Review of Palaeobotany and Palynology 159 (2010) 62–68

Contents lists available at ScienceDirect

Review of Palaeobotany and Palynology

journal homepage: www.elsevier.com/locate/revpalbo

Modifications of the transfer technique for studying complex plant structures

Ignacio H. Escapa [a,b,*], Brian J. Axsmith [c], Thomas N. Taylor [a], Edith L. Taylor [a]

[a] Department of Ecology and Evolutionary Biology, and Natural History Museum and Biodiversity Research Center, The University of Kansas, Lawrence, KS 66045-7534, USA
[b] Museo Paleontológico Egidio Feruglio. Av. Fontana 140, Trelew U9100GYO, Chubut, CONICET, Argentina
[c] Department of Biological Sciences, LSCB 124, University of South Alabama, Mobile, AL 36688, USA

ARTICLE INFO

Article history:
Received 7 August 2009
Received in revised form 14 October 2009
Accepted 26 October 2009
Available online 3 November 2009

Keywords:
techniques
transfer
compressions
Bio-Plastic™

ABSTRACT

The transfer technique is a method for exposing compression fossils that entails embedding the specimen in an adhesive material and dissolving the matrix in appropriate acids. This technique has been used for many years, and played an important role in several classic paleobotanical studies. However, in recent years it appears to have fallen into relative disuse and is not discussed at all in recent compilations of paleobotanical techniques. This is unfortunate, as the method is often extremely effective, especially for revealing the detailed structure of complex plant organs. In this paper, case studies using fossil conifer ovulate cones are presented. The first entails a modification of the classic transfer technique using a polyester resin as the embedding medium on an unnamed cone from the Triassic of Pennsylvania. The second study entails producing serial sections through a polyester resin embedded cone of *Telemachus* from the Triassic of Antarctica in a manner analogous to the classic cellulose acetate peel method. This modification is most useful when the organic material is too fragile for the more classic method. The results of these case studies are presented in the hope of re-stimulating use of the transfer technique in paleobotany.

1. Introduction

The transfer technique is used to expose compressed fossil plant remains by transferring part or all the organic remains to an adhesive material. Removal of the matrix using appropriate acids allows the plant structures to be examined relatively unobstructed. In some cases, the fossil surface that was originally embedded in the matrix can be exposed from the opposite side of the slab. This is particularly useful as this surface is often better preserved than the one exposed on the matrix surface.

The transfer technique was first explicitly described by Walton (1923) and used very effectively in the study of complex cones of conifers and other gymnosperms in several classic studies. For example, a transfer of an ovulate cone scale of the Permian conifer *Pseudovoltzia liebeana* by Walton (1928) provided previously unavailable fossil evidence for the dwarf shoot (i.e., brachyblast) interpretation of confer ovulate scales. This study is particularly illustrative of the value of the transfer technique in that Walton was able to clearly document both the adaxial and abaxial surfaces, thus demonstrating the branch-like three-dimensional structure of the scale. This technique was also used effectively in Harris's (1935) study of the Triassic flora of Greenland. In particular, balsam transfers of the conifers *Swendenborgia* and *Callipitys* were most informative.

Eventually, new varnishes and acetate-based materials, such as nail polish and thin acetate sheets, became available and were used as a transfer medium (Walton, 1923; Abbott, 1950; Abbott and Abbott, 1952). This can be a useful technique for transferring cuticles and smaller structures like fern sporangia (e.g., Axsmith, 2009). These materials generally allow only a relatively thin surface coating, however, which makes it difficult to transfer larger, more complex structures such as conifer cones.

The application of polymer casting resins to the transfer technique was first described by Cridland and Williams (1966), but their contribution provided no actual case studies. Despite the proven effectiveness of the transfer technique for larger cones and, by extension, other complex plant structures, it seems to have nearly fallen out of use. In fact, the technique is not covered at all in the most recent works dealing with paleobotanical research techniques (e.g., Jones and Rowe, 1999). There are a few exceptions, however, in which newer materials have been used in fossil conifer cone studies. For example, Cornet (1977) provided a preliminary study of complete Triassic conifer cones from Pennsylvania using the transfer method. Although the details are not provided in the paper, the author revealed that a commercially available plastic polymer was used as the transfer medium (B. Cornet, personal communication). In a later study of fossils from the same locality by Axsmith and Taylor (1997), Ward's Bio-Plastic™ liquid casting plastic produced excellent results. Axsmith et al. (2000) also used Bio-Plastic™ to examine the three-dimensional morphology of cupules of the pteridosperm *Umkomasia*.

None of the recent papers utilizing Bio-Plastic™ in the context of a transfer medium provided much detail on the technique. In addition,

* Corresponding author. Department of Ecology and Evolutionary Biology, and Natural History Museum and Biodiversity Research Center, The University of Kansas, Lawrence, KS 66045-7534, USA. Tel.: +1 785 864 4255.
E-mail address: iescapa@ku.edu (I.H. Escapa).

0034-6667/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.revpalbo.2009.10.007

we have found that the hardened medium can be easily polished down to produce peels analogous to those made from coal balls and silicified peat in cases where the organic material is too brittle to withstand standard maceration. The goal of this paper is to provide a more detailed description of these modified transfer and peel techniques along with case studies in the hope that the technique will be more widely used and as a result provide additional details about fossil plants than may have been previously used.

2. Embedding in Bio-Plastic™

Polyester-embedding resin is frequently used in certain paleobotanical techniques. While we have used Bio-Plastic™, similar products are sold under various brand names such as Caroplastic, Castolite®, and Vestopal W, which present some differences in composition and basic features. Polyester-embedding resins, used since the 1940s, consist of a polyester prepolymer dissolved in a styrene monomer composes. When they are combined with the catalyst (methyl ethyl ketone peroxide), a uniformly clear solid block is obtained. The polyester resins present several characteristics that facilitate their use for the transfer technique. For example, the high transparency permits the observation of the fossil embedded in the resin. Other beneficial characteristics of polyester resins for paleobotanical work are the ease of preparation and the relatively rapid drying/curing times without heat. While Bio-Plastic™ is mostly resistant to HF, the acid normally used to dissolve the surrounding rock matrix, we have not experimented with other polyester resins, epoxies, and acrylics in combination with various acids to determine if they can also be used effectively in this technique.

Regardless of which of the two techniques discussed here will be used, the embedding process is generally the same. It must be kept in mind that ultimately the matrix will be dissolved or ground down, and thus the process is destructive, so it is essential to first test the technique on expendable specimens. In some specimens there is not sufficient organic material present, or it is present but too brittle to withstand the maceration process. Thus, even with specimens that appear suitable it is still especially important to photograph them extensively before beginning this technique.

We have not tried this technique on carbonate rocks, but suspect that it would work essentially the same way. In terms of clastic sediments, we have found that mudstones and shales produce the best results. Sand grains tend to dissolve and fuse to the fossil and the Bio-Plastic™, rather than falling away during the maceration process. Once suitable specimens are identified and photographed, they are ready to be embedded.

The embedding process can be carried out in the aluminum frames used to make casts that are available from Ward's Natural Science (P.O. Box 92912, Rochester, New York 14692; http://wardsci.com/). We have also obtained satisfactory results, however, using cardboard specimen boxes that can be peeled away and the small disposable

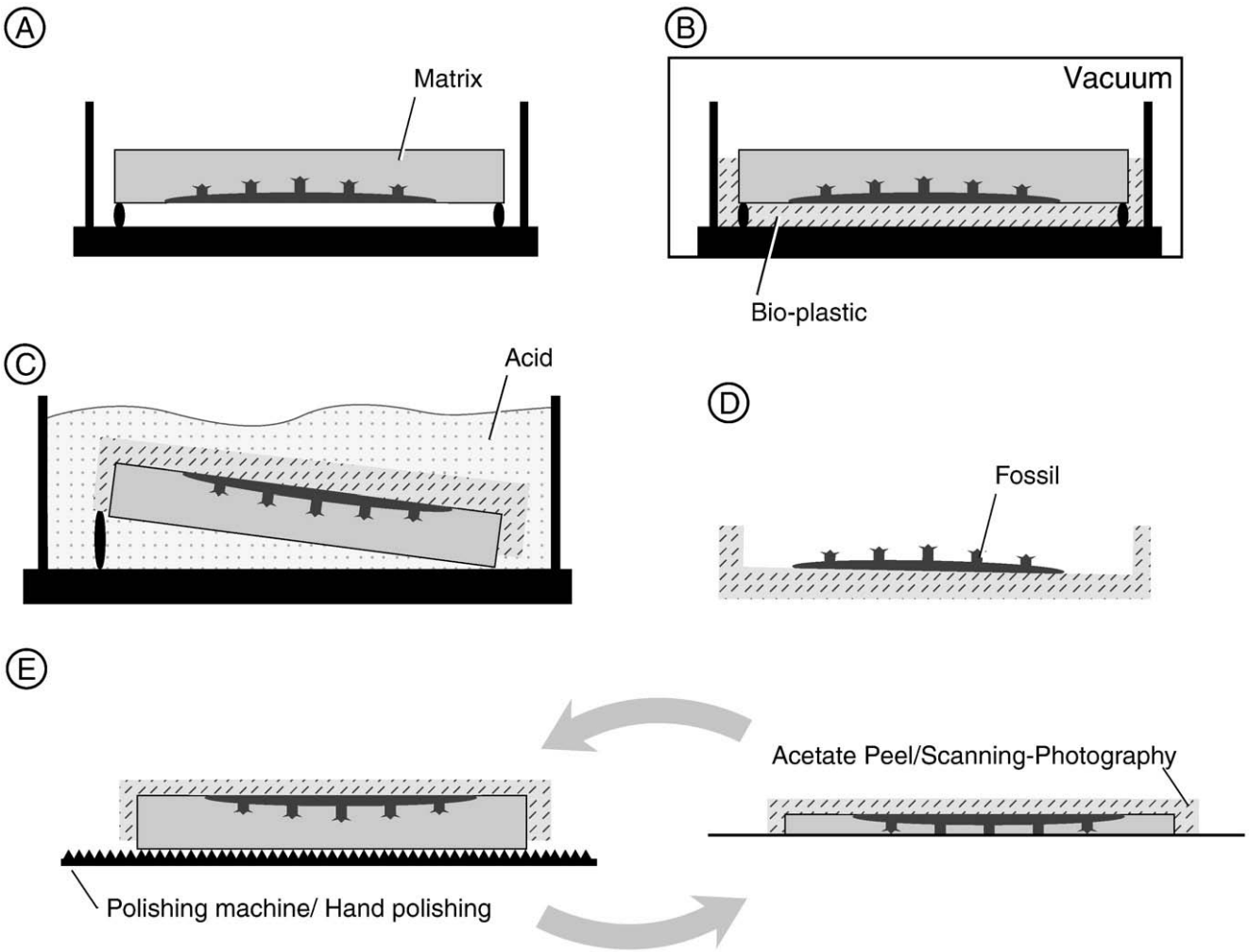


Fig. 1. Schematic diagrams showing the transfer technique and the modification proposed in this contribution. A. Specimen oriented in mold (fossil on lower surface). B. Specimen in mold showing the disposition of the Bioplastic (hatched lines). C. Specimen embedded in Bioplastic that is emersed in acid. D. Specimen in relief after acid maceration. E. Stages in the polishing processes that will reveal different levels of the specimen.

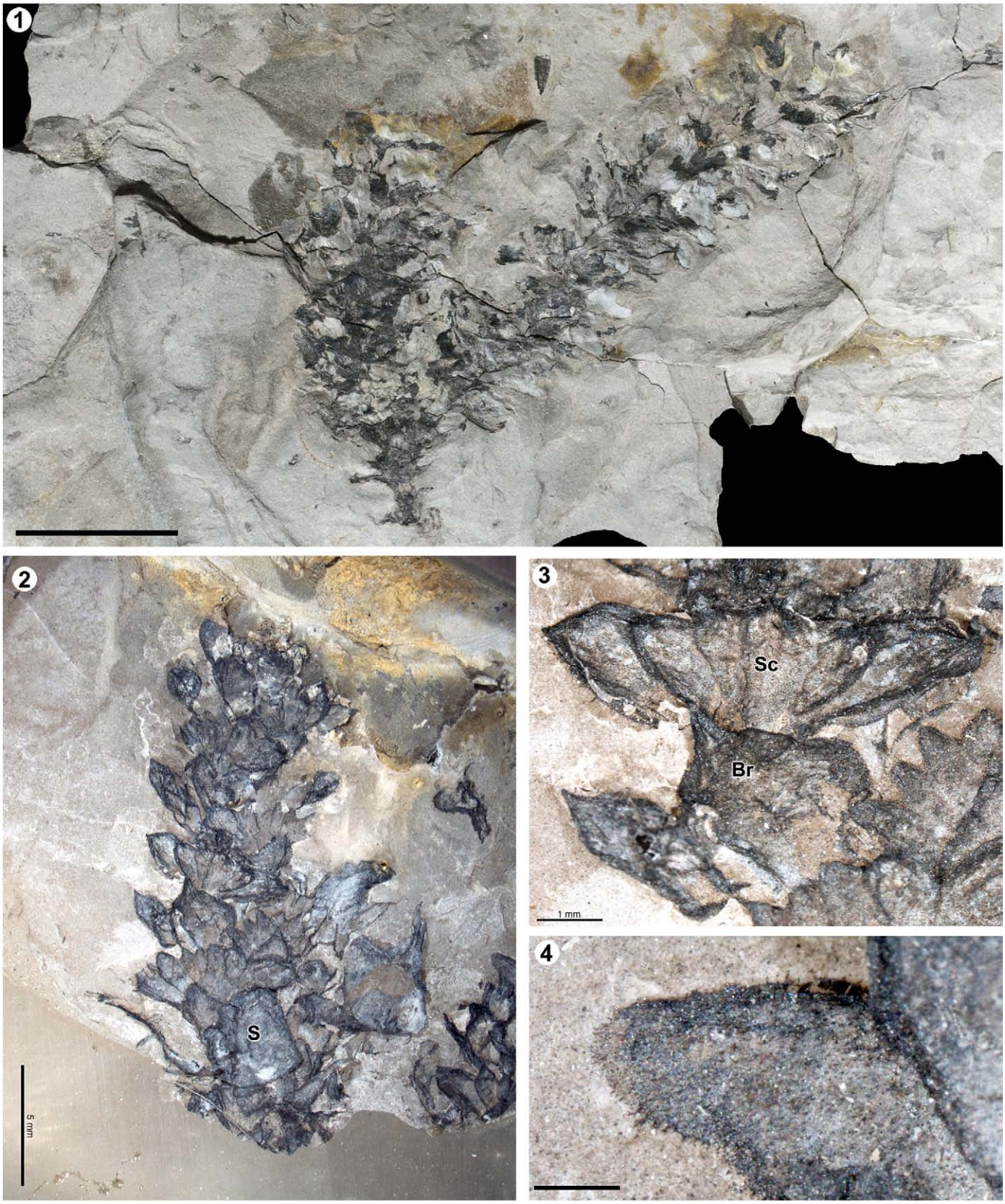


Plate I. Study of unnamed seed cone compressions from the Triassic of Pennsylvania using the transfer technique.

1. Two untransferred cones attached to branch. Note that detailed structure of the ovuliferous complexes cannot be clearly discerned. No. T1-1083. Scale bar = 1 cm.
2. Counterpart of specimen in Fig. 1 (cone on the right) after undergoing a transfer to Bio-Plastic. Note the three-dimensionally preserved axillary complexes and an in situ winged seed (S) Scale Bar = 5 mm. No. T1 – 894. Detail of a bract-scale complex from subpanel 2. Note the bract (Br) and the lobed, axillary cone scale (Sc). Scale bar = 1 mm.
3. Detail of a cone scale lobe from subpanel 2. Note that the delicate papillae and trichomes are intact. They are most easily seen along the margins. Scale bar = 0.5 mm.

aluminum trays used for embedding in electron microscopy. In any case a container of suitable size should be chosen. If possible, break away excess sediment from the specimen; however, do not make it so thin that it cannot be cut parallel to the cone or other plant structure later. It is also good to make the surfaces as flat as possible so the rock sits flat on the bottom of the container and no parts extend above of the Bio-Plastic™ surface.

Our procedure involves soaking the specimen in acetone. This process will help to drive air from cracks within the matrix and will also thin the Bio-Plastic™ allowing it to more completely infiltrate the matrix. This can also be accomplished by using a vacuum to increase penetration of the Bio-Plastic. The wet specimen is then placed in the container with the exposed part of the plant structure facing upward (Fig. 1, A–B). The liquid Bio-Plastic™ is then mixed with the appropriate catalyst. We recommend using half of the amount recommended by the manufacturer, as slower hardening is desirable to maximize infiltration. The mixture is then poured slowly over the specimen until it is completely covered. At least 5 mm of cover is recommended. The Bio-Plastic™ is allowed to harden and then placed under heat (light bulb) to complete the polymerization process according to the manufacturer's instructions. After the Bio-Plastic™ has hardened, the block can be removed from the aluminum mold or the cardboard box can be peeled away. It is useful to place the block under the light again to be sure the surfaces that were in contact with the mold are sufficiently hardened.

3. The transfer method

For making a transfer, the block must be cut parallel to the plant organ using an appropriate rock saw. It is desirable to cut as close to the cone or other plant structure within the matrix as possible. The block containing the plant fossil now has an exposed matrix surface on the opposite side from the originally exposed fossil.

In the case of most clastic sediments, concentrated HF is best as the primary macerating chemical; in some cases HCl is also required depending on the composition of the shale or mudstone. Other combinations of acids can be used in certain instances, but like all techniques, there needs to be some experimentation based on the composition of the matrix and the polyester resin that is being used. These chemicals are extremely dangerous and must be handled strictly according to standard safety procedures and carried out in a appropriate fume hood. To macerate the specimen, place the block in an appropriately sized Nalgene beaker so that the block is situated at about 45° with the exposed matrix facing downward (Fig. 1, C–D). The acid is then carefully poured into the beaker until the entire specimen is fully submersed in liquid. As the sediment dissolves it will fall away from the block and collect at the beaker. While in many techniques the acid/matrix residue is discarded, we believe that this material should be carefully examined as it may contain other fossil materials that were not observed when the initial embedding took place. This residue may also contain various types of microfossils including palynomorphs that may also require the application of other techniques. The amount of time required for the maceration process to be completed will vary depending on the size and exact composition of the specimen. Occasionally remove the specimen and carefully rinse it in an appropriate neutralizing solution followed by rinsing in distilled water to check on the progress of the maceration. In some cases, the matrix can be removed entirely, but it is best to stop the process as soon as the plant structure sufficiently exposed. Once this is achieved, the specimen can be allowed to slowly air dry.

If properly hardened, the Bio-Plastic™ block will withstand the maceration process; however, occasionally the etched surface may become cloudy or in some cases nearly opaque. We are uncertain why this clouding takes place, but it may depend on the length of the maceration process. If only the outermost parts are clouded, the block can be polished to reveal the originally exposed surface. If the etching completely penetrates to the specimen surface, this will not be possible. This underscores the importance of photographing specimens thoroughly before attempting a transfer.

Meurgues (1982) argued that the long-term life of polyester was not appropriate for museum objects, but this suggestion is mostly directed to art objects, in which Bio-Plastic™ has been recurrently used for conservation proposes (see Derrick et al., 1994). In our experience, the etching of the Bio-Plastic™ block does not seem to affect the overall durability or longevity. One of us (Axsmith) has observed specimens in blocks that are over 15 years old and show no signs of brittleness or matrix deterioration. Specimens from the Cornet study are at least 33 years old and also show no deterioration. It is not certain, however, that the casting material used in that research was exactly the same as modern Bio-Plastic™.

3.1. Case study

As discussed above, the technique just outlined was applied to complete seed cones of a Triassic conifer from the New Oxford Formation in York County, Pennsylvania. This conifer remains unnamed, but preliminary study (Cornet, 1977; Axsmith and Taylor, 1997) may indicate affinities with the Permian conifer family Majonicaceae.

Note that the detailed structure of the bract-scale complexes cannot be discerned at all on the original matrix surface (Plate I, 1). They could not be revealed easily by dégaging, as this would entail excavation down through the complexes. After being transferred, however, the entire bract-scale complexes can clearly be seen in three dimensions (Plate I, 2–4). This is a critical observation in that it shows that the lobes of the scale are not exactly in the same plane as for most of the Majonicaceae (Plate I, 3). Note also that the bract can be seen to be free from the expanded part of the cones scale. Attached ovules are also visible. It is remarkable that an impression specimen actually retains this level of three-dimensional structure. It is also noteworthy that the papillae and trichomes are clearly visible on the plant surfaces (Plate I, 4). This level of information would be difficult to acquire from a compression specimen by any other standard techniques, although the use of various procedures in studying fossil plant compressions (e.g., polarizing light, liquids with various defraction indices, etc.) can improve the resolution and contrast of the fossil, and thus informative details.

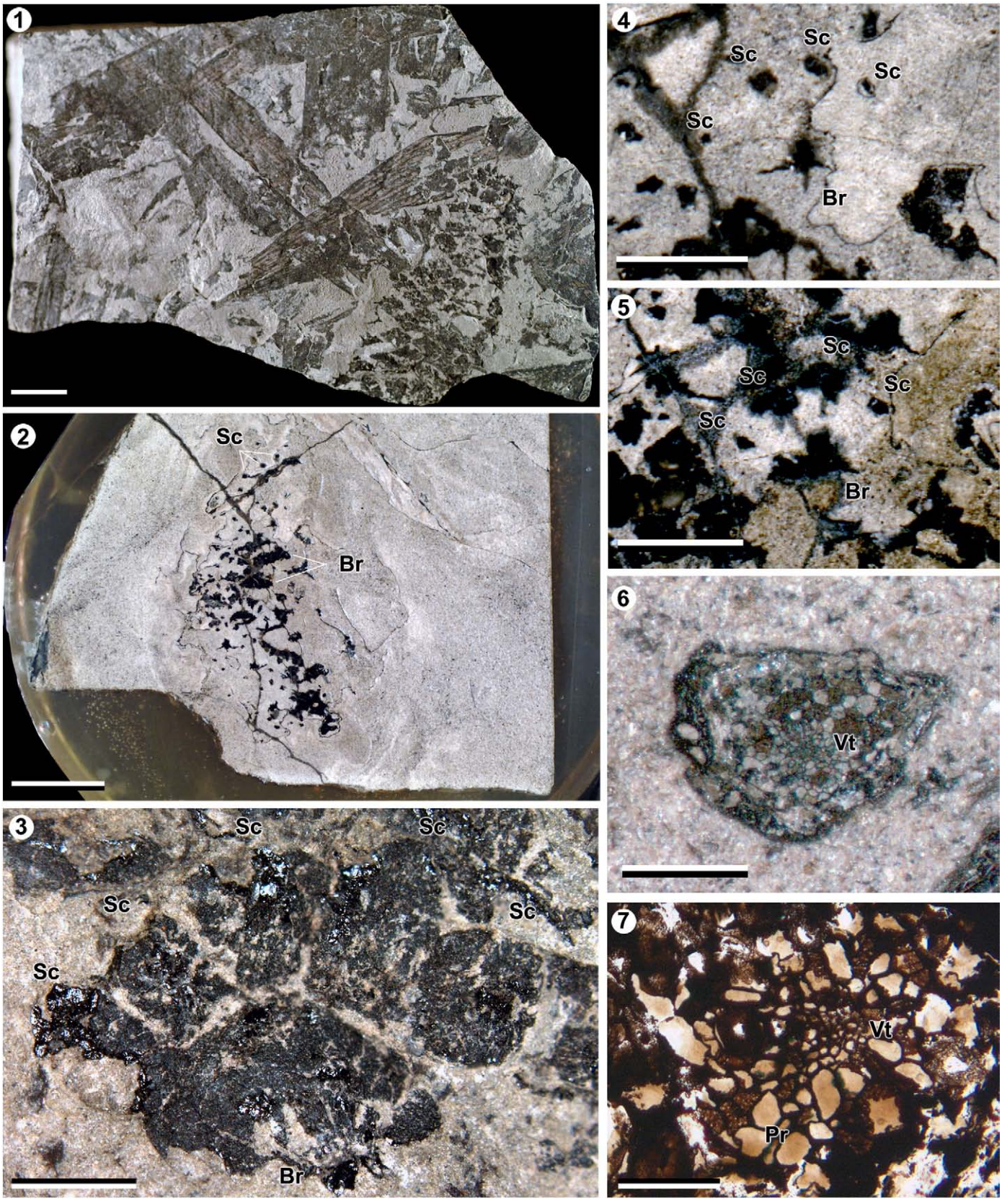
4. Structural features in compressions

The classic transfer method is especially useful when cuticle remains are well preserved and resistant to acid maceration. On the other hand, there are some compressed specimens with preserved cuticles that have characteristics that make it risky or even impossible to use the transfer technique and acid maceration. For example, highly fragmented cuticles could be disaggregated after HF maceration, even with the binding action of the Bio-Plastic™. In such cases, only the fragments in contact with the polymer would remain in the original position, while the remaining organic fragments would be dispersed in the acid at the end of the maceration process.

An alternative method could be useful for complex structures in which additional information of the three-dimensional organization is required. The basic technique consists of the inclusion of the specimen with the visible fossil face embedded in the plastic, following the same basic steps and recommendations explained in Section 2 for the regular transfer technique. After the Bio-Plastic™ dries, the side of the block on the face opposite to the exposed one is polished. Initially, different polishing machines could be used as this is faster than manual grinding, but it is important to stop machine polishing about 3–5 mm above the fossil, and continue the process by hand. Intermediate stops and controls during machine grinding are also suggested, since the rock could contain additional fossil remains that must be registered either by peels or image files (scanning, photography, etc.). A glass plate and

slurry of Carborundum grit can be used for hand grinding the specimen, using 200–400 grit to reach the first evidence of the fossil level. Alternatively, silicon carbide abrasive paper (available in the appropriate grades) can also be used for polishing the specimens.

Continued polishing and production of acetate peels allow for the observation of several sections of the compressed cone (Fig. 1, E). When to next polish the specimen depends on rock hardness, but to obtain the most information obviously requires closely spaced



intervals. Complex structures such as conifer seed cones or other reproductive organs may be only a few millimeters thick when compressed. The use of Bio-Plastic™ support for the fossil permits the sectioning of the entire specimen, even if it presents an irregular surface.

When part and counter-part of the compression are available, one of the parts could be selected for the sectioning. Alternatively, both parts of the fossil could be combined with Bio-Plastic™ before being embedded in order to have the whole compressed structure represented in the sections. The obvious disadvantage is the complete destruction of the specimens, instead of the destruction of only one part.

Treatment with acid was not used in the specimens we analyzed for this case study, since the primary goal of the technique was the observation of different levels of the compressed three-dimensional structures, and previous efforts at observing anatomical details failed. More consolidated rocks, however, could require the use of acids (HF, HCl, etc.) to get good peels. Furthermore, in those cases in which compressions have some anatomical characters preserved, the use of acid could allow the observation of the anatomy in peels, following the same basic principles of the classic rapid peel technique (Joy et al., 1956; Galtier and Phillips, 1999).

The classic transfer technique can be used exclusively for fossil plant compressions containing organic matter, since HF treatment alone might destroy the fossils if they are preserved differently. However, the three-dimensional structure of complex fossil compressions could be studied using only grinding and polishing, even when organic remains are not preserved.

4.1. Case study

The technique just described was used with good results on conifer cones of the genus *Telemachus* (Plate II, 1–7). The cones are compressed on fine-grained black shales from different localities of the Triassic of Antarctica (Yao et al., 1993; Axsmith et al., 1998. The seed cones are compressed and their cuticles are highly coalified and fragile (Plate II, 1). The cones are preserved in different planes of section (longitudinal, oblique, and transverse); however, the number of characters observable from the external view may be limited due to their complex structure. Slabs of shale bearing *Telemachus* cones (Plate II, 1) were reduced in size, embedded in Bio-Plastic™ as described above, and polished down to fossil level (Plate II, 2). After that, a total of 150 repetitions of hand polishing with 600-grit paste and acetate peels of the polished surface were made. Scanning electron microscope observations and pictures of the polished surfaces were also recorded.

The ovuliferous complexes of *Telemachus* comprise a subtending bract and an axillary ovuliferous scale (Plate II, 3). Features such as the number of scale lobes, and the size and shape of the bract can be properly described from the superficial observation of compressed specimens (see, e.g., Yao et al., 1993). Additional characters, such as the fusion of bract and scales, however, cannot be unambiguously reported through observation of superficial characters. In this context, the use of the modified transfer technique permits the description of numerous new details of these ovuliferous cones, which are relevant to understanding its taxonomic position and evolution. For instance, serial cross sections of the ovuliferous complexes (Plate II, 4–5) clearly show the size and shape of ovuliferous scale lobes, as well as their relationship with the subtending bract. The degree of fusion of scale lobes in a simple scale, and its fusion with the bract are relevant characters in conifer systematics, which were unknown in *Telemachus* previously due to preservation only as a compression.

During the preparation of this material we encountered certain sections of the fossil that demonstrated the existence of various cell types within regions of the cone. These included thick-walled cells in the bracts and scales, as well as secondary thickenings in conducting cells (Plate II, 6-7). While information of this type increases our understanding of the complexity of the fossil and provides greater detail about the species, it also suggests that the preservation at a particular locality may provide data that makes it possible to expand information about various fossil plants from several sites in which the preservation is highly variable. For example, there is some information about various groups of Triassic plants based on permineralized specimens from a single locality (e.g. Fremouw Peak). The discovery of anatomical details in certain types of compressions like that demonstrated here for *Telemachus* now may makes it possible to expand our understanding of the geographic, and perhaps stratigraphic distribution, of Triassic plants more accurately.

5. Conclusion

The Bio-Plastic™ transfer technique is useful for studying fossil plant compressions as it significantly increases the number of observable features, especially when the specimens present well-preserved organic remains. The modified transfer technique could be used even when the composition and preservation of fossil remains are not adequate for the standard technique. In cases where anatomical details are preserved in the compressions, this technique will permit the reconstruction of these features as well. In addition, the polyester resins appear to be relatively stable over time, a very important feature for the preservation of the prepared samples.

Like many techniques there are negative aspects that must be considered prior to application. Among these is the obvious fact that the process is irreversible. Therefore it is paramount that there is a recorded record of each surface made during the process we have outlined that can be used to reconstruct and/or demonstrate various levels within the specimen once the fossil is ground away. This is especially important in instances where there may be a small number of specimens available. While there are various techniques being employed to study the three-dimensional aspect of fossil plants (e.g., X-ray CT scanning) not all of these can be used to obtain the same level of resolution with all fossils. Like many paleobotanists before us have noted, it is the mode of preservation and the type of information that is sought that determines the technique(s) that are ultimately used. We hope that the technique outlined here will help to obtain some additional information from a certain type of preservation that will add to our increasing data base about fossil plants.

Acknowledgments

We thank Dr. Rudolph Serbet for his valuable help with both laboratory and collection work, and comments by two anonymous reviewers. The research was partially supported by a National Science Foundation grant (OPP-0635477) to ELT and TNT, and (EAR-0105476) to BJA.

Plate II. Study of *Telemachus* seed-cone compressions from the Triassic of Antarctica by means of the modified transfer technique.

1. External view of *Telemachus* cone and *Heidiphyllum* leaves. No. T-391a.
2. Longitudinal section of compressed *Telemachus* cone embedded in Bio-Plastic™. Ovuliferous complexes in cross section are observed. No. T-391a. Scale bar = 10 mm.
3. Detail of an ovuliferous complex in external view. Note the five lobes of the ovuliferous scale (Sc) disposed on the adaxial side of the subtending bract (Br). Scale bar = 1 mm. 4–7. Serial cross sections of the ovuliferous complexes of *Telemachus* showing the morphology and disposition of bract (Br) and ovuliferous scale lobes (Sc). Note that in the most external section (subpanel 4), just the tips of the scales and bract are observed. The most internal section (subpanel 7) shows the bract and the scale lobes almost totally fused. No. T–391a. Scale bars = 3 mm.

References

Axsmith, B., 2009. A new *Cynepteris* from the Upper Triassic of Arizona: potential implications for the early diversification of schizaealean ferns. Intl. J. Plant Sci. 170, 657–665.

Axsmith, B., Taylor, T.N., 1997. The Triassic conifer seed cone *Glyptolepis*. Rev. Palaeobot. Palynol. 96, 71–79.

Axsmith, B., Taylor, T.N., Taylor, E.L., 1998. Anatomically preserved leaves of the conifer *Notophyton krauselii* (Podocarpaceae) from the Triassic of Antarctica. Am. J. Bot. 85, 704–713.

Axsmith, B.J., Taylor, E.L., Taylor, T.N., Cúneo, N.R., 2000. New perspectives on the Mesozoic seed fern order Corystospermales based on attached organs from the Triassic of Antarctica. Am. J. Bot. 87, 757–768.

Abbott, M., 1950. A paleobotanical transfer method. J. Paleont. 24, 619–621.

Abbott, R.E., Abbott, M., 1952. A simple paleobotanical transfer technique. Ohio J. Sci. 52, 258–260.

Cornet, B., 1977. Preliminary investigation of two Late Triassic conifers from York County, Pennsylvania. In: Romans, R.C. (Ed.), Geobotany. Plenum, New York, NY, pp. 165–172.

Cridland, A.A., Williams, J.L., 1966. Plastic and epoxy transfers of fossil plant compressions. Bull. Torrey Bot. Club 5, 311–322.

Derrick, M., Souza, L., Kieslich, T., Florsheim, H., Stulik, D., 1994. Embedding paint cross-section samples in polyester resins: problems and solutions. J. Am. Inst. Conserv. 33, 227–245.

Galtier, J., Phillips, T.L., 1999. The acetate peel technique. In: Jones, T.P., Rowe, N.P. (Eds.), Fossil Plants and Spores: Modern Techniques. Geological Society of London, London, UK, pp. 67–70.

Harris, T.M., 1935. The fossil flora of Scoresby Sound, East Greenland, Pt. 4: Ginkgoales, Coniferales, Lycopodiales and isolated fructifications. Medd. Gronl. 112 176 pp.

Jones, T.P., Rowe, N.P. (Eds.), 1999. Fossil Plants and Spores: Modern Techniques. Geological Society of London, London, UK. 396 pp.

Joy, K.W., Willis, A.J., Lacey, W.S., 1956. A rapid cellulose peel technique in palaeobotany. Ann. Bot. 20, 635–637.

Meurgues, G., 1982. Synthetic resins can be dangerous. Museum 34, 60–61.

Yao, X., Taylor, T.N., Taylor, E.L., 1993. The Triassic seed cone *Telemachus* from Antarctica. Rev. Palaeobot. Palynol. 78, 269–276.

Walton, J., 1923. On a new method for investigating fossil plant impressions or incrustations. Ann. Bot. 37, 379–391.

Walton, J., 1928. On the structure of a Paleozoic cone-scale and the evidence it furnished of the primitive nature of the double cone-scale in the conifers. Mem. Proc. Manch. Lit. Philos. Soc. 73, 1–6.