

Polishing entire stems and roots using sandpaper under water: An alternative method for macroscopic analyses

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Manuscript received 30 January 2021; revision accepted 22 April 2021.

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Citation: Barbosa, A. C. F., C. S. Gerolamo, A. C. Lima, V. Angyalossy, and M. R. Pace. 2021. Polishing entire stems and roots using sandpaper under water: An alternative method for macroscopic analyses. *Applications in Plant Sciences* 9(5): e11421.

doi:10.1002/aps3.11421

PREMISE: Polishing entire stem and root samples is an effective method for studying their anatomy; however, polishing fresh samples to preserve woods with soft tissues or barks is challenging given that soft tissues shrink when dried. We propose sanding fresh or liquid-preserved samples under water as an alternative, given that it preserves all tissues in an intact and clear state.

METHODS AND RESULTS: By manually grinding the surface of the samples under water using three ascending grits of waterproof sandpapers, an excellent polished sanded surface is obtained. The wood swarf goes into the water without clogging the cell lumina, rendering the surfaces adequate for cell visualization and description. We show results in palms, liana stems, roots, and wood blocks.

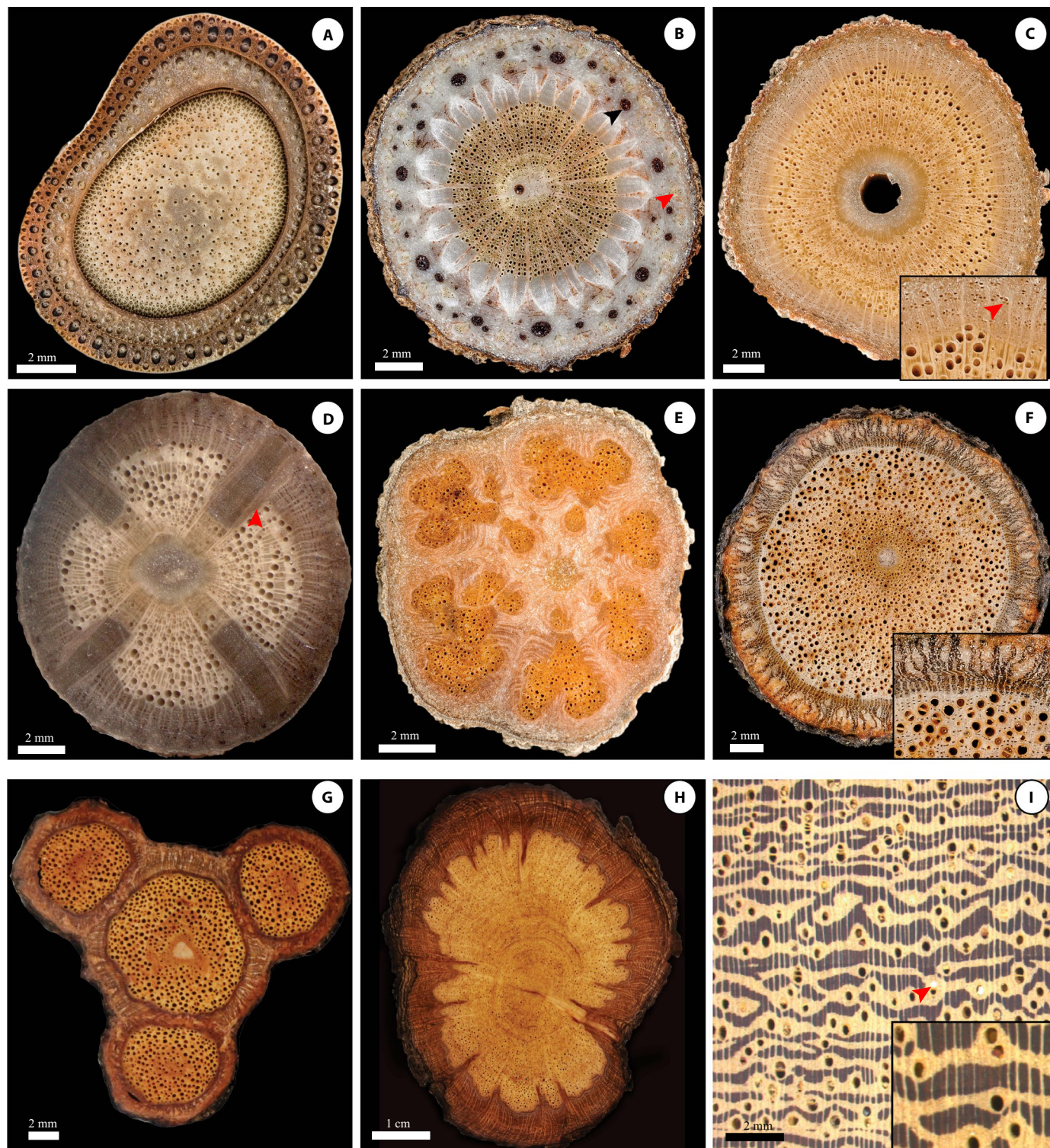
CONCLUSIONS: Using this simple, inexpensive, rapid technique, it is possible to polish either fresh, dry, or liquid-preserved woody plant samples, preserving the integrity of both the soft and hard tissues and allowing for detailed observations of the stems and roots.

KEY WORDS bark; dendrochronology; image visualization; methods; microanatomy; wood anatomy.

Polishing wood samples is perhaps one of the oldest, most common methods for analyzing their anatomical structure. Wood polishing has been used to facilitate the characterization of entire families of woody plants (Koek-Noorman and Westra, 2012), in wood and charcoal identification by both humans and semi-automated machine-based methods (Kisser, 1967; Lantican and Hughes, 1973; Mainieri et al., 1983; Gärtner and Nievergelt, 2010; Botosso, 2011; Hermanson and Wiedenhoef, 2011; Ruffinatto et al., 2019; Ravindran et al., 2020), in dendrochronology (Brandes et al., 2011, 2018; Alves et al., 2013; Gärtner et al., 2015; Locosselli et al., 2016; Arzac et al., 2018), and even to describe parasite–host interactions in the formation of wood galls (Teixeira-Costa and Ceccantini, 2015, 2016; Teixeira-Costa et al., 2020). Despite the importance of this technique in the field, few works describe in detail how to successfully perform wood sanding (Gärtner and Schweingruber, 2013; Arzac et al., 2018). In addition, the polishing of entire fresh stem and root samples when barks are present has been widely neglected. Those few studies that describe wood

sanding typically concur that this technique has two major impediments: (1) the occlusion of cell lumina with swarf, impairing the examination of the transverse surface, especially if using automated software, such as WinDendro (Regent Instruments, Quebec, Canada) or different ImageJ packages (Schneider et al., 2012); and (2) the wood must be dry enough to avoid surface blurring, making the polishing of fresh samples and samples containing barks unfeasible.

The comparative analysis and macroscopic anatomical descriptions of entire cross sections of roots and stems comprise an important step in anatomical studies, and are fundamental to their identification and also to understanding their growth rhythm (dendrochronology), diversity, and evolution. Barks become increasingly fragile as they dry and commonly collapse along with other soft tissues. The same is true for stems and roots with cambial variants, a feature very common in lianas and underground organs (Avetta, 1885, 1887; Carlquist, 1991; Angyalossy et al., 2015). These challenges led us to develop a simple, effective technique to polish



entire samples containing both wood and bark that preserves both components. Our technique was initially developed to polish fresh samples, but here we show that it can also be used to polish dry samples pulled from wood collections. The present method has been used for over a decade in our labs with great success, and is here explained in detail.

METHODS AND RESULTS

Plant materials

To illustrate our technique both for entire organs and blocks of woods alone, we selected seven entire stems approximately 2 cm

FIGURE 1. Stem and root structures of wood samples polished under water. (A) Main stem of *Desmoncus orthacanthos* (Arecaceae), surrounded by two leaf bases. Note the atactostele in the stem, with fewer bundles in the center than on the periphery. The vascular bundles of the leaves are larger than those of the stem. (B) *Manekia obtusa* (Piperaceae) stem, with large rays that dilate in the phloem. Note the phloem axial elements in a whitish color and the cortical bundles in the cortex (red arrowhead). The cortex and pith contain naturally large dark mucilage cavities (dark arrowhead). (C) The stem of the liana *Tetrapterys phlomoides* (Malpighiaceae), which has a hollow pith. The wood axial parenchyma are vasicentric, and the phloem rays are dilating. Note in the inset image the presence of larger cells, which are laticifers (red arrowhead). (D) *Tanaecium pyramidatum* (Bignoniaceae) stem, with four phloem wedges interrupting the wood. The wood is semi-ring porous, with more parenchyma associated with the earlywood. The regular phloem is stratified and has dilating rays. Note the very wide limiting rays (red arrowhead). (E) Fissured stem of *Alicia anisopetala* (Malpighiaceae), with islands of wood immersed in phloem and disruptive parenchyma. (F) Regular stem of the liana *Dioclea rufescens* (Fabaceae), with vessels immersed in a matrix of non-lignified axial parenchyma; the fibers are seen as darker lines. The rays greatly dilate in the phloem forming a flame-like appearance (see inset image). (G) *Serjania lethalis* (Sapindaceae) compound stem with a central cylinder and three peripheral cylinders. The pith is triangular. (H) *Serjania lethalis* root with various phloem wedges. (I) Dry wood sample from the main trunk of *Hymenolobium petraeum* (Fabaceae). Note that some vessels are filled with a whitish gum (red arrowhead). The axial parenchyma is aliform confluent, forming bands on a matrix of thick-walled fibers. The image in panel H was provided by Carolina Lopes Bastos (Universidade de São Paulo).

in diameter, one entire root approximately 6 cm in diameter, and a cube of wood; all samples were approximately 4 cm high (Fig. 1; for collection details see Appendix 1). The selected species were (1) a palm (*Desmoncus orthacanthos* Mart. [Arecaceae]) with a stem surrounded by leaf bases (Fig. 1A); (2) *Manekia obtusa* (Miq.) T. Arias, Callejas & Bornst. (Piperaceae), a soft-stemmed liana with very broad rays separating the axial elements of the secondary xylem and phloem, and the cortical bundles (Fig. 1B); (3) *Tetrapterys phlomoides* (Spreng.) Nied. (Malpighiaceae), a hard-stemmed liana with regular secondary growth (Fig. 1C); (4) *Tanaecium pyramidatum* (Rich.) L. G. Lohmann (Bignoniaceae), a liana with four phloem wedges interrupting the xylem (Fig. 1D); (5) *Alicia anisopetala* (A. Juss.) W. R. Anderson (Malpighiaceae), a liana with a fissured stem (Fig. 1E); (6) *Dioclea rufescens* Benth. (Fabaceae), a liana with regular secondary growth but a large amount of non-lignified axial parenchyma within the wood (Fig. 1F); and (7) *Serjania lethalis* A. St.-Hil. (Sapindaceae), which has a stem with a compound structure (Fig. 1G) and a root with phloem wedges (Fig. 1H). For the cube of wood, we selected the widely commercialized timber of *Hymenolobium petraeum* Ducke (Fabaceae), because it has a combination of very thick-walled fibers and confluent aliform axial parenchyma that form bands, which provide a good contrast of cell types in wood (Fig. 1I).

Any fresh or dry woody sample, whether an entire stem or root cross section or merely a fragment, is suitable for polishing under water. Because the polishing method proposed here is manual, the size of the sample needs to accommodate to the hand of the user or an alternative holding device, but this is the only size limitation of our technique. The costs for this technique are extremely low because waterproof sandpaper is inexpensive and available in hardware stores in any country. From beginning to end, this technique typically takes less than 30 min. In dendrochronology, this technique can be very useful for core samples, although for large trunks our technique may prove impracticable and traditional dry sanding may be superior. Hydrated material is usually easier to polish using this technique, so we advise rehydrating dried samples by boiling in water with glycerol (water : glycerol, 10 : 1) before starting (Pace, 2019). However, in *Hymenolobium* we polished the dry sample, and in this particular case (wood only), it gave excellent results.

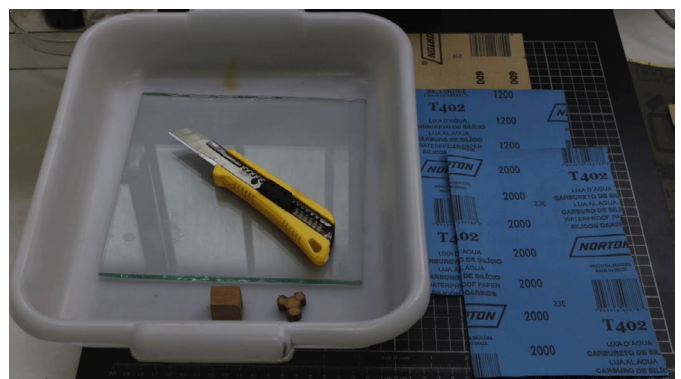
Technique description

Appendix 2 provides a summary of the protocol and the materials used to perform the technique. Appendix 3 presents a flowchart that

guides the reader through the procedure. A video in English is available illustrating the technique (Video 1). The same video narrated in Spanish, Portuguese, German, Italian, French, and Chinese is available from Figshare (<https://doi.org/10.6084/m9.figshare.13656833>).

The entire procedure usually requires 10–25 min, depending on the expertise of the user and the taxon under study. Harder tissues will require longer polishing times. Sawn samples typically present many imperfections, are uneven, and even oblique (Figs. 2, 3); thus, the first step in polishing them is to trim them with a cutter knife and holder to render the surface as flat as possible (Figs. 2, 3). If the user is not comfortable with using a cutter knife, they can alternatively use a very rough sandpaper (e.g., grit grade P220) before the P600 sandpaper, to obtain a similar initial polishing before following the procedure described below.

The P600 (or P220) sandpaper is placed inside a tray on a flat surface (we usually use a glass plate) and covered with tap water. While one hand holds and stabilizes the sandpaper inside the tray, the other holds the sample firmly and grinds the surface of the sample against the sandpaper using mono- or bidirectional movements (Figs. 2, 3). The objective is to render the surface flat and smooth. This may take a while. Typically, 50 movements will provide good results. Every 10 or so movements, rotate the sample to avoid over-grinding one side. The wood swarf automatically disperses into



VIDEO 1. Video illustrating the technique of polishing woody samples under water (in English). To view this version, please visit <https://doi.org/10.1002/aps3.11421>. This video is also available in Spanish, Portuguese, German, Italian, French, and Chinese at Figshare (<https://doi.org/10.6084/m9.figshare.13656833>).

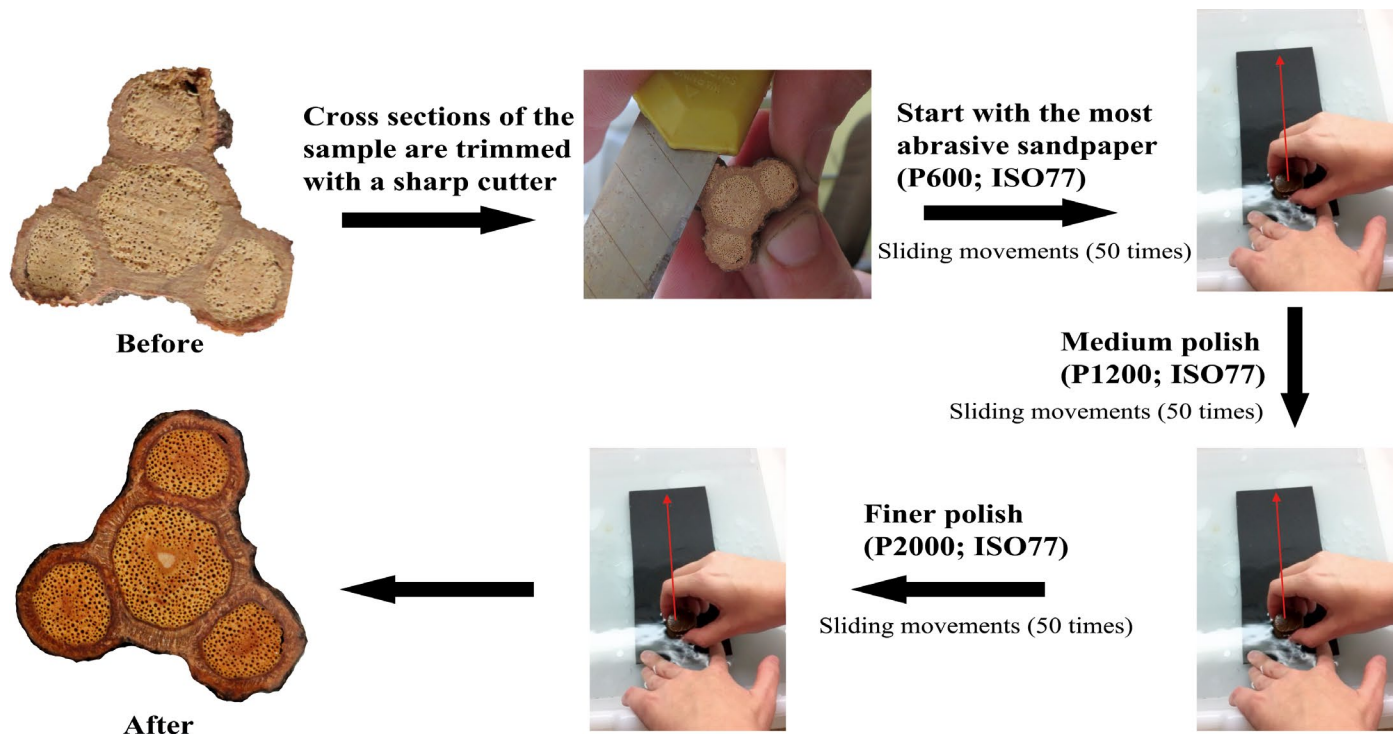


FIGURE 2. Visual guide to sanding samples conserved in 70% ethanol using waterproof sandpapers under water. A *Serjania lethalis* (Sapindaceae) stem is used as a representative example.

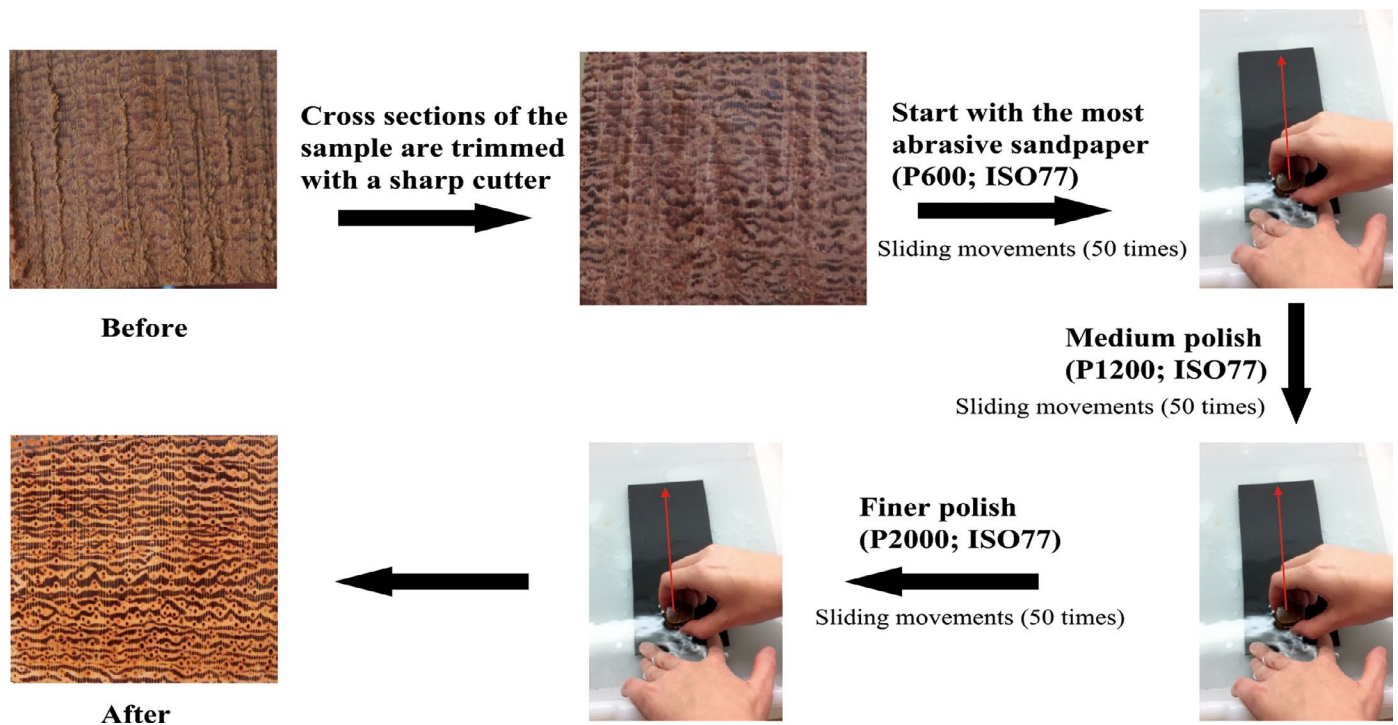


FIGURE 3. Visual guide to sanding dried wood samples using waterproof sandpapers under water. A *Hymenolobium petraeum* (Fabaceae) stem wood is used as a representative example.

the water, avoiding occlusion of the cell lumina. Every so often, the water inside the tray must be replaced, and the sample should be washed under running water.

When the surface is flat and smooth, we move to finishing. Grinding the same sample 50 times using P1200 and then P2000 sandpaper (Figs. 2, 3) will finish the sample and provide optimal

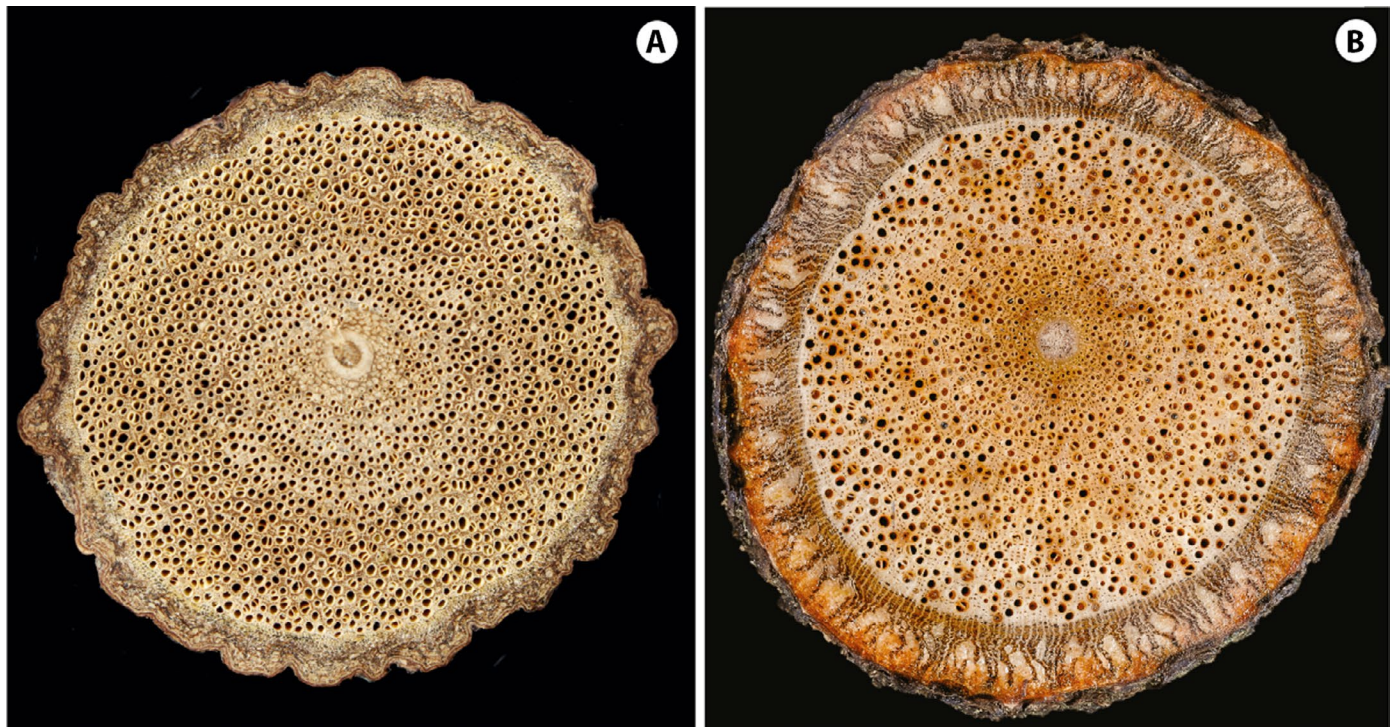


FIGURE 4. The same sample of *Dioclea rufescens* (Fabaceae) subjected to dry polishing in air (A) and our underwater polishing technique (B). In A, we can see that all features related to the bark are lost and some of the swarf particles are retained in the small to medium vessels (see close to pith). This highlights the importance of polishing fresh samples to correctly depict the anatomical features of entire organs containing hard and soft tissues. We performed the dry polishing with a belt electric sander (using ascendant polishing with P100, P150, and P320 sandpaper) and pressurized air to eliminate the wood swarf particles from cell lumina.

results, enabling all different cell types on the stems and roots to be seen. Using sandpaper grits above P2000 does not provide different results in our experience. For optimal results, photograph the samples immediately after sanding. Alternatively, samples can be indefinitely preserved in 70% ethanol, but be aware that some tissues darken and lose contrast once in contact with ethanol. This method also allows the samples to be stained at the end of the process if there is an interest in highlighting specific anatomical characters. The dye must be water soluble for better impregnation.

Results and comparisons with existing techniques

The examples in Fig. 1 illustrate the versatility of the proposed technique and its usefulness in any field that applies polishing for wood and bark anatomical studies. Most of the samples used here would lose most of their informative features if dried; for example, compare the sample of *Dioclea rufescens* polished with our technique under water with a sample polished using the more traditional technique of dry sanding (Gärtner and Nievergelt, 2010; Gärtner and Schweingruber, 2013), in which all features related to the bark are lost (Fig. 4A). In Table 1, we compare the pros and cons of our method with the most commonly used polishing methods. The results achieved for each sample with our proposed technique are summarized below.

The stem of the palm *Desmoncus orthacanthos* is completely surrounded by two leaf bases, and its typical atactostele contains fewer vascular bundles in the center than in its periphery, as is commonly described in palms (Fig. 1A; Tomlinson et al., 2011). All the details of the vascular bundles and the ground tissue can be observed (Fig.

1A), giving a clear overview of its structure. The metaxylem vessel elements are clearly visible in the stem (Fig. 1A), which are swarf free as the water prevents clogging during polishing.

The samples illustrated in Fig. 1B–I all show secondary growth, and in all of them the wood is clearly visible with unclogged vessels, free of swarf. In the very soft stem of *Manekia obtusa*, the large rays greatly dilate within the phloem, and its axial elements are seen in a lighter color (Fig. 1B). A large portion of cortex is also present, with large, naturally dark mucilage cavities. With careful attention, even the cortical bundles can be located. In *Tetrapterys phlomoides*, it is possible to see the partially hollow pith, the inner part of the wood with fewer vessels and more fibers (self-supporting phase, as discussed by Angyalossy et al., 2015), and the remaining wood with its wide vessels and the vasicentric parenchyma (Fig. 1C). In the phloem, it is possible to see the rays dilating and scattered wide cells; these are laticifers (see inset on Fig. 1C), which are commonly found in one of the clades of this genus (Pace et al., 2019). In *Tanaecium pyramidatum*, four equidistant phloem wedges bounded by very wide limiting rays can be clearly seen, which is characteristic of many Bignoniaceae lianas (Fig. 1D; Gasson and Dobbins, 1991; Pace et al., 2015; Gerolamo and Angyalossy, 2017; Gerolamo et al., 2020). It is also possible to clearly see the semi-ring porous wood common in this family (Lima et al., 2010), as well as the dilating rays toward the phloem, which is stratified in its regular portion (sensu Angyalossy et al., 2016). *Alicia anisopetala* forms a remarkable fissured stem, with islands of xylem immersed within a matrix of phloem and disruptive parenchyma (Fig. 1E), similar to what was previously described in *Callaeum* Small (Cabanillas

TABLE 1. Comparison of the present method and other polishing methods commonly used in plant anatomy.

Methods	Pros	Cons	References
Our method (polishing under water)	<ul style="list-style-type: none">• Low cost, simple, and fast (ca. 15–25 min)• Excellent for wood identification and overall anatomical descriptions of the stem and root• Can be used for dry, fresh, and liquid-preserved samples• Can be applied to irregular samples <10 cm in diameter• Does not require electric machines• Perfectly preserves hard (wood) and soft (bark) tissues• Eliminates wood swarf from cell lumina• Good visualization for distinguishing cell types and growth-ring boundaries• Easy to photograph	<ul style="list-style-type: none">• Cannot be used for samples that cannot be made wet or moist• Limited to small- to medium-sized samples (<10 cm diameter)• Drying after polishing typically alters the initial shape of the samples or breaks up the soft tissues	This paper
Dry polishing	<ul style="list-style-type: none">• Median cost (when electric polishing machines are employed)• Simple and rapid (ca. 15 min, but larger samples require a longer amount of time)• Excellent for wood identification• No limitation of sample size• Good visualization for distinguishing growth-ring boundaries• Excellent for dendrochronological analyses (both for cores and entire trunks)	<ul style="list-style-type: none">• Only provides good visualization of wood (secondary xylem) or lignified tissues (hard bast or dry fruits)• Can only be used with dry samples, making it inadequate for entire stems or roots with bark• Electric sanders are preferred• Requires pressurized air to eliminate wood swarf particles from the cell lumina• Typically does not eliminate swarf particles from small to medium vessels• Thin cell walls may break and split during the sanding procedure• Requires skill to use electric sanders and avoid accidents• Takes longer to sand large samples	Gärtner and Nievergelt, 2010; Gärtner and Schweingruber, 2013
Microtome polishing	<ul style="list-style-type: none">• Confers extremely clean sample surfaces, allowing visualization even at higher magnifications (up to 50x)• Rapid execution (ca. 5–15 min)• Eliminates swarf particles from all cell lumina• Excellent for macrophotographs and microscopic analyses• Good visualization for distinguishing cell types and growth-ring boundaries	<ul style="list-style-type: none">• Higher costs because a sliding microtome and either permanent or disposable microtome knives are required• Requires someone trained to use a sliding microtome• Restricted to small samples that fit the sliding microtome holder (0.5 × 6 cm)• Better for dry samples because the microtome holder might compress and break up soft tissues• Higher risks of accidents because of the very sharp microtome knives	Gärtner and Nievergelt, 2010; Gärtner and Schweingruber, 2013

et al., 2017), a lineage sister to *Alicia* (Davis and Anderson, 2010). As can be seen in Fig. 1E, both the stiff xylem and the soft disruptive tissues formed in its interior are perfectly visible. In the soft-stemmed liana *Dioclea rufescens*, the vessels are immersed in a matrix of non-lignified parenchyma, where the fibers appear as darker lines (Fig. 1F). The phloem rays greatly dilate with a flame-like appearance in cross section. Note the increase in vessel width from the center to the periphery. Finally, the stem of *Serjania lethalis* exhibits the typical compound structure of many Sapindaceae lianas (Tamaio and Angyalossy, 2009; Pellissari et al., 2018; Chery et al., 2020a, b), in this particular case with a central cylinder surrounded by three peripheral cylinders (Fig. 1G). The root lacks a compound structure, but possesses phloem wedges (Bastos et al., 2016), which are not damaged or altered by the present technique. This root is 6 cm in diameter, which demonstrates that large organs can also be well-polished with this technique (Fig. 1H). All of these samples contain soft tissue and wood, and would greatly shrink if dried. Sanding them after drying would therefore result in cracked, deformed samples, which do not correctly represent their anatomical structure. The technique here proposed is a simple way to overcome this limitation.

We also included a dry wood sample of *Hymenolobium petraeum*, which was obtained from a wood collection (Appendix 1), to show that our technique can be used for any woody material, and likely even cones, fruits, and seeds (see Benedict, 2015). Here, we see that some of the vessels in *H. petraeum* have a whitish gum obstruction, which is common in the heartwood of many Fabaceae (Fig. 1I). The water eliminates only the swarf, not the gum or other cell inclusions, which is excellent for the purpose of macroscopic descriptions and identification. In the same way, we can clearly see the bands formed by aliform confluent parenchyma in a matrix of thick-walled fibers (Fig. 1I). For dry wood samples (without bark and other soft tissues), we obtained similar results both with the traditional technique of dry sanding followed by the application of pressurized air, and with our underwater sanding technique.

CONCLUSIONS

Using waterproof sandpaper under water, it is possible to polish fresh, fixed, and dry materials with great success. Here, we show that, regardless of the taxonomic group or stem consistency, this

simple, inexpensive, rapid technique enables the polishing of all types of woody materials, and can greatly help anatomists who want to show the entire structures of both wood and bark, or to perform descriptions, dendrochronological analyses, or plant identification.

ACKNOWLEDGMENTS

The first author would like to express his gratitude to the staff and students of the Instituto de Pesquisas Tecnológicas and Universidade de São Paulo, who triggered the creation of this technique across his more than 50 years working on wood anatomy. Special thanks to Joyce G. Chery (Cornell University), Rosa Nejapa Mendoza (Universidad Nacional Autónoma de México), Anne Kaspar (Universidad Autónoma Metropolitana), and Camille Truong (Royal Botanic Gardens Victoria) for lending their voices to the English, Spanish, German, and French audios, respectively. We are indebted also to Carolina L. Bastos (Universidade de São Paulo) for contributing the image of the *Serjania lethalis* root. We also thank Lorena Luna Márquez (Universidad Nacional Autónoma de México) and Rosa Nejapa Mendoza (Universidad Nacional Autónoma de México) for their comments on earlier versions of the manuscript. V.A., C.S.G., and A.C.L. were supported by the São Paulo Research Foundation (FAPESP, 2018/06917-7), and C.S.G. was also supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES, finance code 88882.333016/2019-01). M.R.P. was financed by the Programa de Apoio a Projectos de Investigación e Innovación Tecnológica – Mexico (DGAPA, PAPIIT, Project IA200521). We thank Associate Editor Gregory J. Pec, Reviewing Editor Jordan Dowell, Managing Editor Beth Parada, and two anonymous reviewers for their invaluable comments on the manuscript.

AUTHOR CONTRIBUTIONS

A.C.F.B. created and taught everyone the technique; M.R.P., C.S.G., V.A., and A.C.L. brainstormed, read, and summarized the literature; M.R.P. and C.S.G. wrote the first draft of the manuscript. All authors reviewed, edited, and approved the final manuscript before submission and publication.

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APPENDIX 1. Species, collector (accession number), and location (municipality/state and country) of all samples used in this study. Abbreviations: RBR = Herbarium of the Federal Rural University of Rio de Janeiro; SPF = Herbarium of the University of São Paulo.

Desmoncus orthacanthos Mart.; Pace 117; Paraguay river margins, Corumbá, Mato Grosso do Sul, Brazil; voucher in SPF.

Hymenolobium petraeum Ducke; from the didactic collection of the SPFW wood collection at the University of São Paulo.

Manekia obtusa (Miq.) T. Arias, Callejas & Bornst.; Faleiros-Figueiredo 09, 63; Forest Reserve of the Biosciences Institute, University of São Paulo, São Paulo, Brazil; voucher in SPF.

Serjania lethalis A. St.-Hil.; Somner 1652; Forest Reserve of Poço das Antas, Rio de Janeiro, Brazil; voucher in RBR.

Tanaecium pyramidatum (Rich.) L. G. Lohmann; Pace 14, Pace 35; Forest Reserve of the Biosciences Institute, University of São Paulo, São Paulo, Brazil; voucher in SPF.

Tetrapterys phlomoides (Spreng.) Nied.; Pace 235; Forest Reserve of Poço das Antas, Rio de Janeiro, Brazil; voucher in SPF.

APPENDIX 2. Detailed summary of woody sample polishing under water.

Materials:

1. A cutter knife installed in a holder

2. A large tray at least 5–15 cm high and at least 30 cm long and 20 cm wide
3. A glass plate or an alternative flat surface on which the sandpaper can be situated without sliding, and which fits inside the tray
4. At least three grits of waterproof sandpaper: P600 (ISO77; 24.8–26.8 µm), P1200 (14.3–16.3 µm), and P2000 (8.5–10.5 µm). A coarser grit, such as P200, may be used at the beginning of the process if the surface is exceptionally rough and a cutter knife is not available.
5. Tap water

Procedure:

Step 1: Hold the sample firmly and use a cutter knife installed in a holder to manually remove any rough marks from sawing, knife marks, or to eliminate any visible inclination on the transverse section (Figs. 2, 3). The goal is to render the surface as flat as possible.

Step 2: Once this is done, place the glass plate within the tray and fill it with approximately 1–3 cm of water, so that the surface to be polished is kept under water.

Step 3: Place the roughest waterproof sandpaper, P600 (or P220 if needed), on top of the glass plate under water. If necessary, trim the sandpaper to the desired size. Here, we used sandpaper 20 cm in length and 10 cm wide.

Step 4: With one hand, stabilize the sandpaper on the glass plate. With your other hand, hold the sample firmly between your thumb and index finger, grinding the sample either in a single direction or up and down under water (Figs. 2, 3; see Video 1). Here, we propose grinding the sample 50 times, although different samples might require more or fewer repetitions. Rotate the sample slightly after every 10 or so movements to guarantee uniformity. Do not apply excessive pressure against the sandpaper to avoid marking the sample. Avoid applying uneven pressure to the sample, which could cause tilting of the transverse surface.

Step 5: After ca. 50 movements, wash the sample under running water and change the water if it becomes too tainted with wood swarf.

Step 6: Once the transverse section looks flat and is smooth to the touch, repeat the same operation with waterproof sandpapers of grits P1200 and P2000 to finish (Figs. 2, 3).

Step 7: The length of time spent sanding with each grit of sandpaper will depend on the sample, but most of the time will likely be spent using the P600 sandpaper to make the surface smooth and uniform. As a guide, it took 15 min to polish the *Serjania lethalis* stems, but 25 min to polish *Hymenolobium petraeum*, a much harder wood.

Step 8: We suggest photographing the sample immediately after polishing (Figs. 2, 3), because the oxidation of woods and barks is a common phenomenon that significantly reduces the level of contrast between cell types.

Step 9: The samples can then be preserved in 70% ethanol to avoid cracks due to the shrinkage of soft tissue. This preservation in ethanol may, however, change the wood color and reduce the contrast between different tissues and cell types.

All the main steps are summarized in the flowchart (Appendix 3).

APPENDIX 3. Flowchart showing how to polish woody samples using waterproof sandpapers under water.