Biology of Lichens – Symbiosis, Ecology, Environm. Monitoring, Systematics, Cyber Applications
Thomas H. Nash III et al. (ed.): Bibliotheca Lichenologica ***105****: 183–190.*
J. Cramer in der Gebrüder Borntraeger Verlagsbuchhandlung, Stuttgart 2010

Simulating fossilization to resolve the taxonomic affinities of thalloid fossils in Early Silurian (*ca.* 425 Ma) terrestrial assemblages

ALEXANDRU M.F. TOMESCU, RICHARD W. TATE, NATHAN G. MACK & VANESSA J. CALDER

Department of Biological Sciences, Humboldt State University, Arcata, CA 95521, USA; mihai@humboldt.edu

Abstract: Late Ordovician to Late Silurian (450–420 Ma) fossils of the Appalachian Basin represent land floras that pre-date the advent of vascular plants, but their exact taxonomic affinities are unresolved. This is due to preservation as carbonaceous compressions which precludes direct anatomical comparisons with living organisms. Experiments performed on a broad taxonomic range of organisms to simulate the effects of pressure and heat during fossilization show that, even when highly altered, internal structures can still be used to separate major taxonomic groups. The experiments produced internal structures similar to those of the fossils in some algae, fungi, lichens, and bryophytes. These results emphasize the usefulness of such experimental approaches and corroborate the results of previous microfossil and geochemical studies indicating that pre-tracheophytic terrestrial floras were similar to modern biological soil crusts, consisting of mixed, ground-hugging communities of thalloid and crustose organisms: cyanobacteria, algae, fungi, lichens, bryophytes.

Keywords: thalloid fossils, Early Silurian, cyanobacteria, algae, fungi, lichens, bryophytes.

Introduction

The Appalachian Basin of eastern North America hosts fossil assemblages preserved as carbonaceous compressions in Late Ordovician through Late Silurian (*ca.* 450–420 Ma) terrestrial and marginally marine deposits (TOMESCU et al. 2009). These Appalachian assemblages predate the oldest known vascular plant fossils (*Cooksonia*, mid-Silurian, EDWARDS et al. 1992; *ca.* 425 Ma), thus offering insights into the type of communities that formed a terrestrial groundcover prior to the advent of vascular plants. As illustrated by these assemblages, pretracheophytic terrestrial communities consisted of thalloid and crustose organisms. The Ap-

palachian assemblages most intensively studied occur in the Early Silurian (Llandovery, *ca.* 440 Ma) lower Massanutten Sandstone, at Passage Creek, in Virginia (PRATT et al. 1978; TOMESCU & ROTHWELL 2006).

Passage Creek fossils exhibit diversity of morphologies, textures, and internal structures, which indicates a taxonomically diverse biota. Such diversity, along with the ground-hugging habit of the thalloid and crustose organisms, is reminiscent of modern biological soil crusts, communities formed by mixtures of cyanobacteria, algae, fungi, lichens, and bryophytes in varying proportions. Based on morphology alone, all of these groups of organisms represent potential producers of the fossils, but none of the fossils can be unequivocally assigned to any particular group. The presence of several distinct types of internal organization among the fossils (as documented using light- and electron microscopy; TOMESCU & ROTHWELL 2006), nevertheless indicates that Passage Creek assemblages include organisms representing several distinct taxonomic groups; some of the fossils consist of multiple layers of distinct structure, potentially indicative of eukaryotes with some level of internal differentiation. However, preservation as compressions has altered the original organisms to the extent that their internal structures cannot be identified to any taxonomic group.

In summary, although internal structures allow for differentiation of several fossil types, they cannot be used directly for taxonomic identification. This conundrum could be addressed by simulating fossilization on living representatives of the taxonomic groups that are potential producers of the fossils. Here, we present the results of a first set of experiments aimed at determining whether simulated fossilization of living organisms produces structures similar to those documented in the Early Silurian Passage Creek fossils. Particularly, the questions addressed are: (1) are groups affected differently by treatments simulating fossilization? (2) can different groups still be distinguished and identified? (3) which groups produce structures similar to those of the fossils?

Material and methods

We used fresh material of genera from each major group of organisms characterized by thalloid morphology or mat-forming: cyanobacteria (*Nostoc*), phaeophytes (*Fucus*), rhodophytes (*Mazzaella*), chlorophytes (*Spirogyra*), fungi (sarcosomataceous ascocarp, helotialean basidiocarp), lichens (*Peltigera*, *Parmotrema*), hornwort gametophytes (*Anthoceros*), and thalloid liverwort gametophytes (*Pellia*, *Marchantia*, *Conocephalum*). To simulate fossilization, samples were spread flat between sheets of wax paper sandwiched between two layers of filter paper and compressed between thick plexiglas plates by means of four C-clamps tightened at maximum. The compression setup, with filter paper protruding beyond the plexiglas plate edges was positioned vertically in a tray with water (seawater for the marine taxa) so the filter paper could wick the water and keep the samples humidified; wax paper was used to prevent the soft fresh material from being pushed into the pores of the filter paper during compression. Wet compression lasted 4 days, following which samples, kept between the wax paper sheets, were compressed for 4 hours at 130° C using a clothing iron. Samples were fixed in FPA, dehydrated in an ethanol and then isopropanol series, embedded in paraffin, cross-sectioned on the rotary microtome, and stained with Weigert's hematoxilin, Bismark brown, phloxin, and fast green - orange G.

Results

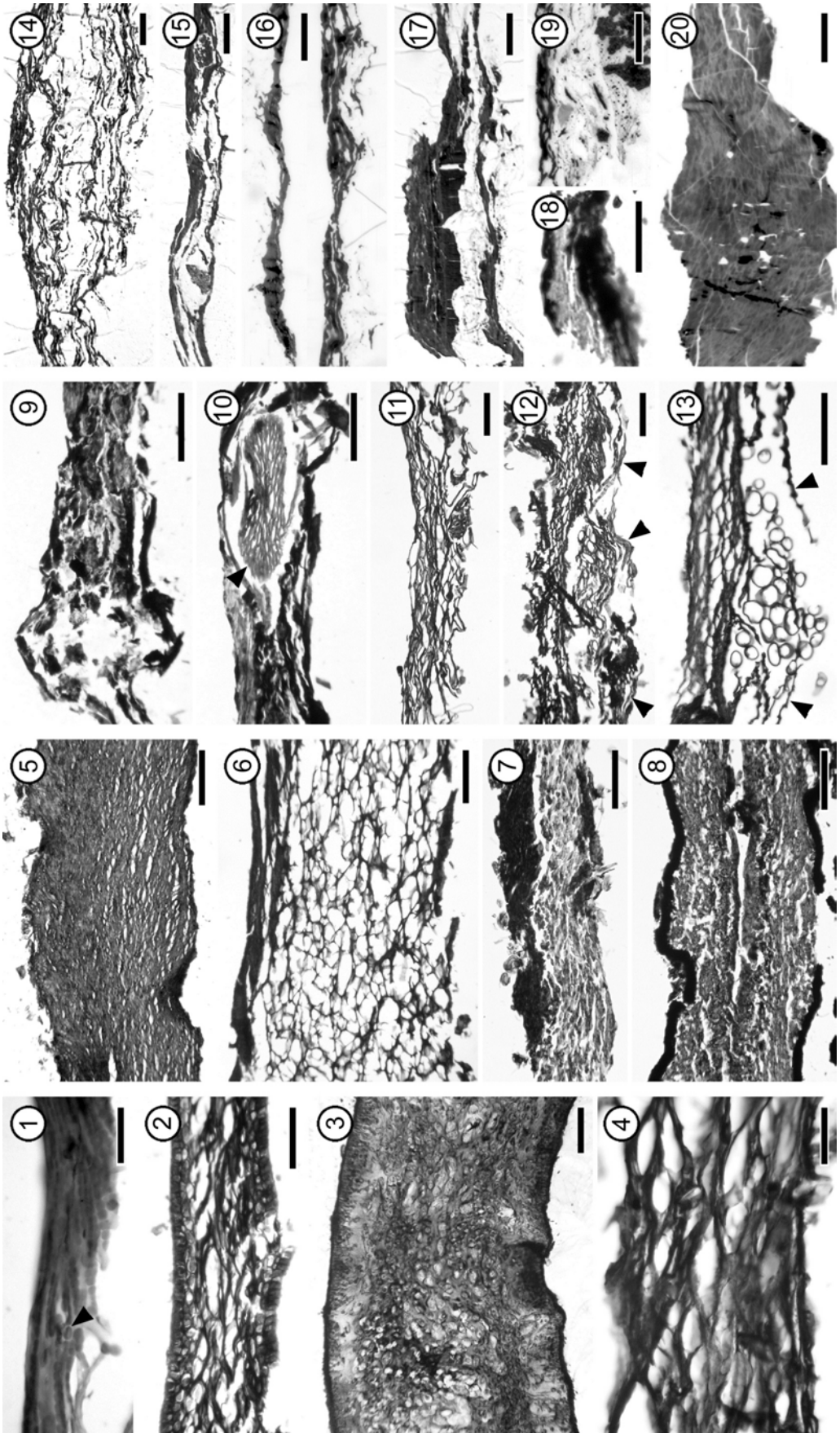
The response of the different taxa to treatments simulating fossilization ranged from modest effects on the internal structures (*Fucus*, *Mazzaella*, *Parmotrema*), to dramatic changes that rendered taxonomic identification difficult (*Spirogyra*, fungal fruiting bodies, *Peltigera*, *Anthoceros*) (Tab. 1, Figs. 1–12). However, even in those taxa with the strongest responses, the treatments did not induce complete obliteration of internal structures.

Tab. 1. Response of different types of organisms to compression and heat simulating fossilization; + weak response, structure virtually not affected; ++ moderate response, tissue layering, cells and other internal structures still recognizable; +++ strong response, cellular structure highly altered, cell walls fused, pseudocellular structures or compression layering sometimes present.

Group	Taxon		Response to treatment
Cyanobacteria	*Nostoc*	Fig. 1	++
Algae	*Mazzaella* (marine Rhodophyta)	Fig. 2	+
	Fucus (marine Phaeopyta)	Fig. 3	+ / ++
	Spirogyra (freshwater Chlorophyta)	Fig. 4	+++
Fungi	sarcosomataceous ascocarp	Fig. 5	+++
	helotialean basidiocarp	Fig. 6	+++
Lichens	*Peltigera* (upper cortex only)	Fig. 7	+++
	Parmotrema (upper + lower cortex)	Fig. 8	+
Bryophytes	*Anthoceros*	Fig. 9	+++
	Pellia	Fig. 10	++
	Conocephalum	Fig. 11	++
	Marchantia	Fig. 12	++ / +++

In *Nostoc* colonies, trichomes oriented perpendicular to the direction of compression form pseudo-layering within the common extracellular slime and heterocysts are still recognizable (Fig. 1). In *Mazzaella* thalli (Fig. 3), the filamentous structure of the medulla is replaced by a pseudocellular pattern generated by changes in the structure and distribution of the intercellular mucilage, which renders recognition of the original layers of the thallus difficult. Thalli of *Fucus* (Fig. 2) retain their distinct layering, with cells of the meristoderm and cortex still recognizable, but the medulla shows a dramatic reduction in volume and its structure changes in the same way as that of *Mazzaella*. In *Spirogyra* mats, the filamentous structure and individual filaments are no longer distinguishable and their fusion generates a pseudocellular tissue-like pattern (Fig. 4).

In ascomycete fruiting bodies (Fig. 5), the hymenium and excipulum become indistinguishable, the hyphal structure is obliterated, generating a rather compact material with small voids elongated perpendicular to the direction of compression,


14
15
16
17
19
20
18
9
10
11
12
13
5
6
7
8
1
2
3
4

forming a pseudocellular pattern particularly in the excipulum. In the basidiocarp pileus, stroma hyphae are shrunken and fused into bundles/sheets that form a pseudocellular pattern with voids much larger than hypal lumens, whereas hymenophore lamellae are compressed and fused together into a thin compact layer (Fig. 6).

Lichen thalli exhibit compaction but the original layering of the thalli is still distinguishable. In *Peltigera* (Fig. 7) the cortex forms a compact layer of varying thickness which is missing in places, whereas hyphae of the medulla are compressed and partly fused into a pattern of discontinuous layers consisting of long lens-shaped units. The cellular structure is also obliterated in *Parmotrema* (Fig. 8). The upper cortex becomes compact and in the medulla hyphae are compressed and fused forming blocks of compact material separated by randomly distributed elongated voids.

In the hornwort *Anthoceros* (Fig. 9, 10), cellular structure is severely obliterated. Collapse of mucilage pockets and fusion of cell packages that separate them lead to formation of discontinuous acellular layers. Sporophyte bases embedded in the gametophyte tissue are still recognizable and retain distinguishable, though compressed, cellular structures. *Pellia* gametophyte thalli (Fig. 11) exhibit mostly recognizable but strongly flattened cellular structure. In *Conocephalum* and *Marchantia* (Fig. 12, 13), midrib tissue consisting of smaller cells retains its cellular structure, but the larger cells of the midrib are compressed and fused into acellular layers. The lamina retains the cellular structure, though strongly compressed. Scales on the underside of the thallus are compressed into thin acellular layers, but the rhizoids are intriguingly not affected by compression.

Discussion

Preservation of plant material as carbonaceous compressions (SCHOPF 1975) involves compression under lithostatic pressure, often combined with heat. For the Passage Creek biota, burial pressures and temperatures in the host rock (Massanutten Sandstone) reached 70 MPa and 200–250°C, respectively (STRAYTON 1987), during part of the 425 Ma time interval between deposition and exhumation. Our treatments simulating fossilization, with 4 days of compression and 4 hours of heating at 130° C, are a very rough approximation of such severe diagenetic history of the fossils. They nevertheless allowed for a first test of the

---

Figs. 1-20. Results of experiments simulating fossilization (in cross sections of compressed specimens) and comparisons with the five types of internal fossil structures of TOMESCU & ROTHWELL (2009). **1.** *Nostoc* colony (arrowhead – heterocyst). **2.** *Fucus*. **3.** *Mazzaella*. **4.** *Spirogyra* mat. **5.** Sarcosomataceous ascocarp. **6.** Helotialean basidiocarp. **7.** *Peltigera* (upper cortex darker). **8.** *Parmotrema* (two compressed thalli with the upper cortical layers back to back). **9.** *Anthoceros* gametophyte. **10.** *Anthoceros* gametophyte with cross section of sporophyte base (arrowhead). **11.** *Pellia* gametophyte. **12.** *Conocephalum* gametophyte (arrowheads – underside scales). **13.** *Marchantia* gametophyte with round rhizoid cross sections and underside scales (arrowheads). **14.** Fossil Type 1. **15.** Fossil Type 2. **16.** Fossil Type 2, part (top) and counterpart (bottom). **17 & 18.** Fossil Type 3. **19.** Detail of fossil Type 2. **20.** Fossil Type 5. Scale bars: 25 µm in Fig. 1, 4; 100 µm in Fig. 2, 3, 5-13; 20 µm in Fig. 14–20.

power of such an experimental approach in view of future research that more accurately simulates fossilization, as well as for first answers to a set of questions relative to fossil diagenesis and taxonomically informative anatomical characters.

Our results show that the internal structures of distinct groups of organisms are affected differently by treatments that simulate compression fossilization. However, it is possible that under more drastic pressure and temperature conditions the differences in response to treatments become less marked or disappear altogether. Under the conditions we generated, the treatments did not induce complete obliteration of internal structures even in taxa that exhibited very strong responses. Overall, major taxonomic groups are still recognizable and the structures induced by experimental treatments can be used to separate them, even in instances where internal structures are very different from those that characterize the uncompressed state.

TOMESCU & ROTHWELL (2006) described five types of internal structures based on cross sections of fossil compressions from Passage Creek (Fig. 14–20). Our experiments produced structures similar to those of the fossils in several groups of organisms. Type 1 organization (Fig. 14) is similar to structures produced experimentally in *Spirogyra* mats (Fig. 4), basidiocarps (Fig. 6), and in thalloid liverwort gametophytes (Fig. 11–13). Type 2 structure (Fig. 15, 16, 19) is comparable to compressed hornwort gametophytes (Fig. 9, 10). Type 3 structure (Fig. 17, 18) is reminiscent of compressed foliose lichen thalli (Fig. 7, 8), whereas type 5 structure (Fig. 20) is comparable to compressed ascocarps (Fig. 5) and lichen thalli (*Peltigera*, Fig. 7). These observations suggest that all these types of organisms were present in pre-tracheophytic terrestrial communities (except for basidiomycetes which are considered to have a post-Silurian origin). This corroborates previous reports from Passage Creek of ascomycete microfossils and embryophyte spores (PRATT et al. 1978), of microfossils comparable to bryophyte conducting strands (NIKLAS & SMOCOVITIS 1983), as well as the results of carbon isotope studies suggesting the presence of liverworts (TOMESCU et al. 2009), and it provides support to the idea that algal mats and lichens may have been present as well.

Conclusions

The experimental approach is a powerful tool in resolution of the taxonomy of enigmatic early terrestrial fossils. However roughly they simulate diagenetic processes, our experiments nevertheless show that (1) severity of the effects of pressure and heat varies widely across a broad taxonomic range of organisms; (2) even when highly altered, internal structures are still different between taxonomic groups and useful in separating them; (3) internal structures comparable to those of Lower Paleozoic thalloid terrestrial fossils can be produced by compression and heating in some algae, fungi, lichens, and bryophytes. This corroborates earlier views that the soil crust-like terrestrial communities which preceded the advent of vascular plants or were contemporaneous with the early phases of tracheophyte and polysporangiophyte evolution, were characterized by broad taxonomic diversity and included cyanobacteria, algae, fungi, lichens, and bryophytes (RETALLACK 2000; TOMESCU & ROTHWELL 2006; TOMESCU et al. 2006; BOYCE et al. 2007).

These results are encouraging and point to directions for future research, such as additional experiments to more accurately simulate fossilization, and detailed characterization of experimentally produced structures using electron microscopy. Together, these can help with (1) better understanding of taphonomic processes acting during the fossilization of different types of organisms; (2) establishing sets of diagnostic characters for taxonomic identification of compressed fossils; (3) generating novel search images for different groups of organisms in the fossil record of early life on land.

Acknowledgements

We thank Karl Niklas and Rosmarie Honegger for stimulating discussions, Gar Rothwell for helpful comments on the manuscript, Jonathan Hill for sectioning of the fungal material, and Laura Cedergreen for support during completion of the project.

References

BOYCE, C.K., HOTTON, C.L., FOGEL, M.L., CODY, G.D., HAZEN, R.M., KNOLL, A.H. & HUEBER, F.M. (2007): Devonian landscape heterogeneity recorded by a giant fungus. – Geology **35**: 399–402.

EDWARDS, D., DAVIES, K.L. & AXE, L. (1992): A vascular conducting strand in the early land plant Cooksonia. – Nature **357**: 683–685.

NIKLAS, K.J. & SMOCOVITIS, V. (1983): Evidence for a conducting strand in early Silurian (Llandoverian) plants: implications for the evolution of the land plants. – Paleobiology **9**: 126–137.

PRATT, L.M., PHILLIPS, T.L., DENNISON, J.M. (1978): Evidence of non-vascular land plants from the Early Silurian (Llandoverian) of Virginia, U.S.A. – Review of Palaeobotany and Palynology **25**: 121–149.

RETALLACK, G.J. (2000): Ordovician life on land and Early Paleozoic global change. – Pp. 21–45. In: GASTALDO, R.A. & DIMICHELE, W.A. (eds.): Phanerozoic terrestrial ecosystems. Paleontological Society Papers, New Haven, CT.

SCHOPF, J.M. (1975): Modes of fossil preservation. – Review of Palaeobotany and Palynology **20**: 27–53.

STRAYTON, J.P. (1987): Microstructural analysis of the Massanutten Sandstone, northwest Virginia. – M.Sc. Thesis, Bowling Green State University, OH.

TOMESCU, A.M.F. & ROTHWELL, G.W. (2006): Wetlands before tracheophytes: thalloid terrestrial communities of the Early Silurian Passage Creek biota (Virginia). – Pp. 41–56. In: GREB, S.F. & DIMICHELE, W.A. (eds.): Wetlands through time. Geological Society of America Special Publication 399.

TOMESCU, A.M.F., ROTHWELL, G.W. & HONEGGER, R. (2006): Cyanobacterial macrophytes in an Early Silurian (Llandovery) continental biota: Passage Creek, lower Massanutten Sandstone, Virginia, USA. – Lethaia **39**: 329–338.

TOMESCU, A.M.F., PRATT, L.M., ROTHWELL, G.W., STROTHER, P.K., & NADON, G.C. (2009): Carbon isotopes support the presence of extensive land floras predating the origin of vascular plants. – Palaeogeography, Palaeoclimatology, Palaeoecology **283**: 46–59.