

A practical technique for preserving specimens of duckmeal, *Wolffia* (Araceae)

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Brunton, D.F. 2019. A practical technique for preserving specimens of duckmeal, *Wolffia* (Araceae). Canadian Field-Naturalist 133(2): 139–143. <https://doi.org/10.22621/cfn.v133i2.2108>

Abstract

Making identifiable herbarium vouchers of the minute aquatic vascular plant duckmeal, *Wolffia* (Lemnoideae; Araceae) has typically required plants to be preserved in transparent, space-consuming vials that are fragile, difficult to work with, and labourious to prepare. An alternative technique for dry-mounting *Wolffia* within a layer of transparent, acid-free glue presents a promising alternative. Although the largely water-filled individual plants still compress substantially, this preparation technique results in specimens that retain their colour, size, and, most important, their shape. This greatly enhances the possibility of confident identification and simplifies both specimen preparation and storage.

Key words: *Wolffia*; Lemnoideae; Araceae; herbarium specimen preparation; storage

Introduction

Dore (1957) described the difficulty collectors face in securing voucher specimens of duckmeal (*Wolffia* spp., Lemnoideae, Araceae; also known as watermeal) in a condition that permits their re-examination and identification. Three species of this minute, simple plant, which is uncommon to rare throughout most of its Canadian range, typically form dense, floating mats (Figure 1) consisting of thousands and even millions of individuals (Brunton 2018; Brunton and Bickerton 2018). At least two *Wolffia* species (Figure 2) are native in southern Ontario and Quebec (Crow and Hellquist 2000). Individuals of this, the smallest flowering plant in the world, shrivel up into greenish-brown dust when air dried as conventional vascular plant herbarium specimens (Figure 3). This deformation eliminates the possibility of further identification, which is heavily dependent on plant shape characteristics (Crow and Hellquist 2000). Accordingly, the potential use of such material for taxonomic or phylogeographic analysis is substantially reduced.

Dore's (1957) solution to that curatorial challenge was to place *Wolffia* samples in a preservative fluid (alcohol) in a sealed glass vial, which was then attached to a standard herbarium sheet. Although this technique permits the voucher plants to retain their shape and size, it is an involved process that results in a slurry of colourless plants floating in a cumbersome, fragile vessel that takes up considerable space in a conventional herbarium arrangement. Individual

plants also move freely within the vial and are difficult to follow or relocate. Unless the vial is opened (necessitating repetition of the entire voucher preparation process), the view of individual plants is also obscured and distorted by the glass container. There is a risk of breakage or leakage of the vials, as is evident in Dore's collection preserved in the Agriculture and Agri-Food Canada herbarium (DAO). In addition, the alcohol-filled vials are sealed with a flame or heat, a process that requires some experience and poses potential hazards (P.M. Catling pers. comm. 14 March 2019). Although preferable to simply drying plants into an unrecognizable condition, the vial solution is a cumbersome and unsatisfying curatorial response.

The easy availability of high-quality digital imagery in the field now provides a practical enhancement of the traditional air-dried voucher technique, because hard-copy images of fresh (pre-dried) voucher material can be affixed to the voucher sheet. However, photographs are only two-dimensional representations and cannot be used for detailed morphological studies.

Discussions with numerous field botanists in eastern Canada have confirmed that the difficulty in securing reasonable quality *Wolffia* vouchers significantly discourages their collection. This represents a floristic and conservation problem in Ontario and Quebec where Northern Duckmeal (*Wolffia borealis* (Engelmann) Landolt & Wildi ex Gandhi, Wiersema & Brouillet) (= *Wolffia punctata* Grisebach) and Co-

A contribution towards the cost of this publication has been provided by the Thomas Manning Memorial Fund of the Ottawa Field-Naturalists' Club.



FIGURE 1. Dense duckmeal (*Wolffia* spp.) mat completely covering creek surface. 8 August 2011, Beachburg, Renfrew County, Ontario. Photo: D.F. Brunton.

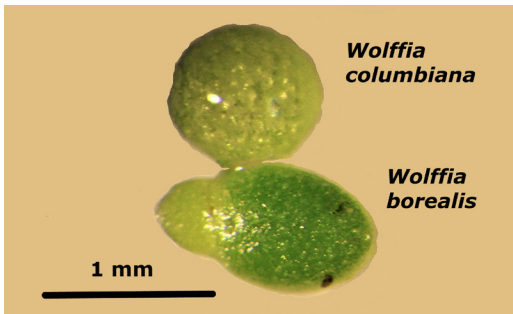


FIGURE 2. Fresh (live) Ontario native duckmeal (*Wolffia*) species. 31 July 2011, Peterborough County, Ontario, D.F. Brunton & K.L. McIntosh 17,896B. Photo: D.F. Brunton.

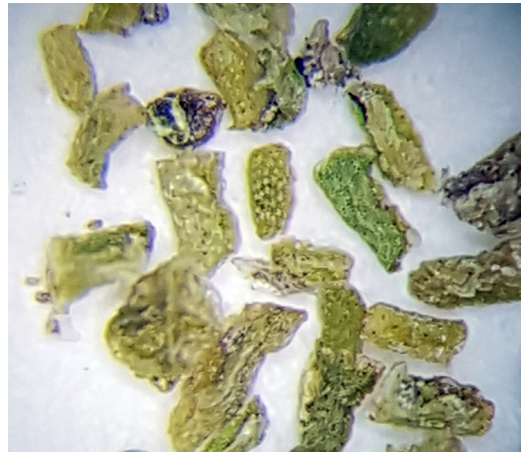


FIGURE 3. Shrivelled air-dried Northern Duckmeal (*Wolffia borealis*). 31 July 2011, Peterborough County, Ontario, D.F. Brunton & K.L. McIntosh 17,896B. Photo: D.F. Brunton.

lumbia Duckmeal (*Wolffia columbiana* H. Kirsten) (= *Wolffia arrhiza* auct., non (L.) Horkel) are almost always regionally uncommon (Dore 1957; Soper 1962; Oldham 2017) and the possibly introduced Papillate Watermeal (*Wolffia brasiliensis* Weddell) is rare (Thomson 2005). Both *W. borealis* and *W. columbiana* are considered rare and of conservation concern in Quebec (Tardif *et al.* 2005). In addition, populations are increasingly being found beyond *Wolffia*'s traditional northern limit along the southern edge of the Canadian Shield in Ontario (Soper 1962), suggesting ongoing range expansion (Brunton 2018).

Does this change represent climate change? In-

creased populations of certain waterfowl species? Transportation by human activity? All of the above? That is not clear, but the range of *Wolffia* unquestionably is enlarging in Canada and new *Wolffia* occurrences must be recorded with voucher specimens to document this and assess its implications. Accordingly, a practical procedure for producing informative vouchers is an important part of addressing these questions.

The dry-mounted specimen preparation technique described in this paper is an adaptation of the method described by Brunton (1990) for securing mass samples of even smaller and more fragile megaspores of the aquatic lycophyte *Isoetes* (quillwort) in a readily examinable state. This new dry-mounting procedure has been used to prepare samples in approximately two dozen *Wolffia* collections to date. It has resulted in vouchers of superior quality and utility in comparison to most vouchers prepared using traditional methods.

Methods

A small (5–10 mL) sample of fresh *Wolffia* plants is placed in a plastic or glass vial with enough of the water-diluted (ca. 75% of full strength), acid-free glue commonly used in herbarium specimen preparation to maintain the sample free floating. The glue used here was commercially available Weldbond Universal Adhesive (Frank T. Ross & Sons Ltd., Markham Ontario, Canada). No attempt was made to assess the performance of other commercial brands of adhesive, but it is suspected that any water-soluble white herbarium glue would perform comparably. The sample is left to soak for ~48 h. The container is covered to prevent the glue from drying and solidifying. Because *Wolffia* plants are exceedingly buoyant, the slurry of plants is stirred periodically (two or three times in the course of the soaking period) to ensure thorough contact with the glue solution.

After about 25–30 h, the colour of the glue solution takes on a distinctively green cast. The *Wolffia* plants also appear to be less buoyant, floating lower in the solution and remaining submerged slightly longer when stirred. This is interpreted as indicating that the plants have become slightly waterlogged and that the glue has penetrated their tissues.

After ~48 h the slurry is spread (poured) onto a sheet of plain paper (a heavier gauge, pH neutral paper is recommended) or directly onto a herbarium sheet (White 100 Percent Rag Bond – neutral pH 20 or 24 lb; Herbarium Supply Co., Bozeman, Montana, USA) in as thin a layer as possible and allowed to air dry. Concentrations of plants on the mounting sheet can be thinned by gently passing a dissection needle or probe through them. This must be done immediately after the slurry has been poured, however, as the solution becomes too tacky for smooth manipulation within as little as 10 min. The sample (Figure 4) is dry and ready for examination or processing as a herbarium specimen within about 2 h.

Results

Applying this voucher preparation technique to over 30 samples of native Ontario *Wolffia* involving

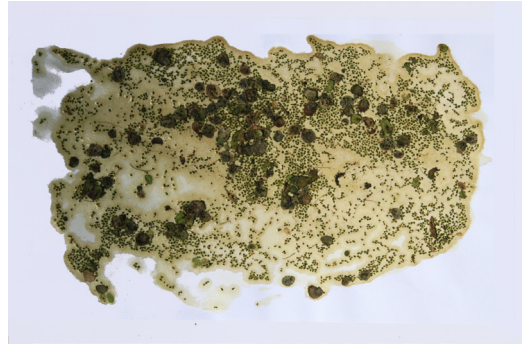


FIGURE 4. Sample of dried, glue-impregnated Columbia Duckmeal (*Wolffia columbiana*); larger intermixed plants are *Spirodella polyrhiza* (L.) Schleiden. 25 August 2017, Renfrew, Renfrew County, Ontario, D.F. Brunton & K. Fleming 19,783. Photo: D.F. Brunton.

23 separate collections (Appendix 1) has produced encouraging results. Although the dry, glue-impregnated plants are completely deflated and flattened, they have, for the most part, retained their original size, shape, and colour (Figure 5). Some aspects of their morphology remain evident: for example, the punctate (dotted) surface of *W. borealis* is clearly visible in the dried plants.

Rehydrating shrivelled, long-dried herbarium material may also be worthwhile. In the two samples tested, the glue solution did not become as green as it did with fresh material, suggesting that the rehydrating plants do not become as completely saturated. Nonetheless, the rehydrated material was more distinguishable than it would have been if specimens had only been air dried.

It is recommended that collectors making *Wolffia* vouchers include a packet of air-dried plants (“green dust”) prepared in the traditional way as well. This typically indistinguishable air-dried material is potentially useful for chemical and molecular analyses. Ideally, photos of fresh plants should be included in the voucher. When used in combination with glue-impregnated plants, these additional specimen components provide the most complete vouchers possible for multidisciplinary investigations.

More extensive experimentation would refine the technique described here. It may well provide similarly satisfying results, for example, for other aquatic plants that occur in vast numbers of minute individuals, such as duckweed (*Lemna*), duck-meat (*Spirodella*), and mud-midget (*Wolffiella*). A variation on this technique to accommodate larger specimens might also prove useful for the preservation of exceptionally delicate and/or fragile water nymph (*Najas*), ditch-grass (*Ruppia*), horned pondweed (*Zannichellia*), and



FIGURE 5. Close-up of glue-impregnated Columbia Duckmeal (*Wolffia columbiana*) retaining shape and size. 25 August 2017, Renfrew, Renfrew County, Ontario, D.F. Brunton & K. Fleming 19,783. Photo: D.F. Brunton.

pondweed (*Potamogeton* and *Stuckenia*) plants in a more natural form. As currently understood, however, this technique provides a logistically simple, space-efficient, permanent preservation alternative for making *Wolffia* vouchers, for which there is an immediate need. The technique has the added benefit of being hazard free for the preparator and posing no curatorial risks to associated herbarium material.

Acknowledgements

This paper benefited significantly from input by Associate Editor, Paul M. Catling, and reviewers, Bruce Ford, University of Manitoba, Winnipeg, Manitoba, Garret Crow, Calvin College, Grand Rapids, Michigan, and Michael J. Oldham, Ontario Ministry of Natural Resources and Forestry, Peterborough, Ontario. Their valuable comments and observations were

based on extensive first-hand field experience with *Wolffia*. The author was inspired to investigate and collect these curious and challenging aquatic plants in the first place by the enthusiastic encouragement and assistance of the late W.G. (Bill) Dore (1912–1996), then of Agriculture Canada, Ottawa.

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Received 20 July 2018

Accepted 25 July 2019

Appendix 1: *Wolffia* specimens prepared, partly or entirely, using the Weldbond technique. Herbarium acronyms of Thiers (continuously updated).

Wolffia borealis

- Ontario: City of Ottawa, 45.36843°N, 75.79522°W, west end of Turtle Bay, southwest portion of Mud Lake, Britannia Conservation Area, D.F. Brunton 17,106, 7 September 2007 (DFB);
- Ontario: City of Ottawa, 45.33572°N, 75.92777°W, in Shirley's Brook along south side of Trillium Woods, South March Highlands, Kanata, D.F. Brunton 17,125, 5 October 2007 (DFB);
- Ontario: City of Ottawa, 45.39846°N, 75.96812°W, south end of Constance Lake by inlet of Constance Creek, West Carleton, D.F. Brunton 18,036, 13 August 2011 (CAN, DFB);
- Ontario: City of Ottawa, 45.34581°N, 75.86959°W, abandoned sewage treatment plant 395 m south southeast of Range Road/Carling Avenue intersection, Nepean, D.F. Brunton 18,913A, 8 October 2014 (WIN, DFB);
- Ontario: Frontenac County, 44.27868°N, 76.59344°W, west side of Collins Creek north side of Creeksford Road, Kingston, D.F. Brunton 18,050A, 24 August 2011 (WIN, DFB);
- Ontario: Peterborough County, 44.76643°N, 78.49064°W, west side of White's Lake Road, north side of Crystal Lake, Galway & Cavendish Township, D.F. Brunton and K.L. McIntosh 17,986B, 12 July 2011 (DFB);
- Ontario: Peterborough County, 44.38742°N, 77.97751°W, east side of mill pond by outlet, north side of Highway 7, east end of Norwood, Asphodel Township, D.F. Brunton 19,916, 6 September 2017 (DFB).
- Ontario: City of Ottawa, 45.33572°N, 75.92777°W, in Shirley's Brook along south side of Trillium Woods, South March Highlands, Kanata, D.F. Brunton 17,126, 5 October 2007 (DFB);
- Ontario: City of Ottawa, 45.39846°N, 75.96812°W, south end of Constance Lake by inlet of Constance Creek, West Carleton, D.F. Brunton 18,035, 13 August 2011 (CAN, DFB);
- Ontario: City of Ottawa, 45.34581°N, 75.86959°W, abandoned sewage treatment plant 395 m south-southeast of Range Road/Carling Avenue intersection, Nepean, D.F. Brunton 18,913B, 8 October 2014 (WIN, DFB);
- Ontario: Frontenac County, 44.27868°N, 76.59344°W, west side of Collins Creek north side of Creeksford Road, Kingston, D.F. Brunton 18,050B, 24 August 2011 (WIN, DFB);
- Ontario: Peterborough County, 44.38742°N, 77.97751°W, east side of mill pond by outlet, north side of Highway 7, east end of Norwood, Asphodel Township, D.F. Brunton 19,917, 6 September 2017 (DFB);
- Ontario: Peterborough County, 44.76643°N, 78.49064°W, west side of White's Lake Road, north side of Crystal Lake, Galway & Cavendish Township, D.F. Brunton and K.L. McIntosh 17,986B, 312 July 2011 (WIN, UBC, DFB);
- Ontario: Renfrew County, 45.5128°N, 76.7591°W, 650 m north of Highway 60 at southeast corner of Clubhouse Lake, west side of Renfrew Golf Club, Horton Geographic Township, D.F. Brunton and K. Fleming 19,873, 27 August 2017 (DFB).

Wolffia columbiana

- Ontario: City of Ottawa, 45.36843°N, 75.79522°W, west end of Turtle Bay, southwest portion of Mud Lake, Britannia Conservation Area, D.F. Brunton 17,107, 7 September 2007 (DFB);

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