FIRST REPORT OF UTAH FRAGILE FERN (*CYSTOPTERIS*: CYSTOPTERIDACEAE) FROM NEW MEXICO, AND NEW NOMENCLATURAL COMBINATIONS IN *CYSTOPTERIS TENNESEENSIS*

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ABSTRACT

Two recently analyzed collections of *Cystopteris* from New Mexico represent the first records of Utah fragile fern for the state. Our morphological reexamination of this taxon reveals a single character (the thickness of lateral cell walls in rhizome and petiole base scales) that consistently distinguishes it from tetraploid *Cystopteris tennesseensis*. Therefore, we propose the new combination **C. tennesseensis** subsp. **utahensis** to accommodate these disjunct, tetraploid populations of the southwestern United States. A second new combination, **C. tennesseensis** subsp. **laurentiana**, is provided for morphologically similar hexaploids from the northeastern U.S. and southern Canada.

RESUMEN

Dos recolecciones estudiadas recientemente de *Cystopteris* de Nuevo México representan los primeros registros de este frágil helecho para el estado de Utah. El reexamen morfológico de este taxón revela un solo carácter, grosor de las paredes celulares laterales en las escamas de la base del rizoma y el pecíolo, que lo distingue consistentemente del tetraploide *Cystopteris tennesseensis*. Por lo tanto, proponemos la nueva combinación **C. tennesseensis** subsp. **utahensis** para acomodar estas poblaciones tetraploides y aisladas del suroeste de los Estados Unidos. Se propone una segunda combinación nueva, **C. tennesseensis** subsp. **laurentiana,** para hexaploides morfológicamente similares del noreste de EE. UU. y el sur de Canadá.

The recent *Flora Neomexicana* treatment of the genus *Cystopteris* Bernh. (Allred & Jercinovic 2020) attributed four species to New Mexico: *C. bulbifera* (L.) Bernh., *C. fragilis* (L.) Bernh., *C. reevesiana* Lellinger, and *C. tenuis* (Michx.) Desv. *Cystopteris bulbifera* is the most distinctive, easily separated from the others by its obviously glandular, often attenuate-triangular fertile leaf blades that commonly produce deciduous, asexual propagules (bulblets) distally on abaxial rachis surfaces. The other taxa are very difficult to distinguish from one another due to phenomenal plasticity in rhizome form, blade size, shape, and dissection, pinna orientation, and the presence or absence of minute epidermal projections on the marginal teeth. Because of the difficulties encountered in separating these three taxa, the senior author has opted to treat them as subspecies of *C. fragilis* in the soon-to-be-published *Flora of New Mexico* (Windham 2022).

While sorting through herbarium specimens to document the distributions of *Cystopteris* taxa in New Mexico, we encountered two unusual specimens at odds with this classification. One distinctive feature of these collections was the presence of minute, gland-tipped trichomes on the leaf blades and rachises (Fig. 1A), which would lead to them being identified as *C. bulbifera* in *Flora Neomexicana* (Allred & Jercinovic 2020). However, the glandular trichomes were sparse compared to those of *C. bulbifera* (Fig. 1B) and the leaves lacked the long-attenuate apices commonly observed in the latter. In addition, although these unusual specimens produced infrequent asexual propagules ("bulblets") near some leaf apices, these were malformed with prominent distal scales (Fig. 1C), quite unlike the plump, largely glabrous bulblets diagnostic of *C. bulbifera* (Fig. 1D). Finally, the spores of these collections averaged 40 µm in length (Fig. 1E)—markedly larger than those of documented diploid specimens of *C. bulbifera* from Arizona (averaging 33 µm; Fig. 1F). Considering the strong correlation between spore size and ploidy reported for *Cystopteris* (Haufler & Windham 1991; Haufler et al. 1993), there is little doubt that the unusual collections discussed here are fertile tetraploids.

The morphological features distinguishing these inferred tetraploids from *C. bulbifera* all point to a hybrid origin involving it and a member of the *C. fragilis* complex sensu Rothfels et al. (2013, 2014). In the *Flora of*

J. Bot. Res. Inst. Texas 16(1): 1–7. 2022
https://doi.org/10.17348/jbrit.v16.i1.1215

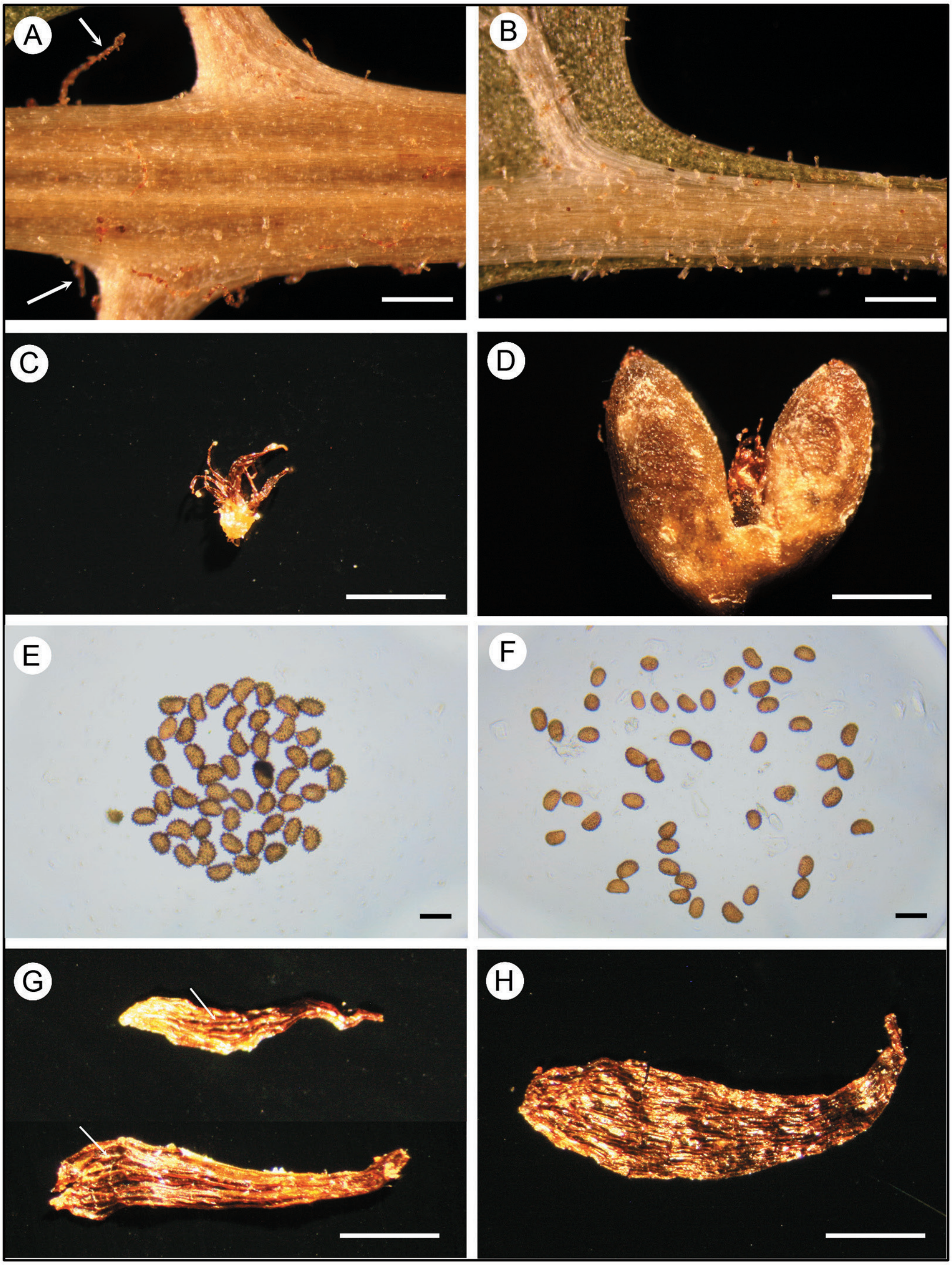

Fig. 1. Morphological comparisons of relevant *Cystopteris* taxa. (A–B) Leaf rachises; scale bars = 0.25 mm; **A** = *C. tennesseensis* subsp. *utahensis* with scattered, minute glands and occasioznal multicellular trichomes (arrows); **B** = *C. bulbifera* with abundant, minute glands. (C–D) Representative leaf bulblets; scale bars = 1 mm; **C** = malformed, distally scaly bulblet of *C. tennesseensis* subsp. *utahensis*; **D** = well-formed, largely glabrous bulblet of *C. bulbifera*. (E–F) Contents of individual 64-spored sporangia; scale bars = 50 µm; **E** = presumed diploid spores of *C. tennesseensis* subsp. *utahensis* averaging 40 µm; **F** = known haploid spores of *C. bulbifera* averaging 33 µm. (G–H) Scales occurring on petiole bases; scale bars = 0.5 mm; **G** = stiff, subclathrate scales with prominently thickened lateral cell walls (arrows) in *C. tennesseensis* subsp. *utahensis*; **H** = papery, non-clathrate scales with less prominent lateral cell walls in *C. tennesseensis* subsp. *tennesseensis*.

North America treatment of *Cystopteris*, Haufler et al. (1993) recognized two allotetraploids with this parentage: *C. tennesseensis* Shaver and *C. utahensis* Windham & Haufler. *Cystopteris tenneseensis*, which is prevalent in east-central U.S., was shown by Haufler et al. (1990) to be a stable allotetraploid with genomes derived from *C. bulbifera* and *C. protrusa* (Weath.) Blasdell (= *C. fragilis* var. *protrusa* in Weatherby 1935). The name *C. utahensis* was applied by Haufler and Windham (1991) to a series of disjunct populations in the southwestern U.S. originally identified as *C.* cf. *tennesseensis* (Windham 1983). Haufler and Windham (1991) reported that this taxon arose through hybridization between *C. bulbifera* and *C. reevesiana* (= *C. fragilis* subsp. *tenuifolia* in Clute 1908; Windham 2022).

Morphological differences between *C. utahensis* and *C. tennesseensis* are few in number and difficult to characterize. The following extended quote from Haufler and Windham (1991: 13, see also their Table 2) summarizes all characters put forward to distinguish them: "… the rhizome scales of *C. utahensis* are dark brown and subclathrate with thick lateral walls whereas scales in *C. tennesseensis* are more uniform in color with tan to light brown lateral walls. In addition, multicellular, gland-tipped trichomes are frequent in the axils of pinnae of *C. utahensis* whereas such trichomes are rare in *C. tennesseensis*. These features may be considered cryptic, but isozyme markers provide clear markers…Further, although frequently considered an inappropriate tool for diagnosing fern species (given the great vagility of their spores), geographic separation of the two tetraploids appears to be absolute. Thus, *C. utahensis* occurs only in the southwestern U.S. and *C. tennesseensis* is confined to the eastern U.S."

Based on geography, the sparsely glandular plants reported here from New Mexico would be expected to represent *C. utahensis*. However, we consider geographic distribution merely as supporting evidence for identifications based on other features. As indicated by Haufler and Windham (1991), the three remaining characters used to distinguish *C. utahensis* from *C. tennesseensis* are all considered "cryptic," with the most diagnostic of these being isozyme markers. Despite their demonstrated value for certain kinds of studies, isozyme data will never play a central role in plant identification due to the cost and limited availability of the technology. Over the last three decades, isozyme analyses have been largely supplanted by DNA studies, and recent molecular phylogenetic research on *Cystopteris* (e g , Rothfels et al. 2013, 2014; Ekrt et al. 2022) offers hope that evolutionary relationships in the genus may one day be better resolved. However, the vast majority of people who need or want to identify plants are unlikely to have access to these technologies, and we therefore advocate a "morphologically-informed" species taxonomy that allows the interested public to recognize the basic units of plant diversity.

The morphological observations of Haufler and Windham (1991) were based on a broad sampling of specimens housed in various US herbaria. Our expanded sampling includes nearly 100 additional specimens from ARIZ, ASU, ASC, BRY, COLO, DES, DUKE, GCNP, KANU, LL, MO, NY, TEX, UNM, UT, UTC (see PteridoPortal 2022; Sernec Data Portal 2022). Our reassessment of collections identified as *C tennesseensis* and *C. utahensis* revealed no additional distinguishing features. Thus, the following discussion addresses only the two morphological characters mentioned by Haufler and Windham (1991). One of these is the relative frequency of multicellular, gland-tipped trichomes in the axils of leaf pinnae, which are described as "frequent" in *C. utahensis* and "rare" in *C. tennesseensis*. Several such trichomes are visible on the leaf rachis illustrated in Fig. 1A. Their occurrence is best characterized as "sporadic", with the frequency of trichomes being highly variable among populations, plants, leaves and even axils of the same leaf. The reported rarity of such multicellular trichomes in *C. tennesseensis*, though generally true, is tempered somewhat by the occasional occurrence of plants in the eastern United States that are indistinguishable from *C. utahensis* in this regard (pers. obs.). This character, though useful in combination with other data, does not consistently separate *C. utahensis* from *C. tennesseensis*.

Since our morphological reassessment of *C. utahensis* and *C. tennesseensis* yielded no new characters to distinguish them, we are forced to depend on the features of the rhizome (and petiole base) scales highlighted by Haufler and Windham (1991). In *C. utahensis*, these are normally stiff and subclathrate, with notably thickened lateral cell walls and prominent luminae (Fig. 1G). By contrast, the rhizome and petiole base scales of *C.*

tennesseensis are papery and non-clathrate, with comparatively thin lateral cell walls and less prominent luminae (Fig. 1H). The scales of *C. tennesseensis* also tend to be broader than those of *C. utahensis* (up to twice as wide on scales of similar length; Figs. 1H vs. 1G), but scale width is highly variable depending on where they are attached to the rhizome or petiole (pers. obs.). For strict comparability between individuals and taxa, we recommend focusing on scales attached to the petioles of fully expanded leaves within 5 mm of the rhizome.

Given that the parentage of the two tetraploid taxa discussed above includes members of the *C. fragilis* complex, their taxonomic treatment depends on how we circumscribe and classify *C. fragilis*. This complex has been called "perhaps the most formidable biosystematic problem in ferns" (Lovis 1978). It encompasses every possible ploidy level between diploid and octoploid, with most of the worldwide range occupied by tetraploids and hexaploids (Hanušová et al. 2019). Diploids are uncommon and there are too few named diploid taxa to account for the documented diversity of polyploids in the group. This has led to the informal naming of a hypothetical diploid (*C. "hemifragilis"*; see Haufler 1985; Ekrt et al. 2022), as well as a bias toward treating known diploids as distinct species with minimal justification (e.g., *C. reevesiana*; Lellinger 1981). Although understandable, given that these diploids often represent the morphological extremes of the complex and play a pivotal role in the origin of the polyploids, this approach ultimately leads to a highly complex species-level taxonomy useable only by experts (Fig. 2).

The taxa discussed in this paper provide a good case in point. Both *Cystopteris tennesseensis* and *C. utahensis* are fertile allotetraploids that originated through hybridization between *C. bulbifera* and diploid members of the *C. fragilis* complex (Haufler et al. 1993). Analyses of select plastid DNA markers indicate that *C. bulbifera* was the maternal parent in both cases (Rothfels et al. 2013). Based on isozyme data, the *C. fragilis* complex parents would appear to be *C. protrusa* and *C reevesiana*, respectively (Haufler & Windham 1991). These two diploid taxa are amply distinct from one another (Haufler et al. 1993), but each is involved in multiple hybridization events that obscure the morphological divergence between them at the tetraploid and hexaploid levels (Ekrt et al. 2022). To further complicate matters, the unique rhizome features that distinguish *C. protrusa* from other *C. fragilis* complex diploids are not apparent in allotetraploids such as *C. tennesseensis* that clearly contain a *protrusa* genome. This, along with potential genetic dominance of the shared maternal genome, undermines our ability to separate *C. utahensis* from *C. tennesseensis*.

The inability to distinguish the various allopolyploid hybrids between *C. bulbifera* × *C. fragilis* s.l. becomes even more acute at the hexaploid level. Isozymes and DNA studies indicate that the taxon currently called *C. laurentiana* (Weath.) Blasdell arose through hybridization between diploid *C. bulbifera* and *C. fragilis* subsp. *fragilis* (Haufler et al. 1993; Ekrt et al. 2022). The latter taxon contributes four of the six genomes present in the allohexaploid and, as a result, the hybrid more closely resembles its *C. fragilis* parent. This taxon, with its mostly ovate-lanceolate leaf blades and dark petioles, is often misidentified as *C. fragilis*. But, in the upper Midwest where *C. laurentiana* overlaps with *C. tennesseensis* s.s., it appears that the only dependable character for distinguishing them is spore size (Haufler et al. 1993). Under our expanded circumscription of *C. fragilis* s.l. (Windham and Pryer, in prep.), these two allopolyploid taxa belong to the same nothospecies, for which the oldest name is *C. tennesseensis*. Within this nothospecies, we recognize each of the three fertile allopolyploid taxa as subspecies to highlight their unique origins and maintain the availability of names for more detailed analyses (which the non-specialist can choose to bypass but still get the species name right). For example, *C. tennesseensis* subsp. *utahensis* is an allotetraploid hybrid between *C. bulbifera* and *C. fragilis* subsp. *tenuifolia* (an older name for *C. reevesiana*) and *C. tennesseensis* subsp. *laurentiana* is an allohexaploid hybrid between *C. bulbifera* and *C. fragilis* subsp. *fragilis*. Under the taxonomy presented here, they both fall within the circumscription of *C. tennesseensis* s.l., thus reducing dependence on cryptic characters (i.e., spore size) and geographically-based assumptions to identify something as central to biological discourse as a species.

To balance the interests of diverse taxonomic stakeholders, we propose recognizing the genetically distinct but morphologically ill-defined entities of the *Cystopteris fragilis* complex as subspecies rather than species. In addition to maintaining the plenitude of names needed by evolutionary biologists, this

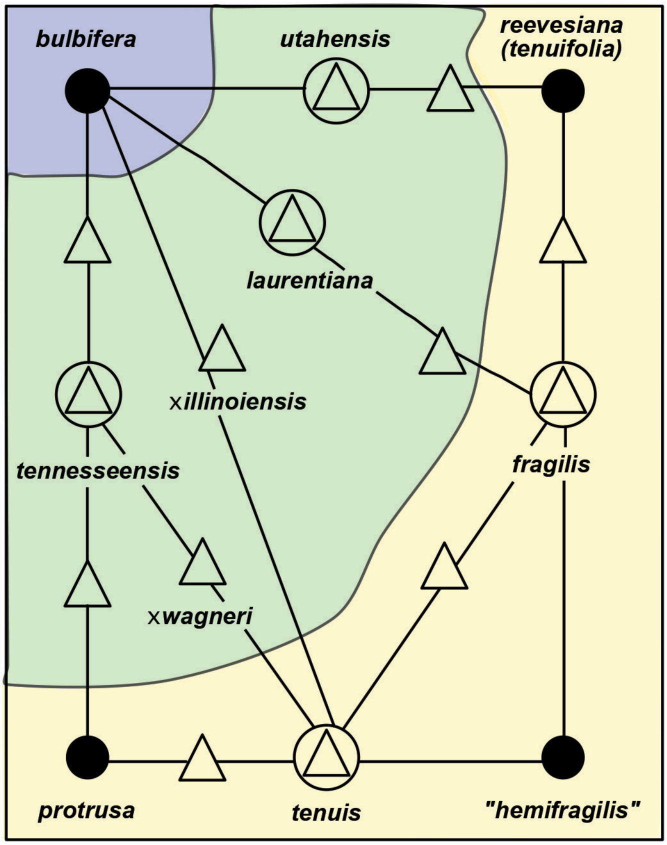

Fig. 2. Hypothesized reticulate relationships among North American *Cystopteris* taxa (figure modified from Haulfer et al. 1993). Solid circles represent parental diploid taxa, triangles represent sterile hybrids, and circled triangles represent fertile allopolyploids. *Cystopteris "hemifragilis"* is a hypothetical diploid progenitor. Blue shading highlights the diploid taxon *C. bulbifera* that is sister to the *C. fragilis* complex s.l. (yellow shading) in all recent molecular phylogenetic analyses (Rothfels et al. 2013, 2014; Erkt et al. 2022). Green shading highlights hybrid taxa between *C. bulbifera* and members of the *C. fragilis* s.l. clade, for which the oldest species name is *C. tennesseensis.*

compromise also permits the description of new taxa whose distinguishing features would be considered insufficient for species recognition. At the same time, this broader view of species focuses attention on the more clearly differentiated levels of the taxonomic hierarchy (the three colored regions of Fig. 2), facilitating species identification by non-specialists. The necessary nomenclatural adjustments for the core *C. fragilis* clade will be presented in an upcoming paper (Windham and Pryer, in prep.). Here, we provide new combinations for the three documented allopolyploids that originated through hybridization between *C. bulbifera* and members of *C. fragilis* s.l.

Cystopteris tennesseensis Shaver subsp. **laurentiana** (Weath.) Windham, **comb. et stat. nov.** Basionym: *C. fragilis* (L.) Bernh. var. *laurentiana* Weath., Rhodora 28:129–130; *C. laurentiana* (Weath.) Blasdell, Mem. Torrey Bot. Club 21:51. 1963. Type: CANADA. Quebec: Rimouski Co.: Bic, headland N of Baptiste Michaud's, limestone-conglomerate cliffs, 18 Jul 1904, *Fernald & Collins s.n.* (holotype: GH!; isotype: GH). $2n=6x=252$ [Haufler et al. 1993].

Cystopteris tennesseensis Shaver subsp. **tennesseensis**, J. Tennessee Acad. Sci. 25:107. 1950. *C. fragilis* (L.) Bernh. var. *tennesseensis* (Shaver) McGregor, Amer. Fern J. 40:202. 1950. Type: U.S.A. Tennessee. Smith Co.: Round Lick Creek at Rome, near the Cumberland River, bluff below quarry, 10 Jun 1944, *Shaver 7885* (holotype: PH; isotypes: F, GH, MO, NY, TENN, US!). $2n=4x=168$ [Haufler et al. 1993].

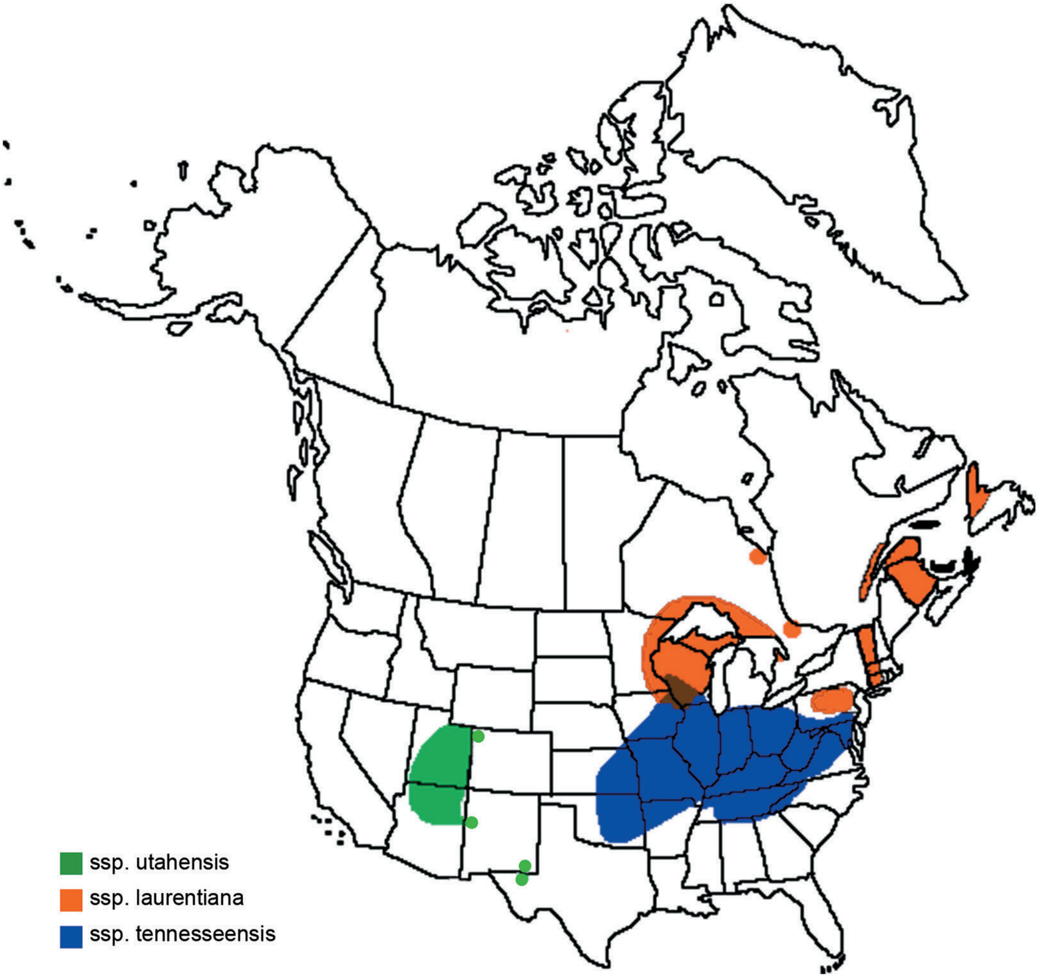

Fig. 3. Geographic distributions of the three subspecies of *Cystopteris tennesseensis*. Map modified from Haufler et al. (1993).

Cystopteris fragilis (L.) Bernh. forma **simulans** Weath. Rhodora 37:376. 1935. *C. fragilis* (L.) Bernh. var. *simulans* (Weath.) McGregor, Amer. Fern J. 40:204. 1950. Type: U.S.A. Missouri: La Grange, collected in woods, 14 Sep 1911, *John Davis s.n.*? (holotype: GH)

Cystopteris tennesseensis Shaver subsp. **utahensis** (Windham & Haufler) Windham, **comb. et stat. nov.** Basionym: *Cystopteris utahensis* Windham & Haufler, Amer. Fern J. 81:13–15. 1981. Type. U.S.A. Utah. Grand Co.: base of Morning Glory Arch in tributary of Negro Bill Canyon 3.93 km SE of its confluence with the Colorado River, 4300 ft, 2 Jul 1990, *Windham (90-282) & Windham* (holotype: UT!; isotypes: ASU!, BRY!, KANU!, MO!, UC!, US!, UTC!) $2n=4x=168$ [Haufler et al. 1993].

Voucher specimens for **NEW MEXICO. Cibola Co.:** NNE of Ramah along Cebolla Creek in the Zuni Mountains ca. 1.88 km WSW of Dan Valley Spring, T11N, R15W, Sec. 5. Lat.: 35°12′51″N, Long.: 108°26′32″W (WGS84 Datum), elev. 7575 ft, cracks and crevices of E-facing sandstone outcrop with *Pinus*, *Juniperus*, *Quercus* & *Pseudotsuga*. 04 Sep 2003, *M.D. Windham 2813* (UT 123563!). Eddy Co.: Guadalupe Mts., Middle Fork of Big Canyon, upper part near 32°02.543N 104°47.063W (T26S, R21E, S15), 6850 ft, mesic wooded limestone canyon bottom, shaded, 20 Oct 2007, *Worthington 35040* (DUKE 397790!).

The largely allopatric distributions of the three subspecies of *C. tennesseensis* are outlined in Fig. 3, and the following key (modified from the *Flora of North America* treatment by Haufler et al. 1993) summarizes the features used to distinguish them.

1. Leaf blades ovate to lanceolate, usually widest above the base; petioles often dark and shiny for much of their length; spores 49–60 µm; ne North America ______ subsp. **laurentiana**
1. Leaf blades deltate to narrowly deltate, usually widest at or near the base; petioles not dark and shiny or, if so, only near the base; spores 38–48 µm; e central to sw North America.
   2. Rhizome scales papery, the lateral cell walls thin and the luminae not prominent; leaves rarely with multicellular, gland-tipped hairs in axils of pinnae; e central United States ______ subsp. **tennesseensis**
   2. Rhizome scales sclerotic, the lateral cell walls thick and the luminae prominent; leaves often with multicellular, gland-tipped hairs in axils of pinnae; sw United States ______ subsp. **utahensis**

ACKNOWLEDGMENTS

We thank Richard Worthington (UTEP) for the many interesting collections he has provided to the senior author over the years, including the Eddy Co., NM specimen of *C. tennesseensis* subsp. *utahensis*. We also express our gratitude to Derick Poindexter, whose help with photographic documentation has been invaluable. We are indebted to an anonymous reviewer for suggested improvements to the manuscript, to Daniel Brunton for his valuable criticisms and for providing a distribution map that we modified to produce Figure 3, and to the editor for carefully guiding our revisions. We are grateful to Sandra Knapp at the Natural History Museum (London) for confirming that our interpretation of the 2018 ICN Shenzhen Code (especially regarding Article H.5.2) was appropriate in making the nomenclatural changes we propose here.

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