Trends in
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Forum

Time to spice-up paleoecological records with bryophyte spores

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Paleovegetation reconstructions rely virtually exclusively on inferences from vascular plants, particularly pollen grains, ignoring other components of the land flora. Artificial intelligence (AI) opens the door to the identification of other microfossils, particularly bryophyte spores, which offer a new, higher magnification lens to characterize past climatic environments.

Quaternary (2.58 million years–present) paleoecological records compose on-site environmental libraries of responses of organisms and ecosystems to climate and land-cover change [1,2]. Pollen grains, in particular, are typically produced in large quantities, are highly resistant to degradation, and carry critical taxonomically diagnostic features to reconstruct detailed paleoecosystems. The analyses of pollen grains accumulated in chronological core sediments provide unambiguous evidence of dynamic shifts in vegetation driven by past global change. These paleoreconstructions constitute the fundamental baseline to weight the impact of the magnitude of human disturbance on ecosystems during the Anthropocene [3]. Fossil pollen identification and counting so far relied on time-consuming examination of slides under light microscopy by experts. The implementation of AI, in much the same way as popular online tools for species identification based on photographs (e.g., Pl@ntNet, https://plantnet.org/en/), has recently emerged as a promising tool to automatize pollen identification, accelerate data acquisition, and enable the analysis of massive amounts of records spanning large geographic regions [4–6].

Other microparticles produced in large amounts and accumulating in sediments are bryophyte spores. With about 22 000 species, bryophytes compose the second most diverse phylum of land plants after the seed plants and contribute significant taxonomic diversity across all ecosystems except marine. While bryophyte macrofossils have been used in paleoecology [7], no key or automated system currently exists to identify bryophyte species from their spores. Consequently, bryophyte spores remain a largely unexploited resource in palaeoecology. Since bryophyte spores exhibit a broad amplitude in size and surface ornamentation across lineages (Box 1), we call for the development of an AI-based identification tool of bryophyte species from their spores, allowing for their integration in paleovegetation reconstructions.

Gaining enhanced resolution of paleoenvironments through a bryophyte lens

Integration of bryophyte spores would substantially boost the amount of data available, contribute to critical complementary perspectives, and enhance the resolution of ecological inferences from the paleoecological records. Bryophytes and seed plants differ substantially in a series of features of their anatomy, ecophysiology, and life-history, which impact their response to variations in environmental conditions:

(i) Water relations. Lacking roots, bryophytes absorb water through the surface of their aerial vegetative body and hence depend on atmospheric precipitation to sustain their needs. Species differ in their ability to withstand water shortage and span a broad physiological spectrum from desiccation avoidance (ephemeral species) to dehydration and even desiccation (i.e., recover following loss of 90% of internal water content) tolerance. Their recovery further varies with the intensity and duration of dehydration or desiccation, such that bryophytes offer a finer resolution of climatic conditions, in particular, in terms of seasonality of precipitation and temperature fluctuations.

(ii) Nutrient uptake and requirements. Bryophytes mostly absorb nutrients from precipitations. Given that bryophytes hold effective mechanism for translocation of nutrients, they display low nutrient requirements and can be globally considered as 'oligotrophic' plants, directly (toxic effects) and indirectly (competition) affected by eutrophication. Because bryophytes absorb water and nutrients through their aerial vegetative body, they are very sensitive to air quality.

(iii) Temperature range. Bryophytes have, on average, lower physiological temperature optima than seed plants. Consequently, species from temperate and boreal environments dramatically suffer from moderate heat, dying when exposed, wet, at >30°C after a few days.

(iv) Dispersal capacity. Unlike in seed plants, the community composition of which is typically characterized by a disequilibrium with climatic conditions due to dispersal limitations, species turnover in bryophyte communities takes place at a much higher rate due to the efficient long-distance dispersal capacities of the spores, which germinate into new vegetative plants. As a result, the composition of bryophyte communities is an almost direct reflection of contemporary climate conditions, potentially allowing paleoenvironmental reconstructions at finer resolution through time.

Box 1. Bryophytes spores: the overlooked homologs of pollen grains

Fundamentally, pollen grains are microspores produced by heterosporous plants, including all seed plants. Bryophytes are isosporous and produce spores in their single sporangium. A single capsule can typically release thousands to hundreds of thousands of spores, making them very abundant in the environment. Like the pollen grains of most seed plants, bryophyte spores are dispersed by wind. As with the spores of all land plants, their wall is impregnated by sporopollenin, a highly resistant biopolymer against physical abrasion, desiccation, decay, and UV radiation. The sporopollenin-impregnated wall accounts for the accumulation and persistence of spores in sediments dating as far back as the Silurian and even older. In both pollen grains and spores, sporopollenin-impregnated walls exhibit striking ornamentation patterns (Figure I), suggesting that, like pollen grains, bryophyte spores offer a hitherto largely unexploited source of taxonomic characters.

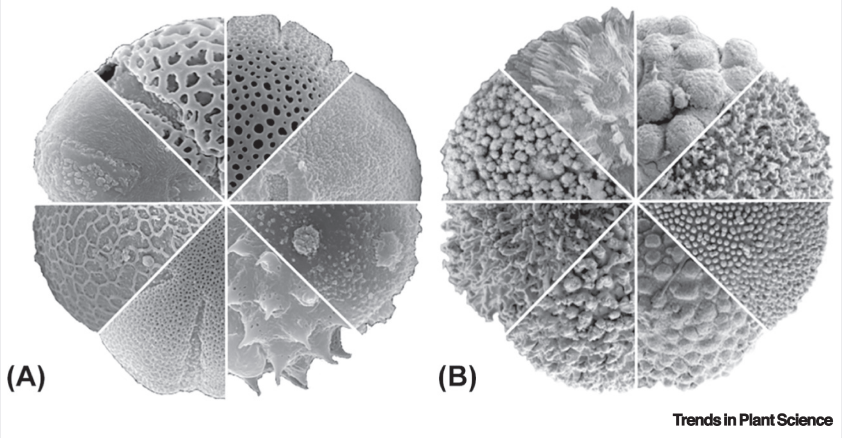

Figure I. Comparison of pollen grain and bryophyte spore ornamentation patterns under scanning electron microscopy. (A) Pollen grains (magnification: 2000×–4000×) of, in clockwise order from the top, *Spermacoce saxatilis, Gramina* sp., *Stellaria media, Bellis perennis, Glechoma hederacea, Iris germanica, Veronica* sp., and *Petunga roxburghii*. (B) Spores (magnification: 2500×–4000×) of, in clockwise order from the top, *Encalypta vulgaris, Sematophyllum subsimplex, Pohlia nutans, Ulota bruchii, Microbryum davallianum, Archidium alternifolium, Neckeropsis disticha*, and *Bruchia flexuosa*. Photographs by I. Van der Beeten, P. Ballings, and M. De Haan.

These features make bryophytes reliable bioindicators that have been used to evidence current major environmental trends, including changes in air quality (e.g., SO_2 pollution resulting from charcoal combustion and acidic rains during the 20th century [8]), eutrophication [8], and climate warming [9]. As a consequence of the high sensitivity of bryophytes to climate conditions and of their high dispersal capacities, the rate of replacement of cold-dwelling by warm-dwelling species (thermophilization) is twice as high in bryophyte than in seed plant communities in mountain environments [9]. Paleo-assemblages of bryophyte spores thus have the potential to contribute significantly to estimating past environmental conditions and impact of human disturbance through time. In practice, such inference can be performed for environmental parameters of interest such as pH, nitrogen content, temperature, or humidity, by averaging species indicator values [10] for these parameters across species within a sample.

Towards the integration of bryophyte spores in paleoecological analyses

Integrating bryophyte spores in paleoecological analysis will require: (i) training AI to identify taxa from scanning electron microscopy (SEM) images of spores. (Figure 1A,B); and (ii) applying the model on sediment cores (Figure 1C). The first step will take place in the context of the fast development of image-based automated species identification, boosted by substantial progress in machine learning algorithms and, in particular, deep neural networks (DNNs). Mounting evidence suggests that bryophyte species can be identified using DNNs based on SEM pictures of their spores at rates ranging between 71% and 100%, depending on species [11]. In seed plants, by comparison, the identification of pollen grains using DNNs allows for taxonomic identification at resolutions ranging between species and families with an accuracy ranging between 70% and 99% [4–6]. Whether spore morphology will allow the identification of bryophytes at species or higher taxonomic level is also likely to vary among genera and families and remains to be tested by extensive taxon sampling. Differences in spore morphology among sibling bryophyte species are, however, recurrently reported in the literature, supporting the idea that spores yield useful characters at shallow taxonomic levels (see [11] for review).

Given the voracity of DNNs in terms of data to train the model and avoid overfitting [5,6], model training will require a large (>100) number of pictures per species for each of the ca. 22 000 species recognized to date. The ongoing massive digitization and online accessioning of preserved specimens within an integrative taxonomic framework [12], such as the Distributed System of Scientific Collections (DISSCO, www.dissco.eu), would provide a unique opportunity to generate, beyond scans of the herbarium specimens themselves, massive amounts of spore pictures under SEM linked to voucher specimens.

To apply the model to reconstruct bryophyte paleovegetation from sediment cores, DNNs will have to be further trained to distinguish bryophyte spores from other non-pollen palynomorphs, using mixes of bryophyte spores and other spores. Core samples will then be mounted on slides after chemical treatment to remove

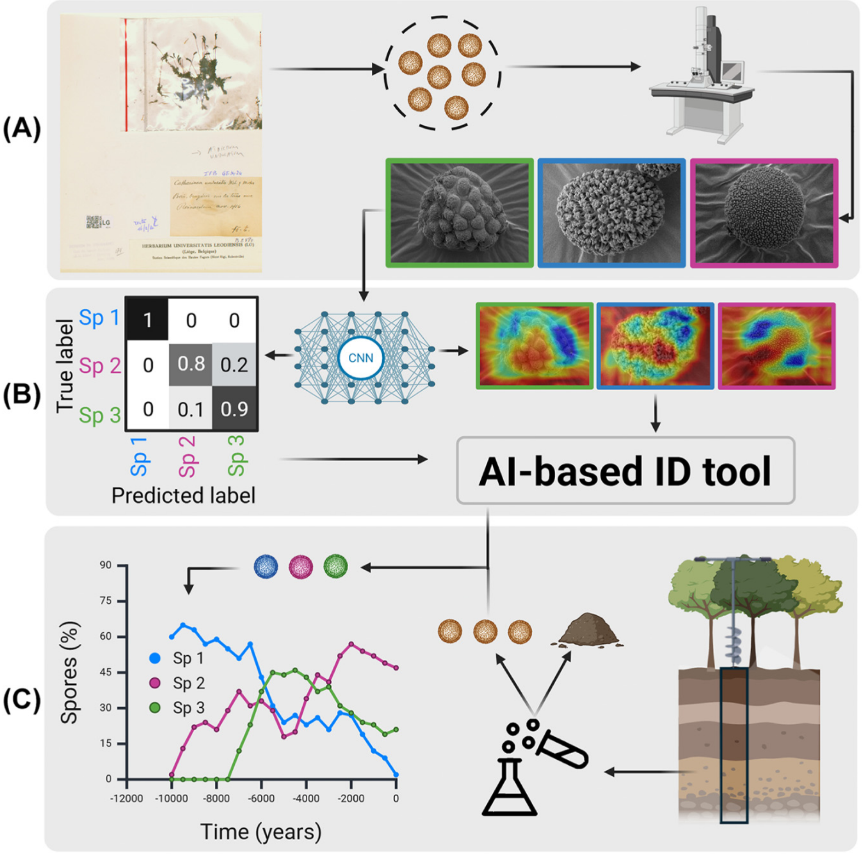

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Figure 1. Towards the automatized identification of bryophyte spores from environmental samples. (A) Spore sampling from herbarium specimens and management of a database of photographs under scanning electron microscopy. (B) Training of deep neural networks for species identification. The pictures represent examples of heat maps, highlighting important regions of an image in terms of relevant diagnostic features, and of confusion matrices, which serve to measure the accuracy of the model. The diagonal represents the percentages of correctly predicted specimens, while the percentage of specimens assigned to a wrong species appears outside of the diagonal. (C) Application to paleoecology. Environmental samples are retrieved from soil cores, from which spores are extracted through physical and chemical treatments and subsequently automatically identified to reconstruct the dynamics of species frequencies through time. Abbreviation: AI, artificial intelligence.

calcium carbonates and silicates and suspended in isolation buffer using standard palynological protocols. Non-pollen palynomorphs will then be photographed under SEM. Finally, bryophyte spores will be sorted and identified at the finest taxonomic resolution possible using DNNs.

Potential pitfalls of this approach, as revealed by automatized pollen grain analysis [13], include degradation of spores in the core sample, differences in the morphology of extant and fossilized spores, and differences in the composition of the extant flora of target sites on which the DNN was trained and fossil floras. In this regard, two features of bryophytes make them good candidates for the application of AI-based identification models calibrated on extant floras to study fossil floras. First, bryophytes include about 22 000 species (vs. 300 000 in seed plants). This makes it possible to envision, in the context of the proposed collaborative effort, generation of a model applicable to the entire group, thereby circumventing the issue that the AI-based tool misses a series of taxa present in the sediment. Second, spores of fossil bryophytes from Quaternary deposits are more likely to share morphologies with extant species and, hence, be confidently identifiable based on a model trained on the latter, than are pollen grains. Such a hypothesis stems from the fact that morphological evolution in bryophytes is slower than in seed plants [14]. This suggests that bryophytes share morphological traits for longer evolutionary periods than seed plants, potentially limiting morphological discrepancies between fossil and extant spores. One complication of the identification of spores as compared with pollen grains is, however, that, while the latter can be identified in light microscopy, the former require examination under SEM, which is more time-consuming and costly than light microscopy. This constraint limits the perspective to automatize the process using multispectral imaging flow cytometry, which opens the door to promising solutions for speeding up the analysis of pollen at 40× magnification [6].

The possibility of identifying bryophyte species from their spores using AI would open the door to a new avenue of research. Beside obvious applications in paleoecology and global change biology, the identification of bryophyte species from their spores would further make it possible to characterize the composition of the air biome, which may hold up to 100 spores m^{-3} [15]. Identifying spores from the air biome would offer a direct means of assessing dispersal, with important consequences for our ability to predict whether species can effectively track the shift of their suitable range due to climate change.

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Declaration of interests

The authors declare no competing interests.

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