, 60, 62, and 87. This species has an altitudinal range of 50 – 1125 m and has been found growing in a variety of habitats on basic cliffs and sandy and peaty ground. This species is relatively scarce and closely related to *P. fontana* (Hedw.) Brid. which is a more widespread species (Blockeel *et al*., 2014). *Philonotis* is a difficult group and species can be difficult to distinguish from one another due to high levels of variations and integrations between species (Atherton *et al*., 2010; Buryová, 2004). However, there seems to be some confusion in the taxonomy of *Philonotis tomentella*. The specimen labels show them to have been originally identified as *P. fontana* and then redetermined as *P. fontana* var. *tomentella* (Molendo) A. Jaeger, before being transferred to *P. tomentella*. It is therefore likely that these specimens all belong to *P. fontana*. Determining the identity of the suspicious specimens is beyond the scope of this study.

4. *Riccia crystallina*

(NMW C96.15.130, C96.15.2959, C96.15.2961 - 67, C96.15.2971 - 78, C97.3.1682, C97.3.1687 - 88, C.1999.028.3603, C.1999.028.3616, C.1999.028.3619, C.1999.028.3942 - 47, C.2000.008.186) and (BBSUK C.2001.020.8617 - 19)

Riccia crystallina is a liverwort which has many different vice county records in the NMW and BBSUK herbaria, compared to the census data (Figure 8). The vice counties recorded for *R. crystallina* not in the census data are: 4, 6, 12, 14, 20, 22, 26, 28, 29, 32, 36, 38, 49, 55, 56, 64, 67, 83, 101 and 110. This species has a distinct ecology, growing in arable fields and sandy soil with an altitudinal range of 0 – 90 m (Blockeel *et al*., 2014). This species was split from *R. cavernosa* Hoffm. in 1966 which has a distribution more closely resembling that of the collection data (Figure 10). The herbarium packets for the mismatched specimens show that the original identifications are *R. crystallina* however many of these specimens are pre. 1966 and are likely to now be considered *R. cavernosa* (Paton, 1999). When these specimens were observed microscopically, many of the specimens resembled other *Riccia* species as the rosettes were not fused together like that of *R. crystallina* with some likely to be *R. cavernosa* whose rosettes are made up of more distinct lobes (Figure 9). As much of the

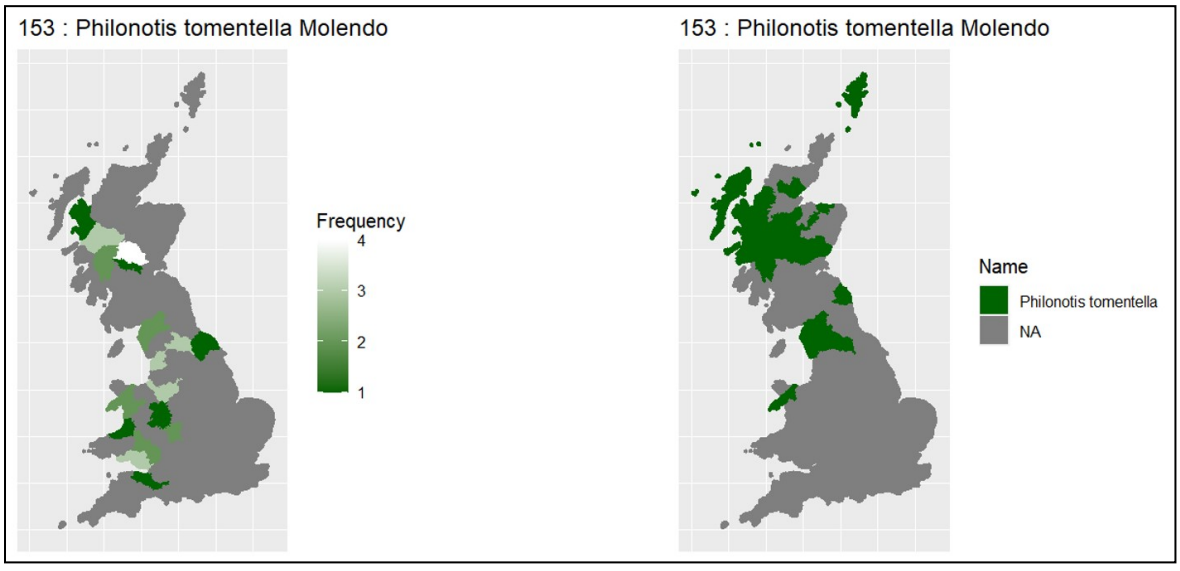


Figure 7. The vice county data for Philonotis tomentella. *The image on the left shows the distribution from the herbaria data: vice counties 5, 35, 37, 40 – 42, 46, 48, 49, 58, 60, 62, 65, 70, 87, 88, 97, 98, 104. The image on the right shows the British Bryological Society 2021 Census Catalogue (Blockeel* et al., *2021b) distribution: vice counties 49, (65, 68 – 70), 88, (89, 90), 94, 96 – 98, 103 – 105, 107, (110), 112. Bracketed vice counties are those that have not been observed since 1969.*

material for these specimens was very fragile, it was decided at this time it should not be hydrated and therefore identification to species level could not be performed for this difficult group within this study.

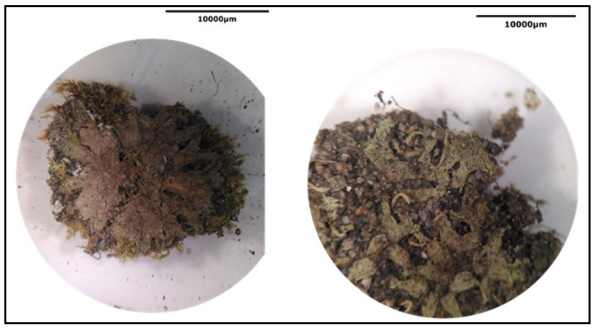


Figure 9. Examples of specimens labelled as Riccia crystallina *(NMW C96.15.2963 and NMW C96.15.261). Specimens show rosettes with more distinct lobes not fused together like that of* Riccia crystallina.

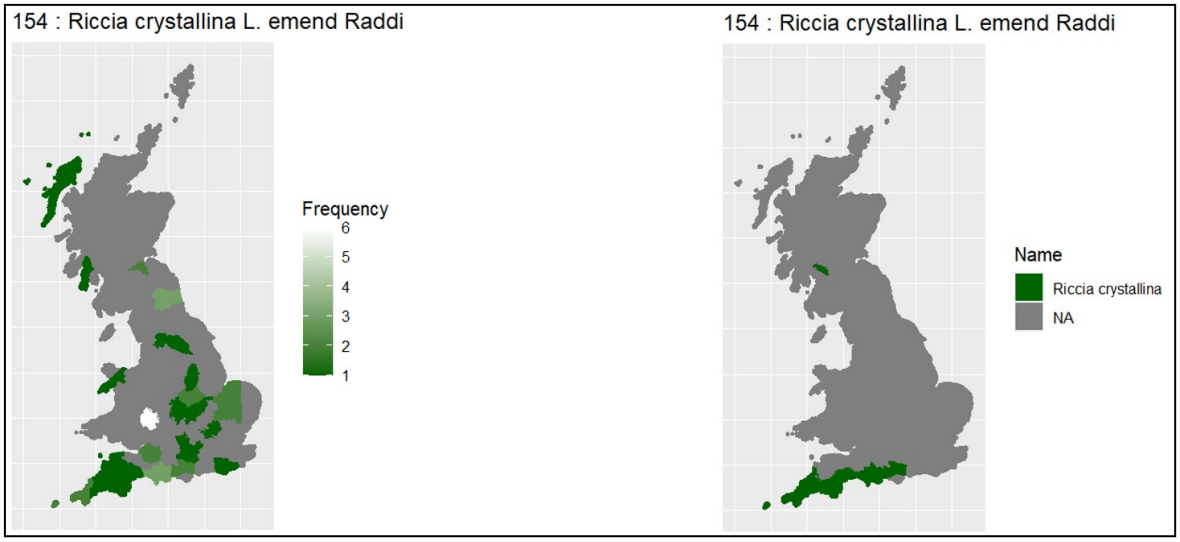


Figure 8. The vice county data for Riccia crystallina. *The image on the left shows the distribution from the herbarium data: vice counties 1 – 4, 6, 9, 11, 12, 14, 20, 22, 26, 28, 29, 32, 36, 38, 49, 55, 56, 64, 67, 83, 101, 110. The image on the right shows the British Bryological Society 2021 Census Catalogue (Blockeel* et al., *2021b) distribution: vice counties 1 – 3, 9, 11, (76). Bracketed vice counties are those that have not been observed since 1969.*

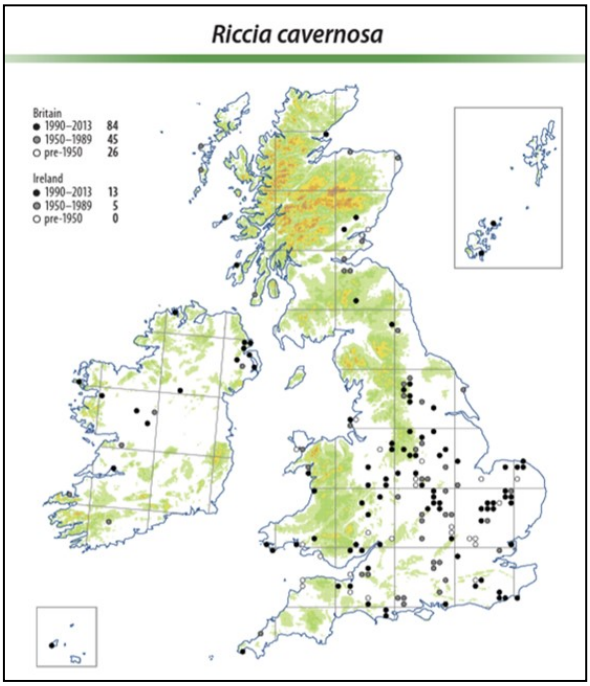


Figure 10. The distribution of Riccia cavernosa *from the Atlas of British Bryophytes (Blockeel* et al., *2014). This species has a greater distribution than* Riccia crystallina *and includes vice counties where the specimens were found.*

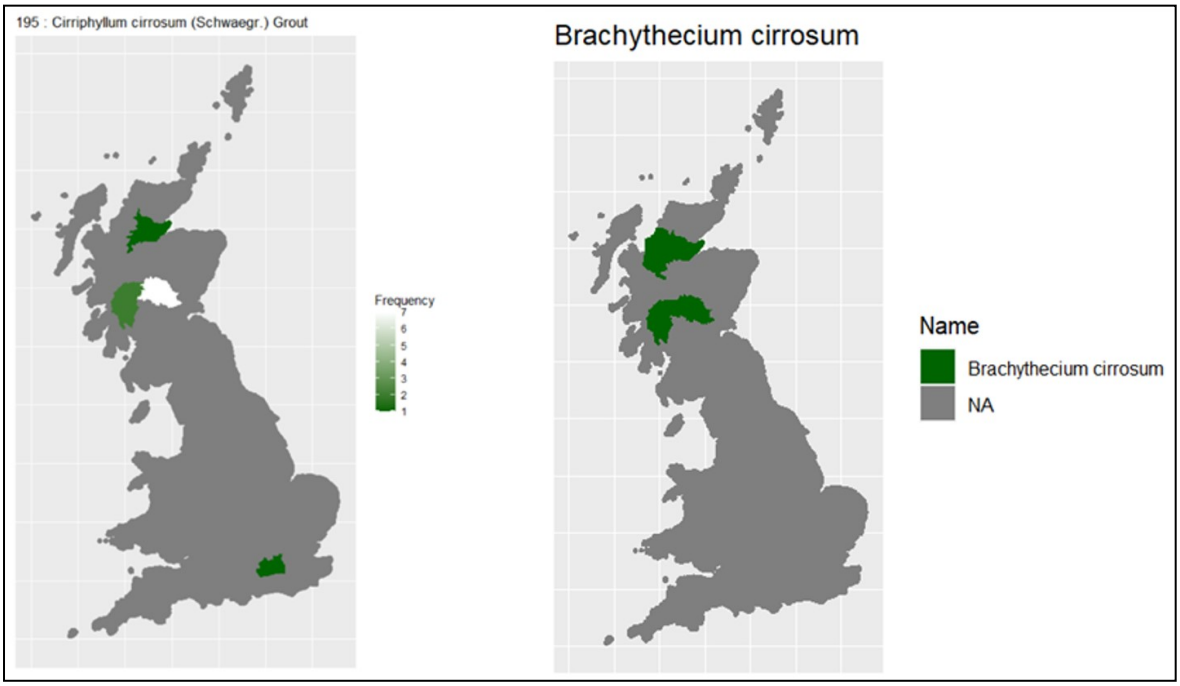
5. *Brachythecium cirrosum*

(NMW C.2000.002.641)

Brachythecium cirrosum (Schwägr.) Schimp. (synonym *Cirriphyllum cirrosum* (Schwaegr.) Grout (Flora of North America Editorial Committee, 2014)) has one specimen from a vice county not found in the census (Figure 11). *B. cirrosum* is found in Scotland on ledges or at the base of crags at higher altitudes (670 – 1070 m). This species is common in the high Arctic and found in many mountain ranges (Blockeel *et al.*, 2014). However, this specimen was found in Denbies in VC 17 (Surrey) which has an altitude of around 50 m (Cucaera, accessed 2023). There is no current record of *B. cirrosum* in Surrey (Blockeel *et al.*, 2021a; Gardiner, 1981). Like most species, bryophytes found at higher altitudes are likely to respond to a changing climate by shifting their elevational range usually so that they are increasingly found at higher altitudes than before (Rumpf *et al.*, 2019). It therefore seems unlikely to find this species at a lower elevation than expected.

When this species was observed microscopically, it was found that the leaves have a rounded apices which tapers to a long acumen (Figure 12). The cells had a width of around 10 µm (Figure 12) and that the lower stem was pinnately branched. *Brachythecium cirrosum* has cells which are 5 – 8 µm wide and are irregularly branched (Smith, 2004). The description of this specimen closely matches that of the more common *Cirriphyllum piliferum* (Hedw.) Grout which has broad a distribution that includes VC 17 where this specimen was found (Figure 13).

Figure 12. Microscope images of (NMW C.2000.002.641). The image on the left shows the leaf shape of this specimen. The image on the right shows the elongated leaf cells around 10 µm wide. These features resemble more closely Brachythecium piliferum.



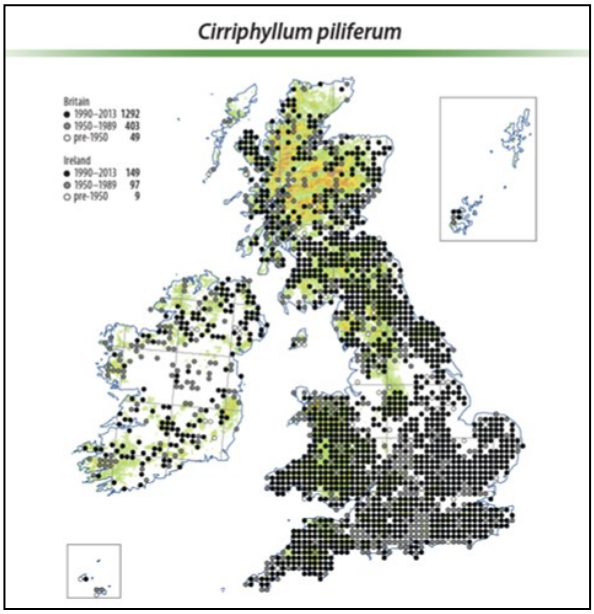


Figure 11. (Above) The vice county data for Brachythecium cirrosum *(NMW C.2000.002.641). The image on the left shows the distribution from the herbarium data: vice counties 17, 88, 98, 107. The image on the right shows the British Bryological Society 2021 Census Catalogue (Blockeel* et al., *2021b) distribution: vice counties 88, 98, 105, 106.*

Figure 13. The distribution of Cirriphyllum piliferum *from the Atlas of British Bryophytes (Blockeel* et al., *2014). This species has a large distribution and includes Surrey where the specimen NMW C.2000.002.641 was found.*

6. *Aongstroemia longipes*

(NMW C96.16.259)

This specimen had a vice county different to that of the census where it was found in VC 67 (Figure 14). *Aongstroemia longipes* is a circumpolar boreal-montane species that has only been recorded in the Scottish Highlands in Britain. Although, as it is a small species it can be easily overlooked in the field (Blockeel *et al*., 2014). This specimen was recorded as having been found on an old lead mine waste tip in Allenheads, Northumberland and when verified microscopically it was found to be *Ditrichum plumbicola* Crundw. which is found on lead-mine spoil. The leaves of the specimen have a larger nerve than that of *Aongstroemia longipes* and are lanceolate rather than oblong-ovate (Figure 15). The leaves have a short apex compared to that of other *Ditrichum* species and are 0.4 – 0.7 mm long (Smith, 2004). The distribution of *Ditrichum plumbicola* includes South Northumberland where this specimen was found (Figure 16). *D. plumbicola* was not described as new species until 1976 (Crundwell, 1976) and this record (NMW C96.16.259) was collected in 1969 with *A. longipes* being the closest morphologically similar species. The collector of this specimen expressed doubt of the original identification on the specimen label. The earliest known record for this species was from 1914 (Blockeel *et al*., 2014) however as this is a scarce species (Smith, 2004), this makes it an important voucher specimen, and could be an older record for this vice county.

Figure 15. Microscope images of (NMW C96.16.259). The image on the left shows the stems of the specimen. The image on the right shows the lanceolate leaf shape with wider nerve than that of Aongstroemia longipes. *These leaf characteristics resemble that of* Ditrichum plumbicola *more closely.*

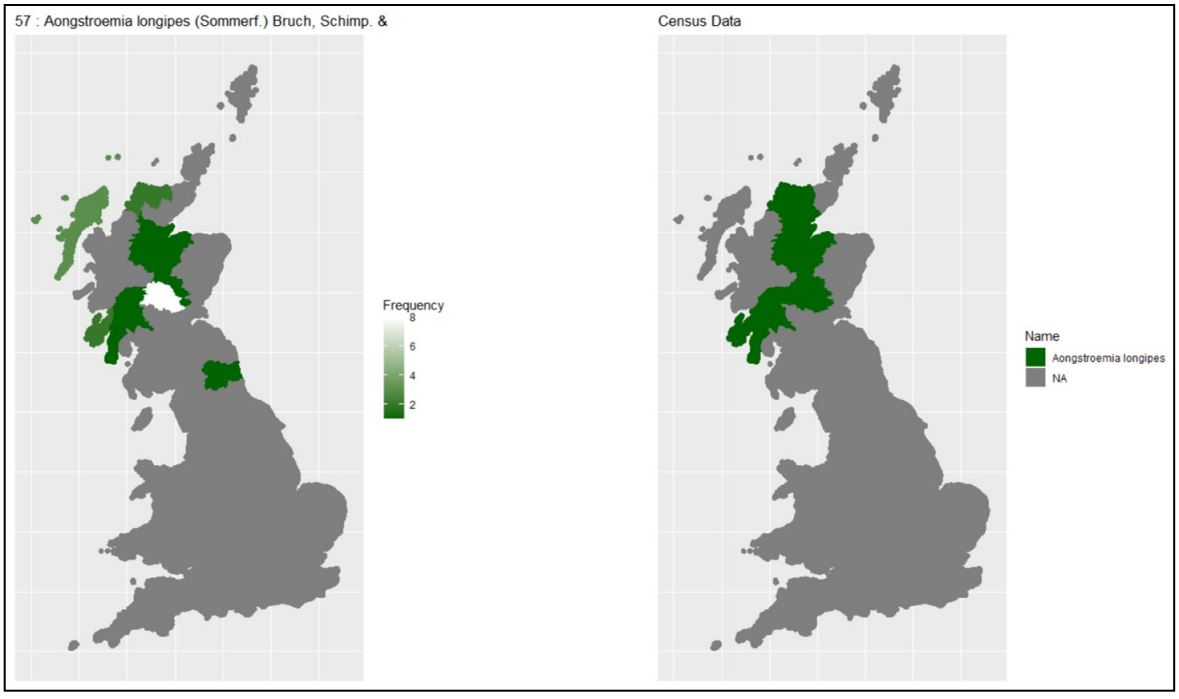


Figure 14. The vice county data for Aongstroemia longipes *(NMW C96.16.259). The image on the left shows the distribution from the herbarium data: vice counties 67, 88, 89, 95, 96, 98, 99, 101, 102, 106, 108, 110. The image on the right shows the British Bryological Society 2021 Census Catalogue (Blockeel* et al., *2021b) distribution: vice counties 88, 89, 95, 96, 98, 99, 101, 102, 106 – 108.*

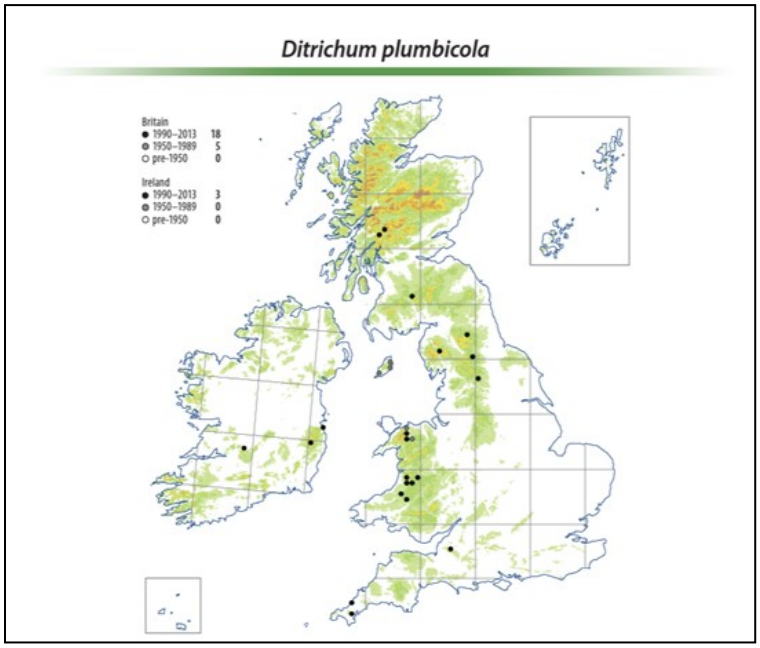


Figure 16. The distribution of Ditrichum plumbicola *from the Atlas of British Bryophytes (Blockeel* et al.*, 2014). This species is found on lead mine spoil and includes South Northumberland where the specimen NMW C96.16.259 was found.*

7. *Heterocladiella dimorpha*

(NMW C.2000.002.528)

This record was found in VC 73 (Kirkcudbrightshire) whereas the census data shows this species is found in the Scottish Highlands (Figure 17). The leaf shape of this specimen is not the same as *H. dimorpha* which have broadly ovate leaves with an acuminate apex (Smith, 2004). The leaves on this specimen are narrowly ovate, gradually tapering to an acute apex. Leaves are smaller than that of *H. dimorpha* with the longest being around 0.4 mm long (Figure 18) and do not show a distinct short double nerve. These leaf characteristics fit more closely with those of *Heterocladium flaccidum* (Schimp.) A.J.E. Sm. which is found in VC 73 (Figure 19).

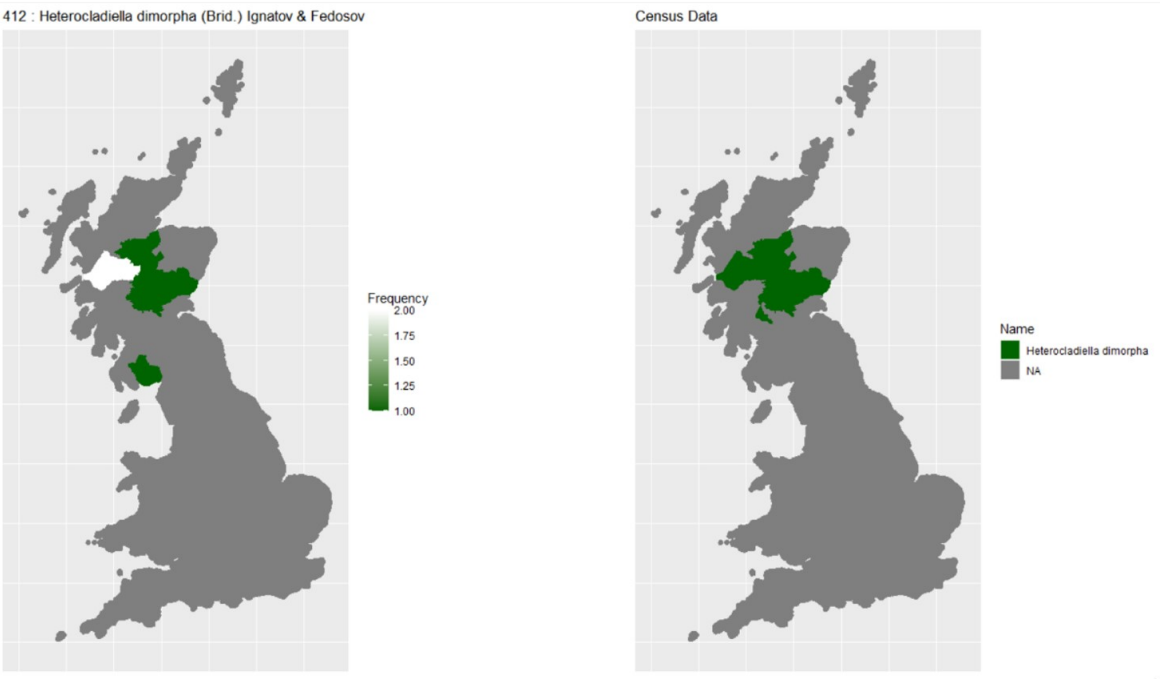


Figure 17. The vice county data for Heterocladiella dimorpha *(NMW C.2000.002.528). The image on the left shows the distribution from the herbarium data: vice counties 73, 87 – 90, 96, 97. The image on the right shows the British Bryological Society 2021 Census Catalogue (Blockeel* et al.*, 2021b) distribution: vice counties 87 – 90, 96, 97, 99.*

Figure 18. Microscopic image of specimen NMW C.2000.002.528. Leaves are narrowly ovate with acute apex no longer than 0.4 mm resembling those of Heterocladium flaccidum.

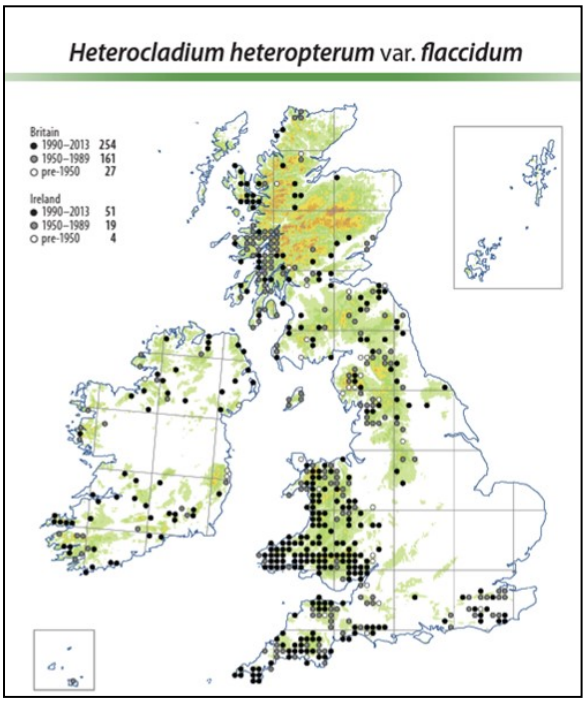


Figure 19. The distribution of Heterocladium flaccidum *(listed as* Heterocladium heteropterum *var.* flaccidum*) from the Atlas of British Bryophytes (Blockeel* et al.*, 2014). This species has a more westerly distribution but found in a variety of locations including VC 73 where NMW C.2000.002.528 was found.*

8. *Homomallium incurvatum*

(NMW C96.18.127)

This specimen was found in VC 107 (East Sutherland) in the north of Scotland, which is not recorded in the census data (Figure 20). After observing this specimen microscopically, it was found that this specimen was correctly identified, having capsules that are horizontal (Figure 21) and leaves that are lanceolate with a long acumen. This species also has distinct basal cells which are elongated but surrounded by small cells in the margin (Figure 22). The mid leaf cells are also small and rectangular (Figure 22). This is believed to be a new vice county record for this species which is Red Listed (endangered, Callaghan, 2022) and the specimen will be sent to the BBS Moss Recorder for confirmation. This is an unexpected outcome which has uncovered a very interesting record of a Red Listed species from a site not included in in the census data. The inclusion of this specimen is important as it allows the site to be targeted for future survey work for the threatened species.

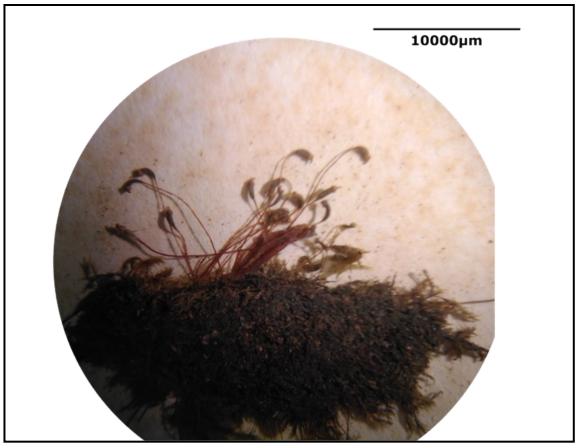


Figure 21. Microscope image of NMW C96.18.127 showing the horizontal capsules.

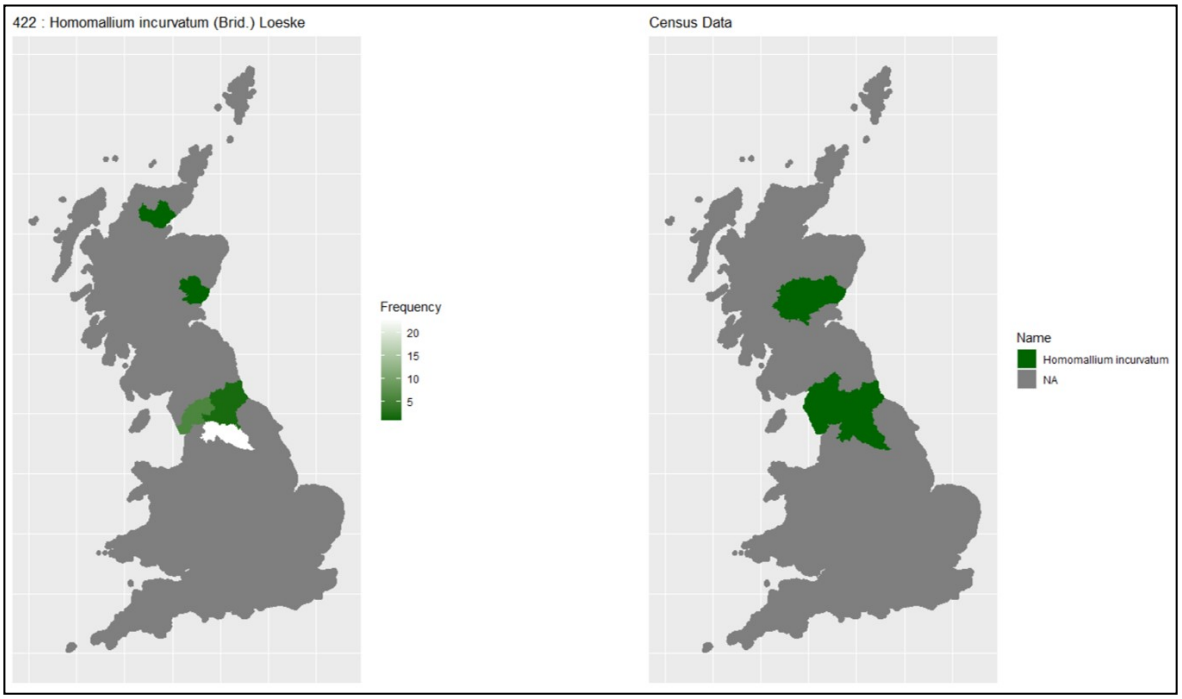


Figure 20.The vice county data for Homomallium incurvatum *(NMW C96.18.127).The image on the left shows the distribution from the herbarium data: vice counties 64 – 66, 69, 90, 107.The image on the right shows the British Bryological Society 2021 Census Catalogue (Blockeel* et al.*, 2021b) distribution: vice counties 64 – 66, 69, 70, 87 – 90.*

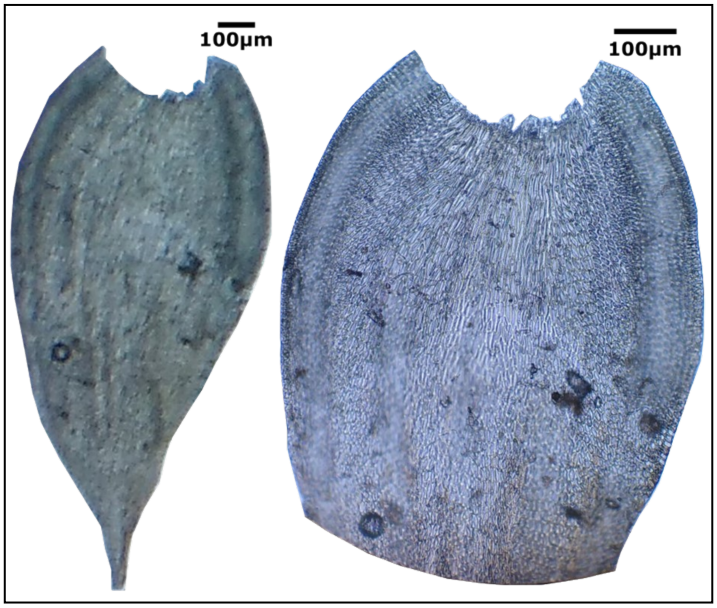


Figure 22. Microscopic image of NMW C96.18.127 leaf. Image on the left shows the lanceolate leaf shape tapering to a long acumen. Image on the right shows the distinctive elongated basal leaf cells surrounded by smaller rectangular cells.The cells in the mid leaf are rectangular rather than elongated.

9. *Paraleucobryum longifolium*

(NMW C97.12.161)

This specimen was found in VC 70 (Cumberland) whereas *Paraleucobryum longifolium* is found in the Scottish Highlands (Figure 23). Initial examination of this specimen revealed it to be a *Campylopus* species due to the long leaf shape with a wide base and tapering to a long, thin acumen (Figure 24). The width of the nerve in *P. longifolium* is greater than that seen in this specimen which is less than a third of the width of the leaf. However, it is larger than that of *Dicranum* species. The auricles of NMW C97.12.161 have a distinctive red-brown colouring and the basal cells are rectangular (Figure 24). The transverse section of the leaf shows small cells with thick walls and closely resembles the transverse section of *Campylopus flexuosus* (Hedw.) Brid. (Figure 24). This is a species that has a wide distribution including Cumberland (Figure 25).

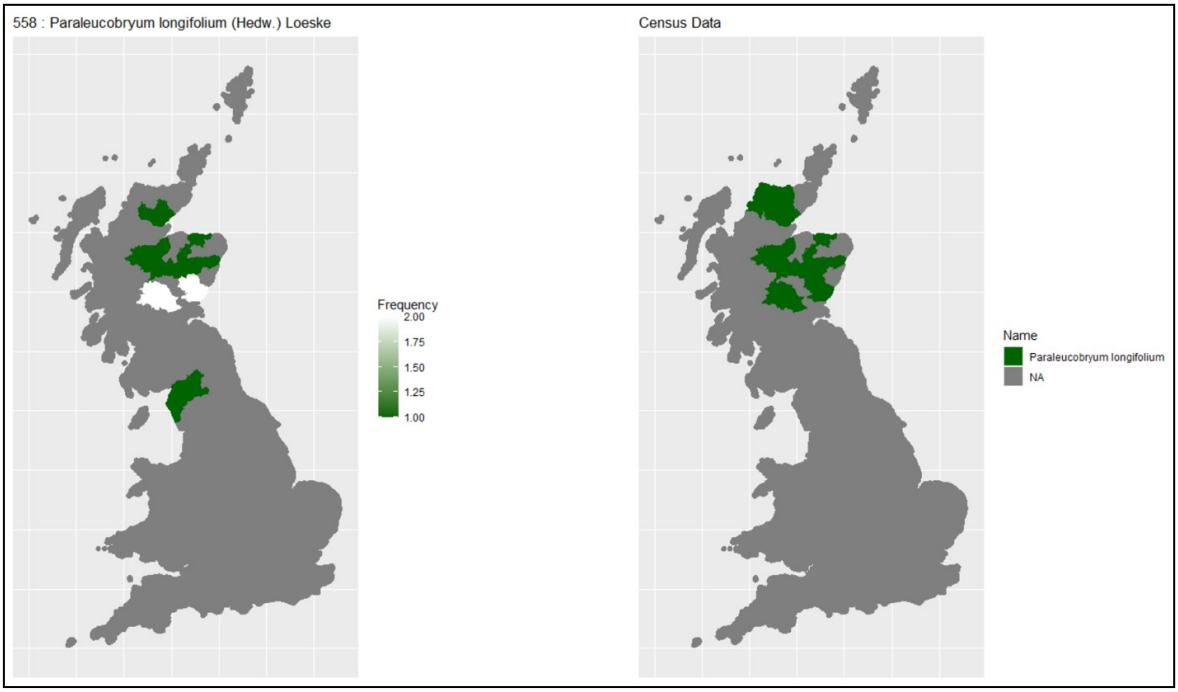


Figure 23. The vice county data for Paraleucobryum longifolium *(NMW C97.12.161). The image on the left shows the distribution from the herbarium data: vice counties 70, 88, 90, 92, 94, 96, 107. The image on the right shows the British Bryological Society 2021 Census Catalogue (Blockeel* et al., *2021b) distribution: vice counties 88, 90, 92, 94, 96, 107, 108.*

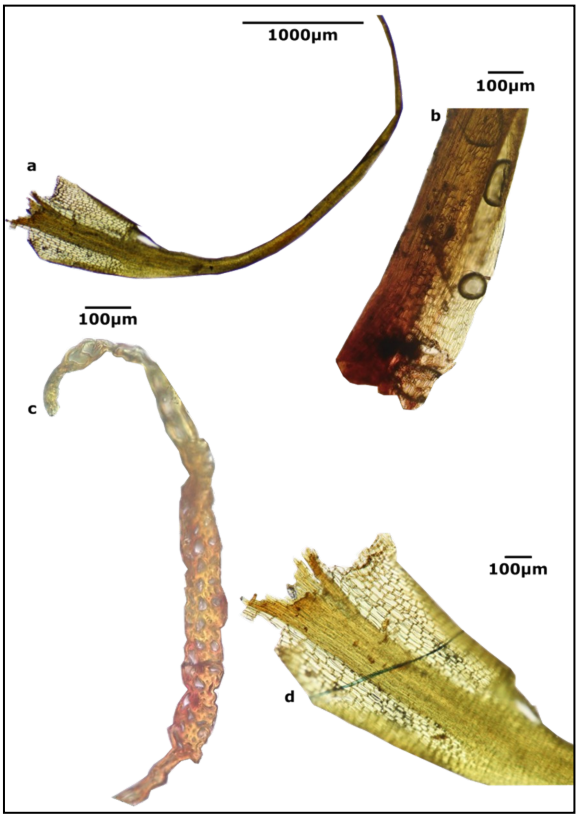


Figure 24. Microscopic images of NMW C97.12.161. a: leaf shape showing wide base and long tapering acumen, nerve less than 1/3 width of leaf. b: red-brown colouring of auricles. c: transverse section of leaf showing small cells in middle with thick cell walls (yellow in colour). d: rectangular basal leaf cells. Leaf characteristics similar to that of Campylopus flexuosus.

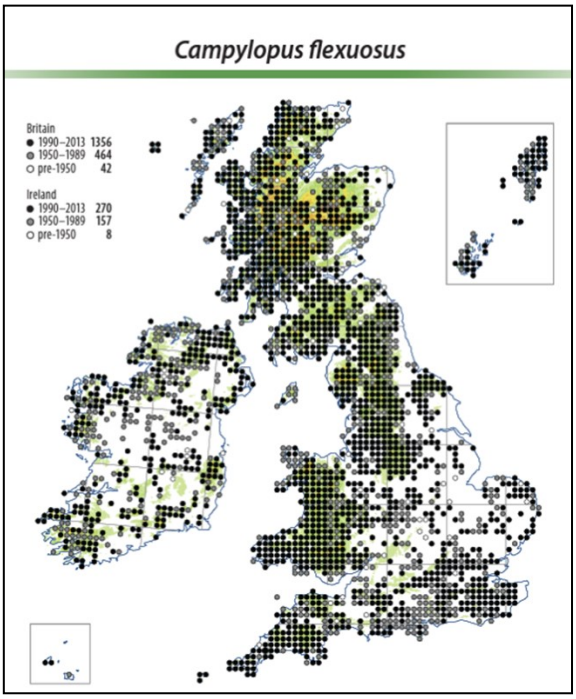


Figure 25. The distribution of Campylopus flexuosus *from the Atlas of British Bryophytes (Blockeel* et al., *2014). This species has a wide distribution including VC 70 where NMW C97.12.161 was found.*

10. *Plagiothecium platyphyllum*

(NMW C.2000.020.28)

This specimen of *Plagiothecium platyphyllum* was found in VC 45 (Pembrokeshire) which is not recorded in the census data (Figure 26). This is a nationally scarce species (Preston, 2006) which can be found in a variety of wet habitats such as springs, rock crevices or by waterfalls in higher altitudes (480 – 870 m).

From microscopic inspection this specimen was found to be in the *Plagiothecium denticulatum* (Hedw.) Schimp. complex. This specimen has an asymmetrical, ovate-lanceolate leaf shape, elongated leaf cells and a double short nerve (Figure 27). *Plagiothecium platyphyllum* is also sharply denticulate near the apex and abruptly tapers to an acumen (Smith, 2004) which is not seen in this specimen. The double nerve of *P. denticulatum* is longer than that of *P. platyphyllum*. *Plagiothecium denticulatum* var. *denticulatum* has a wide distribution that covers Pembrokeshire where this specimen was found and is the more likely variety for this specimen to be (Figure 28) having an acute leaf shape more similar to this specimen.

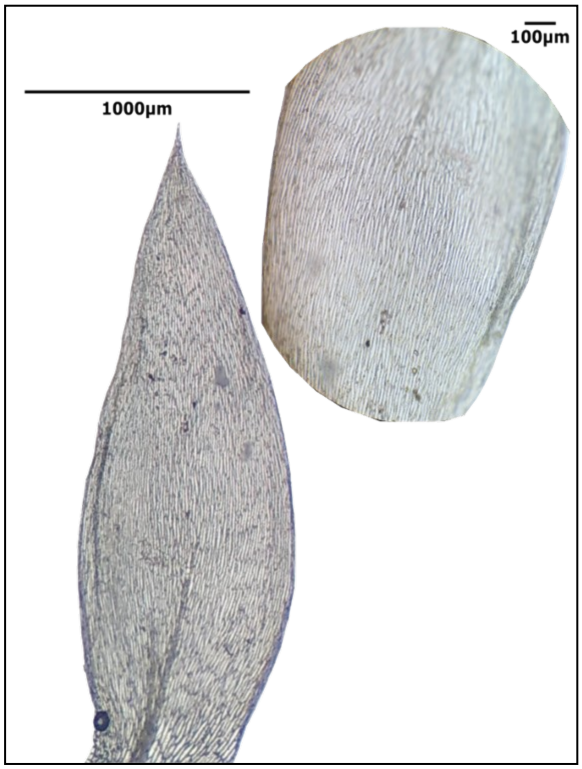


Figure 27. Microscopic images of NMW C.2000.020.28. The image on the left shows the ovate-lanceolate, asymmetrical leaf shape and double short nerve similar to that of Plagiothecium denticulatum. *The image on the right shows the elongated*

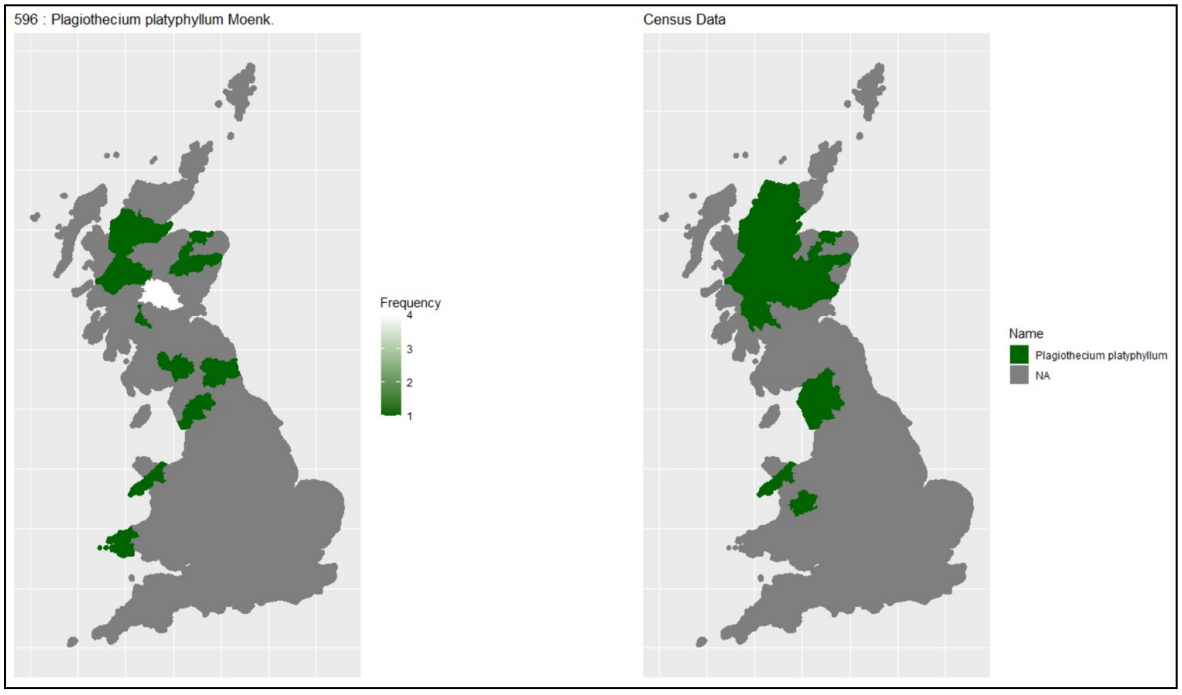


Figure 26. The vice county data for Plagiothecium platyphyllum. *The image on the left shows the distribution from the herbarium data: vice counties 45, 49, 67, 69, 72, 88, 92, 94, 97, 99, 105, 106. The image on the right shows the British Bryological Society 2021 Census Catalogue (Blockeel* et al., *2021b) distribution: vice counties 47, 49, (69), 70, 88, 89, (90), 92, 94, 96 – 99, 105 – 108. Bracketed vice counties are those that have not been observed since 1969.*

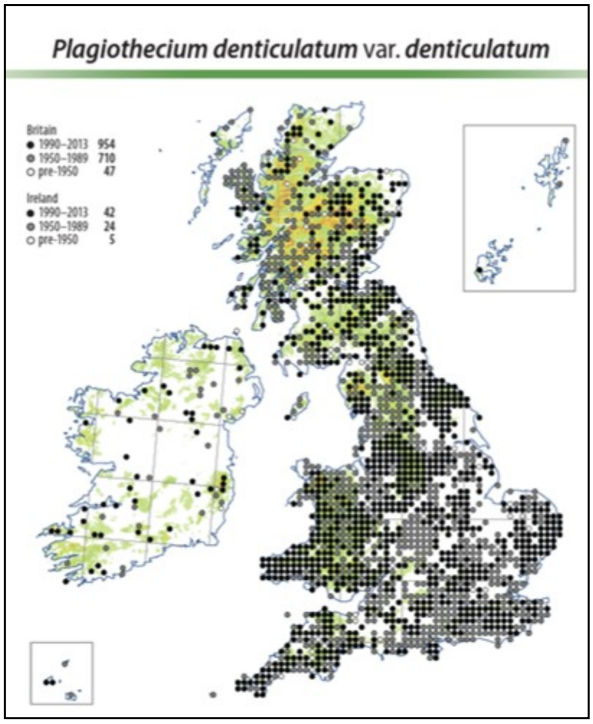


Figure 28. Plagiothecium denticulatum *var.* denticulatum *has a wide distribution including VC 45. This variety of P. denticulatum is more common than* Plagiothecium denticulatum *var.* obtusifolium *which is found in higher altitudes and is not found in Pembrokeshire.*

An overview of the results can be found in Table 2 showing that 70% of the species had specimens that had been misidentified. This can be broken down into 7 specimens requiring reidentification, 53 specimens requiring further work beyond the scope of this study and 1 specimen which had the correct identification.

Table 2. An overview of the top 10 most suspicious species flagged and reviewed and the number of specimens for that species that were suspicious and assessed microscopically. The reidentification column shows new identifications or explanations if no new identifications.

Species	Number of Specimens	Reidentification
Neckera pennata Hedw.	1	*Neckera complanata* (Hedw.) Huebener.
Pseudocampylium radicale (P. Beauv.) Vanderpoorten	1	*Hygroamblystegium varium* (Hedw.) Mönk
Philonotis tomentella Molendo	20	Taxonomic confusion that requires work beyond the scope of this study.
Riccia crystallina L. emend Raddi	33	Fragile material that could not be reidentified during this study.
Cirriphyllum cirrosum (Schwaegr.) Grout	1	*Cirriphyllum piliferum* (Hedw.)
Aongstroemia longipes (Sommerf.) Bruch & Schimp.	1	*Ditrichum plumbicola* Crundw
Heterocladiella dimorpha (Brid.) Ignatov & Fedosov	1	*Heterocladium flaccidum* (Schimp.) A.J.E. Sm.
Homomallium incurvatum (Brid.) Loeske	1	Correct identification
Paraleucobryum longifolium (Hedw.) Loeske	1	*Campylopus flexuosus* (Hedw.) Brid.
Plagiothecium platyphyllum Moenk.	1	*Plagiothecium denticulatum* (Hedw.) Schimp. complex.

Discussion

The list of suspicious specimens produced in this case study, particularly for the mosses, shows that the R package is able to detect specimens that have been misidentified, misnamed, or which have been left behind in changes of taxonomy (particularly in those resulting in splitting of a species into two or more distinct species). 70% of species reviewed in this study had specimens that were misidentified. Of the 10 species (totalling 61 specimens) determined as the most suspicious: 7 specimens were redetermined; 1 specimen was a new regional record not incorporated in the reference dataset for an endangered Red Listed species and 2 species (53 specimens) showed taxonomic reassessment was required. This was only a small subset of the possible species that could be reviewed due to time constraints and although only 11% of specimens assessed were able to be reidentified, 87% of specimens showed potential misnaming that was not possible to be rectified in the study. The only specimen to be correctly identified had been collected in a vice county not included in published data. This proves not only the effectiveness of the tool in identifying specimens labelled with incorrect or outdated specimen labels but also highlights its potential for identifying new vice county records held in collections and opens up further research possibilities of the tool into investigating and evaluating a species' distribution. For example, the specimen NMW C96.16.259 was redetermined as *Ditrichum plumbicola* and thus becomes a record from before the species was described. Even within a vice county where the species has been recorded before, the new record may have been found in a locality that is new within that vice county and can help further understanding of a species ecology and conservation needs.

Bryophytes are an understudied group being part of the 'minority taxa' that receive smaller research interest relative to their abundance (Smith, 2020). Along with other groups such as fungi, lichens, and algae, they can easily be subjected to errors especially as some species require identification microscopically (Atherton *et al*., 2010) and sometimes are only distinguishable from one another if certain morphological features are present. The case study presented here shows that bryophyte specimens had been misidentified and that some groups are difficult to reidentify without expert knowledge and time. However, they are an important group of plants that play a key role in habitat creation and improving biodiversity as well as being indicators of climate change, particularly through assessing changes in their distribution (Gignac, 2001).

The tool presented here has uses beyond bryophytes and can be used to review data for other areas of research that rely on correct identification of specimens and samples and reliable provenances. Thus, it is hoped that it will be an important tool to verify specimen data before it is shared online, particularly because whilst collections are becoming more available online, publicly available data has often been shown to be inaccurate. For example, the fungal sequences deposited in GenBank have been shown to contain a high number of misidentified taxa (Hofstetter *et al*., 2019). For the Agaricomycotina analysed in the study, it was found that around 30% of the fungal sequences in the database were misidentified. Correcting these mistakes in collection databases will ensure a higher quality and reliability of research that uses this data. The tool also has the potential to identify fraudulent records, such as those occurred in the case of Prof. John William Heslop Harrison, who purposefully and deliberately engaged in the collection and recording of specimens that he has planted on the Isle of Rum (VC 104) (Pearman and Walker, 2004).

Furthermore, providing a collection of data which is as accurate as possible is important for studies on how a changing climate is affecting species as well as research into biodiversity loss. Analysing changes in species distributions can be an effective tool, however if the data is formed from misidentified specimens this can both increase and decrease a species' distribution (Costa *et al*., 2015). Producing such distributions can show potential biodiversity hotspots as well as areas where biodiversity is low or areas where more data should be collected (Mannino *et al*., 2020; Meier and Dikow, 2004). For rarer species, distributions can be misleading as these species are more likely to be misidentified (Aubry *et al*., 2017). Species that are more common are less likely to be collected than rarer species and from areas that are easier to collect from which results in a spatial bias (Costa *et al*., 2015; Isaac and Pocock, 2015). The analysis of records presented here also shows the importance of having collections of specimens. Without this evidence, identifications could not be reassessed, biological records could not be updated and finding new regional records would not be possible.

Whilst it has already been suggested that specimen identifications are checked before research is carried out (Kitchener *et al*., 2020), it can be time-

consuming particularly through the need to systematically check collections especially for larger datasets. For example, in a study by Kauserud *et al.* (2008), around 35,000 fungi specimen records were used. It would not be possible to verify the identification for all these records. Similarly, for bryophytes and lichens, this time commitment is particularly high given the need for microscopic identification that precludes automation such as automated image identification tools (Shirai *et al*., 2022), which was able to both select and correct misidentified specimens. However, Shirai *et al*. (2022) only used vascular plant specimens showing further that minority taxa are often forgotten in studies. The tool presented here presents a time-saving procedure to identify samples likely to be misidentified for further reassessment which doesn't rely on photographically identifiable macromorphological changes. The R Package can assess thousands of records at once and only those chosen are reviewed in person. Such a process is only limited by computer power and identification abilities.

The R Package presented here can be used on data of all sizes from collections and observation records of different organisms to find a selection of specimens with a high likelihood of being misidentified or misnamed, as well as detecting new vice county records. This package provides a tool for quick assessment of records which can be evaluated for importance of investigation. As the majority of the specimens reidentified were nationally scarce species, it further highlights the wider potential applications of this tool in informing species conservation measures and wider ecological policy.

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Herbarium specimens: is there a best approach to mount dried plant specimens?

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Abstract

The basic principles for attaching dried pressed plant specimens to a mounting medium have not changed significantly since the 16th century when the first specimens were made. However, a wide range of variations in the practice of plant mounting can be seen today. Based on a survey, the three most common methods are totally adhered, partially adhered and strapped. We evaluated the robustness and efficiency of these approaches, alongside the un-mounted approach, by sending a set of test specimens of vascular plants (seed plants and ferns) on loan to expose them to mechanical risks and recording both failures of the mounting technique and damage to the specimen. In light of the results of this study, we analyzed their stability and suitability for maintaining the material useful for yet unforeseen studies (which can go beyond genetic studies). The present study can help towards determining what might be considered best practices (approach) to mount dried plant specimens, aiming to use a less/or a non-invasive mounting technique.

Keywords: botanical specimens, collections, dried plants, herbarium, plant mounting, preservation.

Introduction

The world's herbaria contain specimens that have been prepared, maintained, and curated for 400 years. According to *Index Herbariorum* there are ca 3095 active herbaria today that collectively are estimated to house more than 396 million specimens (Thiers, 2023). These herbaria provide a vast, distributed resource of specimens that are not only the physical evidence of species occurrences in place and time but that also provide resources of DNA, and associated organisms together with information about cultural heritage and history. Herbarium specimens can help to answer a plethora of questions across disciplines, from conservation to climate change, domestication, and colonial history, though taxonomy remains at the heart of the research using these collections (Carine, *et al.*, 2018; Funk, 2003; Heberling and Isaac, 2017; James, *et al.,* 2018; Lang, *et al.,* 2019; Schindel and Cook, 2018).

The origins of the approach of dried plants being attached to paper can be traced to at least Luca Ghini (1490 – 1556) in the late 15th or early 16th century (Pavord, 2005). However, a range of mounting techniques have evolved and been used over past centuries.

Bridson and Forman (1989) examined two approaches for mounting, namely 'strapping' (the 'straps' being thread, linen tape, archival self-adhesive tape or plastic glue) and 'overall gluing'.

We use the term 'adhered' since most adhesives used in herbaria today are not animal-derived glues but synthetic adhesives.

They focused on the pros and cons of these techniques in terms of stability and the risks of physical damage to the specimens as objects and noted that specimens are susceptible to damage if they are strapped, while adhering reduces damage, and gives them better long-term protection. However, as Yesilyurt (2009) noted that the mounting technique adopted may impact on longevity not only the physical integrity of herbarium specimens but also their value as a scientific resource, given the range of questions they are now used to address (e.g. Gutaker, *et al,* 2019).

In contrast to Bridson and Forman (1989), Grenda-Kurmanow (2021) suggested that the total adhesion of specimens could contribute to their degradation on many levels while Heberling and Isaac (2017) also highlighted the impact of mounting method on the scientific value of the specimens, not least for future, unanticipated uses of herbarium specimens. They concluded that further consideration of the techniques used for mounting specimens is needed to ensure that their scientific value is not compromised.

Aims of this project

Given the importance of mounting technique used for ensuring the long-term preservation of herbarium specimens, both as physical objects and as a scientific resource, this paper has two main aims. First, we aimed to gauge the range of plant mounting techniques in use worldwide.

Second, we tested the hypothesis that a herbarium specimen would suffer greater damage if not well attached ('overall gluing' of Bridson and Forman, 1989) through assessing the damage to specimens included in test loans to institutions to both UK and international locations when specimens that were totally adhered, partially adhered, strapped or un-mounted were dispatched through the post. Finally, we consider the pros and cons of these plant mounting methods, considering both the scientific sustainability of a specimen, protecting its functionality for unanticipated applications and uses, as well as its physical endurance.

Material and Methods

2.1 Herbarium mounting techniques worldwide

To document the mounting techniques used in herbaria worldwide, a questionnaire was sent via e-mail to 175 institutions from 46 different countries in which they were asked about the technique used to mount dried plant specimens. Each institution was additionally asked to provide information on the number of plant mounters and volunteers; the number of working hours; the number of specimens mounted; whether or not the specimens were pressed after adhering, and if so, what it was; the mass of the object/s used to press the specimens and the pressing duration; the adhesives used; mode of application of the adhesive; how long the institution had used the adopted technique; and whether or not institutions sent specimens on loan. Only the data to gauge the range of plant mounting techniques in use worldwide is presented here. The survey was conducted in 2009.

Information on mounting techniques used by other herbaria was also gathered from specimens loaned to Natural History Museum (NHM here and after) for taxonomic research undertaken by Yesilyurt (2004).

2.2. Testing the robustness of methods used to mount dried plant specimens, when sent in transit ('loan-exercise' experiment)

To test the robustness of herbarium specimens in transit, we prepared specimens, using four different approaches, which were sent to five institutions. The selected approaches were: unmounted specimens (leaving them loose inside species covers, made from paper); partially adhered (mounted by applying adhesive in some key point areas of the specimen); totally adhered (mounted by applying adhesive all over the surface of the specimen) and strapped (securing the specimens by adding straps in some parts of the specimen). Many of the specimens selected for the study were particularly vulnerable to mechanical damage such as ferns that were overdried (specimens that have been exposed to (high) heat for long period during the drying process, resulting into a very dark brown to sometimes black colour, e.g. Fig. 7B and 7E), and brittle and very fragile or plants with leaves with long petioles. A number of open three-dimensional fruit specimens were also chosen to be part of the experiment. For some specimens mounted using the strapping approach, we applied straps to areas such as the tips of leaves to investigate the impact of strapping in this way since this approach has been used in the past at the NHM.

A range of adhesives and straps were used in the present study. However, since previous studies (e.g. Croat, 1978; Clark, 1986; Grenda-

Fig 1: Examples of the specimens prepared for the sets.
A: sample of two sets of collections, illustrating that the specimens were, whenever possible, either the same or with similar structures and/or type of plants (Image: J.C. Yesilyurt, 2022).
B: examples of ferns and climbers (Image: J. Jackson, Natural History Museum, Photo Unit, 2024).

Kurmanow, 2021) have examined the use of different adhesives, the present paper will be focusing on the results for subset of the specimens that were mounted using one of two types of adhesives that have been used at the NHM (Polyvinyl Acetate (PVA hereafter) and Methylcellulose (MC hereafter) and of the two types of straps that also have been used at the NHM ('Gunned linen tape' (S-I hereafter) and 'SUALTC 7150' (S-II hereafter)). However, overall figures of the adhesives used during the study will be provided to contextualise the outcomes discussed here. PVA has been used at NHM for mounting specimens during for at least the past 49 years, while MC has been occasionally for repairs. For strapping, the NHM has used S-I for nearly 45 years and S-II for the past 20 years.

Six sets of specimens were prepared with one was retained at the NHM (herbarium acronym BM; acronyms follow Index Herbariorum: IH, here and after) as a control. Specimens were sent to the following herbaria: E (Edinburgh, UK), P (Paris, France), SPF (São Paulo, Brazil), MICH and US (both from United States of America). Acronyms for the herbaria follow the *Index Herbariorum* (IH, here and thereafter). These herbaria- were selected to represent a range of geographical distances from BM (London, UK). All institutions were consulted prior to taking part on the study and agreed to participate and follow the guidelines. Specimens were despatched using courier companies or mail and with label 'Fragile' attached to them.

Fig 2: Examples of the specimens prepared for the loan exercise, with printed copy of the photographs. A: examples of two unmounted specimens, inside flimsies; the herbarium sheet was removed to take this photograph to show the loose material and labels. B: example of mounted specimen. (Images: J. Jackson, Natural History Museum, Photo Unit, 2024).

Specimen sets were selected so that they would include both fragile and flimsy specimens, those that were brittle and those that have thick twigs and bulky structures (e.g. fruits/seeds). Wherever possible the sets were similar regarding the type of vouchers (Fig. 1), so that, each herbarium/ institution received a duplicate of the same type of dried plant specimen. Where this was not possible, herbaria received a specimen selected to have similar characteristics, and whenever possible, they were prepared with the same mounting method.

Each loan comprised between 41 and 49 specimens within which 6-10 were totally adhered, 8-11 were partially adhered, 21-28 were strapped and 2-3 were unmounted. In total, for the study 37 specimens sent on loan were totally adhered, 48 partially adhered, 127 strapped and 11 unmounted.

Specimens were placed inside thin, non-archival quality paper species covers (flimsies here and thereafter; Fig. 2A). Unmounted specimens (Fig. 2A) were placed inside flimsies with a herbarium sheet placed underneath. Photographs of the specimens taken soon after specimens being mounted were also included inside the flimsies, underneath the herbarium sheet (Fig. 2B), and these were used to allow recipients to assess and mark failures (of the materials) and damage (to the specimens) upon receipt.

Specimens were sent on loan as parcels wrapped in two layers of brown paper and similar packaging was requested for the return of the specimens. Cardboard or boxes, that are standard packaging for herbarium specimens, providing support, were not used so to maximise the expose of specimens to possible risk of damage during transit.

Recipients were asked to compare the specimen with the printed image on receipt and to annotate all damage on the printed image, including observation of debris, broken parts and/or lose fragments and any tears of the straps or failure of the adhesive they observed. The specimens were further scrutinised for damages and/or failures upon their return to BM. Changes to the specimen as a result of transit were categorised as *Failure* if the method or the material failed (this would include detachment of the specimen or detachment, tears or breakage of the straps) and *Damage* if the damage was to the specimen itself.

Results

3.1. Herbarium mounting techniques worldwide

83 of the 175 herbaria contacted responded to the survey. Herbaria from all regions defined by Thiers (2023, online) were represented with the exception of the Pacific. Information on a further 15 institutions was based on material loaned to Jovita C. Yesilyurt (JCY hereafter) for taxonomic revisionary work (Yesilyurt, 2004). Table 1, lists the herbaria by geographical region, based on Thiers (2023, online).

In total, 70% indicated that they use a single mounting method, with strapping being predominant (37%) over adhesion (33%). Sewing, stitching or pinning were grouped under strapping and adhesion included key-point adhesion method (Table 2).

Regional variations are evident from Table 2 (see also Fig. 3). Strapping is the most frequently used technique in Europe with a combination of methods also common and few herbaria only using adhesion. In contrast, none of the North American respondents used strapping as their sole (or main) technique and in the Caribbean and Central and South American region and most strikingly in central America (Fig. 3), adhesion was the most popular method among respondents.

Consistent with the survey data, observations on material loaned to Yesilyurt (2004) revealed that strapping was the commonest approach, although this was sometimes supported with stitches, especially in the bulkier parts of the specimens. A wide range of materials were considered strap mounted (e.g. commercial tapes, adhesive; see Fig. 4).

3.2. Testing the robustness of material sent in transit.

Of the 223 specimens sent on loan, 113 (51%) were affected with 64 specimens (29%) presenting a failure (the failure of the material which included detachment of the specimen; detachment, tears or breakage of the straps) and 49 (22%) showing damage to the specimen itself (Table 3).

Damages and failures were not observed on 54% (69 out of 127 specimens) under the strapped approach, 36% (4 out of 11) of unmounted specimens, 29% (14 out of 48) of partially adhered and 11% (4 out of 37) from totally adhered specimens.

Table 1: The distribution of herbaria for which information on mounting method was obtained by geographical region (after Thiers, 2023 [online]). Acronyms follow Index Herbariorum (IH)

Region	Number of herbaria listed in IH	Herbaria providing information (bold = collections loaned to JCY)	Data only from material loaned to JCY	Total number (percentage of regional herbaria surveyed)
Europe	828	34: AIX, BCN, BHUPM, **BR**, BRLU, **C**, CGE, CL, E, FR, GB, H, JE, **K**, KUO, **L**, LE, LEB, MAF, MSM, O, ORT, OXF, **P**, PC, PAL, **PI**, RO, **S**, TFC, TRH, TUR, UPS, WAG	8: B, BOLO, FI, G, M, PR, TCD, U	42 (5%)
Africa	179	4: BOL, EA, J, YA	0	4 (2.2%)
Temperate Asia	785	5: HUJ, IBCA, IBK, KUM, TI	1: PE	6 (0.8%)
Tropical Asia	212	6: **BO**, KEP, LAE, SAN, SING, VNM,	0	6 (2.8%)
Australia and New Zealand	48	6: BRI, CANB, CHR, HO, MEL, WELT	0	6 (12.5%)
Pacific	12	0	0	0 (0%)
North America	844	11: A, AMES, CAN, ECONN, FH, **GH**, **MO**, MT, NEBL, **NY**, QFA,	2: UC, US	13 (1.5%)
Caribbean, Central and South America	416	17: BBS, COL, CONC, CTES, EAP, FCQ, HAC, HULE, IBUG, IEB, INB, LAGU, PMA, SGO, **SPF**, UADY, UB	8: BHBC, FURB, GUA, MBM, OURP, PACA, RB, SP	21 (5.0%)

Failures have been higher under both strapped (36 specimens; 28%) and partially adhered (16 specimens; 33%) approaches compared to totally adhered (12 specimens; 32%).

Although unmounted specimens had the highest number of damages (7 specimens; 63%), among mounted approaches, damages were highest with the totally adhered specimens (16 specimens; 43%), followed by partially (9 specimens;19%) and strapped approaches (17 specimens; 13%). The highest number of specimens with both damages and failures have been recorded to partially adhered (9 specimens;19%), followed by totally adhered (5 specimens; 13%) and strapped (5 specimens; 4%) approaches (Table 3).

Failures were recorded on 15 (7%) specimens following their outward journey and 20 (9%) following their inward journey; none of them were on the same specimen. Damages were recorded on 24 specimens (11%) following their outward journey and 31 (14%) following their inward journey. Damages were reported for the five specimens (2%) following both journeys. One of them was unmounted, two were partially adhered and two strapped mounted. Damages included broken petiole, tips of the leaves and/or fruits.

Damages recorded using the strapped method were largely observed on those areas where the straps have been attached, particularly on/and or near the tips of the leaves (Fig. 5), followed by the petioles (of the leaves, especially if these have also been strapped). We observed that bulky or raised structures (i.e. fruits, thick twigs, bulky inflorescences) were also susceptible to damage (Fig. 6A, B).

Of 14 specimens mounted using PVA, (six totally adhered and eight partially adhered), three failed and one totally adhered specimen presented damages. Of the ten specimens mounted using MC (five each totally and partially adhered), there were two failures under each method but no damages. S-I was used to mount 28 specimens and S-II used to mount 12. Failure was observed using both strap types (S-I had two failures and S-II, one). Three specimens were damaged when mounted using S-I while there was only one damaged specimen mounted using S-II.

Table 2: The number of herbaria using strapped, adhered or a combination of approaches by region (following Thiers (2023 [online]). Results are based on the survey.

Geographical region	Strapped	Adhered (including partially adhered)	Combinations of methods
Europe	16	5	13
Africa	1	1	2
Temperate Asia	2	3	0
Tropical Asia	1	4	1
Australia and New Zealand	4	1	1
Pacific	0	0	0
North America	0	4	7
Caribbean, Central and South America	7	9	1
Total (percentage)	31 (37%)	27 (33%)	25 (30%)

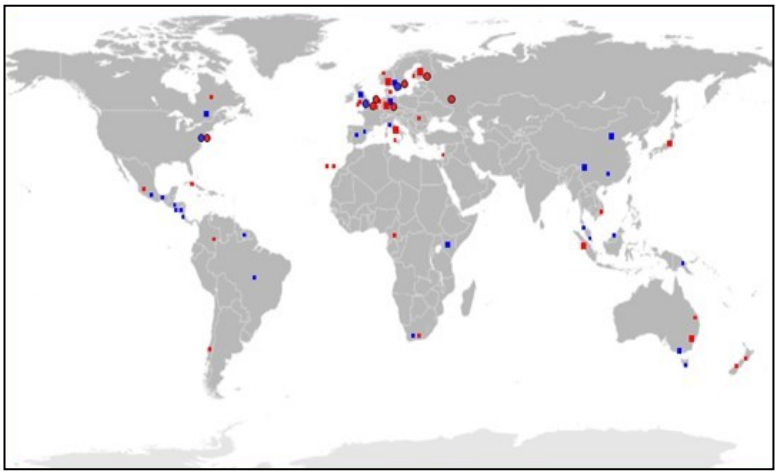

Fig 3: World map showing the distribution of plant mounting approaches displayed into two main techniques: 'strapping' and 'adhesion'. Red circles represent those herbaria that use strapping, sewing/stitching, pinning and/or combinations of one of these methods to mount the specimens. Blue squares represent those herbaria that totally or partially adhere specimens have been adopted. Size of the circles and squares, represents the size of the collection of each institution (based on the survey and data (from Thiers, [online]) gathered on that time: 2009).

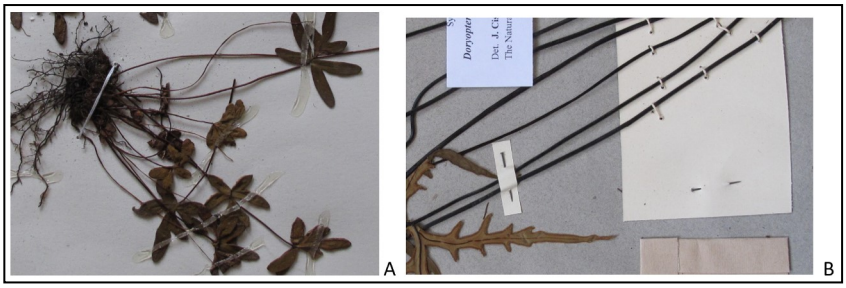

Fig 4: Examples of the strapped method. A: 'straps' have been made of adhesive; rhizome has been sewn. B: straps made of paper attached by pins; petioles being stitched on the paper that has been pinned on the sheet. Both images (from specimens sent on loan to Yesilyurt,2004), are of the fern genus Doryopteris. (Images: J.C. Yesilyurt, 2004).

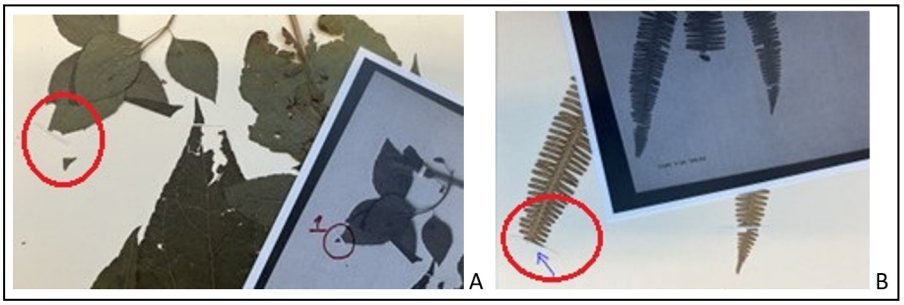

Fig 5: Examples of strap-mounted specimens, demonstrating the damages that occurred as a result of the common practice for strapping specimens at the tip of leaves. A: seed plant specimen; B: fern specimen. (Images: J.C. Yesilyurt, 2022).

Discussion

This paper had two main aims. First, to examine the plant mounting techniques used in herbaria worldwide and second to test the robustness of specimens prepared using different mounting methods through an experiment involving the loan of specimens to a number of different institutions and assessing damage to specimens and material failure of the mounting technique arising from that.

3.1. Herbarium mounting techniques worldwide

The survey suggested that, at a global scale, the proportion of herbaria using strapping (37%), adhesion (33%) and mixed approaches (30%) were broadly similar although at a regional level, there was variation, with the strapped method most common among respondents from Europe whilst in the Americas, the adhered method was predominant. It is interesting to note that while strapping was the most common method among European herbaria, the oldest herbaria, located in Europe, such as those of C. Bauhin (1560 – 1624, Herbarium Basel); U. Aldrovandi (1522 -1625, Bologna University); L. Rauwolf (1535? – 1563, Naturalis Biodiversity Centre); and H. Sloane (1660 – 1753, Natural History Museum) are often totally adhered and there has therefore been a shift in the approach adopted through time. Regional patterns, including the use of adhesion among all but one of the herbaria from Central America for which information was obtained, may reflect the impact of training courses and skills sharing between institutions across a region although it should be borne in mind that within

Table 3: The number of herbaria using strapped, adhered or a combination of approaches by region (following Thiers (2023 [online]). Results are based on the survey.

Method	Total number of specimens	Number of specimens with no observed damage or failure (percentage)	Number of specimens damaged (percentage)	Number specimens with failures (percentage)	Number of specimens with failure and damage (percentage)
Strapped	127	69 (54%)	17 (13%)	36 (28%)	5 (4%)
Partially adhered	48	14 (29%)	9 (19%)	16 (33%)	9 (19%)
Totally adhered	37	4 (11%)	16 (43%)	12 (32%)	5 (14%)
Unmounted	11	4 (36%)	7 (64%)	N/A	N/A
Total	**223**	**91 (41%)**	**49 (22%)**	**64 (29%)**	**19 (9%)**

'adhered' approach, a spectrum exists and the survey results did not seek to differentiate between partially and fully adhered.

3.2. Testing the robustness of material sent in transit ("loan exercise" experiment)

3.2.a. Past studies and the materials used (adhesives and straps)

A number of studies have examined mounting methods with most focused on the performance of the adhesives (e.g. Croat, 1978; Clark, 1986; Grenda-Kurmanow, 2021) and physical risks to the specimens. Croat (1978) raised concerns over the use of adhesives to mount plant specimens. Based on a study of mounting methods across 70 herbaria in the USA, Croat (1978) concluded that strapping the specimens would be better and faster than the total adhesion approach. Egenberg and Moe (1991) reviewed the mounting techniques adopted in four Scandinavian herbaria and similarly concluded that strapping was less time-consuming than applying 'dots of adhesive' to the specimen. The authors also argued that direct gluing should in general be avoided, since straps give the specimen a chance to move without exposing them to stress, although at the National Herbarium of Canada (CAN) have been adhering specimens in order to ensure that they could withstand manual handling for a longer period, Shchepanek (2001) noted that the 'linen strips', used at CAN during the first part of this century, continue to provide excellent durability and protection for specimens. In contrast, Bridson and Forman (1989) stated that specimens would be susceptible to damage if they are strapped, while under the total adhering approach, the damage would be reduced, giving much long-term protection to the specimens.

Grenda-Kurmanow (2021) recently identified three adhesives as particularly suitable for mounting dried plant specimens that are also used in paper conservation (e.g. Borges *et al.,* 2018) and that are therefore considered conservation-approved, namely MC, wheat starch paste, and isinglass. The use of wheat starch paste or isinglass may be problematic due to the possibility of material contamination (e.g. by DNA) since they are of plant origin; they also pose a potential increase in the threat from pests.

We were interested in comparing the performance of PVA and MC, given that both have been used at the NHM. Damages and/or failures mounted using MC were observed mostly for

raised or bulkier specimens. This may be because it can be difficult to create a bond between the surfaces with the adhesive (Clark, 1986; Tillet, 1989). However, MC is known for being readily reversible (and more so than PVA), and for this reason it is widely used in conservation and preservation, particularly for botanical collections. If MC is used, it may be advisable to incorporate extra support (e.g. sewing or adding straps) on key areas of the specimen, particularly if they are raised or bulkier.

In the present study, five specimens were mounted (partially and totally adhered approaches) using MC. While failure was recorded for two specimens under each mounting approach, damages were not observed. The failures were on those specimens with raised and/or bulkier areas.

While other studies have investigated adhesives used for mounting botanical specimens, ours also investigated straps. This is, despite the fact that the strapping method is considered in several studies to be one of the best options. The only statement about the performance of straps was by Shchepanek (2021). If strapping is used, good quality straps, such linen-based straps should be used. Consideration should also be paid to where straps should be added on the specimen. For example, the tips of leaves should be avoided (see below for further discussion).

3.2.b. The robustness of the mounting methods through the loan exercise

As anticipated, in our experimental loan, unmounted specimens presented the highest level of damage; nearly two thirds of specimens were damaged in contrast to levels of damage between 13-43% for other methods.

In contrast with the suggestion of Bridson and Forman (1989), our experimental loan results suggested that the 'totally adhered' method does not prevent damages to specimens. Indeed, only 11% of totally adhered specimens in the study showed no damage or failures during the exercise, in contrast to the 54% of specimens prepared using strapping that were returned in good condition. Full adhesion exposes specimens to much higher stresses, which may result in damages. From the perspective of minimizing risk of physical damage to specimens, our results are consistent with the support for the strapping method suggested by Croat (1978), Egenberg and Moe (1991) and Shchepanek (2001).

It should be noted that in this study, all damages were considered equally. We did not attempt to score damages by severity, size or impact on the specimen: a split on a single petiole, damage to several leaves, the fracture of the fruit (Fig. 6) even though some may be more impactful.

We would also note, however, that damages recorded under the strapped method may have been inflated since the majority of damages recorded were to the tips of leaves (Fig. 5) and to petioles. At the NHM (and also in other herbaria), specimens have sometimes been strapped at the tips of the leaves. These are among the most fragile points of the specimen, and we included specimens prepared in this way in our study to test the assumption that they are fragile points of the specimen. These damages could be considered

Fig 6: Example of specimens with bulky fruit, mounted under strapping approach. A: specimen with fruit half lost with the petiole; B: fruit broken in half, leaf tips damaged; C: specimen with no damages despite failure of the straps (detached or too loose). (Image: J. Jackson, Natural History Museum, Photo Unit, 2024).

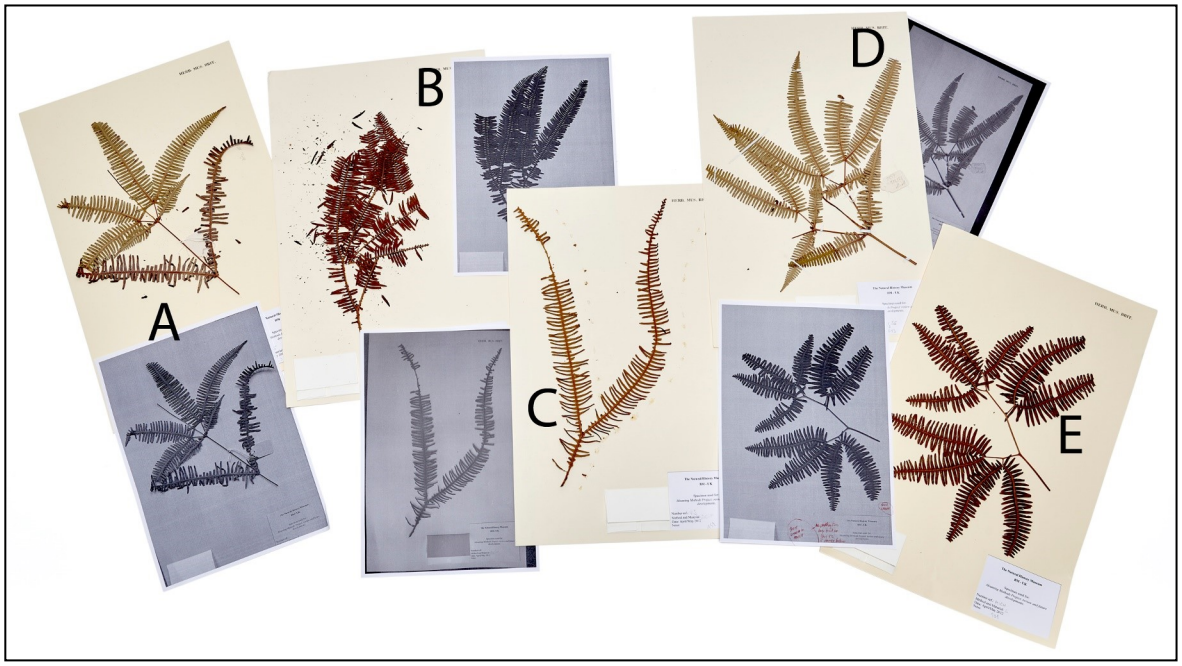

Fig 7: Fern specimens prepared for the loan exercise. A: unmounted, damages recorded to some areas of the leaves; B: overdried specimen, unmounted, specimen severely damaged; C: partially adhered, method failed (specimen detached, as it can be seen the dots of adhesive), no damages recorded to the specimen; D: strapped, method failed (some straps detached), no damages recorded to the specimen; E: overdried, unmounted, no damages recorded to the specimen. (Image: J. Jackson, Natural History Museum, Photo Unit, 2024).

to have resulted from the misuse of the mounting approach rather than the approach itself. The number of damages to strapped specimens would likely have been reduced if the straps have been applied in more appropriate locations on the specimens such as on more robust areas rather or in the middle of long petiole or close to their intersection. This may be particularly true for bulky specimens. Three specimens with bulky fruits were included in the set of strapped specimens in our loan experiment and two were damaged (Fig. 6A, B), a much higher level of damage than overall for this method (13%).

It should also be noted that many of the specimens selected for the study were particularly vulnerable to mechanical damage notably ferns that were overdried, brittle and very fragile and that this may also have increased the levels of damage observed (Fig.7).

3.2.c. Further observations

The opportunity of observing ca 5000 fern specimens from 30 herbaria for *Doryopteris* during the course of revisionary work by JCY also gave insights on how specimens behaved under different mounting methods, including specimens loaned unmounted. *Doryopteris* specimens have naturally a brittle nature, particularly in the petioles that are long and which can break easily even when freshly collected. Moreover, specimens that have been dried for too long or too quickly may be very fragile. Remarkably, among the *Doryopteris* collections that were loaned to BM, those that were unmounted (observed from three herbaria) showed very little damage, and where it did occur, it was typically only to the petiole. While this is at odds with our experimental loan results, physical damage to the specimen is not the only risk that unmounted specimens present since they are also susceptible to other risks such as the dissociation of specimens from labels. An interesting point to make is the comparison of material used (same fern species), where some specimens have been overdried. The experimental loan has shown that those specimens that were overdried, were the ones that suffered most impactful damages (see Fig. 7B) as others, despite also been unmounted, did not present damages (Fig. 7A), including when the method failed, and they have returned loose (Fig. 7C, D). Overdrying seems to be a plausible explanation of their susceptibility to extensive damage/breakage. The damages observed from other loose specimens (unmounted specimens) sent on loan exercise, were much smaller or less impactful (e.g. one leaf was detached, fragile tips of a plant were broken, or a few flowers from the inflorescence detached).

From the loans to JCY, specimens with bulky parts, which were totally or partially adhered or strapped, often had extra re-enforcement by straps, stitches, or even both and while these do not always spare the specimens from damages, the use of additional support needs careful consideration on a case-by-case basis to minimise specimen damage. It should also be noted that sometimes damage resulted from the detachment of straps, leaving the specimen loose in certain areas, which made them prone to move and friction. If strapping is used, the straps must be used correctly, tight to the specimen so they are holding and securing the specimen to prevent movement.

It should also be noted that many of the specimens selected for the study were particularly vulnerable to mechanical damage notably ferns that were overdried, brittle and very fragile and that this may also have increased the levels of damage observed (Fig. 7B). They may be stabilised if totally adhered however, one will need to bear in mind the consequences of this approach, for these kind of material/specimens as they will not be reversible and if so, it will be in several small pieces. Nevertheless, these caveats do not detract from our key finding that strapped specimens experienced fewer damages and failures than those that were adhered.

3.3. Herbarium specimens: is there a best approach to mount dried plant specimens?

A consideration of mounting methods at this time may appear unnecessary, since digitisation efforts, are increasingly making herbaria virtual and digitally available, across the world (e.g. Soltis 2017; Soltis, Nelson and James, 2018), and this already appears to be impacting on the number of requests for loans (e.g. Holstein, 2019). Physical damage through loans may therefore be less likely to occur in the future.

Nevertheless, specimens will still be used and they do still need to be conserved to a high standard. In addition to the support from strapping from our loan experiment, the approach also has other advantages over other mounting techniques. First of all, it is less resource-intensive since it is both faster and easier to strap mount specimens (albeit with skill and expertise still needed as noted above). Strapping also provides the stability needed but is less invasive than other methods and is easier to reverse. Herbaria are increasingly attracting new users undertaking innovative research addressing a wide range of questions and societal issues using the specimens they contain (Carine *et al.*, 2018; Davis, 2023) and both current and potential uses in the future need to be considered.

As the results of our survey revealed, the way in which herbarium specimens are mounted is varied. In the herbarium of the future, the needs of the diverse range of users of botanical specimens are likely to best served by mounting techniques such as strapping that, as our loan experiment suggests, are successful in preventing physical damage while also maximising flexibility in their uses in the future. Similarly, sewing can also be a good option though it would be time consuming and, probably more expensive, as a result.

The findings from the present study hopefully can help towards re-evaluation of the best practices for botanical collections, more precisely, on 'mounting' the vascular plants (i.e. seed plants and ferns) and aim for a non-invasive mounting technique/s, or to at least a less- invasive approach. One could argue that no method would be totally satisfactory, and, in some cases, it might be that more than one approach could work better for certain specimens, to have a stable and sustainable herbarium specimen.

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Author contributions: project design, data tabulation and interpretation of results, writing and discussion, JCY; review of results, writing and discussion, MC; preparation and mounting of the specimens, photographs of the specimens, JCY and FDS. All authors have read and agreed to the publish version of the manuscript.

Conflicts of interest: the authors have declared there are no conflicts of interests.

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Re-curation of the seed plants fluid collection at the Natural History Museum

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Abstract

Over three thousand seed plants fluid collections stored at the Natural History Museum have recently been re-curated. We have addressed the condition and conservation needs of the specimens by replacing broken jars/lids, rewriting labels and topping up the specimens with 70% IMS. The collections are from countries around the world representing 189 families and 1359 taxa (genus and species). There are 41 type specimens as well as some important orchids from the 1930s. An index to the collections has been compiled in an Excel spreadsheet and by making these collections accessible on the Museum's public data portal at (https://data.nhm.ac.uk/dataset/the-seed-plants-fluid-collection-at-the-natural-history-museum), we would like to draw attention to experts in various family groups such as Orchidaceae and families unknown where further taxonomic identifications would help us to determine the collections and enhance our floristic knowledge around the world.

Keywords: Biodiversity, Fruits, Formalin, Fluid preservation, Herbarium specimens, 70% IMS, Re-curation

Introduction

Preserving specimens in fluids is one of the methods of plant preservation (Davies *et al*. 2023). In wet forests, it is extremely useful to preserve specimens in fluids which could be dried later to prepare herbarium sheets. Specimens preserved in fluids help in studying 3D structure of a flower or fruit as compared to dried herbarium sheets. It is also useful for anatomical studies or botanical illustration. Our seed plants fluid collection consists of ca. 3,000 fruits, flowers and seedlings stored in glass jars and plastic bottles of diverse sizes. The collections are stored in an environmentally controlled room at 17 degrees with a relative humidity between 40-50% (cool storage environment maximises the life of fluid preserved specimens. Greater humidity than 65% raises risk of mould and change in preservation concentration. Other factors such as H & S seal to the door are in place to protect from risk of fire. Fire and smoke detectors as well as vapour detector systems are also in place (Collins, 2014)). The collection ranges from late 17th century to the current era and it continues to grow with material incorporated with recent acquisitions.

These collections were previously stored in various fluid mediums such as Formalin, 70% IMS (industrial methylated spirit), and some had unknown liquids. Many bottles have tiny labels of the old family/genus numbers as per Bentham and Hooker system of arrangement. These labels are in distinct colours based on the geographically coloured regions followed in the General Herbarium. (Fig. 1).

Fig. 1. Twenty-six geographical regions used for filing specimens in the folders in storage cabinets in the General Herbarium at NHM. The labels on the folders follow the colours for the countries respectively. These labels are also found on the jars in the fluid collections with family/genus numbers (as per Bentham and Hooker system of arrangement).

Although small, this is an important collection representing different families with some important type specimens. We also have the lectotype of the world's largest flower- *Rafflesia arnoldii* R.Br. (BM001122243) collected by Arnold and Raffles (Fig. 2).

There are some important type specimens of orchids collected by C. E (Cedric Errol) Carr (1892 – 1936), a New Zealand botanist, specialising in orchids. In 1933 and 1934 he worked at the Kew Herbarium before travelling to Papua New Guinea, spending several years collecting there before his death in 1936 due to black water fever. After his death over 4,000 of his orchid collections and detailed descriptions of the specimens were given to the Singapore Herbarium (SING). We house some of Carr's specimens, and some of the duplicate sets of specimens are at ZE Botanischer Garten und Botanisches Museum, Freie Universität Berlin (B),

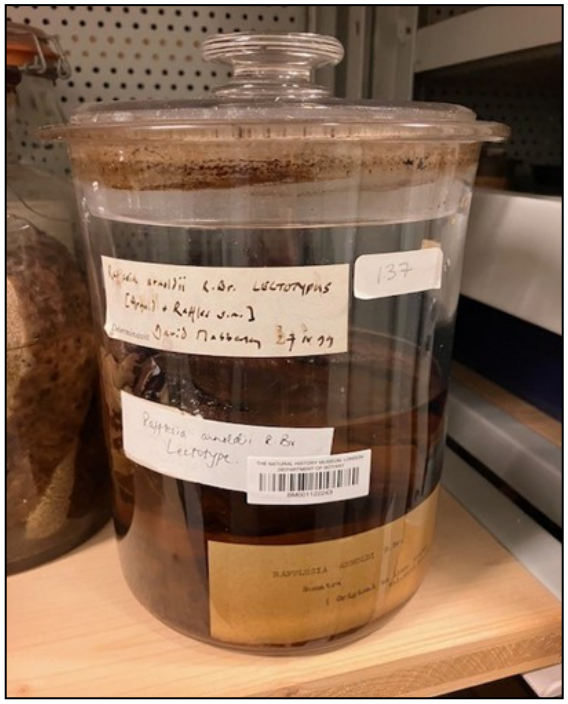

Fig. 2. Lectotype of Rafflesia arnoldii R.Br., (BM001122243) collected by Arnold & Raffles in Indonesia and determined by David Mabberley in 1999.

Naturalis Biodiversity

Center (L), The New York Botanical Garden (NY), and Australian National Herbarium (CANB). These collections are important and provide us knowledge of the flora from Southeast Asia.

There are some specimens which have corresponding herbarium specimens, however there are also some specimens for example *Utricularia gibba* L. (BM013786221) where although there is cross reference to herbarium specimen, there is no herbarium specimen in the General Herbarium, but a label is present with a note "see specimen in spirit". There are some interesting specimens with common names such as Bullock's heart (referring to *Annona squamosa* L., BM000086201) Fig 3., and there's a label of *Solanum mammosum* (BM000642022) used as cockroach poison Fig. 4. The lids of the bottles of type specimens are painted in red (Fig.5) for quick visual inspection and retrieval.

The collections needed Re-curation as pointed out earlier by (Prakash, 2019). Although not enough procedures for re-curating botanical spirit material exist compared to zoological material, we followed Simon Moore (1999), and we have now standardised and re-curated the collections in 70% IMS and use the term "fluid material".

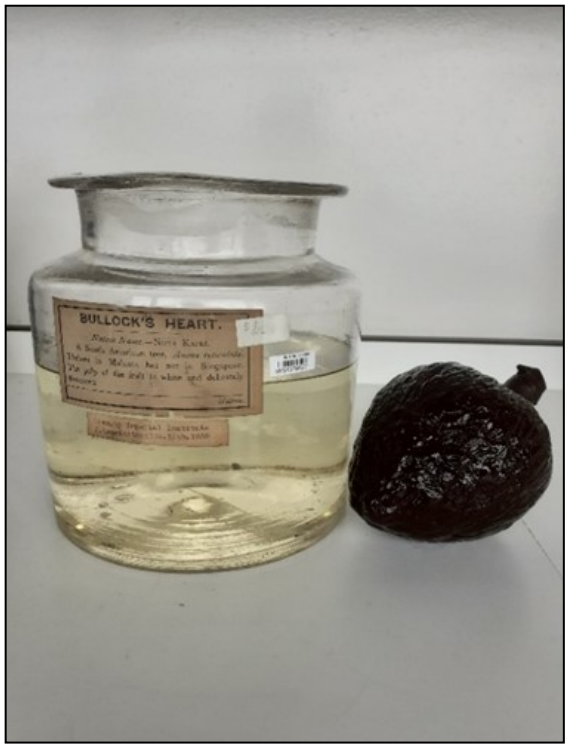

Fig. 3. Bullock's heart (refers to the shape of the fruit of Annona squamosa L., BM00086201)

Fig. 4. Label information showing Solanum jamaicense Mill. determined as S.mammosum L., with the fruit next to it, which is used as cockroach poison (BM000642022).

We provide an update on these collections, how these have been curated and the collections are available on our website (https://data.nhm.ac.uk/dataset/the-seed-plants-fluid-collection-at-the-natural-history-museum). We hope that more people interested in these collections will be able to make use of these collections for their research and help us to update the collections by adding their determinations; for example, some families are well represented such as Orchidaceae, yet many specimens are yet to be determined. We encourage experts from around the world to examine these collections and advance our biodiversity knowledge.

Fig. 5. Lids of bottles containing type specimens painted in red for ease of visualisation and retrieval for physical examination.

Materials and methods

At the outset, a Gantt chart was prepared listing all the tasks/objectives to be achieved in a time bound manner. This project was executed in three stages: first the re-arrangement of specimens as per jar size; then databasing and labelling; and lastly, topping up the bottles and jars with 70% IMS.

Re-arrangement of specimens

The first step was to re-arrange specimens in the storage cabinets. Specimens were organised by jar/bottle size to maximise space and efficiency rather than choosing taxonomic arrangement. The cabinets were labelled 1-5 with arrangement from left to right and the shelves from top to bottom. Numbered trays were used to organise specimens within wooden shelves. The weight of specimens was also considered, placing lighter specimens on top shelves and bigger and heavier jars lower

down for ease and safety. We used blue trays for the easy handling and retrieval of medium sized specimens. Smaller bottles were stored in plastic boxes which were stacked up on shelves. Bottles that did not fit in trays/boxes or which were very heavy were directly placed on the shelves. (Fig.6 & 7).

Fig. 6. Combination of bottles stored in blue trays and without trays directly on shelves.

Fig. 7. smaller bottles stored in plastic boxes.

Databasing

The collection was initially taken through a process of visual examination and recording. This involved inspecting each specimen container, recording the label details present outside and any other information on the inside of the container in an excel spreadsheet. This information often consisted of the collector number and Geographical Region identifiers (Fig. 1). The genus and species names, and any details about the provenance of the specimen were recorded if shown.

In some cases, the collector's number could be used to look up the collection record in the archives at NHM. This was notably found with C. E. Carr's collection from Papua, where NHM holds the collector's records and notes concerning most of the items collected. This additional information was added to the database and included key details about location and environment where the specimen was found, which was often not included on the specimen container label.

A currency detecting microscope x60 and light was used for labels that were difficult to read. Websites such as IPNI (2024), POWO (2024) and Tropicos (2024) were used to ensure currently accepted species names and spellings were correct. POWO (2024) was used most as it lists synonyms too. Tropicos (2024) was consulted when we did not find names in IPNI (2024) or POWO (2024).

The data input method was first to clean the jars/ bottles using a dry tissue removing any dirt or dust collected. Then in some cases where required, wet/damp tissue was used but care was taken to avoid the labels as the water might ruin them. The tissues were disposed safely in relevant bins as hazardous waste in the lab. The jars and bottles were inspected and yellow sticky notes were attached to them if maintenance was needed during the topping up stage, e.g. cracked/broken glass or damaged lids. Barcodes were then placed either vertically or horizontally on jars to ensure ease of scanning. In addition, barcodes were placed close to any other labels to maximise view of the specimen inside the jar. We often washed the lids of the bottles with plain water to get rid of dirt, a special sink was used to avoid water contamination.

Table 1. Key title headings used to capture specimen data in the spreadsheet, their examples and descriptions.

Title Heading	Example	Description
Cabinet Number	1	Explaining which of the five cabinets the specimen can be located. Labelled 1-5 left to right.
Shelf Number	1.1	Explaining which shelf the specimen can be located on. The first number corresponds to the cabinet and the second to the shelf. Labelled top to bottom in ascending order.
Tray/box Number	1	Explaining which tray the specimen can be located in. Labelled from left to right in ascending order per shelf.
Barcode Specimens	BM…..	Giving each specimen an individual barcode number. Barcodes were scanned rather than typed to avoid any human error.
Family/Genus Number	57/1	Some of the jars had old family/genus numbers as per Bentham & Hooker system of arrangement. Often when no label was found, this helped us to identify the specimen. Coloured labels based on geographical regions (1-26) are used in the General Herbarium, e.g. white for Asia, yellow for Central America and green for Australia.
Family Name	Araceae	This is to help identify and categorise the specimen. If someone wanted a plant from a specific family/genus they could search it and find all the species we have of that family.
Taxa Name	Genus and species name	The name to help to identify the taxa. If someone wanted a specific species, they would be able to see if we had it in our data base and any information.
Country	Panama	The place the specimen was collected from. If it was not on the label 'Unknown' would be used.
Locality details	Box Island off Tamian Lake, Titicaca. Alt: 12,500 ft. Island of Taman.	More specific details about where the specimen was collected from. This could help us understand the physical geography of the collection location. If there was no data 'sin.loc.' was used.
Habitat notes	Dry rocky ground	Any notes left on the label to help us understand more about the environment the specimen was collected from.
Plant description	Perennial to 3m; leaves triangular in cross section, c 1/2 m long; fruit long (c 5cm), cylindrical; flowers with 3 brown recurved bracta, 2 green petals upright, 1 large spurred labellum +/- white with pink markings.	We recorded detailed information on the plant describing the habit, leaves, fruit and any other decipherable information from the label.
Name of the Collector	Nancy C. Garwood	Who collected the specimen. 'Anon' for Anonymous would be used if there was no collector name.
Collector Number	1242	The number the collector gave to the specimen. If this information was not on the label we would write 's.n.' (means 'no number').
Collection date	20/10/1988	This is the date the specimen was collected (DD/MM/YYYY). If this information was not on the label we would put 'sin.dat.'
Notes	Corresponding material in general herbarium.	Any other information on the jar or label.
Identification notes	Looks like a pine tree	This recorded information for any specimens that had no identifying details. We recorded any additional notes/labels on the jars which might help curators/researchers identify the specimens.

We captured the data under 16 columns headings in our spreadsheet as shown in Table 1.

Topping up

Decision Making Model for the conservation and restoration of fluid preserved specimens by van Dam (2004) was used to help us decide which specimens and jars needed to be conserved and restored.

Based on the survey by Prakash (2019), we decided to use 70% IMS to preserve the specimens. Practical skills learnt on Fluid preservation course taught by Simon Moore in 2015, literature by Moore (1999), decision-making model by van Dam (2004) and personal visit by Prakash to BR (National Botanic Garden, Belgium) and K (Royal Botanic Garden, Kew) a few years ago helped to decide the best protocols in topping up. A 70% IMS (70 parts IMS and 30 parts deionised water) solution was made from 80% IMS.

We wore protective clothing (lab coats) and used safety glasses while topping up.

Any original alcohol liquid in the jars was poured into a waste bucket, using a small sieve to ensure none of the specimen was lost. The waste alcohol was eventually safely disposed following the protocols of disposing hazardous material (safety data sheets needs to be filled in which provides information on the type of fluids whether it is IMS, Formalin or mixed).

Some jars contained Formalin and were dealt with appropriately and the topping up was checked for a cloudiness which can occur when IMS is added to previous Formalin preserved items/mixed fluids with various densities.

Jars and their lids were inspected to see if new containers were necessary. If so, all labels were transferred. Soaking old jars in warm water helped to remove labels easily, ensuring they were kept in the best condition. Some corks became brittle and disintegrated while trying to remove them, sometimes resulting in cork pieces falling into the jar; however, due to corks low density in comparison to most of the specimens they could be removed easily. Suitable lids were used to replace the cork, if not whole jars were replaced.

Specialised universal stopper jar openers in large and small sizes, designed by van Dam in collaboration with the Natural History Museum (Fig. 8) were used to remove any glass lids which had oblong or circular knobs on the top suitable for being gripped by this tool. Otherwise, the team resorted to various methods for opening lids including manual twisting, levering and hot water soaking. Corks often disintegrated and had to be dug out, Kilner lids sometimes included completely perished sealing rings or welded shut sealing rings which had to be dug out and prised open. Some glass jar knobs broke off when using the lifting tool or the jar rims broke off under the pressure of the lifter on a weakened rim. Many of Carr's specimens were stored in glass tubes within wooden boxes. These tubes had cork stoppers which had become too brittle and usually needed replacing however the tubes were a non-standard size for our modern stoppers, so the specimens often had to be rehoused in modern/new tubes. The wooden boxes were retained for historical reasons and interest (Fig. 9).

Fig. 8. Universal stopper jar opener designed by Andries J. van Dam in collaboration with the Natural History Museum, London.

Topping up was undertaken in a laboratory with an integrated extraction system, using a plastic bottle with a long spout (Fig. 9) for smaller bottles and jars were used for medium/large bottles. Once the new 70% IMS fluid had been added to the specimens the jars or tubes were resealed using existing or replacement tops. Glass stoppers were sealed with a layer of petroleum jelly applied around the rim and around the tops, replacement Kilner seals were used where feasible or sometimes the specimen had to be rehoused if a

Fig. 9. Topping up small vial with a plastic bottle with long spout, the vial is kept in wooden box (likely used for transporting).

suitable seal could not be found. Other screw top, plastic press on tops or rubber corks were largely reused and sealed with petroleum jelly.

Some labels had to be rewritten due to poor handwriting, brittle labels, where jars had broken and when labels would not come off despite soaking in warm water. We used an archival pen and Resistall paper to write these new labels.

Results

Data captured in the spreadsheet is presented as an index to the collections and is now available on the public domain at https://data.nhm.ac.uk/dataset/the-seed-plants-fluid-collection-at-the-natural-history-museum. Index to the collections is arranged as per family alphabetically. One can locate the collections taxa wise and looking at the cabinet/shelf/tray number on the righthand side of the table. Taxa names as recorded originally have been checked for current taxonomy and where names have changed, they are reflected in new determination. Country, locality details, habitat notes, plant description, name of the collector (s) followed by collection number, collection date and registration numbers (barcode numbers) of the specimens databased have also been given. Several specimens have corresponding herbarium sheets in the General Herbarium. Type specimens are highlighted in red.

The data will be eventually ingested in Ke-EMu, the Museum's database system.

Discussion

Around the world, digitisation efforts have increased rapidly in the last few years. By making these collections accessible in the public domain, we hope that interested researchers will be able to make the best use of them and advance our scientific knowledge. Although we found 41 types, it is possible that there are some specimens as yet unidentified type specimens in our collection.

The largest collection is from an American tropical botanist- Nancy Garwood (b.1949) (over 700 bottles) which consists of some carpological material collected from Panama as well as seedlings. She is recorded as having co-collected with many people during this time and specialised mainly in Spermatophytes.

The earliest recorded collection is dating back to 14/01/1803, a *Browniana* (BM013782085) collected by Robert Brown from Australia (Chapman et al. 2001).

We encountered several problems while working on this project such as illegible or undecipherable handwritten labels, missing information, incorrect spelling of species names, degraded labels, no labels, no collector information and difficulties determining geographical area of species collection. Some of these problems were overcome by using museum archives consisting of collectors' notebooks for cross reference purposes. Otherwise, the team resorted to the internet to research using whatever data could be gleaned from the tubes and jars. Following recording issues, the main problems revolved around the jars' condition and accessing samples for topping up with fluid. In instances where some jars were difficult to open, we used lid lifters, levers or hot water slowly poured over the lid to loosen the lid, we also drilled a small hole in the lid of the bottles to release pressure and open the bottles. In some cases, the jars were broken (kept in plastic bags and smashed gently with a small mallet for health and safety) and both the jars and labels had to be replaced. Cork stoppers were usually replaced as were Kilner seals. We soaked the labels in warm water, some came out easily which we stuck on new bottles. However, in some cases, the paper was brittle, and the label was lost,

so we wrote new labels on acid free Resistall paper with archival ink. We envisaged having used ca. 700 litres of IMS to these collections and around 300 new bottles of various sizes. We got some monies (ca. £4500) from the curation budget to curate these collections (buying boxes, bottles and trays, see supplier details listed under references). We also got some supplies of bottles (spare ones) from our other departmental colleagues.

In the near future, we wish to upload all the possible resources such as field notes by C.E. Carr, in the dataset: https://data.nhm.ac.uk/dataset/the-seed-plants-fluid-collection-at-the-natural-history-museum. We hope that these collections will be imaged in the future.

Finally, we feel happy that we have managed to restore the collections to their original glory and make these collections accessible to all.

Conclusion

We have addressed the curation and conservation needs of the seed plants fluid collections. In total 3,072 specimens have been re-curated by replacing broken jars/lids, rewriting labels and topping up the specimens with 70% IMS which are now virtually available on the Museum's public domain at: https://data.nhm.ac.uk/dataset/the-seed-plants-fluid-collection-at-the-natural-history-museum.

189 families are represented with Orchidaceae representing the highest number of specimens followed by unknown families, then Rubiaceae, Melastomataceae, Moraceae, Piperaceae, Fabaceae, Araceae and other families. There are 1359 distinct counts of taxa name (genus and species names) with unknown taxa having a count of 492 taxa, followed by Orchidaceae with 335 taxa, Impatiens with 21 taxa, and other taxa with lower numbers. There are a few gymnosperms as well. 41 types from various countries around the world have also been recorded.

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Preserving colour of botanical wet specimens: bibliographic review and tests of historical recipes

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Abstract

This paper presents the results of a study on colour preservation of botanical specimens in fluid. The article includes: 1 - An introduction on plants pigments and discolouration issues specific to botanical specimens, focusing on the leaching of pigments from the specimen into the fluid, and on the denaturation of the pigments (through photodegradation, oxidation or polymerization) either in the specimen or in the fluid; 2 – An extensive bibliographic review of historical recipes invented specifically to preserve the colouration of plants as a preparation step; 3 – Results of testing some recipes on freshly prepared specimens, and their discolouration rate compared to reference specimens that were kept in 70% ethanol solution. None of the tested recipes gave results that are optimal to preserve all aspects of a specimen. In fact, colour preservation or prevention of fluid opacification came at the cost of either loss of structural stability, changes in chemical composition of pigments preserved, or loss of other colours. Ultimately, the choice of preserving the colour of wet botanical specimens should be made on the intended use of the specimen.

Keywords: Botanical specimens, discolouration, pigments, wet collections, colour preservation

Introduction

This is a follow-up paper to the article "Losing colour: the discolouration of plants in spirit preserved collections" published in 2022 (Granget *et al*., 2022). In our long-term experiment, we collected 3 (2021-2024) to 5 (2019-2024) years of experimental data on fluid preserved botanical specimens with the aim of understanding how to better preserve their colour. In addition, a literature search for historical and modern recipes targeting colour preservation and testing on fresh specimens are included.

Fluid preservation techniques, initially developed in the 17th century for animal and human specimens, have evolved significantly over time (Simmons, 2014; Neumann et al., 2022). The process of preservation generally involves several key steps: the collection of the specimen, followed by fixation through injection or immersion in a solution, rinsing, mounting in a jar, filling the jar with a preservative fluid, and finally, sealing the container. The application of fluid preservation methods gradually became more common in botanical collections in the 19th century (Moore, 2010). Although fluid preservation is not the predominant method for conserving botanical specimens, it is still used as a valuable alternative to drying, freeze-drying, or pressing, especially for specimens with significant volume and complex or fragile three-dimensional structures that are

challenging to preserve by other means (Bridson and Foreman, 1998). Botanical collections encompass a wide range of non-zoological specimens, including vascular plants, fungi, bacterial pathogens, algae, and corals. Despite belonging to different biological kingdoms, these specimens have traditionally been grouped and studied within the field of botany (Morton, 1981).

Historically, alcohol-based solutions were employed as the primary preservative fluids (Moore, 1999; Neumann et al., 2022), until aqueous formaldehyde solutions replaced alcohol as a more effective and cheaper fixative in the late 19th century. Formalin solution remained widely used in collections also for long-term preservation, until concerns over its toxicity prompted a re-evaluation of its use. The primary objectives of fixation include enhancing the mechanical strength and chemical stability of tissues while inhibiting autolytic processes such as enzymatic degradation (Venteo & Velot, 2010). Due to the health risks associated with formaldehyde and its limitations in DNA preservation compared to alcohol (Carter, 2003), there has been a shift back to alcohol-based preservatives in the northern hemisphere, particularly ethanol or denatured alcohol such as Industrial Methylated Spirit (IMS), and a growing interest in using glycerol, a non-toxic but denser alternative to formaldehyde solutions (Neumann et al., 2022).

In botanical collections, most specimens are preserved in 70% alcohol, typically IMS or full-strength ethanol. In case of an initial fixation step, 4% formaldehyde solution or a commercial formaldehyde-acetic-acid (FAA) solution is used (Prakash, 2019).

Due to the fundamental differences between the cell structure of vascular plants compared to those of algae and fungi, fluid preservation methods have been adapted to meet specific requirements. This paper focuses on vascular plants only.

Discolouration phenomena

Colour is a crucial feature of botanical specimens, providing insights into the function of coloured organs, such as attracting pollinators, defence mechanisms, and photosynthesis. Unfortunately, most current preservation methods, whether dry or wet, fail to maintain the in-vivo colour, leading to an inevitable loss of valuable scientific information. This issue highlights the importance of accurately recording by other means (e.g., photography or illustration) the in-vivo colours of specimens (Bedford, 1999). Botanical wet specimens are particularly prone to colour loss, resulting from pigment alteration within the tissues and the migration of pigments into the preservation fluid (Butler, 1918; Granget et al., 2022). The degradation of these pigments also impedes exhibition, as discolouration can obscure public understanding and, in extreme cases, render the specimen unrecognizable. In this paper, we consider three main mechanisms for the degradation of pigments, chemically affecting the colouration of a specimen:

- Leaching is the extraction of the pigments into the preservative fluid. It is accelerated by frequent handling of the specimen (Latty 2021) and causes fading or changes in the colouration of the specimen itself. In most cases, the leached pigments colour the fluid (Butler, 1918; Granget et al., 2022).
- Photodeterioration is the fading of colour of the specimen or the leached pigments in the fluid by exposure to ultraviolet radiation (Groeneveld et al, 2023).
- Oxidation or polymerization of some pigments may cause the yellowing or darkening of the specimen and the fluid, and is accelerated by the presence of oxygen or inappropriate pH-shifts of the solution.

Plant pigments

Plant pigments are diverse, making their conservation during fluid preservation a complex task. Understanding pigments is essential for developing preservation techniques that prevent their discolouration and thus support the visual and structural integrity of botanical specimens. Besides the impracticality of analysing each specimen's individual pigments before preservation, some generalizations on plant pigments are possible. This paper groups the main pigments into two categories: i) Colourful pigments, mostly prone to leaching and photodegradation, and ii) brown or transparent pigments that darken in the specimen or opacify the fluid through polymerization or oxidation. A more comprehensive list of pigments and their solubility is presented in Appendix I.

- Green pigments (i): the term chlorophyll indicates a group of pigments from the tetrapyrrole family, which vary in form and structure. Chlorophyll a and b are the most common forms in vascular plants. It is a primary pigment of chloroplasts, the plastid responsible for photosynthesis of all green plants, and has a pivotal role in this process (Davies, 2004; Ralph et al., 1970). Chlorophyll

has two important parts: a ring-shaped structure (called the chlorin ring) that captures sunlight, and a magnesium ion at the centre that keeps the structure stable. Together, they turn sunlight into energy for the plant.

- Blue-red pigments (i): are hydrosoluble pigments occurring mostly in the vacuoles, their hues may be influenced by shifting pH levels. For example, red cabbage turns blue when put in contact with an acid such as lemon juice. They are either betalains, anthocyanins or other colourful flavonoids (Davies, 2004; Delgado-Vargas et al., 2000). Depending on the plant, the same type of pigment will express a different hue on the blue-red spectrum.

- Yellow, (and orange-red) pigments (i): These colours are more challenging to assess. In photosynthetic organs (leaves) or some fruits, the pigments are likely carotenoids and therefore liposoluble (Delgado-Vargas et al., 2000). However, in flowers, these colours could originate from either carotenoids in plastids or other hydrosoluble pigments in vacuoles (betalains or flavonoids), requiring more research before preservation. If carotenoids are known to be very stable, betalains and flavonoids (such as anthocyanins) are way less stable and very sensitive to pH shifts (Davies, 2004; Delgado-Vargas et al., 2000).

- "Tannins"(ii) often refers to a variety of molecules that may cause browning of the preservation fluid and specimen, such as phlobaphenes, heteropolymers with bound anthocyanins, quinones, and mostly phenolic compounds. Polyphenolic compounds are present in all plant parts and characterized by their ability to bind and precipitate proteins, a property that is exploited in tanning industry. In plants, they play crucial roles as defence mechanisms against herbivores, pathogens, and UV radiation, as well as in regulating growth and development (Arbenz & Avérous, 2015). Tannins are not all colourful while in the plant, but they can darken through oxidation or polymerization (Khanbabaee & Van Ree, 2001). It is worth noting that there are exogeneous sources of tannins in fluid preserved specimens, such as camphor introduced as antiseptic, or resin-based denaturant in ethanol.

The rate at which plant pigments leach into common conservation fluids has been assessed in systematic studies (Dangeon et al., 2020; Granget et al., 2022). They show that none of the tested fluids were effectively preventing discolouration and highlight the various rates at which the fluid can opacify. Moreover, the tests imply that chlorophyll and carotenoid-rich specimens stored in alcohol-based preservatives (full-strength ethanol and rum in these studies) leach most of their pigments within the first few weeks, with no significant difference between those with and without prior fixation (formaldehyde 4% or FAA) (Figure 1A and C). The fluid usually turns yellowish, indicating pigment breakdown. Glycerol and aldehyde-based preservatives preserve colour better, though some yellowing and browning still occur. Betalain specimens leach pigment almost immediately, with colour shifting to yellow-orange in alcohol-based preservatives and brownish-red in formaldehyde-based solutions (Figure 1B). Tannin-rich specimens, such as walnuts, leach dark pigments, with glycerol slowing the process but eventually matching the colour of other fluids after two years (Figure 1D) (Dangeon 2020, Granget 2022).

The experimental set-up for assessing the discolouration rate in common fluids is the same as for the study of colour-preserving recipes (see materials and methods).

Bibliographic review

The recipes tested in this study were selected after an extensive literature review (Figure 2; complete list of references in appendix II). This paper will only give an overview of the chronological appearance of recipes aimed at maintaining the colour information of botanical specimens. The aim of this section is to give context regarding the major principles behind various recipes. Several variations of these recipes exist, only the ones tested are detailed in the material and methods section of this paper.

Attempts to preserve the colours of plants in fluid began in the late 19th century, inspired by a common practice in the canned food industry, particularly in France (Wiley et al., 1887). This practice involved maintaining the green colour of vegetables by boiling them in copper-alloy vessels, which is still applied today on a larger scale with additives such as copper sulphate. Before the various structures of the chlorophyll were discovered and explained by Fischer and colleagues in 1930 (Seely, 1966), scientists including Tschirch and Schunck (Wiley et al., 1887) rightly suggested that copper might be attaching to this molecule rather than just acting as a dye. It is indeed a complexing action.

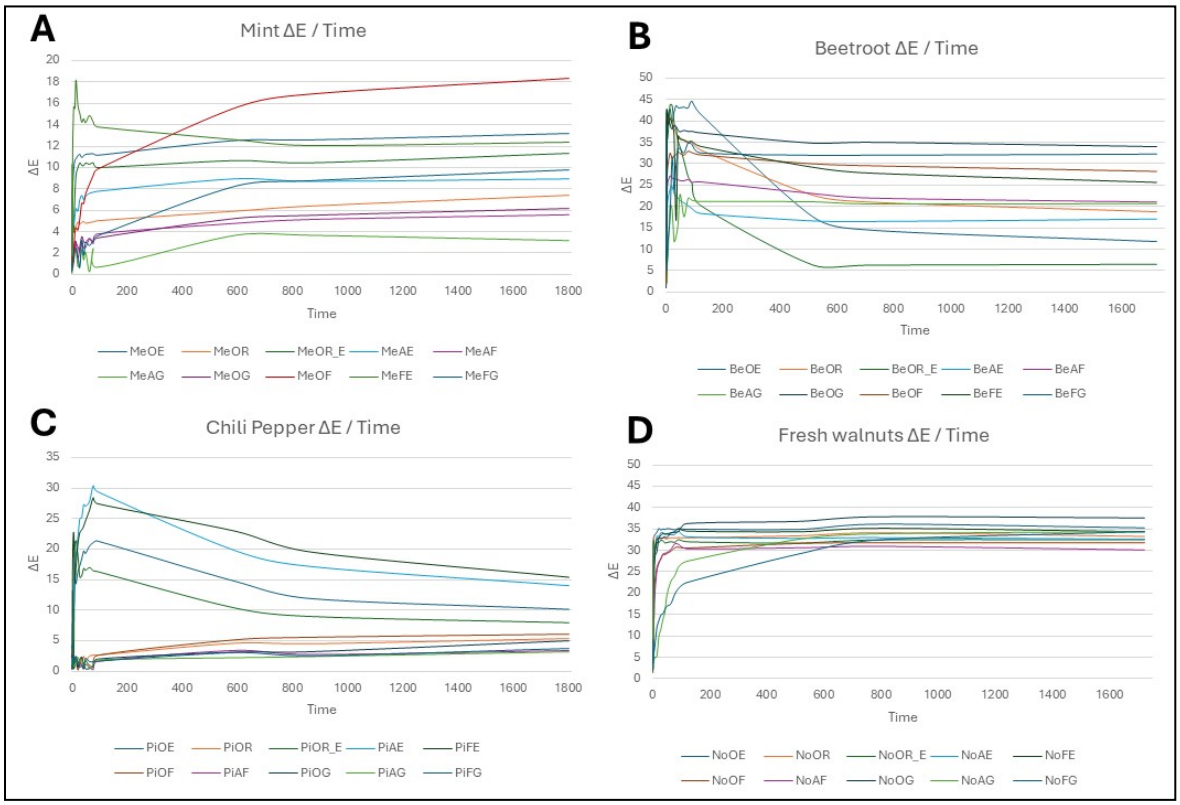


Figure 1: Evolution of Delta E for tested specimens. A. Mint (Me), B. Beetroot (Be), C. Chili pepper (Pi), D. Walnuts (No). Abbreviations are specimen + preparations. OE: no fixation, stored in undenatured EtOH 70%, OR: no fixation, stored in commercial rum, OR_E: temporary storage in rum, stored in EtOH 70%, AE: fixed in FAA, stored in EtOH 70%, AF: fixed in FAA, stored in formaldehyde 4%, AG: fixed in FAA, stored in 70% glycerol, OG: no fix. stored in glycerol, OF: fixed and stored in formaldehyde, FE: fixed in formaldehyde, stored in EtOH 70%, FG: fixed in formaldehyde, stored in glycerol 70%. Summarizing results from Dangeon 2020 and Granget 2022. ©HE-Arc 2024

Chlorophylls are chemical complexes with a cyclical tetrapyrrole as ligand and magnesium (Mg^{2+}), as complexed ion. The ligand acts as a claw, holding the ion in its centre, but this Mg^{2+} ion is easily substituted by two hydrogen ions (H^{+}), through protonation, under the influence of heat or acid, resulting in duller and darker pheophytin. This degradation process eventually ends with cleavage of the porphyrin ring (tetrapyrrole), leading to colourless derivatives. Mg^{2+} can also be substituted by other ions such as zinc (Zn^{2+}) and copper (Cu^{2+}), because they have higher electrophilic affinity with the chlorophyll-complex and replace the Mg^{2+}-ion easily. Thus, the recipes introduced above employ different copper salt solutions to preserve green colour in plants. It seems that the first reports of preserving colour in botanical collections using copper salts date back to around the mid-1890s. The ability of maintaining colours in the original plant material through transformation of their colourising components rather than artificially dyeing the specimens likely influenced the decision to use these methods.

After the fundamental studies by Woods (1897) and Trail (1908), numerous botanists and others have revised, adjusted, or developed new methods to preserve colours of plants in fluid throughout the 20th century (see review in Hangay & Dingley, 1985). The primary focus was on keeping the green shade with copper salts, while only few others investigated the potential of conserving other pigments in plants. Though these copper salts recipes were designed to maintain the green colour of plants, they were also applied to specimens with other colours (Woods, 1897).

Most other attempts to specifically preserve colours were directed towards preventing oxidation. Sulphites were mainly suggested as antioxidants for prevent the browning of clear specimens , e.g., in fruits and parasitical plants (Strasburger, 1911; Butler, 1918; Nieuwland & Slavin, 1928; van Steenis, 1935). Additionally, the use of antioxidant was also proposed to preserve colours, mostly yellow, orange and red (Cruess and Christie, 1922; Adriano and Yonzon, 1933; van Steenis, 1935; Scully 1937). Scully noted that these recipes were not effective for red or blue

colours (Scully 1937). The effectiveness of antioxidants such as sulphites in keeping red colour information in plants likely varies depending on the pigments involved, such as anthocyanins, betalains, or carotenes, which have different structure, solubility, and stability.

The most common red and blue pigments are anthocyanins or betalains, they are hydrosoluble and their colour-information is easily affected by pH shifts. Wagstaffe and Fidler (1968) suggested using a zinc chloride solution ($ZnCl_2$) dissolved in a mixture of formaldehyde and glycerol to preserve these colours (Wagstaffe and Fidler, 1968). Interestingly, they also proposed another solution including a tert-butyl-alcohol, with the addition of a reducing agent, and a complexing agent (thiourea and sodium citrate/citric acid), to preserve red and blue flowers, which contain delicate anthocyanins.

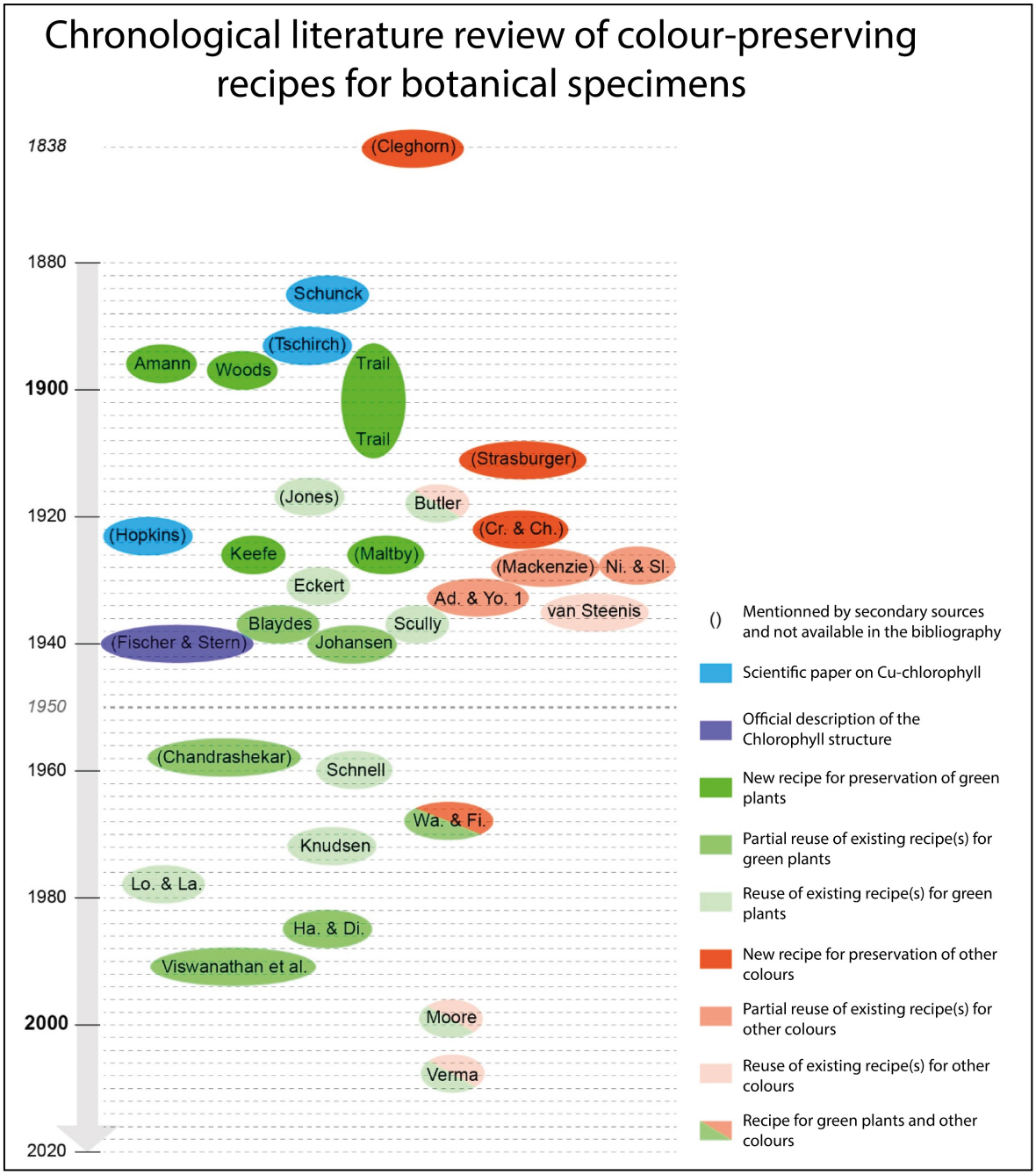


Figure 2: Chronology of colour-maintaining recipes, by author. Translated from Latty 2021. ©HE-Arc 2024

Materials and Methods

To assess how effective the colour-preserving recipes found in literature are, the methodology introduced in Granget et al. (2022), presented below, was followed.

Monitoring

Test-specimens were freshly prepared, following a selection of recipes presented in the next section. They were then kept in a dark environment in a solvent cabinet and underwent both qualitative and quantitative assessments periodically. Test-specimens were monitored daily over the first week, then weekly for 2-3 months and then monthly for 12-18 months. All samples were measured for long-term assessment in 2024 (3-5 years after first measurements). The qualitative and comparative approach consisted of photographic documentation, while the quantitative analysis was conducted on the fluid using a portable spectrophotometer measuring in the visible light range (X-rite® Ci62). For this quantification, 6 mL of the preservative fluid sampled from the individual jars containing the specimens, was put in a special vial for the colourimetric measurements and returned into the jars after measuring.

Fluid colourimetric values in the CIELAB colour space (ISO/CIE 11664-4:2019(E)) were extracted from the spectra acquired.

The CIELAB colour space characterizes colour using three parameters: L* for lightness ranging from 0 (black) to 100 (white), a* for the green-red axis ranging from -a* (green) to +a* (red), and b* for the blue-yellow axis ranging from -b* (blue) to +b* (yellow). These values are relative to a specific illuminant, with D65 (standard daylight) utilized for this protocol.

The extent of colour change was quantified using Delta E (ΔE), representing the Euclidean distance between two points in the colour space. In this case, the comparison was made between the L*a*b* values for the fresh preservative solution (T0) and those on the monitoring day (Tx). A Delta E value approaching zero indicates minimal perceptible colour difference, while a Delta E >2 is perceivable by the human eye. In this study, values exceeding 10 were considered as notable colour deviations (Granget et al, 2022).

Selected recipes

Some of the recipes (Table 1) presented in the bibliographic review have been subjected to close monitoring for at least 3 months, and their usefulness for longer term conservation for 3 to 5 years. For control, fresh specimens were prepared and directly preserved in 70% full-strength ethanol (EtOH) without initial fixation step or other specific colour-preserving treatment. Both the colour retention and the specimen's integrity were evaluated during monitoring.

In the following sections, unless specified differently, "EtOH" or "ethanol" indicates the use of full-strength, non-denatured ethanol, and "water" implies demineralised water.

Table 1. Overview of all tested recipes

Colour	Recipes tested
Green	Copper based recipes: $CuSO_4$ Cu Acetate (II) $Cu(CH_3COO)_2$ $CuCl_2$
Yellow	Scully B: copper salt initial treatment, preservation in sulfuric acid and sodium sulfite solution. Verma 2: propionic acid, formaldehyde, and water for fixation, with glycerol added for preservation
Blue-red	Wagstaffe & Fidler 4: tertiary butyl alcohol, thiourea, and a pH modifier Kew mixture: IMS, water, formaldehyde, and glycerol Copenhagen: IMS, water, and glycerol
Tannins	Antioxidants recipes: aqueous SO_2 Polymerisation recipes: Aldehyde fixatives

Treatments to retain Green colour

Recipes aimed at retaining green colour of pigments in plants are numerous (see Figure 2). Most of these seem to rely on the substitution of metal ions in the chlorophyll complex, thus creating a new and more stable pigment. Variants of these recipes were tested (Latty, 2021) on

young pea leaves (*Pisum sativum* L.). Because they are globally the most effective, only recipes based on copper salts will be presented in this paper. Different copper salt and solvent combinations were tested by using them in an initial step before storing the specimens either in 70% ethanol or leaving them in their initial solution (Table 2).

Table 2. Copper salts recipes used for the preliminary treatment of the samples

Copper salt	Solvent	parts
$CuSO_4$	Water	1:20
	FAA	0.2:100
Cu Acetate (II) $Cu(CH_3COO)_2$	Acetic acid 50% (in water)	19:150
	FAA	11:200
$CuCl_2$	Water	1:100
	FAA	1:100
Control: No salt	Full-strength EtOH 70% in water	

More recipes aimed at maintaining the green colour proposed in the literature were tested rapidly, followed by visual observation and documentation. They included boiling the specimen in Cu acetate (II) before preserving it in 70% EtOH (supposed to enhance and prevent the leaching of pigments) or, in some variants of $CuSO_4$ preparations, adding 5% aqueous SO_2 solution ($SO_{2(aq)}$) (Latty, 2021). These results will not be presented in this paper.

Yellow pigment

Yellow Yarrow (*Achillea millefolium* L.) were used to test two recipes to maintain the yellow colour (Table 3). The yellow colour of yellow yarrow is primarily attributed to carotenoids, though some flavones are co-pigments contributing to their colour (Raudone et al, 2024).

Blue-red pigments

For chemically delicate colours such as blue, red, and purple, tests were carried out on *Glandularia peruviana* L. Small. (red and purple colour) and *Lobelia erinus* L. (blue colour). The vibrant colours of these flowers are mostly due to the presence of anthocyanins and other flavonoids (Chai et al, 2024). Three recipes were tested (Table 4).

Table 3. Recipes tested for the preservation of yellow colour

Scully B	Fixation in 5% copper sulphate; subsequent transfer and preservation in a mixture of 1000 mL water with 16 mL commercial sulphuric acid, and 21 g sodium sulphite.
Verma 2	Fixation in 1 mL propionic acid ($CH_3CH_2CO_2H$), 1 mL formaldehyde, 100 mL water; preservation in same mixture + 2 mL glycerol.
Control	70% EtOH in water

Table 4. Recipes tested for the preservation of blue and red colours

Wagstaffe & Fidler 4	100 g tertiary butyl alcohol, 1 g thiourea, 2 g sodium citrate (for blue flowers) or 2 g citric acid (for red flowers).
Kew mixture	53% IMS, 37% water, 5% formaldehyde and 5% glycerol
Copenhagen	70% IMS, 28% water, 2% glycerol
Control	70% EtOH in water

Tannins

The preservative fluid especially of tannin-rich plant specimens often darken significantly due to extreme leaching of tannins. These include compounds that are either dark in vivo or subsequently darken through polymerization and oxidation as the specimen degrades (this is the case of parasitic plants). To prevent oxidation, a SO_2(aq) solution can be used, prepared by adding $NaHSO_3$ to 70% ethanol, buffering the solution to pH 1.8 with hydrochloric acid, and filtering. Two other methods to prevent pigment migration involve using a non-polar fluid, like liquid paraffin, or polymerizing phenolic compounds with 3.5% formaldehyde and/or phenol. These recipes are only suitable for specimens that are naturally dark, as the fixation process further darkens the tissues (Latty, 2021; Latty et al., 2021).

Table 5. Recipes tested for the prevention of fluid opacification through oxidation of the tannins in parasitical plants.

Fixation step	Preservative fluid
No pre-treatment	$SO_{2(aq)}$ in EtOH 70%
Paraffin impregnation	$SO_{2(aq)}$ in EtOH 70%
Paraffin impregnation	EtOH 70%
Paraffin impregnation	Paraffin
Formaldehyde 3.5%	Glycerol 70% (polar fluid causing less leaching than EtOH)
Control 1: No pre-treatment	Glycerol 70%
Control 2: No pre-treatment	70% EtOH in water

Table 6. Recipes tested to prevent opacification of the fluid through prior fixation of tannins in Pinus *sp. bark.*

Fixation	Recipe	Time
Formaldehyde	35% in water	1 day
	17.5% in water	1 day
	3.5% in water	1 day
	3.5% in water	1 day (kept in the dark)
	3.5% in water	3 days
Formaldehyde / phenol	17.5 + 5%	1 day
	17.5 + 0.5%	1 day
	3.5 + 0.5%	1 day
	3.5 + 5%	1 day
Control	70% EtOH in water	No fixation

Orobanche hederae (Duby) was chosen to test the effects of antioxidants and a non-polar solution, based on studies of browning in parasitic plants. The recipes are summarized in Table 5.

To test the polymerization of phenolic compounds, the bark of *Pinus* sp., known for its high content of condensed tannins (Pizzi, 2008), was used. Various concentration and fixation times, as listed in Table 6, were tested, and all specimens were then stored in ethanol 70%.

Results and discussion

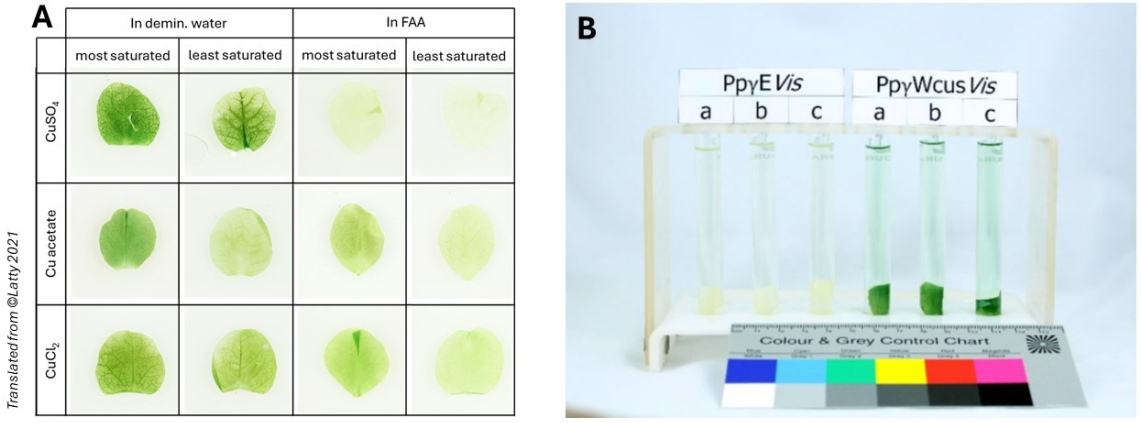
The main observations after several months of monitoring and long-term assessment of all the samples for 3 years (with some results after 5 years) are presented grouped by colour. More detailed results for the green pigment and browning of specimens have been published separately in French (Latty, 2021; Latty et al., 2021). Only main conclusions of these tests are summarised in English in the next section.

Green pigments

Overall, copper salts are efficient at enhancing the colour stability of green leaves, as the specimens subjected to photo-aging demonstrate (Figure 3). However, the hue of the fluid diverges from the untreated specimens kept in 70% ethanol, shifting from the usually observed yellow-green to a bluish -green in the $CuSO_4$-treated samples. This is due to the substitution of the magnesium ion with copper, as confirmed analytically with LC-MS (Latty, 2021), showing the stabilization of tetrapyrrole by Cu^{2+} ions.

The choice of the solvent for the copper salt treatment also affected the results, with FAA solutions permeating faster than the samples in water, but also extracting chlorophylls more rapidly. Thus, FAA recipes yield paler results than water due to this rapid extraction. Regarding the choice of salt, while $CuCl_2$ initially saturates the leaf colour, subsequent pigment loss occurs once the specimen is placed in the preservation fluid. Keeping the specimen in the copper salt solution did preserve the colour better, however, it is important to note that the fluid is noticeably blue and that neither the copper-salts nor the water-based solution provide any fixing or preserving properties of tissues, and does not act as a biocide and thus do not support long term preservation of the tissues.

Additional treatments aimed at enhancing the diffusion of the $CuSO_4$ into the tissues were tested such as prior boiling and submersion in full-strength, concentrated ethanol. Finally, the use of aqueous SO_2 to prevent oxidation was also tested. Boiling the specimen preserved and enhanced its green colour, but caused structural damage, compromising the specimen's integrity. Prior immersion in 95% ethanol removed the



A. Most saturated and least saturated results for Pea leaves after 49 days in 70% ethanol. Prior fixation with various copper salts in water or FAA.

B. Triplicats of pea leaves in ethanol 70%, no colour fixation (left) and $CuSO_4$ fixation (right) after 3 days of light exposure.

Figure 3: Results for Pisum sativum*: A. specimen colour for all tested recipes, B. comparison of specimens stored in ethanol, without and with Cu-salts treatment (dissolved in water). Figure adapted from Latty (2021). ©HE-Arc 2024*

hydrophobic barrier from the leaves, allowing for a more uniform diffusion of the $CuSO_4$ into the specimens. The addition of 5% aqueous SO_2 solution ($SO_{2(aq)}$) as an antioxidant in the 5% $CuSO_4$-water solution enhanced the colour stability, however it was again at the cost of the structural integrity of the specimen. The use of a vacuum pump should also help the penetration of the $CuSO_4$ but could not be explored in this work.

Yellow pigment

The results obtained with the Scully B solutions (Scully 1937) are rather good, and despite a small dulling of the colours, the specimens are still stable and recognizable after 90 days (and after 3 years).
The original Verma 2-recipe (Verma, 2008) requires boiling the specimen in the solution for better colour retention. However, since prior tests demonstrated that boiling compromised the physical integrity of the specimens, making their manipulation outside the fluid perilous for the tissues, the Verma 2 recipe was tested without the boiling step. Even with this modification this method still performed better than the Scully B procedure. The plants keep a vibrant yellow colour during 90 days after the preparation, and are still showing recognisably yellow colour three years after. The specimens floated in the fixation and in the preservation fluids for about 10 days before sinking to the bottom. Using a vacuum pump could help accelerate the diffusion of the fluid and thus further improve the preservation of the colours while probably also avoiding the floating of the specimens.

Yellow yarrows preserved in the ethanol 70% for control showed that both the chlorophyll and the yellow pigment leached, and the specimen stiffened. The flowers were completely bleached after the 90 days of monitoring and the plant stem had browned (Figure 4).

Blue-red pigments

The Wagstaffe & Fidler 4 recipe (Wagstaffe & Fidler 1968) preserved the colour perfectly (Figure 5A), despite showing similar specimen's flotation issues as the Verma 2-recipe. However it embrittles the specimens making them very vulnerable for breakage during manipulation (Figure 6A). It is therefore one of the most suited options to prepare fresh specimens for exhibition but is not recommended for frequently handled specimens.

The Kew mixture (Bridson & Forman 1998) preserves the red verbena's colour, even if a pale pink colouration of the fluid can be observed. However, it failed in preserving the blue and purple pigments. This is probably because these pigments are less stable and highly soluble in alcohol (Figure 5B). This recipe would therefore be a good choice for long term preservation of plant tissues, keeping in mind that some degree of colour loss is inevitable.

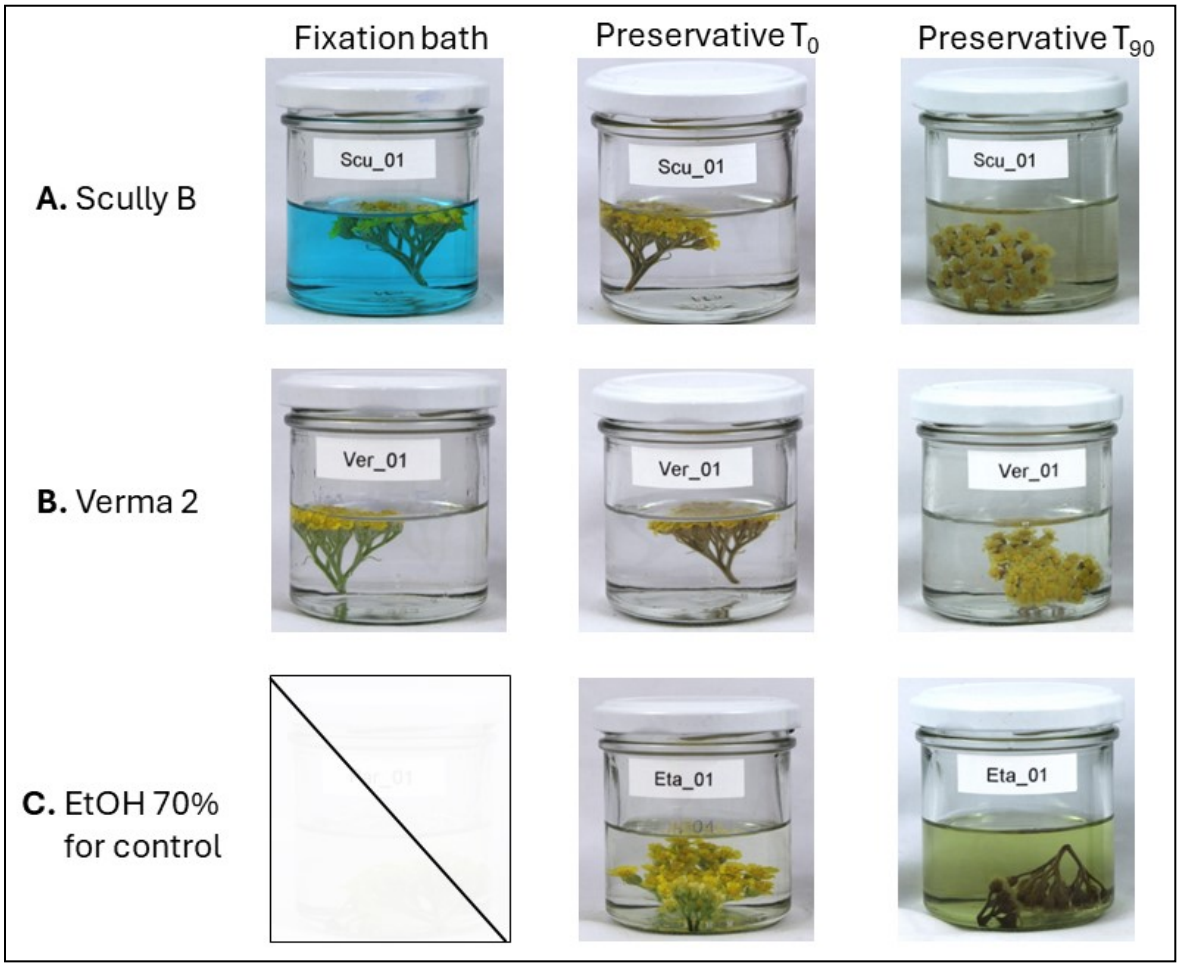


Figure 4. Overview of the tests for yellow colour preservation. ©HE-Arc 2024

The Copenhagen solution (Tredwell 2006) is a widely used recipe in botanical collections supposed to be effective at preserving colours. However, the tests carried out on fresh specimens did not yield satisfying results (Figure 5C). All flowers lost their colours within days and almost as fast as the control specimens in 70% ethanol (Figure 5D).

Tannins

The solution of 70% ethanol saturated with $SO_{2(aq)}$ as an antioxidant avoided the subsequent browning of the specimens but developed a noticeable pink hue just one day after preparation (Figure 7 A1), indicating that anthocyanin pigments of the flower petals leached into the fluid. The colouration of anthocyanins can be modified by adjusting the pH of the solution (Figure 7 A2). The pH of the antioxidant solution as prepared is aimed to be 1.8 to obtain enough $SO_{2(aq)}$. Another solution was tested, saturated with sodium bisulphite in 70% ethanol but without pH adjustment, giving as a result a pH of 4.7 (Figure 7 A2). With increasing pH the anthocyanins normally turn more purple and less red. However, the solution turned out to be colourless, probably due to the reduction of the flavylium cation by the bisulphite anion (Morata et al., 2019), or the formation of a hemiacetal form that usually develops in hydroalcoholic conditions (He et al, 2012). Both are colourless compounds. It should be noted, however, that the fluid will turn back to pink through the continuous drop of the pH to below 3, which also indicates that alcoholic sodium bisulphite solutions are highly susceptible for pH-shifts and should be buffered to a pH>3 to stabilize them. As the pH fluctuates, the $NaHSO_3$ salt can partially precipitate, causing a slight lowering of the transparency of the fluid.

Impregnating the specimens with paraffin was not satisfactory, because the paraffin slowly leached with the darkened pigments into the preservation fluid (Figure 7 B). While preserving the specimens in paraffin prevents opacification of the fluid (Figure 7 D1), the specimens subsequently darken and make them susceptible for mould growth

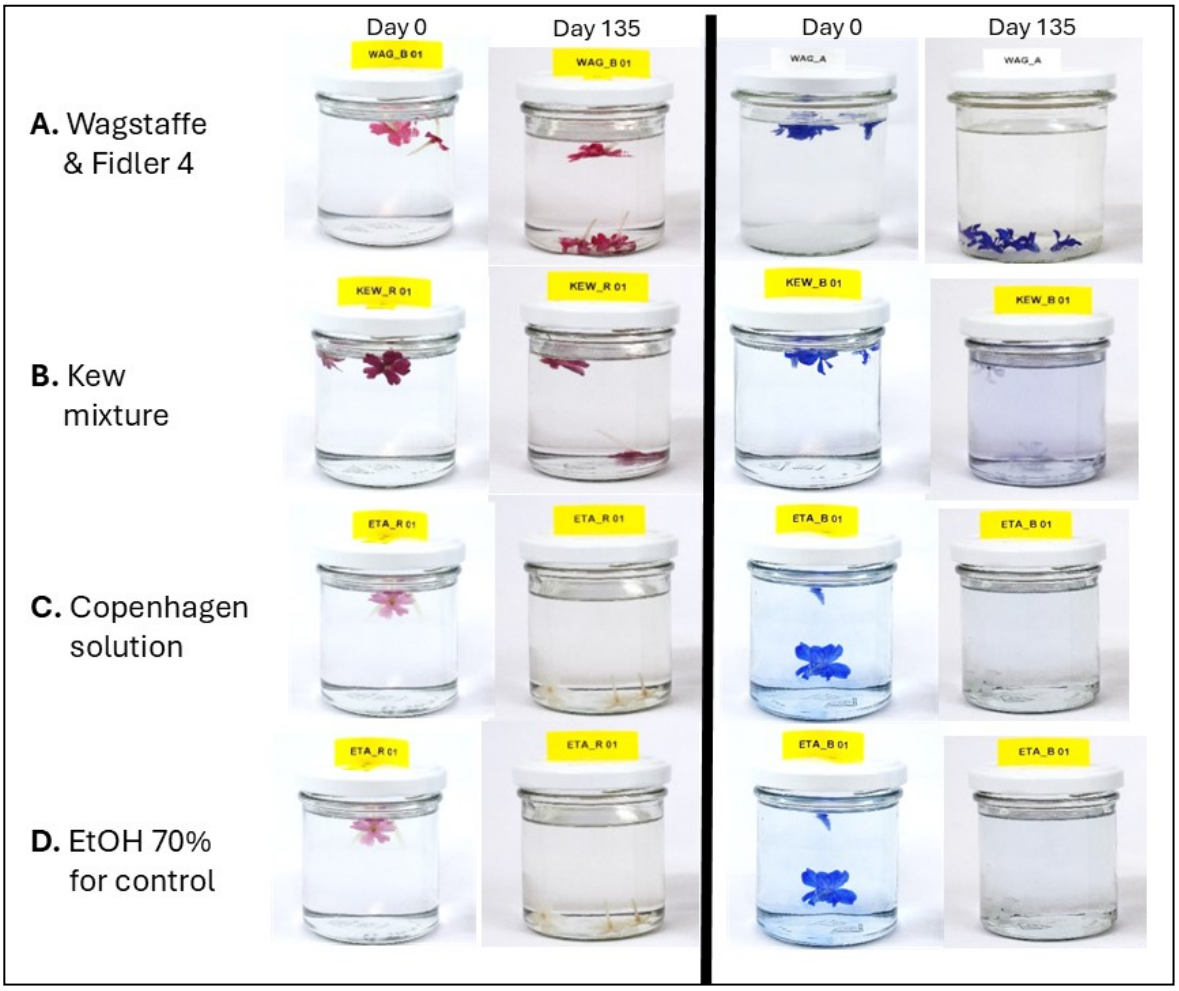


Figure 5. (Above) Overview of the tests for red and blue colour preservation. ©HE-Arc 2024

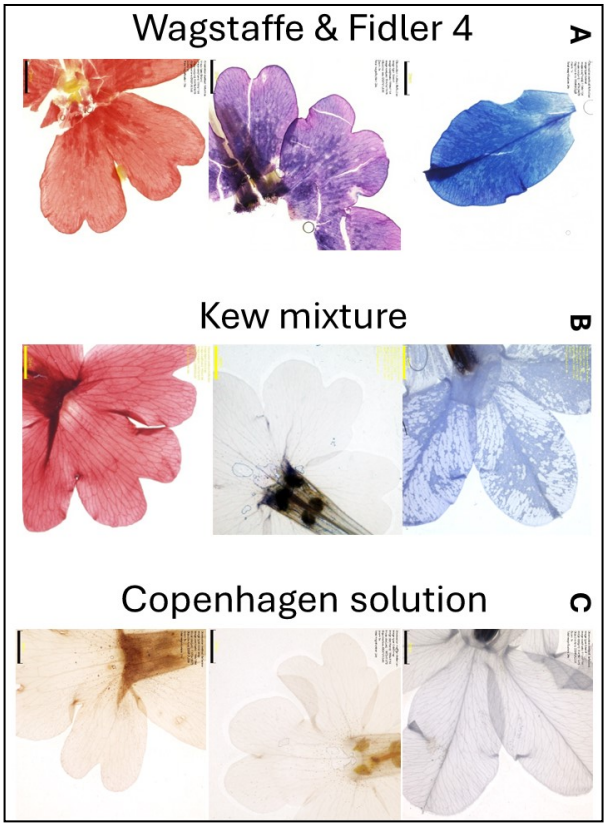


Figure 6. (Right) Optical Microscopy image, Olympus DSX100, x24 magnification. Blue Lobelia erinus *and red and purple* Glandularia peruviana *after 3 months in A. Wagstaffe & Fidler 4, B. Kew mixture and C. Copenhagen solution. ©HE-Arc 2024*

(Figure 7 D2). The high viscosity of paraffin prevents a thorough and close contact with specimens with a complex surface texture. The resulting moist air space between the specimen and the paraffin can accelerate the development of mould. A slow impregnation and surface wetting with paraffin improves the efficacy of this preservation method.

Prior fixation of the *Pinus* bark with formaldehyde and subsequent transfer into ethanol 70% keeps the preservative fluid clear, unlike the non-fixed control samples (Figure 8 A-B). The different concentration or fixation times revealed no noticeable visual differences, probably because the samples were small in size. The recipes mixing

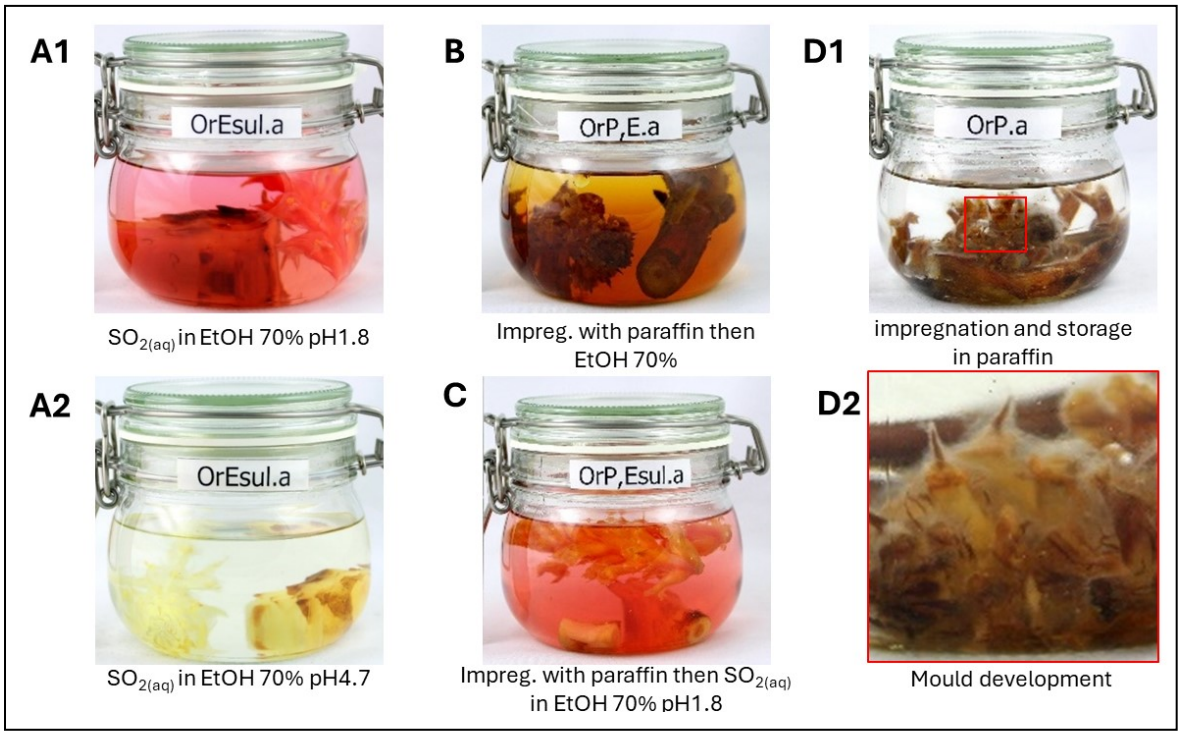


Figure 7. Orobanche hederae *Duby, A1. In $SO_{2(aq)}$ in EtOH 70% at pH 1.8 and A2. pH 4.7, B. Impregnation with paraffin then EtOH 70%, C. Impregnation with paraffin then $SO_{2(aq)}$ in EtOH 70% pH1.8, D1. Impregnation and storage in paraffin, with D2. Mould developing. Figure adapted from Latty 2021. ©HE-Arc 2024*

formaldehyde with phenol cause a slight yellow tint (Figure 8 C) that intensifies with increased concentrations. In addition to worse performances in preventing colouration of the fluid, phenol is highly toxic. Thus, its use should be avoided and we do not advocate for it. Instead, the use of formaldehyde on its own seems more appropriate.

The methods tested are effective to prevent the browning of the preservation fluid, but none preserved the original colours of the specimens. Preparation of fresh specimens therefore requires a choice between accepting the subsequent browning of the tissues because of autolysis or artificial polymerization, and discolouration of less stable pigments in case $SO_{2(aq)}$is added. Like many other light-coloured flowers, the *Orobanche hederae* we used for our tests contains anthocyanins that are prone to pH-induced colour -changes. Highly acidic pH-values turn specimens pink, while less acidic conditions sustain the colourless form(s) of the anthocyanin pigments.

Liquid paraffin, though an interesting alternative for non-toxic preservation fluids was found to be ineffective. It preserved the original colours longer without preventing eventual browning, possibly because it does not stop autolysis or does not diffuse quickly enough into the tissue of specimens. Testing lighter non-polar solvents such as xylene might help to sustain colour information, however, the high toxicity and flammability are major concerns, especially in museums. Using solvents at the beginning of the preservation process and then transferring the specimens to heavier hydrocarbons for long-term preservation could be beneficial, with addition of appropriate antiseptics to prevent microbial growth. This option remains to be tested.

Conclusion

Preserving or maintaining the colour information in botanical specimens in museum collections is to a certain extent achievable when starting with fresh specimens. In the case of historical preparations, the colour loss through leaching and degradation of the pigments is however fully irreversible. Simple application of published recipes does not necessarily return the desired and satisfactory results. Some cases such as the Copenhagen solution or the Kew mixture, though reportedly effective, do not seem to preserve all colours consistently. Some recipes, such as pre-treatment of green specimens with copper salts, lead to chemical transformation and altering of the original chlorophyll pigments, thus changing the natural colour of the specimen even though a colour information close to the original hue can

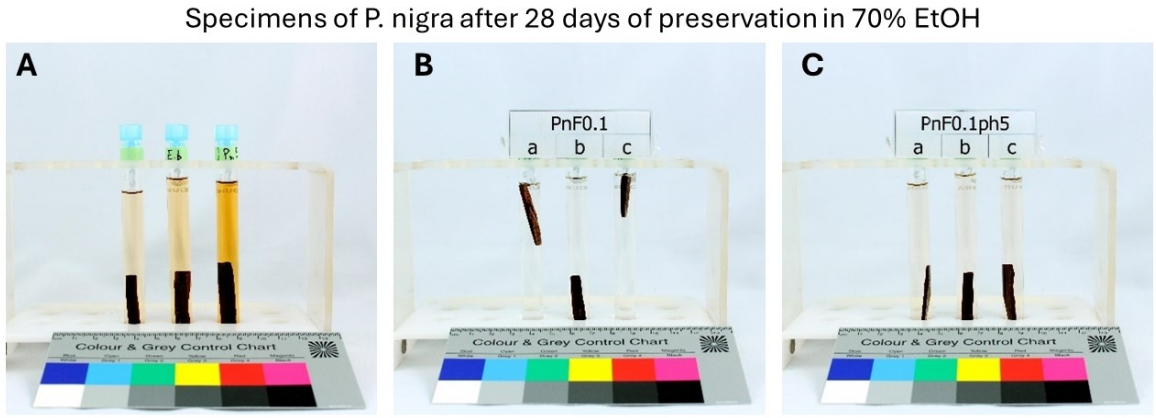


Figure 8. Specimens of P. nigra *after 28 days of preservation in 70% EtOH A No fixation, B. Fixation with formaldehyde, C. Formaldehyde + phenol. Figure adapted from Latty 2021. ©HE-Arc 2024*

be maintained. Wagstaffe & Fidler 4 mixture for blue-red flowers perfectly preserved colour, but may compromise the structural integrity of the specimen, making it fragile and difficult to handle, which causes concern for the long-term preservation and scientific usefulness of the specimens.

The addition of an aqueous solution of $SO_{2\,(aq)}$ to the preservative fluid can prevent the opacification of the preservation fluid in fresh and in historic specimens. However, also this treatment requires a lot of caution. Even if it is essential for maintaining the clarity of the fluid for tannin rich specimens, the use of antioxidants often results in the loss of more delicate pigments such as anthocyanins. It requires a close monitoring of the pH fluid level in specimen jars to avoid precipitation of the salt. It is worth noting that we have no detailed information on how the antioxidant affects future analysis on the preserved tissues.

It is essential to recognize that any intervention during the preparation process inevitably alters the specimen. Each compromise made during preservation comes at the cost of losing certain characteristics, albeit hopefully outweighed by the preservation of more valuable ones. Therefore, a deep understanding of the intrinsic values associated with botanical specimens, their intended use, and their characteristics is paramount when making decisions regarding their preservation. Thus, maintaining the colour of a specimen might not always be worth compromising on other aspects. Nevertheless, some recipes such as the Wagstaffe & Fidler 4 mixture for blue and red flowers, or the addition of antioxidants to tannin-rich specimens, would be a great option for preparations dedicated for display.

Finally, the design and use of individual preservation recipes tailored for specific specimens within an institution may not always be financially or practically viable within the constraints of their budget and resources. This underscores the necessity of employing sound judgment when selecting preservation methods, ensuring that the chosen approach is in line with the goals of the institution as well as with the maintaining and management of its collections. It is also crucial to document and file any interventions made during the preservation process. Transparency regarding these interventions is vital, as it enables researchers to accurately interpret and contextualize their findings when studying the specimens in the future.

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Appendix 1: Summary of pigments and characteristics (translated from Latty 2021 [FR])

Family	Hue	Main location	Stability (qualitative categorization)	Solubility
Chlorophyll		Leaves	-	Liposoluble
Carotenoid		Leaves	+	Liposoluble
Flavonol, aurone, chalcone, flavone	UV	Flowers	+/-	Hydrosoluble
Anthocyanin		Flowers	--	Hydrosoluble
Proanthocyanidin		Young growth	+	Hydrosoluble
Phlobaphene		Bark	++	Liposoluble
Dihydropyran		Ligneous tissues	-	Hydrosoluble
Betalain		Roots, flowers	-	Hydrosoluble
Anthraquinone		Roots	+	Hydrosoluble
Naphtoquinone		Bark	+	Hydrosoluble
Indigo		Leaves	+	Liposoluble (oxidized)
Hydrolysable tannins		Bark and ligneous tissues	+	Hydrosoluble

Appendix II: Colour preservation literature (adapted from Latty 2021 [translated from FR])

The following table presents recipes in chronological order. It is not an exhaustive list regarding vascular plants preserved in fluid. Moreover, this table omits most recipes that are specific for algae and mushrooms. It also omits the recipes intended for green plants but not specifically designed for colour fixation or retention. Reading guide:
Code: This is how the recipe is referred to in the paper. It is often the primary source author's name, and if multiple recipes are listed, numbering is used. This cell is coloured following the main specimen's colour target. A brown colour indicates both brown specimen and white ones prone to browning.
Date: Date of publication of the primary source.
Author: Name of the author of the primary source.
Source: When the primary source could not be consulted, a secondary source in which the detailed recipe can be found is briefly mentioned in this column. An extensive list of references is provided in the bibliography.
Type of specimens: The information provided based on the point of view of the predominantly affected pigments rather than specific species (sometimes specified).
Colour retention: Indicates whether the colour preservation method is based more on "fixation" in a first bath prior to the transfer of the specimen in the preservation fluid (= Fix), or if colour retention is achieved by the same fluid used for

long-term preservation (= Cons). In the case where the principle is unclear (usually in the case of non-detailed recipes), a "?" is used.
Antioxidant: If mentioned, the means of removing oxygen, or generally preventing oxidation, is indicated in this column. The methods can be physical (vacuum), or chemical (addition of sulfites in the solution).
Preservative: Clarification on the preservation fluid, if different from the fixation one, otherwise "Same".

Code	Date	Author	Source	Type of specimens	Colour retention	Antioxidant	Preservative
Cleghorn	1838	Cleghorn	Verma	Coloured plants	?	-	Same
Amann 2	1896	Amann	-	Green plants, mainly algae and bryophytes	Cons	?	Same
Woods	1897	Woods	-	Green plants (also preserves yellows, browns, etc.)	Fix	Physical	Same without Cu, or other than full-strength EtOH
Trail	1908	Trail	-	Green plants (not too fragile, as boiling)	Fix	Physical	Formaldehyde or EtOH base
Strasburger	1911	Strasburger	Butler	Monotropes	?	Sulfites	?
Jones	1917	Jones	Verma	Green plants	Fix	Physical	?
Butler 1	1918	Butler	Trail	Green plants with dark pathologies	Fix	Physical	Formaldehyde
Butler 2	1918	Butler	Strasburger	Colourless or pathological specimens with liposoluble pigments	Fix	Sulfites in fixation	Formaldehyde
Cr. & Ch. 1	1922	Cruess & Christie	Adriano & Yonzon	Yellow fruits and vegetables	Cons	Sulfites in preservation	Same
Cr. & Ch. 2	1922	Cruess & Christie	Adriano & Yonzon	Red and multicoloured fruits and vegetables	Cons	Sulfites in preservation	Same
Maltby 1	1926	Maltby	Verma	Green parts	Cons	Sulfites in preservation	Same
Maltby 2	1926	Maltby	Verma	Green parts	Fix	Physical	?
Maltby 3	1926	Maltby	Verma	Other colours?	?	?	?
Keefee	1926	Keefe	-	Green plants	Fix	-	Same, drying, other?
Mackenzie 1	1928	Mackenzie	Verma	Green leaves	Fix	Sulfites in fixation and preservation	?

Code	Date	Author	Source	Type of specimens	Colour retention	Antioxidant	Preservative
Mackenzie 2	1928	Mackenzie	Verma	Pears, quinces, and soft fruits	?	Sulfites	?
Mackenzie 3	1928	Mackenzie	Verma	Red apples, or yellow or green ones with red spots	Cons	Sulfites in preservation	H2O + sulfites
Ni. & Sl.	1928	Nieuwland & Slavin	-	Monotropes	Cons	Sulfites in fixation and preservation	Same or xylene
Eckert	1931	Eckert	-	Green algae (Intended for microscopy)	Fix	-	-
Ad. & Yo. 1	1933	Adriano & Yonzon	-	Green fruits and vegetables	Fix	Sulfites in preservation	H2O + sulfites
Ad. & Yo. 2	1933	Adriano & Yonzon	-	Yellow or white fruits and vegetables	Cons	Sulfites in preservation	Same
Ad. & Yo. 3	1933	Adriano & Yonzon	-	Red or multicoloured fruits and vegetables	Cons	Sulfites in preservation	Same
van Steenis 1	1935	van Steenis	-	Parasitic plants	Cons	Sulfites in preservation	Same
van Steenis 2	1935	van Steenis	-	Orange fruits of Gonocaryum	Cons	?	Same
Blaydes 1a	1937	Blaydes	-	Green plants, slow fluid penetration	Fix	Physical	FAA, 70% EtOH or other
Blaydes 1b	1937	Blaydes	-	Green plants	Fix	Physical	FAA, 70% EtOH or other
Blaydes 2	1937	Blaydes	-	Green plants	Fix	-	Same?
Scully A	1937	Scully	-	Green plants (with yellow flowers)	Cons	Sulfites in preservation	Same
Scully B	1937	Scully	-	Green plants with yellow flowers	Fix	Sulfites in preservation	H2O + sulfites
Johansen 1	1940	Johansen	-	Green plants?	?	-	Same
Johansen 2	1940	Johansen	-	Green plants	Fix	Physical	?
Johansen 3	1940	Johansen	-	Green plants	Fix	-	?
Johansen 4	1940	Johansen	Blaydes	Green plants	Fix	-	?
Johansen 5	1940	Johansen	-	Green plants	Fix	Physical	70% EtOH + 5% glycerol

Code	Date	Author	Source	Type of specimens	Colour retention	Antioxidant	Preservative
Johansen 6	1940	Johansen	Keefe	Green plants	Fix	-	?
Chandrashekar	1958	Chandrashekar	Verma	Green plants and others	Fix	-	Formaldehyde
Wa. & Fi. 1a	1968	Wagstaffe & Fidler	-	Green plants	Fix	Sulfite in fixation	Formaldehyde
Wa. & Fi. 1b	1968	Wagstaffe & Fidler	-	Green plants	Fix	-	Formaldehyde
Wa. & Fi. 1b'	1968	Wagstaffe & Fidler	-	Green plants	Fix	Physical	Formaldehyde
Wa. & Fi. 1c	1968	Wagstaffe & Fidler	Keefe	Green plants	Fix	-	Formaldehyde
Wa. & Fi. 2	1968	Wagstaffe & Fidler	-	Red apples and other red fruits	Cons	-	Same
Wa. & Fi. 3	1968	Wagstaffe & Fidler	-	Yellow apples with red spots	Cons	Sulfites in preservation	H2O + sulfites
Wa. & Fi. 4	1968	Wagstaffe & Fidler	-	Red and blue flowers	Cons	Thiourea in preservation	Same
Knudsen 1	1972	Knudsen	-	Green plants	?	?	?
Knudsen 2	1972	Knudsen	Scully	Flowering plants, mostly yellow	Fix	Sulfites in preservation	H2O + sulfites
Ha. & Di. 1	1985	Hangay & Dingley	-	Ferns and "seed plants"	Cons	-	Same
Ha. & Di. 2	1985	Hangay & Dingley	-	Green plants	Fix	?	?
Ha. & Di. 3	1985	Hangay & Dingley	-	Green plants	?	Physical	?
Ha. & Di. 4	1985	Hangay & Dingley	-	Green plants	Cons	Sulfites in preservation	Same (except Na silicate used alone before)
Ha. & Di. 5	1985	Hangay & Dingley	-	Green plants	Fix	Sulfites in fixation	Formaldehyde
Ha. & Di. 6	1985	Hangay & Dingley	Knudsen	Coloured plants (green with yellow flowers)	Fix	Sulfites in preservation	H2O + sulfites
Viswanathan et al.	1991	Viswanathan et al.	-	Green plants	Cons	-	Same
Moore	1999	Moore		multiple recipes	multiple recipes	multiple recipes	multiple recipes
Verma 1	2008	Verma	-	Green plants	Fix	-	Propionic acid, formaldehyde
Verma 2	2008	Verma	-	Yellow flowers	Cons	-	Same

Investigating the use of propylene phenoxetol preservation methods in natural history museums

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Abstract

Fluid preservation of organic material requires an initial fixing to prevent tissue breakdown and decomposition, commonly achieved using an aqueous solution of formaldehyde, or using ethanol. Post-fixation, specimens are frequently preserved in solutions of either 4% to 10% formalin, or 70-80% ethanol. Specialist fluids include Steedman's method using propylene phenoxetol (PP), developed for use with small marine invertebrates. Steedman's method was subsequently applied to a much wider range of zoological collections with varying reports of success. In more recent years the use of Steedman's and PP has been questioned and it is now considered inappropriate for long-term storage. Despite this, an audit of the fluid-preserved collections at the Cole Museum of Zoology (REDCZ) showed that many specimens preserved in PP remain in good condition after almost twenty years.

A survey was distributed via the Natural History Collections and Natural Sciences Collections Association mailing lists to determine the variety of preservation fluids used in museum collections worldwide. Only half of the 35 respondents were aware of the recent recommendation to monitor or remove Steedman's from their collections, and only two institutions had already followed this advice. Follow-up interviews with survey participants revealed wider systemic issues that prevent a deeper knowledge of fluid-preserved collections or ability to take appropriate action. These included a lack of financial resources, staffing gaps leading to a loss of institutional knowledge, a lack of suitable laboratory workspaces, and limited to no access to beneficial technology.

Keywords: Steedman, propylene phenoxetol, fluid preservation, zoology museum, fixation, preservation

Introduction

The role of a caretaker of fluid-preserved biological collections is to maintain their specimens in as good a condition as possible, for as long as possible, and to maximise the ways in which these collections can be used for teaching, research, and display.

Techniques used to preserve animal specimens in fluid have not changed drastically from those developed over a hundred years ago (Simmons, 2020). The preservation of whole animals or tissue samples requires an initial fixing to prevent tissue breakdown and decomposition. This is commonly achieved by immersing the specimen into a 4% solution of formaldehyde (10% Formalin – see Appendix II: Formulae) which hardens the

tissues by denaturing or cross-linking chains of proteins. The fixation process takes time, which is variable depending on the type and size of the specimen that is being fixed. Once fixed, specimens are then commonly preserved in aqueous solutions of either 2.5% formaldehyde (5% Formalin), or 70-80% ethanol (Simmons, 2014). Other preservation methods may be used for specific purposes such as tissue clearing, or the stabilisation of particularly delicate specimens (Harris, 1990).

The discovery of formaldehyde (a 37% aqueous solution of formaldehyde gas) as a fixative in the 1890s (Blum, 1893) was enthusiastically espoused by the fluid-preservation community, but it took more than eighty years to discover that long-term exposure to this compound could have not just irritant and sensitising, but also potentially carcinogenic effects on those working with it (Chemical Industry Institute of Toxicology, 1979). Phenoxetol, in varying forms, has been suggested several times as a safer alternative to the use of formaldehyde-based preservatives (Tandon *et al*., 2014, Frølich *et al*., 1984), but in the long term it has not proved to be universally reliable, and has therefore not been widely accepted in the same way that formaldehyde was.

Zoological specimens which have been fixed in formaldehyde, and preserved in either formaldehyde or ethanol, tend to lose much, or almost all, of their natural colour. This can make taxonomic and morphological research difficult, if not impossible. While periodic attempts to maintain colour in fluid-preserved collections have been made (Harris, 1990), it is generally held to be extremely difficult because of the biochemical processes taking place between the specimen and the preservation medium (Stoddart, 1989). Fluid-based preservation methods designed to retain the colours of skin and tissue were developed in the late 19th century by pathologists, including Leonhard Jores and Carl Kaiserling (Jores, 1896, Kaiserling, 1897). Several studies claimed that while good colour had been maintained using these methods for 15-20 years (Jores, 1896), colour degradation attributed to haemolysis had later been observed in those same specimens (Pulvertaft, 1950). However, these methods were developed for specimens where colour was mostly related to haemoglobin. This limits their usefulness in a wide-ranging zoological collection which contains animals that use different respiratory pigments such as haemocyanin or chlorocruorin.

The latter half of the twentieth century saw a resurgence in the development of new methods with the aim of improving colour retention in fluid-preserved specimens. These included work with hydrosulphites (Wentworth, 1957), a survey of potentially useful antioxidants (Gerrick, 1968), and Hugh Steedman's method based on propylene phenoxetol (PP) that was originally designed for preserving marine zooplankton (Owen and Steedman, 1956).

During the 1960s, propylene phenoxetol came into use as a preservation method in some UK museum collections, and Steedman introduced the addition of propylene glycol to propyelene phenoxetol in 1976. (Moore, 1997). However, by the end of the 1980s, some specimens were found to have been insufficiently well preserved by Steedman's method (Crimmen, 1989). In 2022 it was recommended that smaller institutions holding fluid-preserved biological collections should no longer use Steedman's Post-Fixation Preservative as a long-term preservation solution, and that larger institutions and those with the facilities to do so, should monitor their Steedman's specimens monthly for signs of deterioration (Neumann *et al*., 2022).

There are several different phenoxetol-based preservation methods that may be used in fluid-preserved museum collections (Neumann *et al*., 2022), but the one used by the Cole Museum of Zoology (REDCZ) from the early 2000s to the present is the formula given in chapter five of *Care and Conservation of Natural History Collections* (Carter and Walker, 1999) (Table 1).

Table 1: Steedman's Fixation and Preservation Formulae

Steedman's Fixative	**Steedman's Post-Fixation Preservative**
100ml propylene phenoxetol dissolved in 500ml propylene glycol	50ml of propylene phenoxetol dissolved in 500ml of propylene glycol
Add 500ml formaldehyde 37% (Formalin)	
Dissolve 110ml of concentrate in 890ml of distilled water or saline	Dissolve 110ml of concentrate in 890ml distilled or deionized water
pH 6.8-7	pH 7-7.4

There are two parts to the Steedman's process – the first is a fixation concentrate which is made up of propylene phenoxetol, propylene glycol, and formalin, in distilled water or saline solution. The second part is Steedman's Post-Fixation Preservative, which is what is present in the Cole Museum's collection. This does not contain either ethanol or formalin. Instead, it makes use of propylene phenoxetol for its antibacterial and anti-fungal properties, and propylene glycol as a humectant. This keeps the specimens softer and less brittle than other preservatives, which was particularly important to Steedman as he was working primarily with zooplankton and other small marine invertebrates.

Rather than being restricted only for use with similarly small and delicate specimens, Steedman's methods began to be applied to a much wider range of zoological collections. In the early days of its use, the Natural History Museum in London was aware of problems with specimens that had not been properly fixed in formaldehyde before being treated with phenoxyethanol (Nakanishi *et al*., 1969) – something that Steedman was very clear about in his subsequent work (Steedman, 1976). In more recent years the use of Steedman's post-fixation fluid has been questioned, and propylene phenoxetol in general is now considered inappropriate for the long-term storage of fluid-preserved specimens, particularly those of a large size (Neumann *et al*., 2022). This is partly due to the unexpected decomposition of a large (~200 gallons) tank of fishes at the Natural History Museum in London, which had been kept in Steedman's post-fixation preservation fluid (Crimmen, 1989). The Cole Museum currently holds 52 specimens preserved in Steedman's propylene phenoxetol-based preservation solution. Some of these are large fish and densely muscled mammals, which have remained in good condition since their transfer into PP during the early 2000s. It is also being used successfully in modern collections of marine specimens, including the Discovery Collections at the National Oceanography Centre.

The purpose of this paper is to re-examine the use of Steedman's and PP in natural history collections and to determine why the Cole Museum's specimens preserved in PP remain in good condition after almost twenty years in that solution. Those in other museums deteriorated after less than fifteen years (Crimmen, 1989), so this is an ideal time to be carrying out an in-depth investigation into the condition of these specimens. The working hypothesis is that because almost all of the Cole Museum's Steedman's specimens spent up to six decades in other preservation fluids before their transfer to PP, this may have improved their longevity compared with specimens that were both fixed and preserved solely by Steedman's methods.

In order to understand whether the Cole Museum's experience with Steedman's was representative of museum collections overall, a survey was developed to determine the prevalence of Steedman's, and its primary ingredient propylene phenoxetol, in fluid-preserved museum collections across the world (Appendix). This was distributed via the Natural History Collections (NHColl) and Natural Sciences Collections Association (NatSCA) mailing lists, and a link to the online survey was also provided via a QR code at the 2024 NatSCA conference.

Cole Museum survey

The Cole Museum's fluid-preserved collection numbers around two and a half thousand specimens, and 52 of those are currently in Steedman's Post-Fixation Preservative. Additionally, there is a Teaching Collection of around one and a half thousand jars, most containing multiple specimens. In 2007-8 there was an extensive programme of moving many of these specimens out of formaldehyde and into Steedman's, which was probably carried out to make them safer for study and maintenance by undergraduate students. Steedman's specimens make up around 29% of the Teaching Collection, so the total number of Steedman's-preserved specimens across both of the Cole Museum's fluid -preserved collections is approximately 500 jars. Following the SPNHC best practice recommendation, an assessment of the fluid-preserved collections was carried out (Neumann *et al*., 2022).

Cole Museum survey: results

Given that Steedman's preservation methods were developed for use with marine zooplankton, it was expected that marine specimens would be represented in greater numbers in this fluid (Table 2). In fact, mammals represented the largest individual class with a total of 13 specimens. The Steedman's collection overall is made up of 30 vertebrates (including 12 fishes), and 22 invertebrates. This represented a much wider taxonomic range than originally anticipated, which reflects the diversity of the Cole Museum's comparative anatomy collection.

Table 2: Steedman's-preserved specimens, by phylum and class

Phylum	Class	Number of specimens
Chordata	Actinopterygii	5
	Amphibia	2
	Ascidiacea	1
	Aves	1
	Chondrichthyes	6
	Dipnoi	1
	Mammalia	13
	Reptilia	1
Cnidaria	Anthozoa	5
	Myxosporea	1
	Scyphozoa	1
Echinodermata	Asteroidea	2
	Echinoidea	1
Mollusca	Bivalvia	1
	Gastropoda	4
Nematoda	Chromadorea	1
Porifera	Calcarea	1
	Demospongiae	2
Platyhelminthes	Cestoda	1

Most of the Cole Museum's Steedman's specimens have been moved through several different preservation fluids over many decades. Table 3 shows the progression of changes in fluid at two periods of recorded changes – the early 1960s, and the early 2000s. From at least the 1970s until the 2010s, the museum and teaching collections were maintained by technical staff with little oversight by the academic curators. This was because the specimens were seen purely as a teaching collection maintained by teaching technicians. There is no documentation to explain the reasoning behind the decision to transfer large numbers of specimens out of one fluid and into another, or the process by which this was carried out, but archives show that there was a trend during the 1960s for moving specimens out of spirit and into formaldehyde. More recently there was a short period, in 2007-8, of transferring formaldehyde specimens into Steedman's because of Health and Safety concerns. This appears to have been trialled on the Teaching Collection, which now has more than four hundred jars containing Steedman's specimens.

Examples of good and poorly preserved specimens in Steedman's

The general guidelines for Steedman's are that it should not be used for large specimens, and that it

Table 3: Progression of changes in preservation method of the Cole Museum's Steedman's-preserved specimens. Where the strength of a solution is not given, this was not documented in the archives.

Original Preservative 1909-1953	1960s Preservative	2000s Preservative	2024 Preservative	Number of specimens
Not recorded	Not recorded	Steedman's	Steedman's	1
Formaldehyde 2.5%	Formaldehyde 2.5%	Steedman's	Formaldehyde 2.5%	3
Formaldehyde 2.5%	Formaldehyde 2.5%	Steedman's	Steedman's	13
Formaldehyde & glycerol	Formaldehyde & glycerol	Steedman's	Steedman's	1
Methyl salicylate (Oil of Wintergreen)	Methyl salicylate (Oil of Wintergreen)	Steedman's	Paraffin	1
Spirit 70%	Formaldehyde 2.5%	Steedman's	Formaldehyde 2.5%	2
Spirit 70%	Formaldehyde 2.5%	Steedman's	Steedman's	29
Propylene Phenoxetol 1%	Propylene Phenoxetol 1%	Steedman's	Steedman's	1
Dry	Unknown	Steedman's	Steedman's	1

Figure 1a: Rhesus monkey, Macaca mulatta, *REDCZ 2957; 1b: Brown trout,* Salmo trutta, *REDCZ 2188; 1c: Pigeon,* Columbia livia, *REDCZ 2861 Images © The Cole Museum of Zoology*

is inappropriate for densely muscled animals (Moore, 1997). Simon Moore suggests that dense muscle tissue may form a barrier which prevents phenoxetol from entering a specimen, thereby creating only a surface level of preservation. This would allow the specimen to deteriorate from the inside out (Moore, 1997).

While many of the Cole Museum's Steedman's specimens are not large or densely muscled, there are also examples of such specimens that have remained in good condition. Figures 1a and 1c are dissections of the superficial muscles of a rhesus monkey's leg (REDCZ 2957), and a pigeon (REDCZ 2861). Figure 1b is a brown trout with the ovary dissected to show the eggs (REDCZ 2188), and this specimen is a relatively large one at 41 cm tall and 16.4 litres in volume. Despite being both large and densely muscled, there are no signs of turbidity or tissue degradation in these specimens which might suggest they are at immediate risk of damage due to their preservation method, although it is possible that unseen deterioration is taking place inside the specimens. All three were originally preserved in 'Spirit 70%' in the 1930s and 40s, changed to formaldehyde during the 1960s, and moved into Steedman's in 2007-8. It seems likely that the decades which these specimens spent in formaldehyde are a contributing factor to the stability and good condition of the Cole Museum's Steedman's-preserved specimens, in part due to the residual formaldehyde that remains even after the fluid has been changed (Waller and Simmons, 2003).

The trout in Figure 1b, for example, was originally preserved in 70% spirit in 1931. It was changed over to 2.5% formaldehyde in 1965, and then moved into Steedman's at some point prior to 2020. This is the most recent point at which there is documentation about the specimen's conservation treatment. A deteriorating jar seal was replaced, and the fluid was topped up following the resulting evaporation. The loss of preservative fluid due to evaporation can also cause deterioration of specimens, which may then be incorrectly attributed to the preservative itself.

However, not all of the Cole Museum's specimens that were transferred into Steedman's are currently in good condition. A long-eared bat (REDCZ 109 (duplicate)) (Figure 2) that was stored in Steedman's post-fixation preservative, has unexpectedly deteriorated. Although records show that the bat was in good condition in October of 2021, two months later, in December 2021, it was discovered that the most delicate areas of tissue, including the wings, tail, and long ears, had become detached and fallen to the bottom of the jar. The bat was removed from its jar as carefully as possible, and the fallen tissue was retained along with a sample of the fluid. It was re-fixed with 4% formaldehyde and preserved in 2.5% formaldehyde, and this appears to have prevented any further tissue loss.

Figure 2: Long-eared bat, Plecotus auritus*, REDCZ 109 (3/3 with this number), following re-fixation with 5% formaldehyde and preservation in 2.5% formaldehyde Image © The Cole Museum of Zoology, 2024*

The sudden degradation of this specimen is unlikely to be related to dense muscle, since it was the thin wing membranes that have been lost. An alternative hypothesis is that specimen breakdown is related to the lipophilic properties of phenoxetol. Andries van Dam has suggested that phenoxetol migrates towards the lipid-containing parts of the specimen, leaving the surrounding fluid unbalanced, and the less fatty parts of the specimen (e.g. the fins of The Natural History Museum's fishes, and the wings and ears of the Cole Museum's long-eared bat) open to microbial attack (van Dam, 2003). However, there was no evidence of microbial attack to these specific areas of the bat, and van Dam's hypothesis does not address the other signs of deterioration that were seen in the Natural History Museum's specimens (Crimmen, 1989). Checking the fluid sample retained from the bat for phenolic compounds might give an indication as to what extent the propylene phenoxetol had broken down (Carter, 2024). Fourier Transform Infrared (FT-IR) Spectroscopy will be performed on samples to verify this.

The two other issues that have been noted with the Cole Museum's Steedman's specimens are particulates recorded in the fluid, and detachment from their glass backing plates. Five specimens had become detached from their glass mountings. Four of these had originally been tied to the backing plate with an undocumented type of thread, and they were re-attached in 2007 or 2008, with nylon monofilament (REDCZ 738, 2298, 2340 and 2802). It is not clear how long these specimens had been preserved in Steedman's before their detachment and whether this had caused any softening of the tissues which allowed the original mounting thread to tear through the specimens, or whether it was

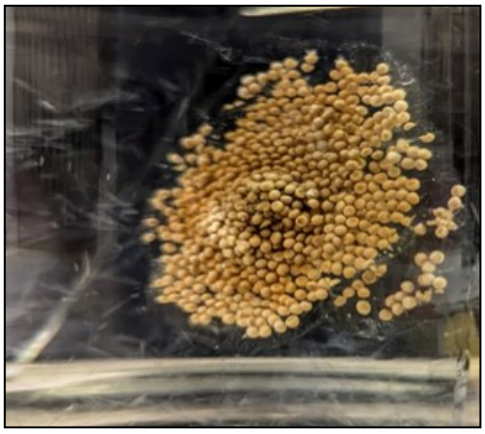

Figure 3: Eggs of albino common frog, Rana temporaria*, REDCZ 2298 Image © The Cole Museum of Zoology*

more likely to be a problem caused by using monofilament thread to mount already soft specimens. It is possible that using a softer and more flexible material, such as cotton or linen thread, could have prevented or minimised this damage. However this would be more visible, which is why monofilament is often preferred.

The fifth specimen, the eggs of an albino frog (REDCZ 2298) were originally fixed in 2.5% formaldehyde (in 1932) and were later moved into Steedman's. In 2007 a long-overdue audit noticed that they had all fallen from the glass plate to the bottom of the jar following the failure of the adhesive used. They were likely to have been attached with gelatine, which forms an effective adhesive when used with formaldehyde-preserved specimens (Carter and Walker, 1999). After the move into Steedman's, the attachment probably weakened. They were reattached with gelatine in 2007 and transferred back into formaldehyde. They remain attached in 2024.

Fixation in fluid-preserved specimens has always been synonymous with firmness, so soft tissue in specimens is generally taken to mean loss of structural integrity and therefore inadequate fixation (Simmons, 2014). Two specimens were removed from Steedman's because they were thought to be improperly fixed due to the softness of the tissues (REDCZ 18, and 2340). These were re-fixed with 5% formaldehyde, and have remained preserved in 2.5% formaldehyde. Specimen REDCZ 18 is one of the Cole Museum's earliest specimens to be accessioned, in 1909. It was originally in 'Spirit 70%' but was transferred to formaldehyde along with many other specimens during the 1960s. As with the albino frog eggs (REDCZ 2298), it was moved from Steedman's into formaldehyde in 2007, when it was also re-

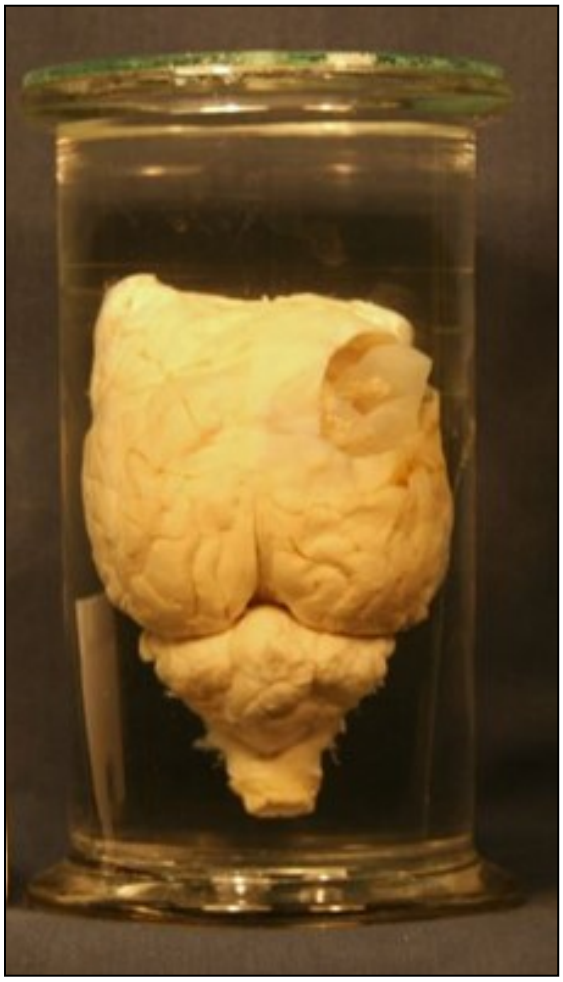

*Figure 4: REDCZ 18, a tapeworm cyst (*Taenia multiceps*) inside the brain of a sheep, following conservation in 2007 Image © The Cole Museum of Zoology*

fixed. The photograph in Figure 4 was taken immediately after this conservation work had been carried out.

This example shows that it is possible that Steedman's can soften previously fixed specimens, as well as keeping them soft when used as a primary preservation method (Frølich *et al*., 1984). It has also been used to keep rehydrated specimens in good condition (Carter and Walker, 1999). Research has not yet been undertaken to determine whether the softness of Steedman's specimens is caused by a modification or even a reversal of the fixation process. These are areas that could benefit from further investigation to determine whether, following appropriate fixation, Steedman's could still be used successfully in the longer term in specimens where softness is a desirable quality. Historic methods of mounting these specimens would also need to be revisited, in order to prevent future damage.

Fluid collections survey

A survey was developed to determine whether the Cole Museum's experiences with Steedman's as a preservation solution were reflected in fluid-preserved collections in other institutions (Appendix). Distributed via the Natural History Collections (NHColl) and Natural Sciences Collections Association (NatSCA) mailing lists, and a QR code at the 2024 NatSCA conference, approximately 1,000 respondents should have been reached through these requests to fill in the survey. During the allotted time period, only 35 individuals completed the survey on behalf of their respective museums. The results therefore represent a qualitative overview of self-selected participants.

Steedman's Post-Fixation Preservative is known by several different names and acronyms. These include Preserving Fluid, PP (propylene phenoxetol), PFP (Post-Fixation Preservative), and 1% (the concentration of propylene phenoxetol in the solution), amongst others. In order to avoid confusion, respondents were asked about Steedman's by name, and about PP as its characteristic ingredient. For the purposes of this analysis, the two have been combined. Other preservation methods were also included in the survey, as these may form the basis of further research about some of the less common preservatives used in fluid-preserved museum collections.

Fluid collections survey: results

The use of Steedman's was most common in European natural history collections, but the 19 respondents from this location made up the largest part of the survey results which has skewed them in this direction. Institutions with multiple types of collections, including comparative anatomy, pathology, and herbaria, were less likely to be aware of Steedman's as a preservation method. Respondents in the United States and Canada were almost entirely unfamiliar with Steedman's or PP as preservation methods, as Steedman's publications were not widely available outside of Europe and the UK. However, these results are an extremely small sample, so this may not represent the wider situation (Figure 5).

Twenty-one respondents were aware of Steedman's, and 13 of these used this preservation method in their own collections (Table 4). Opinions of the efficacy of Steedman's were mixed, with five negative responses, and one very negative. There were five neutral responses, and

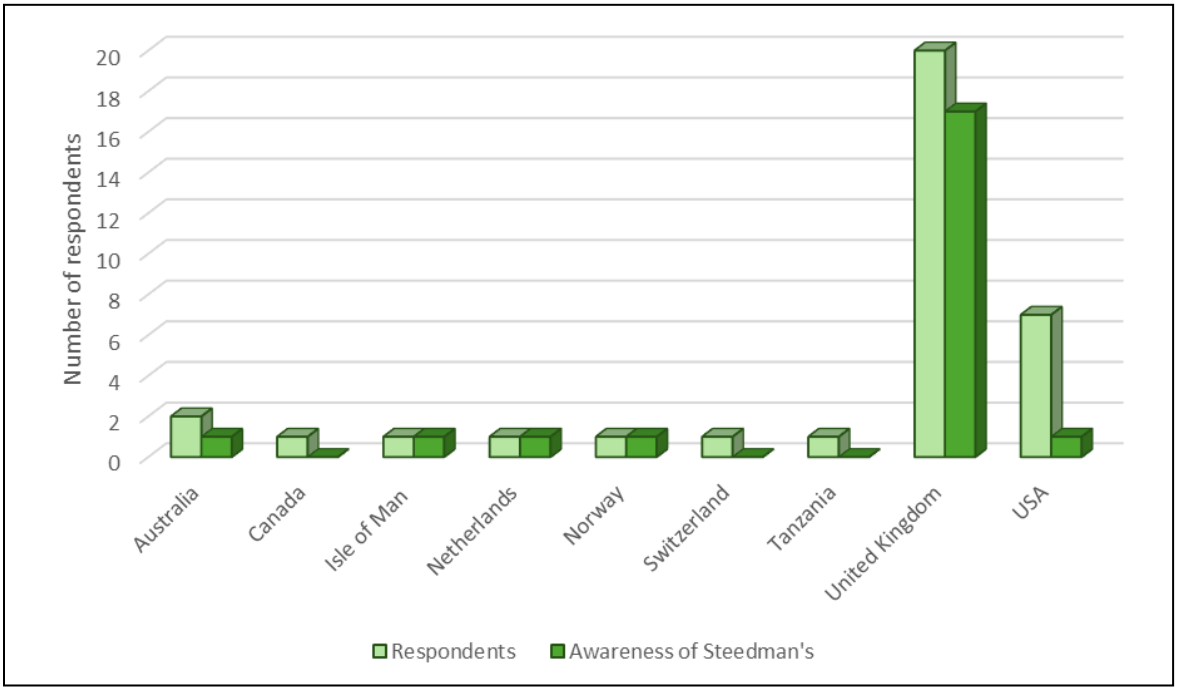

Figure 5: Survey respondents' awareness of Steedman's, by country

	Currently	Historically	No	Don't know	No response
Formaldehyde	26	5	3	0	1
Ethanol / IMS / other spirit	34	1	0	0	0
Steedman's Post-fixation Preservative	5	3	11	10	6
Propylene Phenoxetol (sometimes labelled PP or 1%)	3	6	11	9	6
Propylene Glycol	4	2	9	11	9
Liquid paraffin	2	1	17	7	8
Glycerol	19	1	7	6	2
Glycerol & water	12	0	10	7	6
Jores / Jories	0	1	14	11	9
Kaiserling	6	3	11	10	5
Other	7	3	7	10	8

Table 4: Breakdown of fluids used in natural sciences collections

just two people considered it to be an effective preservative.

Eighteen respondents were aware of the recent recommendation to monitor or remove Steedman's from their collections wherever possible (Neumann *et al*., 2022), and half of these were either currently using or had historically used this method in their collections. Two institutions had carried out the removal process already, three were undecided, and two were making plans to do so in the future. One museum was happy with the quality of their Steedman's specimens and saw no pressing reason to remove it from their collections, and one other institution had purposely moved specimens into Steedman's

rather than out of it. These were both collections of marine invertebrates. The remaining nine respondents did not use Steedman's as a preservative solution in their current collections. Steedman developed his methodology for the study and preservation of marine zooplankton, so it is unsurprising that the institutions whose specimens have been the most successfully preserved in Steedman's are those who hold dedicated collections of siphonophores. These were generally described as being in good condition, although their delicate nature makes them easily damaged by the trawling methods used for their collection. The problem areas illustrated by individual specimens from the Cole Museum were not apparent at other museums – not because their specimens were all in pristine condition, but because a similar audit had not been carried out.

Fluid collections survey: discussion

The survey responses indicated that a variety of preservation methods were used by different types of fluid-preserved biological collections, often for specific purposes. Glycerol, for example, is used not only for tissue clearing and alizarine preparations, but also for the preservation of teeth. Kaiserling is commonly (but not exclusively) used in pathology collections, who may also use methyl salicylate (oil of wintergreen) and turpentine. Fluid-preserved botanical specimens are often stored in Kew Mix, and sometimes temporarily transferred into formaldehyde-free Copenhagen Mix for work that requires handling by researchers. Entomology collections may use Güell & Mendel's Beetle Relaxing Fluid for preservation as well as preparation (Mendel, 1993), and propylene glycol is sometimes used as an additive to ethanol, to prevent embrittlement in small arthropod specimens (See Appendix 2 for formulae). When looking more widely at the range of preservation methods in use, it becomes clear that there is a great deal of nuance in the ways in which these methods can be used, and there may be discrepancies in the ways in which they are understood.

Confusion can also be caused where the same preservation method is known by multiple names. As well as Steedman's being also known as "Post-Fixation Preservative", and "Preserving Fluid", it may also be known only by its component ingredients. For example, three people responded that they had a combination of propylene phenoxetol and propylene glycol in their collections, in addition to specimens that were known to be preserved in Steedman's. This suggests that more people may have Steedman's in their collections than are aware of it, because they know the ingredients but not the name; they might have an alternative name for the same methodology; or the preservation details might not be included in their records. The same is true of Jores' solution: 12 museums said that they had specimens stored in glycerol and water, which is the third stage of preservation by Jores' 1913 method (Jores, 1913), but no respondents were aware of the name of Jores being associated with this methodology. Depending on the availability of documentation and shared knowledge, names associated with fluids can become lost, and they may become known by their constituent parts instead. This type of inconsistency of naming can be confusing both within and between collections.

Given that all respondents had said that they could answer questions about the preservation fluids used in their collections, there was a notable lack of response in some areas. There was a higher degree of certainty when it came to commonly used preservation methods such as formaldehyde and ethanol or other alcohol-based solutions, but less frequently used preservation fluids had a much higher rate of "don't know" or no response answers. There was a mixed response to the survey questions relating to the use of Steedman's. Since fewer than 25% of respondents whose collections included Steedman's were aware of the recommendations to monitor or remove specimens from this preservative, decisions regarding its continued use are largely related to other issues.

The same concerns came up repeatedly, regardless of the size of the institution. Museums are fundamentally under-resourced in terms of both staff, and money (Atkinson, 2024). New staff may be brought in or reassigned to look after sometimes already-problematic fluid collections without access to handover or specialist training, and comprehensive catalogues may not be available, increasing the amount of detective work that needs to be done before changes can be carried out safely. Where this kind of documentation is lacking, it becomes impossible to know which specimens may be at risk. Facilities and expertise to fill these knowledge gaps may not be available.

Individual interviews carried out following the survey indicated that there was frequently a lack of historical and contemporary documentation about the fluids used. Many collections are not fully catalogued and, where they are, there is not always information about the preservation method

assigned to the individual specimens. Sometimes this may be caused by inadequate historical record -keeping, and sometimes by a lack of handover from previous staff, often caused by a gap in funding for personnel. More than one institution shared that they hold collections covering a wide range of zoological specimens that came into their collections between the 1960s and 1980s. Unfortunately, there were no accompanying records to determine which fluids these specimens were preserved in. They are assumed to be in propylene phenoxetol, as it was gathering popularity in the UK during this time, and they are not preserved in either ethanol or formaldehyde. Without documentation the only way to confirm this safely is by chemical testing, and the technology to do this at a detailed level may not be easily or affordably available.

Collections management plans may differ within the same institution, depending on the types of specimens involved and the resources available. Where Steedman's-preserved specimens had been removed from collections, this was generally carried out as an ad-hoc process rather than as a large pre-planned project. When speaking with respondents who stated that they currently or had previously used Steedman's, it became clear that many Steedman's specimens were pre-existing in their collections, and that the curators and conservators who currently care for them would not necessarily advocate for its continued use.

This was not only to do with the degradation of specimens, awareness of the recommendation to remove the fluid from collections (Neumann *et al*., 2022), or the logistical difficulties associated with carrying out that task. There were also considerations concerning ease of use, particularly when compared with ethanol. Steedman's is made up from a concentrate, the ingredients for which need to be sourced and appropriately stored. This takes time, and requires both lab preparation and storage space that may not be available. Ethanol is also easier to use where volunteers carry out much of the topping-up of specimens, particularly when it is purchased pre-diluted to a 70% strength, as less rigorous training may be required. For additional convenience some institutions may be able to buy ethanol or IMS in bulk – or they may even have it readily available on tap. Factors such as these can make the decision to remove Steedman's from fluid-preserved collections perhaps a more pragmatic one than expected. A lack of time and resources for testing historic fluids, particularly in larger collections where this would be an enormous amount of work, suggests that this situation is unlikely to change. Another factor is the expense of specialised equipment relating to fluid-preserved collections, particularly items such as an alcohol density meter, that may be beyond the budget of smaller institutions. that may not be required by an institution on a permanent basis. A system similar to the Library of Things (Library of Things, 2024) might be a means of allowing equipment to be shared, or budgets could be pooled between collections that are relatively local to one another.

Where new staff are taking over fluid-preserved collections without prior training or experience in that area, they may have concerns about safety, particularly when dealing with preservation methods that are less well known than ethanol and formaldehyde. There are also concerns relating to the toxicity of exposure to formaldehyde, and the potential hazards of unidentified chemicals within a collection. Training for fluid preservation has always been difficult to access simply because of its scarcity. Institutions may lack the necessary space and equipment to host such a course themselves, or they may not have the financial means to support staff travel and accommodation as well as the cost of the training itself. This is not to say that such courses are not valuable – quite the opposite. But the fact that they are inaccessible to many means that there is space for additional solutions. Training in how to audit and catalogue a collection, for example, could be provided as a way for those new to wet collections to understand what they have, and how to start planning for their care.

A related issue was being able to find the right information on the subject of caring for fluid-preserved collections. This is not to say that information is unavailable – research is constantly evolving, and new work is frequently published. There is also a dedicated fluid preserved collections conference which was first held in 2018, and which is taking place again in November 2024. This situation could be remedied by making sure that people know where to look. For those who are new to wet collections, the amount of in-depth literature can be overwhelming. A resource dedicated to working with fluid-preserved specimens, such as a web page with a library of links to existing publications divided into themes, would make it a lot easier to find specific information. However, resources such as this require constant maintenance, the resources for which may not be available. This could also be backed up with a dedicated group for those working with fluid-preserved collections. While the NatSCA mailing list is an excellent resource, and an extremely helpful and knowledgeable one, it

may still be perceived as intimidating to ask what might feel like an embarrassingly basic question. There is also shame and embarrassment around having specimens that have deteriorated, even where facilities to investigate the potential causes may not have been available. This can lead to an unwillingness to ask for help from others with experience in the field, for fear of being seen as insufficiently knowledgeable. Sometimes the simplest solution becomes taking the path of least resistance and working with broad spectrum preservation methods, even though this may not be the optimal solution for the long-term safety of individual specimens. Talking openly about the difficulties, and perceived 'failures' of working with fluid-preserved collections can help colleagues to understand that these are complex specimens to care for, and that many of the issues faced are more common than we may think.

Conclusion

Where it is desirable to retain or create softness in a specimen, Steedman's may be used successfully. However, this application requires further research to differentiate between specimens that have remained pliable, and those which are insufficiently fixed. It is also possible to maintain larger and non-marine specimens in Steedman's for at least twenty years, but these specimens should be appropriately formaldehyde-fixed, and perhaps preserved in formaldehyde for an extended period of time before being transferred to Steedman's.

Beyond the original questions of the prevalence of Steedman's in fluid-preserved collections and how it is being used, there are wider issues which affect the type and scale of work that can be carried out. Lack of resources, including money, staff, suitable workspaces, or beneficial technology, prevents appropriate action from being undertaken. This can range from the ability to carry out a project of removing a fluid like Steedman's from an entire collection, to the kinds of monitoring and testing that would allow institutions to build a comprehensive record of the fluids present in their specimens. Without knowing which preservation fluids form the basis of these collections, it becomes extremely difficult to take care of them in the most appropriate way. Potential solutions such as audit training, simplified access to information, and shared equipment could be a beneficial way forward.

It transpires that the Cole Museum of Zoology is extremely fortunate to have a comprehensive manuscript catalogue from its inception in 1909 through to the late 1960s, with additional paper and digital records from the early 2000s onwards. Even so, we still face decades of lost information about our own specimens. Without that recorded infrastructure in place fluid collections are always going to be at risk, as new members of staff may take over without handover or training, and institutional knowledge is lost.

A larger data set would give a clearer picture of the number and types of collections who are using Steedman's or propylene phenoxetol-based preservation methods with their fluid-preserved specimens. This would enable a fuller understanding of the issues that wet collections face, not only regarding Steedman's, but also in terms of the barriers preventing necessary changes to these collections from being carried out. To that end, the survey has been re-opened, and all institutions with fluid-preserved collections are encouraged to respond.

Survey: Fluid Preservation Methods in Biological Collections
https://app.onlinesurveys.jisc.ac.uk/s/reading/fluid-preservation-methods-in-biological-collections

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Appendix 1: Survey

Fluid Preservation Methods in Biological Collections

The purpose of this survey is to determine the frequency of use of different preservation methods in fluid-preserved biological collections. Emphasis is placed on Steedman's Post-Fixation Preservative (Steedman's), sometimes known as PP, or 1% propylene phenoxetol. However, as the purpose of this survey is to gain an overview of methods used, it is requested that you fill in this survey even if this is something that is not used at your institution. The data collected will form part of my PhD research.

Project Description

The aim of fluid-preserved biological collections is to maintain their specimens in as good a condition as possible, for as long a period of time as possible, and to maximise the ways in which these collections can be used for teaching, research, and display. During the 1960s and 1970s a solution known as Steedman's Post-Fixation Preservative became popular, and was widely used in the preservation of these collections. However, by the end of the 1980s, some specimens were found to have been insufficiently preserved by this method, and in 2022 it was recommended that institutions holding fluid-preserved biological collections should no longer use Steedman's Post-Fixation Preservative as a long-term preservation solution. An assessment of the Cole Museum of Zoology's fluid-preserved museum and teaching collections showed that while we do hold specimens preserved by this method, the majority of these remain in excellent condition.

About the survey

No sensitive, impertinent, or distressing questions will be asked, and there is no risk of harm to either participant or researcher.

The data collected will form part of Claire Smith's PhD research, and will be securely stored for five years.

Your participation is voluntary, and your disclosure of identifying details including your name and email address are optional.

Data Protection

The organisation responsible for protection of your personal information is the University of Reading (the Data Controller). Queries regarding data protection and your rights should be directed to the University Data Protection Officer at imps@reading.ac.uk, or in writing to: University of Reading, Information Management & Policy Services, Whiteknights House, Pepper Lane, Whiteknights, Reading , RG6 6UR, UK.

The University of Reading collects, analyses, uses, shares and retains personal data for the purposes of research in the public interest. Under data protection law we are required to inform you that this use of the personal data we may hold about you is on the lawful basis of being a public task in the public interest and where it is necessary for scientific or historical research purposes. If you withdraw from a research study, which processes your personal data, dependent on the stage of withdrawal, we may still rely on this lawful basis to continue using your data if your withdrawal would be of significant detriment to the research study aims. We will always have in place appropriate safeguards to protect your personal data.

The categories of personal data collected are:

- Name of participant
- Email address of participant
- Name of the participant's workplace or institution

- City/town and country of the workplace or institution

These details are collected to enable follow-up contact, where consent is granted for this, and for the analysis of data by location. It is possible to opt out of these questions.

Data will be stored for 5 years as password protected electronic files on the computer of Claire Smith, with paper copies stored for 5 years in the locked office of Amanda Callaghan in the HLS Building, at the University of Reading. You can find out more about your rights on the website of the Information Commissioners Office (ICO) at https://ico.org.uk.
The University of Reading's Data Protection policies can be found at the following link: https://www.reading.ac.uk/imps/data-protection.

Consent

Please tick below to indicate your agreement with the following statements:

1. I understand the purposes of the project.

2. I understand what information will be collected about me, what it will be used for, who it may be shared with, how it will be kept safe, and my rights in relation to my data.

3. I understand that participation is entirely voluntary and that I have the right to withdraw from the project any time, and that this will be without detriment.

4. I understand that the data collected from me in this study may be preserved and made available in anonymised form, so that they can be consulted and re-used by others. This information will be used in a PhD thesis and may be shared with various committees, workshops or presentations, and may contribute towards research publications.

5. I understand that this data will be securely stored for five years, after which it will be deleted. This project has been reviewed by the University of Reading Research Ethics Committee, and has been given a favourable ethical opinion for conduct.

1. I give consent for my data to be used as described above: * ☐

Questions marked * are required.

About your Collections

2. What type of collection do you have at your institution? *

☐ Natural History

☐ Pathology

☐ Biological (other)

☐ None of the above

3. Do you have fluid preserved specimens at your institution? *

- [ ] Yes
- [ ] No
- [ ] Don't know

4. Are you able to answer questions about the types of preservation fluids used at your institution? *

- [ ] Yes
- [ ] No

If you are not able to answer questions about the types of preservation fluids used at your institution, please pass on this survey to other colleagues in the field who work with biological collections.

If you would like to discuss this research further, please contact:

Claire Smith: claire.smith@reading.ac.uk
Professor Amanda Callaghan: a.callaghan@reading.ac.uk

https://app.onlinesurveys.jisc.ac.uk/s/reading/fluid-preservation-methods-in-biological-collections

5. Do you currently have, or have you historically had, specimens stored in any of the following preservatives? *

You may choose multiple responses for each fluid type.

	Currently	Historically	No	Don't know
Formalin	☐	☐	☐	☐
Ethanol / IMS / other Spirit	☐	☐	☐	☐
Steedman's Post-fixation Preservative	☐	☐	☐	☐
Propylene Phenoxetol (sometimes labelled PP or 1%)	☐	☐	☐	☐
Propylene Glycol	☐	☐	☐	☐
Liquid paraffin	☐	☐	☐	☐
Glycerol	☐	☐	☐	☐
Glycerol & water	☐	☐	☐	☐
Jores / Jories	☐	☐	☐	☐
Kaiserling	☐	☐	☐	☐
Other	☐	☐	☐	☐

6. Please add which preservation fluids you use, if they are not listed above:

About your Collections

If you do not know about the balance of different preservation fluids that make up your collection, please skip this question and move on to question 8.

7. Please estimate the % of each type of preservation fluid in your collection

Please indicate ONE response for each fluid type.

	Don't know	up to 20%	up to 40%	up to 80%	over 80%
Formalin	☐	☐	☐	☐	☐
Ethanol / IMS / other Spirit	☐	☐	☐	☐	☐
Steedman's Post-fixation Preservative	☐	☐	☐	☐	☐
Propylene Phenoxetol (sometimes labelled PP or 1%)	☐	☐	☐	☐	☐
Propylene Glycol	☐	☐	☐	☐	☐
Liquid paraffin	☐	☐	☐	☐	☐
Glycerol	☐	☐	☐	☐	☐
Glycerol & water	☐	☐	☐	☐	☐
Jores / Jories	☐	☐	☐	☐	☐
Kaiserling	☐	☐	☐	☐	☐
Other	☐	☐	☐	☐	☐

Knowledge of Steedman's

8. Have you heard of Steedman's / propylene phenoxetol as a preservation fluid? *

☐ Yes

☐ No

9. Which of these options most closely reflects your experience of the use of Steedman's / propylene phenoxetol as a preservation fluid? *

Please indicate ONE response.

☐ Very Positive

☐ Positive

☐ Neutral

☐ Negative

☐ Very negative

☐ I have not used Steedman's / propylene phenoxetol

10. Are you aware of the recent recommendation to remove Steedman's / propylene phenoxetol from fluid-preserved collections? *

Neumann et. al., 2022, *Best Practices in the Preservation and management of Fluid-Preserved Biological Collections* (SPHNC, Chicago, pp66-68)

- [ ] Yes
- [ ] No

11. Are you planning to make any changes to your Steedman's / Propylene Phenoxetol preserved specimens? *

Please indicate ONE response.

- [ ] We have already removed Steedman's from our collection
- [ ] Yes, because of the recommendation above
- [ ] Yes, because we have experienced negative results in our own collection
- [ ] Yes, because we are aware of negative results in other collections
- [ ] Undecided / we do not have a plan either way
- [ ] No, because we are happy with our Steedman's specimens as they are
- [ ] No, but we plan to in the future
- [ ] No, but we are closely monitoring our Steedman's specimens
- [ ] No, we do not have sufficient resources to make this kind of change

12. Have any of your Steedman's / Propylene Phenoxetol specimens ever been transferred into or out of another preservation fluid?

- [ ] Yes - out of Steedman's / PP into another fluid
- [ ] Yes - into Steedman's / PP from another fluid
- [ ] No
- [ ] Don't know

Location details

13. Please provide the name of your institution

_____

14. In which town / city is your institution based?

_____

15. In which country is your institution based? *

_____

Online or digital catalogue

16. Do you have an online or digital version of your catalogue that I would be able to access? *

☐ Yes

☐ No

17. If yes, please provide access details for your online or digital catalogue:

_____

18. Are you willing to be contacted by email, to answer follow-up questions and/or provide access to a digital catalogue? *

☐ Yes

☐ No

Contact information

19. Your name:

_____

20. Your email address:

_____

Thank you for taking the time to participate in this research.

Please pass on this survey to other colleagues in the field who work with biological collections:

https://app.onlinesurveys.jisc.ac.uk/s/reading/fluid-preservation-methods-in-biological-collections

If you would like to discuss this research further, please contact:

Claire Smith: claire.smith@reading.ac.uk
Professor Amanda Callaghan: a.callaghan@reading.ac.uk

Appendix II: Formulae

Formalin

- A saturated solution of 37% formaldehyde gas in water.
- Fixation strength
    - ◊ 10% Formalin solution = 4% formaldehyde
- Preservation strength
    - ◊ 5% Formalin solution = 2.5% formaldehyde

Kaiserling

This is a three-step process, but most survey respondents who used Kaiserling in their collections were using only the preservative step to top up existing specimens. There are many iterations of the Kaiserling process, but the most widely cited was Pulvertaft's modification to remove the arsenious acid (Pulvertaft, 1950).

- 30% Glycerine
- 10% Sodium acetate (B.P.)
- 0.5 % Formalin
- adjust solution to pH 8

Steedman's Fixative (1 litre)

- Concentrate:
    - ◊ 100ml propylene phenoxetol dissolved in 500ml propylene glycol
    - ◊ Add 500ml formaldehyde 37% (Formalin)
- Fixative:
    - ◊ Dissolve 110ml of concentrate in 890ml of distilled water or saline

Steedman's Post-Fixation Preservative (1 litre)

- Concentrate:
    - ◊ 50ml of propylene phenoxetol dissolved in 500ml of propylene glycol
- Preservative:
    - ◊ Dissolve 110ml of concentrate in 890ml distilled water

Kew Mix: fixative for botanical specimens

- 5% formaldehyde
- 5% glycerol
- 53% industrial methylated spirit
- 37% water

Copenhagen Mix: study preservative for botanical specimens

- 70% industrial methylated spirit
- 28% water
- 2% glycerol

Güell & Mendel's Beetle Relaxing Fluid

- Ethyl alcohol (96%), 405ml
- Distilled water, 300ml
- Ethyl acetate, 167ml,
- Ether, 168ml
- Glacial acetic acid, 1ml

UV light as a diagnosis tool for conservation and restoration in natural history collections

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Abstract

The use of ultraviolet light for conservation and restoration in art collections is a common practice. In natural history collections, reaction to UV has been spotted in numerous animal groups; biofluorescence is widespread in the animal kingdom. Here it is the potential of UV light in terms of conservation and restoration of natural history specimens that is explored. UV-induced visible luminescence (UVL) of natural materials are characterized and complemented by actual examples of restored specimens under both regular and UV light. Carpet beetles attack can be spotted due to bright frass, but not other pests and mould in a conclusive way. Restored parts are identified due to glowing inconsistencies. Unusual treatments and dirt may also be distinguishable. It is therefore possible to inspect the integrity and authenticity of specimens, e.g. new acquisitions, and to adapt conservation treatment. As a help to diagnosis, it does not replace the judgement of a conservation specialist.

Keywords: natural history, taxidermy, replicas, conservation, restoration, museum collection, museum specimens, collection management, UV light, fluorescence, luminescence

Introduction

Fluorescence under UV light exposure has been demonstrated in many species across the animal kingdom e.g. invertebrates (Welsh *et al*., 2012; Lagorio *et al*., 2015), fish (Sparks *et al*., 2014), reptiles (Prötzel *et al*., 2018; Gruber and Sparks, 2015), amphibians (Lamb and Davis, 2020), birds (Pearn *et al*., 2001; Dunning *et al*., 2018; Camacho *et al*., 2019), mammals (opossums: Pine *et al*., 1985; flying squirrels: Kohler *et al*., 2019; platypus: Anich *et al*. 2021). Some of these findings were made from museum specimens i.e. taxidermy and study skins (Kohler *et al*., 2019; Anich *et al*. 2021). Research in that field discusses the molecular mechanisms of biofluorescence as well as the evolutionary reasons in terms of adaptation and behaviour. These references are the tip of the iceberg regarding fluorescence in the animal kingdom, and what was considered as an exceptional discovery seems to be widespread. Kohler *et al*. (2019) reports that fluorescent compounds were discovered in bones, feathers, skin, shell and hairs, and emitted colours cover all the visible spectrum except orange.

How can UV light serve conservators and restorers to diagnose specimens they take care of? It is routinely the case in art collections, sometimes in a thorough way; analytical imagery combines complex technologies and computer treatments to transform raw data into exploitable images (Landi and Maino, 2011; Lanteri *et al*., 2019; Webb, 2019). Webb (2019) accurately depicts how

useful UV light is for conservation in art and history museums: characterization and differentiation of materials, state of conservation, identification of past treatments, but also limitations and standardization issues.

Here I want to fill a gap in literature regarding natural history collections that are not specifically taken into account. I present the possibilities offered by UV exposure to help diagnose specimens before implementing conservation or restoration measures and to inspect specimens on loan, subcontracted works and new acquisitions. The following questions underlie the present exploration: What natural materials "glow" under UV light? May UV light help to distinguish original and replica items or parts of them? Can biological attacks, dirt and previous interventions be spotted?

I don't aim to list comprehensively what species react to UV light; by illuminating an entire collection, I want to generalize what conservators and restorers can expect to reveal and hence what is unexpected. These observations are complemented by actual examples that conservators and restorers may encounter.

Material and methods

Ultraviolet rays are invisible and come just after violet. The energy of photons is higher in UV than in the visible light; some materials can absorb this energy and emit lower energy photons among the visible spectrum. This is called fluorescence (Mouchet and Deparis, 2021).

The two wavelengths tested are 375 and 405 nm. The two main commercially available lamps have wavelengths of 365 and 395 nm. Analysis of the spectra (spectroradiometer Apogee model SS-110) reveals that the peaks of emission were slightly different than expected (375 and 405 nm; Figure 1) but results were consistent with preliminary tests using calibrated LED. Ranges of emission include wavelengths used in literature regarding biofluorescence and are the long-wave UV (UVA) used in the field of conservation (Measdey *et al.*, 2017).

The vivid aspect of some materials under UV exposure is not necessarily due to fluorescence in the molecular sense. To avoid any confusion, I use the term "UV-induced visible luminescence" (UVL; Webb, 2019), that groups luminescence (including fluorescence and phosphorescence; Hickey-Friedman, 2002) and total or partial reflectance. Indeed, part of the emitted spectrum of lamps (from 400 nm) belongs to the visible light (Mouchet and Deparis, 2021).

Natural history specimens were inspected and materials divided into different categories: eyes (glass and acrylic), keratinous appendages (beaks, claws, horns, feathers, hairs, etc.), vertebrates skin, bones (including antlers), minerals (shells, bird eggs, fossils and corals), arthropods (insects, arachnids and crustaceans), biological attacks (pests and mould) and fluid collections. I feature what is typically observed without looking for exceptions. Results are given as a guide but not as a rule. Following this goal, characterizing emitted colours is based only on visual perception, consistently

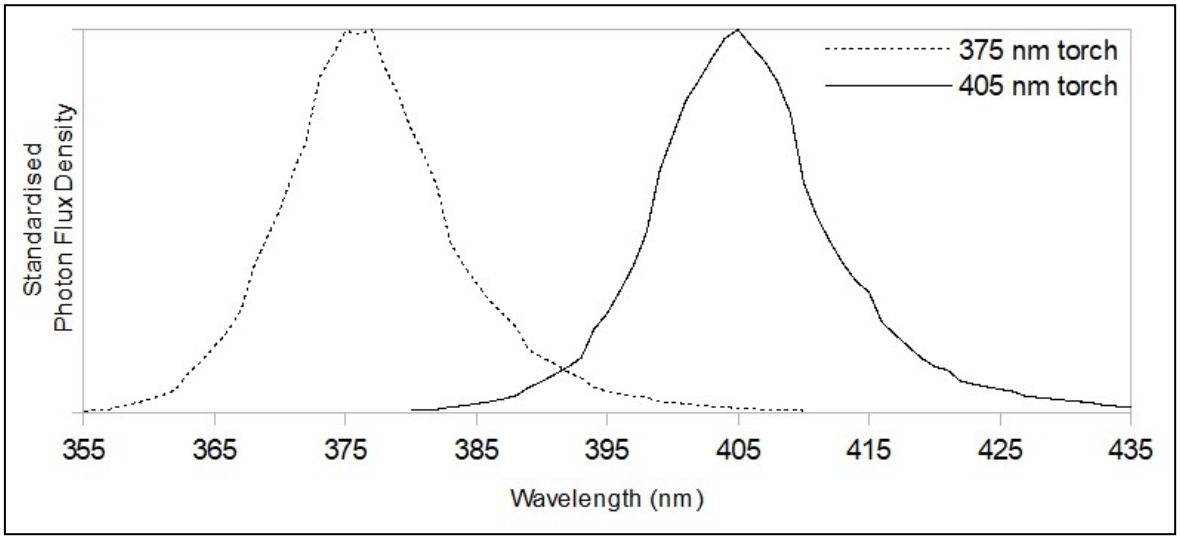

Figure 1. Emission spectra of the two lamps used in the study. Photon Flux Density (in micromoles per square meter per second) was normalized to the maximum value to remove the "intensity" variable that was not specifically controlled.

with previous works, and especially the review of Measdey *et al*., 2017:

- Reactive specimens: yellow, yellow-white and yellow-green (generally light and bright), blue, green and blue-green, purple or violet, red (brownish or purplish), orange, white.
- Non-reactive specimens: black (neither reaction nor reflectance), neutral (no particular reaction observed; only inconsistent reflectance of visible violet wavelengths depending on distance/intensity of the emitting lamp).
- Intensity: more or less bright or dark, milky (diffused).

Investigated specimens come from different museum collections in order to cover all materials. Most were found in the zoological collection of the University of Namur (UNamur), supplemented by the Namur African Museum (MusAfrica) collection for big mammals, the Museum of Zoology of the Free University of Brussels (ULB) for small mammals and a private collection for eggs. It totals more than 5200 specimens or parts of specimens, most of them being shells (1500) and insects (2000). Artificial materials were already investigated in the literature dedicated to conservation and restoration in general (Measdey *et al*., 2017), but paints and artificial eyes have been included because of their omnipresence in natural history collections. Specimens were examined at 405 nm. The 375 nm wavelength showed few interesting/conclusive results.

Photographed specimens display a variety of "inconsistencies" that conservators and restorers could encounter. Considering that it is a tool for everyday use and that we look mainly for surface inconsistencies, standardization is unnecessary. These specimens were photographed under available light (artificial and/or natural) and then under UV light in complete darkness with the same angle of view using a regular triple-camera smartphone. Due to the phone's auto-adjustment settings depending on various light conditions, brightness and contrast may have been edited with GIMP 2.10.8 to achieve a rendering close to reality. The wavelength mainly tested was 405 nm. Some specimens were also inspected at 375 nm. Specimens come from university collections (when specified) and private collections (when unspecified).

Results

Table 1 shows the UV-induced visible luminescence (UVL) at 405 nm of materials found in natural history collections.

Eyes are artificial in taxidermy mounts. Glass and acrylic eyes were tested during this study. Only orange and yellow glass eyes were, in some cases, fluorescent. Some glass eyes are painted at the back so that paint can react independently of glass. Other are neutral. Acrylic eyes glow milky blue or blue-green. Although glass and acrylic resin are currently used to make commercial eyes, other resins, like epoxy, are also used.

Keratin-based materials (dander/appendages or epidermal productions: beaks, bird leg scales, spines, scales, hairs, horns, claws and feather rachis) were reactive when they were light in colour. For darker ones, there was no reaction, and they appeared black or reflect violet, except at the base where they are often lighter and finer. Feathers have particular reactions: some dark plumages glow violet as well as green plumages. One greasy amelanistic raccoon and one greasy wood pigeon rump, both light grey in colour and both recent taxidermy (< 5 years) appeared yellow-green.

The skin of all vertebrate groups also reacted quite vividly. For example, skin around the eyes, nose, mouth and inside of the ear glowed yellow-white, unless the skin was very dark or black (no reaction) or painted (hence appears dark purple). Some birds with black skin (e.g. egrets) were not available but black skin is probably neutral in birds as in mammals. For fish and reptiles, the variety of emission depended on pigmentation: dark parts are neutral while light parts glow yellow-green.

Chitin seemed to react differently between groups: insects and arachnids reacted very little, except for joints, some beetles, butterflies, spider abdomens and light-coloured scorpions. Crustaceans, on the other hand, reacted in more or less dull yellow-green and violet.

Tissue residues were reactive, and this was particularly evident in osteological mounts. Cartilage remnants in joints appeared yellow, while bone varied from white to yellow or violet. Teeth roots were also reactive, but enamel was not, appearing white or reflecting violet.

Shells were fairly neutral on the surface, except for nautiluses and paper nautiluses. The umbilicus and inner surface were reactive. Corals reacted from white to violet. Tested fossils did not fluoresce.

Chicken eggs showed little reactivity at 405 nm, regardless of ground colour and spots. Other species tested were white goose, ostrich, emu, greater rhea, common pheasant, blue tit and great tit, with similar results. In this case, the 375 nm

wavelength had more diverse results than at 405 nm: albumin residues around the blowing holes glowed white, scratches in the pigmented coating and white punctuations stood out, and some brown eggs had areas where UVL was bright (purplish or brownish) red.

Fluid collections glowed green or blue-green, whether the jars were old or new. Only a few old jars from specialist dealers showed no UVL and remained perfectly translucent.

Table 1. UV-induced visible luminescence (UVL) at 405 nm of materials found in natural history collections.

Category	Type	UV-induced visible luminescence	Number of specimens
Eyes	Glass	Neutral. Orange, yellow and white eyes sometimes fluorescent	> 150
	Acrylic	Milky blue or blue-green	> 20
Keratinous appendages	Beaks	Dark parts are neutral, light parts glow yellow-green	> 180
	Claws	Dark parts are neutral, light parts glow yellow-green	> 200
	Horns	Dark parts are neutral, light parts glow yellow-green	13
	Spines and scales (1)	Dark parts are neutral, light parts glow yellow-green	4
	Feathers	Light calamus and rachis generally glow yellow-white while vanes are more or less neutral, dark plumages are generally neutral but some glow dark violet (pigeons, n=11 ; gulls, cormorants, raptors, n=10), green plumages of parakeets glow violet (n=4)	> 180 species
	Hairs (2)	Dark parts are neutral, light parts glow yellow-green, white glows white	20
	Baleens	Dark parts are neutral, light parts glow yellow-green	1
Skin (dried, without paint and varnish)	Mammals	Naked skin glows yellow-green, except when black	20
	Birds	Dark parts are neutral, light parts glow yellow-green (feet and face)	> 180 species
	Reptiles (3)	Dark parts glow (dark) purple, light parts glow yellow-green	14
	Amphibians	Dark parts are neutral, light parts glow yellow-green	1
	Fish and sharks (4)	Dark parts are neutral, light parts glow from white to (yellow-)green	15
Osteology	Bones, skulls and antlers	White, purple and (greenish-)yellow depending on fat and residual tissues	> 100
		Articulations generally glow yellow	
	Enamel	White to purple	> 50
	Teeth plaque and	Purple to black	> 50
	Teeth roots	White, yellow, rarely purple	> 50
	Ivory	White to yellow-green; old yellowed ivory is neutral	4
	Fossil teeth (5)	Neutral	4
Mineral	Shells (bivalves, gasteropods and nautiluses)	No particular glowing, sometimes a bit yellow at ombilic, edges; inside generally white or yellow-green; nautilus and paper nautilus bright at surface ; incrustation of algae or ectoprocta may glow	> 1500 (>450 species)
	Bird eggs (6)	No particular glowing, mat aspect, inner membrane glows white	> 150
	Fossils	Neutral whatever limestone or shale	> 50
	Coral	Yellowish white and violet	34
Arthropods	Insects	Generally neutral, joints often yellow or neutral; some beetles and butterflies glow yellow-green or yellow	> 2000 (all orders)
	Scorpions	Dark ones are neutral (joints sometimes yellow), light ones glow yellow-green	4
	Spiders and amblypygids (7)	Neutral, but joints and abdomen sometimes yellow	23
	Crustaceans (without paint nor varnish) (8)	Yellow-green and violet	10
Biological attacks	Carpet beetle frass	Yellow-green	4
	Moths (larvae, frass, cocoons and adults)	Frass neutral to yellow-green, larvae glow yellow-green but not cocoons and adults	3 degraded specimens
	Booklice frass	Neutral	> 10
	Mould	Neutral	> 10
Fluid specimens	Museum, university and didactical preparations	Milky green or blue-green; 5 ancient development models from professional sellers do not react (UNamur items n°427, 433, 435, 1122, 1124)	> 150

(1) porcupine, hedgehog, echidna, pangolin; (2) raccoon, leopard, wild boar, roe deer, fox, cat, marten, hare, rabbit, buffle, various antelopes; (3) various snakes, lizards, turtles, crocodiles; (4) various percids, salmonids, boxfish, seahorses, sharks; (5) fossil sharks; (6) mostly chicken eggs (from white to dark brown, blue and green, variously spotted from white to dark brown); (7) amblipygids and tarantulas; (8) various crabs and crayfish.

The powdery frass of carpet beetles was easy to spot even on diorama ground as it glowed yellow. For moths, there were no UVLs for cocoons and adults but larvae were very bright, and the droppings varied from neutral to yellow, perhaps depending on the type of the raw material that was nibbled.

Booklice that are found in herbaria and entomological collections typically produce fine powder that is easily spotted without any effort in regular light; UV light only brought more contrast due to white-reflective cardboard, especially at 375 nm. These collections are also subject to mould that doesn't need UV light to be spotted either, but it appeared whiter under 375 nm. Regular dust ("house dust") appeared violet at 405 nm.

Paints (acrylic, watercolour and gouache) were particularly distinctive because they do not react to UV light (except, of course, for fluorescent paints, which are rarely used in this field). Paints therefore appeared very dark, contrasting with the yellow-green or violet of natural materials. They appear dark purple at 405 nm and black at 375 nm. The absence of reaction of paint is essentially what will enable restorations and replicas to be identified. Only the pure red gouache was fluorescent at 375 nm, but of course UVLs may vary among different brands.

Old varnish such as picture varnish was dark yellow-green unlike modern acrylic varnish which is more neutral.

Resins have not been tested because they are very diverse (polyester, epoxy, acrylic and polyurethane) and are often charged to modify texture and colour, making the combinations infinite.

Figures 2 to 9 show specimens under normal and UV light.

Figure 2. ***Upper row.*** *Female gorilla skull from the Maison Tramont on display (UNamur-619). UV light clearly reveals a restoration (arrow) carried out at an undetermined time and had never been spotted before. Examination of the inside of the cranial cavity reveals a round hole, which indicates that the skull must have been base-mounted or once belonged to a complete skeleton. UV at 405 nm.* ***Lower row.*** *Babirusa skull on display (UNamur-602). A missing tooth was replaced by a resin cast of the other one. Under UV light, the roots have different UVLs (arrows). On the right, the two teeth in their entirety: the glowing original (left) and the purple-reflecting replica (right). UV at 405 nm. © Liévin Castelain, 2024.*

Figure 3. Lambis *sp. shell. In visible light, nothing special appears. Under UV light, tips have different UVLs; tips 5 and 6 are original and were cast to restore the other tips in painted plaster resin (1, 2, 3, 4 and 7). UV at 405 nm. © Liévin Castelain, 2024.*

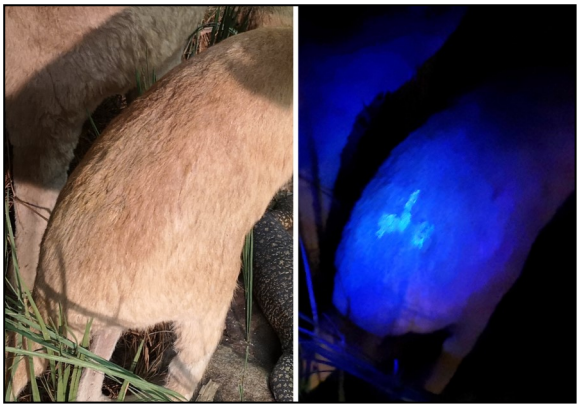

Figure 4. (Above) Kangaroo in a Rowland Ward diorama c1892. Holes in the coat caused by moth attack have been filled with bleached roe deer hair. The inconspicuous restoration is revealed by UV light thanks to the different UVL between the two types of hair. UV at 405 nm. © Liévin Castelain, 2024.

Figure 6. (Right) These two orangutan skulls are virtually identical, but one is actually a replica of the other and the quality of the casting makes it difficult to authenticate the original from the replica. Such a replica could easily be regarded as authentic, or cleaned without caution with solvent. Under UV light, the original skull glows as expected for natural bone, as do the teeth (violet enamel and yellow-green roots) while the polyester resin replica has no UVL and simply reflect violet wavelength. Other resins (e.g. polyurethane) can produce other UVLs closer to bone ones. UV at 405 nm. © Liévin Castelain, 2024.

Figure 5. ***Left column.*** *Tiger from the Center for Scientific Culture, ULB. Top, before restoration. Middle, after restoration. Below, the restoration of the lower canines is clearly visible under UV light. UV at 405 nm.* ***Right column.*** *Mounted whale foetus (Museum of zoology, ULB-RG101A). Top, before restoration. Middle, after restoration; tears and holes of were restored with Japanese paper and painted with watercolour and acrylic paint. Below, location of restored parts are easily identified with UV light. UV at 375 nm. © Liévin Castelain, 2024.*

Figure 7: ***Upper row****. Abnormal illumination of a fox skull indicates that it has been treated differently. Actually this skull has been treated with laundry bleach containing sodium percarbonate and optical brighteners.* ***Middle row.*** *The leg of a kestrel that has been attacked by parasites. Frass is not visible on the rock, but stands out under UV light (arrows). UV light at 375 nm gives a different view, but no clearer than 405 nm. Unpainted parts of toes (underneath) can also be seen, appearing white at 375 nm (right).* ***Lower row.*** *Ratite egg on display (Museum of zoology, ULB-2632). Simply broken at first glance, UV exposure shows that a product has leaked onto the egg. Restoration must be accompanied with appropriate cleaning.* ***Middle column*** *at 405 nm.* ***Right column*** *at 375 nm. © Liévin Castelain, 2024.*

Figure 8. Mounted brown trout. Under UV, the head has a different rendering than the body. In this case the head is artificial and skin and fins are original (and unpainted). Head and body were coated with the same acrylic varnish. UV at 375 nm. © Liévin Castelain, 2024.

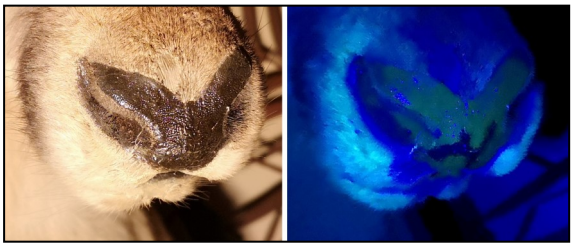

Figure 9. Red kangaroo snout in a Rowland Ward diorama from c1892. The restored tear between nostrils is invisible but the UVL of the restoration looks different than the rest of the nose: original varnish glows dark yellow-green, while restored tear is neutral (acrylic paint and varnish). UV at 405 nm. © Liévin Castelain, 2024.

Discussion

The use of ultraviolet light in art collections has a proven track record (Hickey-Friedman, 2002; Webb, 2019), and its potential is explored here for natural history collections. UV-induced visible luminescence (UVL) has been noted in recent years for various animal groups with molecular and ecological considerations, but it is its use for conservation and restoration purposes that is investigated in the present study.

The reactions observed are in line with what was expected; materials containing keratin, collagen and fat react to ultraviolet light, such as bone, dentine, joints, skin and appendages (Collins, 1992; Bachmann *et al*, 2006; Kollias *et al*., 2002). This is a general summary that should not obscure the fact that fluorophores are very numerous and that identifying their chemical compositions is far from easy (Hughes *et al*., 2022). UVL did not appear to be different for old and recent specimens (except for ivory as noticed by Simpson-Grant, 2000), even though degradation of fluorophores is possible over time ("photochemical damage"; Pearlstein *et al*., 2015) and interactions between molecules are also possible, e.g. lipid oxidation that leads to production of fluorescent proteins (Kikugawa and Beppu, 1987). A few examined specimens were greasy but results suggest that fat stands out at least in recent work and on light hairs and feathers.

Natural materials react very little at 375 nm, giving UVLs in shades of grey, except in a few cases: "normal" dust appeared clearly violet, carpet beetle frass glowed yellow-white, what allows attacks to be spotted at an early stage, and acrylic paint appeared black. Given the absence of visible light in the 375 nm-torch spectrum, there is no light pollution with visible wavelengths close to blue, which gives violet reflection, so the contrast is very sharp and restorations stand out. But this wavelength revealed nothing more than 405 nm.

The reactions of "raw materials" are given as a guide and not as a rule. It is likely that a multitude of exceptions exist, and this is a qualitative evaluation that is not intended to be quantitative. First, the intensity of reflectance and colour varies according to the angle and distance (or intensity) of exposure; white and yellow can tend towards green or violet (for example, enamel appears white or violet). Second, there is a wide range of UVL emission due to the composition, ageing and loading of natural and synthetic materials (Webb, 2019). This is not to mention that commercially available lamps emit on either side of their emission peak (see Materials and Methods) and UV covers a wide spectrum below and between the wavelengths tested. In addition, it is possible that fluorescence occurs at a lower intensity when excitation wavelength is not ideal (Hickey-Friedman, 2002). In short, without reproducible protocols and standardisation of the UV emitting source, it is impossible to produce quantitative studies (Webb, 2019), and it is therefore conceivable that the observed colours and contrasts depend on the equipment. The case of the platypus is illustrative. Anich *et al.* (2021) illuminated specimens with UV light at 385-395 nm, achieving striking results. In the present study, yellow-green UVL was also observed in the platypus, but with much lower intensity. The UV lamp used peaks at 405 nm, meaning it contains a low proportion of 385-395 nm wavelengths, and neither its emitted intensity nor the emitted UVL can be compared with that of Anich *et al.* (2021).

But the quantitative characterisation of UVL is not the central element here; it is a question of differences in reaction, i.e. inconsistencies in UVLs ("surface inconsistencies"; Hickey-Friedman, 2002) because differences in reactions are eye-catching compared to homogeneous reactions. Restored parts stand out and comparison between similar objects give clues if unusual treatment was applied (such a specimen would require special monitoring, or even analysis to detect the presence of a chemical product/residue that could degrade the specimen and contaminate others) or if the specimen is a replica. With this approach, the UV spectrum is useful as a diagnosis tool in natural history collections, as it is in art and history collections (Simpson-Grant, 2000).

Results regarding fossils should be interpreted with caution. In the present study, only a few specimens were tested, all of which were made of shale and limestone. According to Measdey *et al.* (2017), reaction of geological specimens can be highly inconsistent; this is due to the variability of mineral composition that is site-specific (Croft *et al*., 2004). However UV light has already been used to detect restorations and forgeries in palaeontological specimens (Tischlinger and Arratia, 2013), based on the principle of surface inconsistencies.

Conclusion

How can UV light serve conservators and restorers to diagnose specimens they take care of?

The first thing is to detect the presence of pest attacks and other degradations. The dust produced by carpet beetles is clearly visible and different from usual dust. Unfortunately, this is not the case for the other notorious biological attacks with less promising results for booklice and mould. Results for moths are mixed: glowing of frass is inconsistent, cocoons and adults do not react but larvae glow.

The condition also includes the presence of grease, residual tissue and stains. An abnormal reaction may suggest that a substance is present on or in the specimen. UV can help to assess the situation prior to conservation and restoration work. For example, the presence of paint, varnish or artificial parts influences the choice of the treatment. Similarly, the progress and completeness of cleaning can potentially be monitored using UV.

In this respect, newly acquired pieces, whether by purchase, donation or subcontracting, can be examined to determine their condition and integrity; the presence of restorations, non-original elements or fake parts (e.g. bird skull replaced by a copy in a taxidermy mount). In the case of osteological mounts, given the variety of UVL, it is possible to detect whether all the elements originally come from the same specimen, or whether the specimen is composite (a mixture of different specimens).

Examining specimens with UV light does not require costly equipment, is not time-consuming, and allows to spot inconsistencies in the easiest way possible. By revealing the invisible, UV light is presented as a help to diagnosis: the state of conservation, integrity and authenticity are all elements to which conservators and restorers pay attention to. Of course, reactions or lack of reactions and the interpretations that may result from them do not replace but complement the expertise and judgement of an experienced professional.

Acknowledgements

I thank Sébastien Mouchet from the Universities of Exeter and Namur, co-author of *Natural Photonics and Bioinspiration*, for interesting discussion about luminescence in the animal kingdom, and Mark Holmes from the University of Namur who helped me to determine the emission spectra of the UV torches.

I thank the institutions that kindly allowed my curiosity to open their showcases: The University of Namur, the Namur African Museum (MusAfrica) and the Museum of Zoology of the Free University of Brussels.

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NatSCA AGM 2024

1.45pm-2.30pm, 18th April
Oxford University Museum of Natural History
Hybrid meeting
Zoom: link emailed to members

AGENDA

1. Apologies for Absence
2. Matters arising from Minutes of AGM 2023
3. Reports
4. Election of NatSCA Committee
5. Any Other Business
6. Vote of Thanks
7. Next AGM Venue

Close

Annual General Meeting

Please ensure you are a paid-up individual member of NatSCA to propose, second or vote at our AGM. Institutional members are entitled to one vote per institution. Please authorise one individual to vote as a representative of your institution before the AGM. If you are attending via Zoom and would like to propose or second, please write your full name in the Zoom 'Chat' channel. Many thanks.

1. Apologies for absence

Apologies from Eimear Ashe, Clare Booth-Downs and Jan Freedman.

2. Matters arising from Minutes of AGM 2023

Matters arising from minutes of the NatSCA AGM 2023, held in Stoke-on-Trent and on Zoom, as published in: *Journal of Natural Science Collections (2024), NatSCA AGM minutes 2023, Vol.* ***12****: 136-146.*

Proposal to accept the minutes of the 2023 AGM, including any amends from matters arising, as an accurate record:

Proposer: Tannis Davidson

Seconder: Laura McCoy

3. Reports for NatSCA's Annual Year 1st February 2023 to 31st January 2024

Secretary's Report: Yvette Harvey

Nine Zoom committee meetings and one hybrid Away Day (October) have been held between February 2023 and January 2024. With the exception of January 2024, all meetings had over 10 trustees present and lasted c. 1 hr 30 mins. The typed minutes were prepared and distributed amongst the Trustees.

	ii.2023	iii.2023	iv.2023	v.2023	vi.2023	vii.2023	ix.2023	xi.2023	i.2024
Jack Ashby		Y	Y	Y	Y		Y	Y	Y
Eimear Ashe				Y	Y	Y	Y	Y	Y
Clare Booth-Downs				Y	Y	Y		Y	
Clare Brown	Y		Y	Y		Y	Y		
Belle Buchanan-Smith	Y	Y		Y		Y	Y	Y	
Tannis Davidson		Y	Y	Y	Y	Y		Y	
Patti Wood Finkle	Y		Y		Y	Y	Y		Y
Jan Freedman	Y	Y			Y				Y
Jennifer Gallichan	Y	Y	Y	Y	Y	Y	Y	Y	Y
David Gelsthorpe	Y		Y						
Amy Geraghty	Y	Y	Y	Y	Y	Y	Y	Y	Y
Isla Gladstone	Y	Y	Y	Y	Y	Y	Y	Y	Y
Yvette Harvey	Y	Y	Y	Y	Y			Y	Y
Lucie Mascord	Y								
Laura McCoy	Y	Y	Y	Y	Y	Y	Y	Y	
Emma Murphy				Y	Y	Y	Y		Y
Glenn Roadley	Y	Y	Y			Y	Y	Y	
Laura Soul	Y	Y	Y						
Total No. present	**13**	**11**	**12**	**12**	**12**	**12**	**11**	**11**	**9**

Minute taking was shared between Yvette Harvey and Eimear Ashe, the former is very grateful to the latter for stepping in and helping.

Treasurer's Report: Belle Buchanan-Smith

Accounts Summary 01Feb2023 - 31Jan2024							
Income	**2023-24**		**2022-23**	**Expenditure**	**2023-24**		**2022-23**
Institutional Subscriptions				**Running costs**			
Previous Years	£ -		£ -	Committee Expenses	£ (1,957)		£ (333)
Current Year (bank)	£ 1,972		£ 1,880	Website, Zoom etc	£ (467)		£ (815)
Future Years	£ 80		£ 40	Postage	£ -		£ -
		£ 2,052	£ 1,920	Payment Fees	£ (128)		£ (141)
Personal Subscriptions				Data Protection	£ (35)		£ (35)
Previous Years	£ -		£ 20			**£ (2,587)**	£ (1,324)
Current Year	£ 4,135		£ 4,475	**Workshops**			
Wrong amount	£ -		£ -	Catering etc.	£ (286)		£ -
Future Years	£ 20		£ 150			**£ (286)**	£ -
		£ 4,155	£ 4,645	**Conference**			
Workshop Income				2023: So how do we actually do all this?	£ (9,239)		£ -
Mobilising Biodiversity Data	£ 409		£ -	2022: SPNHC	£ -		£ (53)
Natural Science Legislation	£ 243		£ -			**£ (9,239)**	£ (53)
		£ 652	£ -	**Publications & Information Provision**			
Conference Income				2021 Journal print & postage	£ -		£ (2,174)
2023: So how do we actually do all this?	£ 15,235		£ -	2022 Journal print & postage	£ (2,466)		£ -
		£ 15,235	£ -			**£ (2,466)**	£ (2,174)
Donations				**Charitable**			
Donations	£ 5		£ -	Bill Pettit Fund	£ (2,100)		£ (2,528)
		£ 5	£ -	Bursaries	£ (616)		£ (1,504)
Other				Sector support	£ -		£ -
Misc.	£ -		£ -			**£ (2,716)**	£ (4,032)
Publications	£ 6		£ 12	**Other**			
Bank interest	£ -		£ -	Misc.	£ -		
		£ 6	£ 12			£ -	£ -
TOTAL INCOME		**£ 22,105**	£ 6,577	**TOTAL EXPENDITURE**		**£ (17,294)**	£ (7,583)
						2023-24	2022-23
Cash Surplus / (Deficit) for the Year						**£ 4,811**	£ (1,006)

Cash Flow Statement					**OUTSTANDING EXPENDITURE**		
01.02.2023	Current a/c	£ 47,785			2023 Journal Estimate	£ 2,500	
	Paypal a/c						
			£ 47,785				£2,500
31.01.2024	Current a/c	£ 52,596			**EXPECTED INCOME**		
	Paypal a/c						
			£ 52,596				
Balance Including Liabilities			£ 50,096				£ -
Adjusted Surplus/(Deficit)			£ 2,311		Adjusted balance 31.01.2024		£ 50,096

Commentary on the Financial Year:

2023/24 has seen a small decline in subscription income overall, which has been offset by income from workshops.

In April 2023 the first full conference since 2019 took place, with both online and in person delegates. High attendance has led to a healthy surplus for the year. Bursaries were offered for the conference, however uptake was lower than in previous financial year.

No new Bill Petit Grants have been committed to this year; the amount paid out relates to a grant approved in 2020.

Running costs are returning to pre-Covid levels with the largest increase in committee expenses as a result of the full 2 day planning away day.

Reserve Policy:

NatSCA has been cautious in use of reserves over the past 2 years, during and following the COVID-19 pandemic. Prior to this period the CIO general reserves were increasing year on year, largely due to consistent membership levels and good turnout at the annual conference. The trustees have reviewed the level of reserves required, based on spending commitments and timings, and consider that reserves in the region of £20,000 would be sufficient to ensure a secure basis for the continued operation of the charity and delivery of objectives.
Current reserve levels are more than twice this level and the trustees are working on plans to utilise excess funds to the benefit of members and the wider Natural Sciences Collections community. Initial plans over the next 3 years include:
a) Improvement of the online offering via the NatSCA website and resources.
b) Increasing training and workshops to pre-pandemic levels or above.
c) Offering additional support in the form of bursaries and subsidised attendance fees to assist with the cost-of-living crisis, and low training budgets.

The trustees will review reserve levels a minimum of 6 monthly, to monitor available budget for opportunities for additional areas of work, member and public support, and advocacy across the sector.

Draft Accounts for approval:

The draft accounts have been prepared for approval as shown on the following pages

	Charity Name:			No:		CC16a
	Natural Sciences Collections Association (NatSCA)			**1186918**		
	Receipts and payments accounts					
	For the period from	Period start date	**To**	Period end date		
		01/02/2023		**31/01/2024**		

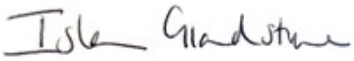
Section A Receipts and payments

	Unrestricted funds		**Restricted funds**		**Endowment funds**		**Total funds**		**Last year**
	to the nearest £		**to the nearest £**		**to the nearest £**		**to the nearest £**		**to the nearest £**
A1 Receipts									
Institutional Subscriptions	**2,052**		**-**		**-**		**2,052**		**1,920**
Personal Subscriptions	**4,155**		**-**		**-**		**4,155**		**4,645**
Workshops	**652**		**-**		**-**		**652**		**-**
Conferences	**15,235**		**-**		**-**		**15,235**		**-**
Donations	**5**		**-**		**-**		**5**		**-**
Publications	**6**		**-**		**-**		**6**		**12**
Bank Interest	**-**		**-**		**-**		**-**		**-**
Sub total(Gross income for AR)	**22,105**		**-**		**-**		**22,105**		**6,577**
A2 Asset and investment sales									
	-		**-**		**-**		**-**		**-**
Sub total	**-**		**-**		**-**		**-**		**-**
Total receipts	**22,105**		**-**		**-**		**22,105**		**6,577**
A3 Payments									
Running costs	**2,587**		**-**		**-**		**2,587**		**1,324**
Workshops	**286**		**-**		**-**		**286**		**-**
Conference	**9,239**		**-**		**-**		**9,239**		**53**
Publications & Information Provision	**2,466**		**-**		**-**		**2,466**		**2,174**

Charitable Activities : Bill Petit Grants	**2,100**	**-**	**-**	**2,100**	**2,528**
Charitable Activities : Bursaries	**616**	**-**	**-**	**616**	**1,504**
Sub total	**17,294**	**-**	**-**	**17,294**	**7,583**
A4 Asset and investment purchases					
	-	**-**	**-**	**-**	
Sub total	**-**	**-**	**-**	**-**	
Total payments	**17,294**	**-**	**-**	**17,294**	**7,583**
Net of receipts/ (payments)	**4,811**	**-**	**-**	**4,811**	**- 1,006**
A5 Transfers between funds	**-**	**-**	**-**	**-**	**-**
A6 Cash funds last year end	**47,785**	**-**	**-**	**47,785**	**48,791**
Cash funds this year end	**52,596**	**-**	**-**	**52,596**	**47,785**

Section B Statement of assets and liabilities at the end of the period				
Categories	Details	Unrestricted funds	Restricted funds	Endowment funds
		to nearest £	to nearest £	to nearest £
B1 Cash funds	Current Account	**52,596**	-	-
	PayPal	-	-	-
		-	-	-
	Total cash funds	**52,596**	-	-
	(agree balances with receipts and payments account(s))	OK	OK	OK
		Unrestricted funds	**Restricted funds**	**Endowment funds**
	Details	**to nearest £**	**to nearest £**	**to nearest £**
B2 Other monetary assets		-	-	-
		-	-	-
		-	-	-
	Details	**Fund to which asset belongs**	**Cost (optional)**	**Current value (optional)**
B3 Investment assets			-	-
			-	-
			-	-
	Details	**Fund to which asset belongs**	**Cost (optional)**	**Current value (optional)**
B4 Assets retained for the charity's own use			-	-
			-	-
			-	-
			-	-
	Details	**Fund to which liability relates**	**Amount due (optional)**	**When due (optional)**
B5 Liabilities	Journal 2023	Unrestricted	**2,500**	

Signed by one or two trustees on behalf of all the trustees

Signature	Print Name	Date of approval
	Isla Gladstone	18.iv.2024
	Yvette Harvey	18.iv.2024

Accounts will be signed when agreed at AGM.

Proposer: Gina Douglas

Seconder: Maggie Reilly

Membership Secretary's Report: Clare Brown

For 2023 the membership statistics are as follows:

- 278 members (56 institutional, 222 personal).
- This is 18 fewer members than 2022-23 (19 fewer individuals and 1 more institution).
- Around 75% of our membership is UK based, we also have members in 17 other countries.
- 103 members chose to receive a hardcopy of the journal.
- In addition there were 13 free/complimentary mailings of the journal either for legal/copyright reasons or networking (British Library LDO, British Library CRO, GCG, Smithsonian Institute Library Gift and Exchanges, ACE, SPNHC, MA, Zoological Record, plus five copies to Agency for the Legal Deposit Libraries).

Membership numbers have dropped very slightly from 2022. However, this was not by a great deal and so the decline in membership numbers seems to be slowing down. Membership rates usually increase with our offer of training events and in-person conferences and so our plans for these in 2024-25 should help with our numbers. Uptake of the hardcopy of the journal is down again this year, with most people choosing to look at it online.

I would like to thank everyone who has supported me with the membership work over the last year, Belle Buchanan-Smith, Eimear Ashe, Glenn Roadley and Justine Aw in particular.

Editor's Report: Jan Freedman

Volume 12 of the *Journal of Natural Science Collections* has been published both in print and online. Members who requested their hard copy will have received it in the post, and the password to access the online articles has been sent by our Membership Secretary. The first three articles are open access as they focus on decolonising work in the sector, and we want these to be accessible to everyone.

The latest Volume includes 10 articles covering decolonisation, community engagement, collections, and conservation. Articles written by international experts from around the world have made my final Volume an impressive one.

Thank you to the amazing Editorial Board, who work so hard behind the scenes to check articles and find reviewers: Paolo Viscardi, Verity Burke, Emilie Pearson, and Lisa Winters. They are patient, fantastic and dedicated, and without them this Volume would not be what it is. Thank you to all the anonymous reviewers, who ensure that each article is to the highest of standards. All the reviewers and the Editorial Board work in their own time to go through the articles in so much detail, and I am very grateful for all of their time they give to the Journal.

Volume 12 will be my last Volume as Editor for NatSCA. I have really enjoyed working for you all as the Editor for the Journal, and although it is an awful lot of work, I will genuinely miss it. The articles, the authors, the editorial board, the committee, the editing, the formatting: everything, I will miss.

Having worked as Editor since 2008, with a small hiatus, I began with *NatSCA News*, and transformed this into the Journal of Natural Science Collections. It has been something I have really enjoyed working on, and something I'm very proud of. I have really enjoyed meeting and working with so many different people along the way.

I will miss being a part of the NatSCA committee: those individuals who work relentlessly out of their working hours to do so much, from the blog to the organising of the conference – there are so many tasks that they all work on, most of which are not seen. One of the greatest things about the committee is their supportive, enthusiastic work ethic. I am proud that I was a small part of this great committee.

And a final thank you to *you*, the members. The workshops, the JISC Mail, the conferences, have all been full of new ideas, support, collaborations, and fun, because of you. This really is such a passionate group to be a part of. Sometimes being the only natural history curator in a smaller regional museum can feel as though there is no support, but being a part of NatSCA shows what an amazing community there is. An amazing community that cares for amazing collections.

Co-Chairs' Report: Jen Gallichan and Isla Gladstone

2023 marked the first year with NatSCA Co-chairs, with the position being divided into two to support capacity and shared leadership. It has been a positive year of transition and learning. NatSCA has worked hard to continue to grow and adapt our activity. We are mindful of difficult situations colleagues are facing globally and locally, and continue to focus on community, support and advocacy.

In April we held our first in-person NatSCA-led annual **conference** since 2019, at The Potteries Museum & Art Gallery in Stoke-on-Trent, Staffordshire. This was hybrid in format for both speakers and attendees, thanks to the hard work of Glenn Roadley and Justine Aw. Titled *"So how do we actually do all this? Hopeful futures and turning theory into practice for big issues in natural history collections",* conference lead Patti Wood Finkle reports that we had 26 presentations and introduced a new "keynote/grandees/discussion" format for the final session on the first day, which was well received. A conference survey compiled and circulated to the NatSCA committee by Jack Ashby showed overwhelmingly positive feedback. Attendees particularly valued: sessions presenting practical approaches as well as sessions on decolonisation; presentation formats including both lightning and longer talks; friendliness for new and seasoned attendees; the range of speakers and sizes of their institutions; the organisation of presentations was well thought out; opportunity for hybrid. The biggest complaints were the audio for virtual attendees on day 1 and the lack of biscuits for the in-person attendees.

Planning for 2024's conference started in summer 2023. Titled *"Trials and Triumphs: sharing practice across the museum sector"* and hosted at Oxford Museum of Natural History and online. By the end of the 2023 NatSCA year we had received 55 papers for 26 speaker slots, as well as organising tours, caterers and AGM.

Training lead Laura McCoy reports that NatSCA delivered two training events this year, one in person and one online, both of which were sold out. "*An Introduction to mobilising your collection's biodiversity data*" was held in July. It was organised in partnership with the Natural History Museum London's DiSSCo UK team as an in-person workshop with 10 participants. Attendees were taken through the steps required to digitise and share specimen occurrence data ('what, where, when, who'), and understand some of the community standards used by the Global Biodiversity Information Facility (GBIF). A mixture of talks and exercises were provided to help people find achievable solutions and aid delegates make the case for future investment. Supplementary online seminars were also offered by the DiSSCo team to deliver more in-depth training and help answer any specific questions. "*An introduction to natural science collections legislation*" was held in November online. With six speakers and 96 attendees, subjects included: CITES relating to plants and animals, poisons in herbaria, loans (including shipping and packing), the Wildlife and Countryside Act 1981, Geological legislation considerations and the Human Tissue Act 2004 in relation to museum collections.

Monthly lunchtime chats have been consistently well attended and of the ten provided in 2023 we have had around 200 attendees cumulatively. Challenges sourcing speakers have been helped in 2024 through offering places to conference speakers who we were unfortunately unable to accommodate due to very high levels of speaker applications. Talks are recorded and the link sent out to members. Please do contact training@natsca.org if you would like to offer a training topic or Lunchtime Chat.

Blog Editor Jen Gallichan reports that visits and views of NatSCA's blog pages are maintaining a steady level. We are consistently attracting between 1700 to over 2000 views a month, with an average of 55 to 75 views per day. We have been successful in attracting submissions from across the sector, including from those in receipt of NatSCA bursaries and through Jisc mail call outs. The highest number of visitors are still coming from the UK, but articles are getting more international coverage than ever before. We continue to attract visitors from the USA, Australia and parts of Europe and have had considerable increase in readers from Canada and India. Over 40 blogs were posted in 2023. Our most read blogs of all time focus on collections management and conservation including 'how to' blogs sharing best practice, as well as decolonial practices. Popular blogs this year include the NHM collections move update and Jazmine Miles-Long's blog on taxidermy and death.

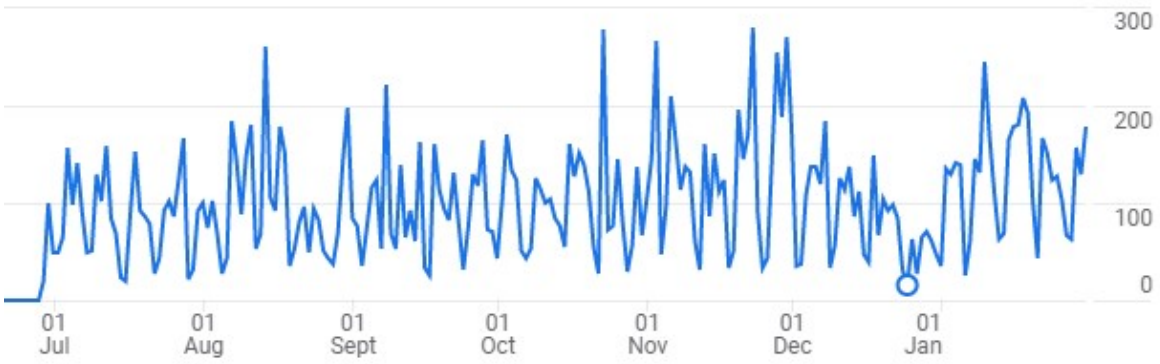
Top Posts
Private Bone Collections: The Good, The Bad and The Illegal
Giant Sequoia at The Natural History Museum
Freezing Specimens and how to Mitigate Freezer Burn
What is Taxidermy? An intimate relationship between death and maker.
How to do decolonial research in natural history museums
Resurrection 101
Preparing collections for a big move
Telling The Truth About Who Really Collected The 'Hero Collections'
The Herbarium Handbook – Sharing best practice from across the globe
Deaccessioning of the non-Manx herbarium in the Natural History Collection, Manx Museum

NatSCA **Website** lead Glenn Roadley reports that a forced switch in analytics platform mid way through 2023 means obtaining a complete picture of website traffic for the year is tricky. Website traffic peaked around late April, coinciding with the NatSCA 2023 conference, with some higher peaks also appearing later in the year. Overall we had 41,435 page views this year, down 12% on the same period last year but a smaller decrease than the one that brought us in line with pre-2020 levels last year. Monthly page view figures range from 2,600 to over 4,600.

Users over time

Feb – June 2023 (Pre-Google Analytics 4)

July – Jan 2024 (Google Analytics 4)



Top Pages (Google Analytics 4)

Rank	Page	Page Views	Users	Avg. Time on Page (seconds)
1	Home	2,911	1,677	16
2	Jobs	1,798	6,33	35
3	Taxidermy	1,149	944	28
4	Journal	848	313	21
5	Nature Read in Black and White	809	574	16
6	Events and Workshops	798	451	20
7	Publications	517	301	38
8	Care and Conservation	516	292	26
9	Resources	439	284	16
10	Membership	429	292	42

Website updates have included: 36 sector job vacancies, committee member profiles and a consolidation of resources relating to Collections and the Law following 2023's training event.

NatSCA's current website has been in use for over 10 years. It lacks many quality-of-life and accessibility features of more modern websites, particularly the ability to respond to a user's screen size – changing shape to ease navigation on tablet or mobile. Its content has also grown substantially over that time, with several areas now requiring consolidation and updating. Furthermore, the NatSCA blog website is a different site entirely, detached from the main site. As such development has started on a new website, which aims to migrate all existing features and content of the current site, while also integrating the blog posts and providing a more modern browsing experience. At time of writing, the design of the website is complete and all content has been migrated with the exception of blog posts and publications, which we aim to complete in the next few weeks. Once confirmed that the basic functionality of the website is in place and working as expected, a review of all content will take place, with the membership consulted on any changes they'd like to see. We aim to launch the new website after the NatSCA 2024 conference.

It remains a difficult time financially for many individuals and organisations. NatSCA has significantly increased our bursary offer both in terms of number of bursaries and total amount offered, to support members to attend our events. We are also able to write letters of advocacy for specific **collections at risk**, please contact: chair@natsca.org. Through platforms such as our conference, we will continue to platform best practice that helps to sustain collections, and offer opportunities for connecting with colleagues for mutual support.

In terms of **national network projects**, NatSCA has continued to participate in pilot work to scope a national digital research infrastructure for natural science collections called DiSSCo UK (Distributed System of Scientific Collections UK). We are excited to see this opportunity develop, and will continue to work to support participation and benefit for small to large collections across the UK.

4. Election of NatSCA trustees

Trustees form a steering committee with obligations to ensure NatSCA meets our mission, ensure good governance and conform to Charity Commission regulations.

Below are the nominees for NatSCA trustee positions standing for election at this AGM. The Membership Secretary has confirmed that those proposed, those proposing and those seconding are all current personal members of NatSCA. No term will exceed three years without re-election.

Below are the nominated candidates standing for **Ordinary Member** positions on the committee:

Nominee	Position	Proposed	Seconded
Tannis Davidson	Ordinary Member	Hannah Cornish	Jack Ashby
Natalie Jones	Ordinary Member	Arianna Bernucci	Emilia Kingham
Patti Wood Finkle	Ordinary Member	Jen Gallichan	Jack Ashby

There are three vacancies for Ordinary Members and three nominees.

Below is the nominated candidate for **Membership Secretary**:

Nominee	Position	Proposed	Seconded
Clare Brown	Membership Secretary	Isla Gladstone	Jen Gallichan

There is one vacancy for Membership Secretary and one nominee.

Below are the nominated candidates for **Editor (Journal)**:

Nominee	Position	Proposed	Seconded
Emilie Pearson	Editor	Abbie Herdman	Nadine Gabriel
Glenn Roadley	Editor	Olivia Beavers	Paolo Viscardi

There is one vacancy for Editor, and there are two nominees for the role.

Proposal 1: An election will be held for the position of Editor.

Membership vote: **Glenn Roadley**

Please remember that only paid up individual members and one authorised individual per institutional member are able to vote at NatSCA's AGM.

Proposal 2: If Glenn Roadley is elected Editor by a simple majority vote, his current Ordinary Member position will become available. In this scenario, we propose Emilie Pearson is elected as an Ordinary Member to support the Editor role. (For the information of members: If Emilie Pearson is elected Editor Glenn Roadley will continue his current term as Ordinary Member to NatSCA's AGM in 2025.)

Proposer: Paolo Viscardi

Seconder: Donna Young

Membership vote: **Yes** / ~~No~~

This will be a hybrid poll, with a greater than 50% vote required to accept the proposal. Please remember that only paid up individual members and one authorised individual per institutional member are able to vote at NatSCA's AGM.

Proposal 3: We propose one 'en bloc' vote for all remaining nominees (three nominees for Ordinary Member, one nominee for Membership Secretary).

Proposer: Erica McAlister

Seconder: Maggie Reilly

Membership vote: **Yes** / ~~No~~

This will be a hybrid poll, with a greater than 50% vote required to accept the proposal. Please remember that only paid up individual members and one authorised individual per institutional member are able to vote at NatSCA's AGM.

5. Any other Business

6. Vote of thanks

NatSCA would like to thank everyone who has been involved in delivering our activities in 2023 for sharing their time, expertise and content.

We would like to thank NatSCA's trustees for all of their hard work leading on key activities and contributing to the overall running of NatSCA.

New trustees welcomed in 2023 were: Eimear Ashe, Clare Booth-Downs, Belle Buchanan-Smith (ratified as Treasurer in 2023; joined in 2022), Emma Murphy.

Committee capacity continues to be affected by wider sector pressures, and as such we are moving to a more collaborative approach to delivering key roles. In addition to the area leads who have provided and are named in annual reports, we would like to express huge thanks to committee members supporting delivery of our activity. Committee members supporting Patti Wood Finkle with conference include: Jack Ashby, Clare Brown, Belle Buchanan-Smith, Jen Gallichan, Amy Geraghty, Yvette Harvey, Emma Murphy, Glenn Roadley; supporting Laura McCoy with training: Amy Geraghty and Emma Murphy; supporting Yvette Harvey Secretary and Clare Brown Membership Secretary: Eimear Ashe; supporting DiSSCo UK work: Jack Ashby, Clare Brown, Isla Gladstone, Emma Murphy, Glenn Roadley. Tannis Davidson has been working on re-releasing the Bill Pettit award in 2024.

We thank Justine Aw for highly valued external technical support.

Jen Gallichan and the trustees would like to pass on heartfelt thanks to the great group of volunteers who compile our monthly Digital Digests including Olivia Beavers and Milo Philipps, with particular thanks to Glenn Roadley who has now had to step down due to other committee duties. We are also pleased to welcome Ellie Clark who joined the team in January.

Editor Jan Freedman has shared thanks to the Editorial Board for their valued support: Paolo Viscardi, Verity Burke, Emilie Pearson, and Lisa Winters.

NatSCA would like to extend special thanks to those trustees stepping down from committee this year:

Jan Freedman for all of his work as NatSCA's Editor over many years, and Amy Geraghty for all of her support for conference and training. Their contributions have been highly valued, and they will be missed.

NatSCA's strength is in being community-led, and we value your contributions towards this past and future. Please do get in touch if you are interested in volunteering for NatSCA or in how our committee works: chair@natsca.org.

7. Next AGM venue: To be announced

8. Close: 2.20pm

NatSCA Committee

Honorary Officers

Ordinary Members

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