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Ecological and Evolutionary Origin of *Costus flammulus* (Costaceae): A New Species from the Montane Cloud Forests of the Volcanic Cordilleras in Northern Costa Rica

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Abstract—*Costus flammulus* is a new herbaceous species endemic to montane cloud forests of the volcanic cordilleras in northern Costa Rica. *Costus flammulus* has been mistaken for *C. wilsonii*, but phylogenetic evidence demonstrates that it is closely related to the widespread lowland species *C. pulverulentus*. Here, we use an integrated framework of species concepts to evaluate whether *C. flammulus* and *C. pulverulentus* are distinct species. First, we re-evaluate prior phylogenetic analyses to assess whether *C. flammulus* bifurcated from or budded off from within *C. pulverulentus* and whether *C. flammulus* is monophyletic. We then compare phenotypic traits to determine which diagnostic vegetative and inflorescence traits can be used to identify species in herbarium specimens and examine whether floral traits may confer floral isolation. We compare pollinator assemblages to examine whether pollinator specificity may contribute to reproductive isolation. Finally, we model species distributions and climatic niche overlap to assess ecogeographic isolation. We found that *C. flammulus* is a monophyletic species phenotypically, ecologically, and geographically distinct from *C. pulverulentus* and may have speciated as a peripheral isolate at the high elevation range edge of *C. pulverulentus*. Several lines of evidence, such as *C. pulverulentus* paraphyly, range size asymmetry, and *C. flammulus'* nested distribution and vegetative traits, suggest that *C. flammulus* budded off from a *C. pulverulentus*-like progenitor species, evolving to tolerate a colder and more seasonal montane environment.

Keywords—Budding speciation, ecogeographic isolation, floral isolation, integrative taxonomy, Neotropical flora, species concepts.

Costus flammulus K.M.Kay & P.Juárez (Costaceae) is a newly proposed plant species restricted to the montane cloud forests of the volcanic cordilleras in northern Costa Rica (Fig. 1). It is a perennial hummingbird-pollinated herb found in forest understory and rocky ravines. This new taxon (hereafter *C. flammulus*) was first identified by phylogenetic analyses of the Neotropical *Costus* L. (Kay et al. 2005) and its phylogenetic placement was confirmed with the recent 756 gene phylogenomic analyses of Vargas et al. (2020). *Costus flammulus* is closely related to the widespread species *C. pulverulentus* C.Presl (Fig. 1), and diverged ~714,000 yr ago (95% confidence interval: 180,000–1,420,000 yr; Vargas et al. 2020). Vargas et al. (2020) suggested that *C. flammulus* may be phylogenetically nested within a paraphyletic *C. pulverulentus* and geographically nested within its range.

Previously, *C. flammulus* had been included in *C. wilsonii* Maas (e.g. Stratton 1989; Haber 2000; Maas and Maas-van de Kamer 2003), apparently due to its similar phenotype and habitat (see Notes). Both species are hummingbird-pollinated understory herbs restricted to montane forests with similar foliage and inflorescence characteristics. However, phylogenetic evidence by Kay et al. (2005) and Vargas et al. (2020) show that *C. flammulus* and *C. wilsonii* are independent evolutionary lineages that may have converged on a similar phenotype. Their most recent common ancestor lived ~3 MYA in the Pliocene when *Costus* first diverged in the Neotropics (Vargas et al. 2020); thus, *C. flammulus* and *C. wilsonii* are not closely related within Neotropical *Costus*.

To understand the ecology, delimitation, and evolutionary origin of *C. flammulus*, we investigate its phylogenetic position, phenotype, distribution, ecological niche, and reproductive isolation from *C. pulverulentus*. Phylogenetic analysis is indispensable for assessing monophyly, the primary criterion for defining species and delimiting taxa under the Phylogenetic Species Concept (reviewed in Baum and Smith 2013). Phylogenetic evidence also may be the most compelling for analyzing modes of speciation, such as budding speciation or splitting cladogenesis (Foote 1996; Sianta and Kay 2022). In plants with low dispersal and strong local adaptation among populations, budding speciation is hypothesized to be a particularly common mode of speciation (Grossenbacher et al. 2014). Budding speciation involves the isolation of marginal populations at the periphery of a species range (Crawford 2010) and initially results in a monophyletic 'derivative' species phylogenetically nested within a paraphyletic 'progenitor' species (Baldwin 2005; Sianta and Kay 2022). Phenotypic distinguishability is essential for species classification and identification (Zapata and Jiménez 2012), and phenotypic comparisons can suggest potential forms of reproductive isolation, which are key criteria for species delimitation under the Biological Species Concept (Mayr 1942). For instance, floral divergence between closely related species may indicate divergence in pollination and/or mating systems that may contribute to reproductive isolation (Van der Niet et al. 2014) and can be investigated with field studies. Moreover, comparing geographic distributions and ecological niches of closely related species can suggest the geographic mode of speciation and whether ecogeographic isolation may be important (Raxworthy et al. 2007; Rissler and Apodaca 2007). This is relevant because divergent adaptation to local environmental conditions often may be the primary source of divergence (Mayr 1947; Futuyma 1998; Hendry 2017). An integrative approach to species delimitation is useful for understanding the roles of ecological divergence and reproductive isolation in speciation (De Queiroz 1998, 2007; Dayrat 2005).

Here, we combine a traditional protologue with an analysis of ecological divergence and putative reproductive isolating mechanisms related to ecogeographic and floral isolation to compare *C. flammulus* with its putative progenitor or sister species, *C. pulverulentus* (Fig. 1). First, we reevaluate the phylogenetic evidence from Vargas et al. (2020) to assess whether

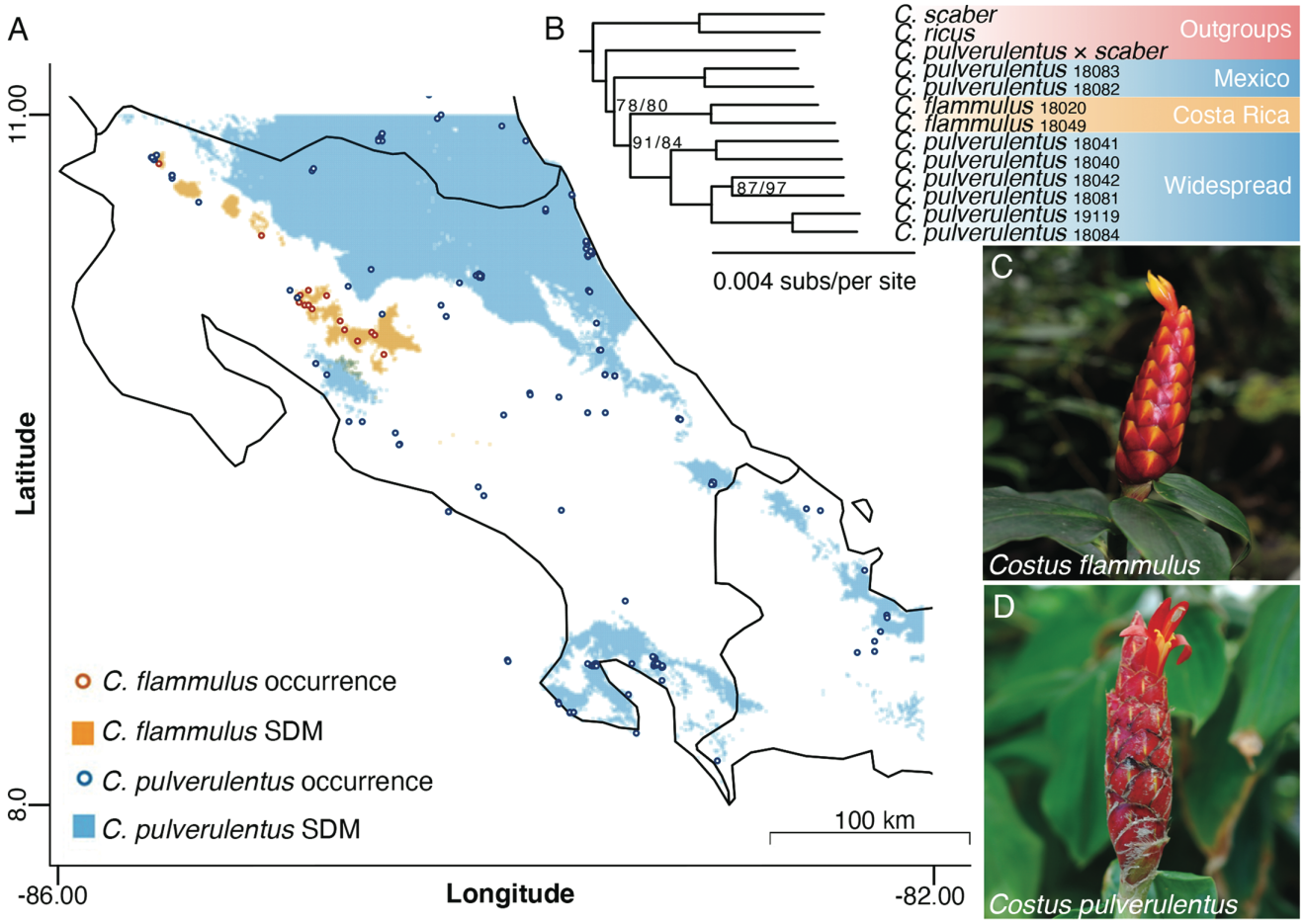

FIG. 1. *Costus flammulus* and *C. pulverulentus*. A. Map of occurrences and the 80% probability of occurrence for each species derived from species distribution models (SDM) based on bioclimatic data. White outline shows southern Nicaragua, Costa Rica, and Northwestern Panama. B. Pruned phylogram inferred from the concatenated matrix of 756 genes for *Costus* (Vargas et al. 2020). Numbers to the right of nodes indicate phylogenetic support, left: ultrafast bootstrap, right: Shimodaira-Hasegawa approximate ratio; nodes without values are fully supported at 100. C–D. Photographs of *C. flammulus* (C) and *C. pulverulentus* (D). Photographs by P. Juárez (C); and K. M. Kay (D).

C. flammulus bifurcated or budded off from *C. pulverulentus* and whether it is monophyletic. We then compare phenotypic traits to determine which diagnostic vegetative and inflorescence traits can be used to identify species in herbarium specimens and examine floral traits that may contribute to reproductive isolation. We compare pollinator assemblages to examine to what extent pollinator specificity may contribute to reproductive isolation. Finally, we model species distributions and climatic niche overlap to assess ecogeographic isolation.

MATERIALS AND METHODS

Species Concepts and Species Delimitation—We use an integrated framework of species concepts to assess whether *C. flammulus* and *C. pulverulentus* are two phylogenetically, morphologically, and ecologically distinct species. First, we use the Phylogenetic Species Concept framework to delimit *C. flammulus* (Donoghue 1985; Mishler 1985). Second, we use the traditional Phenetic Species Concept framework to study phenotypic trait divergence and identify diagnostic phenotypic traits and classify species based on overall similarity (Michener 1970; Sokal and Crovello 1970; Sneath and Sokal 1973). Third, we use the Ecological Species Concept framework to determine whether *C. flammulus* and *C. pulverulentus* are two ecologically distinct species that occupy different climate and pollinator niches (Van Valen 1976; Andersson 1990). Finally, we use the Biological Species Concept framework to study putative reproductive isolating barriers of *C. flammulus*, such as ecogeographic and floral isolation (Mayr 1942; Dobzhansky 1970; Coyne and Orr 2004).

Study System—Neotropical *Costus* comprises approximately 60 named species found in habitats from sea level to montane forests throughout tropical Central and South America (Maas 1972, 1977; Maas and Maas-van de Kamer 2003, 2014; Kay et al. 2005; Vargas et al. 2020). *Costus* are perennial herbs found in forest edges, forest gaps, forest understory, ravines, and riparian habitats. Neotropical *Costus* is a recent and rapid species radiation since dispersing from Africa approximately 3 MYA. Neotropical *Costus* species are primarily pollinated by euglossine bees (tribe Euglossini) or hermit hummingbirds (subfamily Phaethornithinae) (Kay and Schemske 2003; Kay 2006; Kay and Grossenbacher 2022) and there have been many evolutionary transitions from bee to hummingbird pollination (Kay and Grossenbacher 2022). Prior studies on Neotropical *Costus* suggest that ecogeographic and floral isolation play an important role in segregating species (Kay and Schemske 2003; Kay 2006; Chen 2013; Chen and Schemske 2015). Ecogeographic isolation is geographic isolation resulting from genetically based ecological differences between species (Sobel et al. 2010; Sobel 2014). Floral isolation is reproductive isolation mediated by floral morphology and pollinator behavior (Grant 1994; Kay and Sargent 2009; Schiestl and Schlüter 2009).

Specimen Sampling—We studied herbarium specimens from Costa Rica determined as *Costus*, *C. pulverulentus*, and *C. wilsonii* of the following herbaria: CR, HLDG, MO, and USJ. Herbarium acronyms cited in this paper follow Thiers (2022). All specimens we identified as *C. flammulus* were used to extract occurrence data. We selected one representative *C. pulverulentus* specimen from every district where the species occurs in Costa Rica to measure vegetative and inflorescence traits and compare elevation (Supplemental Table S1; Juárez et al. 2023). For the elevation comparison, we excluded those herbarium specimens for which elevation was reported as a range. We also visited the type locality of *C. flammulus* (Estación Biologica Monteverde, Quebrada Máquina, 1,524 m, 10°19′03′′N, 84°48′27′′W) in August 2021 to record pollinator visitation and to collect

and measure flowers for floral trait comparisons. *Costus pulverulentus* flowers were collected and measured from several Costa Rica and Panama locations (Table S2, Juárez et al. 2023).

***Phylogenetic Analyses*—**The concatenated analysis of 756 nuclear genes of Vargas et al. (2020) showed that two samples of *C. flammulus* (*C.* sp. nov. 18020, *C.* sp. nov. 18049) appear monophyletic (support: 100% bootstrap, 161 gene topologies) and nested within *C. pulverulentus* (support: 91% bootstrap, 1 gene topology) (Fig. 1). Precisely, *C. flammulus* appears to be sister to a clade comprising *C. pulverulentus* samples from Costa Rica, Panama, and South America (hereafter widespread); with samples of *C. pulverulentus* from Mexico (hereafter Mexican) sister to a clade containing *C. flammulus* and widespread *C. pulverulentus* (support: 78% bootstrap, 1 gene topology). This topological pattern suggests that *C. flammulus* may be a derivative from a *C. pulverulentus*-like progenitor species (Crawford 2010; Sianta and Kay 2022). Here, we further investigate gene tree support for different phylogenetic placements of *C. flammulus* and *C. pulverulentus* by calculating the support per gene of alternative topologies in the Vargas et al. (2020) results. We asked how many gene trees recover these alternative topologies: a) *C. flammulus* and *C. pulverulentus* as comprising a single clade (regardless of the monophyly of each species), b) *C. pulverulentus* as monophyletic, c) *C. flammulus* as monophyletic, d) *C. flammulus* and widespread *C. pulverulentus* as a single clade, e) *C. flammulus* sister to widespread *C. pulverulentus* (as shown in the concatenated phylogeny), f) *C. flammulus* and Mexican *C. pulverulentus* comprising a single clade, and g) *C. flammulus* sister to Mexican *C. pulverulentus*. We calculated the raw number of gene trees that support each alternative topology, and their percentages relative to the number of gene trees that contain any phylogenetic signal for the position of *C. flammulus* and *C. pulverulentus*. Trees with strong phylogenetic signal are defined as those in which any sample belonging to the focal species forms a well-supported clade (≥ 90 bootstrap support) with any other sample, as calculated by Vargas et al. (2020). We used a custom R script to analyze trees and calculate their gene tree support using the packages phytools (Revell 2012) and MonoPhy (Schwery and O'Meara 2016).

***Phenotypic Trait Measurements and Comparisons*—**We measured five vegetative and five inflorescence traits (Table 1; Fig. 2) on herbarium specimens of *C. flammulus* ($n = 21$) and *C. pulverulentus* ($n = 28$) to determine which diagnostic traits can be used to separate the two species. Vegetative traits include stem diameter (mm), ligule length (mm), petiole length (mm), and leaf length and width (cm). Inflorescence traits include inflorescence length and width (cm), bract length and width (cm), and nectarial callus length (mm). The nectarial callus is located on top of the bracts (Maas 1972) and commonly produces extrafloral nectar that attracts ants that deter florivores and fruit predators (Schemske 1982), although in some species the callus is vestigial.

To determine floral divergence that may contribute to floral isolation, we measured eight floral traits (Table 1; Fig. 2) on fresh flowers of *C. flammulus* ($n = 7$) and *C. pulverulentus* ($n = 17$). Floral traits include corolla length (cm), corolla tube length (cm), corolla dorsal lobe length (cm), labellum length (cm), petaloid stamen length (cm), petaloid stamen exsertion length (mm), anther length (mm), and style length (cm). We separately compared herbarium specimens and floral trait measurements with multiple two-tailed *t* tests and adjusting *p* values within data sets with false discovery rate (FDR) controlling procedures (Benjamini and Hochberg 1995) using the R package Stats (R Core Team 2021).

We used two separate principal component analyses (PCA) to visualize phenotypic distributions for each dataset using the R package Stats; one for vegetative and inflorescence traits measured on herbarium specimens and the other for floral traits measured on fresh flowers. The PCAs allowed us to reduce data complexity and graphically represent differences between the two species (Ringnér 2008). To plot PCAs, we used the R packages ggfortify (Tang et al. 2016) and ggplot2 (Wickham 2009).

***Pollinator Assemblage Sampling*—**We recorded pollinator visitation to six *C. flammulus* individuals at the type locality for seven days in August 2021 to determine pollinator identities and visitation rates. We recorded visitation with video cameras from 6:00 AM to 2:00 PM, when pollinators start activity to about when daily pollinator activity decreases, and flowers wilt. We used Canon PowerShot SX420 IS and Canon PowerShot SX540 HS digital cameras with auto-controlled aperture settings to record visitation. We used the Canon Hack Development Kit (CHDK, https://chdk.fandom.com) to automate motion detection features. We recorded 15-sec videos with the motion detection script MDFB2013 setting the trigger threshold to 5 and burst to 15-sec. Pollinator visitation to *C. pulverulentus* was reported previously (Kay and Schemske 2003; Kay 2006).

***Species Distribution Models*—**To compare suitable habitats, niches, and geographic ranges, we built maximum entropy species distribution models (SDMs) for *C. flammulus* and *C. pulverulentus* with climate data following the methods in Goff et al. (2021). We obtained occurrence data of both species from herbarium databases (CR, https://biodiversidad.museocostarica.go.cr; MO, https://tropicos.org) and field observations by K. M. Kay and P. Juárez. We excluded occurrences with duplicated collection localities and uncertain taxonomic identifications. To avoid potential misidentifications, we considered only occurrences with identifications made by one of four taxonomic experts: P. Maas, D. Skinner, K. M. Kay, and P. Juárez. We used 21 specimens for *C. flammulus* from Costa Rica identified by P. Juárez and 593 for *C. pulverulentus* (Table S3, Juárez et al. 2023). The specimen number disparity was expected since *C. flammulus* is only known from a few locations on volcanic cordilleras in northern Costa Rica (Fig. 1; see Distribution and Habitat), whereas *C. pulverulentus* is a widespread species ranging from Mexico to southern Ecuador (Maas 1972).

We used occurrence data for 29 Neotropical flowering plant genera (Table S4, Juárez et al. 2023) as pseudo-absence data in the SDMs to represent the range of environmental conditions available to *C. flammulus* and *C. pulverulentus* and to correct for geographic variation in plant collecting

TABLE 1. Phenotypic and elevational comparisons between *C. flammulus* and *C. pulverulentus*. Abbreviations is for abbreviations in Fig. 2. ns $p \geq 0.05$, $^{*}p \leq 0.05$, $^{**}p \leq 0.001$, $^{***}p \leq 0.0001$.

Trait	Abbreviations	Mean ± 95% CI (*n*)		*p*
		C. flammulus	*C. pulverulentus*	
Stem diameter (mm)	Stm	6.79 ± 0.90 (21)	8.96 ± 1.13 (28)	*
Ligule length (mm)	Lg	5.90 ± 0.73 (21)	7.32 ± 1.67 (28)	ns
Petiole length (mm)	P	5.09 ± 0.77 (21)	5.17 ± 0.61 (28)	ns
Leaf blade length (cm)	Lvsl	14.67 ± 1.08 (21)	18.23 ± 1.78 (28)	*
Leaf blade width (cm)	Lvsw	4.30 ± 0.45 (21)	5.34 ± 0.47 (28)	*
Inflorescence length (cm)	Infl	6.44 ± 0.80 (21)	9.78 ± 1.16 (28)	***
Inflorescence width (cm)	Infw	3.01 ± 0.22 (21)	3.69 ± 0.82 (28)	ns
Bract length (cm)	Bl	1.76 ± 0.13 (21)	2.56 ± 0.21 (28)	***
Bract width (cm)	Bw	1.56 ± 0.13 (21)	1.87 ± 0.14 (28)	*
Nectarial callus length (mm)	Cal	6.14 ± 0.80 (21)	8.82 ± 1.12 (28)	**
Corolla length (cm)	C	3.44 ± 0.27 (7)	6.19 ± 0.27 (17)	***
Corolla tube length (cm)	Ct	1.11 ± 0.21 (7)	2.06 ± 0.26 (17)	***
Corolla dorsal lobe length (cm)	Cdl	3.47 ± 0.50 (7)	4.20 ± 0.24 (17)	*
Labellum length (cm)	L	3.22 ± 0.35 (7)	4.76 ± 0.23 (17)	***
Petaloid stamen length (cm)	S	3.52 ± 0.21 (7)	6.37 ± 0.26 (17)	***
Petaloid stamen exsertion (mm)	Se	5 ± 0 (7)	16.27 ± 2.30 (17)	***
Anther length (mm)	A	7 ± 0 (7)	8.32 ± 0.73 (17)	***
Style length (cm)	St	2.91 ± 0.21 (7)	5.87 ± 0.21 (17)	***
Elevation (m)	–	1310.7 ± 688.2 (14)	443.7 ± 1045.6 (25)	***

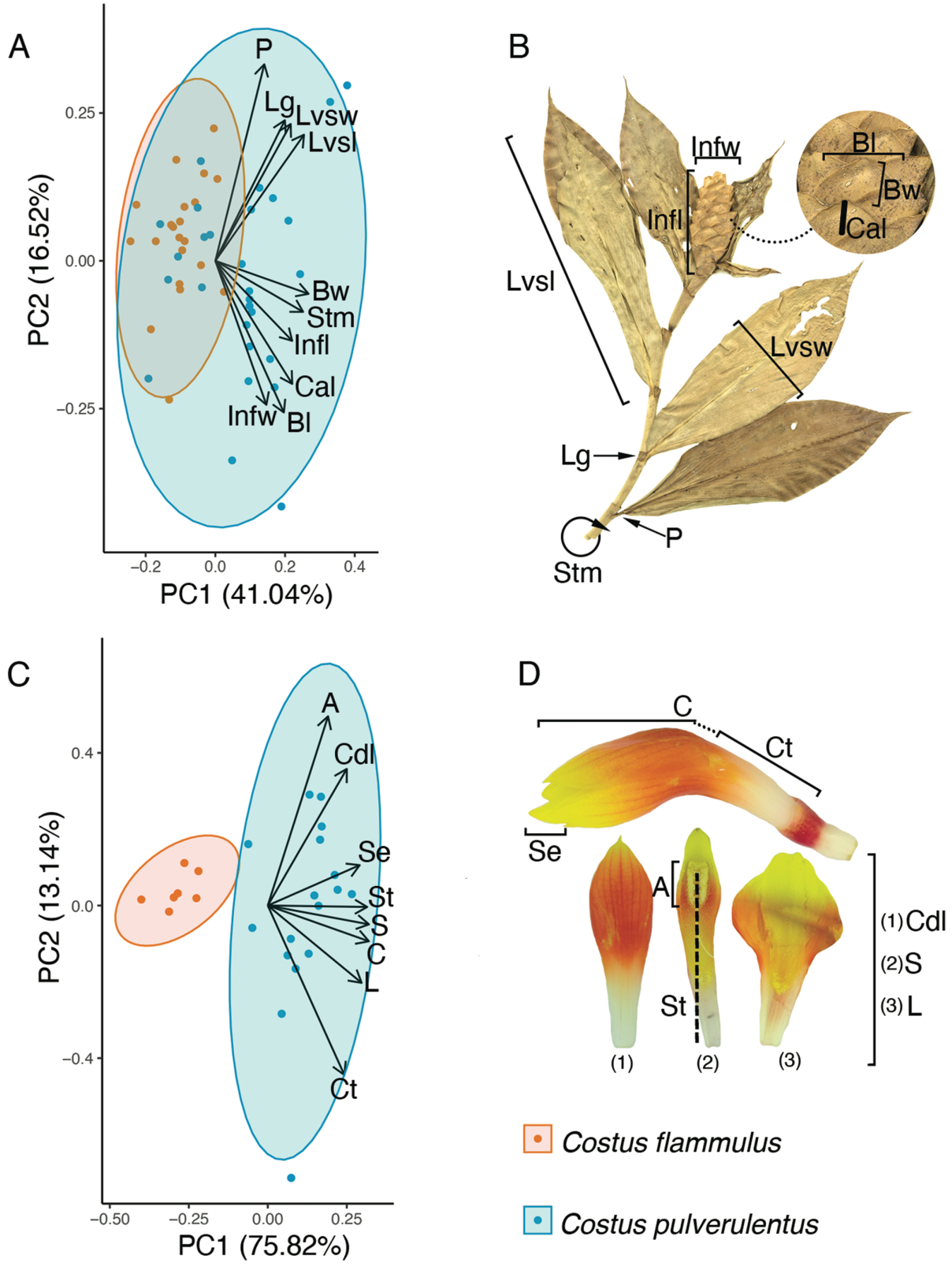

FIG. 2. Phenotypic comparisons between *Costus flammulus* and *C. pulverulentus*. A. Scatterplot from principal component analysis of the vegetative and inflorescence traits measured in herbarium specimens; PC1 and PC2 explain 41.04% and 16.52% of the total phenotypic variance, respectively. *Costus flammulus* vegetative and inflorescence traits appear as a nested subset of *C. pulverulentus*. B. Diagram of the vegetative and inflorescence traits measured on herbarium specimens. Image based on *Haber, Ivey & Zuchowski 10798* (CR, MO). C. Scatterplot from principal component analysis of the floral traits measured on fresh flowers; PC1 and PC2 explain 75.82% and 13.14% of the total phenotypic variance, respectively. *Costus flammulus* and *C. pulverulentus* mainly differentiate along PC1, with high values corresponding to all floral traits measured. D. Diagram of the floral traits measured on fresh flowers. Photographs by M. Morello (B); and C. Girvin (D).

TABLE 2. Number of gene trees that support specific relationships in *C. flammulus* and *C. pulverulentus*. Percentage is calculated over the 443 gene trees that contain phylogenetic signal for the focal species.

Relationship	Number of trees	Percentage
C. flammulus and *C. pulverulentus* are a clade	6	1.4
C. pulverulentus is monophyletic	20	4.6
C. flammulus is monophyletic	243	54.9
C. flammulus and Widespread *C. pulverulentus* are a clade	8	1.8
C. flammulus is sister to Widespread *C. pulverulentus*	2	0.5
C. flammulus and Mexican *C. pulverulentus* are a clade	3	0.7
C. flammulus is sister to Mexican *C. pulverulentus*	3	0.7

effort and accessibility. Using pseudo-absence data that is similarly spatially biased to the actual occurrence data of *Costus* generates less-biased models than when using randomly selected pseudo-absence data (Phillips et al. 2009). We bounded the pseudo-absence points for the coordinates of the terrestrial political borders of Costa Rica (08° to 11° latitude and -86° to -82° longitude) and subsampled them so that there were five pseudo-absence points per 10-min resolution grid cell, totaling 20,424 points (Fig. S1, Juárez et al. 2023). We limited the analysis to the boundaries of Costa Rica, even though the actual *C. pulverulentus* distribution range extends beyond these because we wanted to compare SDMs and ecological niches from co-occurring populations in the same geographical area.

We used 19 BioClim variables at 0.5-min resolution from WorldClim (https://www.worldclim.org) as explanatory variables for the SDMs. To build SDMs, we combined the climate data for *C. flammulus* and *C. pulverulentus* and pseudo-absence data using the default MaxEnt settings with the R package dismo (Hijmans et al. 2017). We used 80% of the occurrence data for each *Costus* species to train the model and 20% to test its performance. We assessed models with the area under the receiver operating characteristic (ROC) curves (AUC) and qualitatively compared the consistency of the percentage contribution of variables to the models.

Climatic Niche Comparisons—We made a multivariate comparison of the climatic niche by circumscribing each *Costus* species' occurrence-based niche relative to the available niche space established by pseudo-absence data using the R package ecospat (Broennimann et al. 2012). We extracted values for the climatic variables from each spatial cell at 0.5-min resolution included in the geographic extent of the pseudo-absence occurrence points used for the SDMs. We used PCA of the 19 BioClim variables to visualize axes of niche overlap, projecting *C. flammulus* and *C. pulverulentus* into an available niche space and tested for niche differentiation. We generated 100 × 100 PCA unit grid cells and used the *Costus* species' occurrence data to project the density of each species into an environmental space. This projection applies a kernel density function to determine the density of occurrences in each cell in the environmental space for each species and lessen sampling strategy bias effects when calculating niche differences (Broennimann et al. 2012). We calculated niche overlap as Schoener's *D* (Schoener 1970) between *C. flammulus* and *C. pulverulentus* and tested for niche equivalency and similarity (Warren et al. 2008) using ecospat. Niche similarity indicates whether two species occupy a similar subset of the total available environment, whereas niche equivalency indicates whether the niches of the two species are statistically distinguishable (Warren et al. 2008; Broennimann et al. 2012).

RESULTS

Phylogenetics—Only 443 genes, out of the 756 genes, have some phylogenetic signal for the position of *C. flammulus*. The monophyly of *C. flammulus* is supported by 243 genes (54.9%) (Table 2). Our support per gene analysis regarding the position of *C. flammulus* relative to *C. pulverulentus* is inconclusive. Twenty genes support the monophyly of all *C. pulverulentus* samples. Only two genes recover *C. flammulus* as sister to widespread *C. pulverulentus*, while three genes show *C. flammulus* as sister to Mexican *C. pulverulentus*. Six genes support the position of *C. flammulus* nested in *C. pulverulentus* and eight genes support *C. flammulus* forming a clade with widespread *C. pulverulentus* (regardless of species-level monophyly). Despite the uncertainty shown by the gene support analysis, our results support that *C. flammulus* samples form a monophyletic clade, possibly nested within *C. pulverulentus*. The concatenated analysis in Vargas et al. (2020), in which *C. flammulus* is nested within *C. pulverulentus* and reciprocally monophyletic with widespread *C. pulverulentus,* may be our best hypothesis, but we only find strong support for the monophyly of *C. flammulus*.

Phenotypic Trait Comparisons—The phenotypic trait comparisons reveal consistent floral trait differences between *C. flammulus* and *C. pulverulentus*, but fewer differences in vegetative and inflorescence traits (Table 1; Fig. 2). *Costus flammulus* has significantly narrower stems and smaller leaves than *C. pulverulentus*. Inflorescence length, bract size, and nectarial callus are significantly different, with *C. flammulus* having shorter inflorescences and smaller bracts than *C. pulverulentus*. The nectarial callus is also shorter in *C. flammulus*, and according to field observations is not functional and does not attract ants, unlike the active extrafloral nectaries of *C. pulverulentus* (Schemske 1982). All floral traits differ significantly; *C. flammulus* has smaller flowers than *C. pulverulentus*. The PCAs demonstrate that *C. flammulus* vegetative and inflorescence traits are a nested subset of C. *pulverulentus*; conversely, floral traits form clearly separate phenotypic clusters (Fig. 2).

Pollinator Assemblage Overlap—We did not detect an overlap in pollinator assemblage between *C. flammulus* and *C. pulverulentus*. *Costus flammulus* was almost exclusively visited by the green hermit (*Phaethornis guy* Lesson) (0.250 visits/hour; 14 legitimate visits) during observations in the type locality; with an additional visit by the black-crested coquette (*Lophornis helenae* Delattre) (0.018 visits/hour; 1 legitimate visit), and several nectar robbing approaches by the white-throated mountaingem (*Lampornis castaneoventris* Gould). Kay and Schemske (2003) and Kay (2006) previously recorded pollinator assemblages for several *Costus* species and determined that *C. pulverulentus* is solely visited by the long-billed hermit (*P. longirostris* Delattre) at all their study sites in Costa Rica and Panama.

Species Distribution Models—The SDMs for *C. flammulus* predicted almost no probability of environmental suitability for *C. pulverulentus* within the range of *C. flammulus* and vice versa (Fig. 1), with the predicted areas of the probability of occurrence closely overlapping the actual species occurrences. The SDMs predict that *C. flammulus* is restricted to the volcanic cordilleras in northern Costa Rica, whereas *C. pulverulentus* occurs primarily in the wet lowlands of Costa Rica, especially in the northern Caribbean plains and southern Pacific region. The SDMs built using 19 BioClim as explanatory variables performed well, as indicated by the area under the receiver operating characteristic curve (AUC of ROC) scores, which are typical measures of SDM performance.

The AUC of ROC was 0.945 for *C. flammulus* and 0.86 for *C. pulverulentus*.

Climatic Niche Overlap—*Costus flammulus* and *C. pulverulentus* occupy significantly different climatic niches (Fig. 3). Within the available climatic niche determined by the pseudo-absence occurrence data, *C. flammulus* and *C. pulverulentus* differentiate along a combination of PC1 and PC2, with *C. flammulus* occurring in colder climates within the drier and more seasonal part of *C. pulverulentus*' precipitation range (Fig. 3). *Costus pulverulentus* occupies a broader climatic niche space, as expected given its widespread distribution, and there is little climate niche overlap between the species. The *D* metric of niche overlap for *C. flammulus* and *C. pulverulentus* was 0.0038, which is significantly lower than when aggregated occurrences were randomly assigned to species (ecospat test of niche equivalency; $p = 0.0099$), indicating that the two species occupy different climatic niches (Fig. 3; Warren et al. 2008). The ecospat test for niche similarity shows that the species' niches are more similar than expected when sampling the total available environment at random ($p = 0.4545$), as expected for closely related taxa (Warren et al. 2008).

Discussion

We describe *C. flammulus*, a new herbaceous species endemic to the montane cloud forests of the volcanic cordilleras in northern Costa Rica. *Costus flammulus* is a monophyletic species that is phenotypically, ecologically, reproductively, and geographically distinct from its closest relative, *C. pulverulentus*. It may have speciated as a peripheral isolate at the high elevation range edge of the widespread, lowland *C. pulverulentus*. *Costus flammulus* can be distinguished from *C. pulverulentus* by its smaller leaves, inflorescences, and flowers and distribution at higher elevations in montane cloud forests.

Our integrative approach allows us to circumscribe *C. flammulus* under multiple species concepts. (a) Under the Phenetic Species Concept framework (Michener 1970; Sokal and Crovello 1970; Sneath and Sokal 1973), *C. flammulus* is a phenotypically distinct species from *C. pulverulentus*, forming a divergent phenotypic cluster in a floral phenotypic space (Fig. 2). Additionally, our phenotypic comparisons show that *C. flammulus* and *C. pulverulentus* significantly differ in leaf, inflorescence, and flower size (Table 1). (b) Under the Ecological Species Concept framework (Van Valen 1976; Andersson 1990), *C. flammulus* is an ecologically distinct species from *C. pulverulentus* because it occupies fundamentally different pollinator and climate niches. Our pollinator observations show that *C. flammulus* and *C. pulverulentus* are consistently visited by different species of hermit hummingbirds with no pollinator assemblage overlap, although that might be because their specific hummingbird pollinators, the green hermit, and the long-billed hermit, also segregate geographically. Our SDMs and ecological niche comparisons show that *C. flammulus* and *C. pulverulentus* niches have diverged and that there is little climatic niche overlap. (c) Under the Biological Species Concept framework (Mayr 1942; Dobzhansky 1970; Coyne and Orr 2004), *C. flammulus* is a reproductively isolated species from *C. pulverulentus* because their effective ecogeographic isolation is nearly complete, such that their populations are unlikely to experience gene flow, and their floral trait divergence could mediate floral isolation if they were to come into geographic contact. (d) Under the Phylogenetic Species Concept framework (Donoghue 1985; Mishler 1985), our conclusions are less certain. Our re-evaluation of Vargas et al.'s (2020) phylogenetic analysis shows strong evidence that *C. flammulus* is a monophyletic species, and possibly nested within *C. pulverulentus*, although its exact position is uncertain, and we do not have strong support for reciprocal monophyly of *C. flammulus* and any subset of *C. pulverulentus* samples.

Costus flammulus' phylogenetic position may make *C. pulverulentus* paraphyletic. This topological pattern is congruent with budding speciation theory (Crawford 2010), in which *C. pulverulentus* and *C. flammulus* may be a progenitor-derivative species pair, i.e. *C. flammulus* may be a derivative from a *C. pulverulentus*-like progenitor species (see below). If true, according to the Phylogenetic Species Concept, *C. pulverulentus* may be considered two separate species, with a possible division between widespread and Mexican samples. Under this scenario, one of these two *C. pulverulentus* phylogenetic entities would need a new name, which would require a detailed examination of the type specimen of *C. pulverulentus* to determine whether it belongs to the Mexican or widespread *C. pulverulentus*. Although Mexico is the cited type locality in the original protologue (Presl 1827), there is uncertainty about the location. The *C. pulverulentus* type specimen was collected in 1790, possibly in Nicaragua, based on the documented itinerary of the collector (D. Skinner pers. comm.). Further investigation of *C. pulverulentus* taxonomy should involve phylogenetic analysis of samples from the putative type locality and could benefit from more extensive genetic data to better resolve relationships. Given our current data, splitting up *C. pulverulentus* is premature.

Phenotypic comparisons detected significant floral divergence with a reduction in floral size in *C. flammulus*, whereas no such pattern was found for vegetative and inflorescence traits. This suggests little diversification of vegetative and inflorescence traits but an evolutionary force acting on flowers. The maintenance of vegetative trait similarity accompanied by floral shifts may be expected between recently diverged species, as observed in other groups (Grant 1949; Van der Niet and Johnson 2009; Grossenbacher and Whittall 2011; Pessoa et al. 2020). This pattern may reflect recent shared ancestry, pollination or mating system divergence, and the establishment of prezygotic reproductive isolation mechanisms.

The quantified floral divergence suggests that floral isolation could be important in segregating *C. flammulus* and *C. pulverulentus* if they were to establish in sympatry. Floral divergence can mediate isolation between closely related species (Grant 1949, 1993, 1994; Fulton and Hodges 1999; Ramsey et al. 2003; Kay 2006; Schiestl and Schlüter 2009). Although we do not have direct measures of floral isolation, we can speculate based on our knowledge of isolating barriers between other species pairs. For instance, the reduction of stamen exsertion beyond the labellum could be particularly relevant for floral isolation. Our pollinator observations show that *C. flammulus* may place its pollen on the distal third of the hummingbirds' beak, whereas *C. pulverulentus* places its pollen on the forehead (Kay 2006; Kay and Surget-Groba 2022). Studies estimating reproductive barriers between sympatric *C. pulverulentus* and *C. scaber* Ruiz & Pav. have shown that divergence in stamen exsertion mediates mechanical isolation by placing pollen in different sites of the hummingbird; a pollen placement shift from the hummingbird beak to forehead produces nearly complete isolation (Kay 2006). Differences

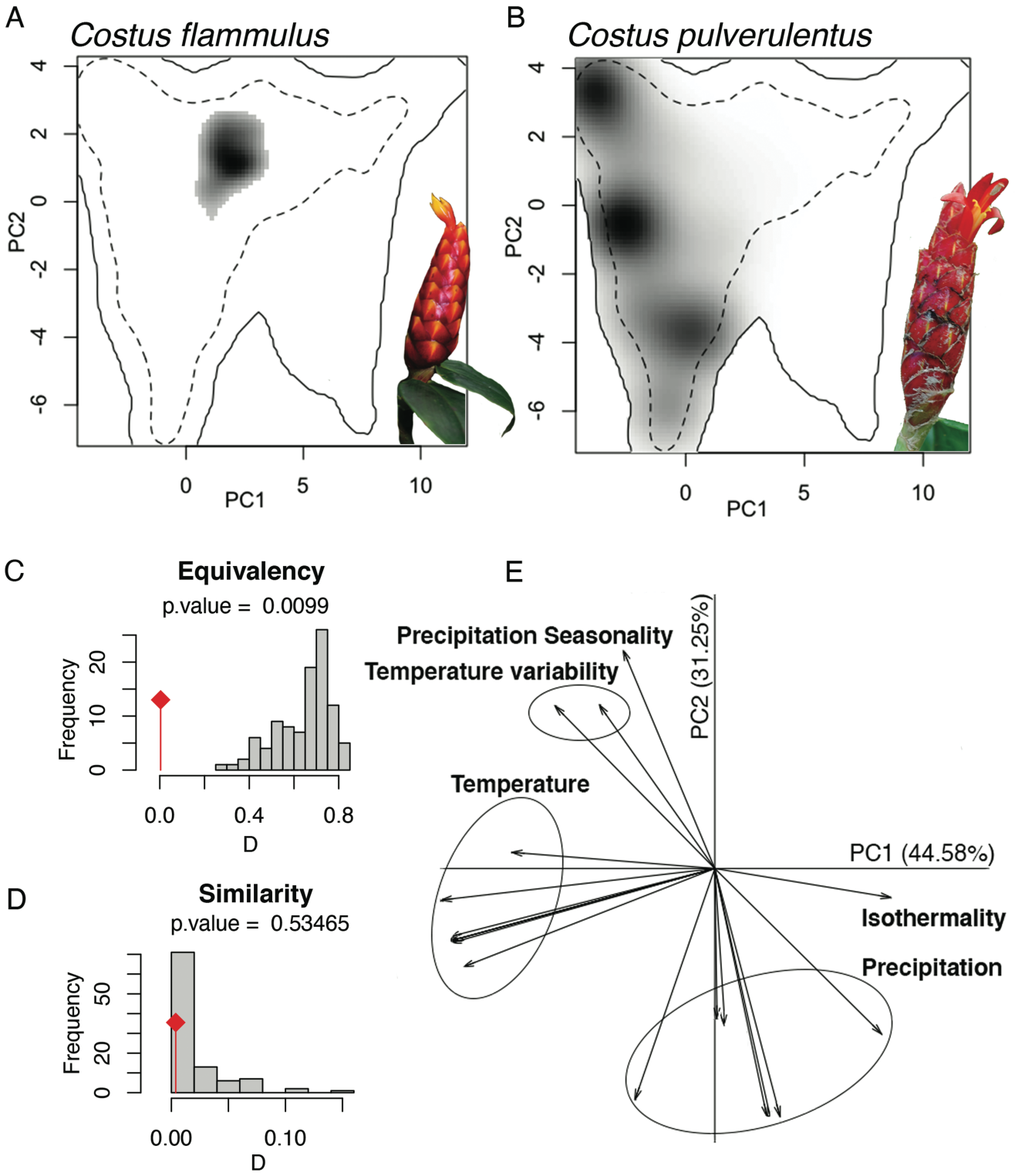

FIG. 3. Climate niches of *C. flammulus* and *C. pulverulentus*. A, B. 100 × 100 gridded environmental spaces representing the climate environment available to *Costus flammulus* and *C. pulverulentus* along these PC axes. Gray shading indicates the density of occurrences within a cell and dotted and solid lines encompass 50% and 100% of the available environment, respectively. Overall, *C. flammulus* inhabits a colder and seasonal habitat, whereas *C. pulverulentus* occupies warmer and very humid environments. The niches of *Costus flammulus* and *C. pulverulentus* are significantly different from each other. C–D. Histograms showing the observed niche overlap *D* between the species' ranges (red bars with a diamond) and simulated niche overlaps (grey bars) on which test of niche equivalency (C) and niche similarity (D) are calculated from 100 iterations. E. Biplot from the principal components analysis of the bioclimatic environmental space defined by pseudo-absence occurrence data used in the species distribution models. Correlated bioclimatic variables are circled and jointly labeled.

in pollen placement sites are important in isolating species in other Neotropical groups as well, such as *Burmeistera* (Muchhala and Thomson 2012), *Heliconia* (Stiles 1975, 1979; Kress 1983), and Orchidaceae (reviewed in Schiestl and Schlüter 2009). Thus, divergent floral traits like stamen exsertion could play a major role in isolating these species by differential pollen placement. However, floral isolation studies are needed to confirm this hypothesis.

Our pollinator assemblage observations show that *C. flammulus* and *C. pulverulentus* occupy non-overlapping pollinator niches. *Costus flammulus* is visited by montane hummingbirds, primarily by the green hermit, whereas *C. pulverulentus* is exclusively visited by the long-billed hermit (Kay and Schemske 2003; Kay 2006). It is well known that pollinator specificity contributes to adaptive divergence and reproductive isolation (Van der Niet et al. 2014). As such, pollinators select on floral traits, and plant populations adapt to pollinators' preferences, behavior, and morphology to maximize visitation and efficient pollen transfer (Grant and Grant 1965; Stebbins 1970; Fenster et al. 2004; Johnson 2006; Schiestl and Johnson 2013). In this way, geographic variation in pollinator-mediated selection can drive floral divergence between plant populations. However, *C. flammulus* speciation may not be related to pollinator specialization, but rather the differences in pollinator identity may be a byproduct of elevational divergence. The green hermit, common at medium to high elevations, and the long-billed hermit, abundant in lowlands and rare above medium elevations (Garrigues and Dean 2014), are ecological equivalents, both being medium-sized forest understory hermit hummingbirds with similar behavior (Stiles 1983; Skutch and Stiles 1989). Thus, it seems likely that *C. flammulus'* pollination niche shift is confounded with climatic adaptation rather than local pollinator availability. It has been hypothesized that climate-mediated divergence in the pollination environment may drive the adoption of a novel pollinator niche (Grant and Grant 1965; Stebbins 1970; Phillips et al. 2020). To better understand the role of pollinator specificity in *C. flammulus* speciation, it is necessary to carry out reciprocal translocation experiments to tease apart the relative contribution of climatic and pollinator niches and their interactions (Miller et al. 2014; Van der Niet et al. 2014; Phillips et al. 2020).

We found almost no geographic and climatic niche overlap, suggesting that strong ecogeographic isolation is the main barrier segregating *C. flammulus* and *C. pulverulentus*. Ecogeographic differentiation is thought to evolve in the early stages of speciation; thus, studying ecogeographic isolation is crucial to understanding incipient species divergence (Schemske 2000, 2010; Sobel et al. 2010). It has been demonstrated that ecogeographic differentiation and geographic isolation are drivers of speciation in several Neotropical plant groups (Kay 2006; Nakazato et al. 2010; Castillo-Cardenas et al. 2015; Chen and Schemske 2015; Cuevas et al. 2018; Maciel et al. 2020; Misiewicz et al. 2020; Vargas et al. 2020). Non-overlapping ranges and climatic niches suggest that *C. flammulus* and *C. pulverulentus* differ significantly in the niches they can occupy.

Together, our results are consistent with the hypothesis of *C. flammulus* having budded off from a *C. pulverulentus*-like progenitor species. Budding, or progenitor-derivative, speciation involves an initially small colonizing population becoming reproductively isolated from a larger-ranged species, generally in a marginal habitat at the range edge (Mayr 1954; Grant 1981). Budding speciation is recognized as an important phenomenon in plants (Richerson and Lum 1980; Gottlieb 2004; Crawford 2010; Anacker and Strauss 2014; Grossenbacher et al. 2014; Sianta and Kay 2022). However, in proportion to its importance, the role of budding speciation in the Neotropical flora is still poorly understood (but see Vargas et al. 2020). Whether *C. flammulus* is derived from a *C. pulverulentus*-like progenitor or not is still uncertain and could benefit from detailed population genomic studies of its demographic and speciation history. Nevertheless, several lines of evidence, such as possible *C. pulverulentus* paraphyly, range size asymmetry, and *C. flammulus'* nested distribution of vegetative traits, suggest that *C. flammulus* budded off from a *C. pulverulentus*-like progenitor species (Crawford 2010; Sianta and Kay 2022), evolving to tolerate a colder and more seasonal montane environment.

Taxonomic Treatment

Costus flammulus K.M.Kay & P.Juárez, sp. nov. Type: Costa Rica. Puntarenas, Monteverde. E. B. Monteverde, Quebrada Máquina (10°19′03′′N, 84°48′27′′W), 1524 m, 18 Aug 2022 (fl), *P. Juárez & C. Girvin 1620* (holotype: USJ!, isotypes CR!, MO!).

Closely related and vegetatively similar to *C. pulverulentus,* it differs in the smaller stem diameter (6.79 mm vs. 8.96 mm in diam), the smaller leaves (14.67 × 4.30 cm vs. 18.23 × 5.34 cm), the shorter inflorescences (6.44 vs. 9.78 cm long), the smaller bracts (1.76 × 1.56 cm vs. 2.56 × 1.87 cm), the shorter nectarial callus (non-functional, 6.14 mm vs. functional, 8.82 mm long), the smaller flowers (Table 1), the antrorse, red and distally yellow corolla lobes (vs. retrorse, red corolla lobes), the yellow to red, antrorse, shortly 5-lobed labellum (vs. yellow, 5-lobed, lobes retrorse), the petaloid stamen shortly exserted by 5 mm (vs. petaloid stamen exserted by 16.27 mm), and geographic distribution (montane cloud forest of the Tilarán and Guanacaste cordilleras in Costa Rica, at elevations of 1000–1650 m vs. lowland wet forests from Mexico to southern Ecuador, at elevations of 0–1400 m). The measurement contrasts are means. See Table 1 for detailed comparisons.

Perennial herb, (0.5) 1.5 (3) m tall, hermaphroditic, geophyte. **Rhizomes** reddish, sympodial, every element ending in a shoot. **Stems** 6.7–9 mm in diam, orthotropic, green to reddish near the base, terete, glabrous, with alternate-spiraled phyllotaxy. **Sheaths** 6.7–9 mm in diam, green, glabrous. **Ligules** 5–5.7 (8) mm long, green, truncate, or slightly two-lobed, glabrous, margins sometimes dilacerating into fibers. **Leaves** 18.5–24 × 4–7 cm; petioles 5–5.7 (8) mm long; blade elliptic-obovate, base rounded to cordate, apex acuminate, margins undulate, adaxial surface glabrous, abaxial surface slightly glaucous, chartaceous, venation parallel. **Inflorescences** strobilaceous-capitate spike, terminal, (4.6) 7.9–13.7 × (2) 3.2–5.8 cm, cylindrical-ovoid. **Bracts** 1.4–2.3 × 1–2.2 cm, red to distally yellow, ovate-triangular, apex acute, margins glabrescent, sometimes dilacerating into fibers, glabrous, coriaceous, subtending 1 flower per bract; callus 4–7 mm long, yellow, linear, non-functional. **Bracteole** 1 (1.5) cm long, red, glabrous. **Flowers** hermaphroditic, sympetalous, zygomorphic; **calyx** connate, 4.2 mm long, red, truncate or slightly 3-lobed, glabrous; **corolla** connate, 2.9–4.1 cm long, proximally white to red to distally yellow, venation red, 3-lobed, lobes antrorse, proximally tubular; **tube** (0.7) 1.2 cm long, white,

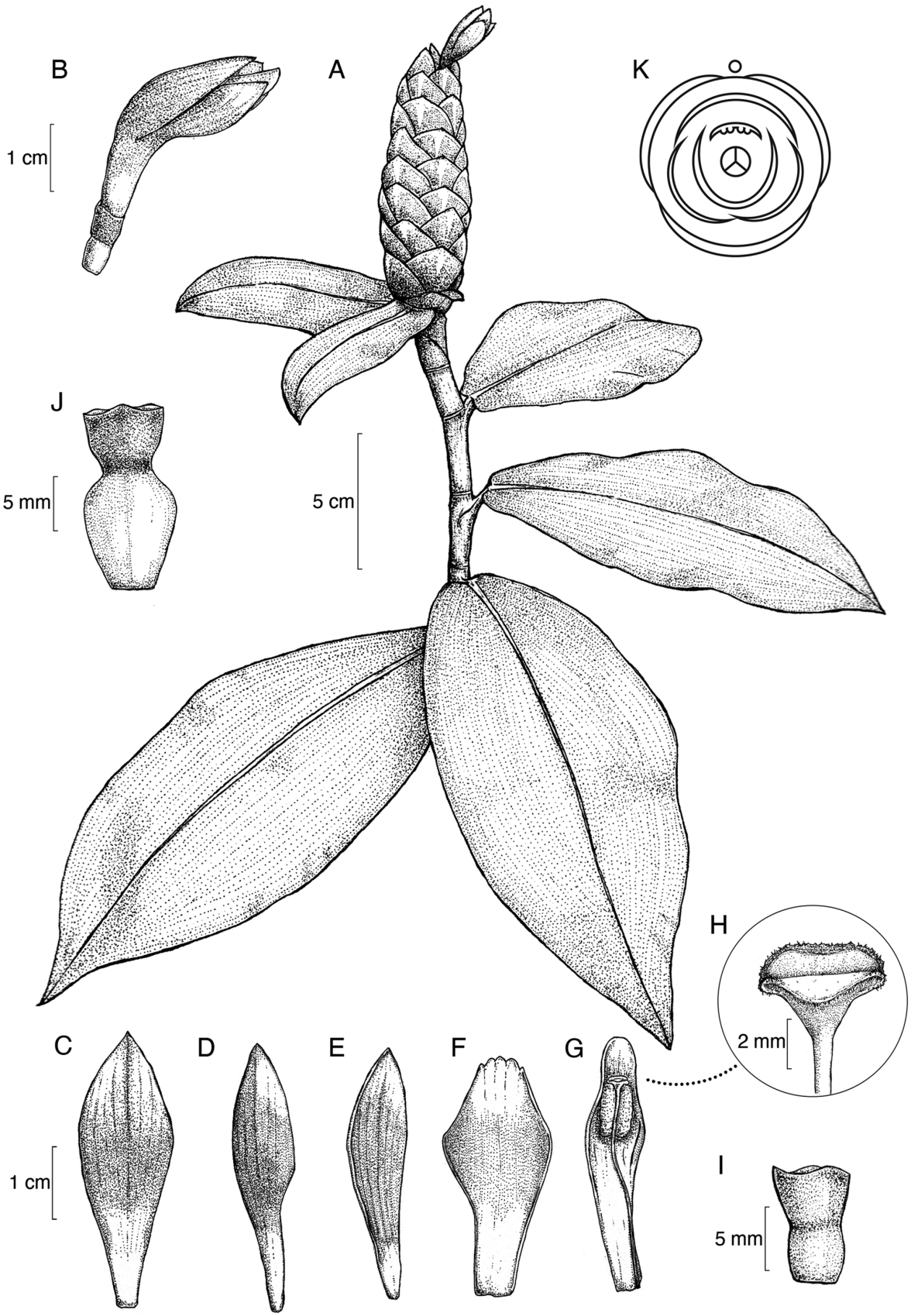

FIG. 4. *Costus flammulus* K. M. Kay & P. Juárez. Line illustration based on *P. Juárez & C. Girvin 1620* (USJ, CR, MO) and live plants from the type locality. A. Stem with terminal spike with flower. B. Flower, side view. C. Dorsal corolla lobe, abaxial view. D. Lateral corolla lobe, abaxial view. E. Lateral corolla lobe, adaxial view. F. Labellum, adaxial view. G. Petaloid stamen, style, and stigma. H. Detail of bilamellated stigma. I. Ovary and calyx. J. Capsule with persistent calyx. K. Floral diagram of *Costus*. Drawn by P. Juárez.

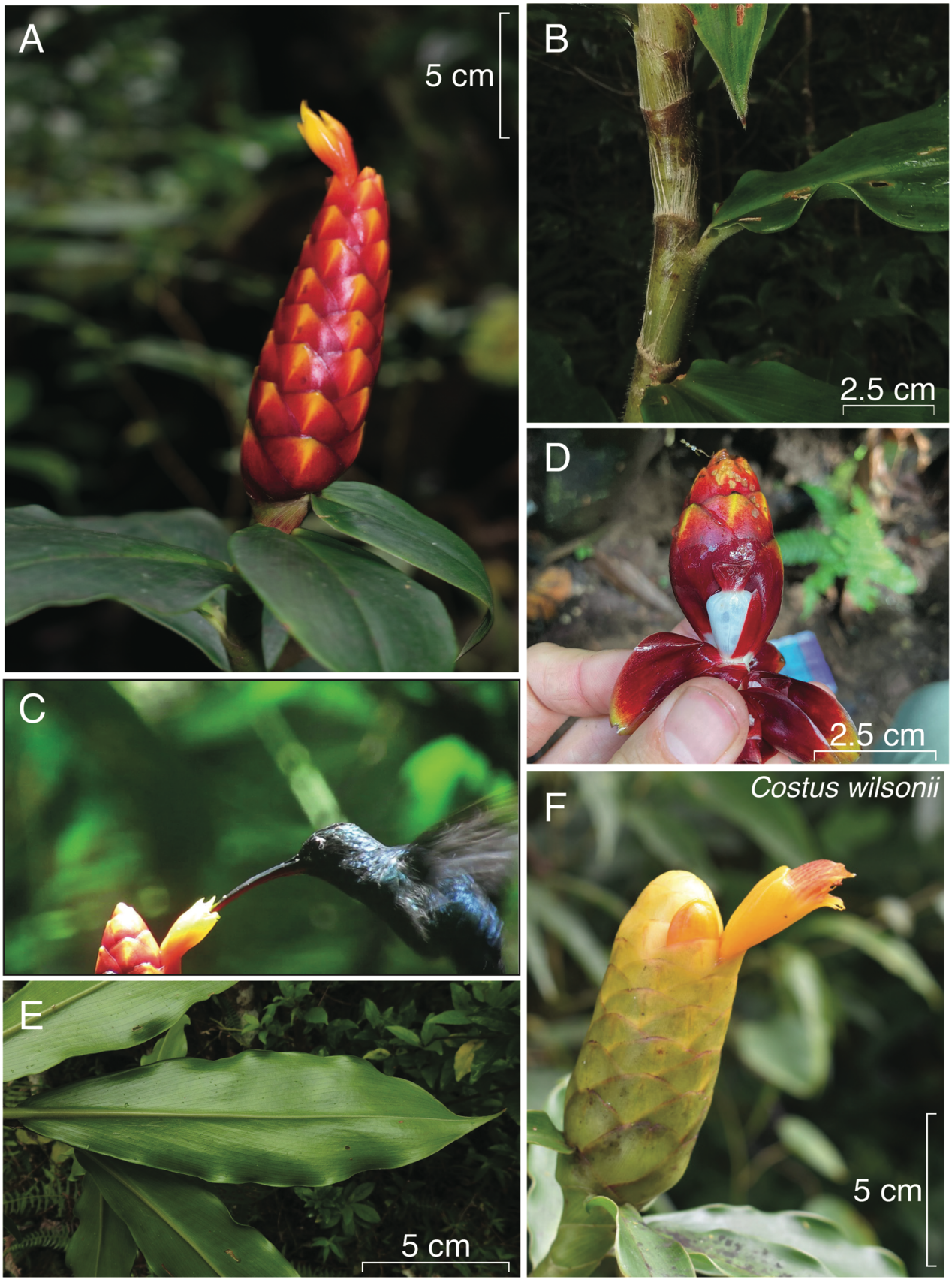

FIG. 5. *Costus flammulus*. A. Terminal spike with flower. B. Stem, petiole, and ligules. Note ligules' margins sometimes dilacerating into fibers. C. *Phaethornis guy* visiting *C. flammulus*' flower. D. Young capsule subtended by bracteole. Note that bracts have been removed. E. Leaves, abaxial view. Note undulate blade margins. F. *Costus wilsonii*, a species with which *C. flammulus* has been commonly mistaken in herbaria. Photographs by P. Juárez (A, C, F); J. E. Jiménez (B, E); and C. Girvin (D).

glabrous, throat adaxially covered with yellow hairs; **dorsal lobe** 2.5–4.1 × 0.7–1.2 cm, obovate, apex mucronate, margin slightly involute; **lateral lobes** 2.4–3.7 × 0.6–0.9 cm, elliptic, apex obtuse, margin involute; **labellum** 3–3.7 × 1.4–1.8 cm, yellow to red, spatulate, apex irregular, antrorse, shortly 5-lobed, lobes < 1 mm long, lateral lobes involute, forming a tube of ca. 4.5 mm in diameter; **stamen** 3.1–3.8 × 0.6–0.8 cm, yellow to reddish, petaloid, narrowly elliptic, apex obtuse, exserted, ca. 5 mm exceeding the labellum, anthers 7 mm long, thecae open in longitudinal slits, pollen white; **ovary** trilocular, 3.4 × 4.2 mm, white, globose, trigonous, many ovules; **style** 2.7–3.3 cm long, whitish, filiform; **stigma** ca. 3 mm wide, whitish, bilamellated, margin ciliate, appressed to anthers. **Capsules** 1–1.3 × 0.7–0.9 cm, white, obovoid, glabrous, calyx persistent. **Seeds** ca. 4 mm wide, black. Figures 4, 5.

Distribution and Habitat*—Costus flammulus* is endemic to the primary and secondary montane cloud forests, at elevations of 1000–1650 m, on the Tilarán and Guanacaste volcanic cordilleras in northern Costa Rica (Fig. 1). The montane cloud forests of the northern volcanic cordilleras are characterized by high edaphic, microclimatic, and topographic diversity, as well as by high inter-annual precipitation variation (Lawton et al. 2016). *Costus flammulus* has been collected in several relevant protected areas, both state and private, such as the Guanacaste, Miravalles-Jorge Manuel Dengo, and Tenorio Volcano national parks, and the Alberto Brenes and Monteverde Cloud Forest biological reserves. Locally, *C. flammulus* is frequently found near streams, in rocky ravines, or in the understory. In the Tilarán Cordillera, *C. flammulus* co-occurs with the distantly related *C. montanus* Maas, which likely hybridize due to microhabitat and pollinator assemblage overlap (P. Juárez pers. obs.). See Table S3 for detailed information on *C. flammulus* occurrences.

Phenology*—Costus flammulus* flowers in the dry and rainy seasons. It has been recorded flowering in February, April, July, August, and October, and fruiting in October and November.

***Etymology*—**The specific epithet is a diminutive of the Latin *flamma* or *flammae,* meaning "flame" (Gledhill 2008), referring to the flower's resemblance to a little flame.

Notes*—Costus flammulus* has been mistakenly included in *C. wilsonii* (e.g. Stratton 1989; Haber 2000; Maas and Maas-van de Kamer 2003). *Costus flammulus* vegetative morphology and habitat affinity resemble that of *C. wilsonii;* thus, distinguishing herbarium specimens could be difficult without flowers. Besides, *Costus* flowers are delicate and generally not preserved well in herbarium specimens. Presumably, *C. flammulus* and *C. wilsonii* have been confused because both species are hummingbird-pollinated herbs from montane forests with truncated or slightly two-lobed ligules and undulate leaf margins.

Costus flammulus and *C. wilsonii* can be differentiated by their dried foliage color, ligule margin, and distribution range in herbarium specimens. *Costus flammulus* foliage usually dries yellowish, whereas *C. wilsonii* foliage dries grayish. *Costus flammulus* ligule margins sometimes dilacerate into fibers (Fig. 5), whereas *C. wilsonii* does not. *Costus flammulus* is endemic to the Tilarán and Guanacaste cordilleras in northern Costa Rica. *Costus wilsonii* occurs in the Talamanca Cordillera, Fila Cruces, and the most southern volcanic peaks of the Central Cordillera, ranging from the southern half of Costa Rica to the north of Panama.

Although *C. flammulus* and *C. wilsonii* herbarium specimens may be confused because of their vegetative resemblance and habitat preferences, their flowers are distinguishably different (Fig. 5). *Costus wilsonii* flowers are yellow and differ from the rest of hummingbird-pollinated *Costus* by their expanded labellum with red nectar guides on the limb. *Costus wilsonii* is the only hummingbird-pollinated *Costus* with nectar guides on the labellum, which potentially is a retained trait related to ancestral euglossine pollination. *Costus wilsonii* is the sister to *C. laevis* (Kay 2006; Vargas et al. 2020), a euglossine-pollinated widespread species inhabiting lowland wet forests of Central and South America.

***Paratypes*—Costa Rica.** —Alajuela: Balsa. 10 km N of San Ramón, 1000 m, 13 Sep. 1974 (fl) *P. J. M. Mass & R. W. Lent 1218* (MO!); San Carlos. Monteverde Reserve, El Valle trail, near continental divide with Atlantic exposure, lower montane rain forest (10°20′N, 84°50′W), 1600 m, 26 Oct. 1985 (fl) *W. Haber & E. Bello 3161* (MO!); Peñas Blancas. 1200 m, 14 Sep. 1985 (fl) *W. Haber & E. Bello 2725* (MO!); San Ramón. Cerro San Isidro, 1260 m, 25 Mar. 1982 (fl), *A. Carvajal 191* (MO!); Ángeles. A la vera del río S. Lorenzo, Faldas de la Fila Volcán Muerto, 1000–1100 m, 31 July 1982 (fl), *J. Gómez-Laurito 8865* (CR!); Reserva de San Ramón, Estación Sn Lorencito, 700–1000 m, 02 July 1986 (fl), *A. Chacón, I. Chacón & V. Mora 1981* (CR!); San Lorenzo. 12 km NNW of San Ramón by road on way to San Lorenzo (10°10′N, 84°29′W), 1100 m, 25 April 1983 (fl), *R. Liesner & E. Judiewicz 14993* (MO!); Piedades Norte. Cuenca del Barranca, Sector Burio. Cerro Azahar (10°08′24′′N, 84°33′40′′W), 1400 m, 04 Oct. 1997 (fl), *B. Hammel & H. Kennedy 21075* (CR!); Cuenca del Barranca, Sector Burio. Cerro Azahar (10°08′24′′N, 84°33′40′′W), 1400 m, 04 Oct. 1997 (fl), *B. Hammel & H. Kennedy 21078* (CR!, MO!). —Guanacaste: Abangares, La Cruz de Abangares, 1400 m, 15 July 1985 (fl) *W. Haber & E. Bello 2027* (MO, digital image); Bagaces, La Fortuna. Parque Nacional Volcán Miravalles, Cabro Muco, sobre sendero camino hacia la catarata, bosque secundario (10°43′27.10′′N, 85°08′38.62′′W), 1246 m, 14 April 2022 (fl) *J. E. Jiménez & M. F. Cordero 6527* (USJ!); La Cruz. SW slope of Volcán Orosí (10°58′00′′N, 85°28′30′′W), 1100–1400 m, 22 Aug. 2007 (fl) *M. H. Grayum & A. Rojas 12835* (MO!); Liberia, Ángeles. SW (Pacific) slope of Cerro Cacao (10°55′53′′N, 85°27′35′′W), 1300–1450 m, 10 Aug. 2007 (fl) *M. H. Grayum & D. García 12580* (MO!); Tilarán, Quebrada Grande. Cuenca del San Carlos, Reserva Biológica Bosque Nuboso, alrededores del laboratorio A. Skutch (10°20′40.67′′N, 84°49′14′′W), 1540 m, 29 Aug. 2011 (fl), *D. Santamaría 8888* (CR!); Tierras Morenas. Z. P. Tenorio, Cabec. Río San Lorenzo (10°36′40′′N, 84°59′45′′W), 1050 m, 23 Aug. 1994 (fl), *G. Rodriguez 307* (CR!, MO!); Tronadora. San Gerardo Biological Station. Road to Río Caño Negro, secondary forest (10°22′00′′N, 84°47′00′′W), 1100 m, 1 Apr. 1995 (fl), *D. Penneys 294* (CR!, MO!). —Puntarenas: Puntarenas, Monteverde. Monteverde Cloud Forest Reserve, 1540–1620 m, 12 Aug. 1982 (fl), *P. Feinsinger et al. 409* (MO!); Santa Elena de Monteverde, Tilarán road, c. 1 km from S. Elena. 1300 m, 17 Feb. 1984 (fl), *R. Khan, M. Tebbs & A. R. Vickery 1091* (CR!); R. B. Bosque Eterno de los Niños, Cuencas del Lagarto y Guacimal, sendero Chomogo y el Roble (10°18′00′′N, 84°48′00′′W), 1600 m, 19 Oct. 1997 (fl), *A. Soto 73* (CR!, MO!); Cloud forest and swamp forest (premontane and lower montane rain forest formations) in and around the Monteverde Nature reserve, mostly on the Pacific watershed (10°18′N, 84°47′W), 1450–1650 m, 31 Oct. & 2 Nov. 1975 (fl) *W. Burger & R. Baker 9720* (MO!); Quebrada Máquina, Pacific slope (10°18′N, 84°48′W), 1400 m, 9 Aug. 1991 (fl), *W. Haber, C. Ivey & W. Zuchowski 10798* (CR!, MO!).

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Author Contributions

PJ and KMK did the fieldwork. PJ analyzed all data, except for OV who did the phylogenetic analysis. PJ prepared the figures and tables. PJ wrote the manuscript with edits of KMK and OV.

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