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Goniopteris ×*tico* (Thelypteridaceae), a New Hybrid Fern from Costa Rica

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Abstract*—*Goniopteris* ×*tico, a new hybrid fern from La Selva Biological Station in Heredia Province, Costa Rica, is described based on morphology and analysis of target-capture DNA sequence data. The hybrid co-occurs with its two putative progenitors, *Goniopteris mollis* and *Goniopteris nicaraguensis,* and is readily recognizable by its intermediate leaf dissection and venation. It is also intermediate in pinnae size and shape, and presents irregularly lobed pinnae. Despite the broad overlap in the geographic distribution of its parental taxa, *Goniopteris* ×*tico* is only known from two collections from a single area of the La Selva Biological Station, highlighting the importance of close observation of ferns from even well-collected areas.

Keywords—Allele phasing, Central America, reticulate evolution, target-capture.

Hybridization is common in ferns and has played an especially important role in the evolutionary history of this lineage, as witnessed both by the complex networks of hybrids and hybrid-derived species documented in many fern genera (Barrington 1990; Haufler et al. 1995; Sessa et al. 2012; Sigel 2016; Liu et al. 2020) and the high percentage of speciation events linked to hybridization and polyploidization (Wood et al. 2009; Schneider et al. 2017). The propensity of ferns to hybridize at an elevated rate compared to other major plant lineages such as angiosperms has been the focus of extensive study (Stebbins 1981; Barrington et al. 1989; Sigel 2016) and is thought to largely reflect ferns' generally weak mechanisms for establishing and maintaining reproductive isolation (Schneller and Liebst 2007; Ranker and Sundue 2015; Testo et al. 2015). The most remarkable evidence for the slow evolution of reproductive isolation in ferns is the existence of naturally forming intergeneric hybrids between lineages that diverged tens or even hundreds of millions of years ago (Rothfels et al. 2015; Lehtonen 2018). These extreme examples are amongst the deepest hybridization events known and have led some authors (Rothfels et al. 2015; Ranker and Sundue 2015) to hypothesize that the slow "speciation clock" (sensu Coyne and Orr 1989) in ferns may help explain the clade's modest species richness in comparison to angiosperms.

Despite the prominence of hybridization in ferns, the frequency of documented hybrids varies considerably both among geographic areas and taxonomic groups. While rates of hybridization vary among fern lineages (Liu et al. 2020), evidence suggests that understanding of the occurrence of hybridization in ferns is skewed by several sampling biases. Perhaps the most prominent of these biases is a tendency for hybrids to be documented in well-studied, relatively species-poor temperate regions compared to highly diverse tropical regions with poorly known floras. Liu et al. (2020) reported that documented hybrids were overrepresented in temperate regions; indeed, the most thoroughly studied and taxon-rich reticulate fern complexes are mostly or entirely temperate, e.g. *Polypodium vulgare* L. group (Haufler et al. 1995; Sigel et al. 2014), North American *Dryopteris* Adans. (Walker 1961; Sessa et al. 2012), and Eurasian *Dryopteris affinis* Kinahan complex, (Fraser-Jenkins 1980, 2007). The relative paucity of tropical fern hybrids could be due to different speciation processes between temperate and tropical lineages (Haufler et al. 2000). More likely, however, is that it reflects a lack of information about many tropical taxa and the difficulty of detecting hybrids in highly diverse tropical groups.

Of the tropical fern hybrids that have been described and whose origins have been characterized, many appear to have originated from hybridization events between taxa with differing levels of leaf dissection. Because hybrids tend to be morphologically intermediate to their progenitors and leaf architecture is amongst the most conspicuous features of fern morphology, hybrids between species with dissimilar leaf division are readily detected and presumably more likely to be described. Such hybrids have been described in an array of tropical fern genera, including: *Adiantum* L. (Moran and Watkins 2002; Prado 2005), *Diplazium* Sw. (Testo et al. 2017), *Pleopeltis* Humb. & Bonpl. ex Willd. (Wagner and Wagner 1975; Anthony and Schelpe 1985; Mickel and Beitel 1987), *Serpocaulon* A.R.Sm. (Sanín and Torrez 2014), *Tectaria* Cav. (Wagner et al. 1978), and even between genera: *Cyclodium* C.Presl and *Polybotrya* Humb. & Bonpl. ex Willd. (Engels and Canestraro 2017; Schwartsburd et al. 2018), *Amblovenatum* J.P.Roux and *Christella* H.Lév. (Almeida et al. 2023). In several cases, these hybrids are known from one or a few collections, but are so distinct morphologically that their origins are readily evident, even at first glance.

By contrast, hybrids between species with similar leaf division are often difficult to identify, especially in the absence of complementary (e.g. DNA, cytological) datasets. This likely

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helps explains the fewer hybrids reported from some principally tropical fern clades that exhibit little variation in leaf division, such as *Elaphoglossum* Schott ex J.Sm. (Dryopteridaceae), which includes at least 600 species but just six reported hybrids (Liu et al. 2020). Similarly, just 50 hybrids have been reported for the hyperdiverse and principally tropical family Thelypteridaceae, which includes approximately 1200 species (Fawcett and Smith 2021), far fewer than some other large fern families such as Aspleniaceae (ca. 700 species, 135 hybrids) or Dryopteridaceae (ca. 2115 species and 211 hybrids) (Liu et al. 2020). The low number of hybrid Thelypteridaceae taxa characterized to date likely reflects in part the fact that, in comparison to these other families, many genera of Thelypteridaceae present little variation in leaf division and "many species appear superficially similar" (Fawcett and Smith 2021). Given that roughly 40% of Thelypteridaceae species are either polyploid or include a polyploid cytotype and that hybridization is prominent in some clades with unusually variable leaf morphology (e.g. Antillean *Goniopteris* C.Presl) (Sánchez 2017; Fawcett and Smith 2021), it is likely that hybridization (and, by extension, allopolyploidy) in the family is considerably underreported on account of the generally subtle variation in leaf morphology.

Here, we describe a hybrid *Goniopteris* (Thelypteridaceae) from northeastern Costa Rica based on morphology and analysis of target-capture DNA sequence data. With approximately 140 species, *Goniopteris* is the third largest genus of Thelypteridaceae, after *Amauropelta* Kunze and *Sphaerostephanos* J.Sm. (Fawcett and Smith 2021). Morphologically, the genus is characterized by the presence of stellate or branched hairs, usually on the leaf surface and/or on the rhizome scales; a small number of species lack such hairs. It is an entirely neotropical group that reaches its highest species richness in wet lowland forests of Central and South America, with an additional radiation of calciphile species in the Antilles. Numerous hybrids have been reported in the genus; almost all of these involve members of the Antillean calciphile clade (Proctor 1989; Sánchez 2017; Fawcett and Smith 2021).

Although the hybrid described here is known only from a single locality and a few collections, it is important to describe it formally for several reasons. First, formal, binomial names are more nomenclaturally stable than formulaic names and are based on a type specimen (Wagner and Wagner 1969). They do not change if one of the parents suffers a later name change, and they do not change if the parentage is later determined to be different than that originally proposed. Second, the hybrid occurs at an important field station in a locality that is frequently the setting for research and educational activities that might incorporate it.

Materials and Methods

Study Site—The study was carried out at the La Selva Biological Station near Puerto Viejo de Sarapiquí, Provincia Heredia, Costa Rica. The putative hybrid has only been recorded in a single small (ca. 5 × 5 m) area of the station's arboretum, approximately 400 m from the start of the SUR trail. The arboretum is highly disturbed due to frequent grass cutting and other activities. Based on examination of specimens at the station's herbarium (LSCR) and digitized herbarium records (www.pteridoportal.org), 11 species of *Goniopteris* have been recorded from La Selva; two of these (*Goniopteris mollis* Fée and *G. nicaraguensis* (E.Fourn.) Salino & T.E.Almeida) are present in the arboretum area where the hybrid is known to occur.

Morphological Analysis—Morphometrics—Morphological measurements were made from 10 leaves each of the hybrid, *G. nicaraguensis*, and *G. mollis*. All plants grew together in an open, disturbed area. The characters measured were: number of pinna pairs, pinna length, pinna width, pinna dissection, and number of vein pairs joined between the costa and pinna margin. Pinna length and width were measured on the second-most distal pinna on the right-hand side of the leaf. Pinna dissection was measured on the middle of the pinna and was calculated as the depth of the sinus as a proportion of the distance between the pinna margin and the costa; the dissection level at five sinuses was measured on a single pinna, and the mean value was recorded. Trait values between species were compared using one-way ANOVA and post-hoc Tukey HSD tests implemented in R 4.2.2. (R Core Team 2022); data were processed and visualized using the packages dplyr (Wickham et al. 2023), magrittr (Bache & Wickham 2022), ggplot2 (Wickham 2016), and ggforce (Pedersen 2022). All measurements and code used to conduct morphological analyses are available on Github (https://github.com/wtesto/GoniopterisHybridMorphology) and Zenodo (DOI: 10.5281/zenodo.7832161).

Imaging and Microscopy—Samples were collected from fully developed specimens of the hybrid, *G. nicaraguensis*, and *G. mollis* growing cultivated and collected from the study site. Spores were removed from living specimens and stored in ethanol (70%), then affixed to 15 mm stubs with double-sided carbon tape and air-dried for 24 hr at 40°C. Dry spores were coated with a 5 nm layer of gold at 20mA for 8 min using a sputter coater (EMS 150RS). Sections of the rachis and lamina were fixed using Karnovsky's solution for 48 hr, then washed twice with 0.2 M phosphate buffer for 15 min each, then post-fixed with 2% osmium tetroxide (OsO4) for 2 h, followed by two washes with 0.2 M phosphate buffer for 10 min each. All samples were dehydrated over an ethanol series (increasing concentrations: 30%, 50%, 70%, 80%, 90%, 95%, and two baths of 100%, for 10 min each). Sections were placed in an isoamyl acetate (C7H14O2)–ethanol bath (1:1, 15 min), followed by a 100% isoamyl acetate bath (15 min). We used a Leica EM-CPD300 critical point dryer to dry sections. Dry samples were mounted and coated with gold following the same procedure for spores. We photographed samples using a ZEISS SIGMA 300 field emission scanning electron microscope (SEM) in the CIEMIC (Research Center on Microscopic Structures, University of Costa Rica).

Phylogenetic Analyses—Sampling—A total of 26 samples representing 19 *Goniopteris* taxa (including the putative hybrid) were included in this study. Raw read paired Illumina Capture-Seq data were obtained from the NCBI Sequence Read Archive (BioProject PRJNA646399); these data were originally published as part of a phylogenomic study of the Thelypteridaceae by Fawcett et al. (2021) using the GoFlag 408 probe set (Breinholt et al. 2021). Taxon sampling was focused on the core "Mexico/Mesoamerican" clade recovered by Fawcett et al. (2021) plus *Goniopteris holodictya* (K.U.Kramer) Salino & T.E.Almeida, which is sister to the remainder of the genus (Appendix 1). This sampling included the putative hybrid and four *Goniopteris* species known from La Selva, including both species present at the study site.

Trimming and Assembly—Illumina primers and low-quality base pairs were trimmed using Trimmomatic (version 0.36, Bolger et al. 2014); settings were: illuminaclip 2:30:8, leading 20, trailing 20, sliding window 10:20, minimum length 40. HybPiper (version 2.0.1, Johnson et al. 2016) was used to assemble sequences, using a custom reference file developed using the *Christella acuminata* (Houtt.) Holttum and *Woodsia scopulina* D.C.Eaton bait sequences from the GoFlag probe set. Individual reads were mapped to the reference file using BWA (version 0.7.17; Li and Durbin 2009) and de novo contig assembly was performed using SPAdes (version 3.15.0, Bankevich et al. 2012); the "intronerate" option was not used. All analyses were run on the Grainger Bioinformatics Center server at the Field Museum of Natural History. All files and custom code used to complete this workflow are available on Github (https://github.com/wtesto/GoFlagHybPipeline) and Zenodo (DOI: 10.5281/zenodo.7832165).

Assessment of Heterozygosity—The HybPhaser v. 2.0 workflow (Nauheimer et al. 2021) was used to identify taxa of putative hybrid origin by identifying accessions with highly divergent sequence variants (hereafter "alleles") across a large proportion of sampled loci and phasing reads from these accessions. The workflow includes four main parts: 1) assessment of heterozygosity, 2) clade association of putative hybrids, 3) read phasing, and 4) re-processing of phased reads.

In the first step, consensus sequences are generated for each locus by mapping reads to de novo assembled contigs and assigning IUPAC ambiguity codes at heterozygous sites. We set a conservative filter for calling SNPs that required $> 10 \times$ coverage, a minor allele frequency of > 0.2, and occurrence of the minor allele in at least 4 reads. The resulting consensus sequences are then used to calculate the percentage of loci with heterozygous sites (locus heterozygosity, LH) and the percentage of total sites that are heterozygous across all loci (allele divergence, AD); these metrics were then used to identify putative hybrids. Based on Nauheimer

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et al. (2021), we anticipated that accessions of hybrid origin would have LH ≥ 80% and AD ≥ 2.0.

In the second step, we used BBSplit (BBMap, v38.47, Bushnell 2014) to map reads from accessions of putative hybrid origin (those with LH ≥ 80% and AD ≥ 2.0) to potential progenitors. To do so, reference accessions of user-defined clades must be selected; we selected a single accession of each taxon that did not exceed our thresholds for putative hybridity. The number of reads that unambiguously mapped to each clade reference accession were recorded; accessions with a high percentage of reads mapped to several clade references were identified as candidates for phasing.

In the third step, the accession of the putative hybrid was selected for read phasing. BBSplit was used to map reads from this accession to the two clade references that had the highest percentage of mapped reads in the previous step of the workflow. Reads mapped unambiguously to each clade reference were then saved to separate files; these groups of phased reads then were treated as independent accessions for downstream phylogenetic analyses.

In the fourth step, the separate groups of read files of the phased accession were assembled using HybPiper, following the same approach as detailed in the *Trimming and assembly* section. The resulting assembled loci for the phased accessions (two separate groups for each phased accession) were then combined with the original dataset; the original, non-phased data from the accessions of the putative hybrid were removed. The final dataset consisted of multiple sequence alignments for each locus, including two separate sequences for the putative hybrid accession. Sequence alignments and supplemental materials have been deposited in the Dryad Digital Repository (Sorojsrisom et al. 2023).

Tree Building—RAxML-VI-HPC (v. 2.2.3; Stamatakis 2006) was used to infer gene trees for each aligned locus; tree inference was run using the GTRGAMMA + I model and the rapid hill climbing algorithm and a random seed number. ASTRAL III (v. 5.7.8, Zhang et al. 2018) was used to infer a coalescence-based species tree from the gene trees inferred using RAxML. The resulting species tree was visualized using FigTree (v. 1.4.4; https://github.com/rambaut/figtree); node support values are local posterior probabilities calculated in ASTRAL (Sayyari and Mirarab 2016).

Results

Morphology—The putative hybrid was intermediate between *Goniopteris nicaraguensis* and *G. mollis* in most of the morphological traits measured (Figs. 1–2; Table 1). Venation patterns differed between the three taxa: *G. nicaraguensis* on average had fewer than 1 vein pair (0.7 ± 0.5) joining below the sinus, whereas the hybrid had 2.5 ± 0.7, and *G. mollis* had 10.0 ± 1.5 joined vein pairs (Fig. 3). The presence, distribution, size, and type of hairs present on the leaves of the three taxa varied conspicuously (Fig. 4A, D, G). *Goniopteris nicaraguensis* had both dense 55–85 μm long furcate hairs and 350–420 μm long acicular hairs sparsely distributed on the petiole, rachis, and costae, as well as 70–95 μm long acicular hairs sparsely distributed on the abaxial veins. The sporangial capsules of *G. nicaraguensis* were also provided with short (40–50 μm) acicular hairs. *Goniopteris mollis* lacked furcate hairs but possessed acicular hairs 340–1230 μm long on the petiole, rachis, costae, veins, and across the laminar surface between the veins. The sporangial capsules of *G. mollis* were glabrous, though hairs were present on the receptacle. The hybrid had acicular hairs 160–365 μm long on the petiole, rachis, costae, veins, and across the laminar surface between the veins and sparse

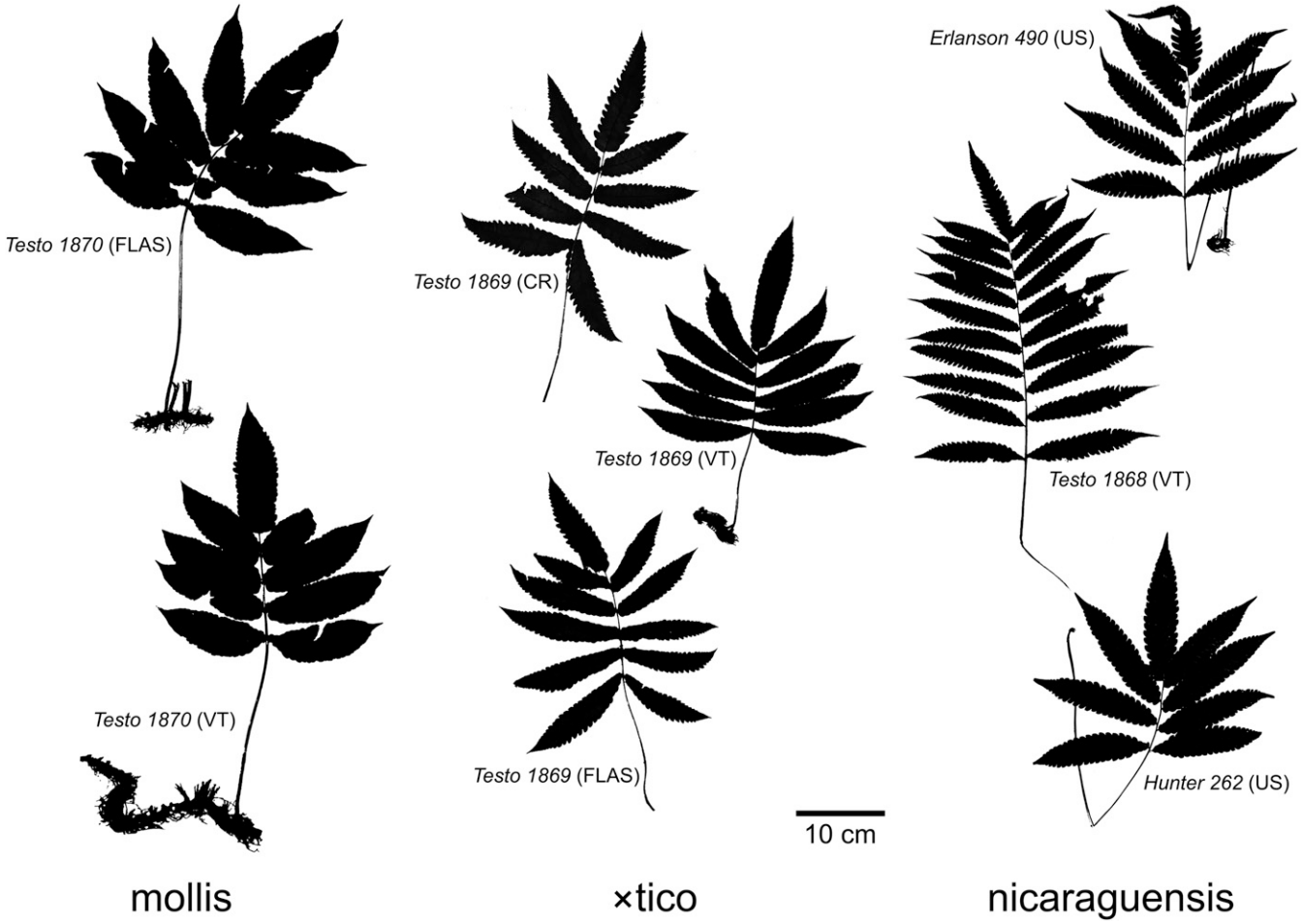

Fig. 1. Shadow diagram comparing gross leaf morphology of *Goniopteris mollis* (left), *Goniopteris ×tico* (center), and *Goniopteris nicaraguensis* (right).

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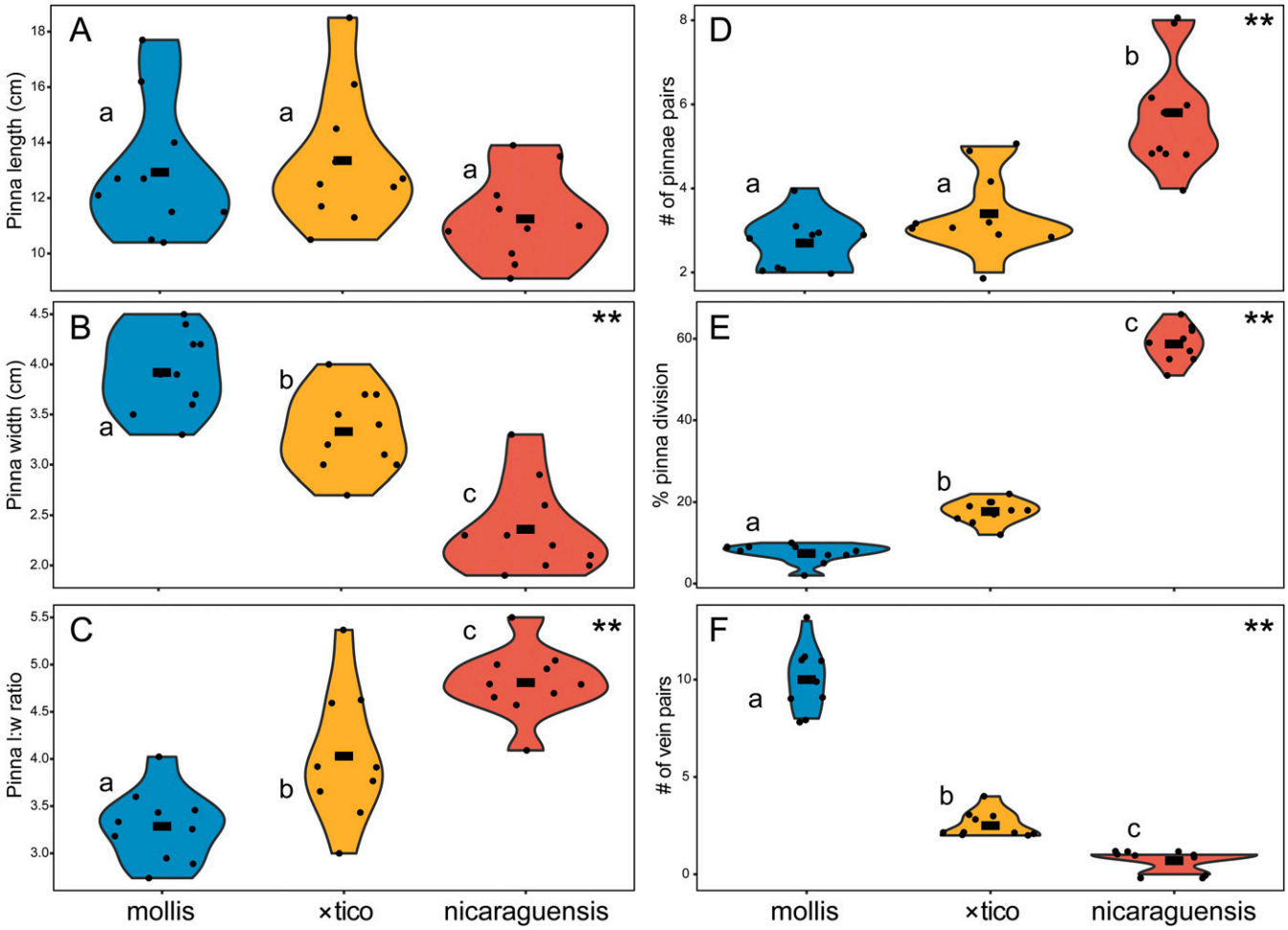

FIG. 2. Violin plots of A) pinna length, B) pinna width, C) pinnae length:width ratio, D) number of pinna pairs, E) level of pinna division, and F) number of vein pairs between the costa and pinna margin in *Goniopteris mollis* (left), *Goniopteris ×tico* (center), and *Goniopteris nicaraguensis* (right). Black dots represent individual measurements, the width of the colored symbol represents the relative frequency of observed values, and the horizontal bar indicates the median of the observed values. Lower case letters indicate assignment to groups based on a one-way ANOVA and post-hoc Tukey HSD test; ** indicate significant differences between groups with $p < 0.01$.

furcate hairs 110–200 μm long on the petiole and rachis. The sporangial capsules of the hybrid were glabrous and no hairs were observed in the sori.

Spores of both *G. nicaraguensis* and *G. mollis* were regularly formed, monolete, and provided with large reticulate crests (Fig. 4C, I). Spores of the hybrid were irregular, with some appearing well-formed and others clearly misshapen and apparently hollow (Fig. 4F).

Phylogenetic Analyses—TRIMMING AND ASSEMBLY—HybPiper recovered up to 356 out of 375 exons (min = 349, median = 352) with an average of 257,906 reads mapped per sample (min = 99,850, max = 448,624) and up to 65,622 bp long (min = 62,634, median = 62,970) (Supplemental Table 1).

ASSESSMENT OF HETEROZYGOSITY—Four accessions (two samples of *Goniopteris poiteana*, one of *Goniopteris* × *rolandii*, and the putative hybrid) had high proportions of loci with heterozygous SNPs (LH ≥ 80%) and highly divergent alleles (AD ≥ 2.0) (Supplemental Table 1). The initial clade association assessment of reads of the putative hybrid accessions revealed strong grouping with *G. mollis* (47% of all reads mapped) and *G. nicaraguensis* (15% of all reads mapped); no other clade reference received more than 8% of mapped reads and most received fewer than 2% (Supplemental Table 2). *Goniopteris mollis* and *G. nicaraguensis* were then selected as the two clade references for read phasing; the total number of reads unambiguously mapped to these references were 19,690 and 16,094, respectively.

TABLE 1. Morphological comparison of *Goniopteris ×tico* and its progenitor species. Numerical values are means and standard deviation (in parentheses). Asterisks (*) denote characters for which values of the hybrid are intermediate to its parents.

Species	Number of pinna pairs	Pinna length (cm)	Pinna width (cm)	Pinna dissection (proportion)	Number of vein pairs below sinus	Spore shape
Goniopteris nicaraguensis	5.8 (1.32)	11.25 (1.57)	2.36 (0.45)	0.587 (0.04)	0.7 (0.48)	Normal
Goniopteris × *tico*	3.4* (0.97)	13.35 (2.42)	3.33* (0.40)	0.177* (0.03)	2.5* (0.71)	Irregular
Goniopteris mollis	2.7 (0.65)	12.93 (2.28)	3.92 (0.38)	0.074 (0.02)	10.0 (1.51)	Normal

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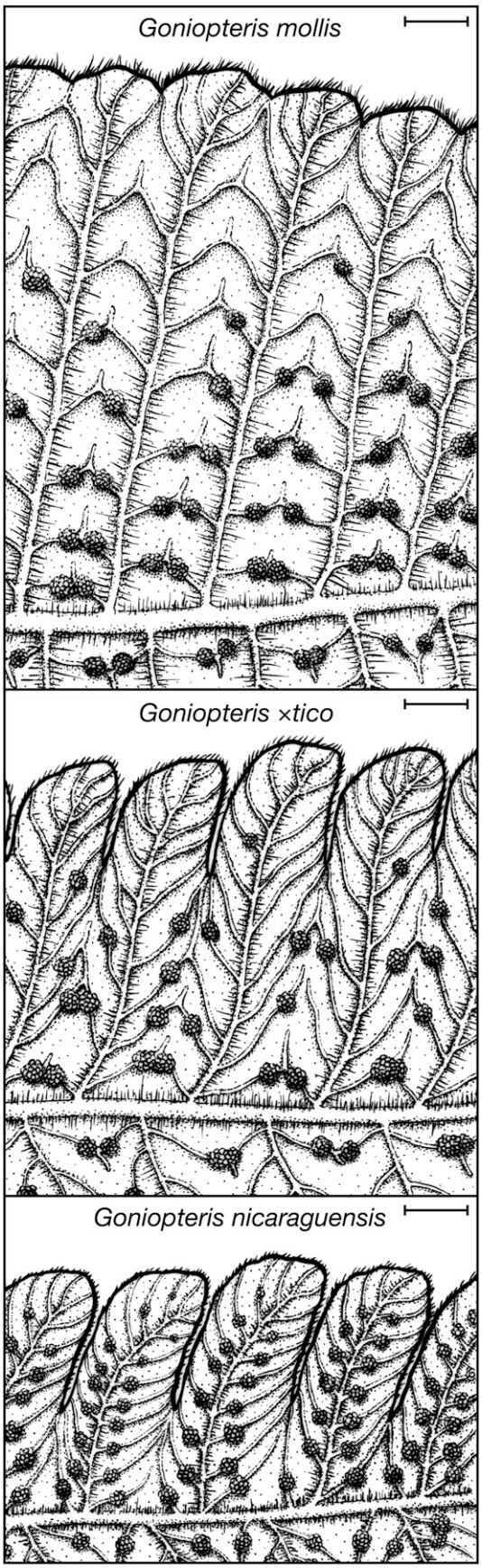

FIG. 3. Line drawing comparison of venation and mature sori of *Goniopteris mollis*, *Goniopteris* ×*tico*, and *Goniopteris nicaraguensis*. All scales = 3 mm.

TREE BUILDING—The ASTRAL phylogeny was generally strongly supported (Fig. 5A, B) and topologically consistent with a recently published phylogeny of the Thelypteridaceae (Fawcett et al. 2021), from which these sequence data were obtained. The phased accessions of the putative hybrid were resolved in two different clades. The first was placed with strong support (PP = 1) as sister to *Goniopteris mollis* in a clade that also included two accessions of *G. poiteana* and *G.* × *rolandii*. The second accession of the putative hybrid was sister to a small clade of three accessions of *G. nicaraguensis* and *Goniopteris oroniensis* (L.D.Gómez) Salino & T.E.Almeida (Fig. 5A, B).

DISCUSSION

Our morphological and phylogenomic analyses support the hypothesis that the putative hybrid is sterile and derived from *G. mollis* and *G. nicaraguensis*, so we propose a binomial here.

TAXONOMIC TREATMENT

Goniopteris* ×*tico Testo, Sorojsrisom, & O.Alvarado. hyb. nov. TYPE: COSTA RICA. Prov. Heredia, Sarapiquí, Puerto Viejo. Estación Biológica La Selva, near Puerto Viejo. 50–150 m elev., 10.428 N, 84.008 W. 16 Jan 2019. *W. L. Testo 1869* (holotype: VT!, isotypes: CR!, FLAS!).

Rhizomes subterranean, short-creeping, 1.0–1.3 cm diameter; rhizome scales concolorous, golden brown, broadly lanceolate, margins entire. **Leaves** 34–50 × 19–37 cm, distichous, arising at 1–1.5 cm intervals from the rhizome, monomorphic; petioles 11–16 cm, with conspicuous acicular hairs 200–365 μm long and sparser furcate hairs 110–200 μm long; rachis with acicular hairs 100–250 μm long and sparser furcate hairs 110–200 μm long; lamina 25–39 × 19–37 cm, ovate to broadly lanceolate, 1-pinnate-pinnatifid, apex conform, with acicular hairs 100–145 μm long on veins, laminar surface, and margins; pinnae 9.5–18.5 × 2.7–4.0 cm, in 3–5 pairs, elliptical to oblanceolate, lobed 0.1–0.3 times the distance to the costa; costae conspicuously pubescent throughout with acicular hairs 0.1–0.2 mm long; veins with sparse acicular hairs 0.1–0.2 mm long, 2–4 pairs of veinlets from adjacent principal veins joining below the sinus and forming excurrent veinlets. **Sori** round, exindusiate, sparsely and irregularly distributed on proximal pairs of veinlets at or below points of union; sporangia regularly formed, glabrous, annulus with 12–13 cells. **Spores** monolete, many irregularly formed, well-formed spores 28–34 × 20–23 μm, perispore cristate, with interrupted folds and sparse conical projections; irregular spores of various sizes. Figures 1, 3, 4D–F, 6.

Ecology and Distribution*—*Goniopteris* ×*tico is known only from a small area of the La Selva Biological Station near Puerto Viejo de Sarapiquí, in the Caribbean lowlands of northeastern Costa Rica (Fig. 7). It grows in highly disturbed secondary forest that is frequently cleared to maintain the station's arboretum and adjacent trails. We have been unable to find additional records of this hybrid despite the study of hundreds of herbarium specimens in Costa Rica and the United States (CR, F, FLAS, GH, NY, UC, US, USJ, VT). The hybrid, however, likely occurs elsewhere, as both putative progenitors grow together throughout much of Mesoamerica.

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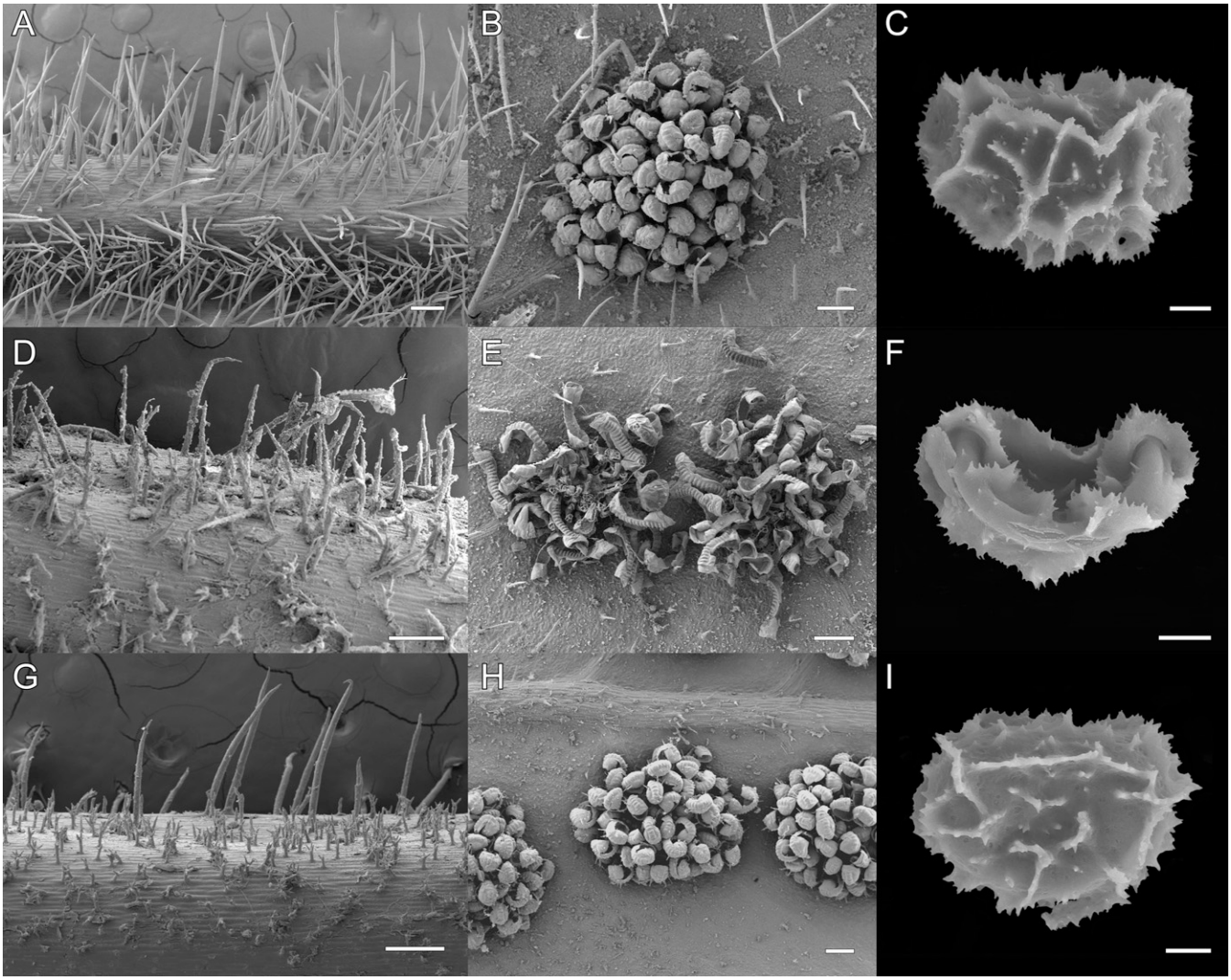

FIG. 4. SEM images of rachis indument (left), sori (center), and spores (right). A–C. *Goniopteris mollis*. D–F. *Goniopteris ×tico*. G–I. *Goniopteris nicaraguensis*. Scale bars = 6 μm for C, F, I; 200 μm for others.

Etymology—"Tico" is a colloquial Spanish-language term commonly used by inhabitants of Costa Rica to refer to themselves. In botanical nomenclature, has been incorporated in both generic names (e.g. *Ticodendron* Gómez-Laur. & L.D.Gómez, Ticodendraceae) and specific epithets (e.g. *Phlegmariurus tico* A.Rojas, Lycopodiaceae) of taxa endemic to Costa Rica, like this hybrid. It is used here as a noun in apposition.

***Additional Specimens Examined*—Costa Rica. —**HEREDIA: Sarapiquí, La Selva Biological Field Station, 10.432°N, 84.0287°W, 100 m, 18 Jan 2015, *M. Sundue 3931* (VT).

Although *Goniopteris ×tico* superficially resembles *G. nicaraguensis*, it is readily distinguishable from that species by its short-creeping rhizome, irregularly lobed pinnae, and partially anastomosing veins. It could also be mistaken for *G. mollis* due to abundant white acicular hairs on both leaf surfaces, but it has much more deeply lobed pinnae, has at least some furcate hairs on the leaf axes, and has fewer and less regular vein anastomoses.

Goniopteris × *rolandii* (C.Chr.) A.R.Sm., which appears to be a hybrid of *G. poiteana* and *Goniopteris tetragona* (Smith 1995), shares the leaf division, irregularly anastomosing venation, and short-creeping rhizome of *G. ×tico*, but differs by prominently setose sporangia and occasional proliferous buds at the base of distal pinnae. It is known from relatively few collections but is apparently widely distributed, with records from the Antilles, Nicaragua, Venezuela, and Ecuador (Smith 1995).

This study also demonstrates the utility of target-capture DNA sequence data obtained with the GoFlag 408 probe set for characterizing hybrid origins in ferns. At least one other study used the same type of data and approach to detect hybrids in Australian Thelypteridaceae (Bloesch et al. 2022). Although our phylogeny resolves one subgenome of the hybrid as sister to a clade comprising *Goniopteris oroniensis* and multiple accessions of *G. nicaraguensis*, we exclude *G. oroniensis* as a candidate progenitor, as it is very rare and only known from a few localities in far southeastern Costa Rica (Gomez 1978). When describing *G. oroniensis*, Gomez (1978) indicated that it was very similar to *G. nicaraguensis* and differed by conspicuously zig-zag rachises. The spores of the type specimen of *G. oroniensis* (*Ocampo 1635*, CR, UC, US) are also irregularly and misshapen, suggesting that it may also be a hybrid with *G. nicaraguensis* as a parent. Along with confirming the hybrid origin of *Goniopteris ×tico*, our phylogenomic analyses also indicate hybrid origins for at least two other taxa in our study set: *G. poiteana* and *G.* × *rolandii*. This approach shows promise for accelerating detection of hybridization in *Goniopteris* and other fern genera.

That this hybrid had not been previously recognized despite occurring in one of the most thoroughly studied field stations in tropical America highlights the cryptic nature of fern hybrids derived from progenitors with similar leaf morphologies. In these cases, study of morphology alone may not be sufficient to distinguish hybrids from their progenitors. Complementary data types such as DNA sequence data can play an important role in hybrid detection, as demonstrated here.

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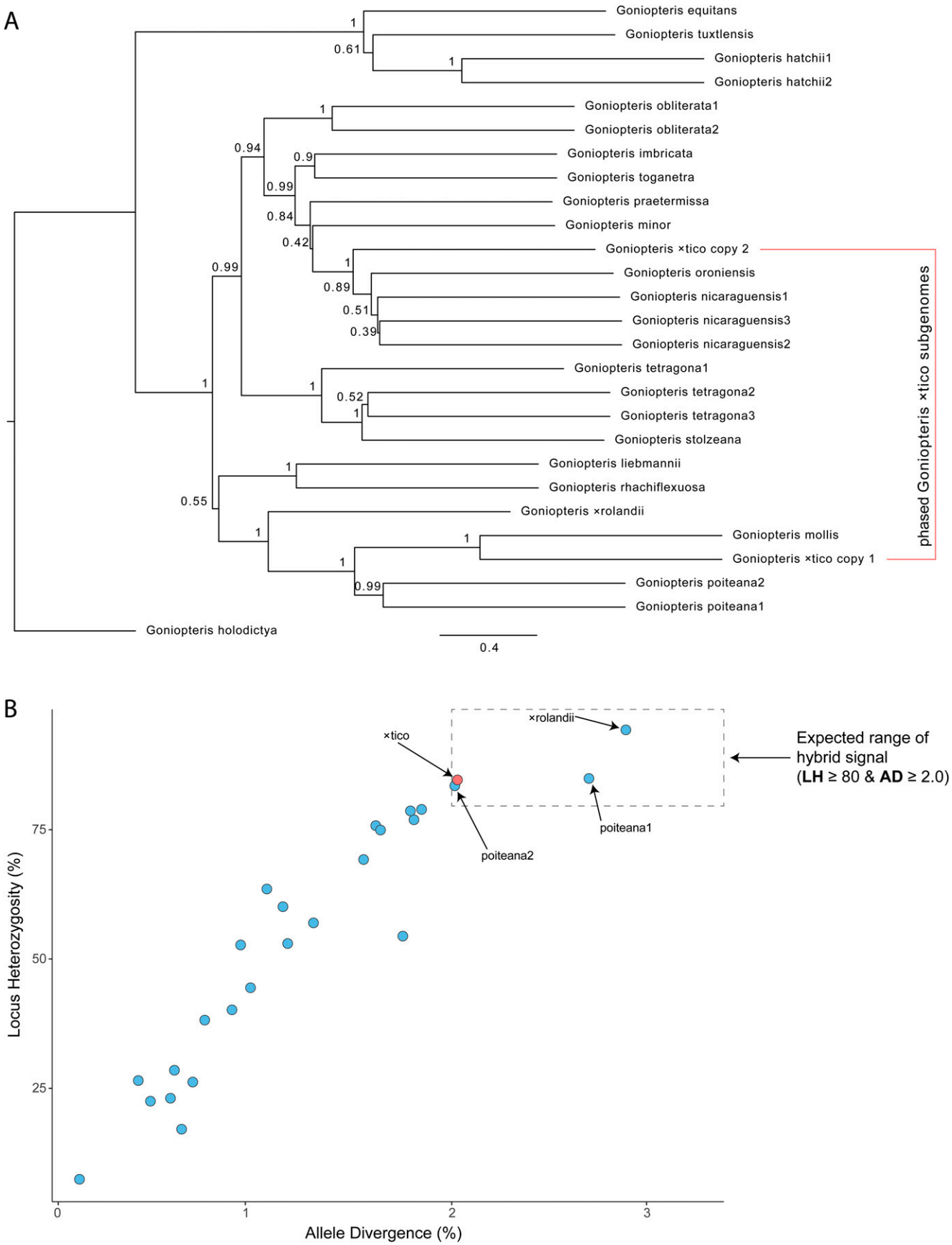

FIG. 5. A. Multispecies coalescent phylogeny of 26 *Goniopteris* samples inferred using ASTRAL from 356 nuclear loci. *Goniopteris* ×*tico* is represented by two terminals, corresponding to parental subgenomes inferred using the HybPhaser workflow. Support values are local posterior probabilities. B. Percentage of heterozygous loci (LH) and percentage of loci with > 0.5% allele divergence (AD) for 26 *Goniopteris* samples inferred from 356 nuclear loci using the HybPhaser workflow. Samples within the range of dashed rectangle (LH ≥ 80 and AD ≥ 2.0) are probable hybrids. *Goniopteris* ×*tico* is indicated with a red circle.

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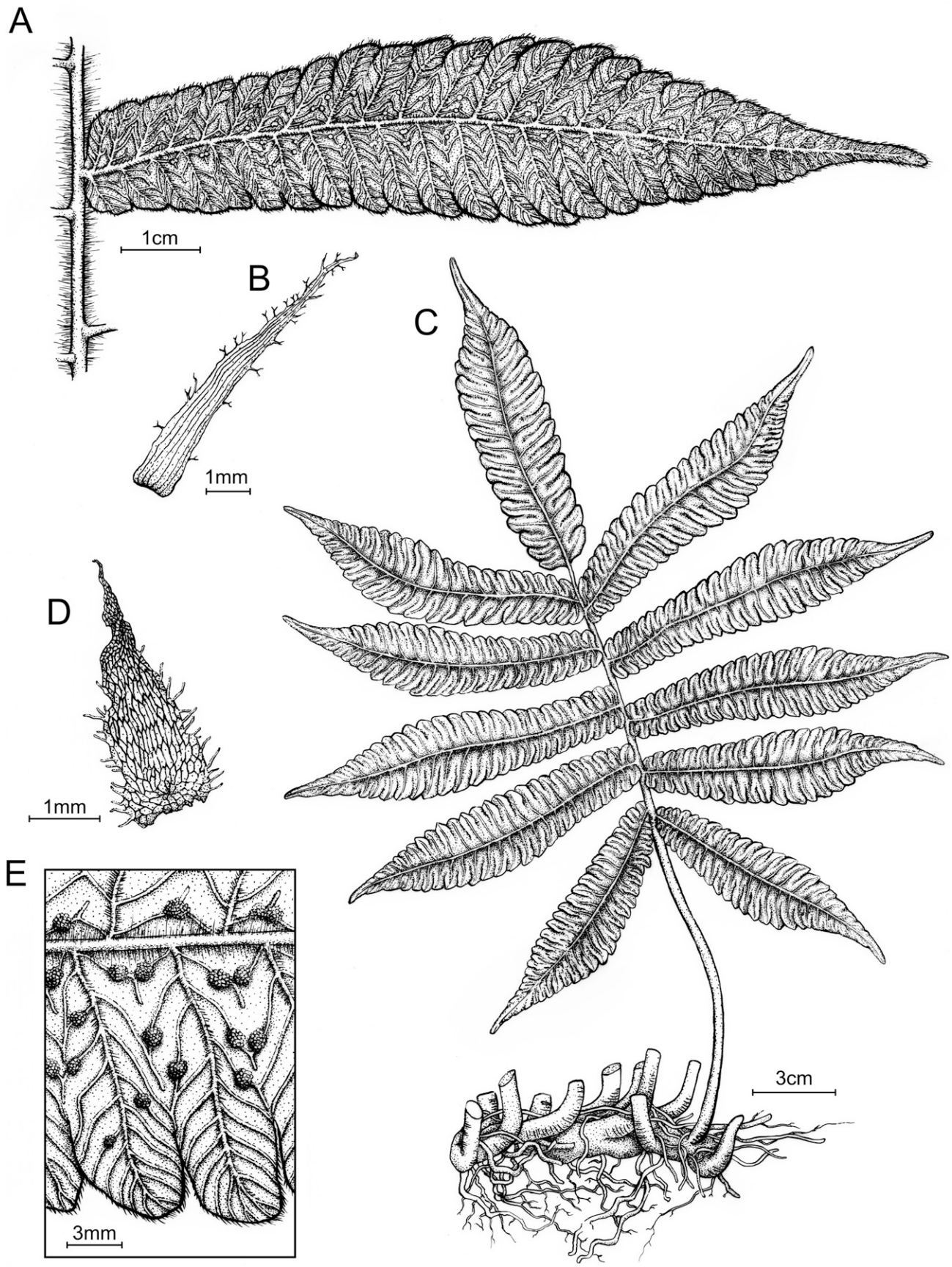

FIG. 6. *Goniopteris ×tico.* A. Pinna with immature sori. B. Petiole scale. C. Habit. D. Rhizome scale. E. Sori and venation detail.

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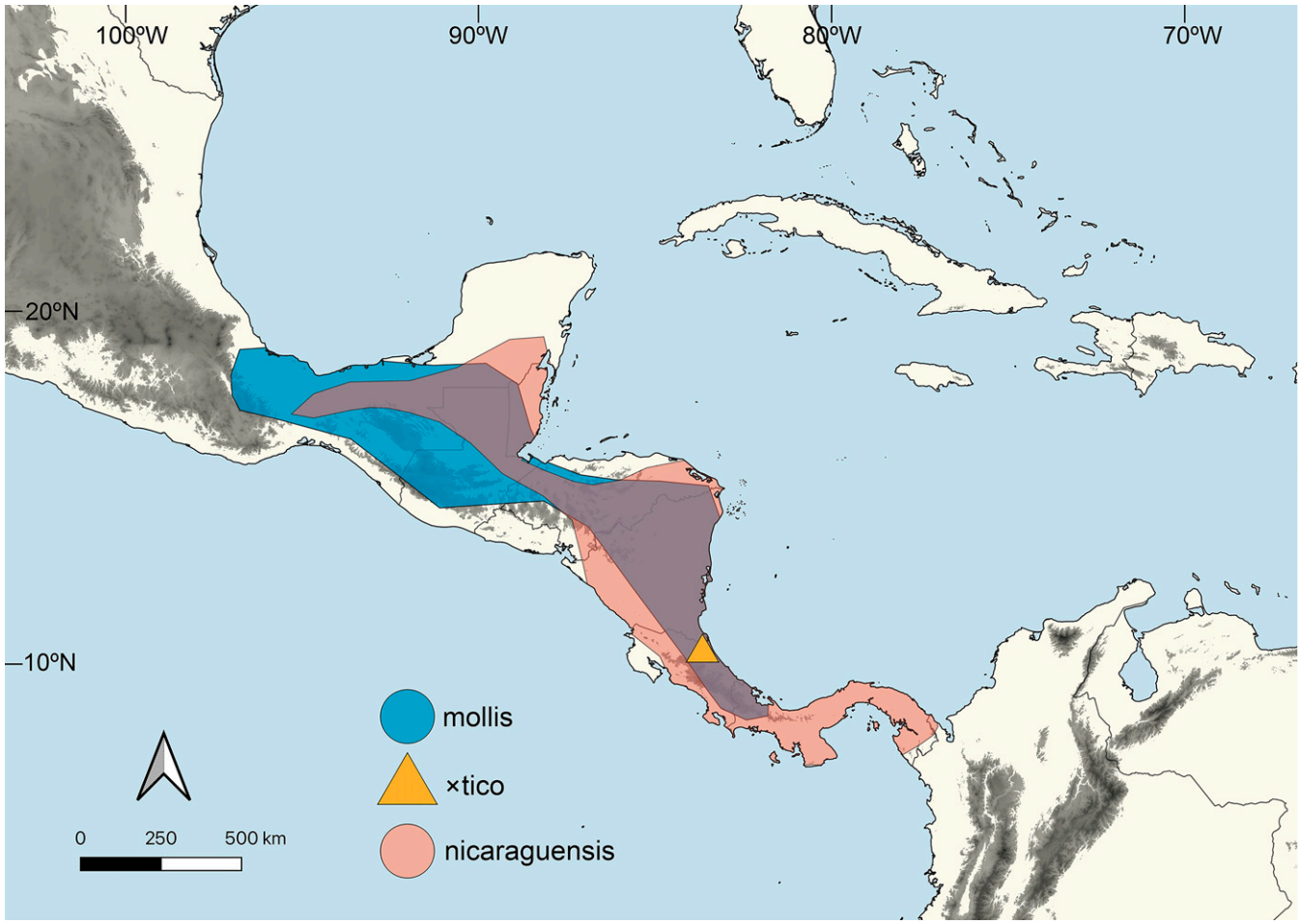

FIG. 7. Distribution map of *Goniopteris mollis* (blue), *Goniopteris nicaraguensis* (red), and *Goniopteris ×tico* (orange triangle).

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AUTHOR CONTRIBUTIONS

WT conceptualized the project and obtained collection permits. OAR and ES performed morphological measurements in the field. OAR prepared material and performed SEM imaging. WT performed phylogenetic and morphometric analyses and prepared the taxonomic treatment. ES created illustration plates. All authors contributed to descriptions and preparation of the manuscript.

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Appendix 1. Voucher data for specimens used in molecular phylogenetic analyses. Specimens are arranged by species in alphabetical order, with collector name and number, acronym of the herbarium where the specimen is deposited, barcode number (in parentheses, when available), and NCBI Sequence Read Archive accession number. All sequence data were generated by Fawcett et al. (2021); additional details can be found in that publication.

Goniopteris equitans (Christ) Salino & T.E.Almeida, *Testo 1252*, VT (UVMVT290301), SRS12394805; *Goniopteris hatchii* (A.R.Sm.) Á.Löve & D.Löve, *Sundue 4007*, VT, SRS12394566; *Goniopteris hatchii* (A.R.Sm.) Á.Löve & D.Löve, *Testo 877*, VT (UVMVT197373), SRS12394566; *Goniopteris holodictya* (K.U.Kramer) Salino & T.E.Almeida, *Jansen-Jacobs 7034*, UC (UC1869861), SRR18496634; *Goniopteris imbricata* (Liebm.) Á.Löve & D.Löve, *González 17141*, UC (UC1534726), SRS12394573; *Goniopteris liebmannii* (Maxon & C.V.Morton) A.R.Sm. & Salino, *Carvajal-Hernández 943*, UC, SRS12394912; *Goniopteris minor* (C.Chr.) A.R.Sm., *Peña-Chocarro 2620*, UC (UC1981609), SRS12394919; *Goniopteris mollis* Fée, *Testo 923*, VT (UVMVT286639, UVMVT286640), SRS12394920; *Goniopteris nicaraguensis* (E.Fourn.) Salino & T.E.Almeida, *Fawcett 722*, VT (UVMVT286845), SRS12394928; *Goniopteris nicaraguensis* (E.Fourn.) Salino & T.E.Almeida, *Testo 530*, VT (UVMVT286638), SRS12394929; *Goniopteris nicaraguensis* (E.Fourn.) Salino & T.E.Almeida, *Testo 791*, VT (UVMVT197472), SRS12394819; *Goniopteris obliterata* (Sw.) C.Presl, *Sander 17618*, UC, SRS12394930; *Goniopteris obliterata* (Sw.) C.Presl, *Testo 1047*, VT (UC2048974), SRS12394795; *Goniopteris oroniensis* (L.D.Gómez) Salino & T.E.Almeida,

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Grayum 3633, UC (UC1506549), SRS12394934; *Goniopteris poiteana* (Bory) Ching, *Fawcett 395*, VT, SRS12395095; *Goniopteris poiteana* (Bory) Ching, *Testo 1053*, VT (UVMVT286646), SRS12395097; *Goniopteris praetermissa* (Maxon) Salino & T.E.Almeida, *Croat 24548*, UC, SRS12395099; *Goniopteris rhachiflexuosa* (Riba) Salino & T.E.Almeida, *Pérez-Farrera 2412*, UC (UC1927079), SRS12395108; *Goniopteris* × *rolandii* (C.Chr.) A.R.Sm., *van der Werff 858*, UC (UC1465700), SRS12394582; *Goniopteris stolzeana* (A.R.Sm.) Salino & T.E.Almeida, *Silva 221*, Z, SRS12394816; *Goniopteris tetragona* (Sw.) C.Presl, *Kromer 4053*, UC, SRS12394820; *Goniopteris tetragona* (Sw.) C.Presl, *Proctor 18875*, GH, SRS12394821; *Goniopteris tetragona* (Sw.) C.Presl, *Testo 1547*, VT, SRS12394814; *Goniopteris* ×*tico* Testo, Sorojsrisom, & O.Alvarado, *Sundue 3931*, VT (UVMVT286641), SRS12394932; *Goniopteris toganetra* (A.R.Sm.) Á.Löve & D.Löve, *Martínez 11754*, UC (UC1605047), SRS12394822; *Goniopteris tuxtlensis* (T.Kromer, Acebey & A.R.Sm.) Salino & T.E.Almeida, *Kromer 3627*, UC, SRS12394829.

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