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Gaga, a New Fern Genus Segregated from *Cheilanthes* (Pteridaceae)

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Abstract—Ongoing molecular phylogenetic studies of cheilanthoid ferns confirm that the genus *Cheilanthes* (Pteridaceae) is polyphyletic. A monophyletic group of species within the hemionitid clade informally called the "*C. marginata* group" is here shown to be distinct from its closest relatives (the genus *Aspidotis*) and phylogenetically distant from the type species of *Cheilanthes*. This group is here segregated from *Cheilanthes* as the newly described genus, ***Gaga***. In this study, we use molecular data from four DNA regions (plastid: *matK*, *rbcL*, *trnG-R*; and nuclear: *gapCp*) together with spore data to circumscribe the morphological and geographical boundaries of the new genus and investigate reticulate evolution within the group. *Gaga* is distinguished from *Aspidotis* by its rounded to attenuate (vs. mucronate) segment apices, minutely bullate margins of mature leaves (vs. smooth at 40×), and less prominently lustrous and striate adaxial blade surfaces. The new genus is distinguished from *Cheilanthes* s. s. by its strongly differentiated, inframarginal pseudoindusia, the production of 64 small or 32 large spores (vs. 32 small or 16 large) per sporangium, and usually glabrous leaf blades. A total of nineteen species are recognized within *Gaga*; seventeen new combinations are made, and two new species, ***Gaga germanotta*** and ***Gaga monstraparva***, are described.

Keywords—Cheilanthoid ferns, hybridization, reticulate evolution, *matK*, Mexico, polyploidy, spore measurements.

Fern systematics has changed radically in the past decade due, in large part, to the rapid pace of molecular phylogenetic studies. Family-level classifications have been overhauled (Smith et al. 2006; Christenhusz et al. 2011; Rothfels et al. 2012), and substantial effort has been directed toward reassessing generic boundaries. This has resulted in the recognition of many new genera across the polypod ferns (e.g. *Moranopteris* R. Y. Hirai & J. Prado; *Alansmia* M. Kessler, Moguel, Sundue & Labiak; *Mickelia* R. C. Moran, Labiak & Sundue; *Leucotrichum* Labiak; *Serpocaulon* A. R. Sm.; *Araiostegiella* M. Kato & Tsutsumi). One notable exception to this trend involves cheilanthoid ferns (Pteridaceae), a monophyletic group of 400 + species that have diversified in semi-arid habitats normally avoided by ferns. Morphological convergence is rampant in this group (Gastony and Rollo 1998), which has been referred to as "the most contentious group of ferns with respect to a practical and natural generic classification" (Tryon and Tryon 1982: 248). Despite a growing number of phylogenetic studies indicating that most of the major genera are not monophyletic (Gastony and Rollo 1995, 1998; Schuettpelz et al. 2007; Kirkpatrick 2007; Prado et al. 2007; Zhang et al. 2007; Rothfels et al. 2008; Windham et al. 2009; Yesilyurt and Schneider 2010; Link-Perez et al. 2011; Eiserhardt et al. 2011), the boundaries of most cheilanthoid genera remain unchanged.

The main impediment to generic reorganization of cheilanthoid ferns is the large and loosely defined genus *Cheilanthes* Sw. Recent phylogenetic studies have documented the extensive polyphyly of *Cheilanthes* s. l., with species traditionally assigned to this genus appearing in every major clade of cheilanthoid ferns (Gastony and Rollo 1995, 1998; Schuettpelz et al. 2007; Zhang et al. 2007; Kirkpatrick 2007; Prado et al. 2007; Rothfels et al. 2008; Eiserhardt et al. 2011). These run the gamut from single, morphologically anomalous species nested within or among other cheilanthoid genera to diverse, well supported clades whose relationships to *Cheilanthes* s. s. are tenuous at best. Among the latter is what has been called the "*Cheilanthes marginata* group" (Mickel and Smith 2004; Li et al. 2011), a clade of about twenty species that represents an early diverging lineage within the hemionitid ferns (Windham et al. 2009; Windham et al. in prep.). This group is sister to the small (four species) genus *Aspidotis* (Nutt. ex Hook.) Copel. and phylogenetically isolated from the type species of *Cheilanthes*, *C. micropteris* Sw. (Kirkpatrick 2007; Eiserhardt et al. 2011; Windham et al. in prep). Given our current understanding of phylogenetic relationships among cheilanthoid ferns, inclusion of the "*C. marginata* group" in *Cheilanthes* is untenable because the resultant clade also encompasses the genus *Hemionitis*, which has priority over *Cheilanthes*. Such an expansion of *Hemionitis* would require hundreds of new combinations, subsume several diverse, well-characterized genera (e.g. *Doryopteris* J. Sm. and *Adiantopsis* Fée), and would be undefinable morphologically (Windham et al. in prep.). Other less disruptive options include subsuming the "*C. marginata* group" under *Aspidotis* (its sister clade) or recognizing it as a distinct genus. Because *Aspidotis* is a relatively distinct and morphologically cohesive group, we have chosen to segregate the "*C. marginata* group" as a new genus herein named *Gaga* (see Taxonomic Treatment).

Although about twenty species have been attributed to this group (Mickel and Smith 2004), only one has been sampled in previous phylogenetic studies (Kirkpatrick 2007; Rothfels et al. 2008; Eiserhardt et al. 2011), and the actual number of taxa involved is uncertain due to extensive hybridization, polyploidy, and apomixis. In this study, we use spore measurements to estimate the ploidy level and reproductive mode of each species in the genus. Sequence data from three plastid regions (*matK*, *rbcL*, and *trnG-R*) are used to assess the monophyly of the group, estimate species numbers and relationships, and assist in identifying maternal progenitors of hybrids. We also use sequences from the nuclear *gapCp* region, in conjunction with the spore data, to examine patterns of reticulate evolution within the genus and propose a new species level taxonomy for this group of cheilanthoid ferns.

Materials and Methods

Taxon Sampling—For spore analyses, a total of 44 samples from the "*Cheilanthes marginata* group" and three *Aspidotis* specimens were included, 42 of which were also sampled for the molecular study (Appendix 1). A total of 75 specimens (Appendix 1) representing all known species from the "*Cheilanthes marginata* group" (including one holotype and five isotypes) were sampled for the molecular phylogenetic study; 58 of these were previously included in a DNA barcoding study by Li et al. (2011). Materials for DNA extraction were obtained either from accessioned herbarium sheets or from the vouchered Fern Lab Database silica-dried tissue archive (fernlab.biology.duke.edu). Sequences for three plastid loci (*matK*, *rbcL*, and *trnG-R*) and one nuclear locus (*gapCp*) were

generated. Based on previous phylogenetic studies of cheilanthoid ferns (Kirkpatrick 2007; Windham et al. 2009; Eiserhardt et al. 2011) eight outgroup species were selected for the plastid analyses, including *C. micropteris* (the type species of *Cheilanthes*); for the analysis, of the nuclear locus, two species of *Aspidotis* were included as outgroups.

Reproductive Mode and Ploidy Assessment—We used the number of spores per sporangium to infer the reproductive mode of each fertile specimen included in the study. Cytogenetic analyses of two species now included in *Gaga* (*G. arizonica* and *G. cuneata*) indicate that, as with most leptosporangiate ferns, sexually reproducing individuals produce a preponderance of 64 spores per sporangium whereas apomicts produce 32 or 16 spores per sporangium (M. D. Windham unpubl. data). To infer reproductive mode for specimens included in the molecular analyses, one to three intact sporangia were removed from each specimen. These were placed in individual droplets of glycerol on a microscope slide and broken open using the tip of a needle. The number of spores per sporangium was estimated using a Leica MZ 125 dissecting microscope. Although rarely totaling exactly 32 or 64 (some spores usually are destroyed or lost in the process of breaking open the sporangium), estimated spore numbers formed two non-overlapping groups congruent with previous determinations of reproductive mode.

We also determined average spore size to assess the ploidy level of each fertile specimen. In cheilanthoid ferns, spore size has been demonstrated to be correlated with ploidy level; diploids have the smallest spores, followed by sexual tetraploids, apomictic triploids, and then higher polyploids (Windham and Rabe 1993; Grusz et al. 2009; Beck et al. 2010; Sigel et al. 2011). Spores from intact or nearly intact sporangia were dispersed in a glycerol drop on a slide. Spore images were then captured at 80 × magnification using a Canon EOS Rebel XSi digital camera mounted on a Leica MZ 125 dissecting microscope. Using ImageJ (Rasband 2011), at least 20 spores per sporangium (for sexual specimens) or 10 spores per sporangium (for apomictic specimens) were measured along their longest axis to determine the average spore length per specimen. Two specimens of *Gaga* and three of *Aspidotis* (Appendix 1; Fig. 1) for which we had both average spore length data and chromosome counts were used to correlate ploidy level with spore size. A Mann-Whitney U test was conducted using R (R Development Core Team 2008) to test the significance of observed differences in spore size.

DNA Extraction, Amplification, Cloning, and Sequencing—Genomic DNA was extracted using the QIAGEN DNeasy plant mini kit (Valencia, California) following published protocols (Schuettpelz and Pryer 2007). Amplification and sequencing of plastid *matK* and *rbcL* followed Li et al. (2011); for plastid *trnG-R*, the protocols of Nagalingum et al. (2007) and Beck et al. (2010) were used with two additional sequencing primers, trnGIF3 (5′-AATAAGGAACGAATTAARG-3′) and trnGIR3 (5′-CYTTAATTCGTTCCTTATT-3′). To amplify the nuclear *gapCp* region, two new internal primers, FWCHgapF1 (5′-TTGCTAAGGGCAATGCCTGCTTTTG-3′) and FWCHgapR1 (5′-CTYCCAGAGCTCAATGGTAAACT-3′), were designed to respectively pair with the published primers ESGAPCP8F1 and ESGAP11R1 (Schuettpelz et al. 2008), to yield two overlapping amplicons. The new primer combinations both yielded a single "short" copy-specific band (Schuettpelz et al. 2008), and the two amplicons had approximately 400 base pairs of overlap (out of a total length of 600–650 base pairs). Both amplicons were obtained from 77% of the specimens in the *gapCp* dataset. Each *gapCp* amplicon was cloned separately using the Promega pGEM-T cloning kit (Madison, Wisconsin). Colony amplifications and subsequent sequencing reactions were done using M13F and M13R primers. The resulting sequences from each specimen were then pooled and manually aligned into a single alignment using PhyDE v0.9971 (Müller et al. 2010). At least seven colony sequences were obtained for each specimen (ranging from 7–18, mean: 12; Appendix 1). Alleles were determined from these colony sequences according to the protocol established by Grusz et al. (2009). A maximum parsimony (MP) analysis was carried out in PAUP* v4.0a123 (Swofford 2002). On the unrooted MP tree, each minimum and recognizable clade with at least two sequences was considered to represent one allele. The consensus sequence that then represented the constituent sequences of that clade was derived from a 50% majority-rule consensus. In the rare event when

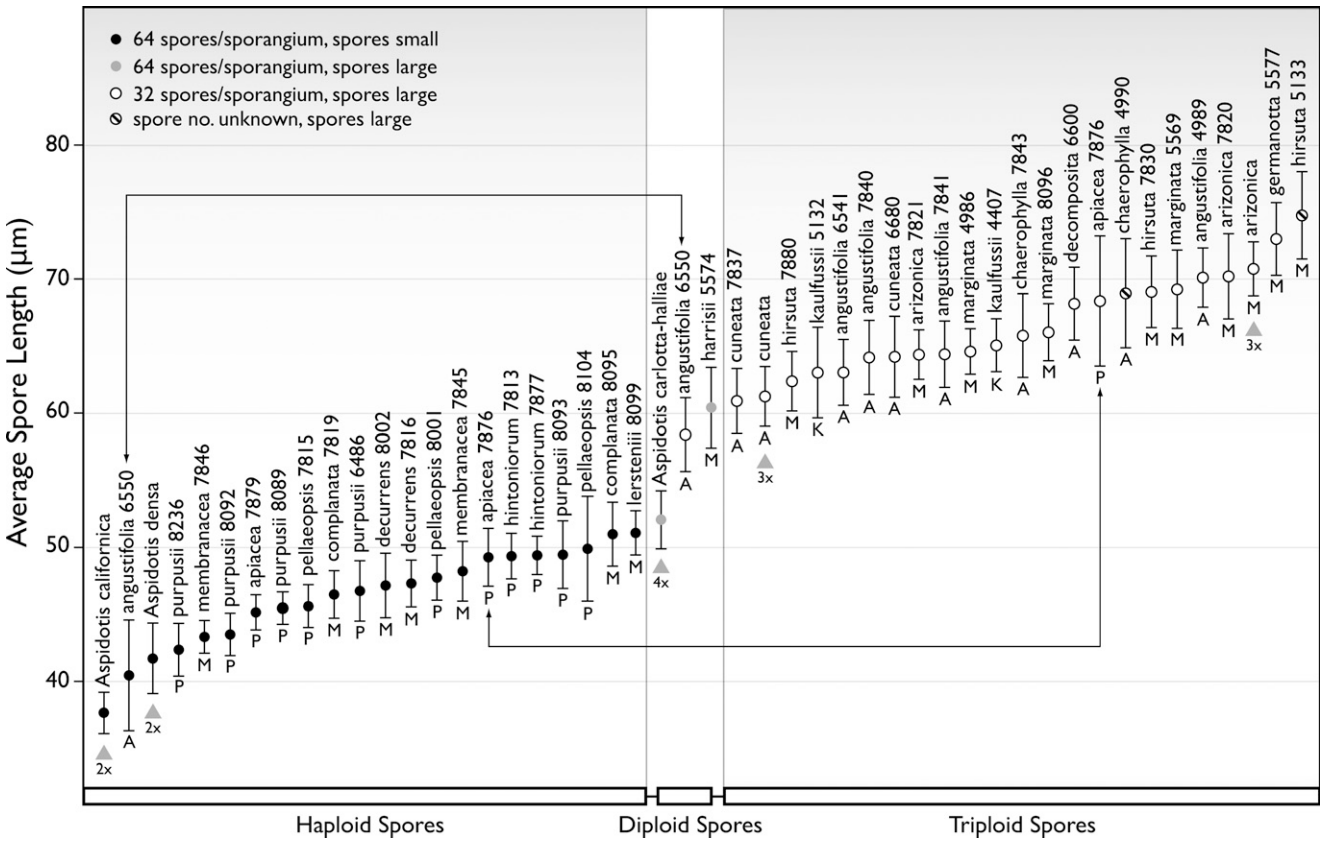

FIG. 1. Range of average spore lengths (µm) in species of *Gaga* and *Aspidotis*, with error bars indicating one standard deviation. 64- and 32-spored sporangia produced by a single individual are connected by fine lines terminating in arrowheads. Grey arrowheads identify voucher specimens for chromosome counts used to calibrate ploidy level determinations. The numbers associated with species names are the Fern Lab Database accession numbers (fernlab.biology.duke.edu), and the capital letters below the error bars identify the major *Gaga* clade within which the specimen is located (A = angustifolia clade, M = marginata clade, P = purpusii clade, K = kaulfussii clade).

there were chimeric colony sequences, these were easily identified in the alignment and removed prior to making a consensus sequence.

Phylogenetic Analyses—For each dataset, all sequences were aligned manually; only the *trnG-R* dataset has ambiguous alignment regions and these were excluded prior to phylogenetic analyses. Sequence evolution models for each dataset (*matK*, *rbcL*, *trnG-R*, and *gapCp*) were chosen using Modeltest (Posada and Crandall 1998; Swofford 2002) under the Akaike information criterion. When the best-fit model was not available in MrBayes v3.2 (Huelsenbeck and Ronquist 2001), the second-best-fit model was used. Maximum likelihood (ML) analyses were carried out using Garli v2.0 (Zwickl 2006) with "genthreshfortopoterm" set to 1,000,000 (100,000 for bootstrapping). Bootstrapping (BS) was done with 1,000 replicates to determine branch support. Because no topological incongruence (using a threshold BS > 70) was observed among the *matK*, *rbcL,* and *trnG-R* datasets, the three regions were combined into one dataset that was then subjected to ML and Bayesian inference (BI). For the combined plastid dataset (*matK* + *rbcL* + *trnG-R*), ML was run with three partitions, with the best-fit model assigned to each locus. BI was performed using MrBayes v3.2 (Huelsenbeck and Ronquist 2001). Two independent MCMC runs were carried out, each with four chains (one heated and three cold) running for 13 million generations. Priors followed the default settings with a flat Dirichlet distribution for both the stationary state frequencies and the substitution rates, and trees were sampled every 1,000 generations. For the combined plastid dataset, substitution parameters were unlinked across the loci partitions and the rate prior was set to vary among the partitions. After the MCMC runs, the output parameters were inspected in Tracer v1.5 (Rambaut and Drummond 2009) to ensure convergence. The first 25% of the sample was discarded as burn-in and the rest was used to calculate posterior probabilities (PP). For the nuclear *gapCp* dataset, ML and BI analyses were carried out as described above but as a single partition. The plastid datasets were congruent and therefore combined to provide the overall maternal phylogenetic framework. Because the nuclear dataset serves to assist in determining biparental contributions and multiple alleles were present for most of the polyploid taxa, it could not be combined with the plastid dataset. The complete sequence alignments are deposited in TreeBASE (study number TB2:S12433).

Results

Reproductive Mode and Ploidy Assessment (Fig. 1)—Forty-three specimens of *Gaga* and three of the sister genus *Aspidotis* were analyzed to assess reproductive mode and ploidy level. All *Aspidotis* and 21 of the *Gaga* specimens were categorized as sexually reproducing based on their production of 64-spored sporangia. Two of the latter (*G. angustifolia* 6550 and *G. apiacea* 7876; identified by long black arrows in Fig. 1) also produced rare, 32-spored sporangia containing larger, presumably unreduced spores. Twenty *Gaga* specimens produced only the 32 large spores per sporangium characteristic of apomictic taxa among most cheilanthoid ferns. Spore measurements form two sharply-defined groups ($p < 0.0001$ in Mann-Whitney U test; Fig. 1), one with a mean length of 46.54 μm (from *Aspidotis californica* to *A. carlotta-halliae*; Fig. 1), and the other with 66.26 μm (from *Gaga angustifolia* 6550 to *G. hirsuta* 5133). The small-spored group (left side of Fig. 1) includes 20 *Gaga* samples all of which were 64-spored. Although diploid chromosome counts have not yet been reported from *Gaga*, spore sizes in this group are only slightly larger than those of known diploids in the sister genus *Aspidotis* (Fig. 1) and well within the range observed for known sexual diploids in other closely related genera (Windham et al. in prep.). All members of this group have spores smaller than those of the documented tetraploid *A. carlotta-halliae* (Fig. 1), and we hypothesize that most (probably all) of these samples represent sexual diploid sporophytes producing haploid spores.

Among the 23 *Gaga* samples with known spore numbers belonging to the large-spored group (right side of Fig. 1), all but one (*G. harrisii* 5574) are 32-spored. Specimens with 32 spores per sporangium averaging more than 60.5 μm long (excluding the anomalous *G. apiacea* 7876) are interpreted as triploid apomicts because the voucher specimens of known triploids (indicated by grey arrowheads in Fig. 1) in *Gaga* span this size range. The two *Gaga* specimens at the lower end of the large-spored distribution (*G. angustifolia* 6550 and *G. harrisii* 5574) deserve special mention. Both have spores significantly larger than those of tetraploid *A. carlotta-halliae*, yet smaller than those of known apomictic triploids (Fig. 1). This particular spore sample from *G. angustifolia* 6550 derives from a rare 32-spored sporangium produced by a plant that showed a predominance of 64-spored sporangia (indicated by long black arrow in Fig. 1). Because *G. angustifolia* 6550 also produces spores at the low end of the sexual diploid range (similar in size to sexual diploid *Aspidotis*), we hypothesize that the contents of this 32-spored sporangium represent unreduced, diploid spores. As such, they should be comparable in size to the diploid spores produced by sexual tetraploids, which makes the data for *G. harrisii* 5574 even more interesting. This sample of *G. harrisii* is unique among the specimens examined in producing 64 large spores per sporangium (Fig. 1). Based on the available data, we hypothesize that this plant of *G. harrisii* is a sexual tetraploid.

Phylogenetic Analyses—Five DNA alignment data matrices (four plastid and one nuclear) were analyzed phylogenetically for this study; a summary of sequence characteristics, best-fit evolution models, and tree statistics appears in Table 1. The monophyly of the new genus *Gaga* (formerly the "*Cheilanthes marginata* group") is robustly supported in both plastid (Fig. 2) and nuclear (Fig. 3) analyses, as is its sister relationship to *Aspidotis*. The plastid *matK* gene, only recently added to the fern phylogenetics toolkit (Kuo et al. 2011), proves to be a particularly useful marker with as many variable sites as the non-coding *trnG-R* region (Table 1). Four major monophyletic groups, here designated the marginata, purpusii, angustifolia, and kaulfussii clades, are resolved within *Gaga* (Figs. 2, 3). Both the plastid (*matK* + *rbcL* + *trnG-R*) and nuclear (*gapCp*) datasets strongly support a sister relationship between the marginata and purpusii clades. Relationships between this larger clade and the other two monophyletic groups (i.e. the angustifolia and kaulfussii clades) are unsupported, though the data suggest that the latter may be more closely related to each other than to the marginata/purpusii clade (Figs. 2, 3).

Table 1. Sequence characteristics, best-fit sequence evolution models, and resulting tree statistics. Missing data does not include indels.

	plastid *matK*	plastid *rbcL*	plastid *trnG-R*	plastid (combined)	nuclear *gapCp*
Alignment length	1,302	1,309	1,279	3,890	781
Characters included	1,302	1,309	1,136	3,747	781
Missing data (%)	0.08	0.47	2.52	0.96	13.27
Variable sites (%)	28.73	9.24	28.08	21.72	27.78
Model used	TVM + G	GTR + I + G	GTR + G	Mixed	TIM + G
Best ln*L*	–4,802.2783	–2,981.6344	–4,297.8015	–12,156.1157	–3,074.8286

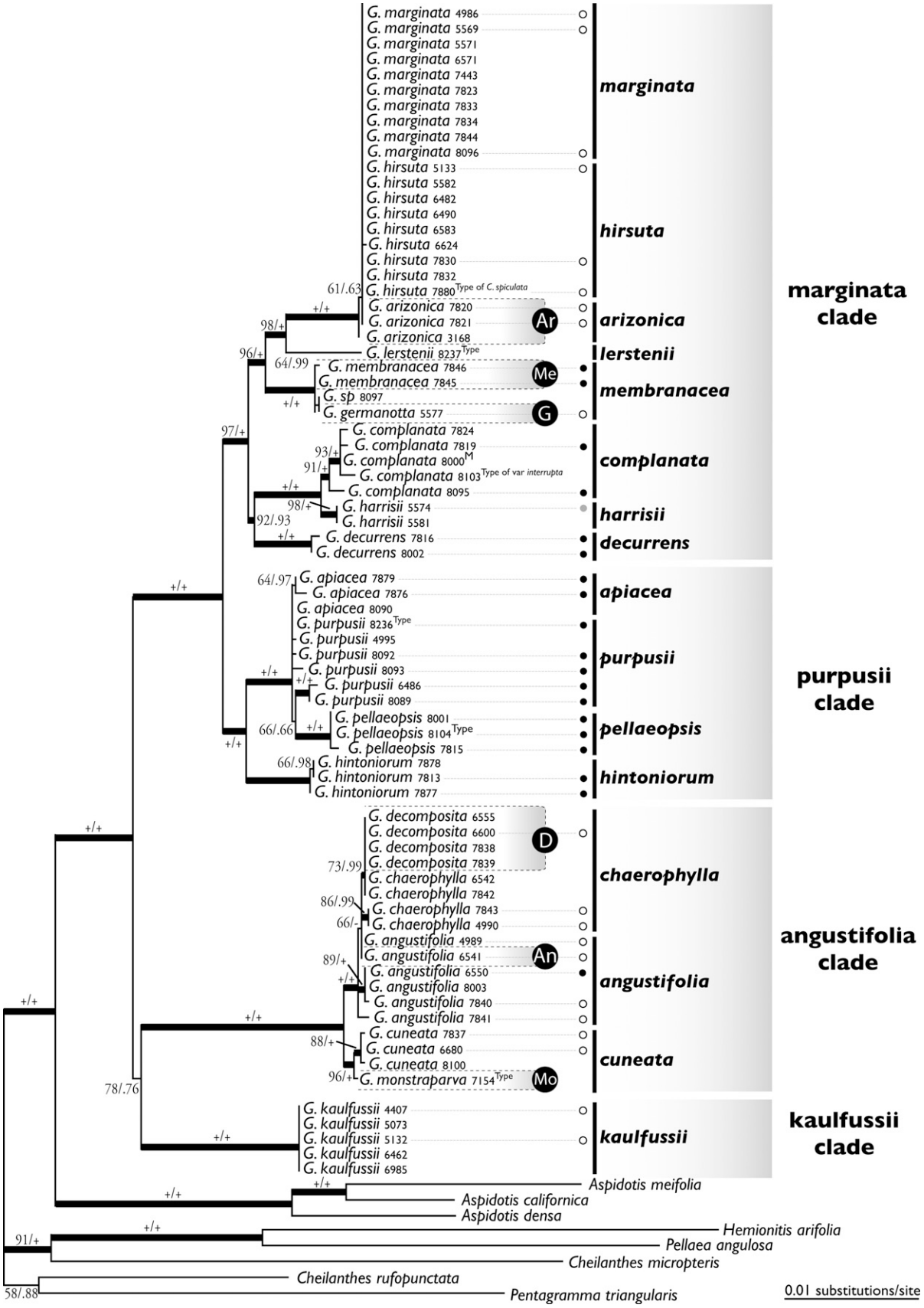

FIG. 2. The best ML tree from the analysis of the plastid *matK* + *rbcL* + *trnG-R* dataset. Branch support is shown at each node as ML bootstrap/Bayesian posterior probability; "+" equals 100% or 1.00. Numbers associated with species names are Fern Lab Database accession numbers (fernlab.biology.duke.edu); nomenclatural type specimens are identified by the superscript "Type." Black, grey, and open circles indicate inferred sexual diploid, sexual tetraploid and apomictic triploid, respectively. Abbreviations: Ar = *Gaga arizonica*, Me = *G. membranacea*, G = *G. germanotta*, D = *G. decomposita*, An = *G. angustifolia* 6541, Mo = *G. monstraparva*.

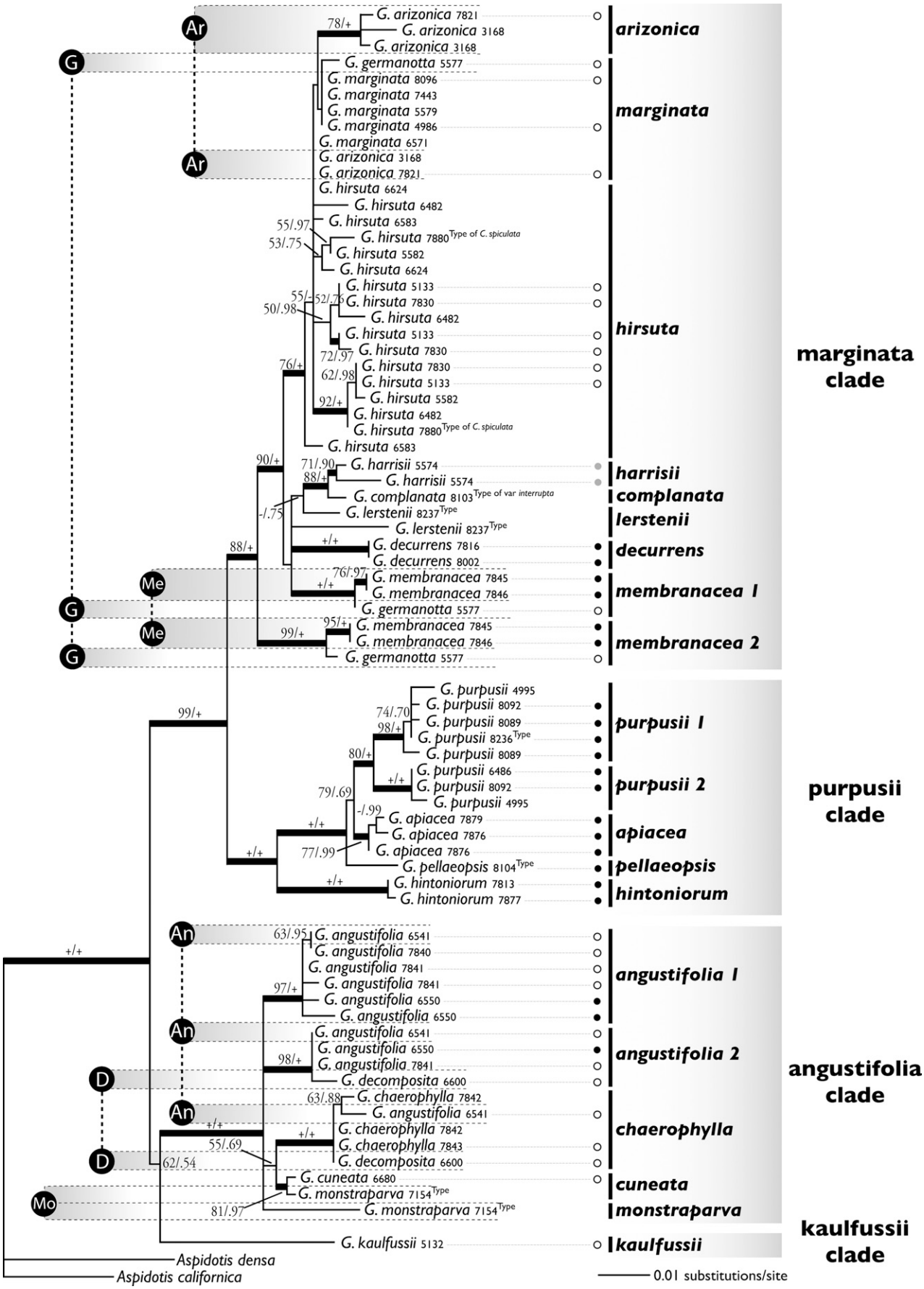

FIG. 3. The best ML tree from the analysis of the nuclear *gapCp* dataset. Symbols as in Fig. 2. Terminals sharing the same taxon name and accession number represent different alleles recovered by cloning.

Hybridization has been reported to be common in both the marginata and angustifolia clades (Mickel and Smith 2004). We used a combination of plastid and nuclear data to investigate potential reticulate evolution in the genus. Because plastids have been shown to be maternally inherited in cheilanthoid ferns (Gastony and Yatskievych 1992), plastid data are useful for identifying maternal progenitors. The nuclear data, on the other hand, can reveal information about both parents involved in a hybridization event. Here we separately present the phylogenetic results inferred from the plastid and nuclear datasets; the patterns of reticulate evolution observed and their implications for taxonomy are elaborated in the Discussion.

Plastid Phylogeny (Fig. 2)—Within the marginata clade, plastid sequences are nearly identical across *G. marginata*, *G. hirsuta*, and *G. arizonica*, despite their considerable morphological differences. On the other hand, *G. lerstenii*, recently treated as a synonym of *G. arizonica* (Mickel and Smith 2004), is shown here to be phylogenetically distinct. *Gaga germanotta*, a new hybrid species described here ("G" in Fig. 2; see Taxonomic Treatment), is minimally divergent from *G. membranacea* ("Me" in Fig. 2), indicating that *G. membranacea* is its maternal progenitor. Because *Gaga* sp. 8097 shares identical *matK*, *rbcL,* and *trnG-R* sequences with *G. germanotta*, it too may be a hybrid involving *G. membranacea* but with a different paternal progenitor. The isotype of *Cheilanthes complanata* var. *interrupta* (*G. complanata* 8103 in Fig. 2) is nested among collections identified as var. *complanata* and thus provides no support for the recognition of infraspecific taxa within this species. *Gaga complanata* and *G. harrisii* are closely related, consistent with their morphological similarity, and these two species are, in turn, sister to *G. decurrens*.

Within the purpusii clade, neither *G. apiacea* nor *G. purpusii* is resolved as monophyletic in the plastid dataset. Both *G. pellaeopsis* and *G. hintoniorum* are monophyletic, with *G. pellaeopsis* forming a polytomy with accessions of *G. apiacea* and *G. purpusii*, and *G. hintoniorum* strongly supported as sister to the other three species. Within the angustifolia clade, a well-supported early dichotomy separates *G. angustifolia* + *G. chaerophylla* + *G. decomposita* from *G. cuneata* + *G. monstraparva*. Neither *G. angustifolia* nor *G. chaerophylla* are resolved as monophyletic, and the probable hybrid between them (*G. decomposita*; "D" in Fig. 2) groups with *G. chaerophylla*. All *G. cuneata* specimens form a monophyletic group that is sister to a new species, *G. monstraparva* ("Mo" in Fig. 2). The kaulfussii clade consists of a single species, *G. kaulfussii*, and its plastid sequences are identical across all five specimens sampled. Although the phylogenetic position of the kaulfussii clade as sister to the angustifolia clade is likely, this relationship is poorly supported (78% MLBS/ 0.76 PP; Fig. 2).

Nuclear Phylogeny (Fig. 3)—Within the marginata clade, the *gapCp* data provide significantly more phylogenetic resolution than that observed in the plastid phylogeny. Only one *gapCp* allele was recovered in each of the five *G. marginata* specimens sampled (Fig. 3). Two of these apparent homozygotes produced 32 large spores per sporangium, suggesting that they are apomictic triploids that originated through autopolyploidy. By contrast, all nine samples of *G. hirsuta* (including an isotype of *Cheilanthes spiculata*) showed at least two, distinctive alleles (Fig. 3), indicating that this "species" is an assemblage of apomictic allotriploids with diverse combinations of progenitor genomes. The morphologically distinctive *G. arizonica* also appears to be an apomictic allotriploid. Both samples of this species have alleles that fall into two groups ("Ar" in Fig. 3); one forming a monophyletic group unique to *G. arizonica*, the other identical to an allele occasionally observed in *G. marginata* and *G. hirsuta*. Two *gapCp* alleles were recovered from our only sample of *G. harrisii*, congruent with the hypothesis that this species may be a sexual tetraploid. The only specimen of *G. complanata* yielded a single *gapCp* allele whereas the only sample of *G. lerstenii* (an isotype) had two, neither of which was related to *G. arizonica* (under which this species recently has been synonymized). *Gaga decurrens*, inferred to be a sexual diploid, is strongly supported as monophyletic though its relationships to other members of the marginata clade are unclear. The two samples of *G. membranacea* are identical to one another and have two distinct allele types ("Me" in Fig. 3), one of which is sister to all members of the marginata clade. *Gaga germanotta* has three alleles ("G" in Fig. 3), two grouping with *G. membranacea* and one with *G. marginata.*

Within the purpusii clade, *G. purpusii* and *G. apiacea* are each robustly resolved as monophyletic (Fig. 3), in contrast to the polytomy presented by the plastid dataset (Fig. 2). *Gaga purpusii* alleles fall into two highly supported groups (Fig. 3); some specimens appear to be reciprocally homozygous whereas others draw alleles from both groups. Phylogenetic relationships of *G. apiacea, G. purpusii,* and *G. pellaeopsis* remain uncertain, although *G. hintoniorum* is supported as sister to all other species in the clade, consistent with the plastid phylogeny. Within the angustifolia clade, four highly supported groups are resolved, two of which correspond to *G. angustifolia*, one to *G. chaerophylla*, and the other to *G. cuneata*. *Gaga decomposita* ("D" in Fig. 3) has alleles that group both with *G. angustifolia* and with *G. chaerophylla*, suggesting a hybrid origin. *Gaga monstraparva* has two alleles ("Mo" in Fig. 3), one slightly divergent from *G. cuneata* and the other unaffiliated with any of the well-supported groups in the angustifolia clade. The nuclear *gapCp* dataset provides no additional information regarding the unresolved phylogenetic position of the kaulfussii clade.

Discussion

Cheilanthes (Pteridaceae) is notorious for its lack of morphological distinctiveness and its constantly changing circumscription (Stolze 1981; Tryon and Tryon 1982). Recent phylogenetic studies have documented the extensive polyphyly of *Cheilanthes* s. l., with species traditionally assigned to this genus appearing in every major clade of cheilanthoid ferns (Gastony and Rollo 1995, 1998; Kirkpatrick 2007; Prado et al. 2007; Schuettpelz et al. 2007; Zhang et al. 2007; Rothfels et al. 2008; Eiserhardt et al. 2011). Unless we choose to treat all 400 + cheilanthoid ferns as a single highly polymorphic genus (which would bear the name *Hemionitis*), many of the species groups recognized as *Cheilanthes* in the past will need to be transferred to other genera or segregated as new genera. Here we focus on one of the larger potential segregates, informally known as the "*C. marginata* group."

The relative distinctiveness of the "*C. marginata* group" was alluded to in the past. An invalidly published generic name, *Othonoloma*, was applied by Link (1833) to *C. hirsuta* Link, a member of this group (Figs. 2, 3). Christensen (1906) treated *Othonoloma* as a synonym of *Cheilanthes*, associating it

with the species *C. marginata* and *C. angustifolia*. Lellinger (1965) and Reeves (1979) later used *Othonoloma* as a section and as a subgenus, respectively, of *Cheilanthes*. Tryon and Tryon (1982) circumscribed the "*C. marginata* group" to include *C. marginata* Kunth, *C. angustifolia* Kunth, *C. membranacea* (Davenp.) Maxon, *C. kaulfussii* Kunze, *C. siliquosa* Maxon, *C. poeppigiana* Kuhn, and *C. intramarginalis* (Kaulf. ex Link) Hook. Of the seven species mentioned by Tryon and Tryon, the first four are closely related to one another (Figs. 2, 3), the fifth [= *Aspidotis densa* (Brack.) Lellinger] is sister to this lineage, and the last two are only distantly related (Kirkpatrick 2007; Eiserhardt et al. 2011; Windham et al. in prep).

Our extensive taxonomic sampling and molecular phylogenetic reconstructions robustly demonstrate the monophyly of the "*C. marginata* group." Because it is phylogenetically isolated from the type species of *Cheilanthes* (Kirkpatrick 2007; Eiserhardt et al. 2011; Windham et al. in prep) and easily distinguished from its sister clade *Aspidotis*, we have chosen to segregate it as a newly described genus, *Gaga* (see Taxonomic Treatment). We recognize a total of nineteen species in the genus, including two taxa new to science (*G. germanotta* and *G. monstraparva*) and one resurrected from synonymy (*G. lerstenii*). Our plastid and nuclear phylogenies revealed congruent topologies with four highly supported clades: marginata, purpusii, angustifolia, and kaulfussii (Figs. 2, 3); the relationships among these major clades are well resolved, except for the position of the kaulfussii clade. Reproductive mode and ploidy level show a clear phylogenetic pattern in *Gaga*; while all species in the purpusii clade appear to be sexual diploids, apomictic triploids dominate in the marginata, angustifolia, and kaulfussii clades (Figs. 1–3). Morphology, polyploidy, reproductive mode, hybridization, and taxonomy are discussed below for each of these clades.

Marginata Clade—Seven of the nine species assigned to this clade (Figs. 2, 3) have bicolored rhizome scales with a dark central stripe and conspicuous brown margins. These scales differ from the concolored or inconspicuously bicolored scales observed in the angustifolia, kaulfussii, and purpusii clades. Most taxa in this clade also have long decurrent pseudoindusia that extend along the costae or rachises (Fig. 4A-B, D, indicated by black arrowheads); the exceptions here include *G. arizonica* (Fig. 4C) and *G. lerstenii*.

GAGA MARGINATA/HIRSUTA COMPLEX—The morphological diversity observed in this complex is significant, with leaf blades ranging from ovate to broadly deltate, and pseudoindusial margins that vary from hairy to spiculate or only slightly pebbly (Fig. 4A-B). These variants are mostly continuous, however, and species boundaries appear to have been seriously obscured by hybridization, apomixis, and polyploidization (Mickel and Smith 2004), leading to inevitable taxonomic conflicts. Smith (1981), Mickel and Beitel (1988), and Yatskievych and Moran (1995) accepted two species in this complex, *Cheilanthes hirsuta* and *C. marginata*, whereas Mickel and Smith (2004) treated *C. hirsuta* as a synonym of *C. marginata*, and also recognized *C. pyramidalis* and *C. spiculata*. After examining the type specimens for each of these four names in light of the molecular data (Fig. 2, 3), we advocate a return to the pre-2004 species taxonomy. Although some intermediates exist, *G. marginata* appears to be cohesive and distinct from *G. hirsuta*; its non-hairy, non-spiculate pseudoindusia (Fig. 4A) with pebbly surfaces are different from those observed on the type specimen of *G. hirsuta*. On the other hand, we see *G. hirsuta*, *C. pyramidalis*, and *C. spiculata* as completely intergradient, with each name representing extremes in pseudoindusial morphology (e.g. *G. hirsuta* being the most hairy, *C. pyramidalis* the least decurrent, and *C. spiculata* the most spiculate). Here we recognize only *G. hirsuta*, and synonymize the other two (see Taxonomic Treatment).

Although the plastid sequences for *G. marginata* and *G. hirsuta* are essentially identical across all specimens (Fig. 2), the nuclear *gapCp* data recover some informative phylogenetic clusters (Fig. 3). Only one allele was recovered for each of the *Gaga marginata* specimens and, importantly, these alleles do not mingle with those of *G. hirsuta* (Fig. 3), suggesting *G. marginata* is indeed a separate entity. All seven of the *G. hirsuta* specimens that we sequenced show two or more *gapCp* alleles, and the alleles for each individual do not group together but are intermixed with other *G. hirsuta* individuals, suggesting rampant hybridization and providing no justification at this point to recognize more than one species. Unfortunately, our *gapCp* data could not resolve *G. hirsuta* and *G. marginata* into two reciprocally monophyletic groups; additional nuclear markers will be needed to better understand the relationships of these taxa. In addition, no diploid individuals have been discovered so far in this species complex (Fig. 1). Diploids in cryptic species complexes are likely extant in nature, though usually overlooked or rare, but they are critical to disentangling patterns of reticulate evolution (e.g. Grusz et al. 2009; Beck et al. 2010). Future studies on this complex should focus on locating these putatively missing diploids.

GAGA ARIZONICA—This is one of the most distinctive species in the marginata clade, with unique reddish glands distributed on the abaxial blade surface (Fig. 4C, arrow). In the *gapCp* analysis, it presented two types of alleles ("Ar" in Fig. 3): one forms a well-supported clade only with other *G. arizonica* alleles, while the other allele is located in a polytomy with alleles from *G. marginata* and *G. hirsuta*. Our samples of *G. arizonica*, including material collected near the type locality, represent an apomictic triploid ($n = 2n = 90$; Windham and Yatskievych 2003) that arose through hybridization between a sexual diploid progenitor of the *G. hirsuta/marginata* complex and an as-yet-undiscovered *G. arizonica*-like species that must have contributed the unique gland character.

GAGA LERSTENII—The isotype of *G. lerstenii*, which was treated as a synonym of *G. arizonica* by Mickel and Smith (2004), is shown here to be phylogenetically distinct (Figs. 2, 3). *Gaga lerstenii* differs from *G. arizonica* in lacking reddish glands on the abaxial blade surface, and from all other species in the marginata clade by its non-decurrent pseudoindusia. Although the isotype examined has no mature spores that could be measured, spore measurements from a Honduran *G. lerstenii* 8099 (*Rafael Calderón C. 066*, MO 3315427) suggest it might be a sexual diploid (Fig. 1). Unfortunately, we were unable to obtain DNA sequence data from this Honduran specimen to compare with the isotype. Based on the data we do have, however, *G. lerstenii* is resurrected as a distinct species.

GAGA MEMBRANACEA—*Gaga membranacea* (Fig. 4D) is the only species in the genus to have light brown rhizome scales (in contrast to either lustrous black or dark brown scales), and it is also one of the few putatively diploid species in the marginata clade (Fig. 1). *Gaga membranacea* specimens have two groups of *gapCp* alleles that are not sister to one another ("Me" in Fig. 3); one of these groups (*membranacea 2*) is resolved as sister to the rest of the marginata clade including

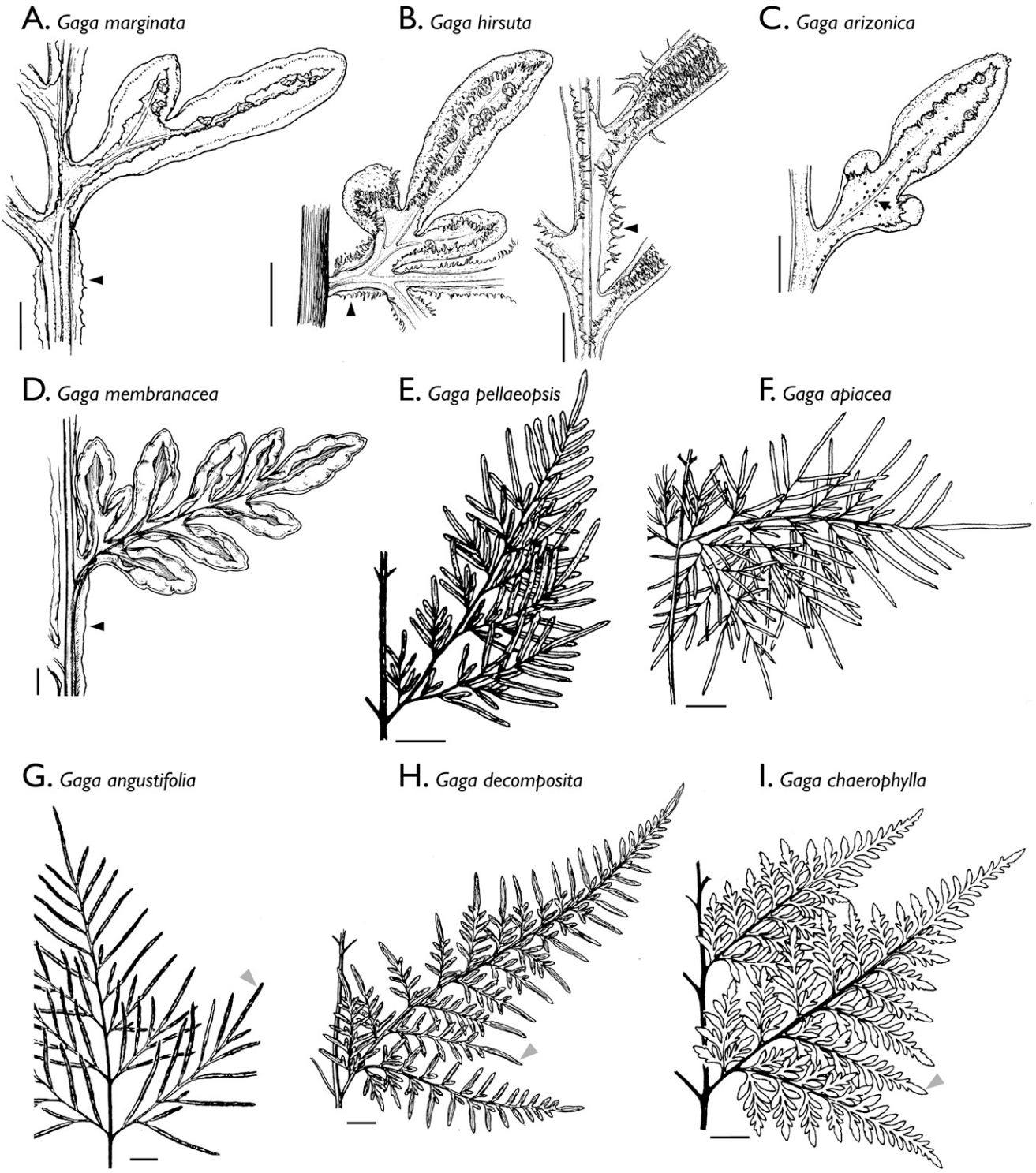

FIG. 4. Morphology of selected *Gaga* species. A. *G. marginata* (*Brade 847*, NY). B. *G. hirsuta* (Right: *Hinton 6837*, LL; isotype of *Cheilanthes spiculata* Mickel. Left: Hort. berol., PH). C. *G. arizonica* (*Gentry and Arguelles 18163*, NY). D. *G. membranacea* (*Mickel 7057*, NY; Fern Lab Database accession number: 7846). E. *G. pellaeopsis* (*Anderson and Laskowski 4394*, NY; DB7815). F. *G. apiacea* (*Nesom 7716*, NY; holotype of the species). G. *G. angustifolia* (*Mickel 4782*, NY). H. *G. decomposita* (*Pringle 2588*, NY). I. *G. chaerophylla* (*Mickel 6254*, NY). Black arrowheads in A, B, and D point to decurrent pseudoindusia, grey arrowheads in G–I to terminal segments, and arrow in C to sessile reddish glands. Vertical bars = 1 mm; horizontal bars = 1 cm. Line drawings modified from Mickel and Smith (2004), with permission.

membranacea 1. Such a phenomenon may be the result of a gene duplication followed by multiple losses, but this would require further study to confirm.

GAGA GERMANOTTA (FIG. 5)—This is a new species from Costa Rica that groups with *G. membranacea* in our plastid analysis ("G" in Fig. 2). In the nuclear *gapCp* analysis, *G. germanotta* alleles appear in three locations ("G" in Fig. 3), suggesting that it is a triploid hybrid between *G. membranacea* (maternal parent) and an undetected sexual diploid cytotype of *G. marginata* (paternal parent). The morphology of

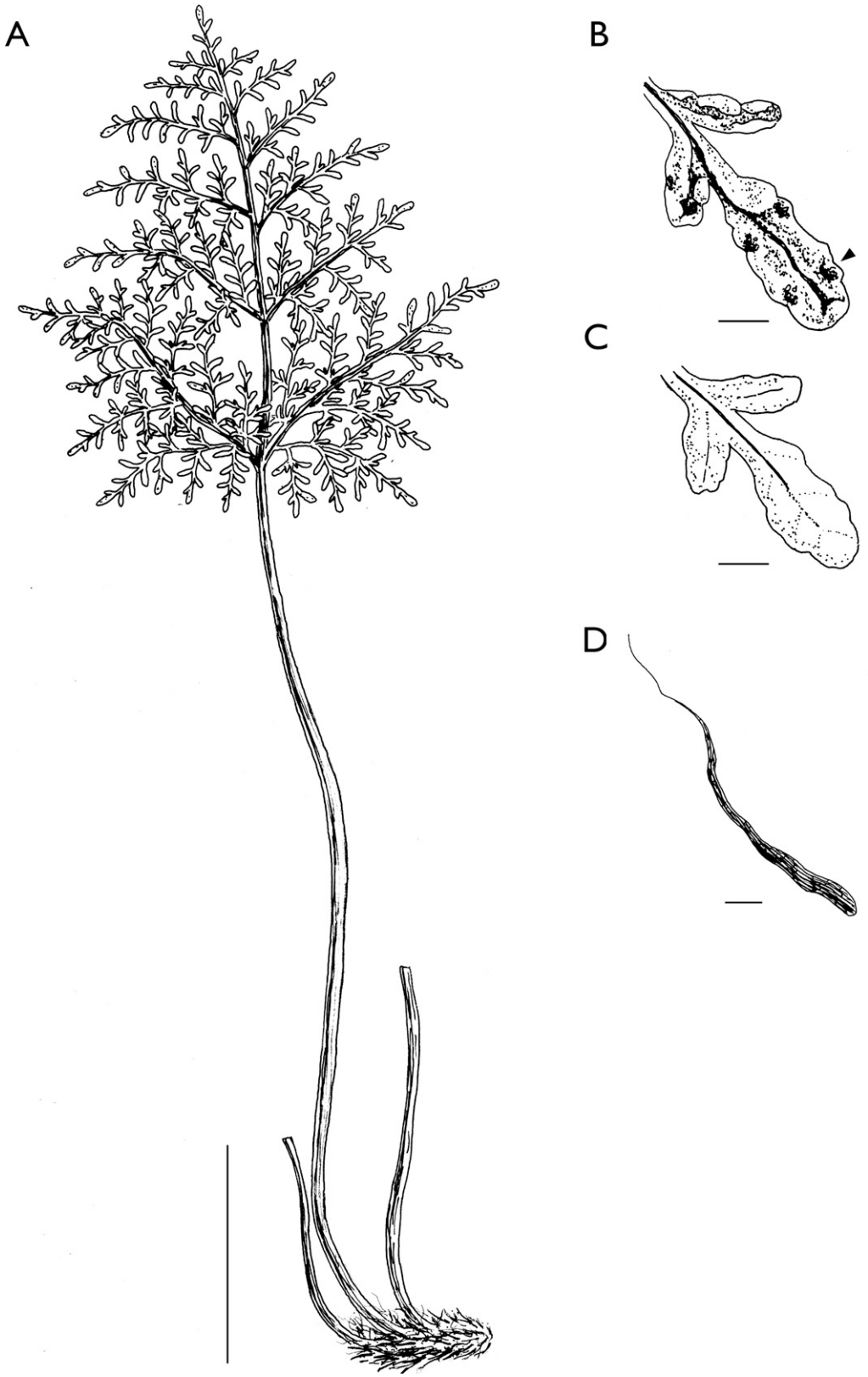

FIG. 5. *Gaga germanotta* (*Rothfels et al. 2618*, DUKE). A. Habit. B. Abaxial view of segment. Arrowhead points to a conspicuous vein ending. C. Adaxial view of segment. D. Rhizome scale. Vertical bar = 5 cm; horizontal bars = 1 mm. Illustrated by Anne Johnson.

G. germanotta is more-or-less intermediate between its two putative parents (see Taxonomic Treatment), and one of the paratype specimens (*Smith & Béliz 2081*, MO 337501) was originally identified as *Cheilanthes marginata* by A. R. Smith and subsequently annotated as *C. membranacea* by Moran and Yatskievych.

GAGA SP. 8097—This specimen, also from Costa Rica, may represent a new species. Although its plastid sequences are

identical to those of *G. germanotta* (Fig. 2), it is distinct from that species in having entire pseudoindusia and light brown rhizome scales. We suspect that it may represent a hybrid between *G. membranacea* (the source of the plastid shared with *G. germanotta*) and *G. decurrens*, which has similar entire and decurrent pseudoindusia. Unfortunately, we were unable to obtain nuclear *gapCp* sequences from this specimen, and no intact sporangia were available for spore measurement. As such, we are hesitant to formally describe this specimen as a new species and, thus, determination of its taxonomic status awaits further study.

GAGA COMPLANATA/HARRISII—*Gaga complanata* and *G. harrisii* are morphologically similar, and both the plastid and nuclear datasets indicate that they are sister species (Figs. 2, 3). Mickel and Smith (2004: 189) provide the clearest contrast, indicating that "*C. harrisii* differs in its rhizome scales being somewhat flexuous with kinky hair-tips, frond axes darker (atropurpureous vs. castaneous), and blades more dissected (to quadripinnate vs. tripinnate to tripinnate-pinnatifid." Although based on only one specimen for each species, our spore measurements suggest another possible distinction, that *G. complanata* is a sexual diploid, whereas *G. harrisii* may be tetraploid (Fig. 1). The isotype of *Cheilanthes complanata* var. *interrupta* (*G. complanata* 8103 in Fig. 2) is nested among samples assigned to var. *complanata* in the plastid analysis (Fig. 2), and we therefore treat this as a synonym of *G. complanata*.

GAGA DECURRENS—This species is similar to *G. marginata* in having strongly decurrent pseudoindusia, but it differs in that the pseudoindusial margin is entire (compared to pebbly in *G. marginata*). Our spore measurements suggest that *G. decurrens* is a sexual diploid species (Fig. 1) whereas the sampled specimens of *G. marginata* appear to be apomictic triploids.

Purpusii Clade—In contrast to the other three clades in which apomixis predominates, the four species of the purpusii clade (*G. purpusii*, *G. apiacea*, *G. pellaeopsis*, and *G. hintoniorum*) appear to be sexual diploids (Fig. 1). Defining characters for this clade include pentagonal leaf blades and acicular rhizome scales that are uniformly dark or, if bicolored, with light brown margins only one cell wide. This clade is entirely endemic to Mexico.

GAGA PURPUSII—The monophyly of the putatively diploid *G. purpusii* is not recovered in the plastid phylogeny (Fig. 2), but is clearly supported by the nuclear data (Fig. 3), where two groups of *G. purpusii* alleles are resolved. A plausible explanation for these two allele groups is that gene duplication may have occurred in *G. purpusii*, with subsequent loss of one copy in some populations.

GAGA APIACEA/G. PELLAEOPSIS—*Gaga apiacea* and *G. pellaeopsis* are morphologically similar and easily confused. Mickel and Smith (2004) distinguished the two species based primarily on the observation that the leaf blades of *G. apiacea* tend to be more divided (quadripinnate proximally) with more elongate ultimate segments (Fig. 4E-F). The margin of the pseudoindusia provides an additional feature seemingly overlooked by previous authors. In *G. apiacea*, this margin is thickened and smooth to slightly granular; in *G. pellaeopsis*, the pseudoindusial margins are thin and conspicuously papillate. Despite their morphological similarity, the two species are phylogenetically and geographically distinct. *Gaga pellaeopsis* specimens, including the isotype, form a well-supported clade in the plastid phylogeny (Fig. 2), and its distribution is confined to southwestern Mexico (Jalisco, Guerrero, and Oaxaca). On the other hand, *G. apiacea*, although intermingled with *G. purpusii* in the plastid phylogeny (Fig. 2), forms a well-supported clade in the nuclear phylogeny that is distinct from *G. pellaeopsis* (Fig. 3). The distribution of *G. apiacea* is restricted to northeastern Mexico (Nuevo León and Tamaulipas). *Gaga apiacea* was described only recently, based on two collections from Zaragoza, Nuevo León (Mickel and Smith 2004). Here we add two new records from Tamaulipas, both of which were previously misidentified as *G. pellaeopsis* (Appendix 1).

GAGA HINTONIORUM—*Gaga hintoniorum* is perhaps the most recognizable and distinctive species in the genus, with its combination of acicular rhizome scales, elongate segments, and strongly deltate leaf blades. The pseudoindusial margins have regular papillate protrusions (irregular in all other species, if papillate). This species has only been reported from Nuevo León, Mexico, on gypsum substrates.

Angustifolia Clade—This clade is unique in that all members have black (rather than brown or tan) spores, nondecurrent pseudoindusia, and veins that are visible on both blade surfaces. Here, as in the *Gaga marginata/hirsuta* complex, apomixis, polyploidy, and hybridization are prevalent. Mickel and Smith (2004) noted that "there seem to be no sharp boundaries between the members of the complex," and this observation is reflected in the plastid phylogeny (Fig. 2). In the nuclear phylogeny (Fig. 3), all *G. chaerophylla* alleles form a well-supported clade, and the *G. angustifolia* alleles fall into either one or two groups (*angustifolia 1* and *angustifolia 2* in Fig. 3). *Gaga decomposita* is shown to be a hybrid between *G. chaerophylla* (putative maternal parent; "D" in Figs. 2, 3) and *G. angustifolia* (*angustifolia 2*, as putative paternal parent; "D" in Fig. 3). Morphologically, *G. decomposita* is intermediate between its two progenitors (Fig. 4 G-I) with tripinnate to tripinnatifid leaves (as in *G. chaerophylla*, Fig. 4I), yet with conform and linear-lanceolate terminal segments (as in *G. angustifolia*, Fig. 4 G, grey arrowheads). One specimen identified as *G. angustifolia* (6541) has three *gapCp* alleles, two from *G. angustifolia* (one each from *angustifolia 1* and *angustifolia 2* in Fig. 3) and one from the *G. chaerophylla* group ("An" in Fig. 3). This suggests that *G. angustifolia* 6541 is a hybrid similar to *G. decomposita* but with an additional genome from the *angustifolia 1* group. Interestingly, the morphology of this specimen is much closer to *G. angustifolia*, congruent with its inferred genomic constitution (two genomes from *G. angustifolia* and one from *G. chaerophylla*).

Gaga monstraparva, a new species described here (see Taxonomic Treatment), appears to be a hybrid between *G. cuneata* (putative maternal parent; "Mo" in Figs. 2, 3) and an unknown diploid species (putative paternal parent; "Mo" in Fig. 3). Although *G. cuneata* was clearly involved in the origin of *G. monstraparva*, the two species are rather easily separated. *Gaga monstraparva* differs from *G. cuneata* in having ovate (vs. lanceolate) leaf blades that are 4-pinnate (vs. 2-pinnate-pinnatifid or 3-pinnate) proximally, as well as having much longer basal pinnae (15 vs. 3.2–6.5 cm). It is superficially more similar to *G. chaerophylla* and *G. decomposita* but differs from both these species in having ovate (vs. deltate-pentagonal) leaf blades and basal basiscopic pinnules only slightly longer than the acroscopic pinnules. This new species is known from only one sterile collection, and its ploidy level is unknown.

Kaulfussii Clade—*Gaga kaulfussii* is the only species currently assigned to this clade, and it is unique in the genus in having dense glandular hairs on petioles, rachises, and leaf blades. It is weakly supported as sister to the angustifolia

clade (Figs. 2, 3) and members of both clades have non-decurrent indusia and concolored rhizome scales, a character combination not seen in the marginata or purpusii clades. The two specimens of *G. kaulfussii* included in our spore study are inferred to be apomictic triploids (Fig. 1).

Answers to the mysteries of *Gaga* have just begun to unfold. Hybridization, polyploidization, and apomixis have greatly complicated the taxonomy of the genus and species boundaries are particularly vague in the *Gaga marginata/hirsuta* complex and in the angustifolia clade. By combining plastid and nuclear molecular data with spore data, we were able to partly resolve past hybridization events and clarify several taxonomic issues. However, it should be noted that our sampling is far from complete and many morphological intermediates were not included. Furthermore, putative sexual diploids were found in less than half of the accepted species. Without knowing the morphology of sexual diploids, we are unable to fully delimit species boundaries and provide a stable taxonomy. *Gaga* is a prime candidate for additional systematic study and careful taxonomic revision. Identifying sexual diploids and incorporating additional nuclear loci (or microsatellite data) will be critical to these efforts.

Taxonomic treatment

Gaga Pryer, Fay-Wei Li, & Windham, gen.nov.—TYPE: *Gaga marginata* (Kunth) Fay-Wei Li & Windham. Basionym: *Cheilanthes marginata* Kunth

Most similar to *Aspidotis* but differing in its rounded to attenuate (vs. mucronate) segment apices, minutely bullate margins of mature leaves (vs. smooth at 40×), and less prominently lustrous and striate adaxial blade surfaces; differing from *Cheilanthes* s. s. in its strongly differentiated, inframarginal pseudoindusia, the production of 64 small or 32 large spores (vs. 32 small or 16 large) per sporangium, and usually glabrous blades (except on pseudoindusia in several species and blade surfaces in *Gaga kaulfussii*).

Plants terrestrial. Rhizomes short and compact, ascending or horizontal, scaly. Rhizome scales lanceolate to acicular, dark brown to black and often lustrous (pale brown in *G. membranacea*), concolored, or bicolored with dark central stripe and brown margins. Petioles castaneous to black, mostly glabrous (glandular hairy in *G. kaulfussii*), occasionally scaly at base. Rachises usually grooved adaxially, occasionally with scattered hairs or hair-like scales. Blades 2- to 4-pinnate, ovate-deltate to pentagonal, the basal basiscopic pinnules usually exaggerated, the costae and costules often grooved adaxially; usually glabrous (except for *G. kaulfussii*). Segments ovate, oblong, linear to elongate, the vein endings forming prominent hydathodes. Sori protected by strongly differentiated inframarginal pseudoindusia, the sporangia clustered at vein tips. Pseudoindusia glabrous to hairy; decurrent (i.e. extending along costae and rachises) in some species, the margins entire, pebbly, papillate, fimbriate, or spiculate. Sporangia 32-spored or 64-spored. Spores globose; tan, brown or black. Chromosome numbers: $n = 2n = 90$ (apomictic triploid) in *G. arizonica* and *G. cuneata* (as *Cheilanthes* in Windham and Yatskievych 2003); the base number presumably $x = 30$.

Distribution—The distribution of *Gaga* ranges from the southwestern United States (Arizona and Texas) to southern Bolivia. *Gaga marginata* is the most widely distributed species, having been recorded from Mexico, Honduras, Guatemala, El Salvador, Nicaragua, Costa Rica, Colombia, Ecuador, Peru, and Bolivia. Mexico is the center of species diversity for the genus; 17 of the 19 species can be found in Mexico, and six of these are endemic.

Etymology—The genus *Gaga* is named in honor of the American pop singer-songwriter-performer Lady Gaga, for her articulate and fervent defense of equality and individual expression in today's society. Because Lady Gaga speaks to the need for humanity to celebrate broad differences within its own species, we hereby provide her with a scientific namesake that characterizes the struggle to understand the intricate biology underlying cryptic patterns of diversity. Because public funding supports basic research, this naming honor allows us to acknowledge the confluence between science and public interests, and to make our findings more accessible and relevant to the diversity of individuals who fund our work. The name *Gaga* also echoes one of the molecular synapomorphies that characterizes the genus. At nucleotide positions 598–601 in the *matK* gene alignment, all *Gaga* species have "GAGA" (Fig. 6), a sequence pattern not seen at this site in any other cheilanthoid fern sampled [e.g. the closely related *Aspidotis densa* shows GAGG, and the type species of *Cheilanthes* (*C. micropteris*) has CAGG].

Morphological Affinities—Although *Gaga* is easily distinguished from its sister genus *Aspidotis* using a combination of macroscopic and microscopic features (see diagnosis above), it is nearly impossible to separate from *Cheilanthes* s. l., within which it and most other cheilanthoid fern genera are nested. The recircumscription of *Cheilanthes* is a work in progress (Windham et al. in prep.), and we are still determining exactly where to draw the boundaries. Our unpublished molecular analyses identify a series of nested

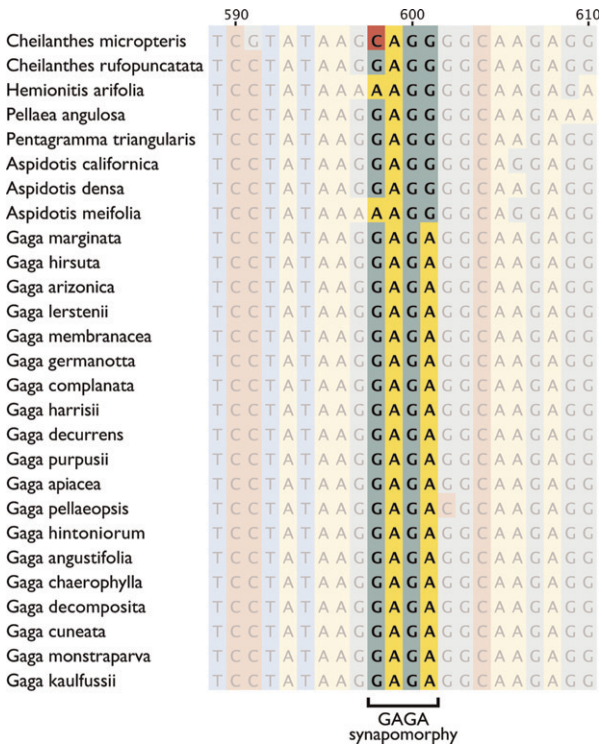

Fig. 6. Part of the *matK* alignment showing a defining molecular synapomorphy of *Gaga*. Only *Gaga* species have "GAGA" at positions 598–601.

clades, all of which meet the minimum requirements for a revised concept of *Cheilanthes* in that they include the type species *C. micropteris*, are reasonably well supported, and show some degree of morphological coherence. One of the most defensible circumscriptions of *Cheilanthes* emerges from the phylogenetic analyses of Eiserhardt et al. (2011; Clade C in their Fig. 2B). In addition to the clade of African species identified by these authors (Clade 1 in their Fig. 2B), our analyses suggest that this revised concept of the genus would encompass all Australian *Cheilanthes* plus a group of South American species including *C. micropteris* (the type species), the *C. squamosa* Hook. & Grev. species group of Tryon and Tryon (1982), *C. obducta* Mett. ex Kuhn, and *C. fractifera* R. M. Tryon.

The *Cheilanthes* s. s. clade outlined above is distinguished from *Gaga* based on a combination of morphological characters, none of which is absolute. Foremost among these is the nature of the pseudoindusium, which is strongly differentiated and clearly inframarginal (recessed from the leaf edge) in *Gaga* and its sister genus *Aspidotis*. Nearly all species of the *Cheilanthes* s. s. clade have a poorly defined pseudoindusium (many previously resided in *Notholaena*, which often was defined by this character), and only in *Cheilanthes* (formerly *Aspidotis*) *schimperi* does the pseudoindusium appear to be inframarginal. Our research focus on spore number per sporangium and spore size has led to the discovery of another feature that distinguishes all species of *Gaga* from many taxa in the *Cheilanthes* s. s. clade. Like most leptosporangiate ferns, sexually reproducing species of *Gaga* produce a preponderance of 64 small spores per sporangium whereas apomicts produce 32 large spores per sporangium. This stands in stark contrast to the South American and Australian (but not African) species of *Cheilanthes* s. s. In the latter clade, sexually reproducing species produce 32 small spores per sporangium and apomicts produce 16 large spores per sporangium due to the loss of a premeiotic mitosis in the cell lineages generating the spore mother cells (Windham et al. in prep.). A third morphological character distinguishing *Gaga* from *Cheilanthes* s. s. as defined above relates to the pubescence of the leaf blades. These are largely glabrous in *Gaga*, with the following exceptions: 1) the leaf blades of *G. kaulfussii* are covered with multicellular, gland-tipped trichomes; 2) the pseudoindusia of several species are pubescent on their surfaces and/or margins, and 3) the leaf blades of a few species (most notably in the marginata clade) produce sessile glands or distinctive conical, glassy projections referred to as spiculae. By contrast, species of the *Cheilanthes* s. s. clade generally have hairy and/or scaly leaves, though the blade surfaces of some of the African species are nearly glabrous.

Within *Cheilanthes* s. l., the species most similar to *Gaga* are *C. rufopunctata* Rosenst., *C. glauca* (Cav.) Mett. and *C. bolborrhiza* Mickel & Beitel. *Gaga* is distinguishable from each of these in having a compact or short-creeping rhizome without swollen protuberences. The rhizomes of *C. rufopunctata* and *C. glauca* are long-creeping and those of *C. bolborrhiza* have prominent swollen root bases. Furthermore, in contrast to the glabrous leaves of most *Gaga* species, both *C. glauca* and *C. rufopunctata* have reddish, stalked, glandular trichomes on the abaxial surfaces. When glands are present on *Gaga*, they are either sessile (*G. arizonica*) or not at all reddish (*G. kaulfussii*). Unpublished molecular results indicate that none of these species are closely related to *Gaga* (Windham et al. in prep.; F. W. Li et al. unpubl. data).

New Combinations—

1. **Gaga angustifolia** (Kunth) Fay-Wei Li & Windham, comb. nov., *Cheilanthes angustifolia* Kunth, Nov. Gen. Sp. 1: 21. 1815. *Allosorus angustifolius* (Kunth) C. Presl, Suppl. Tent. Pterid. 152. 1836. *Onychium angustifolium* (Kunth) Kunze, Farrnkräuter 2: 11. 1848. *Pellaea angustifolia* (Kunth) Baker in Hooker & Baker, Syn. Fil. 150. 1867.—TYPE: MEXICO. Michoacán, *Humboldt & Bonpland s. n.* (B-Willd. 20116, P-Humb.).

Allosorus angustifolius (Kunth) C. Presl var. *minus* Liebm., Mexic. Bregn. 219 (reprint 67). 1849. —TYPE: MEXICO. Oaxaca: Yavesia, *Liebmann s. n.* (lectotype: C, chosen by A. R. Sm. in Fl. Chiapas 2: 71. 1981).

Cheilanthes venusta Fée var. *aurata* Fée, Mém. Foug. 9: 12. 1857, nom. nud.—TYPE: MEXICO. Oaxaca: San Pedro de Nolasco, *Galeotti 6560* (BR).

2. **Gaga apiacea** (Mickel) Fay-Wei Li & Windham, comb. nov., *Cheilanthes apiacea* Mickel, Mem. New York Bot. Gard. 88: 182. 2004.—TYPE: MEXICO. Nuevo León: Zaragoza, *Nesom 7716* (holotype: NY!; isotypes: CAS!, TEX).

3. **Gaga arizonica** (Maxon) Fay-Wei Li & Windham, comb. nov., *Cheilanthes pyramidalis* Fée subsp. *arizonica* Maxon, Am. Fern J. 8: 116. 1918. *Cheilanthes pyramidalis* Fée var. *arizonica* (Maxon) Broun, Index N. Amer. Fern 51. 1938. *Cheilanthes arizonica* (Maxon) Mickel, Phytologia 41: 433. 1979.—TYPE: U. S. A. Arizona: Huachuca Mts, *Goodding 1327* (holotype: US!; isotype: NY).

4. **Gaga chaerophylla** (M. Martens & Galeotti) Fay-Wei Li & Windham, comb. nov., *Allosorus chaerophyllus* M. Martens & Galeotti, Mém. Foug. Mexique 47, pl. 11. 1842, *Cheilanthes chaerophylla* (M. Martens & Galeotti) Kunze, Linnaea 23: 243, 307. 1850.—TYPE: MEXICO. Oaxaca: Juquila, *Galeotti 6367* (holotype: BR; isotype: P).

5. **Gaga complanata** (A. R. Sm.) Fay-Wei Li & Windham, comb. nov., *Cheilanthes complanata* A. R. Sm., Am. Fern J. 70: 19. 1980.—TYPE: MEXICO. Chiapas: Motozintla de Mendoza, *Breedlove 41747* (holotype: DS!; isotypes: MEXU, MO!).

Cheilanthes complanata A.R. Sm. var. *interrupta* Mickel, Mem. New York Bot. Gard. 88: 189. 2004.—TYPE: MEXICO. Guerrero, *Espin M. 149* (holotype: NY!; isotypes: ENCB, FCME).

6. **Gaga cuneata** (Link) Fay-Wei Li & Windham, comb. nov., *Cheilanthes cuneata* Link, Hort. Berol. 2: 40. 1833. *Cassebeera cuneata* (Link) J. Sm., J. Bot. (Hook.) 4: 159. 1841. *Pellaea cuneata* (Link) J. Sm., Hist. Fil. 286. 1875. *Cheilanthes angustifolia* Kunth var. *cuneata* Brause, Verh. Bot. Vereins Prov. Brandenburg 51: 5. 1910.—TYPE: Described from cultivated plants (holotype: B; isotype: PH).

7. **Gaga decomposita** (M. Martens & Galeotti) Fay-Wei Li & Windham, comb. nov., *Allosorus decompositus* M. Martens & Galeotti, Nouv. Mém. Acad. Roy. Sci. Bruxelles 15: 48, pl. 10, f. 2. 1842. *Pellaea decompositus* (M. Martens & Galeotti) Hook., Sp. Fil. 2: 151. 1852. *Cheilanthes decomposita* (M. Martens & Galeotti) Fée, Mém. Foug. 9: 11. 1857.—TYPE: MEXICO. Oaxaca: Juquila, *Galeotti 6362* (BR).

8. **Gaga decurrens** (Mickel) Fay-Wei Li & Windham, comb. nov., *Cheilanthes decurrens* Mickel, Mem. New York Bot. Gard. 88: 191. 2004.—TYPE: GUATEMALA. Volcán de Agua, *Maxon & Hay 3709* (NY!).

9. Gaga harrisii (Maxon) Fay-Wei Li & Windham, comb. nov., *Cheilanthes harrisii* Maxon, Contr. U. S. Natl. Herb. 24: 51. 1922.—TYPE: JAMAICA. *Harris 12902* (US).

10. Gaga hirsuta (Link) Fay-Wei Li & Windham, comb. nov., *Cheilanthes hirsuta* Link, Hort. Berol. 2: 40. 1833.—LECTOTYPE (designated here): Described from cultivated plants (B!; herbarium barcode: B 20 0036984, labeled as 'Hort. Berol. 1832').

Cheilanthes crenulata Link, Hort. Berol. 2: 41. 1833.—TYPE: Described from cultivated plants (holotype: B; isotype: PH!).

Cheilanthes rufescens Link, Hort. Berol. 2: 39. 1833.—TYPE: Described from cultivated plants (holotype: B; isotype: PH!).

Cheilanthes pyramidalis Fée, Mém. Foug. 7: 38, t. 25, f. 3. 1857.—TYPE: MEXICO. Valley of Mexico, *Schaffner 88* (syntype: P); MEXICO. Guatimalpan, *Schaffner 304* (syntype: P!); MEXICO. San Agostín, *Schaffner 305* (isosyntype: K).

Cheilanthes spiculata Mickel, Mem. New York Bot. Gard. 88: 210. 2004.—TYPE: MEXICO. México: Temascaltepec, *Hinton et al. 6837* (holotype: NY!; isotypes: F, LL!, MO!).

Pellaea angustifolia (Kunth) Baker var. *elongata* Rovirosa, Pteridogr. Sur México 130. 1909.—TYPE: MEXICO. Chiapas: Mesa de Coapilla, *Rovirosa 1059* (PH, frag. US).

11. Gaga hintoniorum (Mendenh. & Nesom) Fay-Wei Li & Windham, comb. nov., *Cheilanthes hintoniorum* Mendenh. & Nesom, Sida 14: 551, f. 1, 1991.—TYPE: MEXICO. Nuevo León: Galeana, *Hinton et al. 18765* (holotype: TEX; isotypes: MEXU, NY, UC).

12. Gaga kaulfussii (Kunze) Fay-Wei Li & Windham, comb. nov., *Cheilanthes viscosa* Link nom. illeg., non Carmich 1818, Hort. Berol. 2: 43. 1833. *Cheilanthes kaulfussii* Kunze, Linnaea 13: 145. 1839.—TYPE: Described from cultivated plants (B, BM).

Cheilanthes glandulifera Liebm., Mexic. Bergn. 258 (reprint 106). 1849.—TYPE: MEXICO. Oaxaca, *Liebmann s. n.* (lectotype: C; isolectotype: K, chosen by A. R. Sm., Fl. Chiapas 2: 72. 1981).

13. Gaga lerstenii (Mickel & Beitel) Fay-Wei Li & Windham, comb. nov., *Cheilanthes lerstenii* Mickel & Beitel, Mem. New York Bot. Gard. 46: 117. 1988.—TYPE: MEXICO. Oaxaca: Sola de Vega, *Mickel 6238* (holotype: NY; isotype: UC!).

14. Gaga marginata (Kunth) Fay-Wei Li & Windham, comb. nov., *Cheilanthes marginata* Kunth, Nov. Gen. Sp. 1: 22. 1815, *Pellaea marginata* (Kunth) Baker, Syn. Fil. 151. 1865.—TYPE: ECUADOR. Quito, *Humboldt & Bonpland s. n.* (holotype: P, high resolution photo!; isotype: B-Willd. 20126).

Allosorus ciliatus C. Presl var. *minor* C. Presl, Reliq. Haenk. 1: 59. 1825.—TYPE: MEXICO. *Haeke s. n.* (PR or PRC).

15. Gaga membranacea (Davenp.) Fay-Wei Li & Windham, comb. nov., *Pellaea membranacea* Davenp., Bot. Gaz. 21: 262, t. 18, f. 5, 6. 1896. *Cheilanthes membranacea* (Davenp.) Maxon, Am. Fern J. 8: 119. 1918.—TYPE: MEXICO. Oaxaca: Sierra de San Felipe, *Pringle 5963* (GH).

Cheilanthes selinoides Mickel in McVaugh, Fl. Nov.-Gal. 17: 243. 1992.—TYPE: MEXICO. Jalisco: Nevado de Colima, *McVaugh 12869* (holotype: MICH; isotype: US).

16. Gaga pellaeopsis (Mickel) Fay-Wei Li & Windham, comb. nov., *Cheilanthes pellaeopsis* Mickel in McVaugh, Fl. Nov.-Gal. 17: 241. 1992.—TYPE: MEXICO. Jalisco: Zapotitlán, *Iltis et al.* 29543 (WIS, frag. NY!).

17. Gaga purpusii (T. Reeves) Fay-Wei Li & Windham, comb. nov., *Cheilanthes purpusii* T. Reeves, Rhodora 84: 293. 1982.—TYPE: MEXICO. San Luis Potosí: Minas de San Rafael, *Purpus 4881* (holotype: GH; isotypes: F, UC!, MO!).

New Species—

1. Gaga germanotta Fay-Wei Li & Windham, sp. nov.—TYPE: COSTA RICA. Provincia de Cartago, NE-side of Panamerican Hwy 2, Cerro de la Muerte, Cerro Zacatales, near the trailhead to Cerro Sakira, W-facing slope, paramo vegetation, 18 June 2012, N9.57983, W83.76144, 3334 m elevation, *Fay-Wei Li 1520* (holotype: DUKE; isotypes: CR, INB, MO, UC, US, K, NY).

Most similar to *Gaga marginata* but differing in having thicker petioles (> 2 mm in diameter), and larger (to 10 × 1 mm), mostly concolored, black rhizome scales lacking obvious brown margins; similar to *Gaga membranacea* but differing in having black (vs. castaneous) rhizome scales, and somewhat coriaceous (vs. thin-textured) leaf blades with obscure venation.

Plants terrestrial. Rhizomes short and compact. Rhizome scales 7–10 mm long, to 1 mm wide; lanceolate, lustrous, black, concolored or with a lighter base. Petioles to 30 cm long × 2.5 mm in diameter, castaneous, glabrous except for sparse linear scales at base. Rachises grooved adaxially, with pale ridges on both sides of groove, glabrous. Leaf blades to 15 cm long, 11 cm wide, somewhat coriaceous; deltate to pentagonal, 3- to 4-pinnate; largest pinnae ca. 9 cm long, 6 cm wide; pinnae and pinnules often with lobed, non-conform, terminal segment; basal basiscopic pinnules slightly longer than acroscopic pinnules. Ultimate segments 3–8 mm long, ca. 1 mm wide, oblong to linear; veins obscure. Pseudoindusia covering more than half the abaxial segment surface; strongly decurrent along costules; margin with irregular one-celled protrusions. Sporangia 32-spored. Spores globose, brown. Chromosome number unknown. Figure 5.

Paratypes—COSTA RICA. Prov. San José, Perez Zeledon, side of Panamericana, at beginning of Sendero Cerro Zacatales-Cerro Sabila-Cerro Sakira, pie de Cerro Estaqueros, edge of Parque National Tapanti-Macizo de la Muerte, N9.57983, W83.76144, 3,334 m elevation, 23 June 2008, *C. J. Rothfels et al. 2618* (DUKE). Lugar de hallazgo: Camino de San Gerardo al Chirripó, 3,250 m elevation, 26 April 1991, *K. Mehltreter 129* (CR 160010). Prov. San José/Cartago, road from Cartago to San Isidro del General (Pan American Hwy, Rt 2), Km 87–88, ca. 1 km NW of Asunción, páramo vegetation, 3,200 m elevation, on steep road bank. 29 Jan 1986, *A. R. Smith 2018 & T. Béliz* (CR 122854, MO 3337501).

Distribution—The species is known only from páramo of Cerro de la Muerte and Cerro Chirripó, Costa Rica.

Etymology—The epithet "germanotta" is named in honor of Lady Gaga's parents, Cynthia and Joe Germanotta. On February 29, 2012, in partnership with The John D. & Catherine T. MacArthur Foundation, The California Endowment, and Harvard's Berkman Center for Internet & Society, the Germanotta family launched the Born This Way Foundation (BTWF), a new non-profit charitable organization (bornthiswayfoundation.org). The foundation is dedicated to fostering a more accepting society, where differences are embraced and individuality is celebrated. Its mission is to empower youth and inspire bravery in order to make the world a kinder, braver place.

Relationships—Spore and DNA data suggest that *Gaga germanotta* is an apomictic triploid that originated through hybridization between *G. membranacea* (the maternal progenitor) and an undetected sexual diploid cytotype of *G. marginata*. The large, lustrous, black rhizome scales of *G. germanotta* immediately distinguish it from both *G. membranacea* (with concolored, brown scales) and *G. marginata* (with smaller, usually bicolored scales with a dark central stripe and narrow brown margins). Additional distinguishing features are provided in the species diagnosis.

2. Gaga monstraparva Fay-Wei Li & Windham, sp. nov.—TYPE: MEXICO. Mexico, Municipio de Ocuilán, approximately 3.9 km west of México/Morelos border on Ocuilán de Arteaga/Cuernavaca road, N18.98474, W99.33072, 2,295 m elevation, 09 August 2009, *J. B. Beck 1229* (holotype: DUKE).

Similar to *Gaga cuneata* but differing in having ovate (vs. lanceolate) leaf blades that are 4-pinnate (vs. 2-pinnate-pinnatifid or 3-pinnate) proximally and much longer basal pinnae (15 vs. 3.2–6.5 cm); similar to *G. chaerophylla* and *G. decomposita* but differing in having ovate (vs. deltate-pentagonal) leaf blades and basal basiscopic pinnules only slightly longer than the acroscopic pinnules; similar to *Gaga angustifolia* but differing in having the pseudoindusia predominantly continuous, the segments narrowly lanceolate (vs. linear), and a complete lack of scales on leaf blades.

Plants terrestrial. Rhizomes short and compact. Rhizome scales 3–4 mm long, ca. 0.7 mm wide, lanceolate, concolored, lustrous black. Petioles ca. 35 cm long and 2.7 mm in diameter, castaneous, glabrous. Rachises grooved adaxially, with pale ridges on both sides of groove, glabrous. Leaf blades ca. 36 cm long, 16 cm wide; somewhat coriaceous, ovate, 4-pinnate proximally, largest pinnae ca. 15 × 8 cm; pinnae and pinnules with a conform, terminal segment; basal basiscopic pinnules at most 1.5 times longer than acroscopic pinnules. Ultimate segments to 18 × 3 mm, narrowly lanceolate; primary and secondary veins visible both adaxially and abaxially. Pseudoindusia covering half to one-third of the abaxial segment surface; non-decurrent and occasionally discontinuous proximally; margin more or less entire and smooth. Spores unknown. Chromosome number unknown.

Distribution—This species is only known from the holotype.

Etymology—The epithet "*monstraparva*" honors Lady Gaga's fervent and loyal fans, her "little monsters." The official little monster greeting is the outstretched "monster claw" hand, which bears a striking resemblance to a tightly inrolled young fern leaf prior to unfurling.

Relationships—Our phylogenetic reconstructions suggest that *G. monstraparva* is a hybrid involving *G. cuneata* and an unknown diploid from the angustifolia clade. In the plastid dataset (Fig. 2), the only accession of *G. monstraparva* is well-supported as sister to the three samples of *G. cuneata*. In the nuclear dataset (Fig. 3), *G. monstraparva* shows two alleles, one minimally divergent from *G. cuneata* and the other unaffiliated with any of the well-supported groups in the angustifolia clade.

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Appendix 1. Sampling information. Taxon name Fern Lab Database accession number (fernlab.biology.duke.edu), collection site, collector and collection number (voucher location); *matK* GenBank accession, *rbcL* GenBank accession, *trnG-R* GenBank accession, *gapCp* GenBank accessions (number of clones); average spore diameter in μm ± one standard deviation; number of spores counted per sporangium. NA = not available.

Ingroup—Gaga angustifolia (Kunth) Fay-Wei Li & Windham 4989, Sonora, Mexico, *van Devender 97-1244* (MO); JN647719, JN647777, JX313452, NA; 70.11±2.21; 31. ***Gaga angustifolia*** (Kunth) Fay-Wei Li & Windham 6541, Jalisco, Mexico, *Rothfels 3109* (DUKE); JN647720, JN647778, JX313453, JX313550, JX313551, JX313552 (14); 63.03±2.46; 25. ***Gaga angustifolia*** (Kunth) Fay-Wei Li & Windham 6550, Jalisco, Mexico, *Rothfels 3116* (DUKE); JN647721, JN647779, JX313454, JX313553, JX313554, JX313555 (12); 40.45±4.14/58.40±2.74; 57/32. ***Gaga angustifolia*** (Kunth) Fay-Wei Li & Windham 7840, Sinaloa, Mexico, *Anderson 12567* (NY); JN647722, JN647780, JX313455, JX313556, JX313557 (11); 64.15±2.77; 30. ***Gaga angustifolia*** (Kunth) Fay-Wei Li & Windham 7841, Jalisco, Mexico, *McVaugh 17184* (NY); JN647723, JN647781, JX313456, JX313558, JX313559 (8); 64.39±2.48; 31. ***Gaga angustifolia*** (Kunth) Fay-Wei Li & Windham 8003, Chiapas, Mexico, *Breedlove 20549* (CAS); JN647724, JN647782, JX313457, NA; 72.23±6.74; 21. ***Gaga apiacea*** (Mickel) Fay-Wei Li & Windham 7876, Tamaulipas, Mexico, *Webster and Webster 18* (TEX); JX313639, JX313539, JX313505, JX313594, JX313595 (8); 49.26±2.16/68.36±4.85; 60/27. ***Gaga apiacea*** (Mickel) Fay-Wei Li & Windham 7879, Nuevo León, Mexico, *Hinton et al. 23441* (TEX); JN647725, JN647783, JX313459, JX313560 (11); 45.16±1.31; 64. ***Gaga apiacea*** (Mickel) Fay-Wei Li & Windham 8090, Tamaulipas, Mexico, *Diggs and Nee 2378* (F); JX313640, JX313540, JX313507, NA; NA. ***Gaga arizonica*** (Maxon) Fay-Wei Li & Windham NA, Arizona, U. S. A., Windham 0301A; NA, NA, NA, NA; 70.76±2.01; 32. ***Gaga arizonica*** (Maxon) Fay-Wei Li & Windham 3168, Arizona, U. S. A., *Schuettpelz 461* (DUKE); JN647726, JN647784, EU268673, JX313561, JX313562, JX313563 (12); NA. ***Gaga arizonica*** (Maxon) Fay-Wei Li & Windham 7820, Jalisco, Mexico, *Koch and Fryxell 89200* (NY); JN647727, JN647785, JX313460, NA; 70.20±3.18; 31. ***Gaga arizonica*** (Maxon) Fay-Wei Li & Windham 7821, Sinaloa, Mexico, *Breedlove 18570* (NY); JN647728, JN647786, JX313461, JX313564, JX313565 (9); 64.36±1.85; 29. ***Gaga chaerophylla*** (M. Martens & Galeotti) Fay-Wei Li & Windham 4990, Michoacán, Mexico, *Steinmann 4721* (MO); JN647730, JN647788, JX313463, NA; 68.94±4.07; NA. ***Gaga chaerophylla*** (M. Martens & Galeotti) Fay-Wei Li & Windham 6542, Jalisco, Mexico, *Rothfels 3110* (DUKE); JN647731, JN647789, JX313464, NA; NA. ***Gaga chaerophylla*** (M. Martens & Galeotti) Fay-Wei Li & Windham 7842, Colima, Mexico, *McVaugh 16108* (NY); JN647732, JN647790, JX313465, JX313566, JX313567 (16); NA. ***Gaga chaerophylla*** (M. Martens & Galeotti) Fay-Wei Li & Windham 7843, Sinaloa, Mexico, *Bartholomew 2516* (NY); JN647733, JN647791, JX313466, JX313568 (12); 65.78±3.13; 32. ***Gaga complanata*** (A. R. Sm.) Fay-Wei Li & Windham 7819, Chiapas, Mexico, *Breedlove 55870* (NY); JN647734, JN647792, JX313467, NA; 46.49±1.77; 64. ***Gaga complanata*** (A. R. Sm.) Fay-Wei Li & Windham 7824, Guatemala, *Lloyd 4040a* (DUKE); JX313633, JX313533, JX313468, NA; NA. ***Gaga complanata*** (A. R. Sm.) Fay-Wei Li & Windham 8000, Chiapas, Mexico, *Breedlove 40335* (CAS); JN647735, JN647793, JX313469, NA; NA. ***Gaga complanata*** (A. R. Sm.) Fay-Wei Li & Windham 8095, Lempira, Honduras, *Moran 5638*; JX313634, JX313534, JX313470, NA; 50.99±2.39; 64. ***Gaga complanata*** (A. R. Sm.) Fay-Wei Li & Windham 8103, Guerrero, Mexico, *Millan 149* (NY, isotype of *Cheilanthes complanata* A. R. Sm. var. *interrupta* Mickel); JX313635, JX313535, JX313471, JX313569 (9); NA. ***Gaga cuneata*** (Link) Fay-Wei Li & Windham NA, Oaxaca, Mexico, *Windham et al. 566* (DUKE); NA, NA, NA, NA; 61.25±2.23; 29. ***Gaga cuneata*** (Link) Fay-Wei Li & Windham 6680, Oaxaca, Mexico, *Rothfels 3288* (DUKE); JN647736, JN647794, JX313472, JX313570 (12); 64.20±3.04; 32. ***Gaga cuneata*** (Link) Fay-Wei Li & Windham 7837, Oaxaca, Mexico, *Mickel and Leonard 4881* (NY); JN647737, JN647795, JX313473, NA; 60.90±2.42; 32. ***Gaga cuneata*** (Link) Fay-Wei Li & Windham 8100, Francisco Morazán, Honduras, *Montoya 53* (MO); JX313632, JX313532, JX313458, NA; NA. ***Gaga decomposita*** (M. Martens & Galeotti) Fay-Wei Li & Windham 6555, Nayarit, Mexico, *Rothfels 3119A* (DUKE); JN647738, JN647796, JX313474, NA; NA. ***Gaga decomposita*** (M. Martens & Galeotti) Fay-Wei Li & Windham 6600, Nayarit, Mexico, *Rothfels 3183* (DUKE); JN647739, JN647797, JX313475, JX313571, JX313572 (12); 68.16±2.72; 26. ***Gaga decomposita*** (M. Martens & Galeotti) Fay-Wei Li & Windham 7838, Oaxaca, Mexico, *Mickel 6064* (NY); JN647740, JN647798, JX313476, NA; NA. ***Gaga decomposita*** (M. Martens & Galeotti) Fay-Wei Li & Windham 7839, Nayarit, Mexico, *McVaugh 16427* (NY); JN647741, JN647799, JX313477, NA; NA. ***Gaga decurrens*** (Mickel) Fay-Wei Li & Windham 7816, El Salvador, *Seiler 1209* (NY); JN647742, JN647800, JX313478, JX313573 (9); 47.31±1.74; 61. ***Gaga decurrens*** (Mickel) Fay-Wei Li & Windham 8002, Chiapas, Mexico, *Breedlove 55788* (CAS); JN647743, JN647801, JX313479, JX313574 (9); 47.16±2.41; 47. ***Gaga germanotta*** Fay-Wei Li & Windham 5577, San José, Costa Rica, *Rothfels et al. 2618* (DUKE); JX313646, JX313546, JX313522, JX313617, JX313618, JX313619 (12); 72.99±2.72; 25. ***Gaga harrisii*** (Maxon) Fay-Wei Li & Windham 5574, Heredia, Costa Rica, *Rothfels 2689* (DUKE); JN647744, JN647802, JX313480, JX313575, JX313576 (11); 60.41±3.00; 64. ***Gaga harrisii*** (Maxon) Fay-Wei Li & Windham 5581, San José, Costa Rica, *Grusz 105* (DUKE); JN647745, JN647803, JX313481, NA; NA. ***Gaga hirsuta*** (Link) Fay-Wei Li & Windham 5133, San José, Costa Rica, *Rothfels 08-023* (DUKE); JN647755, JN647813, JX313492, JX313583, JX313584, JX313585 (14); 74.75±3.26; NA.

Gaga hirsuta (Link) Fay-Wei Li & Windham 5582, San José, Costa Rica, *Rothfels 2696* (DUKE); JN647769, JN647827, JX313515, JX313605, JX313606 (11); NA. ***Gaga hirsuta*** (Link) Fay-Wei Li & Windham 6482, Hidalgo, Mexico, *Rothfels 3023* (DUKE); JN647770, JN647828, JX313516, JX313607, JX313608, JX313609 (18); NA. ***Gaga hirsuta*** (Link) Fay-Wei Li & Windham 6490, Hidalgo, Mexico, *Rothfels 3032* (DUKE); JN647771, JN647829, JX313517, NA; NA. ***Gaga hirsuta*** (Link) Fay-Wei Li & Windham 6583, Jalisco, Mexico, *Rothfels 3154* (DUKE); JN647772, JN647830, JX313518, JX313610, JX313611 (12); NA. ***Gaga hirsuta*** (Link) Fay-Wei Li & Windham 6624, Jalisco, Mexico, *Rothfels 3212* (DUKE); JN647773, JN647831, JX313519, JX313612, JX313613 (7); NA. ***Gaga hirsuta*** (Link) Fay-Wei Li & Windham 7830, Chiapas, Mexico, *Breedlove 22395* (NY); JN647774, JN647832, JX313520, JX313614, JX313615, JX313616 (14); 69.05±2.67; 29. ***Gaga hirsuta*** (Link) Fay-Wei Li & Windham 7832, Oaxaca, Mexico, *Mickel and Hellwig 3891* (NY); JN647775, JN647833, JX313521, NA; NA. ***Gaga hirsuta*** (Link) Fay-Wei Li & Windham 7880, Mexico, Mexico, *Hinton et al. 6837* (LL, isotype of *Cheilanthes spiculata*); JN647776, JN647834, JX313524, JX313622, JX313623 (16); 62.37±2.22; 32. ***Gaga hintoniorum*** (Mendenh. & Nesom) Fay-Wei Li & Windham 7813, Nuevo León, Mexico, *Hinton et al 22695* (NY); JN647746, JN647804, JX313482, JX313577 (14); 49.35±1.70; 61. ***Gaga hintoniorum*** (Mendenh. & Nesom) Fay-Wei Li & Windham 7877, Nuevo León, Mexico, *Hinton et al* 20157 (TEX); JN647747, JN647805, JX313483, JX313578 (8); 49.41±1.43; 53 ***Gaga hintoniorum*** (Mendenh. & Nesom) Fay-Wei Li & Windham 7878, Nuevo León, Mexico, *Hinton et al 28587* (TEX); JN647748, JN647806, JX313484, NA; NA. ***Gaga kaulfussii*** (Kunze) Fay-Wei Li & Windham 4407, Morelos, Mexico, *Windham et al. 519* (DUKE); JN647749, JN647807, JX313485, NA; 65.05±1.97; 24. ***Gaga kaulfussii*** (Kunze) Fay-Wei Li & Windham 5073, Morelos, Mexico, *Yatskievych and Gastony 89-286* (MO); JN647750, JN647808, JX313486, NA; NA. ***Gaga kaulfussii*** (Kunze) Fay-Wei Li & Windham 5132, San José, Costa Rica, *Rothfels 08-022* (DUKE); JN647751, JN647809, JX313487, JX313579 (12); 63.02±3.38; 32. ***Gaga kaulfussii*** (Kunze) Fay-Wei Li & Windham 6462, Mexico, Mexico, *Rothfels 2988* (DUKE); JN647752, JN647810, JX313488, NA; NA. ***Gaga kaulfussii*** (Kunze) Fay-Wei Li & Windham 6985, San Luis Potosí, Mexico, *Larsson 109* (DUKE); JN647753, JN647811, JX313489, NA; NA. ***Gaga lerstenii*** (Mickel & Beitel) Fay-Wei Li & Windham 8099, Francisco Morazán, Honduras, *Calderon 066* (MO); NA, NA, NA, NA; 51.09±1.65; 63. ***Gaga lerstenii*** (Mickel & Beitel) Fay-Wei Li & Windham 8237, Oaxaca, Mexico, *Mickel 6238b* (UC, isotype); JX313636, JX313536, JX313490, JX313580, JX313581 (13); NA. ***Gaga marginata*** (Kunth) Fay-Wei Li & Windham 4986, Tarija, Bolivia, *Serrano et al. 6089* (MO); JN647754, JN647812, JX313491, JX313582 (9); 64.59±1.70; 32. ***Gaga marginata*** (Kunth) Fay-Wei Li & Windham 5569, Heredia, Costa Rica, *Rothfels 2693* (DUKE); JN647756, JN647814, JX313493, NA; 69.24±2.92; 32. ***Gaga marginata*** (Kunth) Fay-Wei Li & Windham 5571, Heredia, Costa Rica, *Rothfels 2692* (DUKE); JN647757, JN647815, JX313494, NA; NA. ***Gaga marginata*** (Kunth) Fay-Wei Li & Windham 5579, Heredia, Costa Rica, *Rothfels et al. 2688* (DUKE); NA, NA, NA, JX313586 (10); NA. ***Gaga marginata*** (Kunth) Fay-Wei Li & Windham 6571, Jalisco, Mexico, *Rothfels 3137* (DUKE); JN647758, JN647816, JX313495, JX313587 (14); NA. ***Gaga marginata*** (Kunth) Fay-Wei Li & Windham 7443, Pichincha, Ecuador, *Rothfels 3731* (DUKE); JN647759, JN647817, JX313496, JX313588 (12); NA. ***Gaga marginata*** (Kunth) Fay-Wei Li & Windham 7823, Mexico, Mexico, *Tejero-Diez 2839* (NY); JN647729, JN647787, JX313462, NA; NA. ***Gaga marginata*** (Kunth) Fay-Wei Li & Windham 7833, Puebla, Mexico, *Ventura 1393* (NY); JN647760, JN647818, JX313497, NA; NA. ***Gaga marginata*** (Kunth) Fay-Wei Li & Windham 7834, Oaxaca, Mexico, *Mickel 7432* (NY); JN647761, JN647819, JX313498, NA; NA. ***Gaga marginata*** (Kunth) Fay-Wei Li & Windham 7844, Jalisco, Mexico, *Boutin and Brandt 2306* (NY); JN647762, JN647820, JX313499, NA; NA. ***Gaga marginata*** (Kunth) Fay-Wei Li & Windham 8096, Cartago, Costa Rica, *Moran 2361* (MO); JX313637, JX313537, JX313500, JX313589 (9); 66.03±2.13; 32. ***Gaga membranacea*** (Davenp.) Fay-Wei Li & Windham 7845, Oaxaca, Mexico, *Mickel 7431* (NY); JN647763, JN647821, JX313501, JX313590, JX313591 (9); 48.22±2.23; 64. ***Gaga membranacea*** (Davenp.) Fay-Wei Li & Windham 7846, Oaxaca, Mexico, *Mickel 7057* (NY); JN647764, JN647822, JX313502, JX313592, JX313593 (15); 43.33±1.22; 64. ***Gaga monstraparva*** Fay-Wei Li & Windham 7154, Mexico, Mexico, *Beck 1229* (DUKE); JX313647, JX313547, JX313523, JX313620, JX313621 (13); NA. ***Gaga pellaeopsis*** (Mickel) Fay-Wei Li & Windham 7815, Guerrero, Mexico, *Anderson and Laskowski 4394* (NY); JN647765, JN647823, JX313504, NA; 45.62±1.60; 62. ***Gaga pellaeopsis*** (Mickel) Fay-Wei Li & Windham 8001, Oaxaca, Mexico, *Breedlove 59767* (CAS); JN647766, JN647824, JX313506, NA; 47.74±1.68; 60. ***Gaga pellaeopsis*** (Mickel) Fay-Wei Li & Windham 8104, Jalisco, Mexico, *Iltis et al 29543* (NY, isotype); JX313641, JX313541, JX313508, JX313596 (10); 49.88±3.91; 59. ***Gaga purpusii*** (T. Reeves) Fay-Wei Li & Windham 4995, Querétaro, Mexico, *Steinmann 2528* (MO); JN647767, JN647825, JX313509, JX313597, JX313598 (13); NA. ***Gaga purpusii*** (T. Reeves) Fay-Wei Li & Windham 6486, Hidalgo, Mexico, *Rothfels 3027* (DUKE); JN647768, JN647826, JX313510, JX313599 (17); 46.75±2.25; 62. ***Gaga purpusii*** (T. Reeves) Fay-Wei Li & Windham 8089, Hidalgo, Mexico, *Kenoyer 586* (F); JX313642, JX313542, JX313511, JX313600, JX313601 (14); 45.48±1.20; 60. ***Gaga purpusii*** (T. Reeves) Fay-Wei Li & Windham 8092, Guanajuato, Mexico, *Ventura and Lopez 9059* (NY); JX313643, JX313543, JX313512, JX313602, JX313603 (16); 43.51±1.58; 60. ***Gaga purpusii*** (T. Reeves) Fay-Wei Li & Windham 8093, Querétaro, Mexico, *Barriga 6598* (NY); JX313644, JX313544, JX313513, NA; 49.46±2.53; 63. ***Gaga purpusii*** (T. Reeves) Fay-Wei Li & Windham 8236, San Luis Potosí, Mexico, *Purpus 4881* (UC, isotype); JX313645, JX313545, JX313514, JX313604 (9); 42.37±1.97; 59. ***Gaga* sp.** 8097, San José, Costa Rica, *Davidse 24959* (MO); JX313638, JX313538, JX313503, NA; NA.

Outgroup—Aspidotis californica (Hook.) Nutt. ex Copel. 3831, California, U. S. A., *Metzgar 178* (DUKE); JX313624, JX313525, JX313445, JX313548 (11); NA. ***Aspidotis californica*** (Hook.) Nutt. ex Copel. NA, California, U. S. A., *Windham 856* (DUKE); NA, NA, NA, NA; 37.602±1.548; 64. ***Aspidotis densa*** (Brack.) Lellinger 3870, Oregon, U. S. A., *Pryer 06-02* (DUKE); JX313625, JX313526, JX313446, JX313549 (9); NA. ***Aspidotis carlotta-halliae*** (W.H. Wagner & E.F. Gilbert) Lellinger NA, California, U. S. A., *Ranker 902* (DUKE); NA, NA, NA, NA; 52.004± 2.161; 58. ***Aspidotis densa*** (Brack.) Lellinger NA, Washington, U. S. A., *Windham 823* (DUKE); NA, NA, NA, NA; 41.663± 2.613; 62. ***Aspidotis meifolia*** (D. C. Eaton) Pic. Serm. 5056, Tamaulipas, Mexico, *Yatskievych and Gastony 89-219* (MO); JX313626, JX313527, JX313447, NA; NA. ***Cheilanthes micropteris*** Sw. 3709, Misiones, Argentina, *Deginani et al. 1363* (MO); JX313627, EF452145, EU268683, NA; NA. ***Cheilanthes rufopunctata*** Rosenst. 7829, Carrasco, Bolivia, *Sundue 613* (US); JX313628, JX313528, JX313448, NA; NA. ***Hemionitis arifolia*** (Burm. f.) T. Moore 5318, Bolikhamsai, Laos, *Wu et al. WS-296* (MO); JX313629, JX313529, JX313449, NA; NA. ***Pellaea angulosa*** (Bory ex Willd.) Baker 4711, Iringa, Tanzania, *Kayombo 2654* (MO); JX313630, JX313530, JX313450, NA; NA. ***Pentagramma triangularis*** (Kaulf.) Yatsk., Windham & E. Wollenw. 3869, Oregon, U. S. A., *Pryer 06-01* (DUKE); JX313631, JX313531, JX313451, NA; NA.