Diplazium hybrids involving *D. plantaginifolium* and *D. ternatum* from Mexico and Central America

Weston L. Testo[1], Michael Sundue[1], and Alejandra Vasco[2]

[1] Pringle Herbarium, Plant Biology Department, University of Vermont, 27 Colchester Ave., Burlington, VT 05401, USA; e-mail: wtesto@uvm.edu; sundue@gmail.com
[2] Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Circuito Exterior s/n, Ciudad Universitaria A.P. 70-367, C.P. 04510, Ciudad de México, DF, Mexico; e-mail: avascog@gmail.com

Abstract. Here we evaluate the origins and relationships of Mexican and Central American *Diplazium* hybrids derived from crosses involving either *D. plantaginifolium* or *D. ternatum*. Based on study of live plants and herbarium specimens, we distinguish *D.* ×*verapax* from the similar *D. riedelianum* and present evidence that the former is a sterile hybrid derived from a cross between *D. plantaginifolium* and *D. werckleanum*. We also describe new hybrids, **D.** ×**torresianum** and **D.** ×**subternatum** from Mexico and northern Central America. Both involve *D. ternatum* as one parent. *Diplazium. cristatum* is the other putative parent of *D.* ×*torresianum,* and *D. plantaginifolium* is the second parent of *D.* ×*subternatum*. We also designate lectotypes for D. cordovense and D. dissimile.

Keywords: Athyriaceae, evolution, fern, hybridization, Neotropics, pteridophyte.

Resolving reticulate evolution is important for delineating species relationships in many fern genera, especially in species-rich groups such as *Asplenium* (Wagner, 1954; Werth et al., 1985; Dyer et al., 2012), *Dryopteris* (Walker, 1959; Sessa et al., 2012a, b; Hori et al., 2014) and *Polystichum* (Wagner, 1973; Barrington, 1990; Mayer & Mesler, 1993). Most studies of reticulate evolution, however, have focused on temperate genera; few examples from tropical genera have been published, even for groups in which hybridization appears to have played an important evolutionary role (but see Conant, 1975; Conant & Cooper-Driver, 1980 for Cyatheaceae; Barrington, 1990 for *Polystichum*; and Gabancho et al., 2010; Dyer et al., 2012 for *Asplenium*).

Among the taxonomically challenging fern genera is *Diplazium*, which comprises about 150 species in the American tropics. Despite its high incidence of polyploidy (Jermy & Walker, 1985; Wood et al., 2009) and widespread hybridization (Smith & Mickel, 1977; Mickel & Smith, 2004), reticulate evolution in *Diplazium* remains little studied.

One prominent feature indicating the occurrence of hybridization among Neotropical *Diplazium* is the existence of taxa with irregularly lobed leaves, which are indicative of hybridization between simple-leaved and divided-leaved species. These taxa provide a useful system to study reticulation because the intermediate morphology of the hybrids is readily discernable. Among Neotropical *Diplazium*, four taxa with irregularly lobed leaves have been described: *D. cordovense* (Baker) C. Chr. (type from Mexico), *D. dissimile* Fée (type from Brazil), *D. riedelianum* (Bong. ex Kuhn) Kuhn ex C. Chr. (type from Brazil), and *D.* ×*verapax* (Donn. Sm.) Hieron. (type from Guatemala). These taxa have similar leaf morphology (Fig. 1), with elliptic or narrowly lanceolate laminae and acute to attenuate apices. Also, leaf division is variable but mature leaves typically have one to several free pinnae proximally and increasingly shallow dissection distally. Because of their irregularly lobed leaves, these taxa have been hypothesized to be of hybrid origin (Lorea-Hernández & Smith, 1999; Mickel & Smith, 2004). The simple-leaved species *D. plantaginifolium* has been indicated as a parent of these taxa; however, the other parent taxa, and the reproductive status of the hybrid taxa (e.g., whether sterile hybrids or fertile allopolyploids) remain uncertain. These taxa are

Brittonia, DOI 10.1007/s12228-017-9476-2
ISSN: 0007-196X (print) ISSN: 1938-436X (electronic)

Published online: 25 April 2017

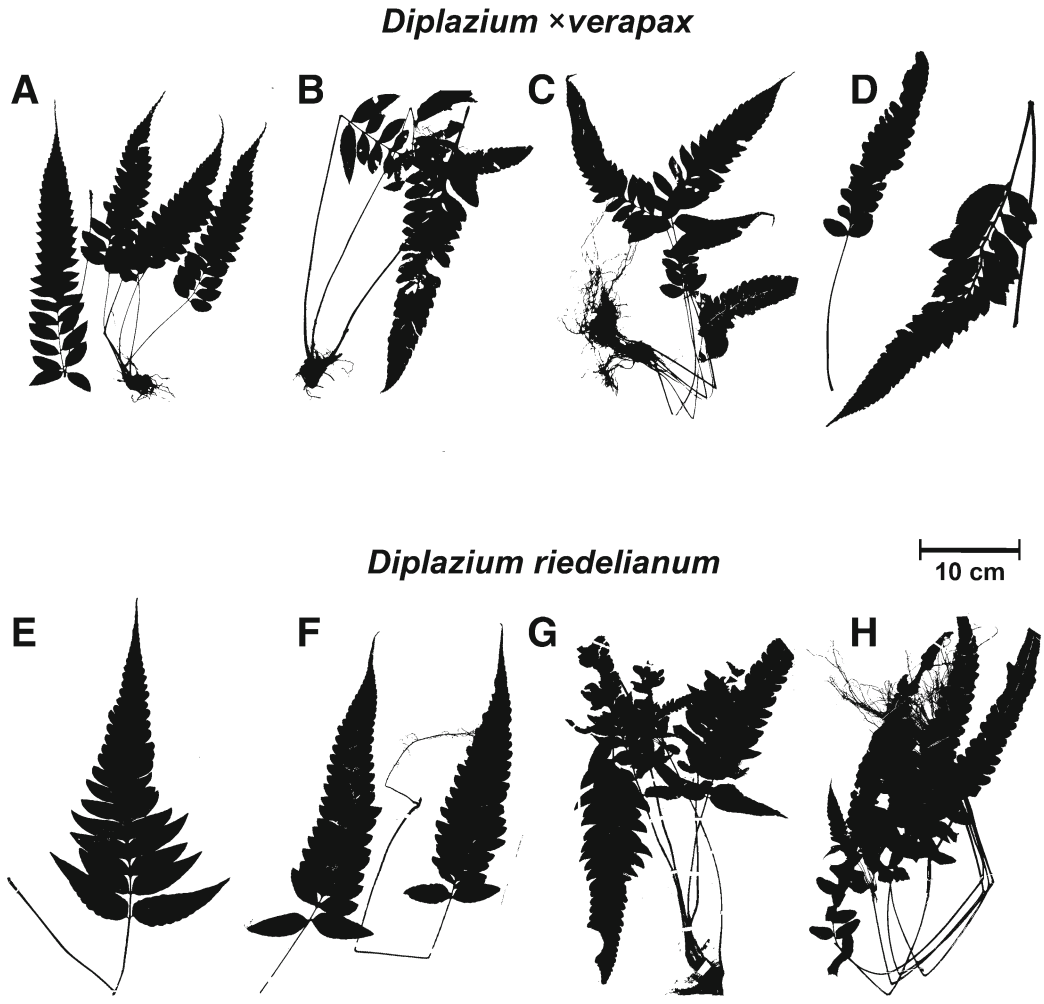

Fig. 1. Silhouettes of two *Diplazium* taxa. **A–D.** *D. ×verapax.* **E–H.** *D. riedelianum*. (A. *Holst 5712,* MO; B. *Rojas 4932*, MO; C. *Brenes s.n.*, NY; D. *Cornman 951*, VT; E. *Schmalz s.n.*, VT; F. *Schmalz 170*, MO; G. *Riedel s.n.*, isotype, K; H. *Zardini* 48832, MO.)

found in two regions in the Neotropics: (1) southern Mexico, Central America, and the West Indies, and (2) southeastern South America. Here, we focus on Mexico and Central America, but we also discuss taxa described from Brazil because Brazilian names have historically been applied to Mexican and Central American taxa in floristic treatments. We address their taxonomy and infer their hybrid origins using morphology and spore condition (normal vs. aborted). In this process, we also describe two new *Diplazium* hybrids with irregularly lobed leaves from Mexico and Central America.

Materials and methods

Field work was carried out on Isla Ometepe, Departamento de Rivas, Nicaragua, and in Distrito Ixtlán, Oaxaca, Mexico, in 2015 and 2016. We examined herbarium specimens at CR, HULE, INB, MEXU, NY, UC, and VT, and viewed high-quality images of specimens from AS, ENCB, K, MO, P, RB, and SEL. Type material of all relevant published taxa were studied. Morphometric measurements were taken directly from specimens when possible. In some cases, leaf measurements were obtained from high-quality images. To

determine whether taxa were sterile hybrids or fertile species, sporangia and spores (10–20 per specimen) were examined using a light microscope at 200× magnification.

Taxonomic treatment

DIPLAZIUM ×VERAPAX GROUP

Diplazium ×verapax (Donn. Sm.) Hieron. (pro sp.) Hedwigia 59: 322, 1917. *Asplenium verapax* Donn. Sm. Bot. Gaz. 13(4): 77, 1888. Type: Guatemala, Alta Verapaz: Pansamalá forest, 1220 m, Sep 1886, *H. von Tuerckheim 850* (lectotype, designated by Smith, 1981: US [US00066966]; isolectotypes: F [F0075627F], K – 2 sheets [K000632717], NY [NY00149408], P – 2 sheets [P00220172, P00220173]). (Figs. 1A, 2F).

Diplazium cordovense (Baker) C. Chr. Ind. Fil. 230, 1905. *Asplenium cordovense* Baker Ann. Bot. 8:125, 1894. Type: Mexico, Veracruz: "Cordova" [Córdoba], 1889, *H. Finck 143* (lectotype, **here designated**: US [US00066920]; isolectotypes: K [K000632717], UC [UC678458]).

Plants terrestrial; rhizome erect; rhizome scales 2–5 × 1–1.5 mm, lanceolate, dark brown, lustrous; fronds 30–80 × 6–15 cm, arching; petiole about ½ frond length, stramineous to brown; petiole 10–40 cm; blade 15–40 cm long, elliptic to narrowly lanceolate, proximally with 1–8 pairs of free pinnae, followed by 2–6 pairs of adnate pinnae, with an elongate pinnatifid apex; proximal pinnae to ca. 7 cm long, 1.5–3 cm broad, lanceolate with cuneate base and acute apex, sometimes with small acroscopic auricle, pinna margins entire to crenate; buds present in the axils of most free and adnate pinnae; venation free; lamina herbaceous; sori to 3 cm long, usually on first acroscopic branch of veins, single or back-to-back along veins; indusium present, inconspicuous, subentire or occasionally lacerate, brown; sporangia and spores irregularly formed.

Distribution.—Mexico, Guatemala, Belize, Nicaragua, Costa Rica, Panama; 650–1690 m (Fig. 3A).

Additional specimens examined. **BELIZE. TOLEDO**: Southwestern Maya Mountains, Columbia River Forest Reserve, Little Quartz Ridge, 16°24'25"N, 89°06'07"W, 700–800 m, 10–11 Apr 1992, *Holst 4317* (MO); Columbia River Forest

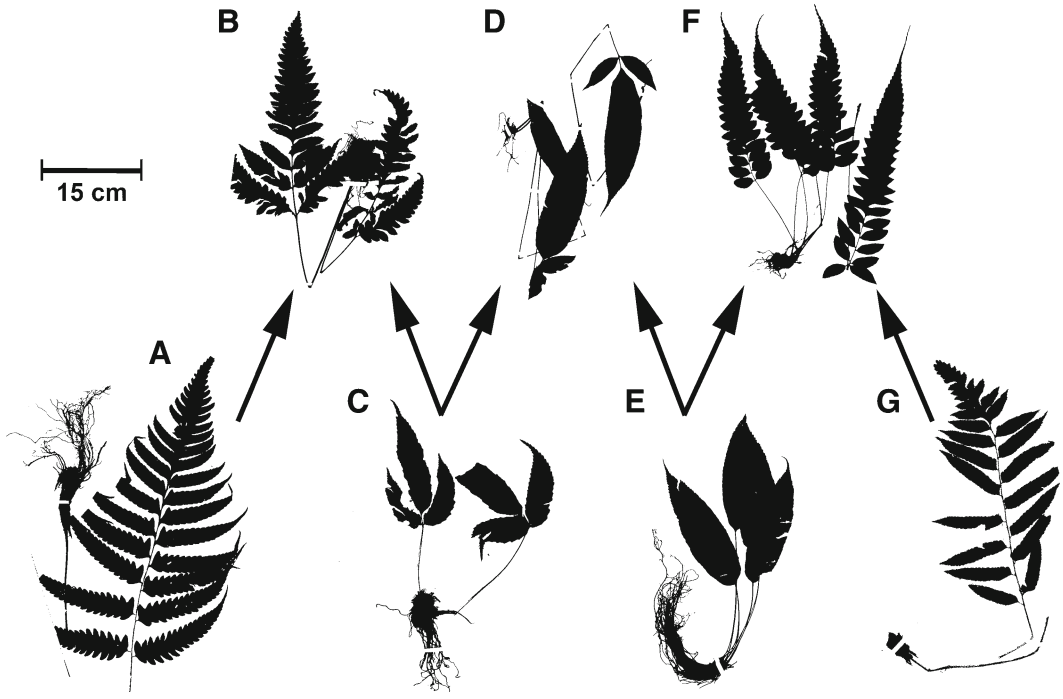

FIG. 2. Hypothesized reticulation network. **A.** *Diplazium cristatum*. **B.** *D.* ×*torresianum*. **C.** *D. ternatum*. **D.** *D.* ×*subternatum*. **E.** *D. plantaginifolium*. **F.** *D.* ×*verapax*. **G.** *D. werckleanum*. (A. *Windisch 2492*, VT; B. *Testo 880*, VT; C. *Torres-Colín 17822*, VT; D. *Holst 5758*, MO; E. *Conant 330*, VT; F. *Holst 5712*, MO; G. *Martínez 58*, MO.).

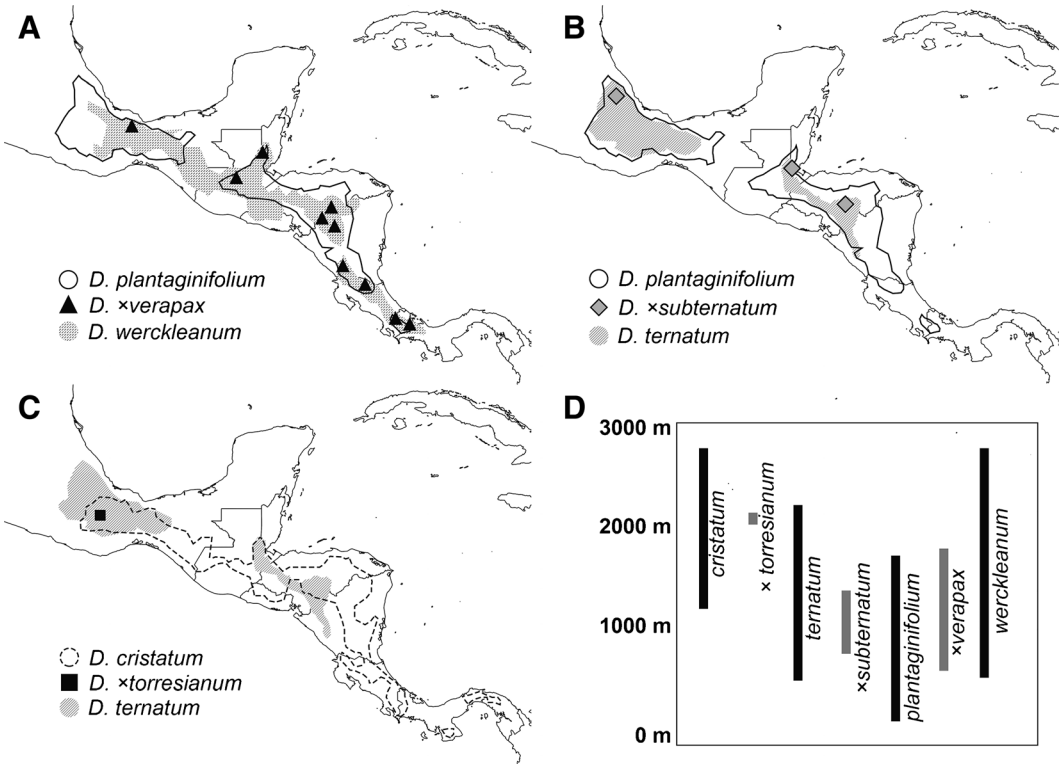

Fig. 3. Distribution of hybrids and their parents in Mexico and Central America. Symbols represent localities of described hybrid taxa; shaded areas represent the approximate range of parental species. **A.** *D.* ×*verapax*. **B.** *D.* ×*subternatum*. **C.** *D.* ×*torresianum*. **D.** Elevational ranges of species (black bars) and hybrids (gray bars).

Reserve: ca. 2 km SW of SW end of Little Quartz Ridge, 16°22'58"N 089°07'10"W, 700 m, 12 Feb 1997, *Holst 5712* (MO, SEL, UC).

COSTA RICA. Alajuela: San Ramón, Potrerillo, [10° 6' 45.36"N, 84° 34' 29"W], Apr 1902, *Brenes s.n* (NY). **Puntarenas:** Cantón de Coto Brus, Z. P. Las Tablas, Cuenca Terraba-Sierpe, 8°58'30"N, 82°46'15"W, 1960 m, 18 Feb 1998, *Rojas 4324* (CR, INB, MO, NY); Cantón de Coto Brus, Z. P. Las Tablas, Cuenca Terraba-Sierpe, sendero entre Cotoncito y sitio Coto Brus, 8°56'47"N,82°46'38"W, 1680 m, 13 Feb 1999, *Rojas 4932* (CR, INB, MO, NY).

NICARAGUA. Madriz: Municipio de Telpaneca, Comunidad de San Jerónimo, Cerro Canta Gallo, 13°26'04"N, 86°13'52"W, 800–900 m, 7 Aug 2007, *Cárdenas Cruz 39* (HULE, MO). **Matagalpa:** 9 km N of Matagalpa on Highway 3, 12°55'N, 85°54'W, 1350–1400m, 14 Apr 1978, *Vincelli 335* (HULE, MO, UCA); Municipio de Matagalpa, camino a la comunidad de Aranjuez, 13°02'N 85°53'W, 1100–1300 m, 28 Nov 2002, *Rueda* et al. *17518* (HULE, MO); Matagalpa, ca. 0.8 km from Hwy 3 on road to Aranjuez, 13°02'N, 82°55'W, 1360–1380 m, 7 May 1980, *Stevens* et al. *17092* (HULE, MO). **Rivas:** Isla Ometepe, Volcán Concepción, 800–1100 m, 7 Aug 1982, *Martínez* et al. *1526* (MEXU); Isla Ometepe, Volcán Maderas, N slope, 11°27'N, 85°31'W, 800–1000m, 24 Feb 1978, *Neill & Vincelli 3274* (HULE, MO, UCA); Isla Ometepe, trail to Volcán Maderas from Balgüe, northern slope, 11.471°N, 85.506°W, 650 m, 26 Feb 2016, *Testo 1045* (HULE, NY, VT).

PANAMA. Chiriquí: El Boquete, Dexter's trail, 13 Feb 1918, *Cornman 951* (MO, VT).

Diplazium ×*verapax* appears to be a hybrid between *D. plantaginifolium* and *D. werckelanum. Diplazium plantaginifolium* is the only simple-leaved *Diplazium* present in the range of *D.* ×*verapax*, and both taxa at least occasionally produce buds at the base of their lamina. *Diplazium werckleanum* is the only species of *Diplazium* found throughout the range of *D.* ×*verapax* that possesses pinnate leaves with entire to slightly lobed margins, as would be expected based on hybrid intermediacy. Involvement of *D. werckleanum* in the origin of *D.* ×*verapax* is further supported by similarities in rhizome scale and indusium morphology (Table I). Label data indicate that *D.* ×*verapax* at least occasionally co-occurs with its proposed progenitors, *D. plantaginifolium* (*Testo 1045*,

TABLE I COMPARISON OF MORPHOLOGICAL CHARACTERS AND DISTRIBUTION OF *DIPLAZIUM* TAXA IN THIS STUDY.

Species	Leaf length (cm)	Leaf width (cm)	Leaf division	Number of pinna pairs	Venation	Leaf buds	Spore shape	Distribution	Elevation (m)
D. cristatum	60–110	20–30	Pinnate–pinnatifid	6–12	Free	Rare; distal	Regular	Mexico to Brazil	1300–2700
D. ×torresianum	40–90	16–30	Pinnate–pinnatifid	2–5	Mostly free	Proximal	Irregular	Mexico	2100–2150
D. ternatum	20–55	8–18	Ternate	1	Mostly free	Proximal	Regular	Mexico to Nicaragua	500–2200
D. ×subternatum	45–75	8–14	Ternate	1	Mostly free	Proximal	Irregular	Mexico, Belize, Honduras	700–1300
D. plantaginifolium	30–60	4–8	Simple	0	Mostly free	Occasional; proximal	Regular	Mexico to Brazil	150–1600
D. ×verapax	30–80	6–15	Proximally pinnate, lobed	1–8	Mostly free	Throughout	Irregular	Mexico to Panama	650–1690
D. werckleanum	40–100	8–20	Pinnate	5–10	Free	Occasional;distal	Regular	Mexico to Panama	550–2700
D. riedelianum	35–90	15–25	Proximally pinnate, lobed	1–5	Mostly free	Proximal	?	SE South America	200–800

HULE, NY, VT) and *D. werckleanum* (*Holst 4317*, MO). Both spores and sporangia of *D.* ×*verapax* from Costa Rica (*Brenes s.n.*, NY, *Rojas 4932*, NY), Guatemala (*von Tuerckheim 850*, NY), Nicaragua (*Testo 1045*, VT), and Panama (*Cornman 951*, VT) were malformed, suggesting that this taxon is sterile and should be treated as a hybrid rather than an allopolyploid fertile species.

As evidenced by the small number of collections, *D.* ×*verapax* is apparently uncommon; however, it can be locally abundant, presumably due to vegetative reproduction from leaf buds. Mickel and Smith (2004) reported it from Chiapas, Mexico, on the basis of two collections (*Breedlove 33110,* DS, and *Breedlove 67989*, CAS); however, we have been unable to locate these specimens. It was reported for El Salvador by Seiler (1980) and by Monterrosa and Monro (2008) on the basis of a single collection (*Aguilar 659*, MHES) from Departamento Santa Ana, but we have not seen this specimen. Some specimens from southern Costa Rica (*Rojas 4232, 4932,* CR, NY) possess unusually large basal pinnae and appear to approach Brazilian *D. riedelianum*; however, they resemble typical *D.* ×*verapax* with respect to rhizome scale morphology and have abortive spores. We maintain them here under *D.* ×*verapax* but acknowledge that it is possible that these collections may represent a different hybrid involving *D. plantaginifolium* and another member of the *D. cristatum* complex.

Diplazium riedelianum (Bong. ex Kuhn) Kuhn ex C. Chr. Index Filic. 238. 1905. *Asplenium riedelianum* Bong. ex Kuhn Linnaea 36: 102. 1869. Type: Brazil, [state unknown]: 1824. *L. Riedel s.n.* (lectotype, designated by Adams, 1995: B [B 20 0049408]; isolectotypes: K [K000632763], NY [NY00149394] – fragment and drawing). (Fig. 1B).

Diplazium dissimile Fée, Crypt. Vasc. Brésil 1: 76, pl. 21. 1869. Type: Brazil, Santa Catarina: 1860. *Mors 16* (lectotype, **here designated:** P [P00696338]; isolectotype: RB).

Plants terrestrial; rhizome erect; rhizome scales 4–7 × 1–2.5 mm, lanceolate, brown, lustrous; fronds 35–90 × 15–25 cm, arching; petiole about ½ frond length, stramineous to brown; blade 15–50 cm long, elliptic to narrowly lanceolate, proximally with 1–5 pairs of free pinnae, followed by 2–5 pairs of adnate pinnae, with an elongate pinnatifid apex; proximal pinnae to ca. 12 cm long, 1.5–3.5 cm broad, lanceolate with broadly cuneate to truncate base and acute apex, sometimes with small acroscopic auricle and slightly excavate basiscopically, pinna margins crenate to lobed ¼ distance to pinna rachis; buds present usually only in axils of proximal pinnae; venation free; lamina chartaceous; sori to 3 cm long, on multiple veins of a single vein group, single or back-to-back along veins; indusium present, inconspicuous, entire, brown; sporangia and spores unknown.

Distribution.—Argentina, Brazil, Paraguay; 200–800 m.

Additional specimens examined. **ARGENTINA. Misiones:** San Pedro, Parque Provincial Piñalito, Arroyo Sidra, Salto Paca, 26°25'S, 53°50'W, 750 m, 11 Mar 2002, *Múlgura de Romero 3243* (MO, SI).

BRAZIL. Santa Catarina: Joinville, 1904, *Schmalz 170* (MO, NY); Joinville. 1905, *Schmalz s.n.* (NY, VT); Hammonia, 1 Aug 1911, *Luederwaldt 691* (NY); Joinville, 16 Apr 1906, *Muller 166* (NY). **Paraná:** Antonina, Reserva Natural Rio Cachoeira, Trilha do Mirante, 200 m, 12 Sep 2008, *Matos 1600* (NY).

PARAGUAY. Canindeyú: Mbarabayú Natural Reserve, 1 km SE of Jejui Mí, 24°07'59"S, 55°31'41"W, 12 Jun 1998, *Zardini & Chaparro 48832* (AS, MO); around Jejui-Mí, 24°07'59"S, 55°31'41"W, 26 Aug 2000, *Zardini & Franco 54875* (MO).

Although we suspect that *Diplazium riedelianum* is a hybrid, its parentage and fertility are uncertain. *Diplazium plantaginifolium* appears to be one parent, and a member of the *D. cristatum* group is likely the other. *Diplazium riedelianum* is distinct from *D.* ×*verapax* based on differences in leaf shape and division, lamina texture, pinna number, position of proliferous buds, and sorus distribution (Table I, Fig. 1). The geographic distribution of the progenitor species supports this conclusion as well; *D. werckleanum* (the apparent divided-leaved progenitor of *D.* ×*verapax*) does not occur within the range of *D. riedelianum* (Fig. 3A). The isolectotype at NY had insufficient spore material to determine whether the spores were aborted. Thus, although the plant appears to be of hybrid origin, we treat it here as a species. Further work on this

species group in southeastern South America is necessary to resolve the origin and status of this taxon.

TWO NEW HYBRIDS

Diplazium ×torresianum Testo, Sundue & A. Vasco, **hybrid nov.** Type: Mexico, Oaxaca: Distrito Ixtlán, Municipio San Pedro Yolox, San Francisco la Reforma II, ravine in dry *Pinus*- and *Quercus*-dominated montane forest above logging camp, 17°40'15"N, 96°35'13"W, 2100–2150 m, 19 Mar 2015, *W. Testo 880* (holotype: VT; isotypes: AAU, MEXU, MO, NY, UC). (Figs. 2B, 4A, 5).

Hybrid between *Diplazium cristatum* and *D. ternatum*. Plants terrestrial with erect rhizome, rhizome scales 3–6 × 0.8–1.3 mm, dark brown, concolorous. Frond to 90 cm, petiole to 50 cm, petiole scales 5–11 × 0.7–1.5 mm, dark brown, concolorous. Lamina 25–40 cm long and 16–30 cm wide at base; proximal pinnae prominent, to 22 cm long and 6 cm wide, variably lobed 1/4–3/4 the distance to the costa. Number of free pinna pairs 2–5. Veins mostly free, with some anastomoses proximally. Fertile and sterile leaves similar. Proliferous buds often present in axils of pinnae. Sori elongate, along veins, single or back-to-back along veins; indusia present, subentire, 5–15 mm long; sporangia and spores irregular.

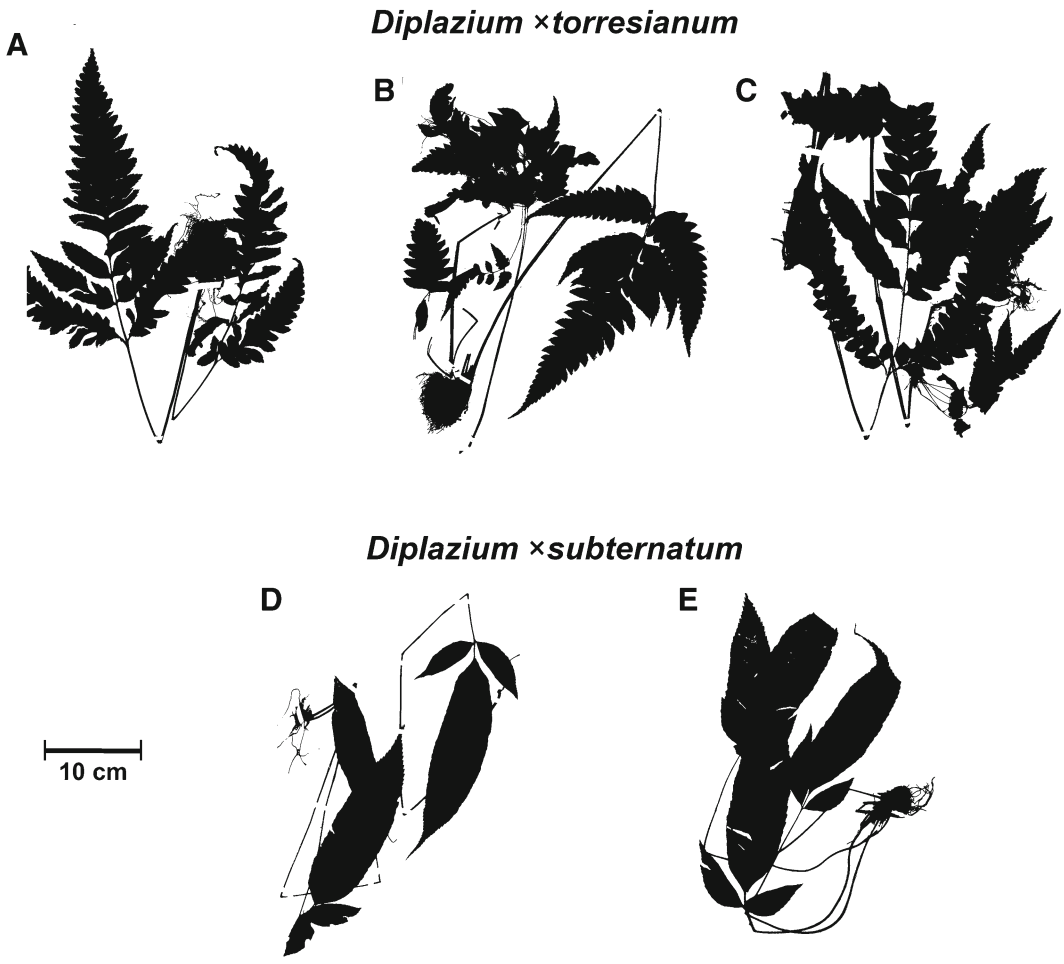

FIG. 4. Silhouettes of two *Diplazium* hybrids. **A–C.** *D. ×torresianum.* **D–E.** *D. ×subternatum*. (A. *Testo 895,* VT; B. *Testo 880*, holotype, VT; C. *Sundue 4028* VT; D. *Croat & Hannon 64,218* UC; E. *Holst 5758* NY).

Fig. 5. *Diplazium ×torresianum*. **A.** Typical leaf showing enlarged proximal pinnae and abruptly contracted pinnatifid apex. **B.** Abaxial surface of pinna showing arrangement of sori. **C.** Growth habit of plant.

Distribution and ecology.—*Diplazium ×torresianum* is known only from two humid ravines in otherwise dry pine-oak forest at an elevation of 2100–2150 m near the village of San Francisco la Reforma II, municipality of San Pedro Yolox, in Distrito Ixtlán of Oaxaca, Mexico (Fig. 3C). It was found growing on soil banks and along streams, and is capable of reproducing locally via leaf buds.

Etymology.—The hybrid epithet is given in honor of Rafael Torres-Colín, Mexican botanist and colleague of the authors. Rafael's extensive collecting efforts have advanced our understanding of plant diversity and distributions in Mexico, including the fern flora of Oaxaca and especially the Sierra Juárez (Torres-Colín et al., 2009). Without his efforts and knowledge, the botanical expedition that resulted in the discovery of this hybrid would not have been possible.

Additional specimens examined. **MEXICO. Oaxaca:** Distrito Ixtlán, Municipio San Pedro Yolox, San Francisco la Reforma II, second ravine above logging camp, 17°40'15"N, 96°35'13"W, 2100–2150 m, 19 Mar 2015, *Testo 895* (MEXU, VT); Distrito Ixtlán, Mun. San Pedro Yolox, Agencia San Mateo [Francisco] Reforma, on rocky streambank and in soil, 17°40'15"N, 96°35'13"W, 2100 m, 19 Mar 2015, *Sundue 4028* (MEXU, NY, UC, VT).

Diplazium ×torresianum was discovered during fieldwork in March, 2015, related to a study of the fern flora of Oaxaca, Mexico. We encountered two populations of a *Diplazium* with irregularly lobed leaves that did not match the descriptions of any species reported by Mickel and Beitel (1988) or Mickel and Smith (2004). The plants in question were terrestrial, medium-sized, and had 2–4 pairs of free pinnae, the pinnae irregularly lobed proximally and with an elongate deeply lobed apex. Some plants possessed a single bud on the rachis at the base of the lamina. These buds occasionally formed plantlets that rooted in the ground when the leaf upon which they were borne senesced. Because of their conspicuously elongate basal pinnae with axillary buds, the plants resembled an odd form of *D. ternatum*, a distinctive species with ternate leaves bearing buds that was common at the collection locality. In total, approximately 20 plants were found growing along streams and on

disturbed soil banks in two small, humid ravines in an otherwise dry pine-oak forest at approximately 2100 m near the village of San Francisco la Reforma II, within the limits of San Pedro Yolox, in Oaxaca's Sierra Juárez mountains. Extensive searches of the surrounding area failed to uncover additional suitable habitat or populations. Despite their small size and fragmented nature, these ravines harbored considerable pteridophyte diversity; more than 20 species of ferns and lycophytes were observed.

The overall appearance of these plants and their variable leaf dissection suggested that they were of hybrid origin. This was later confirmed by inspection of their sporangia and spores, which were malformed. Considering the other species of *Diplazium* at the site, a close affinity to the distinctive *D. ternatum* was evident, as these plants possessed leaves with elongate proximal pinnae with buds at the base of the lamina. The identity of the other progenitor was not as obvious, though the leaf morphology of the unknown taxon indicated that it should have inequilateral pinna bases and once-pinnate to once-pinnate-pinnatisect laminae. Among the remaining species of *Diplazium* present at the locality, only *D. cristatum* fulfilled these criteria. Both *D. franconis* and *D. puberulentum* have leaves that are too divided to be morphologically coherent progenitors of the unknown taxon. Also, *D. puberulentum* can be distinguished from other Neotropical *Diplazium* by its abundant papillae in the sulcae of the rachis and costae, which are absent from the hybrids in question.

Among Mexican *Diplazium* species not found at the site, two other species have a combination of once-pinnate to once-pinnate-pinnatisect laminae and inequilateral pinna bases: *D. errans* and *D. werckleanum*. We do not believe that either of these species is involved in the origin of *D.* ×*torresianum*. *Diplazium errans* can be excluded because it possesses more than 20 pairs of pinnae with buds in the axils of distal pinnae, whereas the unknown hybrid has ternate leaves with a lobed apical pinnae and buds restricted to the axils of the proximal pinnae. Also, as mentioned previously, *D. errans* is only known from a single collection in the neighboring state of Guerrero at an elevation 1100 m lower than *Diplazium* ×*torresianum*.

Though more similar to the unknown hybrid than *Diplazium errans*, *D. werckleanum* was probably not a parent. Like *D. ternatum*, the pinnae of *D. werckleanum* are less-divided than those of the hybrid (Fig. 2). If the latter species were a parent, it would require that the leaf division of the hybrid to not be intermediate to its progenitors. As fern hybrids are consistently morphologically intermediate to their progenitors with respect to leaf division (Wagner, 1954; Conant, 1975; Moran, 1981; Smith & Grayum, 1988; Barrington et al., 1989; Chaves-Fallas et al., 2015; Testo et al., 2015), we strongly doubt that *D. werckleanum* is the second progenitor of this hybrid. Also, *D. werckleanum* is distinct by suberose indusia margins, whereas the hybrid has entire margins. The hybrid also possesses longer rhizome scales (3–6 mm) than those found in *D. werckleanum* (2–4 mm) (Table I).

With *Diplazium werckleanum* excluded as a possible parent, only *D. cristatum* remains a morphologically defensible second progenitor of this hybrid. This is evidenced by hybrid's intermediate leaf division between the ternate-leaved *D. ternatum* and the pinnate-pinnatifid leaf of *D. cristatum*. Additional characters supporting this proposed relationship include rhizome scale size (6–9 × 0.8–1.3 mm in the hybrid, 5–7 × 1–1.5 mm in *D. cristatum*) and indusium margin (subentire in both the hybrid and *D. cristatum*). In these characters and in gross morphology, these plants overwhelmingly appear intermediate between *D. ternatum* and *D. cristatum* (Table I, Fig. 2).

Despite examination of material in several relevant herbaria (HULE, MEXU, NY, US, VT), we have not encountered any other specimens of *Diplazium* ×*torresianum*. This is surprising because both *D. cristatum* and *D. ternatum* are widespread and occupy similar habitats throughout southern Mexico and northern Central America, where their ranges overlap. Although no suitable material was available for chromosome squashes. We expect *D.* ×*torresianum* to be a tetraploid, as cytological examination of both *D. cristatum* and *D. ternatum* have yielded counts of $2n = 164 = 4\times$ (Walker, 1966; Smith & Mickel, 1977).

Diplazium ×subternatum Testo, Sundue & A. Vasco, **hybrid nov.** Type: Honduras, Olancho: Road between San Francisco la Paz and Gualaco, 13.6 mile SW of Gualaco, in disturbed virgin forest on steep slope ca. 1/2 mile east of main road, 15°00'N, 86°07'W, 1300 m, 6 Feb 1987, *T. Croat & D. Hammon 64218* (holotype: UC; isotypes: MO). (Figs. 2D, 4B).

Hybrid between *Diplazium plantaginifolium* and *D. ternatum*. Plants terrestrial with erect

rhizome, rhizome scales 0.8–1.2 × 0.6–0.9 mm, concolorous, dark brown to black. Petiole 30–50 cm long, petiole scales sparse, 2–3 × 0.4–0.6 mm concolorous, dark brown to black. Laminae 18–26 cm long and 8–14 cm wide at widest point, ternate, lateral pinnae 4–7 cm long and 1–2.5 cm wide, apical pinna 14–24 cm and 3–6 cm wide. Leaf margins serrulate to doubly serrate. Veins mostly free, with some anastomoses near the costae. Fertile and sterile leaves similar. Proliferous buds occasionally present in axils of pinnae. Sori elongate along veins, single or back–to–back along veins, with subentire indusia, 8–20 mm long; sporangia and spores irregular.

Etymology.—The hybrid is named for its morphological similarity to one of its progenitor species, *Diplazium ternatum*. These two taxa are the only ternate–leaved *Diplazium* in the Neotropics. The epithet *subternatum* refers to the conspicuous size difference between its lateral pinnae and apical pinna, which contrasts with the nearly equal-sized pinnae of *D. ternatum*.

Distribution and ecology.—This hybrid is known currently from three collections: one each from Mexico, Belize, and Honduras (Fig. 3B). It is likely to be more widespread and should be looked for in humid forests from Mexico to Nicaragua at elevations from 500–1600 m, where its progenitor species co–occur.

Additional specimens examined. **BELIZE. Toledo:** Columbia River Forest Reserve, Little Quartz Ridge, lower slopes on southern flank, 16°23'N, 89°05'W, 700–750 m, 15 Feb 1997, *Holst 5758* (MO, NY, SEL).

MEXICO. Puebla: [without exact locality], *Ventura A. 20222* (ENCB).

Diplazium ×*subternatum* is a sterile hybrid derived from crosses between *D. plantaginifolium* and *D. ternatum*. Like both of its progenitors, it possesses buds at its lamina base. It is likely that these buds allow the hybrid to form colonies, but this was not mentioned on the collection labels of the material seen by us. The hybrid most resembles *D. ternatum* but differs by its relatively smaller lateral pinnae (20–40% as long as the apical pinnae, vs. 50–80% as long in *D. ternatum*) and malformed sporangia and spores. Considering their similar leaf morphology, it is likely that additional collections of *D.* ×*subternatum* are present in herbaria, misidentified as *D. ternatum*. Rarely, *D. plantaginifolium* possesses small, free lobes at the base of the lamina. These lobes are typically less than 1 cm long and round to ovate. We interpret these as vestigial pinnae because simple leaves are derived in the genus (Wei et al., 2013). Specimens of *D. plantaginifolium* bearing such lobes should not be easily confused with *D.* ×*subternatum*.

During herbarium work, we encountered several specimens of the putative hybrid *Diplazium plantaginifolium* × *D. ternatum*. Although all specimens were similar by having ternate leaves with sparse vein anastomoses and spores malformed, additional study of indicated that two distinct morphologies were represented. Whereas several plants (*Croat & Hannon 64218*, MO, UC; *Holst 5758*, MO, NY, SEL; *Ventura A. 20222,* ENCB) possessed leaves with serrulate margins and an apical pinna 3–5 times longer than the lateral pinnae, one specimen (*Hallberg 1615*, NY, US) had smooth to undulate-margined leaves with an apical pinna that is roughly the same size as the lateral pinnae. These differences were noted by Mickel and Smith (2004), who reported two collections of this hybrid from Mexico but considered plants with both morphologies to be the same hybrid.

Examination of the three specimens with small lateral pinnae and serrulate leaf margins indicated that these plants were intermediate between *D. plantaginifolium* and *D. ternatum* (Fig. 4B). As would be expected of hybrid between a simple-leaved (*D. plantaginifolium*) and a ternate-leaved (*D. ternatum*) species, these plants possessed ternate leaves with relatively small lateral pinnae. These collections—one each from Mexico, Belize, and Honduras—represent the newly described hybrid *D.* ×*subternatum*.

Several lines of evidence led us to believe that *Hallberg 1615* does not represent *Diplazium* ×*subternatum* (=*D. plantaginifolium* × *D. ternatum*), but instead a different hybrid involving *D. plantaginifolium*. The specimen possesses lateral pinnae approximately the same size as the apical pinna. This is inconsistent with our hypothesis of hybrid morphological intermediacy, as *D.* ×*subternatum* is a cross between a simple-leaved species (*D. plantaginifolium*) and a ternate-leaved species with lateral pinnae that are smaller (0.5–0.8× the length) than the apical pinna. Second, because the parents possess leaves either crenulate to weakly serrulate (*D. plantaginifolium*) or strongly and doubly serrate (*D. ternatum*), the hybrid should have serrulate to serrate margins. *Hallberg 1615,* however, is entire to merely undulate. Further evidence against the involvement of *D. ternatum* in this hybrid's origin is a difference in

elevational range (Fig. 3D). As noted by Mickel and Smith (2004), *Hallberg 1615* was collected at 150–250 m, which is considerably below the 500–2200 m range reported for *D. ternatum* in Mexico. Based on leaf morphology and elevation, *Hallberg 1615* probably represents a hybrid between *D. plantaginifolium* and a once-pinnate species of lowland forests, possibly *Diplazium grandifolium* (Sw.) Sw. or a close relative. *Diplazium grandifolium* has not been reported for Oaxaca but likely occurs there because it is known from the neighboring states of Chiapas and Veracruz.

Acknowledgments

We thank the community of San Francisco la Reforma II in Oaxaca, Mexico, for permission to collect on their communal lands and for being exceptionally gracious hosts during our stay with them. In Nicaragua, Dra. Indiana Coronado provided logistic support during fieldwork. Conversations with Drs. David Barrington, Robbin Moran, and Alan Smith were critical in developing the ideas presented in this work; we also thank Dr. James Solomon for providing access to herbarium material. We thank Art Gilman, Susan Fawcett, Nikisha Patel, and Morgan Southgate for helpful comments on an early version of this manuscript, and Claudine Mynssen and one anonymous reviewer for comments that greatly improve this manuscript. This work supported in part by an NSF Graduate Research Fellowship and a Graduate Student Research Award from the American Society of Plant Taxonomists awarded to W.T., a grant from the Programa UNAM-DGAPA-PAPIIT (IA201416) awarded to A.V., and a CONACyT grant (convocatoria INFR-2016-01, proyecto No. 269382) awarded to the National Herbarium of Mexico (MEXU).

Literature cited

Adams C.D. 1995. *Diplazium*. In Davidse G., Sousa S., M. & Knapp S. (eds.), Flora Mesoamericana: 228- 246. México, Universidad Nacional Autónoma de México.

Barrington, D. S. 1990. Hybridization and allopolyploidy in Central American *Polystichum*: cytological and isozyme documentation. Annals of the Missouri Botanical Garden 77: 297–305.

———, **C. H. Haufler & C. R. Werth**. 1989. Hybridization, reticulation, and species concepts in the ferns. American Fern Journal 79: 55–64.

Chaves-Fallas, J. M., R. C. Moran & F. Oviedo-Brenes. 2015. *Serpocaulon ×rojasianum* (Polypodiaceae): A new fern hybrid from Costa Rica. Brittonia 67: 185–190.

Conant, D. S. 1975. Hybrids in American Cyatheaceae. Rhodora 77: 441–455.

——— **& G. Cooper-Driver.** 1980. Autogamous allohomoploidy in *Alsophila* and *Nephelea* (Cyatheaceae): A new hypothesis for speciation in homoploid homosporous ferns. American Journal of Botany 67: 1269–1288.

Dyer, R. J., V. Savolainen & H. Schneider. 2012. Apomixis and reticulate evolution in the *Asplenium monanthes* fern complex. Annals of Botany 110: 1515–1529.

Gabancho, L. R., C. Prada & J. G. Galán. 2010. Sexuality and apogamy in the Cuban *Asplenium auritum-monodon* complex (Aspleniaceae). Plant Systematics and Evolution 289:137–146.

Hori, K., A. Tono, K. Fujimoto, J. Kato, A. Ebihara, Y. Watano & N. Murakami. 2014. Reticulate evolution in the apogamous *Dryopteris varia* complex (Dryopteridaceae, subg. *Erythrovariae*, sect. *Variae*) and its related sexual species in Japan. Journal of Plant Research 127: 661–684.

Jermy, A. C. & T. G. Walker. 1985. Cytotaxonomic studies of the ferns of Trinidad. 3. Descriptions of new species and hybrids and a new combination. Bulletin of the British Museum 13: 251–276.

Lorea-Hernández, F. G. & A. R. Smith. 1999. Two new fern species from southern Mexico. American Fern Journal 89: 181–186.

Mayer, M. S. & M. R. Mesler. 1993. Morphometric evidence of hybrid swarms in mixed populations of *Polystichum munitum* and *P. imbricans* (Dryopteridaceae). Systematic Botany 18: 248–260.

Mickel, J. T. & A. R. Smith. 2004. Pteridophytes of Mexico. New York Botanical Garden Press, Bronx, NY.

——— **& J. M. Beitel**. 1988. Pteridophyte flora of Oaxaca, Mexico. Memoirs of the New York Botanical Garden 46: 1–568.

Monterrosa, J. & A. K. **Monro.** 2008. An annotated checklist of the monilophytes (ferns) and lycophytes of El Salvador. Fern Gazette 18: 120–215.

Moran, R. C. 1981. × *Asplenosorus shawneensis*, a new natural fern hybrid between *Asplenium trichomanes* and *Camptosorus rhizophyllus*. American Fern Journal 71: 85–89.

Sessa, E. B., E. A. Zimmer & T. J. Givnish. 2012a. Unraveling reticulate evolution in North American *Dryopteris* (Dryopteridaceae). BMC Evolutionary Biology 12: 104.

———, **E. A. Zimmer & T. J. Givnish**. 2012b. Reticulate evolution on a global scale: A nuclear phylogeny for New World *Dryopteris* (Dryopteridaceae). Molecular Phylogenetics and Evolution 64: 563–581.

Seiler, R. 1980. Una guía taxonómica para helechos de El Salvador. Ministerio de Educación. San Salvador.

Smith, A. R. 1981. Pteridophytes. Pp. 1–370 in Flora of Chiapas, Part 2, ed. D. E. Breedlove. San Francisco: California Academy of Sciences.

Smith, A.R. & M. H. Grayum. 1988. *Cyathea stolzei* × *ursina*, a distinctive tree fern hybrid from Costa Rica. American Fern Journal 78: 105–108.

——— **& J. T. Mickel**. 1977. Chromosome counts for Mexican ferns. Brittonia 29: 391–398.

Testo, W. L., J. E. Watkins, J. Pittermann & R. Momin. 2015. *Pteris ×caridadiae* (Pteridaceae), a new hybrid fern from Costa Rica. Brittonia 67: 138–143.

Torres-Colín, R., D. Lorence, M. P. Ramírez-De Anda & R. E. Villa. 2009. Flora de la Sierra de Juárez, Oaxaca: Distrito de Ixtlán y áreas adyacentes (Sierra Norte de Oaxaca). Listados Florísticos de México XXV. Instituto de Biología, Universidad Nacional Autónoma de México, México D. F.

Wagner, W. H. 1954. Reticulate evolution in the Appalachian Aspleniums. Evolution 8: 103–118.

———. 1973. Reticulation of holly ferns (*Polystichum*) in the western United States and adjacent Canada. American Fern Journal 63: 99–115.

Walker, S. 1959. Cytotaxonomic studies of some American species of *Dryopteris*. American Fern Journal 49: 104–112.

Walker, T. G. 1966. A cytotaxonomic survey of the pteridophytes of Jamaica. Earth and Environmental Science Transactions of The Royal Society of Edinburgh 66: 169–237.

Wei, R., H. Schneider & X. C. Zhang. 2013. Toward a new circumscription of the twinsorus-fern genus *Diplazium* (Athyriaceae): A molecular phylogeny with morphological implications and infrageneric taxonomy. Taxon 62: 441–457.

Werth, C. R., S. I. Guttman & W. H. Eshbaugh. 1985. Electrophoretic evidence of reticulate evolution in the Appalachian *Asplenium* complex. Systematic Botany 10: 184–192.

Wood, T. E., N. Takebayashi, M. S. Barker, I. Mayrose, P. B. Greenspoon & L. H. Rieseberg. 2009. The frequency of polyploid speciation in vascular plants. Proceedings of the National Academy of Sciences 106: 13875–13879.