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Phylogenetic Relationships of *Brachistus* and *Witheringia* (Solanaceae)

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Abstract—The neotropical physaloid genera *Witheringia* and *Brachistus* have long been subject to taxonomic confusion. We obtained tissue from well-documented species of these genera, including multiple accessions from widespread taxa, to generate a molecular systematic framework. We present Bayesian phylogenies based on sequences of one chloroplast (*trnL-trnF*) and two nuclear loci (ITS and *waxy*). The major findings are that: 1) *Witheringia* and *Brachistus* together form a well-supported monophyletic group, 2) *Brachistus* is nested within *Witheringia*, 3) two Mexican endemic species represent the earliest diverging lineages of the clade, 4) several proposed synonyms represent distinct species, and 5) sequenced accessions include several new species awaiting description. The four currently recognized species of *Brachistus* are transferred to *Witheringia*, necessitating the new combination ***Witheringia knappiae***, and *Athenaea affinis* is lectotypified. A list is also provided for names now excluded from *Brachistus* and recognized as belonging to other genera.

Resumen—Los géneros neotropicales de physaloid *Witheringia* y *Brachistus* han sido durante mucho tiempo objeto de confusión taxonómica. Obtuvimos tejidos de especies bien documentadas de estos géneros, incluyendo múltiples adiciones de taxones muy extendidos, para generar un marco sistemático molecular. Presentamos filogenias bayesianas basadas en las secuencias de un cloroplasto (*trnL-trnF*) y dos loci nucleares (ITS y *waxy*). Los principales hallazgos son que: 1) *Witheringia* y *Brachistus* forman juntos un grupo monofilético bien apoyado, 2) *Brachistus* sale dentro de *Witheringia*, 3) dos especies endémicas mexicanas representan los linajes divergentes más tempranos del clado, 4) varios sinónimos propuestos representan especies distintas, y 5) las adiciones secuenciadas incluyen varias especies nuevas pendientes de descripción. La filogenia proporcionará un valioso marco para futuros trabajos sobre sistemática, evolución de los caracteres y biogeografía. Las cuatro especies de *Brachistus* actualmente reconocidas se transfieren a *Witheringia*, lo que requiere la nueva combinación ***Witheringia knappiae***, y *Athenaea affinis* está lectotipificada. También se proporciona una lista de nombres ahora excluidos de *Brachistus* y reconocidos como pertenecientes a otros géneros.

Keywords—ITS, Mesoamerica, Physalideae, Physalidinae, *trnL-trnF*, *waxy*.

The neotropical genus *Witheringia* L'Her. encompasses about 20 species, with a geographic range extending from Mexico and the Antilles to Bolivia and Brazil (Hunziker 2001; Bohs 2015). Taxonomic confusion has accompanied *Witheringia* from the outset. It was first described in 1788 based on a single specimen grown in cultivation, and named *W. solanacea* L'Her. (L'Heritier 1788). Sixty years later, Miers (1849a) transferred the type species to the genus *Saracha* Ruiz & Pav. He retained the name *Witheringia* but applied it instead to species within the recently described *Athenaea* Sendtn. For the next twelve decades, specimens of *Witheringia* were assigned to a plethora of genera, including *Acnistus* Schott, *Athenaea, Bassovia* Aubl., *Brachistus* Miers, *Capsicum* L., *Cuatresia* Hunz., *Lycianthes* Hassl. (Dunal), and *Saracha.* Hunziker (1969) reinstated the genus based on its original type species. Since that foundational monograph, *Witheringia* has been considered only in regional treatments (e.g. D'Arcy 1973, 2001; Gentry and Standley 1974; Bohs 2015) and in a Ph.D. thesis (Sousa-Peña 2001). The focus on regional floras has led to confusion about species circumscriptions. Various authors have designated new species, proposed merging species, or resurrected previously synonymized species. Therefore, the number of species and their delimitation are currently unclear. For example, Hunziker (1969) considered *W. coccoloboides* and *W. fuscoviolacea* to be distinct species whereas D'Arcy (1973) synonymized them in his Flora of Panama, and Bohs (2015) in the Manual of Plants of Costa Rica was unsure whether the primarily Panamanian *W. correana* should be considered a synonym of *W. macrantha*.

Phylogenetic studies of the Solanaceae establish that *Witheringia* lies within the tribe Physalideae and subtribe Physalidinae [formerly subtribe Physalinae; Olmstead et al. 2008; Särkinen et al. 2013; Deanna et al. 2019]. Deanna et al. (2019) examined phylogenetic relationships in the tribe Physalideae using four gene regions and extensive taxonomic sampling. *Cuatresia*, a genus previously thought to be closely related to *Witheringia,* is instead part of a clade with *Aureliana* Sendtn. and *Deprea* Raf. in a recircumscribed subtribe Withaninae. The subtribe Physalidinae forms a clade that includes *Witheringia*, *Physalis* L., and several small "physaloid" genera [e.g. *Leucophysalis* Rybd., *Chamaesaracha* (A.Gray) Benth. & Hook.f., and *Tzeltalia* E.Estrada & M.Martínez], whose relationships are still unclear.

A major systematic problem with *Witheringia* above the species level is its relationship with the genus *Brachistus*. Miers (1849b) created *Brachistus* and included fifteen species within it. All of these are now assigned to different genera (*Capsicum, Cuatresia*, *Physalis,* and *Witheringia*) except *B. stramoniifolius,* which has been placed in *Brachistus* by many authors. Species of *Brachistus* have been distinguished from *Witheringia* by their lobed and often accrescent calyces, whereas those of *Witheringia* are truncate or shallowly lobed and not or slightly accrescent in fruit (Hunziker 1969; D'Arcy et al. 1981; Bohs 2015). Hunziker (1969) originally considered *Brachistus* to be a section within *Witheringia* and recognized three species within it, although he later elevated it as a separate genus (Hunziker 2001), as did D'Arcy et al. (1981) and Bohs (2015). A molecular phylogeny of the Solanaceae based on cpDNA markers placed *B. stramoniifolius* nested in a clade containing four species of *Witheringia* (Olmstead et al. 2008). Subsequent analyses (Särkinen et al. 2013; Zamora-Tavares et al. 2016) have found *B. stramoniifolius* to be sister to *Witheringia*, but these analyses sampled only two to three *Witheringia* species and did not include other *Brachistus* species.

Deanna et al. (2019) included two *Brachistus* species in their phylogenies; these emerged in a clade with multiple *Witheringia* species and *Schraderanthus viscosus* (Schrad.) Averett. Whether *Witheringia* and *Brachistus* are each monophyletic has been unknown (Bohs 2015).

The center of diversity for *Witheringia* is currently considered to be Costa Rica (Hunziker 2001). According to the time-calibrated phylogeny of Särkinen et al. (2013), the clade including *Brachistus* and *Witheringia* diverged from *Leucophysalis* ca. 3.72 million years ago, although subsequent discoveries such as *Physalis* fossils from the early Eocene of Argentina dated at ca. 52 million years suggest that the Physalideae diversified much earlier (Wilf et al. 2017; Deanna et al. 2020, 2023). Therefore, the origin and diversification of the genus is largely contemporaneous with the uplift of the northern Andes ca. 15–35 Ma and the formation of the Central American land bridge ca. 3–20 Ma (Coates and Obando 1996; Blandin and Purser 2013; Bacon et al. 2015), providing an interesting example of diversification in a geologically dynamic context.

Our goal in this study was to create a well-supported phylogenetic analysis of *Witheringia* and *Brachistus*, including three of the three to four currently recognized species of *Brachistus* and 13 of the approximately 20 species of *Witheringia*, as well as multiple accessions of widespread species. The molecular phylogeny will provide support for a thorough taxonomic treatment. Increased resolution of the phylogeny of *Witheringia* will also permit investigations of character evolution and biogeographic questions.

MATERIALS AND METHODS

Taxon Sampling—The ingroup consists of 31 accessions belonging to 16 putative species, including three species of *Brachistus* and 13 species of *Witheringia*, as well as multiple accessions of widespread species and several putative new species (Appendix 1). All but two of the ingroup accessions were collected or propagated by the authors. For *B. nelsonii*, DNA was extracted from an herbarium specimen collected in 2000 (Appendix 1). DNA for *W. correana* from Panama was shared by R. Olmstead from the BIRM Solanaceae collection. All new specimens gathered for the study were collected legally with all necessary valid permits; see Acknowledgments for permit information.

Several of the *Witheringia* accessions used here are provisionally identified pending increased taxonomic study of the genus. *Witheringia* cf. *asterotricha* refers to a Colombian collection with branched hairs that is morphologically similar to *W. asterotricha* accessions from Costa Rica. The accession labeled *W.* sp. *3858* from southwestern Colombia was identified in the field as *W. killipiana*; upon further study, we think it probably does not belong to *W. killipiana*, but its identity is currently unknown. *Witheringia* cf. *solanacea* refers to accessions from Costa Rica that are similar to *W. solanacea* but have 5-merous flowers, are self-compatible, and may represent a distinct taxon (Stone and Jenkins 2008; Bohs 2015). Finally, the name *W. coccoloboides* is used provisionally here. Although material sequenced here from Costa Rica and Panama is identified as *W. coccoloboides* using Bohs (2015), the type of *W. coccoloboides* is from Colombia; ongoing studies indicate that there may be more than one species represented in this morphological complex.

Outgroup taxa were chosen with reference to Särkinen et al. (2013), focusing on species within the tribe Physalideae: six from subtribe Physalidinae (*Chamaesaracha, Leucophysalis, Physalis,* and *Tzeltalia*) and two from subtribe Iochrominae (*Iochroma* and *Saracha*). Most outgroup taxa were previously sequenced for all three loci (Appendix 1) and these sequences were retrieved from GenBank. For the *waxy* locus, full-length sequences in GenBank were available only for *Leucophysalis* and *Saracha.*

DNA Extraction, Amplification, and Sequencing—Genomic DNA was extracted from fresh, silica-dried, or herbarium leaf tissue using the Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia California), the CTAB procedure (Doyle and Doyle 1987), or the Qiagen kit with CTAB substituting for the first buffer. PCR was carried out in 25 μL volumes in standard buffer with 1.5 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 μM each primer, 2 U *Taq* polymerase, and 10–20 ng genomic DNA. For recalcitrant samples from herbarium tissue, BSA was added at a final concentration of 0.5 g/L.

We used primers c and f and standard cycling conditions to amplify the chloroplast *trnL-trnF* region (Taberlet et al. 1991). Amplification of the ITS region was done using primers ITSleu1 (Andreasen et al. 1999) and ITS4 (White et al. 1990), and standard cycling conditions (Bohs and Olmstead 2001). For a subset of taxa, these conditions produced either fungal or pseudogene amplicons, and a more specific primer was required. Primer ITSSol1 was designed based on GenBank records for Solanaceae sequences of the 18S region from *Capsicum frutescens* L. (JF766710), *Lycium barbarum* L. (JN8354580), *Physalis heterophylla* Nees (KT179698.1), and *Solanum carolinense* L. (KT179700). In each case, the sixth base was a T rather than a C as in ITSLeu1. The ITSSol1 primer sequence is therefore 5′-GTC CA**T** TGA ACC TTA TCA TTT AG-3′. In addition, the annealing temperature was increased to 55°C, and 5% DMSO was added to the PCR reaction. Amplification of the granule-bound starch synthase gene (GBSSI or *waxy*) was carried out as in Levin et al. (2006), amplifying the gene in two sections. Primers waxyF and 1171R (Walsh and Hoot 2001) were used to amplify from exons two to seven; primers 1058 and 2R were used to amplify from exons six to ten (Levin et al. 2006). In cases where spurious bands appeared, the magnesium concentration was reduced to 1.5 mM and annealing temperatures for the touchdown PCR were raised two degrees, to begin at 54°C and end at 50°C.

PCR products were cleaned using the QIAquick PCR purification kit (Qiagen, Valencia California). Sequencing was done in both directions using an ABI 3130 automated sequencer (Applied Biosystems, Foster City California). For *trnL-trnF* and *waxy*, primers used for sequencing were the same as those used in initial amplifications. For ITS, internal primers ITS2 and ITS3 (White et al. 1990) were also used in sequencing. In addition, we designed a new internal primer based on sequences from a range of Solanaceae species for the 5.8S region. The ITSSol3 primer differs from ITS3 in having a T rather than a C in the fourth-to-last position (5′-GCA TCG ATG AAG AAC G**T**A GC -3′). Sequences were obtained for all accessions at all loci except for ITS for *Brachistus knappiae* (Appendix 1). In this case, repeated efforts produced pseudogene amplicons as revealed by their GC content < 57%, in contrast to the 62–69% GC content found for ITS amplicons in this group. Accordingly, the ITS sequence for *B. knappiae* was coded as missing data in the matrix. The data matrix from the combined analysis as well as the individual trees from each of the three loci are deposited in Dryad (see Stone et al. 2024).

Phylogenetic Reconstruction—Sequences were aligned using Geneious 11.1.4 (Kearse et al. 2012) and adjusted by eye. Phylogenetic analysis was carried out using Bayesian inference (BI; Mr. Bayes 3.2; Ronquist et al. 2011), with gaps treated as missing data.

The appropriate nucleotide substitution rate model for each locus was evaluated by the Akaike Information Criterion (AIC) as implemented in JModeltest2 (Guindon and Gascuel 2003; Darriba et al. 2012). The GTR + I model had a delta AIC < 2 for all three loci and was applied to all, with each locus treated as a separate partition. Two simultaneous runs of four MCMC chains were run for 20 million generations, each starting with a random tree and saving every 1000 generations. Convergence among independent runs was indicated by standard deviation of split frequencies < 0.01 and by Potential Scale Reduction Factors near 1.0. The first 25% of the trees were discarded as burn-in before creating a majority-rule consensus tree. Trees were displayed using IcyTree (Vaughan 2017).

Incongruence between the individual trees from each locus and the combined tree was evaluated using posterior probability (PP) values of ≥ 0.95 indicating well-supported incongruence.

RESULTS

In total, 84 new sequences were obtained: 28 for ITS, 26 for *trnL-trnF,* and 30 for *waxy*, with the remaining sequences obtained from GenBank (Appendix 1). The combined data matrix contained 3640 aligned characters: 649 for ITS, 1216 for t*rnL-trnF,* and 1775 for *waxy.* According to Bayesian Inference, most branches were supported by PP of 1.0 (Fig. 1). There were a few well-supported conflicts between phylogenetic hypotheses constructed from the three individual loci and the combined tree (Figs. 1, S1–S3). These concerned the positions of *W. mexicana* (sister to a well-supported clade of 24 taxa in the *waxy* tree and part of a larger clade containing

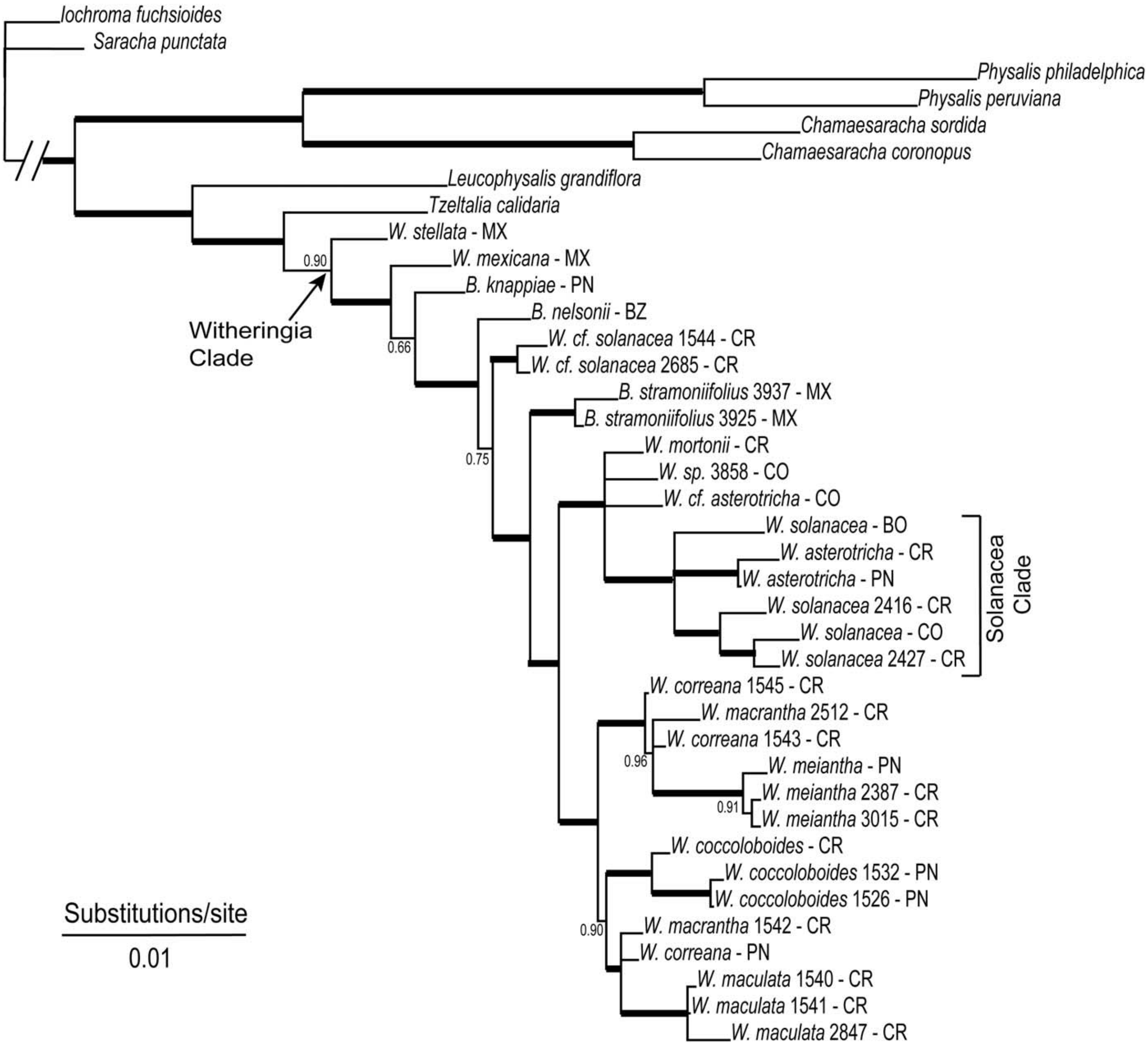

FIG. 1. Majority-rule consensus tree for *Witheringia*, *Brachistus*, and outgroup taxa based on the combined data set from *trnL-trnF*, ITS, and *waxy* sequences. Thick branches represent Bayesian posterior probabilities greater than or equal to 0.98. Country code is indicated for species accessions spanning geopolitical boundaries (BO = Bolivia; BZ = Belize; CO = Colombia; CR = Costa Rica; MX = Mexico; PN = Panama). Accession numbers are indicated for species with more than one accession per country.

30 taxa in the combined tree) and *B. nelsonii* (sister to a well-supported clade of 23 taxa in the *waxy* tree and part of a larger clade containing 28 taxa in the combined tree). Conflicting positions were also seen in the relationships among the three accessions of *W. meiantha* and among the accessions of *W. solanacea* from Costa Rica and Colombia in the *trnL-trnF* vs. *waxy* and *waxy* vs. combined trees. We present the individual and combined data trees for comparison but consider the combined tree (Fig. 1) as the most complete phylogenetic hypothesis for relationships within *Witheringia* and *Brachistus* at this time.

The phylogeny recovered previously documented relationships among *Witheringia* and outgroup taxa published by Olmstead et al. (2008), Särkinen et al. (2013), Zamora-Tavares et al. (2016), and Deanna et al. (2019), with *Witheringia* + *Brachistus* included with other members of the subtribe Physalidinae. Where sampled, *Tzeltalia* and *Schraderanthus viscosus* (Schrad.) Averett [as *Leucophysalis viscosa* (Schrad.) Hunz. in some trees] were closely related to the two genera, although their positions varied depending on the taxa sampled and regions sequenced.

In our trees, *Witheringia* and *Brachistus* together form a monophyletic group (the *Witheringia* clade). *Brachistus* species appear to have originated multiple times within the *Witheringia* clade, but several of these branches have low support. The Mexican endemic species *W. stellata* and *W. mexicana* are the earliest diverging lineages of the *Witheringia* clade. Beyond these, *B. knappiae*, *B. nelsonii*, and *B. stramoniifolius* form a paraphyletic group with *W.* cf. *solanacea*. The remainder of the sampled species form two well-supported clades. The first includes *W. asterotricha*, *W. solanacea*, *W. mortonii*, *W.* sp. *3858*, and *W.* cf. *asterotricha* from Colombia. Within this clade, all accessions of *W. asterotricha* and *W. solanacea* form a well-supported group (the Solanacea clade of Fig. 1). The second clade includes *W. coccoloboides*, *W. correana*, *W. macrantha*, *W. maculata*, and *W. meiantha*. All accessions of *W. coccoloboides*, *W. maculata*, and *W. meiantha* form monophyletic groups, but accessions of *W. correana* and *W. macrantha* are interspersed throughout this clade.

Discussion

Taxonomic Implications—The phylogenetic framework established here provides a useful construct for the taxonomic treatment of this challenging genus. Most importantly, it resolves the longstanding debate about the status of *Brachistus. Brachistus* was initially described as a genus distinct from what is now *Witheringia* based on its lobed (versus truncate) calyx (Miers 1849b). Our phylogeny shows that *Brachistus* is nested within *Witheringia*, and, although some of these nodes have only moderate support, it is clear that *Brachistus* is not a monophyletic group. At best, it is paraphyletic and should be merged within *Witheringia.* The characters used to separate *Brachistus* from *Witheringia* (lobed and accrescent calyces in *Brachistus* vs. unlobed and non-accrescent calyces in *Witheringia*) have arisen several times within this clade, as was also shown in the analyses of Deanna et al. (2019). A list of *Brachistus* species now included in *Witheringia* is given below, with one new combination, as well as an enumeration of *Brachistus* names now attributed to other genera.

The phylogeny verifies the similarity of *W. asterotricha* and the widespread *W. solanacea*, which together occupy a well-supported clade. These taxa are interfertile, and several authors have suggested that perhaps they should be synonymized (Hunziker 1969; Bohs 2000). Nevertheless, the two *W. asterotricha* sampled here form a well-supported monophyletic group. Furthermore, *W. asterotricha* and *W. solanacea* are morphologically and perhaps geographically distinct, so we advocate keeping them separate at this time. *Witheringia solanacea* is exceedingly widespread, ranging from Mexico to Andean South America. It is also widely collected and displays substantial morphological variation across its range. In our phylogeny, accessions from Costa Rica and Colombia form a single clade, but the placement of the accession from Bolivia is ambiguous. More sampling will be required to determine whether southern accessions belong to the same species as northern collections.

The phylogeny sheds partial light on the persistent confusion among *W. maculata, W. macrantha,* and *W. correana.* These taxa differ in several characteristics but are similar in all having an elongated peduncle, which is lacking in the other taxa of the *Witheringia* clade. In our phylogeny, the sampled accessions of *W. maculata* form a well-supported clade distinct from *W. macrantha*, thus contradicting their synonymization by D'Arcy (1973). *Witheringia maculata* is also clearly morphologically distinct from these other two taxa by its possession of a frilled calyx visible both in flower and in fruit. However, the three accessions of *W. macrantha* do not cluster together on the tree and distinguishing between *W. macrantha* and *W. correana* morphologically is difficult. Bohs (2015) treated these taxa as possible synonyms, differentiating between them by the size of the flowers, specifically the calyces, which are easily compared in most herbarium specimens. A major challenge in delineating these two species is the great variation within each (Fig. 2). Individuals within *W. macrantha* may bear flowers that have no spots, a pattern of green spots, or dense purple spots at the base of corolla lobes. Similarly, individuals within *W. correana* may bear spotted or unspotted flowers. In the phylogeny, accessions of these two species form clades with either *W. meiantha* or *W. maculata*. Even within these subclades, accessions of these two species do not form monophyletic groups. Further study will be required to determine whether these phylogenetic relationships reveal cryptic species within one or more of these taxa or whether this is a case of incomplete lineage sorting in a recently diverged group.

Finally, the phylogeny supports the existence of two new species. The accessions denoted as *W.* cf. *solanacea* were collected in Costa Rica and resemble *W. solanacea* except for their 5-merous flowers (vs. typically 4-merous flowers in *W. solanacea*), more woody habit, and more ovate leaves with cordate bases. Pollination studies have shown these plants to be self-compatible, in contrast to most *W. solanacea* accessions that have been tested (Bohs 2000; Stone and Jenkins 2008). Bohs (2015), in the treatment of *Witheringia* for Costa Rica, noted that these geographically and morphologically distinctive populations may represent a distinct species. The molecular results (Fig. 1) support this view; the two accessions sampled of this form do not cluster with other *W. solanacea* collections from Costa Rica, Colombia, and Bolivia.

The accession *W.* cf. *asterotricha* was collected in southeastern Colombia in Dept. Caquetá near Florencia. Morphologically, it is most similar to *W. asterotricha*, a species known only from Central America. Although this could indicate a geographical disjunction, the *Witheringia* species of Colombia are poorly known, and the identification of this collection was regarded as provisional. This collection does not cluster with *W. asterotricha* accessions from Central America in the combined tree (Fig. 1) or in any of the individual gene trees (Figs. S1–S3) and apparently represents an undescribed species.

Character Evolution—The *Witheringia* phylogeny can shed light on the evolution of several morphological characters. There has evidently been a transition from woody to herbaceous habit; early diverging taxa such as *W. stellata*, *W. mexicana, B. knappiae, B. nelsonii,* *W.* cf. *solanacea,* and *B. stramoniifolius* are usually small trees or subshrubs (> 2 m in height) with somewhat woody trunks, whereas the remainder of the *Witheringia* clade consists of herbaceous species that are typically less than 2 m tall. Several of these species (*B. knappiae, B. stramoniifolius*, and maybe *B. nelsonii*) can have leaf blades with lobed margins, in contrast to the entire leaf margins seen in most species of the *Witheringia* clade. Interestingly, the monotypic physaloid genus *Schraderanthus* Averett can be a woody shrub with lobed leaf blades. An ITS sequence of *Schraderanthus viscosus* was included in the phylogenies of Deanna et al. (2019) and it emerged in a clade with species of *Witheringia* and *Brachistus*. Unfortunately, we were not able to include *Schraderanthus* on our trees. Data available in GenBank include an ITS sequence the same length as ours (AY665848), but ITS is not useful in resolving that part of the tree (Fig. S1). *Waxy* is a more useful locus, but the *waxy* sequence in GenBank (AY665927) contains only 325 bp of nucleotides, spanning exon and intron 9. Obtaining additional genomic data for *Schraderanthus* should be a priority for resolving relationships in the *Witheringia* clade and in the broader context of the tribe Physalideae.

FIG. 2. Variation within and among Costa Rican accessions of *W. correana* (A–C) and *W. macrantha* (D–F). Both species have flowers and fruits borne on peduncles (B, E), and dense stiff hairs throughout. Flowers may be spotted or unspotted in plants of either species. Flower size is variable in both taxa, but flowers are consistently smaller in *W. correana*. Scale bars = 1 cm. A, B. *Stone & Flores 1543*, Limon Province; C. *Stone & Flores 1545*, Guanacaste Conservation Area; D, E. Photo voucher only but cf. *Bohs 2512*, Monteverde; F. *Stone & Flores 1548*, Las Tablas. Photos by J. Flores.

Calyx lobing and accrescence have evolved repeatedly within this group. The presence of toothed or lobed rather than truncate calyces has previously been used to delineate *Brachistus* as a separate section within *Witheringia* (Hunziker 1969) or as a separate genus (D'Arcy et al. 1981). The emergence of multiple *Brachistus* lineages within *Witheringia* indicates that calyx lobing is more evolutionarily labile than previously thought, presumably arising at least twice within the group. Calyx accrescence also displays evolutionary lability. *Witheringia* clade species with accrescent calyces include *B. stramoniifolius*, *W. correana*, and *W. macrantha*, and *B. nelsonii* has accrescent-inflated calyces that completely envelop the ripe fruit. In an analysis of character-state evolution across the Physalideae, Deanna et al. (2019) inferred that accrescent calyces have arisen three times within the *Witheringia/Brachistus* clade. This estimate is consistent with our more fully sampled phylogeny.

Biogeography—*Witheringia* occupies a pivotal biogeographic position in the evolution of the physaloid genera, near the juncture where the ancestors of the large genus *Physalis* are hypothesized to have dispersed from the Andean region to current-day Mexico and thereby the North

American continent (Zamora-Tavares et al. 2016). This scenario is supported by our finding that the lineages represented by the two narrow Mexican endemics, *W. stellata* and *W. mexicana*, diverged at the base of the *Witheringia* clade. Early diverging lineages also include *B. stramoniifolius*, *B. nelsonii*, *B. knappiae*, and *W.* cf. *solanacea*. *Brachistus stramoniifolius* ranges from Mexico to Panama, with the highest density of collections in southern Mexico, Guatemala, and El Salvador. *Brachistus nelsonii* occurs primarily in Mexico, Belize, and Guatemala. *Brachistus knappiae*, in contrast, is a narrow endemic that has been found only in the Talamanca mountains near the border of Costa Rica and Panama, and *W.* cf. *solanacea* appears to be a Costa Rican endemic. Thus, the clade may have originated in Mexico and subsequently dispersed southward over a landscape characterized by mountainous and geologically dynamic terrain (Coates and Obando 1996). However, recent field and herbarium work in South America, particularly Colombia, has unearthed numerous accessions that may represent undescribed species. More accurate species delimitation, thorough inter- and intraspecific sampling, and a more completely resolved phylogeny is needed for a more complete picture of the evolutionary relationships of the *Witheringia* clade.

In sum, the phylogeny presented here provides a systematic foundation for a taxonomic revision of *Witheringia* and its relatives. It also indicates avenues for future study of character evolution and the biogeographic context of diversification in the *Witheringia* clade.

Transfer of Brachistus to Witheringia—The phylogenetic results presented here show that four currently recognized species of *Brachistus* are nested within the genus *Witheringia*, which was published earlier and has priority. Three of these already have published names within *Witheringia*; one new combination is required to transfer *Brachistus knappiae* to *Witheringia*. *Athenaea affinis* C.V. Morton, the basionym of *Witheringia affinis* (C.V.Morton) Hunz. is lectotypified here. In the following section the accepted name in *Witheringia* is listed first, followed by the basionym (if necessary), and any homotypic synonyms.

A list is also provided for *Brachistus* names now assigned to other genera under Excluded Names.

Brachistus Miers, Ann. Mag. Nat. Hist., ser. 2, 3: 262. 1849. Lectotype: *B. stramoniifolius* (Kunth) Miers, based on *Witheringia stramoniifolia* Kunth, designated by Hunziker, 1969, p. 153 [as "especie lectotípica"].

1. Witheringia affinis (C.V.Morton) Hunz., Kurtziana 5: 162. 1969. *Athenaea affinis* C.V.Morton, Contr. Univ. Mich. Herb. 4: 24. 1940. *Brachistus affinis* (C.V.Morton) D'Arcy, J.L. Gentry & Averett, Ann. Missouri Bot. Gard. 68(1): 227. 1981. Type: Belize. El Cayo District: Cohune Ridge, 10 Jul 1936, *C. L. Lundell 6452* (lectotype, designated here: US acc. # 1688342 [barcode 00027303!]; isolectotypes: A, F, US acc. # 1633341 [barcode 00027304!]).

Morton in Lundell (1940) designated two sheets at US as the "Type," but these sheets are not cross labeled as belonging to the same specimen, so lectotypification is necessary (Art. 8.3; Turland et al. 2018). The sheet of US-1688342 is a slightly better specimen and is designated here as the lectotype.

Brachistus affinis was regarded as a synonym of *B. nelsonii* in Knapp et al. (2005) and Bohs (2015).

2. ***Witheringia knappiae*** (Mont.-Castro & Sousa-Peña) Bohs, comb. nov. *Brachistus knappiae* Mont.-Castro & Sousa-Peña, Brittonia 60: 167. 2008. Type: Panama. Prov. Chiriquí: Bugaba, Santa Clara, Cerro Pando, 8°50′N, 82°44′W, 28 Feb 1985, *H. van der Werff & J. Herrera 7196* (holotype: MO acc. # 3902425 [barcode MO-037930]!).

3. Witheringia nelsonii (Fernald) Hunz., Kurtziana 5: 160. 1969. *Athenaea nelsonii* Fernald, Proc. Amer. Acad. Arts 35 (25): 567. 1900. *Brachistus nelsonii* (Fernald) D'Arcy, J.L.Gentry & Averett, Ann. Missouri Bot. Gard. 68(1): 227. 1981. Type: Mexico. Chiapas: between Tumbala and El Salto, 460–1385 m, 29 Oct 1895, *E. W. Nelson 3395* (lectotype, designated by Hunziker, 1969, p. 160 [as "holotipo"]: GH [barcode 00057708!]; isolectotype: US acc. # 233172 [barcode 00027306!]).

4. Witheringia stramoniifolia Kunth, Nov. Gen. Sp. Pl. [H.B.K.] 3: 11 (folio), 13 (quarto). 1818, as "*stramonifolia*." *Brachistus stramoniifolius* (Kunth) Miers, Ann. Mag. Nat. Hist., ser. 2, 3:263. 1849, as "*stramonifolius*." *Capsicum stramoniifolium* (Kunth) Kuntze, Rev. Gen. Pl. 2: 450. 1891, as "*stramonifolius*." *Bassovia stramoniifolia* (Kunth) Standl. Contr. U.S. Natl. Herb. 23: 1303. 1924. *Capsicum stramoniifolium* (Kunth) Standl., Publ. Field Mus. Nat. Hist., Bot. Ser. 18: 1044. 1938.Type: Mexico. "Crescit frequentissime in Regno Mexicano prope La Banderilla et urbem Xalapae, alt. 750 hex," *Humboldt & Bonpland 4440* (lectotype, designated by Hunziker, 1969, p. 154 [as "tipo"]: P-Bonpl. [barcode P00157179!]; isolectotype: B [destroyed; F neg. 2878, B!]).

Excluded Names—

Brachistus actinocalyx H.J.P.Winkl., Repert. Spec. Nov. Regni Veg. 7: 245. 1909 = Lycianthes leptocaulis (Rusby) Rusby.

Brachistus ceratocalycius Donn.Sm., Bot. Gaz. 48: 297. 1909 = Lycianthes ceratocalycia (Donn.Sm.) Bitter.

Brachistus ciliatus (Kunth) Miers, Ann. Mag. Nat. Hist., ser. 2, 3: 263. 1849 = Capsicum rhomboideum (Dunal) Kuntze.

Brachistus coccineus Rusby, Bull. New York Bot. Gard. 8: 117. 1912 = Capsicum coccineum (Rusby) Hunz.

Brachistus cuspidatus (Dunal) Werderm. & Diels, Bibliogr. Bot. Cracow 116: 130. 1937 = Cuatresia cuspidata (Dunal) Hunz.

Brachistus dimorphus Miers, Ann. Mag. Nat. Hist., ser. 2, 3: 267. 1849 = Capsicum dimorphum (Miers) Kuntze.

Brachistus diversifolius (Klotzsch) Miers, Ann. Mag. Nat. Hist., ser. 2, 3: 268. 1849 = Capsicum rhomboideum (Dunal) Kuntze.

Brachistus dumetorum (Dunal) Miers, Ann. Mag. Nat. Hist., ser. 2, 3: 265. 1849 = Capsicum rhomboideum (Dunal) Kuntze.

Brachistus escuintlensis J.M.Coult., Bot. Gaz. 16: 144. 1891 = Lycianthes heteroclita (Sendtn.) Bitter.

Brachistus fasciculatus Rusby, Bull. New York Bot. Gard. 4: 423. 1907 = Lycianthes fasciculata (Rusby) Bitter.

Brachistus feddei Reinecke, Bot. Jahrb. Syst. 25: 674. 1898 = Lycianthes vitiensis (Seem.) A.R.Bean.

Brachistus fendleri (Rusby) Rusby, Bull. New York Bot. Gard. 4: 470. 1907 = Lycianthes inaequilatera (Rusby) Bitter.

Brachistus fuscoviolaceus Cufod., Arch. Bot. Sist. 10(1): 20. 1934 = Witheringia coccoloboides (Dammer) Hunz.

Brachistus haughtii Svenson, Amer. J. Bot. 33: 481. 1946 = Capsicum rhomboideum (Dunal) Kuntze.

Brachistus hebephyllus Miers, Ann. Mag. Nat. Hist., ser. 2, 3: 266. 1849 = Witheringia solanacea L'Her.

Brachistus hispidus Rusby, Bull. Torrey Bot. Club 26: 198. 1899 = Lycianthes pauciflora (Vahl) Bitter.

Brachistus hookerianus Miers, Ann. Mag. Nat. Hist., ser. 2, 3: 268. 1849 = Capsicum hookerianum (Miers) Kuntze.

Brachistus inaequilaterus (Rusby) Rusby, Bull. New York Bot. Gard. 4: 470. 1907 = Lycianthes inaequilatera (Rusby) Bitter.

Brachistus lanceifolius Miers, Ann. Mag. Nat. Hist., ser. 2, 3: 267. 1849 = Capsicum pubescens Ruiz & Pav.

Brachistus lanceolatus Greenm. ex Donn.Sm., Bot. Gaz. 37: 212. 1904 = Capsicum lanceolatum (Greenm. ex Donn.Sm.) C.V.Morton & Standl.

Brachistus lasiophyllus (Dunal) Rusby, Bull. Torrey Bot. Club 26: 198. 1899 = Lycianthes lasiophylla (Dunal) Bitter.

Brachistus leptocaulis Rusby, Bull. Torrey Bot. Club 26: 199. 1899 = Lycianthes leptocaulis (Rusby) Rusby.

Brachistus lindenii (Dunal) Pittier, Cat. Fl. Venez. 2: 358. 1947 = Cuatresia riparia (Kunth) Hunz.

Brachistus linnaeanus Miers, Ann. Mag. Nat. Hist., ser. 2, 3: 269. 1849 = Physalis campechiana L.

Brachistus macrophyllus (Dunal) Miers, Ann. Mag. Nat. Hist., ser. 2, 3: 263. 1849 = Witheringia solanacea L'Her.

Brachistus meianthus Donn.Sm., Bot. Gaz. 57: 424. 1914 = Witheringia meiantha (Donn.Sm.) Hunz.

Brachistus mollis (Kunth) Miers, Ann. Mag. Nat. Hist., ser. 2, 3: 264. 1849 = Capsicum rhomboideum (Dunal) Kuntze.

Brachistus neesianus Miers, Ann. Mag. Nat. Hist., ser. 2, 3: 268. 1849 = Physalis campechiana L.

Brachistus oblongifolius Miers, Ann. Mag. Nat. Hist., ser. 2, 3: 266. 1849 = Cuatresia riparia (Kunth) Hunz.

Brachistus physocalycius Donn.Sm., Bot. Gaz. 40: 8. 1905 = Cuatresia riparia (Kunth) Hunz.

Brachistus poasensis Cufod., Arch. Bot. Sist. 10: 45. 1934 = Witheringia coccoloboides (Dammer) Hunz.

Brachistus pringlei S.Watson, Proc. Amer. Acad. 25: 159. 1890 = Capsicum rhomboideum (Dunal) Kuntze.

Brachistus pubescens Stewart, Proc. Calif. Acad. Sci., ser. 4, 1: 137. 1911 = Capsicum galapagoense Hunz.

Brachistus rhomboideus (Dunal) Miers, Ann. Mag. Nat. Hist., ser. 2, 3: 264. 1849 = Capsicum rhomboideum (Dunal) Kuntze.

Brachistus riparius (Kunth) Miers, Ann. Mag. Nat. Hist., ser. 2, 3: 265. 1849 = Cuatresia riparia (Kunth) Hunz.

Brachistus sancti-caroli H.J.P.Winkl., Repert. Spec. Nov. Regni Veg. 7: 245. 1909 = Lycianthes sancti-caroli (H.J.P.Winkl.) Bitter.

Brachistus solanaceus (L'Her.) Benth. & Hook.f. ex Hemsl., Biol. Cent.-Amer., Bot. 2: 424. 1882 = Witheringia solanacea L'Her.

Brachistus spruceanus (Hunz.) D'Arcy, Monogr. Syst. Bot. Missouri Bot. Gard. 45: 1259. 1993 = Darcyanthus spruceanus (Hunz.) Hunz.

Brachistus strigosus Rusby, Bull. Torrey Bot. Club 26: 198. 1899 = Lycianthes radiata (Sendtn.) Bitter.

Brachistus subfalcatus Rusby, Bull. New York Bot. Gard. 8: 117. 1912 = Lycianthes inaequilatera (Rusby) Bitter.

Brachistus vargasii (Dunal) Pittier, Cat. Fl. Venez. 2: 358. 1947 = Capsicum rhomboideum (Dunal) Kuntze.

Brachistus virgatus H.J.P.Winkl., Repert. Spec. Nov. Regni Veg. 7: 245. 1909 = Lycianthes leptocaulis (Rusby) Rusby.

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Author Contributions

LB, JF, and JS collected accessions, LB and JS amplified and sequenced DNA, JS analyzed the data, and JF prepared the photographs. LB resolved the *Brachistus* nomenclature, and all authors contributed to writing the manuscript.

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APPENDIX 1. Species, geographic origin, collector and collection number (herbarium acronym), and GenBank accession numbers for sequences included in this study. GenBank accession numbers are listed in the following order: ITS, waxy, *trnL-trnF*.

Ingroup: *Brachistus knappiae* Mont.-Castro & Sousa-Peña, Panama, *Stone & Flores 1547* (USJ), —, OP137233, OP137241; *Brachistus nelsonii* (Fernald) D'Arcy, J. L.Gentry & Averett, Belize, *Whitefoord 10544* (MO), MH683571, MH760378, MH558593; *Brachistus stramoniifolius* (Kunth) Miers, Mexico, *Bohs & al. 3937* (UT), MH747470, MH760379, MH558594; *Brachistus stramoniifolius* (Kunth) Miers, Mexico, *Bohs & al. 3925* (UT), MH683589, MH760398, MH558605; *Witheringia asterotricha* (Standl.) Hunz., Costa Rica, *Bohs 3007* (UT), MH683572, MH760380, MH752674; *Witheringia asterotricha* (Standl.) Hunz., Panama, *Stone & al. 1525* (MO), MH683573, MH760381, MH558595; *Witheringia* cf. *asterotricha*, Colombia, *Orejuela & al. 2641* (COL), MH683574, MH760382, MH647766; *Witheringia coccoloboides* (Dammer) Hunz., Costa Rica, *Bohs 2978* (UT), MH683575, MH760383, MH558596; *Witheringia coccoloboides* (Dammer) Hunz., Boquete, Panama, *Stone 1526* (MO), MH683576, MH760384, MH558597; *Witheringia coccoloboides* (Dammer) Hunz., Chiriquí, Panama, *Stone 1532* (MO), MH683577, MH760385, MH558598; *Witheringia correana* D'Arcy, Panama, *D'Arcy 16415* (MO), MH683578, MH760386, MH558599; *Witheringia correana* D'Arcy, Costa Rica, *Stone & Flores 1543* (USJ), OP162412, OP137234, OP137242; *Witheringia correana* D'Arcy, Costa Rica, *Stone & Flores 1545* (USJ), OR666665, OP137235, OP137243; *Witheringia macrantha* (Standl. & C.V.Morton) Hunz., Costa Rica, *Bohs 2512* (UT), AY665857, MH796627 and AY665925, EU581071; *Witheringia macrantha* (Standl. & C.V.Morton) Hunz., Costa Rica, *Stone & Flores 1542* (USJ), OR666664, OP137236, OP137244; *Witheringia maculata* (Standl. & C. V.Morton) Hunz., Costa Rica, *Bohs 2487* (UT), MH683580, MH760388, MH558600; *Witheringia maculata* (Standl. & C. V.Morton) Hunz., Costa Rica, *Stone & Flores 1540* (USJ), OP162410, OP137238, OP137246; *Witheringia maculata* (Standl. & C. V.Morton) Hunz., Costa Rica, *Stone & Flores 1541* (USJ), OP162411, OP137239, OP137247; *Witheringia meiantha* (Donn.Sm.) Hunz., La Selva, Costa Rica, *Bohs 3015* (UT), MH683581, MH760389, EU581072; *Witheringia meiantha* (Donn.Sm.) Hunz., La Selva, Costa Rica, *Bohs 2387* (UT), MH683582, MH760390, MH558601; *Witheringia meiantha* (Donn.Sm.) Hunz., Panama, *Stone 1523* (MO), MH683583, MH760391, MH558602; *Witheringia mexicana* (B.L.Rob.) Hunz. Mexico, BIRM S.1199 (BIRM), MH763750, MH760392, EU581073; *Witheringia mortonii* Hunz., Costa Rica, *Bohs 2594* (UT), MH683584, MH760393, MH752678; *Witheringia solanacea* L'Her., Bolivia, *Bohs 3007a* (UT), MH683587, MH760396, MH647768; *Witheringia solanacea* L'Her., Colombia, *Orejuela & al. 2637* (COL), MH683588, MH760397, MH647769; *Witheringia solanacea* L'Her., Las Cruces, Costa Rica, *Bohs 2427* (UT), MH683585, MH760394, MH558603; *Witheringia solanacea* L'Her., Rio de la Paz, Costa Rica, *Bohs 2416* (UT), MH683586, MH760395, MH558604; *Witheringia* cf. *solanacea*, Costa Rica, *Bohs 2685* (UT), MH683591, MH760399, MH558606; *Witheringia* cf. *solanacea*, Costa Rica *Stone & Flores 1544* (USJ), OP162413, OP137240, OP137248; *Witheringia stellata* (Greenm.) Hunz., Mexico, *Stone 1522* (MO), MH683590, MH760400, MH752679; *Witheringia* sp. 3858, Colombia, *Orozco & al. 3858* (COL), MH683579, MH760387, MH647767.

Outgroup: *Chamaesaracha coronopus* (Dunal) A.Gray, U.S.A, *Turner 15854* (TEX), AY665860, AY665937.1, EU580978; *Chamaesaracha sordida* (Dunal) A.Gray, U.S.A, *Olmstead 92-245* (WTU), AY665861, AY665938.1, EU580979; *Iochroma fuchsioides* (Bonpl.) Miers, Ecuador, *Smith 488* (WIS), DQ314203, DQ309514.1, —; *Iochroma fuchsioides* (Bonpl.) Miers, Colombia, *Olmstead S-29* (WTU), —, —, EU581001; *Leucophysalis grandiflora* (Hook.) Rydb., U.S.A., *Smith 217* (WIS), DQ314161, DQ309471, —; *Physalis peruviana* L., Peru, *Olmstead S-69* (WTU), —, —, EU581044; *Physalis philadelphica* Lam., in cult., *Bohs 2433* (UT), AY665871, AY665955, EU581045.1; *Saracha punctata* Ruiz & Pav., Bolivia, *Nee 51804* (NY), DQ314182, DQ309492, —; *Saracha punctata* Ruiz & Pav., South America, *Plowman 4651* (UC), —, —, EU581053; *Tzeltalia calidaria* (Standl. & Steyerm.) Estrada & Martínez, *Lundell 19625* (LL-TEX), AY665855, AY665930, MH752670.

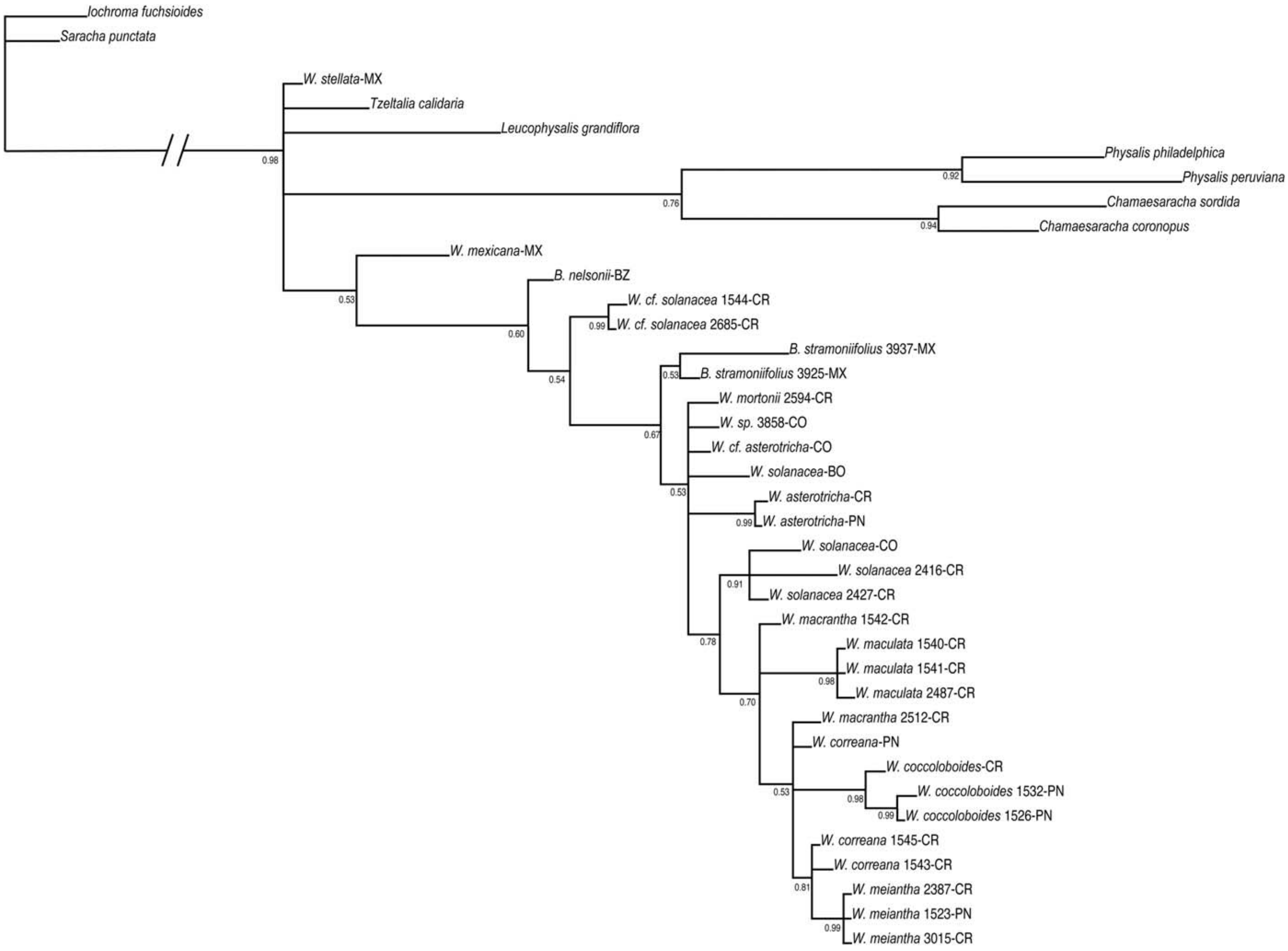

FIG. S1. Bayesian inference tree for *Witheringia*, *Brachistus*, and outgroup taxa inferred from ITS data. Numerals after names are collection numbers. Posterior probabilities are shown at nodes. Country code is indicated for species accessions spanning geopolitical boundaries (BO = Bolivia; BZ = Belize; CO = Colombia; CR = Costa Rica; MX = Mexico; PN = Panama). Accession numbers are indicated for species with more than one accession per country.

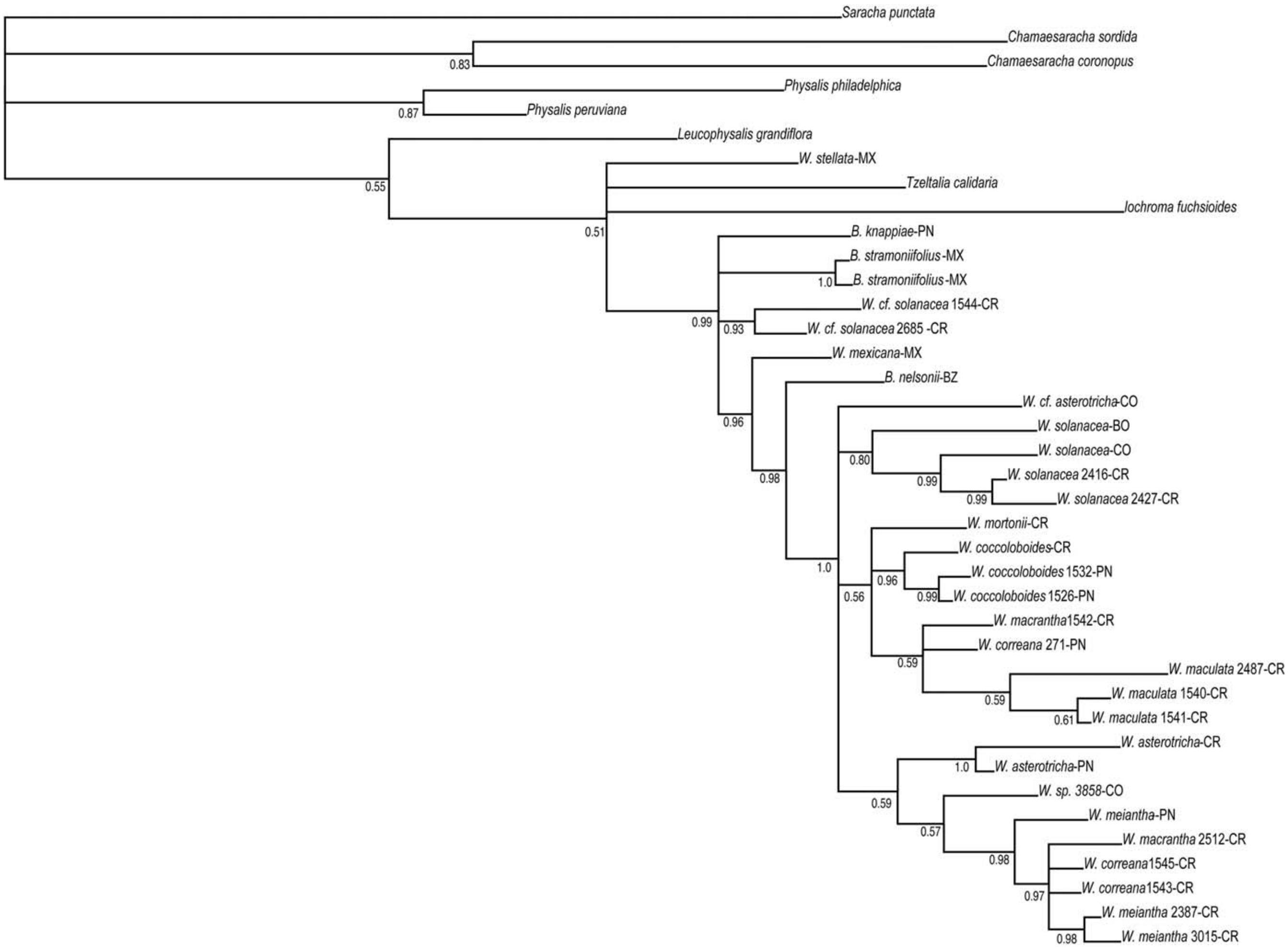

FIG. S2. Bayesian inference tree for *Witheringia*, *Brachistus*, and outgroup taxa inferred from *waxy* data. Numerals after names are collection numbers. Posterior probabilities are shown at nodes. Country code is indicated for species accessions spanning geopolitical boundaries (BO = Bolivia; BZ = Belize; CO = Colombia; CR = Costa Rica; MX = Mexico; PN = Panama). Accession numbers are indicated for species with more than one accession per country.

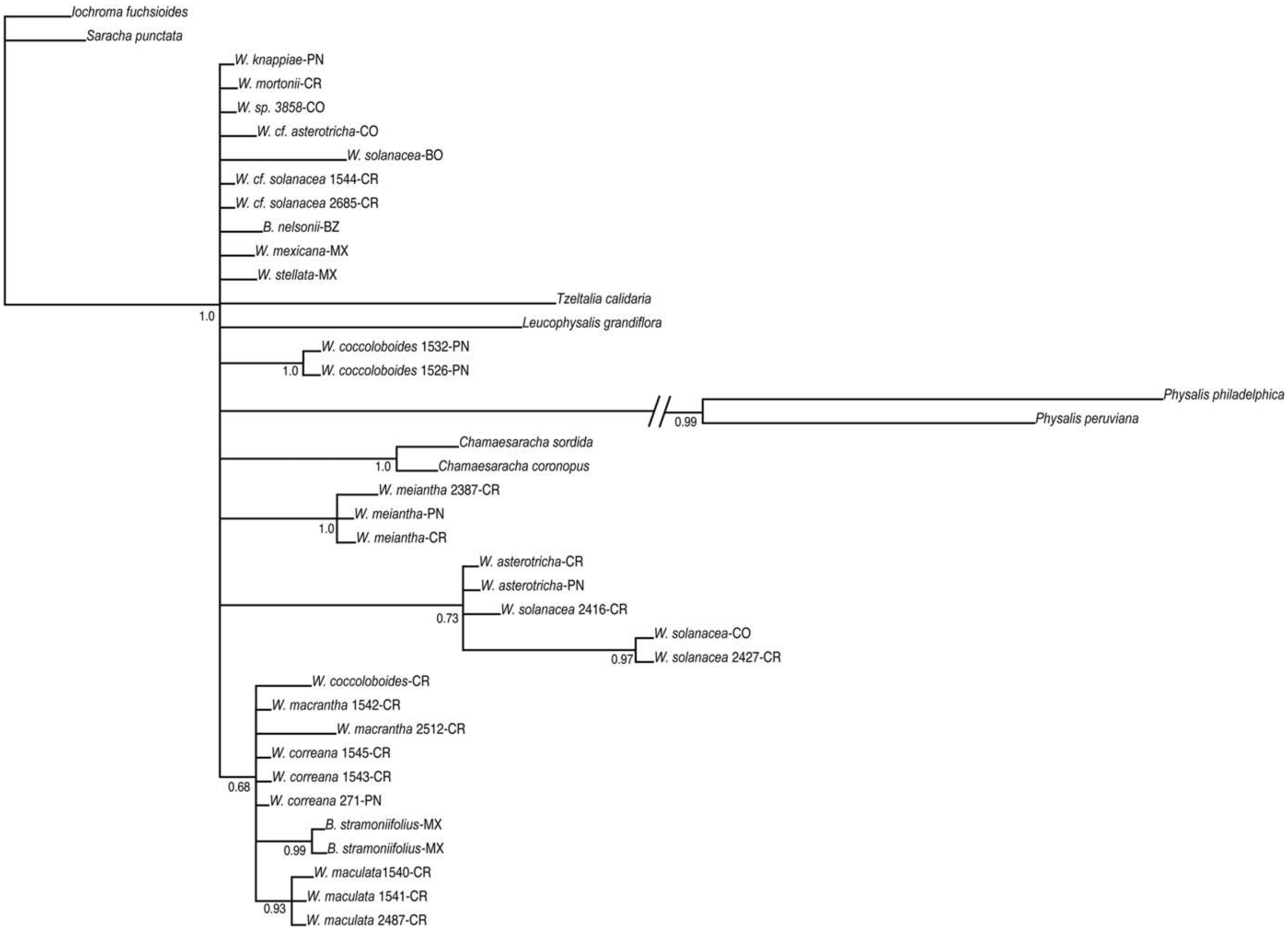

FIG. S3. Bayesian inference tree for *Witheringia, Brachistus,* and outgroup taxa inferred from *trnL-trnF* data. Numerals after names are collection numbers. Posterior probabilities are shown at nodes. Country code is indicated for species accessions spanning geopolitical boundaries (BO = Bolivia; BZ = Belize; CO = Colombia; CR = Costa Rica; MX = Mexico; PN = Panama). Accession numbers are indicated for species with more than one accession per country.