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The Bryologist 83(2), 1980, pp. 228-233
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Acid-Base Color Reactions: The Status of *Triquetrella ferruginea*, *Barbula inaequalifolia* and *B. calcarea*

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Abstract. *Triquetrella ferruginea* (*Schimp. ex Besch.*) *Thér. of Mexico* is a new synonym of *Didymodon fallax* var. *reflexus* (*Brid.*) *Zander, comb. nov.* *Triquetrella californica* (*Lesq.*) *Grout* is morphologically similar but can be distinguished by color reactions to strong acids and bases. *Bryoerythrophyllum calcareum* (*Thér.*) *Zander, comb. nov.*, and *Bryoerythrophyllum inaequalifolium* (*Tayl.*) *Zander, comb. nov.*, are transfers from the genus *Barbula* based in part on the different acid-base color reactions of the genera *Bryoerythrophyllum* and *Barbula*.

Encouraged by recent reports (Hill 1976, Lane 1978) of the utility of color reactions to acids and bases in *Sphagnum* taxonomy, I made a survey of the response of the leaves of various species of Pottiaceae to a number of histochemical and chromatographic spray reagents given by Haas and Hill (1928), Hais and Macek (1968), Jensen (1962), Mueller (1973) and Seikel (1964). The only reactions that seemed to be commonly specific to taxa and therefore potentially useful in classification were simply changes in leaf color on application of strong mineral acids and bases. Such color reactions are usually reversible. Leaves of Pottiaceae species readily blacken in 10% ferric chloride solution and become orange-brown in 1% *p*-nitrobenzenediazonium fluroborate solution—two tests for phenolic compounds (Robinson 1967). The observed acid-base color responses are possibly a result

of the presence of various anthocyanins or flavonoids, which are phenolics commonly exhibiting changes in coloration with changes in pH (Geissman 1955, Robinson 1967). Flavonoids are apparently common and diverse in acrocarpous mosses (McClure & Miller 1967) and have proven to be of considerable value in vascular plant taxonomy because of their chemical stability and general lack of variation in populations, even in environmentally modified plants. Mårtensson and Nilsson's (1974) review of the chemical bases of morphological color in bryophytes emphasized that to this date few color reactions to chemical reagents have been shown to be potentially important in bryophyte taxonomy. Stone (1976) used differing reactions to staining by Toluidine Blue followed by a lactic acid rinse to discriminate, in part, two new species of *Acaulon*. Koponen (1968, 1974) reviewed color responses of Mniaceae species to ferric chloride and to potassium hydroxide solutions. Crundwell (1979) found color changes in moss rhizoids after application of acids and bases to be often strong, distinctive and valuable as taxonomic characters for some species and groups. Several papers compiled by Suire (1978) emphasize the utility of phenolic analyses in taxonomy of certain bryophyte groups, but these involve comparatively lengthy chromatographic methods, not simple color reactions of the plants themselves.

I now routinely test herbarium specimens with concentrated hydrochloric acid ("Cl"), a 10% solution of potassium hydroxide ("K"), concentrated nitric acid ("N"), and a 2 : 1 mixture of concentrated sulphuric acid and ethanol ("SE"). The alcohol in the last reagent (add acid to alcohol *slowly* in a cold water bath) retards the dissolution of the leaf cell walls by the acid—dilution of the acid by water weakens or stops the color reactions of the moss plants, which is the reason blotting of wet plants is important before testing. The pink partial reaction product of the acid and alcohol has no evident effect on the color reactions. The four reagents are corrosive but are safely kept in small, squeezable, plastic dropper bottles or in glass dropper bottles with rubber bulbs replaced by plastic bulbs when attacked by acids. Mixtures of both young and mature leaves are prepared from cleaned plants that have been soaked in hot water for 1–5 seconds, then lightly blotted. Portions of these mixtures are placed in the slots of depression slides and saturated with 1–3 drops of each reagent. Extra acid reagent is added whenever calcareous soil is adherent to the leaves. When chlorophyll appears to mask the color response, as is commonly the case in fresh plants, a lactic acid (concentrated) presoak of 15–30 seconds, followed by a hot water rinse, "clears" the plants and allows easy recognition of color response in K but often not in other reagents for which mature, achlorophyllose leaves must be examined. The lactic acid apparently does not affect the subsequent response to reagent. The colors of the leaves (not the costae or the stems) are then recorded against a white background under a dissecting microscope (up to two minutes are allowed to elapse if there is no immediate reaction), and the slides are then cleaned of the reagents by rinsing with tapwater from a washbottle into a large, glass canister. This technique is fast and, with some care, safe to clothes and person. A small fan may be used to disperse acrid fumes.

Color reactions do not appear to lessen in intensity with aging of the herbarium specimen; indeed, the fading of chlorophyll allows better color discernment. Young plants or plants that grew in unusually moist conditions often exhibit a less strong response than do older plants or older portions of plants, or plants from more exposed stations. But sometimes young leaves have brighter color responses than do mature leaves. Some variation in color response in herbarium specimens may be due to leaching by preservatives after collection or degradation of chemicals by heat (see Coradin & Giannasi 1980, Culberson et al. 1977). But, in spite of some variation, many taxa appear to have color responses that may be used to distinguish them from other taxa. This can be of importance in separating taxa with morphologically similar gametophytes that are easily confused when sterile.

For example, sterile *Anoetangium aestivum* (Hedw.) Mitt. is easily distinguished from *Hymenostylium recurvirostrum* (Hedw.) Dix. by the N deep red reaction—the latter species is N light to medium orange-brown, seldom light to medium reddish-brown. Also,

small plants of *Didymodon acutus* (Brid.) K. Saito and *D. rigidulus* Hedw. are readily distinguishable from morphologically similar small specimens of species of *Didymodon* sect. *Vineales* (Steere) Zander by the K light red-brown or light to strong yellow- or red-orange reaction of the first two species versus the K medium to deep red response of sect. *Vineales*. Of course, similar color reactions may be produced by different organic substrates, but acid-base color reactions are here suggested only as characters additional to those of morphology.

An instance in which acid-base color testing has proved of particular importance is in a review of the genus *Triquetrella* in Mexico. I have examined all reported (Bescherelle 1872, Crum 1951, Thériot 1931) Mexican collections (NY, PC, TENN) of this genus, including the type of *T. ferruginea* (Schimp.) Thér., and these proved to be "*Barbula reflexa* (Brid.) Brid." This weak segregate of *D. fallax* (Hedw.) Zander is treated as *D. rigidicaulis* (C. Muell.) K. Saito by Saito (1975) and Zander (1978a). Although morphological intergradation with typical *D. fallax* is not uncommon, "*B. reflexa*"—characterized by small plants with strongly papillose, recurved leaves that reach 2.0 mm in length—may be better treated as follows:

***Didymodon fallax* var. *reflexus* (Brid.) Zander, comb. nov.**

Tortula reflexa Brid., *Musc. Recent. Suppl.* 1: 255. 1806, basionym. — *Barbula reflexa* (Brid.) Brid., *Mant. Musc.* 93. 1819. — *Barbula fallax* var. *reflexa* (Brid.) Brid., *Bryol. Univ.* 1: 558. 1826.

Barbula ferruginea Schimp. ex Besch., *Mém. Soc. Nat. Sci. Nat. Cherb.* 16: 181. 1872, **syn. nov.** Type: Mexico, San Cristobal, Müller s.n. (PC—holotype; BM, NY—isotypes). — *Triquetrella ferruginea* (Schimp. ex Besch.) Thér., *Smithsonian Misc. Coll.* 85(4): 9. 1931.

Additional synonyms, including three other combinations in *Triquetrella* are given by Saito (1975, p. 502); however, Saito's citation of "*Tortula fallax* var. *recurvifolia* Wils., *Musc. Hib.*: 48 (1804)" is incorrect (see van der Wijk et al. 1969).

Judging from the two specimens (including the type) at NY, *Triquetrella californica* (Lesq.) Grout is a good species of *Triquetrella*, similar to other species of the genus in that the stem transverse section is triangular and shows a central strand; the leaves are trifarious and have strongly recurved lower margins; the laminal papillae are sharp, single over the cell lumina; and the costa lacks an adaxial epidermis and is abaxially bulging and sharply papillose. It has not yet been collected in Mexico and is apparently restricted to California (but note that California: *Bolander 61*—CU, distributed by the U.S. National Herbarium under the basionym *Anomodon californicus* Lesq., is actually *Leskeella arizonae* (Williams) Flow.). *Didymodon fallax* var. *reflexus* is quite similar in general appearance but differs by the stem pentagonal in transverse section, the leaves in 3–5 rows and having weakly recurved lower margins, the laminal cells less strongly papillose, and the costa not bulging abaxially and only weakly papillose. It may well be that only specialists with access to authentic material for comparison purposes can readily distinguish these two taxa on morphological bases alone. However, the acid-base color reactions of the two taxa are quite different. *Triquetrella californica* responds strongly to all reagents: Cl deep yellow, K deep orange, N deep red, SE deep yellow-brown. These same strong colors are also found in the related species *Leptodontium flexifolium* (Dicks. ex With.) Hampe in Lindb. and *L. viticulosoides* (P. Beauv.) Wijk & Marg. But *Didymodon fallax* var. *reflexus* (and also the var. *fallax*) has the following color reactions: Cl negative or pale yellow-brown to light orange, K light to deep red-brown, N light tan to light red, SE light to medium red-brown.

Another difficult taxonomic case "solved" with the aid of acid-base color reactions involves the delimitation of the genera *Barbula* and *Bryoerythrophyllum*. In a recent syn-

TABLE 1. Comparison of acid-base color reactions of New World species of *Barbula* and *Bryoerythrophyllum* after lactic acid pre-soak. Br = brown, G = green, O = orange, R = red; l. = light, med. = medium, brt. = bright, dk. = dark, dp. = deep; neg. = negative, occ. = occasionally.

	Cl (conc. HCl)	K (10% KOH)	N (conc. HNO ₃)	SE (H ₂ SO ₄ -ethanol, 2: 1)
<i>Barbula</i> , 9 species	med. G to brt. YG usually present and predominating + l. to med. YB to OB, occ. l. Br	G seldom present + l. to dp. YBr to OBr	l. to dp. R or RBr, occ. l. to dk. O or l. Br	G usually present + l. to med. YBr to OBr or l. Br., seldom l. R throughout or with dp. R blotches
<i>Bryoerythrophyllum</i> , 9 species including <i>B. calcareum</i> and <i>B. inaequalifolium</i>	G seldom present or other colors predominating + l. to med. YBr to OBr, occ. neg. or l. Br	G seldom present + l. to dp. R or RBr, occ. OBr to ROBr or neg.	l. to med. R or RBr, occ. l. to med. OBr or l. Br	G usually absent or weak, when present often blackened + med. to dp. R or RBr, or often med. to dk. RO to ROBr, seldom dk. Br

opsis of *Barbula* in North America (Zander 1979), I noted that *Barbula inaequalifolia* Tayl. and *Barbula calcarea* Thér. had the red coloration in nature (in some collections) and hollow laminal papillae typical of *Bryoerythrophyllum* species, but had the long, twisted peristome teeth characteristic of *Barbula* species. All *Bryoerythrophyllum* species have rudimentary or short, straight peristome teeth or are, rarely, eperistomate (Zander 1978b). A survey of acid-base reactions of nine *Barbula* and seven *Bryoerythrophyllum* species was conducted (Table 1). When available, several specimens of each were tested. The color reactions of these two *Barbula* species are the same as those typical of *Bryoerythrophyllum* species. Salient differences between the two genera follow: *Barbula* is Cl green to bright green usually present and predominating, K light to deep yellow- to orange-brown, SE green usually present plus light to medium yellow- to orange-brown in mature leaves; *Bryoerythrophyllum* is Cl green seldom present or other colors predominating, K light to deep red or red-brown, SE green usually absent or weak, when present usually blackish, plus medium to deep red or red-brown in mature leaves. With this, it is apparent that some *Bryoerythrophyllum* species have long, twisted peristome teeth, just as a few *Barbula* species at least occasionally have short, straight peristome teeth. In both *Bryoerythrophyllum* and *Barbula*, peristome length and degree of torsion are less conservative characters than are acid-base color reactions.

***Bryoerythrophyllum inaequalifolium* (Tayl.) Zander, comb. nov.**

Barbula inaequalifolia Tayl., *London J. Bot.* 5: 49. 1846, basionym. — *Tortula inaequalifolia* (Tayl.) Mitt., *J. Linn. Soc.* 12: 153. 1869. For additional synonyms, see Zander (1968).

***Bryoerythrophyllum calcareum* (Thér.) Zander, comb. nov.**

Barbula calcarea Thér., *Smiths. Misc. Coll.* 85(4): 20. 1931, basionym. — *Barbula linguaeifolia* Bartr., *THE BRYOLOGIST* 50: 204. 1947.

Crundwell (1979) found that there appeared to be two main groups of pigments in moss rhizoids: red to purple or violet pigments that become red in acid but bluer in alkali, and orange to orange-red or red-brown pigments that become more yellow in acid and more red in alkali. The presence of various amounts of these pigment groups in different taxa might also account for the differential color changes in moss leaves discussed above; of course, chemical isolation and analysis is needed to demonstrate this conclusively. In any event, acid-base color reactions do have utility as additional taxonomic characters in dealing with some difficult moss groups.

I thank the curators of BM, CU, NY and TENN for loan of specimens, and M. O. Hill, R. Magill, G. Pierce and W. C. Steere for helpful suggestions and information.

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