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Morphometric Analyses and Taxonomic Revision of the North American Holoparasitic Genus *Conopholis* (Orobanchaceae)

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Abstract—Members of the small genus *Conopholis* are perennial holoparasites. They are found growing in eastern and southwestern North America and in Central America, where they attach to the roots of their oak hosts. Two species were recognized in the last taxonomic revision of the group based on geographic range and differences in floral, capsule, and bract morphology. Due to the overlapping nature of the characters used to distinguish between taxa, no single morphological feature can be relied on to differentiate between the species. A recent molecular phylogenetic study of the genus recovered three well-supported lineages, none of which corresponds entirely to the current subdivision of the genus into two species. We undertook a fine-scale morphometric study of the genus, emphasizing calyx and bract morphology. Unweighted pair-group method using arithmetic averages and principal coordinate analyses corroborate molecular data and strongly support the distinction of three separate lineages within *Conopholis*. A taxonomic re-alignment is proposed for the genus including three species, *C. americana*, *C. panamensis*, and *C. alpina*, each with various degrees of overlap with previously described taxa.

Keywords—Disjunction, holoparasite, hybrid swarms, morphometrics, principal coordinate analysis, UPGMA.

Conopholis Wallr. is a small holoparasitic genus distributed throughout eastern and southwestern North America and Central America. The genus was established by Wallroth (1825) based on a specimen from South Carolina (eastern U. S. A.) described originally by Linnaeus (1767) as *Orobanche americana*. Since then, four other species have been described: *C. alpina* Liebm., *C. sylvatica* Liebm., *C. mexicana* Gray ex Watson, and *C. panamensis* Woodson. *Conopholis* belongs to Orobanchaceae (Young et al. 1999; Olmstead et al. 2001; Angiosperm Phylogeny Group 2009), one of the largest and most prominent families of parasitic plants, containing approximately 1,800 species, one-half of all known parasitic angiosperms (Nickrent 2012). Within Orobanchaceae, *Conopholis* is closely related to other North American parasites in the holoparasitic clade III (as defined by Bennett and Mathews 2006), specifically *Epifagus* Nutt., *Boschniakia* C. A. Mey ex Bong, and *Orobanche* L. It can be distinguished from *Epifagus* by its chasmogamous flowers and from *Orobanche* by its exserted stamens. Following the descriptive terminology as applied traditionally to this genus (see Woodson and Seibert 1938; their Fig. 2), *Conopholis* possesses calyces that are lobed (i.e. with rounded margins) or toothed (i.e. with pointed margins), with tubes split deeply longitudinally along the anterior side, while those in *Boschniakia* are often zygomorphic but not split longitudinally.

In plants, parasitism is defined by the presence of haustoria. These are the organs that connect the parasite to the vascular system of its host. With the evolution of advanced parasitism, many holoparasitic species exhibit what is known as the "parasitic reduction syndrome" (Colwell 1994), a suite of correlated morphological and physiological changes, including the loss/reduction of chlorophyll production, photosynthesis, and vegetative structures, along with the complete reliance on their haustorial connection to hosts, from which they acquire carbon, water, and nutrients. Due to this syndrome, there are a limited number of morphological characters that can be relied upon to potentially differentiate between species of *Conopholis*. This has led to disagreement regarding the number of species in the genus among early floristic treatments, and the genus has been variously treated as having one to four species. For example, Beck (1930) accepted two species; Small (1933) assigned three species to this genus; Fernald (1950) reduced it to only one; and Gleason (1952) accepted four species.

In 1971, Haynes determined that the genus was in need of a revision given the taxonomic uncertainty and the high degree of similarity among taxa. After studying the relevant type specimens, he concluded that the individuals assigned to *C. alpina*, *C. sylvatica*, and *C. panamensis* represented only intraspecific variability and did not warrant separation into three different species. Therefore, these three entities were combined under *C. alpina*. His classification is based on a combination of presence/absence of characters along with a number of quantitative traits such as the relative proportion of bracts and scales as well as the shape of the calyx (Haynes 1971). Ultimately, Haynes (1971) recognized only two species, *C. americana* and *C. alpina*, with the latter being divided into two varieties, *C. alpina* var. *alpina* and var. *mexicana* (Gray ex Watson) R. R. Haynes. The two species were separated because of their partial morphological distinctiveness, and perhaps most importantly, because of their geographic isolation and apparent host specificity (Haynes 1971). Figure 1 summarizes the relationship between the five species of *Conopholis* that were described before Haynes' work in 1971 and the two species proposed by Haynes following his taxonomic treatment.

Conopholis americana parasitizes red oaks (*Quercus* section *Lobatae* Loudon; Manos et al. 2001) in moist deciduous or mixed forests and is found today across eastern North America, from Nova Scotia to Wisconsin in the north and from Florida to Alabama in the south. Compared to *C. alpina*, *C. americana* has a looser inflorescence, broader bracts nearly or wholly concealing the calyx, and styles mostly persistent in fruit. *Conopholis alpina* parasitizes various oak species, but predominantly those of white oaks (*Quercus* section *Quercus*) in oak woodlands and mixed montane forests found in southwestern

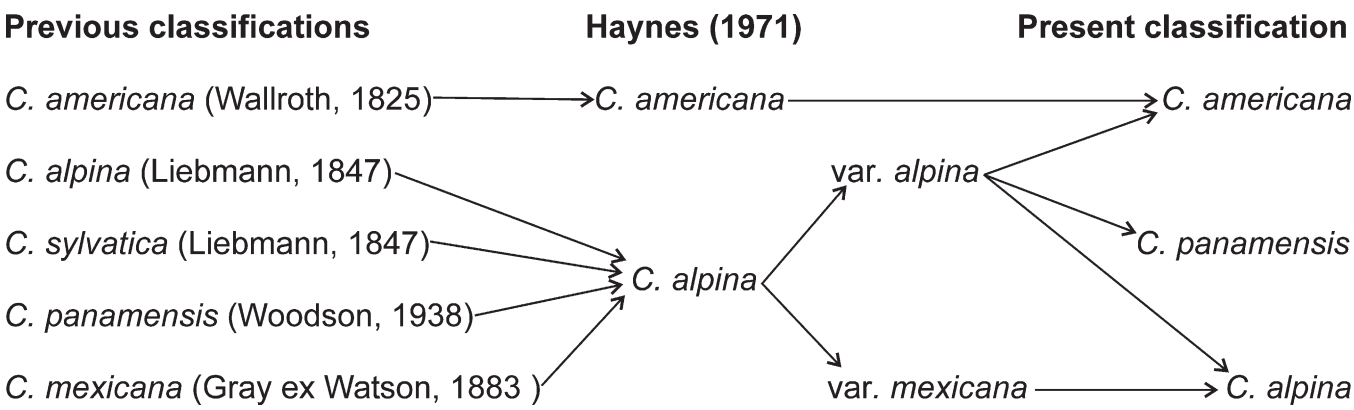

Fig. 1. A summary of the relationships between the various names applied to taxa in the genus *Conopholis* according to the various authors before 1971, by Haynes in his monograph in 1971, and by our revised classification following this morphometric study.

North America. *Conopholis alpina* var. *alpina* occurs in the central area of Mexico across the Trans-Mexican volcanic belt (TMVB) south to Costa Rica and Panama. *Conopholis alpina* var. *mexicana* is distributed from the Trans-Pecos area of Texas through northern New Mexico and central Arizona south to central Oaxaca, including the same central area of Mexico as *C. alpina* var. *alpina*. The distribution of both varieties thus overlaps in the central region of Mexico along the TMVB where identifying a specimen to a particular variety is especially challenging. The features that distinguish the two varieties, apart from their geographic range, are the texture and venation of scales, and whether or not the bract conceals the calyx.

In a recent molecular phylogenetic study (Rodrigues et al. 2011), *Conopholis* was found to contain three major lineages. Regardless of the source of data (plastid or nuclear sequences) and phylogenetic method utilized (distance or character-based methods), none of the analyses resulted in the strict subdivision of the genus into the two currently recognized species. Each of the three distinct and well-supported clades recovered had varying degrees of overlap with previously proposed taxa. In addition, the three clades showed much greater genetic differentiation among them than among individuals within each of those clades. Altogether, taking into account the composition of these clades and the branch lengths subtending them, the molecular results were interpreted as lending support to three distinct lineages within *Conopholis*, potentially at the species level (Rodrigues et al. 2011).

Given the overlapping distribution of variation in morphological traits used to assign individuals of *Conopholis* to their respective species, combined with the recent molecular findings suggesting three distinct lineages, a morphometric study of this genus is necessary. The specific objectives are to (1) examine the patterns of morphological variation among *Conopholis* taxa, (2) conduct morphometric analyses, and (3) provide taxonomic realignment for the genus. We present new morphological evidence to expand upon the previous molecular phylogenetic study, and based on these combined lines of evidence we provide a comprehensive systematic treatment for *Conopholis*.

Materials and Methods

Taxon Sampling—Approximately 600 *Conopholis* herbarium specimens from ARIZ, ASU, AUA, F, IEB, INBIO, MEXU, NMC, NY, RSA, TEX/LL, TRTE, UNM, US, and XAL were examined. Many of these specimens could not be included in this morphometric study owing primarily to their inappropriate ontogenetic stage (e.g. young emerging inflorescence that had not yet expanded, flowers in buds, late fruiting specimens) or their poor condition. In total, 105 individuals sampled from across the geographic range of the two currently recognized species of *Conopholis* (including the two varieties of *C. alpina*), were used in this study (Appendix 1). This sampling includes 27 individuals of *C. americana*, 40 individuals of *C. alpina* var. *alpina*, and 38 individuals of *C. alpina* var. *mexicana*. The initial names applied to these accessions follow the species delimitations by Haynes (1971), which emphasizes the geographical distinctions between the species. Given the difficulty of distinguishing the two varieties of *C. alpina* at their parapatric boundaries along the TMVB, it was important to investigate multiple individuals from a single herbarium sheet, when available. Of all examined sheets attributed to *C. alpina* var. *alpina*, seven collections included two plants on a single sheet. This allowed for an attempt to assess variation within populations, assuming that the collected specimens are representatives of different individuals and not coming from the same tubercle.

Morphology and Morphometric Analysis—States for seven characters derived from bract and calyx morphology were recorded, five qualitative and two quantitative (Table 1; Supplemental Appendix 1). These characters were chosen based on (1) primary differences noted in previous species descriptions (Beck 1930; Small 1933; Woodson and Seibert 1938; Fernald 1950; Gleason 1952), (2) the fact that they were deemed most taxonomically useful in the last comprehensive monograph of the genus (Haynes 1971), and (3) personal observations made during a pilot study. Descriptions and measurements are based on rehydrated herbarium material. Material was rehydrated, fixed in FAA, and then stored in 70% ethanol. The character states were recorded at two positions along the inflorescence of the specimens: the observations and measurements made at the 'top' were always located four to six bract positions below the youngest bract subtending a flower while those made at the 'base' were always from the first bract that subtends a flower found just above basal stem scales that do not surround a flower. These landmark locations, depicted in Figure 2, were established to ensure that observations and measurements would be made at the same relative position across all specimens, regardless of their exact ontogenetic stage or environmental conditions. Quantitative characters (bract width and length) were measured from digitally acquired bract outlines and computer-based measurements using MorphoSys (Meacham and Duncan 1991) and an image capture system based on the PCvision*plus* framegrabber from Imaging Technology Inc., Woburn, Mass., U. S. A. Length was measured from the base of the bract to its apex, and width was measured at the widest point of the bract (always at the base of the bract; Fig. 2).

To assess overall morphological variation, the data were visualized with clustering and ordination methods implemented in R (R Core Team 2012).

Table 1. Characters and character states used to make observations/measurements at the 'top' and 'base' of the plant (see Fig. 1) and used in morphometric analyses.

1. Bract shape (0 = does not conceal calyx; 1 = conceals calyx), **2.** Calyx shape (0 = lobed; 1 = toothed), **3.** Bract tip (0 = acute; 1 = obtuse), **4.** Calyx tooth shape (0 = acute; 1 = obtuse), **5.** Bract margin (0 = glabrous; 1 = with hair), **6.** Bract width (cm), **7.** Bract length (cm)

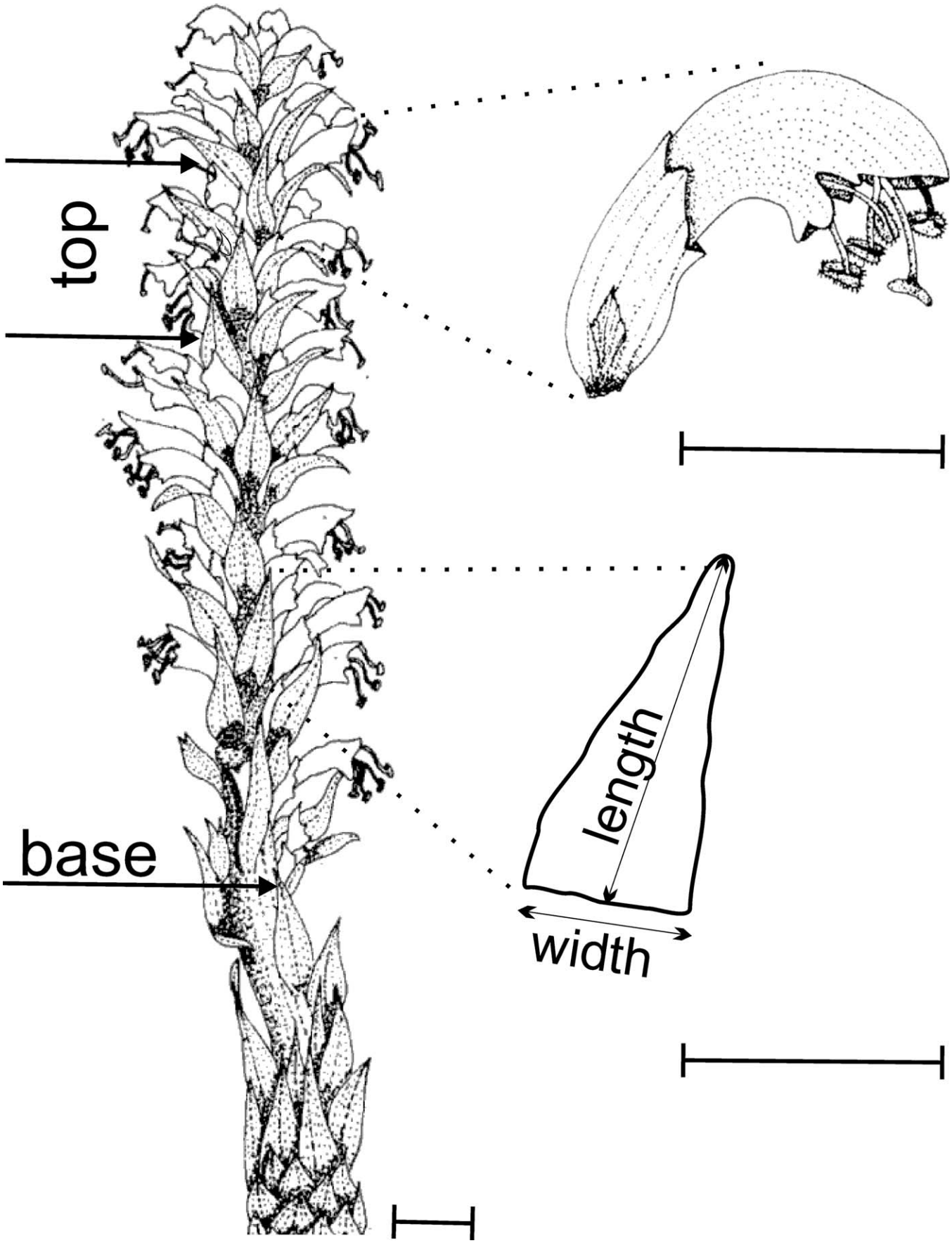

FIG. 2. A composite sketch of a stylized *Conopholis* specimen (adapted from Haynes 1971). The two positions along the inflorescence from where the morphometric observations and measurements were taken are labeled 'top' and 'base.' Scale bars equal 1 cm.

We first calculated the pairwise dissimilarities (distance) between observations in the data using Gower's coefficient (Gower 1971; function *daisy* in the R package *cluster*; Maechler et al. 2012). For binary characters, 0/0 matches were treated as negative matches. Gower's coefficient was used because it allows for the combination of qualitative and quantitative data. Phenograms were then constructed using the unweighted pair-group method using arithmetic averages (UPGMA; Sneath and Sokal 1973; function *hclust* in the R package *vegan*; Oksanen et al. 2013) on the Gower's coefficient matrix. The cophenetic correlation coefficient was calculated to determine how well the hierarchical structure of the dendrogram represents the actual distances. Finally, we applied principal coordinate analysis (PCoA) to the distance matrix (function *pcoa* in the R package *ape*; Paradis et al. 2004). This form of analysis is more appropriate than principal component analyses when there are missing values in the data matrix (Rohlf 1972). This allowed us to include calyx morphology in the analysis despite its absence by the time an individual bears mature fruit. In this study, the distances amongst specimens are illustrated by plotting the first two principal coordinates. Both UPGMA and PCoA analyses were performed on observations and measurements made from the 'top' and 'base' along the inflorescence of the specimens, as indicated in Figure 2. Two sets of measurements were made and analyzed because (1) for a number of specimens we could not establish what the 'base' on the inflorescence was, (2) the 'top' data set had more observations than the 'base', and (3) we wanted to determine if the individuals would cluster in the same manner, regardless of where observations were made.

Results

The UPGMA cluster analysis using the Gower's coefficient matrix produced from measurements obtained from the 'top' of the inflorescence shows a clear separation of three backbone clusters (Fig. 3A–C). The majority of specimens from southwestern North America used in this study (*C. alpina* var. *alpina* and *C. alpina* var. *mexicana*) are found in two separate clusters (A and B). Cluster A contains individuals from the southwestern portion of the U. S. A. and throughout Mexico (*C. alpina* var. *mexicana* and *C. alpina* var. *alpina*). Cluster B comprises the lineage found in Costa Rica and Panama (*C. alpina* var. *alpina*). Cluster C, sister to B, contains all samples from eastern North America (*C. americana*) along with one individual identified a priori as *C. alpina* var. *mexicana* from Texas and nine accessions named a priori as *C. alpina* var. *alpina* from the southern Mexican states of Vera Cruz, Puebla, Distrito Federal, Michoacán, and Hidalgo. These ten samples of *C. alpina* are positioned within this predominantly eastern North American cluster C, instead of being more closely related to other specimens of *C. alpina* (clusters A and B), as would be expected based on traditional classification. The cophenetic correlation coefficient of the analysis was 0.92. The UPGMA cluster analysis performed on the 'base' observations and measurements produced a topology consistent with that from the 'top' UPGMA analysis, recovering identical backbone clusters (dendrogram not shown; cophenetic correlation coefficient of the analysis was 0.93).

Both the 'top' and 'base' ordination analyses (PCoA) revealed three clearly separated clusters (Fig. 4A–C). The compositions of species and populations within each group were identical to the three clusters obtained by UPGMA analysis described above. The first coordinate axis for each analysis separates clusters B and C from cluster A while the second axis separates B from C. In both plots of Fig. 4, individuals in cluster C marked by an arrowhead are found to be outliers within this group. These individuals are from sympatric populations in southern Mexico where the two varieties of *C. alpina* overlap in distribution. Character states and measurements were recorded for two individuals per herbarium sheet. For these three particular populations, one individual from each herbarium sheet was found to group with cluster C while the other was found in cluster A.

Discussion

This work represents the first fine-scale morphometric study of *Conopholis*. The clustering and ordination analyses performed in this study failed to reveal groupings corresponding to the subdivision of the genus into the two species recognized by Haynes (1971), *C. americana* and *C. alpina*. Instead, our results demonstrate the morphological differentiation that has occurred between the three lineages detected in our molecular study (Rodrigues et al. 2011). The clear morphological separation among the three clusters recovered here stands in contrast with the traditional classification (synopsis provided in Fig. 1). These new morphological results complement our molecular findings (Rodrigues et al. 2011) and reinforce the distinction of three species within *Conopholis*. Figure 5 summarizes our understanding of the circumscription of species and their relationships based on all available morphological and molecular data. This best estimate of phylogeneny is also used to map morphological synapomorphies and autapomorphies as well as to illustrate the relationship between the current classification of the genus (Haynes 1971) and the revised classification being proposed here (Fig. 5).

Multivariate analyses of morphological data delineated three separate and distinct clusters. *Conopholis alpina*, as defined traditionally, is shown to be polyphyletic as was the case with molecular data (Rodrigues et al. 2011). Its representatives belong to all three clusters (A, B, and C; Figs. 3, 4). Cluster A consists of all members of *C. alpina* var. *mexicana* found in the southwestern portion of the U. S. A. and north of the TMVB as well as several individuals from southern Mexico (*C. alpina* var. *alpina* in part). Members of group A can be identified by their acute bract that does not conceal the calyx, pubescence along the margin of bracts, and obtusely toothed calyx. This species definition encompasses descriptions previously put forth for *C. alpina* (Liebmann 1847), *C. sylvatica* (Liebmann 1847), and *C. mexicana* (Gray ex Watson 1883). Described from Puebla, Mexico, *C. alpina* was deemed to be different from *C. americana* by its unibracteolate calyx, corolla that is twice as long as the calyx, lobes of its lower lip that are short with much exserted stamens, and styles hardly longer than the stamens. *Conopholis sylvatica* from Veracruz, Mexico, was described at the same time as *C. alpina* by Liebmann in 1847 and was defined as having a slender stem, small calyx, slender corolla that was twice as long as the calyx, and short, more obtuse lower lip. *Conopholis mexicana* was described from Coahuila, Mexico, and was said to differ from *C. americana* by its less deeply toothed calyx, larger corolla, and longer and more rigid, lanceolate and acuminate scales. No defining characters were indicated to distinguish it from either *C. alpina* or *C. sylvatica*. For all three of these species, the differences were only noted relative to *C. americana* and not to each other. Based on name precedence, the specific epithet to be applied to this lineage corresponds to *C. alpina* (Liebmann 1847).

Cluster B consists of all individuals sampled from Costa Rica and Panama. Members of this group can be identified by their acute bract that conceals the calyx, lack of pubescence along the margin of bracts, and lobed calyx. This lineage corresponds to the previously described species, *C. panamensis* (Woodson and Seibert 1938) from Chiriqui, Panama. In its

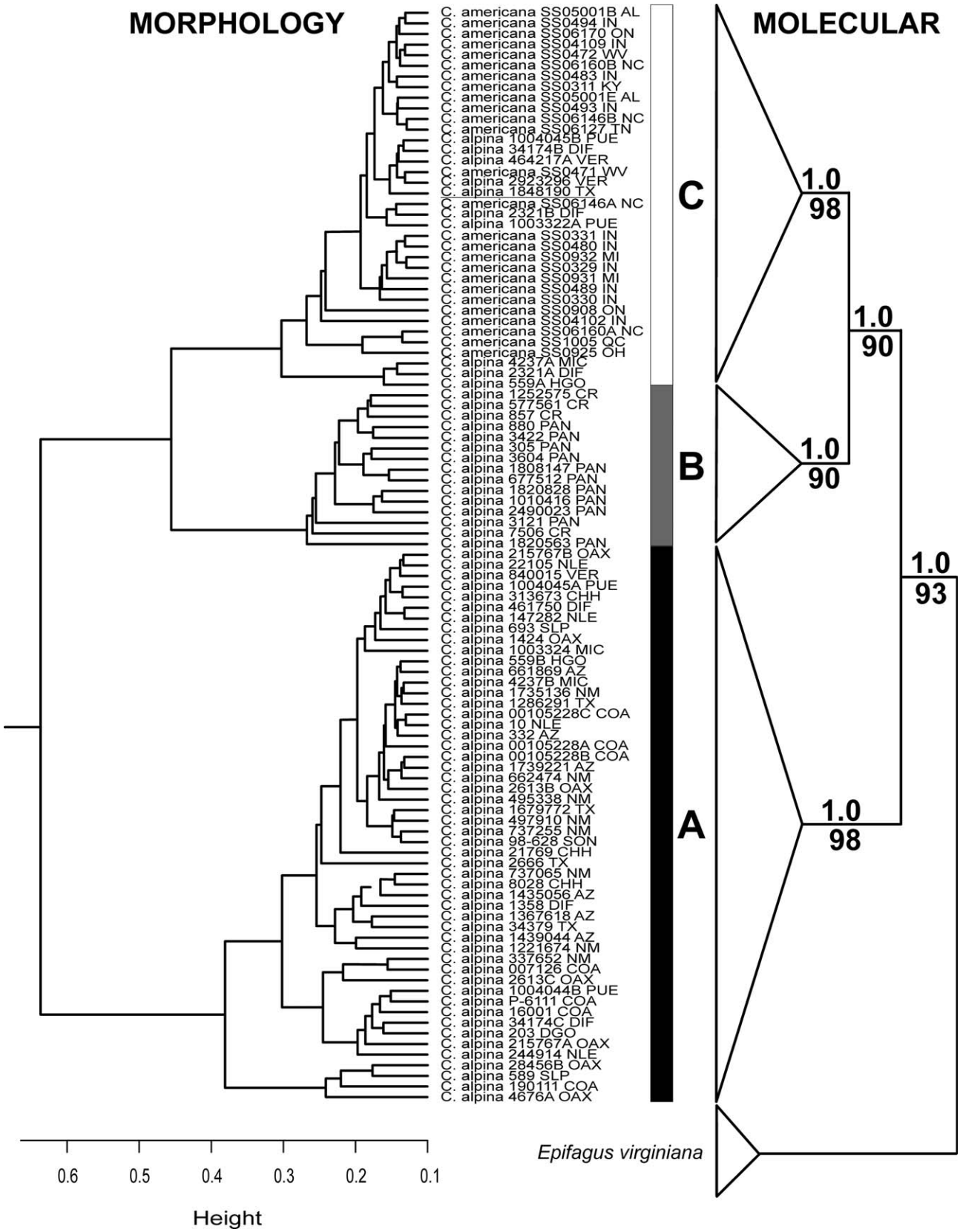

FIG. 3. Illustration of the congruence between morphological and molecular data. On the left is the phenogram resulting from the UPGMA analysis performed in this study using Gower's coefficient matrix on the observations and measurements made from the top of the inflorescence on 103 specimens. Major clusters recovered and discussed in this study are labeled A–C. Species names are followed by their respective accession label/collector numbers and abbreviations of states/provinces in which they were collected (Appendix 1). Underlined is an individual with an anomalous position, see text for discussion. On the right is a summary phylogenetic tree showing the relationships among the three major lineages within *Conopholis* inferred from a combined analysis of plastid (*trnfM-E* and *clpP*) and nuclear (*PHYA*) sequences (adopted from Rodrigues et al. 2011, which used *Epifagus* as the outgroup). Bayesian posterior probabilities and parsimony bootstrap support values are indicated above and below the branches respectively.

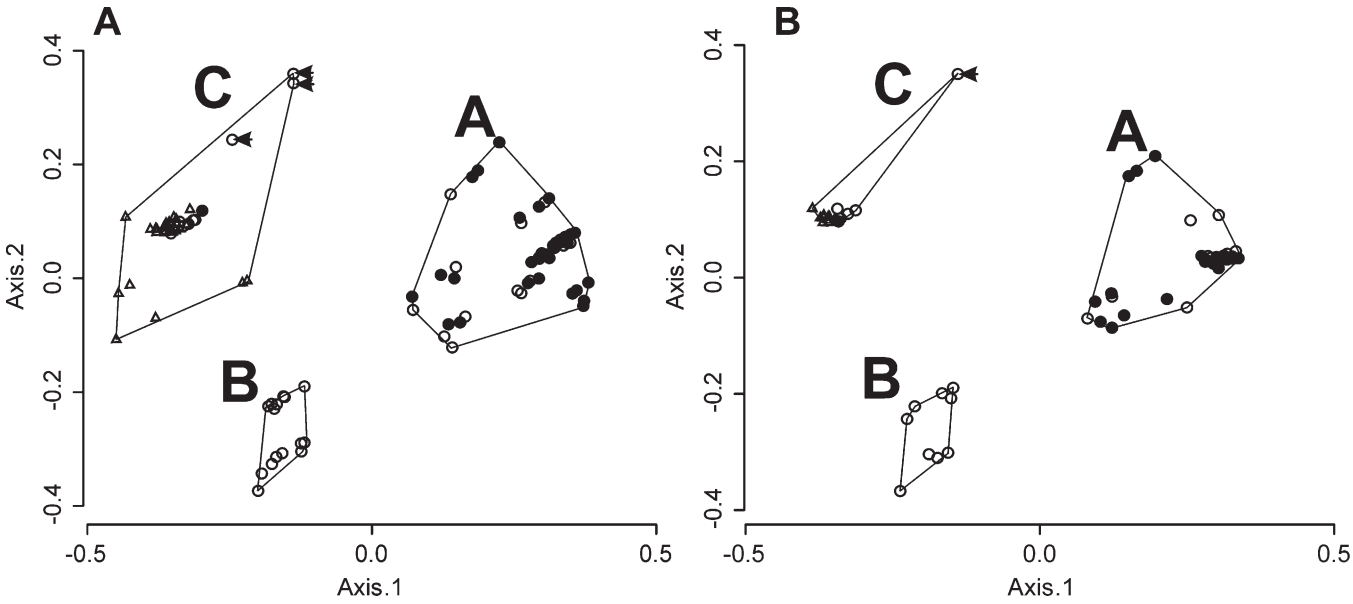

FIG. 4. Principal coordinates analysis (PCoA) for the specimens of the genus *Conopholis*. A. Plot of the first two axes following analyses utilizing observations and measurements made from the 'top' of the inflorescence on 103 specimens. PCoA axes 1 and 2 explain 14.33% and 4.21% of the variation, respectively. B. Plot of the first two axes following analyses utilizing observations and measurements made from the 'base' of the inflorescence on 59 specimens. PCoA axes 1 and 2 explain 25.47% and 7.52% of the variation, respectively. Major clusters recovered and discussed in this study are encased by convex hulls and labeled A–C. Triangles represent individuals traditionally identified as *C. americana*, open circles represent those of *C. alpina* var. *alpina*, while closed circles are those of *C. alpina* var. *mexicana*. Arrowheads highlight outliers in cluster C (see text for discussion).

original description, *C. panamensis* was said to differ from both *C. americana* and *C. mexicana* by its shallow, broadly obtuse lobed calyx. The broad bracts of *C. panamensis* were similar to those of *C. americana*, while its loss of style in fruit resembled that of *C. mexicana*. For this lineage to correspond with the description of *C. alpina* var. *alpina*, it would have to contain not only individuals from Costa Rica and Panama, but also all individuals occurring in southern Mexico. However, nine individuals from southern Mexico are confined to cluster C, therefore rendering *C. alpina* var. *alpina* polyphyletic. Along with these disjunct individuals from southern Mexico, cluster C contains all accessions of *C. americana* from eastern North America (and one member of *C. alpina* var. *mexicana* from Texas; see below). Members of cluster C can be distinguished by their obtuse bract that conceals the calyx, lack of pubescence along the margin of bracts, and acutely toothed calyx.

In his description, Haynes (1971) views eastern and western species as morphologically distinct, yet states that "*No single character can be relied upon to determine all specimens encountered*…" (p. 252). Haynes (1971) saw this as challenging, but implied that any given specimen can be placed to the correct taxon when several morphological features are considered in combination with geographic distribution, an extrinsic character. In light of this current morphometric study and previous molecular work, this problem he encountered can be explained by the fact that *C. alpina*, as he defined the species, is polyphyletic. Some *Conopholis* populations found in Mexico are actually disjunct members of *C. americana,* and hence do not belong to *C. alpina*, as solely expected by their geographic distribution. The persistence of both continuous and disjunct species distributions between Mexico and eastern North America are not uncommon. *Epifagus*, the monotypic sister genus to *Conopholis*, also exhibits this east-west disjunction. *Epifagus virginiana* (L.) W. P. C. Barton is predominantly found across eastern North America, but it does have small disjunct populations found in Mexico (Thieret 1969; Tsai and Manos 2010). Other examples of Mexican disjunct lineages include *Liquidambar styraciflua* L. (Graham 1973; Morris et al. 2008), *Nyssa sylvatica* Marshall (Miranda and Sharp 1950), *Fagus grandifolia* Ehrh. (Morris et al. 2010), and two members of the *Corallorhiza striata* species complex (*C. bentleyi* and *C. striata* var *involuta*; Barrett and Freudenstein 2009).

In addition to the delimitations of populations and lineages described above, there were three unsuspected features discovered as a result of this study. The first is the anomalous presence of a single individual from Jeff Davis County, Texas (*C. alpina* 1848190; Fig. 3), found to group with cluster C instead of with cluster A. This can be explained in one of two ways. There is the possibility this unusual result stems from a herbarium sheet that was mislabeled for the sampling locality. However, a more likely alternative is that this individual comes from an as yet undocumented, disjunct population of *C. americana* in Texas. Namely, we observed another specimen (*C. alpina* 1679772) from the same herbarium (US), collected by the same individual (Sperry) who collected *C. alpina* 1848190, and at the same locality in Texas (Jeff Davis County), but three years earlier (1936). This specimen could not be included in the morphometric analyses due to its deterioration and inability to sample at landmark locations ('top/base') along the inflorescence but it also appears to share general morphological features with *C. americana*. Taken together, these findings suggest the presence of another disjunct population of *C. americana* in southwestern Texas, analogous to those discovered in southern Mexico. All other samples in this study from Texas (and the rest of the southwestern U.S.A.) are found in cluster A.

Second, sampling of localities where the two varieties of *C. alpina* occur in sympatry (at and just south of the TMVB) indicates the presence of mixed populations, containing individuals from both clusters A and C (e.g. accessions *C. alpina*

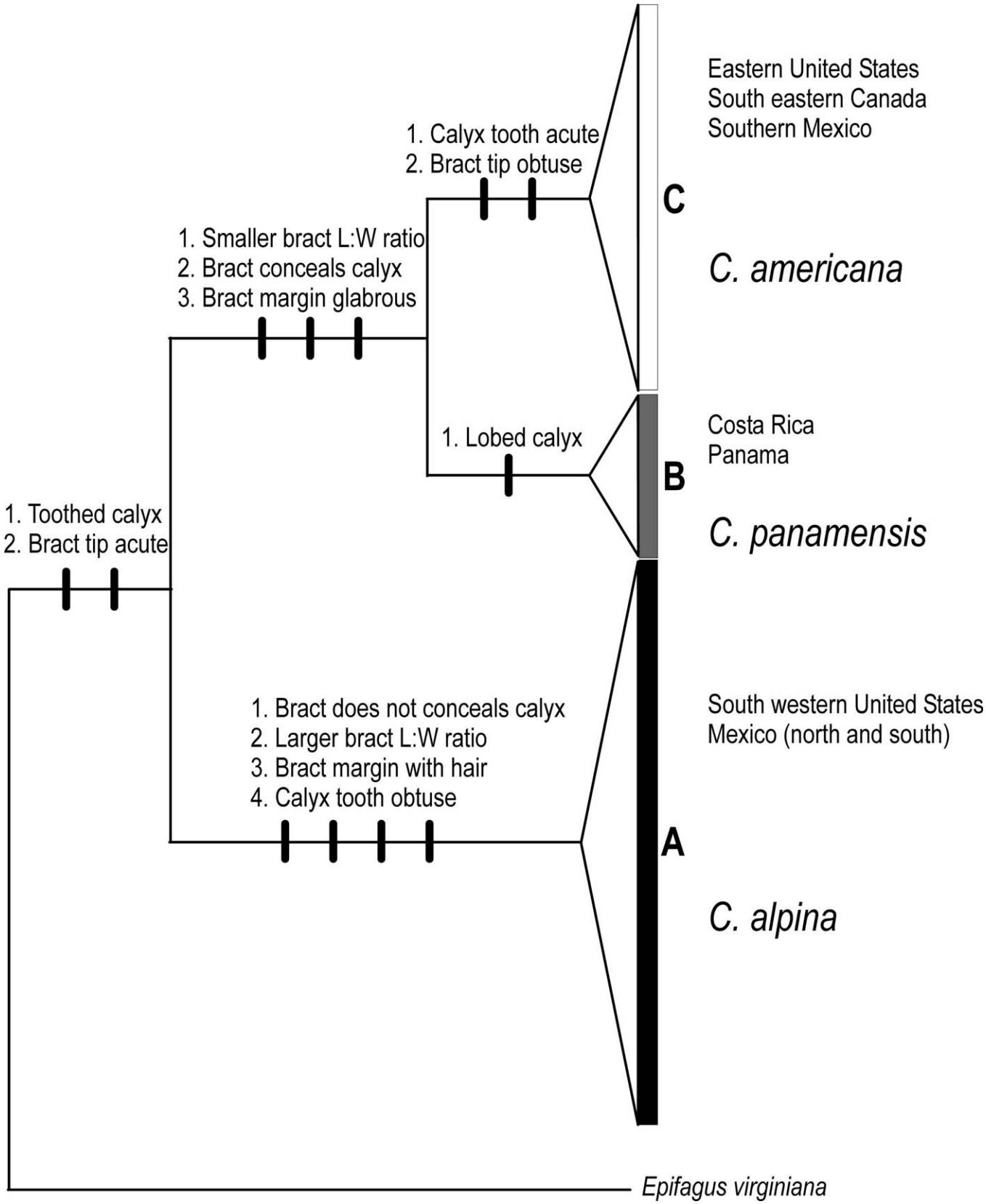

FIG. 5. Stylized phylogenetic tree, based on morphological and molecular data, showing the relationships between the three proposed species of *Conopholis*. Character state transformations for the morphological characters examined in this study are indicated above branches. Characters and character states are listed in Table 1.

1004045A and B, 559A and B, 4237A and B, and 34174B and C; Fig. 3). Referring in particular to these areas of overlap, Haynes (1971) stated that "*some specimens cannot confidently be placed into either taxon and for this reason I consider these two taxa to be varieties of one species.*" (p. 255). Members of populations that occur in southern Mexico, including the TMVB, are shown here to belong to two separate species, *C. alpina* and disjunct members of *C. americana*. In view of the fact that Haynes (1971) considered geographic distribution to be of overwhelming importance, he did not consider *C. americana* as a possibility. Instead he considered individuals from this region to be placed into one of the two varieties of *C. alpina* (var. *alpina* or var. *mexicana*).

Finally, two of these four mixed populations (i.e. *C. alpina* 559 and 4237) as well as one additional disjunct *C. americana* population/individual (specimen labeled as *C. alpina* 2321) exhibit mixed morphological characters, as evidenced by their outlier position within cluster C in the PCoA (Fig. 4; highlighted with arrowheads). These three samples have narrow bracts that do not entirely conceal their calyces, normally a diagnostic trait for *C. alpina*. Other than this feature, their remaining character states are all shared with *C. americana*. The discovery of populations from southern Mexico that have two species and show some individuals with intermediate morphology suggest the existence of hybrid swarms in zones of overlap. Individuals that possess this intermediate morphology may also have been another reason why Haynes (1971) was not able to confidently assign them to a particular taxon. To confirm whether hybridization is occuring between *C. alpina* and *C. americana*, further investigations involving multiple single or low copy nuclear genes, are required.

Taxonomic Treatment

Conopholis Wallroth, Carl Friedrich Wilheml. Orobanches Gen. Diask. 78.1825.—TYPE: *Conopholis americana* (L.) Wallr.

Low, glabrous, yellow, cream, yellow-brown, or brown simple herbs, fleshy at first but becoming brittle, flowering stems arising from a brown to black subterranean tubercle. Leaves scale-like, of 2 types, the lower very tightly imbricate and wide at base; the upper alternate, glandular pubescent or not along the margins, ovate to ovate-oblong or lanceolate to narolowly elongate triangular, widest at base, apex acute or obtuse. Inflorescence a compact raceme, each flower subtended by a bract, bract longer than the calyx and may or may not entirely conceal the calyx. Calyx irregular, tube cylindrical, 4- to 5-toothed or 2-lobed, teeth acute or obtuse to apiculate, lobes obtuse. Corolla cream colored, tubular, 2-lipped. Stamens 4, inserted above the ovary, exserted. Style apically reflexed, persistent with stigma on or deciduous from fruit. Fruit 2- halved, nonfleshy, brown to black capsule, ovoid, dehiscing regularly or irregularly. Seeds oval, triangular, rhomboidal, and quadrangular, brown to dark brown.

Key to Species of *Conopholis*

1. Bracts narrow, not concealing the calyx; bract margin glandular pubescent; calyx toothed and teeth obtuse; plants of southwestern U. S. A. and Mexico ..... *C. alpina*
1. Bracts wide, concealing the calyx; bract margin glabrous; calyx either lobed or toothed; plants of eastern North America, southern Mexico, Costa Rica and Panama 2
   2. Bract tips acute; calyx lobed (not toothed and lobes rounded); plants of Costa Rica and Panama ..... *C. panamensis*
   2. Bract tips obtuse; calyx toothed and teeth acute; plants of eastern North America and southern Mexico ..... *C. americana*

Conopholis americana (L.) Wallr., Orob. Gen. Diask. 78. 1825. *Orobanche americana* L. Mant. Pl. 88. 1767.—TYPE: U. S. A., Carolina. No date recorded. *Anon., s. n.* (lectotype: LINN scanned image!, designated by Haynes)

Conopholis alpina Liebm. var. *alpina* sensu R. R. Haynes pro parte (excluding type).

Stem erect, simple, glabrous, 6–20 cm tall; bracts glabrous along the margins, ovate to ovate-oblong, widest at the base, conceal calyx, 10.5–20 mm long, (2) 4–8 mm wide, apex obtuse; calyx irregular 4- to 5-toothed, tube cylindrical, teeth acute; corolla 8–14 mm long; filaments 6–10.5 mm long; anthers glabrous; style 5–13 mm long; capsule ovoid, 5–13 × 5.5–11 mm, style and stigma persistent; seeds irregularly oval, triangular, and quadrangular, 0.5–1.5 mm long.

Distribution and Ecology—Found parasitizing oaks (*Quercus* section *Lobatae*) in moist, deciduous, or mixed forests from central Florida west to Alabama, north to Wisconsin, west to Nova Scotia, central and southern Mexican states. In the eastern U. S. A., flowering mid-February in the south to mid-June in the north. Flowering in central and southern Mexico April to late July.

Conopholis panamensis Woodson, Ann. Missouri Bot. Gard. 25: 835–836, Fig. 2. 1935. *Conopholis alpina* var. *alpina* R. R. Haynes, SIDA 4 246–264. 1971. pro parte—TYPE: Panama, Chiriqui, Trail from Bambito to Cerro Punta, April 1937, *P. H. Allen 305.* (holotype: MO scanned image!; isotypes: F, MICH, MO, NY, US scanned images!).

Stem erect, simple, glabrous, 5–20 cm tall; bracts glabrous along the margins, ovate to ovate-oblong, widest at the base, concealing calyx, 11–22 mm long, 3.5–8.5 mm wide, apex obtuse; calyx irregular 2-lobed, tube cylindrical, lobes obtuse; corolla 12–16 mm long, filaments 10–15 mm long, anthers glabrous; style 10–14 mm long; capsule ovoid, 7–16 × 5.5–12 mm, style and stigma deciduous; seeds irregularly oval, triangular, and quadrangular, 0.3–1.5 mm long.

Distribution and Ecology—Found parasitizing oaks (*Quercus* spp.) in high elevation forests in Costa Rica and Panama. Flowering mid-December to May.

Conopholis alpina Liebm., Fohr, Skand. Naturf. Mode 4: 184. 1847.—TYPE: Mexico, Puebla, March 1841, *F. M. Liebmann 3719* (lectotype: C; isolectotype: F scanned image!, designated by R. R. Haynes).

Conopholis sylvatica Liebm., Fohr, Skand. Naturf. Mode 4: 185. 1847.—TYPE: Mexico, Vera Cruz, *Liebmann* s.n. (holotype: illustration s. n. no date, Mexico (C))

Conopholis alpina Liebm. var. *mexicana* (A. Gray ex S. Watson) R. R. Haynes Sida 3(5) 347 1969. *Conopholis mexicana*

A. Gray ex S. Watson, Proc. Amer. Acad. Arts 18: 131, 1883.—TYPE: Mexico, Coahuila, In the Sierra Madre, south of Saltillo, 1880, *Palmer 996*. (holotype: GH photographed image!, isotypes: F, NY, PH, US, VT, K scanned image!).

Conopholis alpina var. *alpina* sensu R. R. Haynes pro parte (excluding type)

Stem erect, simple, glabrous, 11–33 cm tall, bracts pubescent along margin, lanceolate or narrowly elongate triangular, widest at the base, not entirely concealing the calyx, 9–21 mm long, 2–5.5 mm wide, apex acute; calyx irregular 4- to 5-toothed, tube cylindrical, teeth less deeply toothed and obtuse to apiculate; corolla 14–20 mm long, filaments 7–12 mm long, anthers sparingly pilose; style 5–12 mm long; capsule ovoid, 8–15 × 6–12 mm, style and stigma deciduous; seeds irregularly oval, triangular, and quadrangular, 0.5–1.3 mm long.

Distribution and Ecology—Found parasitizing oaks (*Quercus* spp.) in oak woodlands and mixed montane forests in the Trans-Pecos area of Texas, through northern and central Arizona, and south to Oaxaca, Mexico. Flowering from mid-February to late-July.

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Literature Cited

Angiosperm Phylogeny Group. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 161: 105–121.

Barrett, C. F. and J. V. Freudenstein. 2009. Patterns of morphological and plastid DNA variation in the *Corallorhiza striata* species complex (Orchidaceae). *Systematic Botany* 34: 496–504.

Beck, G. 1930. Orobanchaceae. Pp. 1–348 in *Das Pflanzenreich. Regni Vegetabili Conspectus IV. 261*. Vol. 96, ed. A. Engler. Leipzig: Verlag von Wilhelm Engelmann.

Bennett, J. R. and S. Mathews. 2006. Phylogeny of the parasitic plant family Orobanchaceae inferred from phytochrome A. *American Journal of Botany* 93: 1039–1051.

Colwell, A. E. 1994. *Genome evolution in a non-photosynthetic plant*, Conopholis americana. Ph. D. Dissertation. St. Louis, Missouri: Washington University.

Fernald, M. I. 1950. *Grey's manual of botany*. Ed. 8. New York: American Book Company.

Gleason, H. A. 1952. *The new Britton and Brown illustrated flora of the northeastern United States and adjacent Canada*. Volume 3. Lancaster: Lancaster Press Incorporated.

Gower, J. C. 1971. A general coefficient of similarity and some of its properties. *Biometrics* 27: 857–871.

Graham, A. 1973. History of the arborescent temperate element in the northern Latin American Biota. Pp. 301–314 in *Vegetation and Vegetational History of Northern Latin America*, ed. A. Graham. New York: Elsevier Scientific Publishing Company.

Haynes, R. R. 1971. A monograph of the genus *Conopholis* (Orobanchaceae). *SIDA* 4: 246–264.

Liebmann, F. M. 1847. To nye arter af slaegten *Conopholis* Wallr. Pp. 184–186 in *Förhandlingar; Skandinaviske Naturforskeres Möte* 4. Stockholm: P. A. Norstedt and Soner, Kongl Boktryckare.

Linnaeus, C. 1767. Pp. 88–89 in part 1 of *Mantissa Plantarum*. Stockholm: Impensis direct. Laurentii Salvii.

Maechler, M., P. Rousseeuw, A. Struyf, M. Hubert, and K. Hornik. 2012. cluster: Cluster Analysis Basics and Extensions. R package version 1.14.3.

Manos, P. S., Z.-K. Zhou, and C. H. Cannon. 2001. Systematics of Fagaceae: Phylogenetic tests of reproductive trait evolution. *International Journal of Plant Sciences* 162: 1361–1379.

Meacham, C. A. and T. Duncan. 1991. MorphoSys. Berkeley, California: Regents of the University of California.

Miranda, F. and A. J. Sharp. 1950. Characteristics of the vegetation in certain temperate regions of eastern Mexico. *Ecology* 31: 313–333.

Morris, A. B., S. M. Ickert-bond, D. B. Brunson, D. E. Soltis, and P. S. Soltis. 2008. Phylogeographical structure and temporal complexity in the American sweetgum (*Liquidambar stryaciflua*; Altingiaceae). *Molecular Ecology* 17: 3889–3900.

Morris, A. B., C. H. Graham, D. E. Soltis, and P. S. Soltis. 2010. Reassessment of phylogeographical structure in eastern North American trees using Monmonier's algorithm and ecological niche modelling. *Journal of Biogeography* 37: 1657–1667.

Nickrent, D. L. 2012. The parasitic plant connection. Website http://www.parasiticplants.siu.edu/ (accessed June 3, 2012).

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. Henry, H. Stevens, and H. Wagner. 2013. vegan: Community Ecology Package. R package version 2.0–7. http://CRAN.R-project.org/package=vegan.

Olmstead, R. G., C. W. DePamphillis, A. D. Wolfe, N. D. Young, W. J. Elisons, and P. A. Reeves. 2001. Disintegration of the Scrophulariaceae. *American Journal of Botany* 88: 348–361.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* 20: 289–290.

R Core Team. 2012. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Rodrigues, A. G., A. E. L. Colwell, and S. Stefanović. 2011. Molecular systematics of the parasitic genus *Conopholis* (Orobanchaceae) inferred from plastid and nuclear sequences. *American Journal of Botany* 98: 896–908.

Rohlf, F. J. 1972. An empirical comparison of three ordination techniques in numerical taxonomy. *Systematic Zoology* 21: 271–280.

Small, J. K. 1933. *Manual of the southeasten flora*. Chapel Hill: University of North Carolina Press.

Sneath, P. H. A. and R. R. Sokal. 1973. *Numerical taxonomy: The principles and practice of numerical classification*. San Francisco: W. H. Freeman and Company.

Thieret, J. W. 1969. Notes on *Epifagus*. *Castanea* 34: 397–402.

Thiers, B. 2012. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/ (accessed November 2, 2012).

Tsai, Y. H. E. and P. S. Manos. 2010. Host density drives the postglacial migration of the tree parasite *Epifagus virginiana*. *Proceedings of the National Academy of Sciences USA* 107: 17035–17040.

Wallroth, K. F. W. 1825. Pp. 80 in *Orobanches Generis Diaskene*. Frankfurt: Francofurti ad Moenum.

Watson, S. 1883. Contributions to American botany. I. List of plants from southwestern Texas and northern Mexico. II. Gamopetalae to Acotyledons. *Proceedings of the American Academy of Arts and Sciences* 18: 96–191.

Woodson, R. E. and R. J. Seibert. 1938. Contributions towards a flora of Panama II. Miscellaneous collections during 1936–1938. *Annals of the Missouri Botanical Garden* 25: 823–840.

Young, N. D., K. E. Steiner, and C. W. DePamphilis. 1999. The evolution of parasitism in Scrophulariaceae/Orobanchaceae plastid gene sequences refute an evolutionary transition series. *Annals of the Missouri Botanical Garden* 86: 876–893.

Appendix 1. List of herbarium specimens examined for morphometric analyses of the genus Conopholis. Country, locality, collectors, and herbaria in which the specimens are deposited are provided for each individual. Entries follow the following format: Species name Authority: accession label, locality, voucher information (Herbarium acronym). Accession labels are the unique alphanumeric codes applied to the specimen indicated on dendrogram (see Fig. 3). Abbreviations of herbaria follow Index Herbariorum (Thiers 2012).

Conopholis alpina Liebm. var ***alpina*** sensu R. R. Haynes. **1820563**, PANAMA. Chiriquí: Boquete, *Davidson 399* (US); **1010416**, Chiriquí: Potrero, *Killip 3605* (US); **2490023**, Chiriquí: Boquete, *Stern 2033* (US); **577561**, COSTA RICA. Santa Rosa, *Pittier 12212* (US); **857**, San José:

Utley 857 (NY); **7506**, Canaan, *Burger 7506* (NY); **1252575**, San José: *Standley 42022* (US); **677512**, PANAMA. Chiriquí: Boquete, *Pittier 3122* (US); **1808147**, Chiriqui: *White 66 (*US); **1820828**, Chiriqui: *Davidson 956* (US); **3422**, Chiriqui: Jurutungo, *Aranda 3422* (NY); **3604**, Chiriqui: *Killip 3604* (NY); **3121**, Chiriquí: Boquete, *Pittier 3121* (NY); **880**, Chiriqui: Casita Alta, *Woodson 880* (NY); **305**, Chiriqui: Cerro Punta, *Allen 305* (NY); **215767A**, MEXICO. Oaxaca: Sierra De San Felipe, *Pringle 4676* (US); **215767B**, Oaxaca: Sierra De San Felipe, *Pringle 4676* (US); **4676A**, Oaxaca: Sierra De San Felipe, *Pringle 4677* (US); **2613C**, Oaxaca: Cerro de San Felipe, *Camp 2613* (NY); **1424**, Oaxaca: Ixtlan, *Mickel 1424* (NY); **28456B**, Oaxaca: *Matuda 28456* (NY); **2613B**, Oaxaca: Cerro de San Felipe, *Camp 2613* (NY); **2321A**, Distrito Federal: *Fryxell 2321* (NY); **2321B**, Distrito Federal: *Fryxell 2321* (NY); **34174B**, Distrito Federal: *Davidse 34174* (NY); **34174C**, Distrito Federal: *Davidse 34174* (NY); **461750**, Distrito Federal: *Pringle 13153* (US); **1358**, Distrito Federal: *Ventura 1358* (NY); **840015**, Veracruz: Santiago Tuxtla, *Brandegee 1851* (US); **464217A**, Puebla: *Arsine 1062* (US); **2923296**, Veracruz: *Ventura 4913* (US); **1003322A**, Puebla: *Arsene 1062* (US); **1004044B**, Puebla: *Arsene 1004044* (US); **1003324**, Michoacán: Morelia, *Arsene 5229* (US); **1004045A**, Puebla: Manzanilla, *Nicolas 109* (US); **1004045B**, Puebla: Manzanilla, *Nicolas 109* (US); **4237A**, Michoacán: Tingambato, *Steinmann 4237* (NY); **4237B**, Michoacán: Tingambato, *Steinmann 4237* (NY); **559A**, Hidalgo: *Galvan 559* (NY); **559B**, Hidalgo: *Galvan 559* (NY).

Conopholis alpina Liebm. var. ***mexicana*** (A. Gray ex S. Watson) R. R. Haynes. **337652**, U. S. A. New Mexico: Hidalgo Co., *Turner 97–90* (ARIZ); **21769**, MEXICO. Chihuahua: Majalca, *Correll 21769* (NY); **8028**, Chihuahua: Ocampo, *Spellenberg 8028* (NY); **313673**, Chihuahua: Temosachi, *Laferriere 355* (ARIZ); **3194**, U. S. A. Texas: Brewster Co., *Moore 3194* (NY); **203**, MEXICO. Durango: San Ramon, *Palmer 203* (NY); **98–628**, Sonora: Yecora, *Van Devender 98–628* (NY); **497910**, U. S. A. New Mexico: *Metcalfe 1022* (US); **495338**, New Mexico: Socorro Co., *Metcalfe 241* (US); **34379**, Texas: Jeff Davis Co., *Palmer 34379* (NY); **693**, MEXICO. San Luis Potosí: *Parry 693* (NY); **589**, San Luis Potosí: Sierra De Alvarez, *Palmer 589* (NY); **244914**, Nuevo León: Cola De Caballo, *Yatskievych 83–81* (ARIZ); **737255**, U. S. A. New Mexico: Albuquerque, *Herrick 262* (US); **662474**, New Mexico: Bernalillo Co., *Ellis 48* (US); **1735136**, New Mexico: Grant Co., *Studhalter S3000* (US); **1221674**, New Mexico: Hidalgo Co., *Lee 161* (US); **737065**, New Mexico: Dona Ana Co., *Wooton 737065* (US); **1739221**, Arizona: Gila Co., *Peebles 13272* (US); **1439044**; Arizona: Cochise Co., *Peebles 5862* (US); **1435056**, Arizona: Cochise Co., *Peebles 5387* (US); **332**, Arizona: Greenlee Co., *Rusby 332* (NY); **1367618**, Arizona: Graham Co., *Peebles 4404* (US); **1679772**, Texas: Jeff Davis Co., *Warnock T97* (US); **1848190**, Texas: Jeff Davis Co., *Sperry T744* (US); **661869**, Arizona: Cochise Co., *Gooding 1048* (US); **1286291**; Texas: Jeff Davis Co., *Orcutt 1085* (US); **10**, MEXICO. Nuevo León: San Isidro, *Fryxell 10* (NY); **22105**, Nuevo León: Galeana, *Henrickson 22105* (NY); **147282**, Nuevo León: *Pringle 13746* (ARIZ); **007126**, Coahuila: Cuatro Cienegas, *Henrickson 16000* (NMC); **190111**, Coahuila: Cuatro Cienegas, *Pinkava 10472* (ARIZ); **P6111**, Coahuila: Sierra De San Marcos, *Pinkava P-6111* (NY); **16001**, Coahuila: Canon Desiderio, *Henrickson 16001* (NY); **00105228A**, Coahuila: *Johnston 10824* (TEX/LL); **00105228B**, Coahuila: *Johnston 10824* (TEX/LL); **00105228C**, Coahuila: *Johnston 10824* (TEX/LL); **85**, Durango: Santiago Papasquiaro, *Palmer 85* (NY).

Conopholis americana (L.) Wallr. **SS0330**, U. S. A. Indiana: Monroe Co., *Stefanović SS.03.30* (TRTE); **SS04102**, Indiana: Monroe Co., Bloomington, *Stefanović SS.04.102* (TRTE); **SS0489**, Indiana: Martin Co., *Stefanović SS.04.89* (TRTE); **SS0480**, Indiana: Lawrence Co., *Stefanović SS.04.80* (TRTE); **SS0494**, Indiana: Crawford Co., *Stefanović SS.04.94* (TRTE); **SS0331**, Indiana: Monroe Co., Hickory Ridge Lookout, *Stefanović SS.03.31* (TRTE); **SS0493**, Indiana: Crawford Co., *Stefanović SS.04.93* (TRTE); **SS0311**, Kentucky: McCreary Co., Gulf Bottom Trail, *Stefanović SS.03.11* (TRTE); **SS0329**, Indiana: Monroe Co., *Stefanović SS.03.29* (TRTE); **SS0472**, West Virginia: Kanawha Co., *Stefanović SS.04.72* (TRTE); **SS0483**, Indiana: Perry Co., German Ridge, *Stefanović SS.04.83* (TRTE); **SS04109**, Indiana: Monroe Co., Bloomington, *Stefanović SS.04.109* (TRTE); **SS06127**, Tennessee: Blount Co., Sugarlands Valley, *Stefanović SS.06.127* (TRTE); **SS0932**, Michigan: Muskegan Co., *Stefanović SS.09.32* (TRTE); **SS06146A**, North Carolina: Swain Co., *Stefanović SS.06.146A* (TRTE); **SS06146B**, North Carolina: Swain Co., *Stefanović SS.06.146B* (TRTE); **SS1005**, CANADA. Quebec: Gatineau Park, *Stefanović SS.10.05* (TRTE); **SS06170**, *Stefanović SS.06.170* (TRTE), Halton Co., Ontario, Canada; **SS0925**, Ontario: Summit Co., *Stefanović SS.09.25* (TRTE); **SS0471**, U. S. A. West Virginia: Kanawha Co., *Stefanović SS.04.71* (TRTE); **SS0908**, CANADA. Ontario: Niagara Co., *Stefanović SS.09.08* (TRTE); **SS0931**, U. S. A. Michigan: Allegan Co., *Stefanović SS.09.31* (TRTE); **SS05001B**, Alabama: Lee Co., *Stefanović SS.05.001B* (TRTE); **SS05001E**, Alabama: Lee Co., *Stefanović SS.05.001E* (TRTE); **SS06160A**, North Carolina: Jackson Co., *Stefanović SS.06.160A* (TRTE); **SS06160B**, North Carolina: Jackson Co., *Stefanović SS.06.160B* (TRTE); **SS06133A**, Tennessee: Blount Co., *Stefanović SS.06.133A* (TRTE).