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Botrychium furculatum (Ophioglossaceae), a New Moonwort Species from the Rocky Mountains of North America

Steve J. Popovich
37 Cambridge Ct.
Coto de Caza, CA 92679
stevepopovich@hotmail.com

Donald R. Farrar[1]
Department of Ecology, Evolution and Organismal Biology
Iowa State University, Ames, IA 50011
dfarrar@iastate.edu

Arthur V. Gilman
Gilman & Briggs Environmental
1 Conti Circle #5, Barre, VT 05641
avgilman@together.net

Abstract.—***Botrychium furculatum*** S. J. Popovich & Farrar is a new species widespread in the central and southern Rocky Mountains from Alberta and Montana south to New Mexico, with additional populations in the Cypress Hills of Saskatchewan and the Black Hills of Wyoming and South Dakota. Based on allozyme banding patterns, it is inferred to be an allotetraploid with *B. pallidum* W. H. Wagner as one parent and another, as yet undescribed, diploid species (*B.* "farrarii") as the other parent. Genetically, *B. furculatum* is distinguished from *B. pallidum* by exhibiting fixed heterozygous loci in which expressed alleles of one of the genomic contributions matches those of *B. pallidum*, whereas many alleles of the other genomic contribution have not been detected in *B. pallidum*, but are displayed by *B.* "farrarii." Morphologically, a suite of leaf characters differentiates *B. furculatum* from *B. pallidum*, particularly a more pronounced bowed or wishbone-like junction of sporophore and trophophore. Gradations in morphology and color between the two species have led to erroneous reports of *B. pallidum* in the Rocky Mountains. A key to differentiate *B. furculatum* from similar species is presented.

Key Words.—*Botrychium*, allozymes, Rocky Mountain flora, allotetraploid speciation

W. H. "Herb" Wagner was exceptional in his ability to recognize diagnostic morphological characters differentiating cryptic species within *Botrychium* s.s. As a result, he and Florence Wagner from 1981 through 2002 described 16 of the 27 taxa known in North America at that time. Herb and Florence also made initial studies on several more putative taxa, often assigning them informal names. They applied the provisional specific epithet "adnatum" to a group of plants collected by Peter Lesica in 1996 in two prairies of western Glacier National Park, Montana, the name reflecting the broad, sometimes decurrent, attachment of pinnae to the rachis in those populations. In an unpublished 1998 field study of these sites, the Wagners (accompanied by

[1] Corresponding author

Gilman) examined *B.* "adnatum" and also what they believed to be the diploid species *B. pallidum* and *B. simplex* in the same area. They considered a possible allopolyploid origin of "adnatum" through hybridization between those two species and sent specimens of each taxon to Farrar for genetic analysis of this possibility via enzyme electrophoresis.

Based on the allelic composition of "adnatum" at 20 gene loci and the presence of fixed heterozygosity at most loci, Farrar (unreported prior to this paper) concluded that "adnatum" was probably an allotetraploid and that *B. pallidum* was likely one of its parents. However, the non-*pallidum* complement of alleles detected in "adnatum" did not match those of *B. simplex*. Farrar also concluded from allozyme analysis that the plants the Wagners had identified as *B. pallidum* were not that species. Rather, a preponderance of fixed heterozygosity in their allelic constitution also indicated allopolyploidy and matched that of *B. gallicomontanum*, an allopolyploid with *B. pallidum* as one of its parents (Farrar and Johnson-Groh, 1991). Because of these genetic results, and because of a perceived failure to find additional populations, formal publication of "adnatum" was withheld.

In 2005, in cooperation with Glacier National Park botanists, we initiated further study of "adnatum" at the original localities, and a broader search for this taxon in the Rocky Mountains of southern Canada, Colorado, and the Black Hills of South Dakota. Field visits and genetic analysis of the Glacier National Park plants confirmed Farrar's initial conclusions, and plants of similar morphology and yielding identical genetic profiles were also detected in southern Alberta, central Colorado, and in the Black Hills. Surprisingly, we found that many of the Colorado plants with the allelic composition of "adnatum" correlated in their morphology, pallid appearance, and location to plants reported by Wagner and Wagner (1990) as Colorado paratypes of *B. pallidum*. Less pallid plants in Colorado of the same morphology had been given the provisional name "Colorado" by Peter Root (affiliated with the Denver Botanic Gardens) and were thought to be possibly a form of *B. minganense*. Also in 2005, plants resembling *B. minganense* but with the genotype of "adnatum" were detected at Redbank Spring in the Black Hills of South Dakota and informally named "redbank." Subsequently, Popovich continued survey efforts in Colorado and other western states through the following decade while a Botanist for the US Forest Service. Other Forest Service Botanists and moonwort enthusiasts augmented those surveys and collections.

With increasing familiarity regarding the range of morphologies associated with this genotype, subsequent collections revealed morphologies additional to "adnatum", "Colorado" and "redbank," including plants much larger and plants lacking conspicuously adnate pinnae. In retrospect, it is clear that Wagner and others did see populations of "adnatum" outside of its original site in Glacier National Park but did not recognize them as the same taxon. Because of the widely varying and not universally adnate morphology of this taxon,

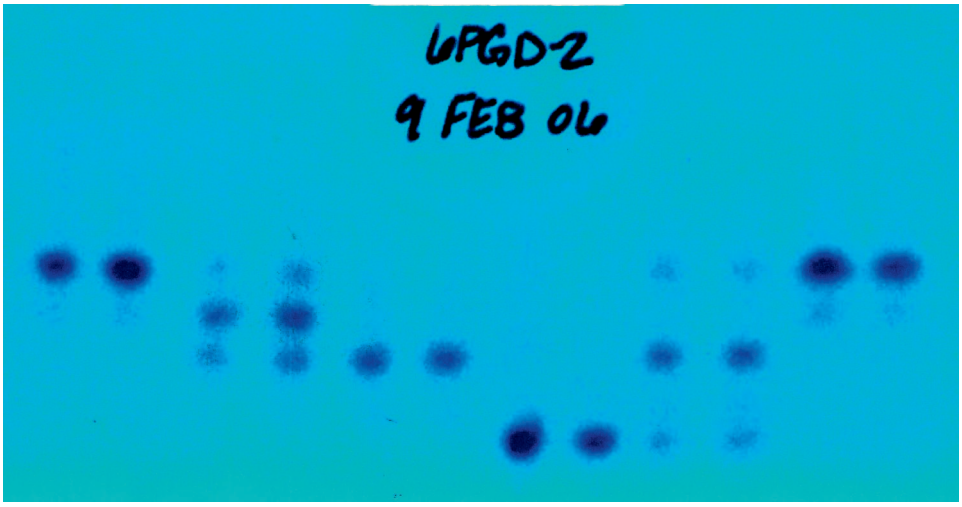

FIG. 1. Typical electrophoresis banding patterns of the enzyme 6-pgd displaying 3 banded allelic patterns in allopolyploid *Botrychium* taxa combining single allele patterns of their diploid parents. From left to right, *B. campestre* (diploid), *B. campestre* × *B. tunux* (probable allotetraploid, undescribed), *B. tunux* (diploid), *B. crenulatum* (diploid), *B. crenulatum* × *B. campestre* (allopolyploid *B. ascendens*), *B. campestre* (diploid).

Farrar and Popovich (2012) applied a different informal name, *B.* "furculatum," which we formalize below as *B. furculatum* S. J. Popovich & Farrar.

Important range extensions for this species were added in southern Saskatchewan by the authors and in New Mexico and Wyoming by Ben Legler. In Legler's Wyoming collections sent to Farrar, he included specimens he considered "similar to, but possibly different from *B.* 'furculatum'," provisionally naming these other plants *B.* "farrarii." Farrar's genetic analyses of those plants indicated not only were they a new diploid taxon, but one possessing the alleles of *B. furculatum* not contributed by *B. pallidum*. A formal description of *B.* "farrarii" is in progress.

Recognition of allopolyploidy as a significant element in understanding *Botrychium* evolution began with cytological determination by W. H. Wagner (1955) of "doubled" chromosome numbers in *B. matricariifolium* and what he later concluded (Wagner and Lord, 1956) was *B. minganense*. As subsequent publications by Wagner and co-workers added taxa to the list of *Botrychium* allopolyploids, detections of their probable diploid parents were greatly aided by genetic evidence derived through enzyme electrophoresis (*e.g.,* Hauk and Haufler, 1999; Farrar and Johnson-Groh, 1991; Stensvold, Johnson-Groh, and Farrar, 2002; Stensvold and Farrar, 2016), a technique revealing sets of alleles distinctive to each diploid and with both parental contributions being visualized in derived polyploids (see Materials and Methods, and Fig. 1). Inclusion of allozyme-confirmed taxa in chloroplast DNA analyses of all known species (Dauphin *et al.*, 2017) allowed detection of the maternal parent of each polyploid taxon through alignment with that diploid. Lastly,

development of procedures allowing visualization of codominant nuclear markers (Dauphin *et al.*, 2018) produced results congruent with morphology, cytology, allozymes, and chloroplast DNA in depicting an array of 15 allotetraploid and one allohexaploid derived from 10 diploid taxa from the three principal clades of *Botrychium* (Dauphin *et al.*, 2018) with only the diploids *B. tunux* and five species of the *B. simplex* subclade excluded from participation in allopolyploid parentage.

Materials and Methods

Samples of putative *B. furculatum* were collected by the authors and other field botanists from sites in Alberta, Saskatchewan, Montana, Wyoming, South Dakota, Colorado, and New Mexico and sent to the Farrar laboratory at Iowa State University for genetic analysis and herbarium curation. For use in genetic analyses, each specimen and each collection were assigned a unique tracking code. These codes, appearing in our figures as numbers attached to individual plants, indicate genetic verification of the identification.

Morphological measurements were taken from live plants, from dried, pressed specimens, and from high-resolution color scans of pressed specimens enlarged to optimize measurement accuracy. Depending upon attribute examined, between 82 and 256 pressed individuals from across the range were measured. Qualitative characters that are not well-captured in pressed specimens, *e.g.*, longitudinal folding of the trophophore, fresh color and glaucousness, and the orientation ("bowing out") of the trophophore stalk at its junction with the sporophore stalk, were scored on living or freshly collected specimens. Spore sizes were measured in the longest diameter on spores mounted in Hoyer's chlorohydrate clearing solution. A minimum of 20 spores was measured for each sample plant (n=40 plants for *B. furculatum*, 15 for *B. pallidum*, and 14 for *B.* "farrarii") selected from populations representing the range of *B. furculatum*. Spores were extracted from multiple sporangia for each plant and measured under a compound microscope.

Enzyme electrophoresis followed procedures described in Stensvold and Farrar (2016). Patterns of allele distributions were analyzed for 269 plants of *B. furculatum*, 175 plants of *B. pallidum* and 18 plants of the rare diploid, *B.* "farrarii," each sampled from across its known range. Plants were analyzed for 22 gene loci from 10 enzyme systems using three buffer systems from Soltis *et al.* (1983): buffer system 7 (0.038M LiOH, 0.188M boric acid) for resolving enzyme systems, aspartate aminotransferase (*Aat*) and triose-phosphate isomerase (*Tpi*); buffer system 9 (0.065M L-Histidine, 0.015–0.016M citric acid, anhydrous) for resolving enzyme systems, malate dehydrogenase (*Mdh*), phosphoglucomutase (*Pgm*), 6-phosphogluconate dehydrogenase (*6-Pgd*), and phospho-glucoisomerase (*Pgi*); and buffer system 11 (0.4M citric acid, trisodium salt) for resolving enzyme systems, aconitase (*Acn*), diaphorase (*Dia*), isocitrate dehydrogenase (*Idh*), and shikimate dehydrogenase (*Skdh*). Alleles are expressed as descending numbers relative to distance of migration on the starch gels, with "1" being the most distal from the origin.

FIG. 2. Specimens of *B. furculatum* from the type locality, Grand County, Colorado.

RESULTS

Morphological measurements and shapes that characterize *B. furculatum* are presented in the species description with diagnostic characters highlighted in italics and shown in Figs. 2–5 and Fig. 7. Relevant comparisons to similar

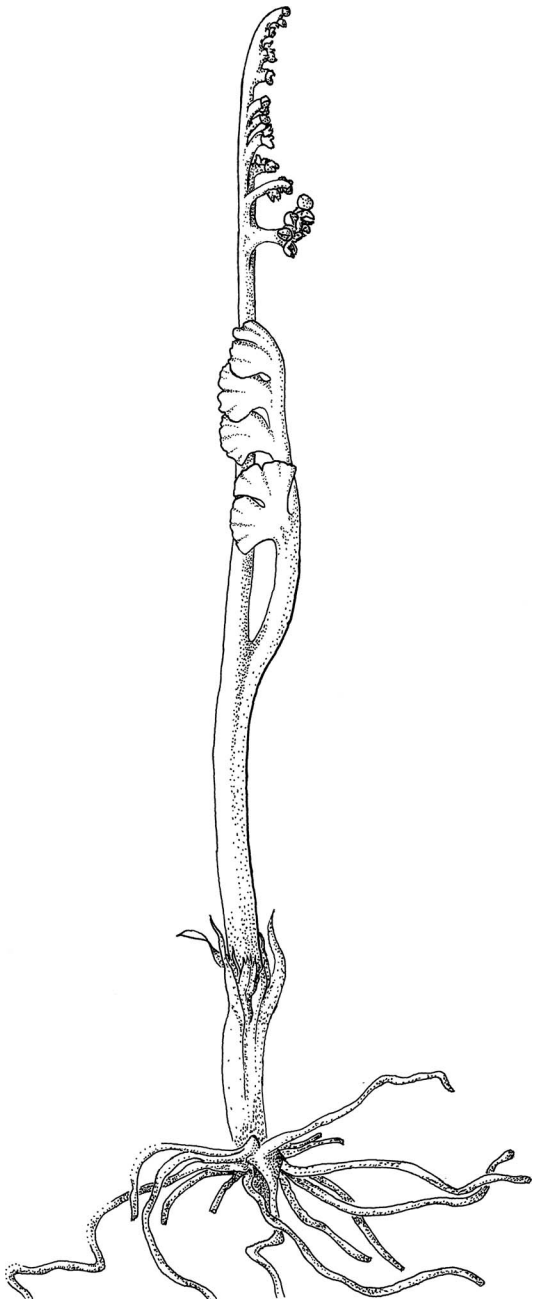

FIG. 3. *Botrychium furculatum*, illustrating in-field stature and morphology. Line drawing by Daryl E. Mergen and modified by the authors. Above-ground leaf from a high-resolution image of a typical plant from near Monarch Pass, Colorado.

FIG. 4. A small individual of *B. furculatum*, showing trophophore bowed outward at base. This character may be difficult to assess on pressed specimens. Cypress Hills, Saskatchewan. Photo: Glen Lee.

species are presented in the accompanying key and Fig. 6. Additional, less diagnostic, measures not included in the formal description are available from the authors. A range map is provided as Fig. 8.

In electrophoretic analysis of enzyme extracts, both *Botrychium pallidum* and *B.* "farrarii" expressed single alleles at 21 of 22 gene loci and two alleles at one locus each (Table 1). Of 20 loci visualized for *B. furculatum,* only one, *Skdh*, expressed an allele not also present in either *B. pallidum* or *B.* "farrarii." At 11 loci where the expressed allele differed between the diploid taxa, plants of *B. furculatum* expressed heterozygosity combining alleles present in the putative parents. Of the 11 loci not expressing heterozygosity in *B. furculatum*, 9 expressed the single allele that was expressed in both *B. pallidum* and *B.* "farrarii." At two loci no activity was expressed in *B. furculatum* plants.

DISCUSSION

Evidence from morphology and allozymes demonstrates that *B. furculatum* is a new allotetraploid species derived in part from parentage by *B. pallidum*, as first hypothesized by Wagner for plants at Glacier National Park. The pale coloration of the plant in life, particularly as expressed by Colorado populations, and the commonly asymmetrical lobing of the pinnae appear to be directly inherited from that species. This close relationship is also

FIG. 5. Range-wide morphological variation in *B. furculatum*. Major sites arranged from north to south across the species range. Numbers attached to specimens were assigned by the Farrar laboratory for tracking individuals during enzyme extraction and analysis; all specimens at ISC. Southwestern Saskatchewan, Cypress Hills Interprovincial Park: 20019, 21, 22, 28, 34, 38, 50, 52 (missing sporophores in 20019 and 21 used for enzyme extraction); Northwestern Montana, Glacier Nat. Park, Big Prairie: 2868, 71, 73; Northern Wyoming, Bighorn Mountains: 17976, 84, 86, 18396a, 97a; Northeastern Wyoming, Black Hills, Warren Peak: 15016, 17220; Western South Dakota, Black Hills: 15322 (Northern Hills), 17219 (Eagle Cliffs), 18594 (Hat Mountain), 17113, 14 (Reynolds Prairie); Central Colorado, Rocky Mountains: 15697 (Boreas Pass), 15780, 86 (Marshall Pass), 17696 (Weston Pass), 17472 (Vail Pass), 17225 (Grizzly Gulch), 18199 (Winter Park), 17483 (Brainard Lake); Northern New Mexico, Vermejo Park Ranch: 16855, 61, 17585, 88, 87, 91, 92.

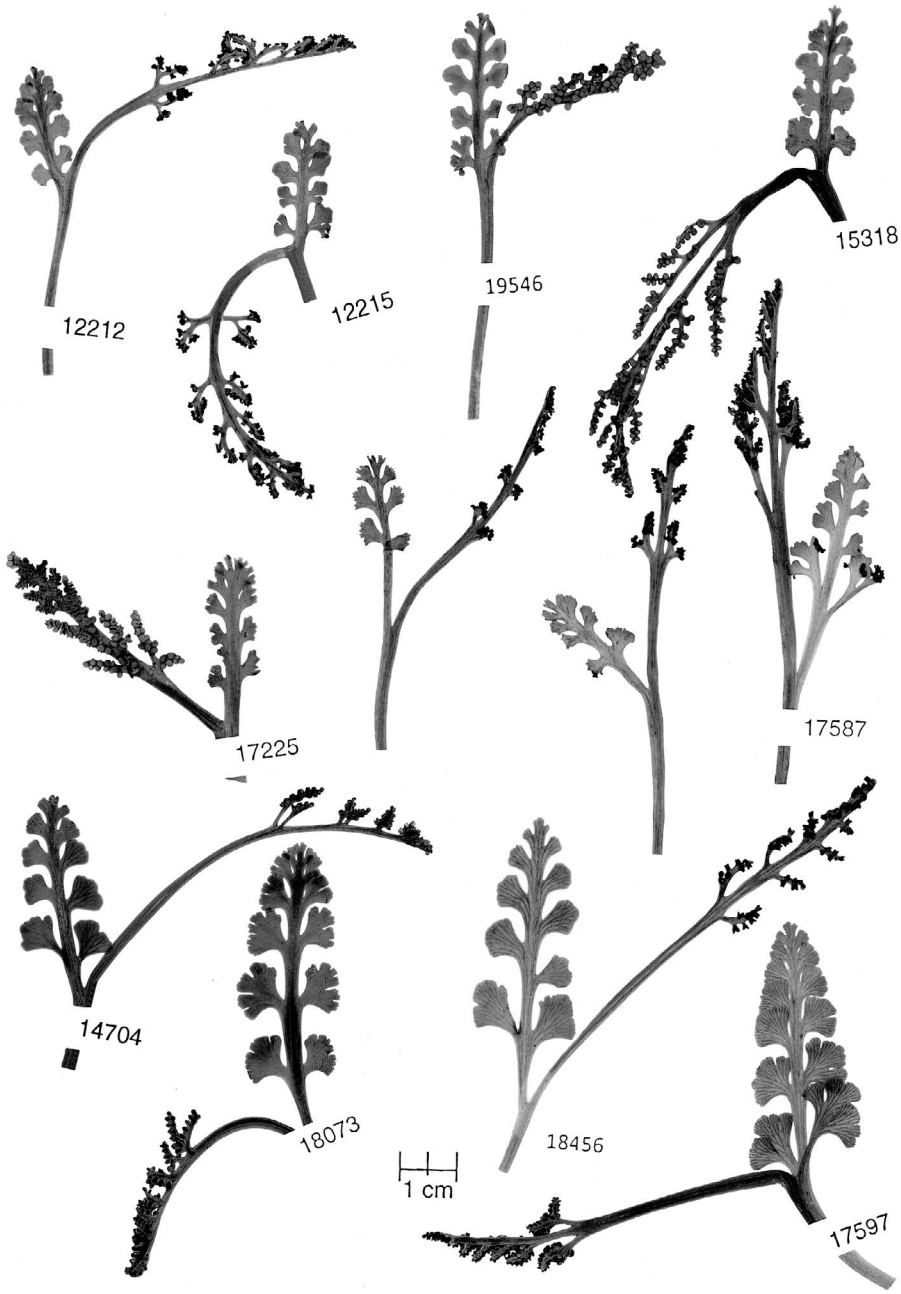

FIG. 6. Comparison of *Botrychium furculatum* to *B. minganense* and *B. pallidum*. Top row: *B. pallidum*: 12212, Elk Island Nat. Park, central Alberta; 12215, Elk Island Nat. Park, central Alberta; 19546, northwestern Minnesota; 15318, Black Hills, South Dakota. Middle row: *B. furculatum*: 17225, Grizzly Gulch, Colorado; (two un-numbered plants) Rocky Mtn. Nat. Park, Colorado (type locality); 17587, Vermejo Park Ranch, northern New Mexico. Bottom row: *B. minganense*: 14704, Rocky Mtn. Nat. Park, Colorado; 18073, Black Hills, South Dakota; 18456, Bighorn Mts., Wyoming; 17597, Vermejo Park Ranch, northern New Mexico.

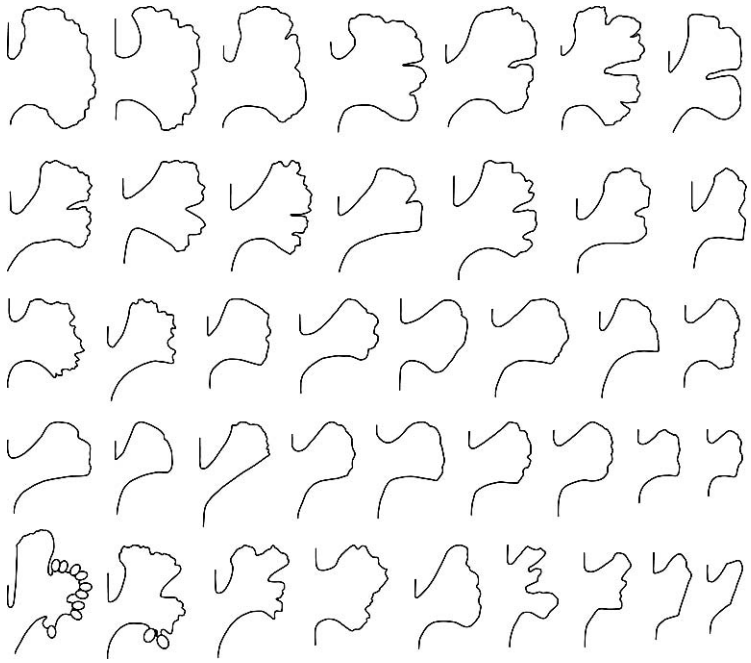

FIG. 7. Outlines of basal pinnae of *B. furculatum* illustrating high variation of lobing, margins, size, and width. Outlines were traced from enlarged, high-resolution images of pressed specimens across the species range exclusive of Canada.

evidenced by the initial identification (Wagner and Wagner 1990) of some Colorado plants as "Rocky Mt. *B. pallidum*." Allozyme evidence also supports parentage by an as yet undescribed diploid, *B.* "farrarii." The adnate pinna attachment of some plants described by Wagner and alluded to in his provisional name "adnatum" may be derived from *B.* "farrarii."

Establishing ploidy level by direct chromosome counts in *Botrychium* is difficult because meiosis, which is the optimum stage of the fern life cycle to observe chromosomes (Manton, 1950), occurs while leaves are emerging from underground and are undetectable or, at best, unidentifiable in the field. Increased spore size and disproportionately high frequencies of heterozygosity provide alternate evidence of allopolyploidy. Although a rigorous comparison of spore size in all *Botrychium* allopolyploids relative to their parent diploids has not been published, a search of published *Botrychium* species descriptions including spore size, supplemented by our own spore measurements, yields an estimate of spore size in 13 allopolyploid species averaging 42.3 μm (39–45), and a 7.1 μm (3.2–11.2) increase in size relative to the average size of their assigned diploid parents (avg. 35.2 μm). The spore size of *B. furculatum,* with an average spore size of 43.0 μm and diploid parent average of 35.2 μm, fits this pattern. Furthermore, in *B. furculatum*, heterozygosity is displayed in most plants at 11 of 22 loci, and no plants were observed without heterozygous loci.

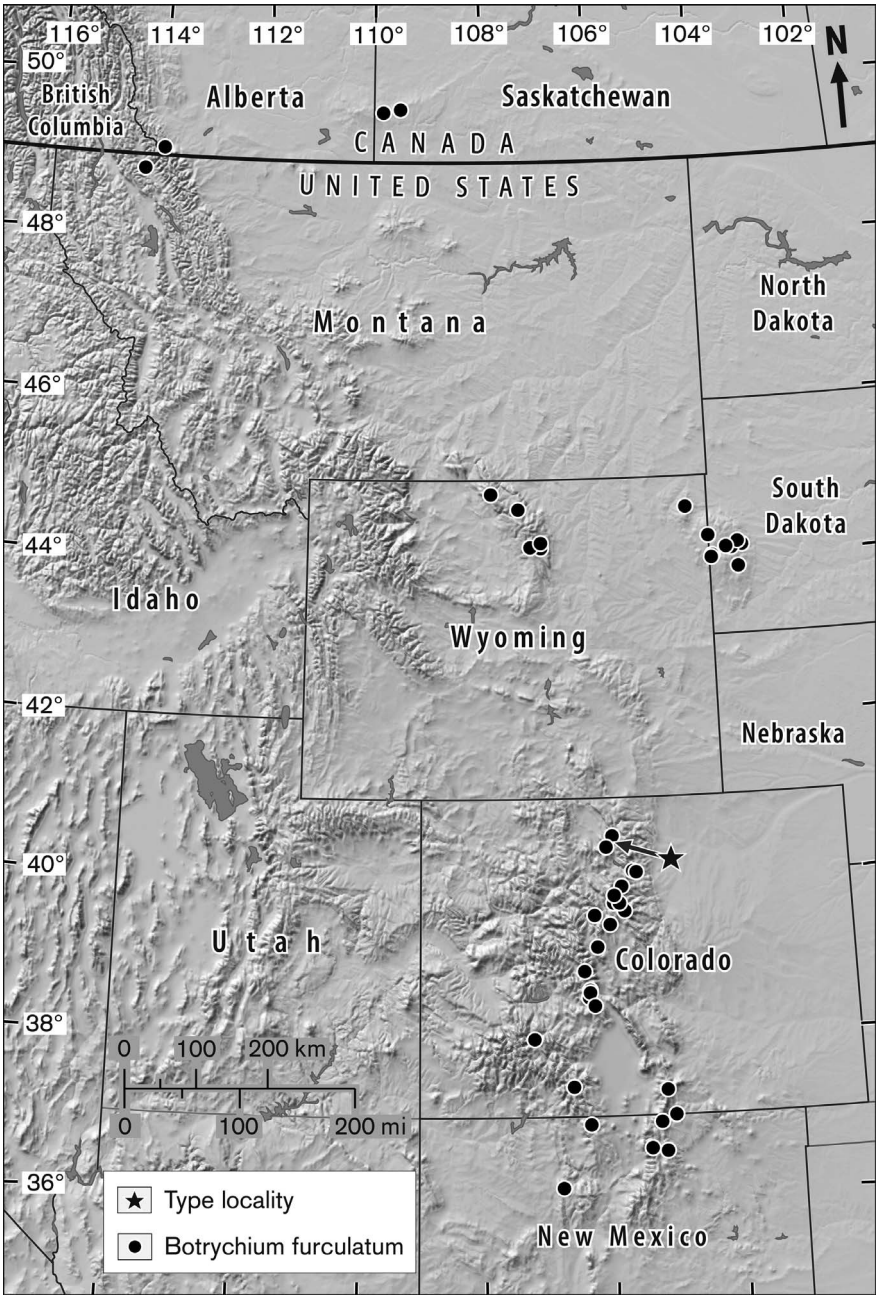

FIG. 8. Range of *Botrychium furculatum*. A dot may represent more than one locality or collection. The star symbol indicates the type locality, offset by arrow.

TABLE 1. Alleles expressed at enzyme-coding loci in *B. furculatum* and its putative ancestral diploid parent species. Allele numbers joined by "+" are alleles contributed by parent taxa expressing different alleles at a given locus. Expression of a single allele in *B. furculatum* (where two are possible) presumably results from failed expression (silencing) of one of its two inherited loci. Allele numbers reflect relative migrating positions within genus *Botrychium*. A "null" listing reflects characteristic non-expression of enzyme activity at a locus for a given taxon.

Locus	***B. pallidum***	***B. furculatum***	***B.* "farrarii"**
	n = 175	n = 269	n = 18
Aat-1	1	1+3 or 3	3
Aat-2	3	3	3
Aat-3	2	1.5+2 or 2	1.5
Aat-4	3	1.5+3 or 3	1.5
Acn-1	1	–	2
Acn-2	2 or 3	3	3
Dia-1	2	2+4 or 4	4
Dia-2	1	1	1
Dia-3	null	3	3
Dia-4	8	7.5+8 or 7.5 or 8	7.5
Idh-1	1	1+4 or 4	4
Mdh-1	1	1	1
Mdh-2	3	3+4.5 or 3	4.5
Mdh-3	2	2	2
Mdh-4	2	–	null
6Pgd	1	1	1
Pgi-2	2	1.6+2 or 2	1.6
Pgm-1	1	1+3 or 3	3
Pgm-2	2	2+4 or 2	2 or 4
Skdh	1	1+3 or 1 or 3	1
Tpi-1	3	3	3
Tpi-2	3	3	3

Co-dominant allele expression at heterozygous loci in allotetraploid species provides evidence of their probable diploid parentage. If at least one allele at each locus is contributed by each parent, a majority of alleles present in the allopolyploid should be present as well in one or the other of the proposed diploid parents. In *B. furculatum*, alleles at 19 of 20 loci expressing activity are present in either *B. pallidum* or *B.* "farrarii" (Table 1). One allele, *Skdh*-3, was not detected in either putative parent although it is present in several diploid *Botrychium* species at a low frequency and may yet be detected in further sampling of *B. pallidum* or *B.* "farrarii." Many of the alleles expressed in *B. pallidum* are common among diploid *Botrychium* and individually do not exclude other species from consideration, but the consistent expression of *B. pallidum* alleles in the 20 expressed loci of *B. furculatum* constitutes strong support for its parentage of *B. furculatum*. Alleles of *B.* "farrarii" are also expressed in each of the 20 visualized loci of *B. furculatum,* and its lack of expression at *Mdh*-4 is also shared with *B. furculatum*. In our analyses of North American *Botrychium*, the alleles *Dia*-4 = 7.5, *Mdh*-2 = 4.5, and *Pgi*-2 = 1.6 have been detected only in *B.* "farrarii" and *B. furculatum*. The presence of these

private alleles in *B. furculatum* strongly supports the hypothesis of its parentage by *B.* "farrarii."

To determine the female (chloroplast donor) parent of *B. furculatum* we included allozyme-determined plants of all *Botrychium* species in a worldwide phylogenetic study (Dauphin *et al.*, 2017) using chloroplast DNA sequencing. Results clustered *B. furcul*atum with *B.* "farrarii" indicating that species to be the female parent of *B. furculatum*.

Current knowledge, including information on morphology, allozymes, nuclear and chloroplast DNA, ploidy, parentage, range, and habitats provides convincing evidence that *B. furculatum* warrants recognition as a new allotetraploid species.

Taxonomic treatment

Botrychium furculatum S. J. Popovich & Farrar, sp. nov.

TYPE: USA. Colorado: Grand County, Rocky Mountain National Park, Kawuneeche Valley, Lulu City townsite, 40°26′48.47″N, -105°50′51.40″W, 2857m, common in herb-graminoid forest opening along Colorado River trail, with congeners *B. echo, B. hesperium, B. lanceolatum,* and *B. minganense*, 17 Aug 2011, *Popovich 8580 & Connor* (holotype: COLO!; isotypes: ALTA!, BRIT!, CAN!, CS!, ID!, ISC!, KHD!, LM!, MICH!, MO!, MONT!, NY!, Rocky Mtn. Nat. Park working herbarium!, RM!, US!, VT!, WTU!). FIGS. 2–4.

Etymology.—Latin *furcula*, little fork, the zoological term for the avian wishbone, referring to the bowed junction of the sporophore and trophophore. A suitable common name is wishbone moonwort.

Diagnosis.—Differs from other members of the *Botrychium* "Campestre clade" in its combination of pallid color, irregular pinna outline, and trophophore bowed out at base.

Description.—Perennial herb, producing a single aboveground leaf annually, this divided dichotomously into a sterile, laminate *trophophore* and a fertile, spore-bearing *sporophore*, dying back following spore release; plant endomycorrhizal.

Underground stem erect, unbranched, bearing 5–18+ fleshy roots up to 1.2 mm wide; spherical gemmae sparingly present or absent, 0.5–1.0 mm in diameter, attached directly to the stem. Depth from soil surface to belowground apical bud 2.3–7.4 cm.

Aboveground leaf erect, fleshy, glabrous to glaucous; color variable, pale green, whitish-green, or yellow-green to extremely pale yellow-white, rarely glaucous blue-green; total leaf height from ground to top of sporophore (1.7) 4–10 (16.4) cm; common stalk (petiole) (0.7) 1.5–6.5 (8.8) cm long, (0.3) 0.5–1.5 (5.0) times the length of the mature sporophore; *trophophore stalk often outwardly bowed, joining sporophore stalk in a spreading to narrow wishbone-like* "junction".

Trophophore (0.6) 1.2–3.6 (5.3) cm long; *stalk (0.1) 0.3–1.4 (2.9) cm long, (0.3) 0.7–2.1 (3.8) times the entire length of the rachis;* blade erect, (0.4) 0.7–2.3

(4.4) cm long, (0.2) 0.5–1.0 (1.7) cm wide at basal pinnae, narrowly ovate-oblong to ovate or linear-oblong in outline, once pinnate, *more or less folded longitudinally and trough-like in life* with apex prow-like and inflexed to 90° toward sporophore.

Pinna pairs (2) 4–5 (7), *proximal 1–2 pairs usually clasping the sporophore stalk at maturity*. Pinnae ascending to spreading, remote to approximate. Lower pinnae usually well-spaced with upper pinnae approximate to overlapping, distance between basal and 2nd pairs (0.6) 1.8–6.5 (11.5) mm, distance between 2nd and 3rd pairs (0.3) 0.5–3.0 (5.8) mm, the former distance (1.0) 1.2–3.0 (5.0) times the latter; basal pinnae usually largest (0.6) 1.8–5.0 (9.0) mm long, (0.5) 1.6–4.8 (7.5) mm wide, subsequent pairs gradually reduced in size and lobing, short-stalked to adnate, sometimes decurrent; pinna margins not overlapping the rachis.

Pinna outline fan-shaped to spatulate, sometimes ± rhombic, often asymmetric, widest at outer margin, *span (24) 70–120° (148)*, 50–70° in small plants; pinna palmately lobed to entire, *lobes often asymmetric and shallow, sometimes cleft ± halfway to pinna base; principal lobes usually two, spreading, with upper lobe usually larger (or longer) and more developed*; sinuses usually rounded, sometimes angular in deeper lobing; *pinna and lobe outer margins irregularly crenate to erose or lacerate,* tending toward ± entire in small plants; pinna side margins slightly to moderately concave, sometimes strongly concave-recurved or more or less straight; junction of outer and side margins usually rounded, occasionally sharp-cornered; midrib lacking, veins dichotomous, 2 or 4 (6) major veins entering the pinna base, (4) 11–31 (45) ending near the margins. *Often some pinnae appearing malformed, unevenly truncate, or terminating abruptly as if cut, or stubby. Stalks of lower pinnae (1/6) 1/3–2/3 (9/10) as wide as the pinnae*. Basal pinnae occasionally bearing sporangia on their margins.

Sporophore erect, sometimes arching away from the trophophore, (1.0) 1.9–7.6 (10.3) cm long, (*1.0*) *1.4–2.6* (*3.4*) *times the length of the trophophore*; stalk (0.2) 0.8–3.9 (6.1) cm long, *(0.2) 0.5–1.3 (1.9) times the entire length of the trophophore*; sporangia-bearing portion branched, narrowly ovate to linear in outline, 1–2-pinnate (2-pinnate usually restricted to lowermost branches); branches in 4–9 (11) pairs (rarely absent in small plants with sessile sporangia), usually short-stalked to sessile, 0.2–11.0 mm long, strongly ascending, *twisted inward in life and appressed to the rachis such that sporangia often partially obscure the rachis*; lowermost branches the longest, *often conspicuously so in large plants*, to 4.6 cm long and 0.8 times the rachis length; proximal branchlets of basal branches often descending; *sporophore often terminating in an unbranched rachis bearing 2–7 (10) sessile sporangial clusters*; sporangia (8) 40–150 (200+), 10–70 in small plants, partially embedded, *not crowded*, dull yellow at spore release, opening in June at low elevations through early August at high elevations; *spores (39) 41–45 (49) avg. 43.02 μm in longest diameter*. Apparently tetraploid.

Habitat.—Open montane to subalpine meadows, forest openings, and high-latitude prairies, especially where exhibiting historic disturbance by natural or

anthropogenic processes, *e.g.* fire scars, snow slides, retired mining districts, roadsides and ski runs; often in older (>20 years) disturbed areas where vegetational succession has proceeded to dominance by perennial herbs and grasses but not to canopy closure by woody plants. Elevation ranges from 1235m in the Cypress Hills of Saskatchewan (*Popovich & Farrar FL160716*, ISC!) to 3609m in New Mexico (*Legler 11522*, RM!).

Distribution.—Populations of *B. furculatum* have been morphologically and genetically confirmed in the central and southern Rocky Mountain region of western North America from Alberta, Canada, south to Montana, Wyoming, Colorado, and New Mexico, USA, with easterly outlying populations in the Cypress Hills of Saskatchewan, Canada, and the Black Hills of Wyoming and South Dakota, USA. Fig. 8.

Wagner and Wagner (1990) listed paratype specimens of *B. pallidum* from Colorado. We examined images of cited collections from Boulder County (*W. H. Wagner 84204, 84205*, MICH-images!) and found them to exhibit the morphologies of *B. furculatum*. We did not locate *Root-89-11* from El Paso County. Our collections from Wagner's paratype areas also display the morphologies as well as genotype patterns of *B. furculatum*. *Botrychium pallidum* reported from Colorado by Heil *et al.* (2013) is *B. campestre* (*Stewart s.n.*, COLO!). Barring further evidence, we suggest that *B. pallidum* be excluded from the Colorado flora.

Conservation.—With more than 65 known localities (45 in Colorado alone), many of them with populations numbering 100's of individuals, we believe that *Botrychium furculatum*, although perhaps locally rare in portions of its range, is secure in viability overall. Fortunately, many populations occur on managed or protected public lands, including Waterton-Glacier International Peace Park, Cypress Hills Interprovincial Park, Glacier National Park, Rocky Mountain National Park, and the following National Forests: Arapaho-Roosevelt, Bighorn, Black Hills, Carson, Gunnison, Pike-San Isabel, Rio Grande, Routt, Santa Fe, and White River.

Additional Specimens Examined.—CANADA. Alberta: Waterton-Glacier International Peace Park, Red Rock Canyon, 23 Jun 2005, *Farrar FL050623* (ISC). Saskatchewan: Cypress Hills Interprovincial Park ("CHIP"), Centre Block, Ben Vannock Dr SW of Visitor Centre, 15 Jul 2016, *Popovich & Farrar FL160715* (ISC); CHIP, West Block, Battle Creek Ranger Station Rd, 16 Jul 2016, *Popovich & Farrar FL160716* (ISC).

UNITED STATES. Colorado: Boulder Co., Roosevelt National Forest ("RNF"), Brainard Lake, 15 Jun 2006, *Popovich 8352–8355* (ISC); 29 Jul 2009, *Popovich & Farrar et al. s.n.* (COLO); RNF, Coney Flats, 11 Aug 1999, *Steinmann s.n.* (KHD); Cty, Grassy Top S of Ward, 15 Jun 2009, *Steinmann 2009-2* (ISC). Chaffee Co., San Isabel National Forest ("SINF"), Boss Lake, 10 Jul 2007, *Popovich 8494c* (ISC); SINF, Monarch Ski Area, talus slope, 27 Jul 2009, *Popovich & Farrar FL090727* (ISC); SINF, North Fork Reservoir, natural bank, 20 Jul 2006, *Popovich & Farrar FL060720* (ISC); SINF, Old Monarch Pass, summer 2007, *Kirkpatrick s.n.-4* (ISC). Clear Creek Co., Arapaho National Forest ("ANF"), Eisenhower Tunnel, 22 Jul 2010, *Popovich s.n.* (ISC); ANF,

Grizzly Gulch, 10 Jul 2009, *Popovich 8551, 8552* (ISC); ANF, Jones Pass, 28 Aug 2008, *Popovich 8527* (ISC). Conejos Co., Rio Grande National Forest, 2 mi W of Stunner Campground, 13 Jul 1991, *Root 91-28* (KHD). Costilla Co., Vermejo Park Ranch ("VPR"), Culebra Range, NE side State Line Peak, 20 Jul 2009, *Legler 11522* (RM); VPR, Culebra Range, head of W Frk Costilla Crk, 24 July 2009, *Popovich & Farrar FL090724* (ISC); VPR, 24 Jul 2009, *Popovich & Farrar FL090724* (ISC). Grand Co., Rocky Mountain National Park, Kawuneeche Valley, Lulu City townsite, meadow in forest opening, type locality, 14 Jul 2012, *Popovich 8587* (COLO, ISC; genetically confirmed topotype); ANF, St. Louis Creek Rd, 5 Aug 2009, *Smith s.n.* (ICS); ANF, Winter Park Resort, Jack Kendrick ski run, 20 Aug 2008, *Popovich 8535*, *8536* (ISC). Gunnison Co., Gunnison National Forest ("GNF"), W side Cottonwood Pass, 15 Jul 2008, *Popovich 8525* (ISC). Hinsdale Co., GNF, W of Slumgullion [Pass] Campground, 19 Aug 1999, *Root 1253* (KHD). Huerfano Co., SINF, Culebra Range, Blue Lakes, 28 Jul 2019, *Popovich & Olson 04* (COLO). Jackson Co., Routt National Forest ("RNF"), Cameron Pass, rest area, 8 Aug 2006, *Popovich 8377–8389* (ISC); RNF, Hi Ho Mine, 15 Sep 2008, *Proctor 80915-1* (ISC); RNF, Medicine Bow, 16 Sep 2008, *Proctor s.n.* (ISC); RNF, switchback on Forest Service Rd 758, 31 Jul 2009, *Popovich & Farrar FL090731* (ISC). Park Co., Pike National Forest ("PNF"), E side Weston Pass, 28 Jul 2009, *Popovich & Farrar FL090728* (ISC); PNF, Guanella Pass, Duck Creek, 5 Aug 2009, *Popovich 8364–8366* (ISC); PNF, above Leavick townsite, 10 Aug 1990, *Root 90-88* (COLO). Pitkin Co., White River National Forest ("WRNF"), Snowmass Ski Area, 29 Aug 2010, *Elliott 1612a* (RM). Saguache Co., GNF, Marshall Pass, 13 Jul 2007, *Popovich 8505a-g* (ISC). Summit Co., PNF, Boreas Pass, Windy Point, 18 Jul 2007, *Popovich & Farrar FL070718* (ISC); WRNF, Vail Pass, old burn scar, 18 Jul 2009, *Popovich & Farrar FL090718* (ISC). Montana: Flathead Co., Glacier National Park ("GNP"), Big Prairie, burned 1988, 26 Jun 1996, *Lesica 7251* (MICH-image!); 19 Jun 1998, *W. H. Wagner s.n.* (ISC); 19 Jun 1998, *Gilman et al. 98038* (VT); GNP, Round Prairie, 13 Jun 2003, *Carolin et al. s.n.* (ISC). New Mexico: Colfax Co., VPR, flat top of volcanic ridge, old logging road, 7 Aug 2008, *Legler 10425* (ISC, RM); 8 Aug 2008, *Legler 10426* (ISC, RM); VPR, Site 582, 23 Jul 2009, *Popovich & Farrar FL090723* (ISC); Colin Neblett State Wildlife Area, 30 Jul 2009, *Legler 11569* (RM). Taos Co., VPR, Costillo Creek, 27 Jun 2008, *Legler 9153* (ISC); Carson National Forest ("CNF"), Wheeler Peak Wilderness, 28 Jul 2009, *Legler 11554* (RM). Rio Arriba Co., Santa Fe National Forest, San Pedro Parks Wilderness, 5 Aug 2009, *Legler 11593* (RM); CNF, Cruces Basin Wilderness, 7 Aug 2009, *Legler 11616* (RM). South Dakota: Custer Co., Black Hills National Forest ("BHNF"), Cathedral Spires, 26 Jun 2011, *Mayer 1393* (ISC); BHNF, Hell Canyon, 5 Jun 2007, *Mergen 07B016A* (ISC). Lawrence Co., BHNF, Eagle Cliff, montane grassland, 19 Jun 2009, *Farrar 090619* (ISC); BHNF, Northern Hills, 24 Jun 2007, *Mergen 070524A* (ISC); BHNF, Riflepit Canyon, grassland, 15 Jun 2010 *Farrar FL100615* (ISC). Pennington Co., BHNF, Hat Mountain, 21 Jun 2011, *Farrar FL110621* (ISC); BHNF, Castle Peak, openings in ponderosa pine forest, 9 Jun 2007, *Mergen 07PC04C* (ISC); BHNF, Redbank Spring, 28 Jun 2006, *Farrar FL060628* (ISC);

BHNF, Reynolds Prairie, open prairie, 6 Jun 2009, *Mergen 09P006A* (ISC); BHNF, N of Moon, 5 Jun 2007, *Mergen 07B008A* (ISC). Wyoming: Big Horn: Bighorn NF ("BNF"), NE side Bald Mtn, 18 Aug 2010, *Legler 11813A* (RM, WTU). Crook Co., BHNF, Warren Peak, montane grassland, 1 Jun 2007, *Farrar FL070601* (ISC). Johnson Co., BNF, Forest Service Rd 31, 19 Aug 2010, *Popovich & Farrar FL100819* (ISC); BNF, Forest Service Rd 448 E of Munkres Pass, 18 Aug 2014, *Legler 13267* (ISC); BNF, Leigh Creek, 8 Aug 2012, *Legler 12473* (ISC); BNF, Pole Creek Rd, under roadside *Pinus contorta* saplings, 17 Aug 2009, *Legler 11631* (ISC); BNF, Powder River Pass, 22 Jul 2008, *Spann s.n.* (ISC). Sheridan Co., BNF, Forest Service Rd 26, 3 air-mi NW of Twin Lakes, 16 Aug 2010, *Legler 11805* (RM).

Supplemental data are available at the Farrar lab at Iowa State University or by contacting the authors, including additional specimens examined but not listed above, measurement data used in deriving the species description, and the geospatial data used for Fig. 8.

With the exception of *B.* "farrarii," an illustrated key that separates the below species from other *Botrychium* occurring within the known distributional range of *B. furculatum* is available (Farrar and Popovich, 2012).

Key to *B. furculatum* and similar species in the Rocky Mountain Region

1. Plants usually green when fresh; pinnae entire to symmetrically shallowly 3- or 5-lobed; stalks of lower pinnae appearing narrow, approximately 1/4 the pinna width; lower sporophore branches distinctly stalked, sporangia not obscuring the sporophore rachis . . . . . *B. minganense*
1. Plants usually pallid, yellow- to white-green to glaucous blue-green when fresh; pinnae entire to crenate to asymmetrically cleft into two principal lobes, upper lobe often larger and more developed; stalks of lower pinnae not appearing narrow, approximately 1/3 or more the pinna width; lower sporophore branches usually short-stalked to sessile, sporangia partially obscuring the rachis 2
2. Plants glaucous blue-green; pinnae dome-shaped in outline, outer margins of pinnae (or if lobed, of pinnae lobes) entire; sporophore and trophophore stalks straight, joining in a straight-sided junction; spore size 31–39 μm (avg. 34) in longest diameter; plants of boreal North America and boreal areas in the Black Hills of South Dakota and Wyoming, not known from the Rocky Mountains . . . . . *B. pallidum*
2. Plants glabrous to glaucous, yellow- to white-green, seldom glaucous blue-green; pinnae fan-shaped to spatulate in outline, sometimes ± rhombic, pinnae sometimes appearing stubby, truncate, or malformed, outer margins of pinnae and pinnae lobes often irregularly toothed or crenate; trophophore stalk often ± outwardly bowed, joining sporophore stalk in a spreading to narrow wishbone-like "V"; spore size 41–45 μm (avg. 43) in longest diameter; plants of the Rocky Mountains and the Black Hills . . . . . *B. furculatum*

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