

×*Dryostichum singulare* (Dryopteridaceae), a new fern nothogenus from Ontario

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A previously unknown fern, here named ×*Dryostichum singulare* nothogen. et nothosp.nov. W. H. Wagner, has been discovered in several localities in the Georgian Bay region of Ontario, Canada. The plants are found in shaded northern hardwood forests on dolomitic outcrops. First thought to be a bizarre mutation, evidence is presented here that it is a recurring natural hybrid between *Dryopteris goldiana* and *Polystichum lonchitis*. Chromosome and spore studies indicate that the hybrid is sterile and has abortive spores; the chromosome number is $2n = 82$ with no pairing at meiotic metaphase.

Key words: fern, ×*Dryostichum*, *Dryopteris*, *Polystichum*, hybrid, Ontario.

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Les auteurs ont découvert dans plusieurs localités de la région de la baie Géorgienne en Ontario au Canada, une fougère inconnue jusqu'ici qu'ils nomment ×*Dryostichum singulare*, nothogen. et nothosp.nov. W. H. Wagner. Ces plantes se retrouvent sur des effleurements dolomitiques dans des forêts nordiques ombragées d'espèces feuillues. Croyant d'abord qu'il s'agissait d'une curieuse mutation, les auteurs présentent la preuve qu'il s'agit d'un hybride naturel récurrent entre le *Dryopteris goldiana* et le *Polystichum lonchitis*. L'examen des chromosomes et des spores indique que l'hybride est stérile et que ses spores sont avortées; le nombre de chromosomes est de $2n = 82$ et il n'y a pas de jumelage à la métaphase de la méiose.

Mots clés : fougère, ×*Dryostichum*, *Dryopteris*, *Polystichum*, hybride, Ontario.

[Traduit par la rédaction]

Introduction

Two of the best known and most studied fern genera are the wood ferns, *Dryopteris*, and the holly ferns, *Polystichum*, each subcosmopolitan and comprising around 200 species. They are most common in the north temperate regions and have been studied extensively in Eurasia and North America. They are noted for reticulation among their species. In North American *Dryopteris* there are 10 orthospecies plus 30 putative or proven nothospecies, and in *Polystichum* 10 orthospecies plus 10 nothospecies. Two past reports of hybrids between these two genera have been questionable. Greene (1913) described what he called "×*Polystichum acrostichoides* × *Dryopteris cristata*" from Great Falls, Virginia, U.S.A., but the specimen (US) is merely a form of *Polystichum acrostichoides*. Domin (1942) published a presumed hybrid of *Dryopteris filix-mas* and *Polystichum lobatum* from Strasov, Slovakia, eastern Czechoslovakia, which he named *Dryopolystichum mirabile*. This name was not validly published and efforts to find Domin's specimens have failed (G. Vida, personal communication). What is described below is apparently a true intergeneric hybrid of *Dryopteris* and *Polystichum*. It has been found several times in the Georgian Bay region of Ontario, where it grows in association with *Polystichum lonchitis* and five species of *Dryopteris*.

The first specimen of the hybrid was discovered near Devil's Glen Provincial Park, Simcoe County, Ontario in 1973 by Heather Mackey. It was an overwintered frond, somewhat damaged (Fig. 9, S₄), and was sent in 1981 by Robert C. Simpson to W. H. Wagner, who mistakenly identified it as *Polystichum acrostichoides* f. *multifidum* Clute, a

bipinnate form of the common Christmas fern (Fig. 10, M). Later, however, Simpson sent a well-developed summer frond. Simpson had suggested that it was possibly an ecological or pathological morph, or a hybrid, of *P. lonchitis* and *Dryopteris filix-mas* (R. C. Simpson, personal communication).

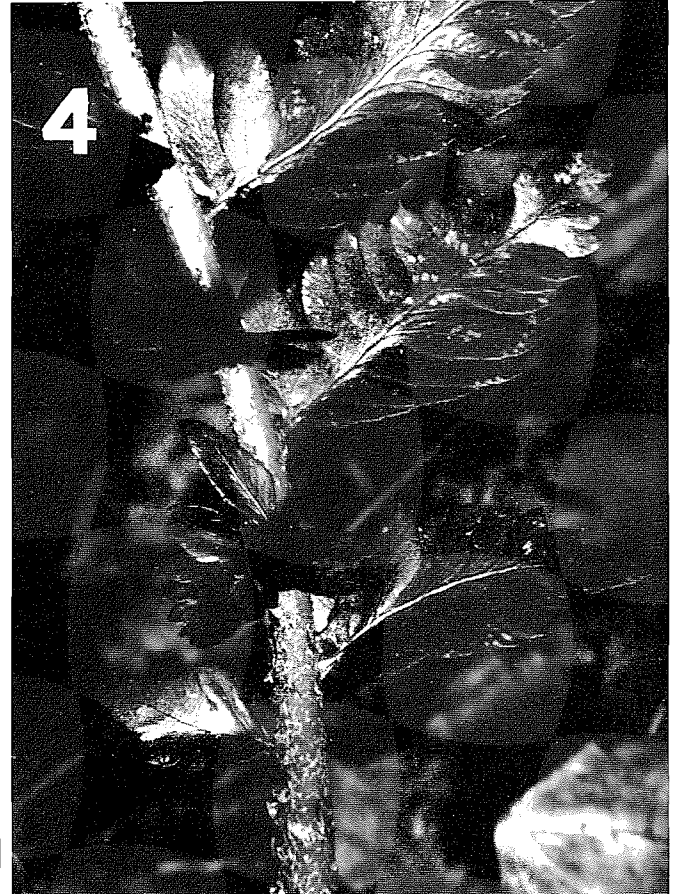
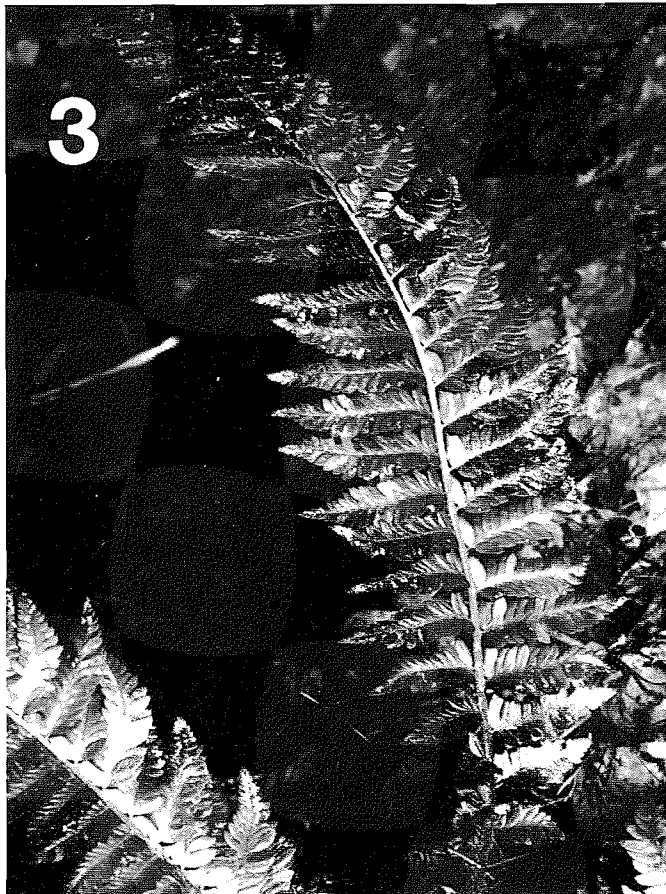
Materials and methods

We revisited the area a number of times between 1981 and 1991 to study the habitats, to search for additional examples of the hybrid, and obtain comparative materials for various purposes. The field work involved three main foci: (i) determination of what species coexist with the hybrid as possible parents; (ii) observations on associated flora to characterize the habitat; and (iii) locating other sites to find new hybrids where similar associations occur. Herbarium investigations (mainly at MICH) were made of all North American relatives, both in *Dryopteris* and *Polystichum*. Detailed comparisons were made of the hybrids and their potential parents, using dried herbarium specimens collected in Ontario, supplemented by modern descriptions at the generic level given in Kramer (1990) and at the species level in Cody and Britton (1989). Venation patterns (Fig. 10) were examined in pinnae cleared in 5% aqueous sodium hydroxide for 24 h and traced using a microprojector. To examine meiotic chromosomes (Fig. 11), proper stages were collected in the field and fixed in Newcomer's solution; the procedure for making squashes described by Wagner (1987). For comparisons of isozymes (Table 2), the methods and interpretations follow those outlined by Werth (1991).

Results

The results of this research are given below in the following order: description of the new taxon, its ecology, and comparisons with the parents morphologically, cytologically, and enzymatically.

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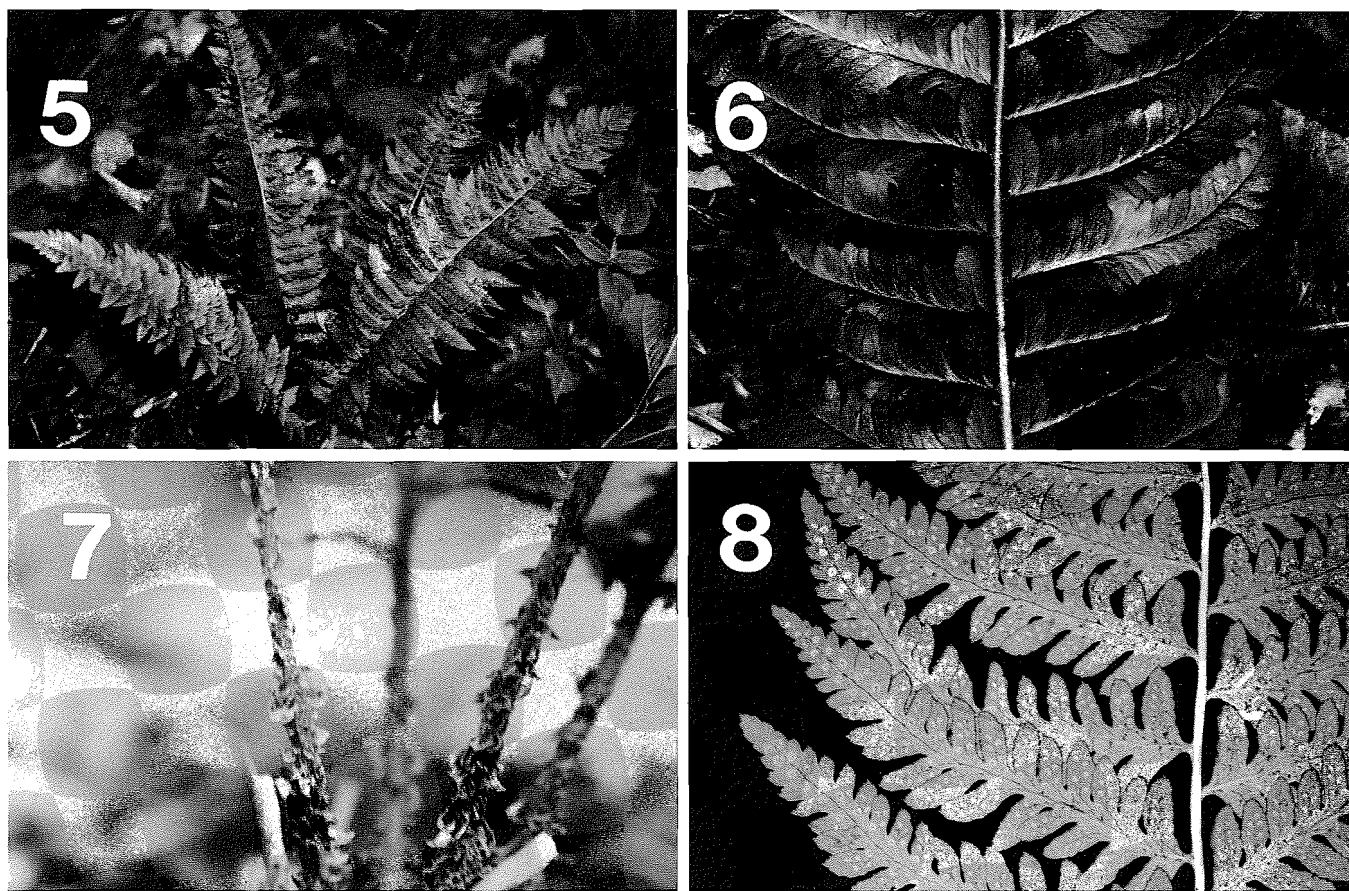


FIG. 5. Frond orientation of living plant of *×Dryostichum*. (Same locality as Fig. 1.) FIG. 6. Pinnae of living frond showing orientation and subfalcate outline (Nottawasaga Twp.). FIG. 7. Scales at stipe bases of living *×Dryostichum* plants showing black centers, pale margins, truncate tips, and orientation. (Same as Fig. 5.) FIG. 8. Dried pinnae of *×Dryostichum* showing submedial sori and petiolules. (Same as Fig. 5.)

×Dryostichum singulare W. H. Wagner, nothogen. et nothosp. nov.

Figs. 5, 9

Hybrida inter *Dryopteris goldiana* et *Polystichum lonchitidis* plus minusve intermedia. Frondes semideciduae, 30 – 48 cm longae petiolis 5 – 11 cm longis inclusis. Squamae praecipue nigrae, forma variabiles, 1.0 – 6.3 × 1.5 – 3.3 mm, Pinnae aliquantum falcatae, usque ad 6.0 × 2.0 cm, in $\frac{2}{3}$ proximalibus lobatae vel pinnatae, oblique lanceolatae vel oblongo-lanceolatae. Stipites pinnarum 1.5 – 3.0 cm longi. Lamina subcoriacea. Margines paulo crenulatae praeter spinas apicibus pinnularum loborumque usque ad 0.3 mm longas. Sori in medio pinnularum vel affines, in pinnulis maximis 6 – 10. Indusia praecipue reniformia, interdum peltata vel intermedia. Spora abortivae. Chromosomatum numerus $2n = 82$.

Rhizome short-creeping, the shoot apex, petioles, and lower rachises covered with numerous blackish, pale margined, more or less convex pointed to truncate scales 1.0 – 6.3 × 1.5 – 3.3 mm (Fig. 7). Leaves half-evergreen, lying on the ground in winter and early spring (Fig. 9, S₄). Vascular strands in petiole base 5. Petioles 8(5 – 11) cm. Blades linear-lanceolate, 30(25 – 40) × 10(8 – 12) cm, tapering gradually

to tip, abruptly reduced basally, the basal 2 – 4 pairs of pinnae $\frac{1}{2}$ to $\frac{1}{3}$ the length of the medial pinnae (Figs. 7, 9, S). Pinnae falcate, the largest medial pinnae 5.3(4.5 – 6.0) × 1.6(1.4 – 2.0) cm, lobed in the upper third, pinnatifid to pinnate in lower two-thirds (Figs. 3, 10, S). Pinnules obliquely lanceolate to oblong-lanceolate. Basal pinnules nearly equal in size, the lower ones only slightly shorter on average than the upper. Pinna stalks 2.1(1.5 – 3.0) mm long (Fig. 8). Lamina texture subcoriaceous, color medium green, darker above and paler below, luster shiny, white spotted in summer and fall (Figs. 2, 3). Margins nearly smooth except for short spines 0.1 – 0.3 mm long on pinnule and lobe tips. Vein branch tips from costa to margin in the pinnules of lower half of medial pinnae 15 – 30 (Fig. 10, S). Sorus number in the largest pinnules 6 – 10. Sorus position medial to slightly submedial (Figs. 8, 10, S). Indusial shapes variable, mainly reniform with open sinuses or with sinus margins touching to overlapping, a few symmetrically or asymmetrically peltate. Sporangia commonly poorly developed and rudimentary. Spores abortive, highly irregular in size and shape. Chromosomes 82 singles at meiotic metaphase (Fig. 11).

FIG. 1. Typical rich dolomitic forest habitat for *×Dryostichum singulare*, Nottawasaga Twp., Simcoe Co., Ontario. FIG. 2. Site of natural hybridization. g, plant of *Dryopteris goldiana*; l, *Polystichum lonchitis*; s, hybrid. (Locality same as Fig. 1.) FIG. 3. Blade of living *×Dryostichum* showing white laminar flecks like those of *D. goldiana*. (Locality same as Fig. 1.) FIG. 4. Reduced basal pinnae of living *×Dryostichum*. Note white laminar flecks. (Same as Fig. 1.)

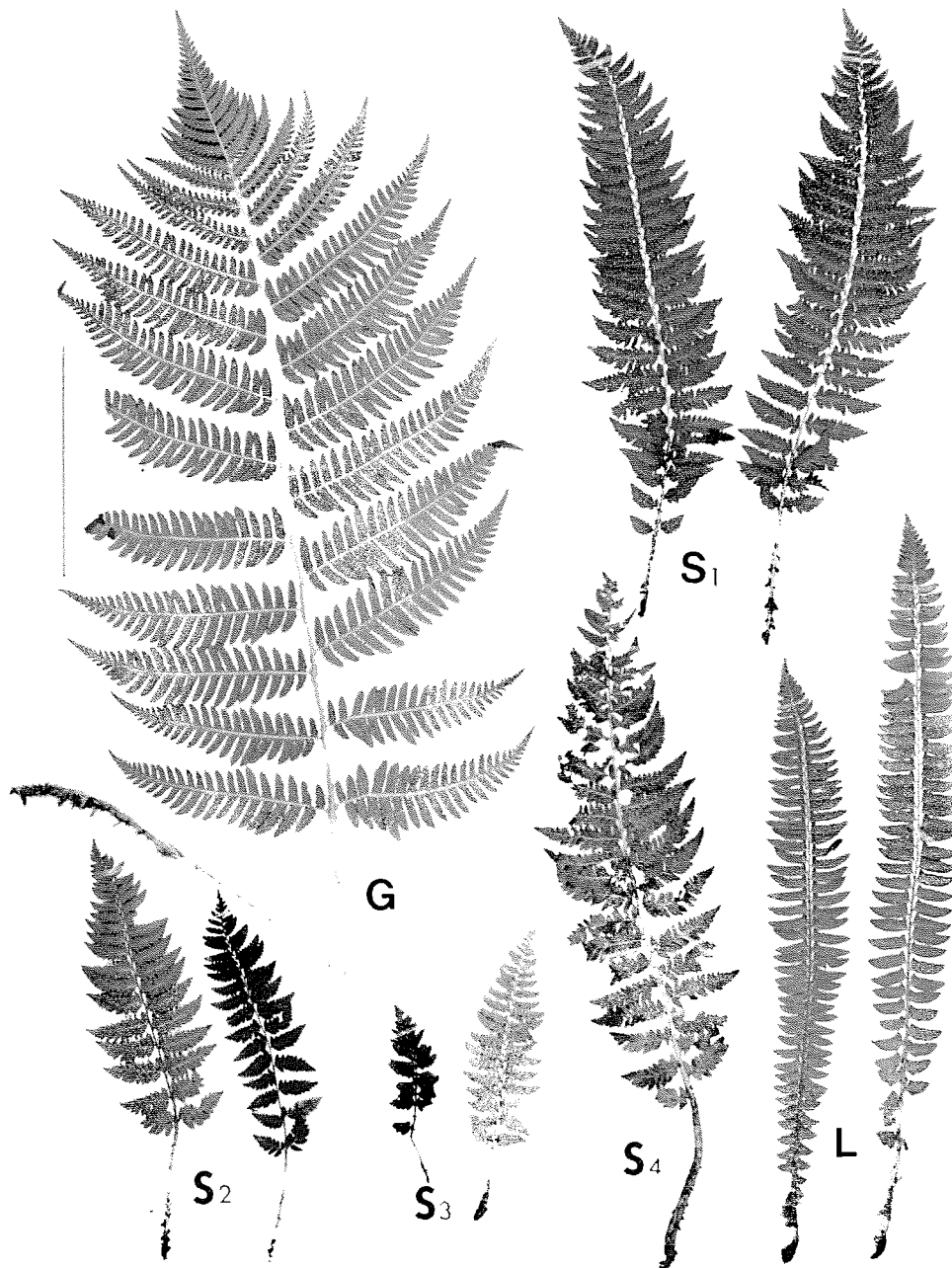


FIG. 9. Comparison of whole dried fronds (all numbers W. H. Wagner, Jr., deposited in MICH): G, *Dryopteris goldiana*, 81038b; L, *Polystichum lonchitis*, 81039; S, \times *Dryostichum singulare*: S₁, large plant, type collection, Ontario, Simcoe Co., 21 Aug. 1990, 90030a; S₂, small plant, Grey Co., 21 Aug. 1990, 90034a; S₃, small plant, note dark brown previous year's leaf, Simcoe Co., 17 June 1983, 83213; S₄, type locality, frond taken 22 April 1981 showing only partial evergreen state, the color mainly brown and blotched, 81024. Scale bar = 15 cm.

HOLOTYPE: CANADA: ONTARIO: Simcoe Co., Nottawasaga Twp., east of Singhampton, 21 August 1990, Wagner 90030 (MICH) (large plant) (Fig. 9, S₁).

OTHER SPECIMENS EXAMINED: TYPE LOCALITY: Large plant: H. Mackey in 1973 (MICH, Herb. D. F. Brunton); 24 April 1981, Wagner 81024 (MICH) (Fig. 9, S₄); 17 June 1983, Wagner 83213 (MICH); 24 May 1985, Reznicek 7479 (MICH); 7 November 1987, Reznicek 8059 (MICH). Small plant: (Fig. 9, S₃) 13 June 1986, Wagner (cytological voucher specimen, MICH). Simcoe Co., Nottawasaga Twp., west of Stayner, 17 November 1987, Reznicek 8060 (MICH) one large plant. Ontario, Grey Co., Collingwood Twp., east of Gibraltar,

21 August 1990, Wagner 90034 (MICH) two small plants (Fig. 9, S₂).

A total of five plants at three sites are now known of this hybrid. All of the habitats so far known are in cool shaded disturbed rich hardwood forest (Fig. 1) among dolomitic limestone boulders at the sloping bases of escarpment cliffs (Fig. 2). The vegetation is luxuriant. A number of the fern species here grow on rocks and in mossy rock crevices; some are confined to more or less mucky seeps. The following are composite lists of species associated with \times *Dryostichum singulare* in three localities. The dominant trees and shrubs are *Acer saccharum*, *Tilia americana*, and *Acer spicatum*. Other species include

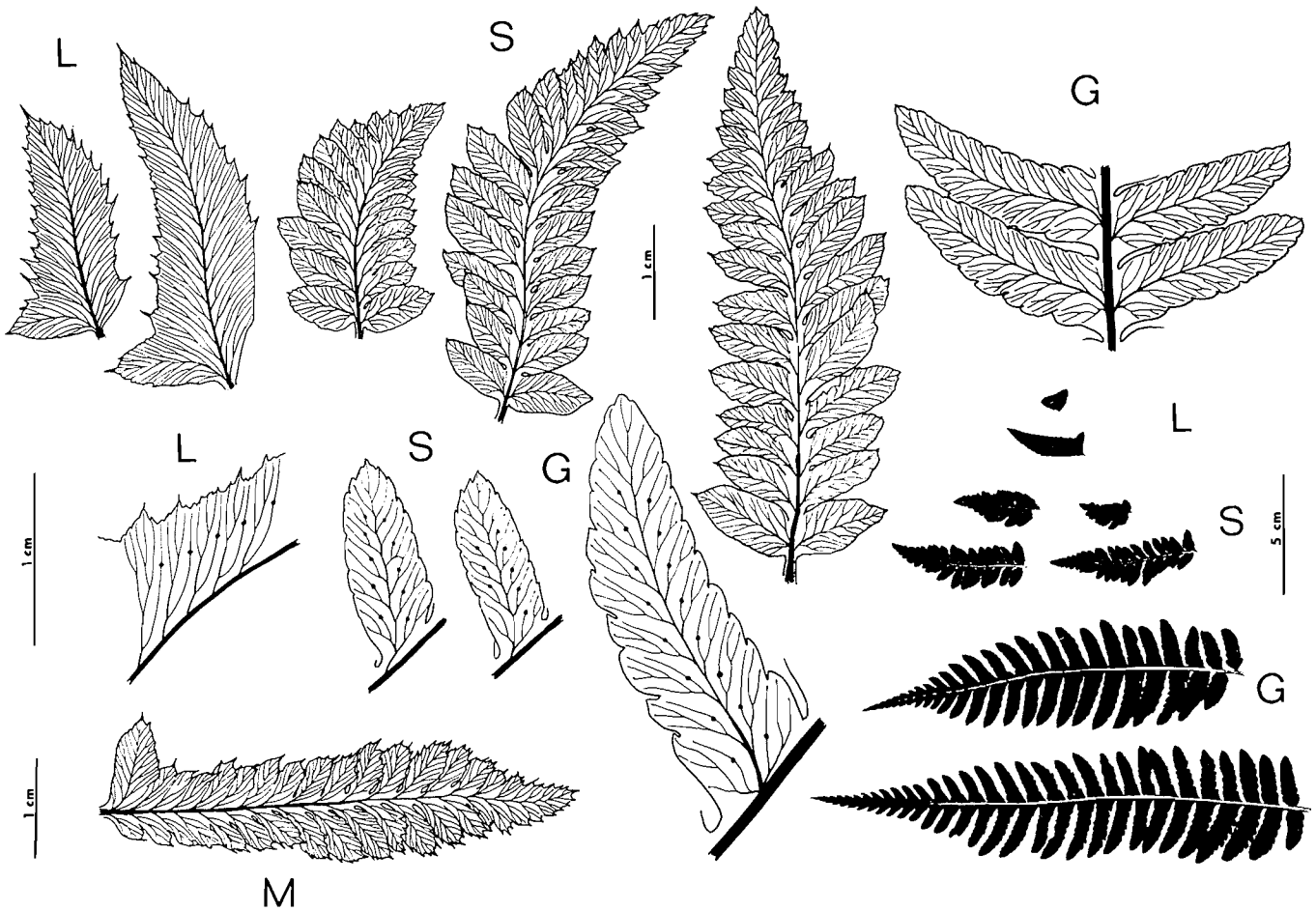


FIG. 10. Silhouettes and vein patterns of pinnae; parents and nothospecies from Simcoe Co., Ontario shown at different scales. L, *Polystichum lonchitis*; G, *Dryopteris goldiana*; S, \times *Dryostichum singulare*; M, *Polystichum acrostichoides* f. *multifidum*.

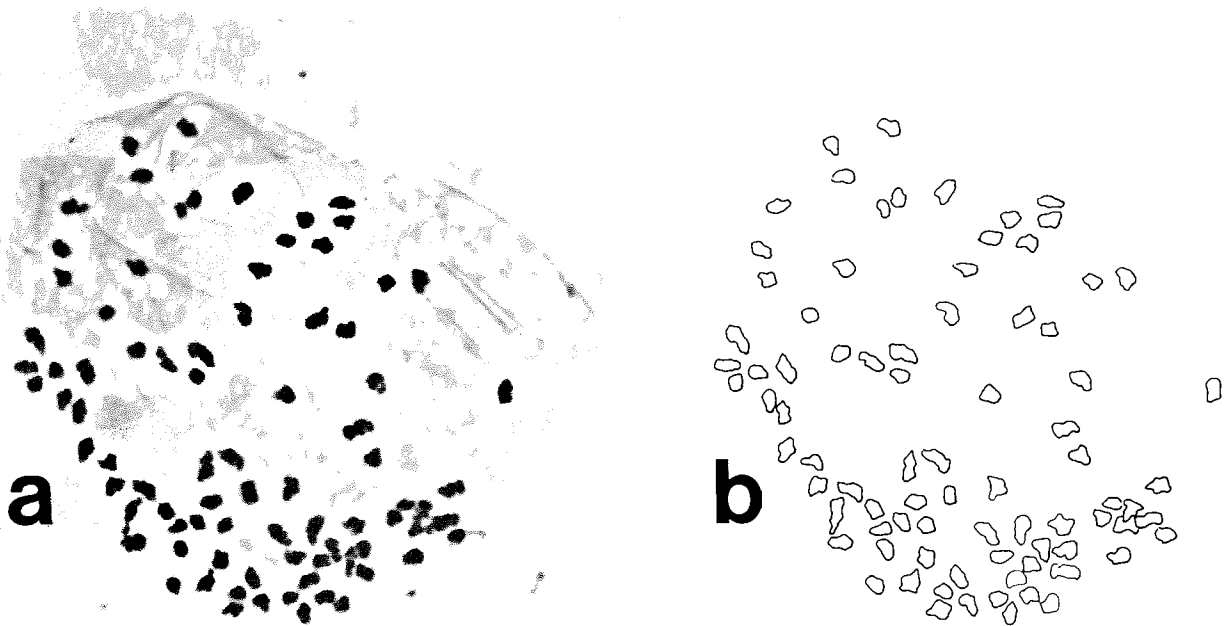


FIG. 11. Meiotic metaphase chromosomes of \times *Dryostichum singulare* showing no pairing of 82 chromosomes (voucher, Wagner on 13 June 1986). (a) Squash of a single spore mother cell. (b) Interpretation.

TABLE 1. Comparison of three associated dryopteroid ferns

	<i>Polystichum lonchitis</i>	<i>×Dryostichum singulare</i>	<i>Dryopteris goldiana</i>
Substrate	Mainly boulders	On ground and boulders	Mainly on ground
Fronds and (or) shoot apex			
Number in summer	10 (8–14)	3–5	1–3
Duration	Evergreen	Semideciduous	Deciduous
Orientation	Spreading	Suberect	Suberect
Length (cm)	43 (30–60)	40 (30–48)	88 (60–110)
Petioles			
Base duration—spring	Alive	Dead	Dead
Length (cm)	6 (2–11)	8 (5–11)	38 (30–52)
Ratio of petiole to frond (%)	13.3	20	42.2
Scales (stipe bases)			
Color	Brown, black center stripe	Mainly black	Mainly black
Shape	Lanceolate to ovate	Ovate, often truncate, irregular	Linear, gradually narrowing
Contour	Nearly plane	Commonly ruffled or bullate, concave	Nearly plane
Size (mm)	11.3(6–15) × 4.6(3–7)	6.3(4–9) × 3.3(3–4)	16(13–20) × 3.5(3–6)
On blade axes	Numerous semipersistent small scales	Numerous semipersistent small scales	Sparse, early abscising, narrow fibrils
Blade			
White laminal spots (Aug.–Sept.)	Absent	Present	Present
Cutting	1-pinnate	Pinnate-pinnatifid	Pinnate-pinnatisect
Shape	Linear	Lanceolate	Broadly ovate
Length (cm)	37.4 (30–50)	31.8 (25–38)	49.6 (19.8–59)
Width (cm)	5.4 (3.5–7.1)	9.9 (8–12)	33.5 (25.5–39)
W:L ratio (%)	14.4	31	67.5
Basal reduction	Very gradual	Rapid (2–4 reduced pinna pairs)	Abrupt (no dwindling pinnae)
Pinnae			
Number	44 (36–50)	28 (24–32)	11.3 (9–14)
Bottom pinna			
Length (cm)	1.1 (0.8–1.6)	2.2 (1–3)	15.3 (13–18.5)
Curvature	Strongly falcate	Somewhat falcate	Straight
Medial pinna			
Length (cm)	2.9 (1.7–3.6)	5.3 (4.7–6)	17.7 (13.5–18.5)
Width (cm)	0.84 (0.7–1)	1.6 (1.4–2)	4.9 (3.8–5.6)
W:L ratio (%)	30	30	28
Petiolule length (mm)	1.5 (1–2)	2.1 (1.5–3)	4.5 (3.8–5.6)
Segment attachment	—	Mostly pinnatifid	Mostly pinnatisect
Pinna base (anterior auricle)	Wider than adjacent portion	Slightly wider than adjacent portion	Usually narrower than adjacent portion
Base shape	Strongly inequilateral	Nearly equilateral	Equilateral
Texture	Extremely coriaceous	Subcoriaceous	Chartaceous
Laminar luster (alive)	Very shiny	Shiny	Dull
Margins	Spinulose denticulate	Shallowly crenulate	Shallowly crenate
Elongate marginal spinules	Large, present on sides and tips	Small, present only on tips	Absent
Basal pinna arrangement	Mainly alternate	Mainly opposite	Mainly opposite
Veins			
Number ultimate branches from costal veins (medial pinnae)	4 (3–6)	22 (15–30)	47 (35–60)
Vein anastomosis	Very rare	Occasional	Frequent
Sorus			
Number per vein truss or medial segment	1	6–10	10–15
Position	Medial to supramedial	Medial to slightly inframedial	Inframedial
Indusial shape	Mainly peltate, some with wide sinuses	Mainly round-reniform, sinuses narrow or wide, a few completely peltate	Round-reniform with margins overlapping sinuses
Spores	Normal	Abortive	Normal
Chromosomes (2n)	82	82	82
Meiotic metaphase pairing	Present	Absent	Present

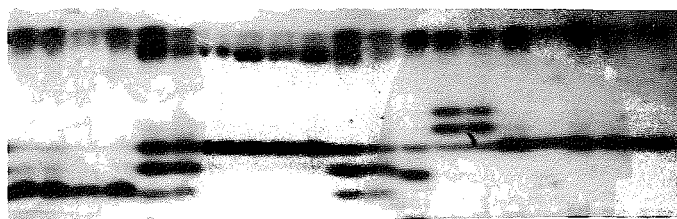
Abies balsamea, *Betula papyrifera*, *Populus grandidentata*, *Rubus occidentalis*, *Sambucus pubens*, *Thuja occidentalis*. Herbs include *Actaea pachypoda*, *Actaea rubra*, *Arctium minus*, *Arisaema triphyllum*, *Asarum canadense*, *Carex pedunculata*, *Carex rosea*, *Circaea alpina*, *Erythronium americanum*, *Eupatorium rugosum*, *Galeopsis tetrahit*, *Geranium robertianum*, *Hystrix patula*, *Impatiens pallida*, *Laportea canadensis*, *Melica smithii*, *Oryzopsis racemosa*, *Pilea fontana*, *Poa alsodes*, *Ranunculus recurvatus*, *Solanum dulcamara*, *Trillium grandiflorum*, and *Viola cf. sororia*. The habitats are all rich in ferns, including collectively all of the following: *Adiantum pedatum*, *Asplenium scolopendrium* var. *americanum*, *A. trichomanes*, *Athyrium filix-femina*, *Deparia thelypteroides*, *Botrychium virginianum*, *Cystopteris bulbifera*, *Cystopteris fragilis*, *Dryopteris carthusiana*, *Dryopteris filix-mas*, *Dryopteris goldiana*, *Dryopteris intermedia*, *Dryopteris marginalis*, *Dryopteris ×neo-wherryi* (= *D. goldiana* × *D. marginalis*), *Polypodium virginianum*, and *Polystichum lonchitis*.

The sites in which *×Dryostichum singulare* grows support a distinctive flora confined to the Niagara Escarpment outcropping areas from Simcoe to Bruce counties. This flora is thus far well preserved because of the rocky, often sloping, substratum unsuitable for development. A conspicuous feature of the *×Dryostichum* habitats is the remaining hulks of white elms, *Ulmus americana*, killed over the years by Dutch elm disease. Although hybridization in plants is often stimulated by disturbances in habitat, this hybrid is found in a habitat that has remained relatively unchanged. Possibly the death of the elm trees has fostered hybridization by creating changes in the microhabitats and in the amount of light coming into the forest.

One possibility for the identity of the plant that we considered early in our study was that it might be a bizarre mutation of some associated species. It is, however, hard to conceive of a single mutation that could affect the many characters that distinguish this plant (cf. Table 1). We concluded that it was more likely a hybrid on the basis of its morphology, sterility, and association at each occurrence with certain other nonhybrid orthospecies.

The plant clearly expresses, at least partially, characters that we associate with *Polystichum* (Table 1) (and in fact we first thought that the plant was a *Polystichum*): the narrow frond (Fig. 9, L) with numerous pinnae, which are falcate, the slightly spinulose tips of pinnae and pinnules, and the presence of at least some peltate indusia. Of the associated ferns, only *P. lonchitis* seemed obviously to be one parent. Although known in the Georgian Bay region, the related *P. acrostichoides* is not present at any of the three localities for the hybrid. The greater problem was to determine the other parent, and although it appeared clearly to be a species of *Dryopteris*, five species of that genus are nearby: *D. filix-mas*, *D. goldiana*, *D. carthusiana*, *D. intermedia*, and *D. marginalis*.

We determined that the chromosome number of *×Dryostichum singulare* is diploid ($2n = 82$, Fig. 11), so that two of the possible parental species, *D. carthusiana* and *D. filix-mas*, both of which are tetraploid, were automatically eliminated. Of the three associated *Dryopteris* diploids, two (*D. intermedia* and *D. marginalis*) are evergreen, as is *P. lonchitis*. The hybrid is only semievergreen, however, and the fronds are mostly brown and dying by the end of winter (Fig. 9, S₄), suggesting that one parent would be deciduous. Also the hybrid lacks the very distinctive epidermal glands of *D. intermedia*, and the sori of the hybrid are submedial, rather



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FIG. 12. Photograph of gel stained for phosphoglucose isomerase as a representation of the isozyme comparison of *×Dryostichum* (X) and its possible ancestors *Polystichum lonchitis* (l), *Dryopteris goldiana* (g), *D. marginalis* (m), and *D. intermedia* (i). Anode is toward top of photo. Uppermost bands, Pgi-1, were not considered interpretable (although they do suggest additivity in the hybrid). For Pgi-2, (lower set of bands) *×Dryostichum* shows three-banded pattern, as expected in heterozygotes for this dimeric enzyme. Pgi-2^B, shared by *P. lonchitis*, *D. marginalis*, and *D. intermedia*, apparently represents the *P. lonchitis* contribution to *×Dryostichum*, combined with Pgi-2^C of *D. goldiana*. Pgi-2 bands anodal to the dark staining Pgi-2^C allozyme in *D. goldiana* lanes are interpreted as secondary isozymes (ghosts).

than supramedial or submarginal as would be expected if *D. marginalis* were a parent. Therefore, *D. goldiana* was left as the most likely *Dryopteris* candidate. The late summer white flecks or spots in the leaves of the hybrid and *D. goldiana* are absent in both *D. intermedia* and *D. marginalis*.

The hybrid shows character states that indicate relationship to *D. goldiana* (Table 1). These include the frond duration in winter (Fig. 9, S), orientation of the leaves, petiole to frond ratio, scale color, basal pinna arrangement, and the white laminar spots in late summer and fall (Figs. 3, 4). Many of the character states of *×Dryostichum* are intermediate between *D. goldiana* and *P. lonchitis*: the curvature (Fig. 6), cutting (Fig. 10), width to length ratio and number of the pinnae (Figs. 9, 10), petiolule length (Fig. 4), laminar texture, reduction in marginal spinules (Fig. 10 S), vein branches, sorus number and position, and indusium shape. The character contrasts are given in detail in Table 1. Some characters are not intermediate, including frond length, pinna length, scale size and shape, and the sori, which are mainly like *Dryopteris*. Most of these are smaller than either of the parents; moreover, the average scale shape with truncate tips is unusual for either *Dryopteris* or *Polystichum*. The individual hybrid plants themselves are, however, remarkably similar to each other. Once learned, they can be readily recognized in the field.

Comparisons of isozymes using starch gel electrophoresis, a technique for analyzing parentage of hybrid ferns (Werth 1989), were made by C. R. Werth of the two individuals of *×Dryostichum* plus 10 each of the possible diploid parents associated with them near Singhampton (Fig. 12). Using standard electrophoretic procedures (Soltis et al. 1983), 15 loci coding the following enzymes were resolved: aldolase (ALD), glucose-6-phosphate dehydrogenase (G-6-PDH), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), 6-phosphogluconic acid dehydrogenase (6-PGDH), phosphoglucose isomerase (PGI; see Fig. 12), phosphoglucomutase (PGM), shikimic acid dehydrogenase (SKDH), and triose-phosphate isomerase (TPI). Isozyme methodology and interpretation of banding patterns parallels that reported in Werth (1991).

TABLE 2. Comparison of alleles of *×Dryostichum singulare* with those of *Polystichum lonchitis* and three possible diploid *Dryopteris* ancestors

Locus	<i>×Dryostichum singulare</i>	<i>Polystichum lonchitis</i>	<i>Dryopteris goldiana</i>	<i>Dryopteris marginalis</i>	<i>Dryopteris intermedia</i>
<i>Ald</i>	A/B	A	B	B,C	B
<i>G6pdh</i>	A	A	A	A	A
<i>Idh-1</i>	C/D	C	D	C,B	B,A
<i>Idh-2</i>	A/C	A	C	C,D	B
<i>Lap-1</i>	B;A/B	B	B	D	C,D
<i>Mdh-1</i>	B/C	C	B	D	A
<i>Mdh-2</i>	A	A	A	A	A
<i>Mdh-4</i>	A/B	B	A	A	A
<i>Pgi-2</i>	B/C	B	C	A,B,D	B
<i>Pgm-1</i>	D	D,F	D	C,E	C,B,A
<i>Pgm-2</i>	A/B	B	A	C	B
<i>6Pgdh-2</i>	A/B	B	A	A,B,C,D	A
<i>Skdh</i>	B/C	C	B,A	D	B
<i>Tpi-1</i>	A/B	A	B	D,B	C
<i>Tpi-2</i>	A/B	B	A	A	B

NOTE: Alleles are designated by letters with A representing the allele that codes the most anodally migrating allozyme at each locus, B the next most anodal, and so on. Heterozygous genotypes of *×Dryostichum* are indicated by two alleles separated by a solidus. Alleles of putative parental species are listed in order of descending frequency.

Table 2 indicates the allozyme composition of *×Dryostichum singulare* in comparison with possible parental species. Alleles, designated by letters for convenience, correspond to those previously reported as numerical mobility ratios for *D. goldiana* and *D. marginalis* (Werth 1991). Both individuals of *×Dryostichum* were allozymically identical with the exception of *Lap-1* where one was homozygous and the other heterozygous. These hybrids were heterozygous at most loci (11 and 12, respectively), reflecting the combination of genetically divergent genomes. At each locus, an allozymic contribution from *P. lonchitis* was apparent, further verifying the hypothesized parentage by this species.

By subtracting the *P. lonchitis* allele from the *×Dryostichum* genotype at each locus, the genotype of the *Dryopteris* parent may be inferred. This genotype matches that of *D. goldiana* at all 15 loci, while parentage by *D. marginalis* and *D. goldiana* and *D. intermedia* can be supported by only eight and six loci, respectively. The only complication in the data set is the *Lap-1*^{AB} genotype of one of the *×Dryostichum* individuals. The *Lap-1*^A allele was not discovered in a large sample of *D. goldiana* previously surveyed (Werth 1991) and most likely represents a polymorphism in *P. lonchitis* that escaped detection in the small sample of this species analyzed for the present study. *Dryopteris goldiana* has a very uniform genotype, and among the loci sampled only *Skdh* is polymorphic (Werth 1991). Both *×Dryostichum* plants received *Skdh*^B from the *Dryopteris* parent, which is the commoner of the two alleles present in *D. goldiana*. Since each locus represents an independent test among hypothesized parental species, these isozyme data can be interpreted as very strong evidence that *D. goldiana* is the second parent of *×Dryostichum*. Of more general interest is the conclusion that *×Dryostichum* is indeed the hybrid of a *Dryopteris* and a *Polystichum* species and thus represents a new nothogenus.

Discussion

The taxon described here is noteworthy in several respects. Hybrids within the genera *Dryopteris* and *Polystichum* are well known and numerous in Japan, North America, and

Europe. *Polystichum lonchitis* is known to hybridize with *P. acrostichoides* (Wagner and Hagenah 1954) and with *Polystichum lemmonii* (Wagner 1973), as well as numerous other species in the Old World, including *Polystichum braunii* (Sleep and Reichstein 1967), a species occurring only very rarely in Southern Ontario (Cody and Britton 1989). *Dryopteris goldiana* is known to hybridize with *D. marginalis*, *D. intermedia*, *D. carthusiana*, *D. cristata*, *D. celsa*, *D. clintoniana* (Wagner 1971) and *D. ludoviciana* (Werth 1991). *×Dryostichum singulare* is confined to a narrow region where the two parent species occur together in the same distinctive rich forest type on dolomite where some disturbance, at least, may be due to the death of the white elm. The region of Simcoe and Grey counties, Ontario, is the only area where we know these two species to be associated, unlike *D. intermedia* and *D. marginalis* that coincide with *P. lonchitis* in many places (Cody and Britton 1989). *Dryopteris intermedia* and *D. marginalis* coexist with *P. acrostichoides* over a wide range in eastern North America, but there has been no authentic report of intergeneric hybridization.

The characters of *×Dryostichum singulare* produce a nondescript-looking fern that without analysis would be difficult to place. Although most of its characters are intermediate (Table 1), a few are not, such as the smallness of the fronds, the mainly *Dryopteris*-like sori, and the smallness and the shape of the scales. The hypothesis of hybridization between *P. lonchitis* and *D. goldiana* receives strong support from comparisons of isozymes electrophoretically, as all 15 of the loci examined corroborate the hypothesis. Parentage by the two other possible diploid *Dryopteris* species proved to be much less likely.

The most intriguing aspect of this hybrid is that it involves two universally recognized genera of ferns. The question arises whether *Polystichum* should any longer be considered a good genus, or should it be reduced to subgeneric status under *Dryopteris*. The question is not only whether hybridization by itself should be a governing factor in future generic applications, but also whether most of the genera in the *Dryopteris* complex may not already be too finely divided on other

grounds in comparison to generic classification of ferns at large. Such dryopteroid genera as *Rumohra*, *Acrophorus*, *Stenolepia*, *Dryopteris*, *Lithostegia*, *Maxonia*, *Polystichum*, *Cyrtomium*, *Phanerophlebia*, and so on (cf. Kramer 1990) are usually recognized on the basis of only one or a few character differences that elsewhere in the system are accepted in members of the same genus. *Dryopteris* and *Polystichum* are supposed to differ mainly in the reniform (*Dryopteris*) versus peltate (*Polystichum*) indusia and the catadromous (*Dryopteris*) versus anadromous (*Polystichum*) arrangement of the blade segments and veins. However, these characters do overlap. In fact, *D. goldiana* itself is heterodromous, i.e., anadromous at base, changing to catadromous (cf. Kramer 1987, Fig. 3), and many of its reniform indusia are actually subpeltate. We do not doubt that the ferns presently treated in *Dryopteris* and in *Polystichum* probably do represent distinct lines of evolution. The more important issue is whether, in light of the broad systematics of ferns, it might not be more consistent to designate these two taxa as subgenera, a topic beyond the scope of this paper.

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