The Systematics of *Monotropis* (Ericaceae)

Thesis

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By

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Abstract

Monotropsis Schweinitz is a genus of leafless, achlorophyllous, mycoheterotrophic plants in the subfamily Monotropoideae of the blueberry family, Ericaceae. The Monotropoideae is small, comprised of 14 species and 10 genera, collectively referred to as the monotropoids. The monotropoids are currently viewed as a monophyletic group, embedded deeply within the Ericaceae and most closely related to the woody subfamily Arbutoideae. Monotropsis is one of the more uncommonly encountered monotropoids. The genus in currently monotypic and occurs in mixed woods in the Appalachian Mountains from West Virginia south and west to Alabama, on the Coastal Plain in Virginia, and as a disjunct in northern peninsular Florida.

Despite containing only one species as currently circumscribed, four species have been described in the genus; all treated as synonyms of the type species Monotropsis odorata. Monotropsis reynoldsiae, found exclusively in Florida, was distinguished from M. odorata based on several characters including flowering period, flower color, and its sepal length to width ratio. Monotropsis lehmaniae was distinguished based in part on flowering period, sepal color, and its sepal length to width ratio. Another genus and species, Cryptophila pudica, was distinguished by fruit type and internal floral anatomy. All species were placed as synonyms of Monotropsis odorata based on supposed variability or
misinterpretation of all distinguishing features. This study reevaluated the status of all synonyms of *Monotropsis odorata* using a holistic approach to species delimitation emphasizing quantitative differences. The study incorporated four major lines of evidence: phenology, morphology (including anatomy), geography, and DNA.

Phenological, morphological, and geographical differences between the described species were assessed by examining over 200 herbarium specimens, supplemented with collections from 16 populations made by the author and others.

Phenological data, as measured by the percent of specimens in a particular phenological condition per month of collection, shows that *Monotropsis lehmaniae* does not have a flowering period distinct from that of *M. odorata*, while *M. reynoldsiae* does.

The morphological data were scored as quantitative measures. The data as viewed using Principle Components Analysis, box plots, and scatter plots shows *Monotropsis reynoldsiae* forming a distinct cluster relative to all specimens of *Monotropsis odorata*. Further, box plots and scatter plots indicate that the absolute size of many organs differs between the two species. Therefore, two species within *Monotropsis* are distinguishable based on organ size.

Anatomical data were collected by making thin sections of paraffin-embedded flowers. Evidence supports the results of the phenological and morphometric data. *Monotropsis lehmaniae* represents immature individuals of
*M. odorata*, while *M. reynoldsiae* is distinguishable based on the presence of an epidermal layer impregnated with a substance which stains positively in Safranin.

The geographical evidence supports two units separated by a wide geographic space. One is found in the Appalachians and another in Florida. These units correspond to *Monotropsis odorata* and *M. reynoldsiae*, respectively.

Lastly, the DNA data evidence was gathered using material from the 16 collections made by the author, plus one additional collection. DNA was extracted from all accessions and three loci examined. Two nuclear loci *(ITS/26S and Xdh 1296-1869)* and one chloroplast locus *(rpl32-trnL)* were sequenced. These were analyzed separately and together using the Maximum Parsimony optimality criterion. Support values using jackknife resampling and branch lengths were plotted onto the strict consensus tree. The results for using all loci were uninformative *(Xdh 1296-1869)* or gave two well-supported (greater than 90 percent support) clades *(ITS/26S and rpl32-trnL)*. These clades were supported by a total difference of six base pairs and corresponded to *Monotropsis odorata* or *M. reynoldsiae*, supporting their recognition as two distinct species.

All results support the recognition of two species of *Monotropsis*: *M. odorata* and *M. reynoldsiae*. The taxonomic history and ecology of these species is also summarized.
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Chapter 1: Phylogenetic Placement and Taxonomic History of *Monotropis*

*Monotropis* Schweinitz is a genus of leafless, achlorophyllous plants belonging to the subfamily Monotropoideae of the Ericaceae (the monotropoids). *Monotropis* is endemic to the southeastern United States, and is found in the Appalachian Mountains, including the Piedmont, Blue Ridge, and Cumberland Mountains, the Coastal Plain of Virginia, and in Florida. The Monotropoideae is small, comprised of only 10 genera and 14 species (Wallace 1975a, 1975b, 1987). These genera are for the most part quite distinct from one another.

**Evolution of the monotropoids**

The phylogenetic placement of the monotropoids has long been contentious. At various times they have been placed in the Ericaceae, the Pyrolaceae, or their own family, the Monotropaceae. Recognition of the Monotropaceae was based primarily on its achlorophyllous habit and stamen morphology (Wallace 1975a, Cronquist 1981). However, Copeland (1941) and Wallace (1975a) presented convincing arguments for the recognition of the Monotropaceae as a subfamily within the Ericaceae, noting in particular the
strong similarity of *Pterospora andromeda* Nutt. to the Arbutoideae of the Ericaceae because of its distinctly urceolate corolla and appendaged anthers. Cladistic analyses using DNA and morphology have supported the subordination of the Monotropaceae within the Ericaceae (Cullings 1994, Kron et al. 2002). Furthermore, these studies have confirmed that the subfamily is sister to the Arbutoideae (Cullings and Bruns 1992, Freudenstein & Broe, unpubl.).

The monotropoids were long thought to be saprophytic either because they are achlorophyllous and colored similarly to mushrooms or because the fungal hyphae with which their roots are associated were assumed to be found with the plants merely because of their similar ecologies. However, anatomical and ecological studies (MacDougal and Lloyd 1900; Bjorkman 1960, Furman and Trappe 1971) have shown that fungal hyphae penetrate the roots and cell walls. These studies have convincingly demonstrated that the monotropoids are mycoheterotrophic (Bidartondo 2005). In fact, no plants are known to be saprophytic; they are either mycoheterotrophic or directly parasitic on the root systems of other plants (i.e. the Rafflesiaceae and Orobanchaceae).

The species within the Monotropoideae have long been known to science, with some described as early as 1753 in Linnaeus’s *Species Plantarum*, but many species are much less familiar. Some of these lesser-known species, such as *Cheilotheca*, are only documented in one or a handful of collections (c.f. Wallace 1975b). Infrequency of collection may be due in part to their mycoheterotrophic habit and associated infrequency of emergence as opposed
to true rarity since plants only appear above ground to flower. The emergence of inflorescences may be limited by fungal nutrient availability and, indirectly, by the nutrient production of the trees with which the fungal hosts are associated. The amount of nutrients available to the parasite is likely determined by how well the indirect host (the tree species) fared several years before.

Recent studies have shown that the monotropoids utilize fungal hosts from a broad phylogenetic spectrum (Bidartondo and Bruns 2001), but that within a species specific fungal groups are targeted, usually a single species complex (Cullings et al. 1996; Bidartondo and Bruns 2002). The fungal species utilized by the host may be even more specific at the local level (Kretzer et al. 2000). Fungal symbionts are not shared between species, even sibling species (Bidartondo and Bruns 2001). In the case of Monotropis, species in the genus Hydnellum have been identified as the fungus parasitized (Bidartondo and Bruns 2001).

In parasitic plants, complex signaling is involved in obtaining a viable connection with the host plant and the host must not be able to recognize the parasite as such, so there should be strong selection for host recognition and avoidance of detection as a parasite (Bruns and Read 2000, Bruns 2005, Cardoso et al. 2011). Bidartondo and Bruns (2002) suggested that recognition of the monotropoid as parasite by the host may fuel a host shift. Therefore, host shift may be an important speciation mechanism within the subfamily.
Several species (or species complexes) within the Monotropoideae are common and widespread such as *Monotropa hypopitys* and *M. uniflora*. However, others are extremely rare and may be extirpated such as the tropical Asian genus *Cheilotheca*. *Monotropsis* appears to be intermediate in rarity. It is not a common plant, but the genus is represented by at least a few collections in all major herbaria in the eastern United States and may even be locally common throughout its range (Plitt 1909; Baldwin 1957). The generally uncommon occurrence of the monotropoids may be attributed to their mycoheterotrophic habitat, as discussed above, as the distribution of the plant is presumably determined by the distribution of its host fungus. Given the specificity of their symbiosis and the fact that complex interactions must be successful for development, it is not surprising that the monotropoids are uncommon.

### Phylogenetic placement of *Monotropsis*

The phylogenetic position of *Monotropsis* within the monotropoids is unclear. The first person to conduct a detailed study of the subfamily was H.F. Copeland in the 1930’s and 1940’s. From his anatomical observations he concluded that *Monotropsis* was clearly a member of a clade composed of *Hemitomes*, *Pityopus*, *Hypopitys* (*Monotropa h.*), and *Monotropa*. Within this clade, Copeland (1941) believed that *Monotropsis* was most closely related to *Monotropa hypopitys*. Takahashi (1987), in a palynological study of the
monotropoids, supported Copeland’s conclusions but found that the pollen of
Monotropis was most similar to that of Hemitomes congestum Gray in being 2-
colporate. However, he dismissed the similarity in morphology as “superficial” (p.
398). New World Monotropa hypopitys was also found to have 2-coporate
pollen, which points to a potentially close relationship between this species and
Monotropis in light of Copeland’s findings.

There have been four cladistic analyses published which have sought to
uncover the phylogenetic history of the Monotropoideae. Those of Cullings
(1994, 1996) presented a paraphyletic Monotropoideae in which Monotropis
represents a second derivation of achlorophyllous plants within the
Vaccinioideae. This is not only interesting in that it would be a second derivation
of mycoheterotrophy in the Ericaceae, but that if true, there would also have
been a large amount of morphological change on this branch as the gynoecium
of Monotropis is hypogynous while that of Vaccinium is epigynous, at least in
temperate regions.

Cullings (1994) stated that this second derivation was supported by root
morphology, as Copeland (1939) noted patchy hyphal covering over the roots,
which is atypical for the other monotropoids but similar to that of members of the
Vaccinioideae. However, Cullings presented cladograms with low support values
for taxa outside the traditionally recognized Monotropoideae although
Monotropis is strongly supported as sister to Vaccinium in these studies. In
addition, the ingroup sampling was low, with one representative for each species, including *Monotropsis*.

Conversely, the studies of the relationships within *Monotropa uniflora* in Neyland and Hennigan (2004) and those within *Pityopus californicus* in Neyland (2005) suggested that *Monotropsis* is embedded within the Monotropoideae. *Monotropsis* forms a clade with *Monotropa uniflora, M. hypopitys, Monotropastrum, Pityopus, and Allotropa*. This clade is well supported (bootstrap value of 93) and is sister to *Pleuricospora*.

The study of Bidartondo and Bruns (2001) is the largest published to date on the Monotropoids in terms of intra- and inter-specific taxon sampling. Two plant phylogenies were presented in it, one using the locus *rps2* (ribosomal protein 2) and the other using ITS + 28S. In the *rps2* tree, *Monotropsis* was sister to *Allotropa*, with this clade sister to a *Pityopus*/North American *Monotropa hypopitys* clade. In the ITS tree it was sister to a clade comprised of *Monotropa hypopitys, Pityopus, Allotropa, and Hemitomes*, with this whole clade (*Monotropsis* + others) sister to *Monotropa uniflora* and *Monotropastrum*. While the authors concluded that the two dendrograms are incongruent, both showed *Monotropsis* deeply embedded within the monotropoids, contrary to the assertion of Cullings (1994). Recent observations (Broe and Freudenstein unpubl.) suggest that there are likely paralogous copies of *rps2* in *Monotropa hypopitys*, which is not surprising given the achlorophyllous habit of these plants and associated generation of pseudogenes, loss and/or duplication of chloroplast
genes. Therefore, the ITS phylogeny is likely closer to reality than the rps2 phylogeny.

Unpublished data of Broe and Freudenstein using an even larger sample of monotropoid taxa than that in Bidartondo and Bruns (2001) found that *Monotropsis* is sister to *Monotropa uniflora* for ITS/26S. It seems clear from a summary of the currently available morphological and molecular data that *Monotropsis* is likely most closely related to *Monotropa uniflora* as supported by Broe and Freudenstein, Copeland, Bidartondo and Bruns, and Neyland and Hennigan.

**Taxonomic History of Monotropsis**

Within *Monotropsis*, four taxonomic entities have been recognized. However, only three of these units have any semblance of being distinct taxa, though the fourth will be discussed briefly.

*Monotropsis* was first described by the famous mycologist and cyperologist Lewis David von Schweinitz in Stephen Elliott’s *A Sketch of the Botany of South Carolina and Georgia* (Elliott 1817), and was so named based on its resemblance to *Monotropa*, the only other monotropoid genus described at the time (likely *M. hypopitys* since the inflorescence is also a raceme). In the original description, *Monotropsis* was described as flowering in spring, being noticeably fragrant (smelling of violets), having sepals about as long as the
petals, and having a maroon corolla. Because of the fragrance of the plant, it was given the specific epithet _odorata_. In the same publication, Elliot proposed the generic name _Schweinitzia_ instead in commemoration of Schweinitz as the collector.

Asa Gray (1885) distinguished _Monotropsis reynoldsiae_ (as _Schweinitzia r._) from _M. odorata_ s.s. by its winter flowering period, white corolla, more slender habit, and sepals more narrow and half as long as the corolla lobes.

_Monotropsis lehmaniae_ was described by Stuart Burnham in 1906. He differentiated this species from _M. odorata_ s.s. based on the overall color of the plant (purple as opposed to brown), its fall-flowering habit, lack of fragrance, sepals much longer than the petals, and deeper corolla lobes.

The last (and most dubiously distinct) taxon synonymized with _Monotropsis odorata_ is _Cryptophila pudica_ (Wolf 1922). This material was described as an entirely new genus based on supposed differences in placentation, locule number, and fruit type despite being identical to _Monotropsis odorata_ s.s. in gross morphological features. The species soon faded into taxonomic obscurity as it was quickly synonymized by Small (1933). A summary of the key characters used to differentiate the first three putative species is presented in Table 1.

The only monographic treatment of _Monotropsis_ is that of Wallace (1975b). In this publication, as in his treatment for the _Flora of North America_ series (2009), Wallace recognized _Monotropsis_ as a monotypic genus. Previous
floristic works such as those of Small (1914, 1933), Spawn (1940), and Gleason (1952) recognized three species within the genus. It is unclear how critical these authors were in assessing variation within the genus as opposed to merely compiling a list of currently recognized species. However, prior to Wallace (1975b) Wood (1961) also raised doubts as to whether several species existed within *Monotropis*. Wallace placed *Monotropis reynoldsiae* as well as *M. lehmaniae* and *Cryptophila pudica* as synonyms of the type species, *Monotropis odorata* Schweinitz.

*M. reynoldisae* was placed into synonymy by Wallace because of an apparent overlap in all of the characters used to differentiate it from *M. odorata* s.s. Wallace (1975b) stated that a range in character states “may be seen on the type specimen of *S. [M.] reynoldsiae,*” (p. 53) and reiterated this assertion in his treatment for the *Flora of North America* (Wallace 2009).

Wallace placed *Monotropis lehmaniae* into synonymy because he believed that it was merely a seasonal phase of *M. odorata*. This synonymy is supported by the observations of previous botanists. Baldwin (1957) used the notes of Wolf (1922) as well as personal collections and field observations to conclude that Burnham “based his new species on immature plants of *M. odorata*, and the characters he used to separate the plants change with the ontogeny of the individual…” (p. 260). Ahles (1967) considered *Monotropis lehmaniae* simply to be inflorescences of *M. odorata* that had been exposed above the leaf litter, only blooming in rare circumstances when conditions were
ideal. These aberrant individuals were not given any taxonomic status. The arguments presented by Baldwin and Ahles were summarized by Wilbur (1970).

The distinctness of Cryptophila pudica was almost instantly dismissed by botanists (Small 1933) and it was subsumed under M. odorata s.s. Wolf described it as a distinct genus based on imperfect descriptions of Monotropsis in the extant literature. Earlier authors such as Gray (1846) either misinterpreted the anatomy of Monotropsis or were not clear enough in their descriptions. Gray assumed that because all monotropoids known at the time had capsular fruit, Monotropsis did as well. Gray, in addition to other authors, stated that Monotropsis had a 5-lobed ovary and axile placentation. However, Wallace (1975b) clarified that the fruit is indeed a berry. Olson (1994) demonstrated that the ovary of Monotropsis odorata was five celled at the apex but became more or less one-celled at the base. Olson (1994) and Wallace (2009) stated that the placentation is intruded-parietal, which can easily be confused with axile placentation. Therefore, Monotropsis odorata is actually identical to Cryptophila pudica in the areas where they were described as differing.

While the brief discussion by Wood (1961) and the more detailed observations of Wallace (1975b) have resulted in the recognition of Monotropsis as monotypic, lingering questions remain as to how many taxonomic entities exist within Monotropsis. These questions primarily concern populations assignable to M. reynoldsiae, which are endemic to Florida. Certainly the presence of a highly endemic species in Florida would not be surprising (cf. Estill
and Cruzan 2001). Indeed, some floristic treatments such as Wunderlin (1998) have considered *M. reynoldsiae* to be a distinct species. Images such as those presented in Weakley (2009) support the segregation of this taxon from *M. odorata* based on gross morphology, but each figure could obviously represent extremes in a continuum of variation, as stated by Wallace (1975b). The decision by Wunderlin to recognize *M. reynoldsiae* as a distinct species was apparently not made in association with a critical analysis of variability within the genus.

Having been described by Gray in 1885, *M. reynoldsiae* was only recognized from the type locality along the Indian River near St. Augustine, Florida, and one other locality also near St. Augustine (Gray 1885, Wallace 1975b). Since then, however, populations have been discovered at a few other localities in the northern peninsular region of the state, from the coast along the Atlantic Ocean to the coast along the Gulf of Mexico. Nevertheless, the occurrence of the genus in Florida appears to be less frequent than in the Appalachian Mountains and it is currently listed as endangered in the state (Ward *et al.* 2003). In addition, the ecology of *Monotropis reynoldsiae* appears to be strikingly different than that of the other described species. *M. odorata* and *M. lehmaniæ* are found in identical habitats: rich, well-drained mixed pine-oak woodlands in the Appalachian Mountains and along the Coastal Plain in Virginia, whereas *M. reynoldsiae* is found in scrub oak thickets and hammocks in Florida. Geographically, the closest populations of *M. odorata* s.s. to those of *M.*
Monotropsis reynoldsiae are found in Northern Georgia. Recognition of what has been called *Monotropsis reynoldsiae* as either a distinct species or variety of *M. odorata* will likely require that action be taken to protect the few extant populations, including floristic work to determine how common the species actually is. This is discussed in slightly more detail in a popular article by Weakley (2009).

*Monotropsis lehmaniae*, while presumed to be a seasonal form of *M. odorata* s.s. similar to the red and yellow color forms of the closely related *Monotropa hypopitys* L., could conceivably be distinct species. These color forms of *Monotropa hypopitys* have been described as distinct species in the past (Wallace 1975b, Neyland 2004, Klooster and Culley 2010). The yellow form of *Monotropa hypopitys* ceases flowering about a month earlier than the beginning of flowering for the red form, which suggests reproductive isolation, at least in some parts of the eastern United States (Klooster and Culley 2009). However, in the case of *M. lehmaniae*, there is a much greater difference in phenology between this species and *M. odorata* s.s. (fall versus spring, a total period of six to seven months). For *Monotropa hypopitys* there may be some regions not studied by Klooster and Culley in which the time of anthesis overlaps between the red and yellow color forms, thus permitting gene flow. Wallace (1975b) noted this in his taxonomic treatment of the species. Investigations into possible genetic differences between these color forms have been conducted by Neyland (2004) and Klooster and Culley (2010). While Neyland found that the red and yellow color forms did not form distinct clades within *Monotropa*
*hypopitys*, Klooster and Culley, using microsatellite data found that the red and yellow color forms were genetically distinct from one another, even when the color forms were in sympatry. Given the similarity of flowering times for both color forms of *M. hypopitys* and the possibility of genetic differentiation associated with the morphological color differentiation, it seems plausible that *Monotropis lehmaniae* may be very distinct from *M. odorata*.

All three of the described species within *Monotropis* appear at first glance to differ significantly enough to warrant recognition on the species level. The possibility of a fourth taxon (*Cryptophila pudica*) also exists, but it is doubtfully distinct in light of the anatomical findings of Olson (1994). Of particular concern are the assertions of Wallace (1975b, 2009) that there is a significant range in overlap between *M. reynoldsiae* and *M. odorata* s.s. This claim appears to be completely qualitative in nature, as no numeric values are given to demonstrate any overlap in character range. Due to this lack of quantitative data, it seems possible that close study in both the herbarium and field could reveal the existence of several species within *Monotropis*.

This study seeks to answer the question of how many species exist within *Monotropis* in several ways by taking an integrative approach to species delimitation similar to that used by Barrett and Freudenstein (2011). Yang and Rannala (2010) argue for this pluralistic approach to delimiting species, stating that multiple lines of evidence should be used “at a minimum” (p. 9269). In this
study, four lines of evidence were used: morphological, molecular, phenological, and geographical.

Attempts to discover distinct clusters corresponding to described taxa were carried out in the following ways. First, phenological data was collected in order to see how distinct flowering periods were between described species in order to assess the probability of sexual reproduction between segregates. Second, morphological variation was assessed in order to see if any of the segregates of *Monotropsis* formed distinct clusters and, if so, if they differed from each other in such a way that these characters had little overlap and were consistent within previously described taxa. Third, molecular data were used to see if previously described taxa were at all variable, and if these taxa formed genealogically exclusive groups. Lastly, the geographical range of individuals and how this corresponded (if at all) to any morphological or molecular distinctness was assessed.
Table 1: Key characters differentiating described species of *Monotropsis*. Because of its very dubious taxonomic status *Cryptophila pudica* is excluded (see text).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Bracts</th>
<th>Corolla</th>
<th>Calyx</th>
<th>Perianth Ratio</th>
<th>Habit</th>
<th>Ecology</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. odorata</em> s.s.</td>
<td>chaffy; brown/tawny</td>
<td>pink; lobes shorter than tube</td>
<td>broad-ovate; 8-12 mm</td>
<td>Calyx as long as corolla</td>
<td>imbricate flowers</td>
<td>mixed uplands; fragrant, flr. Feb.-May</td>
</tr>
<tr>
<td><em>M. lehmaniae</em></td>
<td>fleshy; lavender</td>
<td>pink; lobes as long as tube</td>
<td>oblong; 7-8 mm</td>
<td>Calyx twice as long as corolla</td>
<td>imbricate flowers</td>
<td>mixed uplands, not fragrant, flr. Sep.-Nov.</td>
</tr>
<tr>
<td><em>M. renolydsiae</em></td>
<td>?</td>
<td>white; lobes shorter than tube</td>
<td>ovate-lanceolate; 2-4 mm</td>
<td>Calyx half as long as corolla</td>
<td>flowers not very imbricate</td>
<td>scrub oak thickets; usually fragrant, flr. Nov.-Dec.</td>
</tr>
</tbody>
</table>
Chapter 2: Morphological and Phenological Variation Within Monotropsis

Species within Monotropsis have been recognized both on the basis of morphological and phenological differences. However, given that Wallace (1975b) did not quantitatively assess the distinctness of groupings based on these traits, a key objective of this study was to do so. Several methods were employed to accomplish this, including morphometric analysis to: 1) find new morphological differences between putative taxa and 2) assess the stability of characters which have historically been used to differentiate the taxa, and 3) provide hard data on any differences in flowering period.

Methods and Materials

Morphometrics

Sampling: All members of the Monotropoideae are fleshy, so herbarium specimens are generally not preserved well. Characters may be lost or distorted by the drying process. For example, specimens of Monotropa uniflora press almost completely flat and blacken upon drying. Unless noted on the label, the color of the live plants is unknown. This complicates monographic work as color
has often been used to differentiate between monotropoid taxa (e.g. red or yellow color forms of *M. uniflora* or *M. hypopitys*). While the example of color change in *M. uniflora* is an extreme case, this also happens to some degree to other taxa, including *Monotropsis*, although *Monotropsis* usually becomes discolored rather than distorted when dried. However, the use of herbarium specimens is important when attempting to get a sense of variability within this genus because of its infrequent to rare occurrence within its known range. In this study, 196 herbarium specimens were borrowed from the following institutions: A, AUA, BH, CLEMS, DUKE, FLAS, FSU, GA, GH, MO, NCSC, OS, PH, TENN, UNA, US, USF, USCH, VPI, and WVA. These herbaria were selected in an attempt to survey herbaria that would have a large number of recent collections as well as to examine the specimens seen by Wallace (1975b). Herbarium specimens were supplemented by 16 fresh collections made by the author and others (Table 2). In addition, liquid preserved collections were made from every live population sampled by the author in order to get a sense of variability in the three dimensional floral structure which may be lost on herbarium specimens. Small population sizes observed in the field prevented sampling of many individuals within the population. When found, populations normally consisted of one or several clumps of about 20 inflorescences at most (oftentimes fewer). Individual clumps of inflorescences were considered to be one clonal individual. Therefore, the number of individuals sampled from each population is quite low. Aberrant
individuals were noted and collected along with “typical” individuals from a given population.

All herbarium specimens were used in these analyses insofar as was possible given their quality. By using as much of the entire sample as possible rather than a subsample or “representative” sample, it was hoped that a more complete sample of the variability present in the genus would eliminate any bias in selecting individuals. It was also hoped that any disproportionate contribution of a singularly different individual to the data set would be eliminated by using the full sample. In addition, a full sampling allowed for the inclusion of duplicate collections and therefore a representation of within-population variability in the analyses. The methodology and analyses employed generally follow those of Costea et al. (2009) and Barrett and Freudenstein (2009) who also studied parasitic plant genera.

From the herbarium and field collections, measurements were made of 23 characters (see Table 3). These characters also were used to generate potentially useful derived measures (e.g. ratios) for a total of 31, largely quantitative, characters. These data were used in both the morphometric analyses and in furnishing species descriptions for the taxonomic treatment (see Chapter 4). Because of a concern that incorporating characters which used repeated measures would bias the results (for example; using both sepal length and ratio of sepal length to sepal width includes some information about sepal
length twice), several measurements were excluded from analyses. However, these measures were still used in the taxonomic treatment.

Since Monotropis is parasitic, only inflorescences are produced above ground. Therefore, the characters selected for measurement almost completely encompass the full range of characters available for study on most herbarium specimens. All herbarium specimens, unless in extremely poor condition, were sampled. Quantitative measures were to the nearest one-tenth of a unit and were measured using a stereo microscope. When multiple inflorescences were present, an inflorescence deemed representative of the variation present on the sheet was selected. Vegetative characters were consistently measured on herbarium specimens, but internal floral characters were measured only if there were multiple inflorescences on the sheet. This ensured continued utility of the collection since flowers would have to be removed and dissected in order to observe these features. Internal floral characters were measured by removing a medial flower from the sheet and rehydrating it by boiling in water in a microwave oven for approximately 20 seconds. This was done primarily to restore flexibility to the outermost tissues so that the flower could be easily dissected, rather than to restore volume.

When sheets representing multiple collections from a single population were encountered, individuals were sampled in such a way that a sense of the extremes of variability present in that particular population could be gained. For example, an extremely robust, many flowered individual, and a depauperate,
few-flowered specimen were selected on different sheets of the same collection number in order to define the limits of the variability within a population, even though neither individual inflorescence represented the “typical” individual in that population.

Before any analyses were conducted, care was taken to ensure that all specimens included in any data matrix were in equivalent developmental states. The majority of specimens were collected in spring and corresponded to Monotropis odorata s.s. These specimens were used only if they were collected between 15 February and 15 May to ensure that they were either in anthesis or very early fruit. All specimens collected in fall (identifiable to M. lehmaniae) were in bud and therefore excluded (these specimens were analyzed phenologically as discussed below). Specimens identifiable to M. reynoldsiae were collected in winter and were scarce, but all were in flower or early fruit. The specimen quality and ontogeny filters resulted in a subset of 166 of 212 total specimens (196 herbarium plus 16 fresh collections) used in the analyses.

In order to avoid using repeated measurements (those having a high correlation with other characters because they incorporated some information about the same character twice), measures such as ratios were excluded from all morphometric analyses. Based on field experience, characters 1-2 and 5 (on Table 3) are possibly correlated with environmental factors such as the depth of the humus layer, but in order to avoid biasing the matrix, only character 1 was excluded from all analyses. Characters 6-7 and 12-13 were variable even within
an individual and were therefore excluded from all analyses. Characters pertaining to the fruit were not assessed due to a low sample size and because the fleshy fruits were, in general, distorted or destroyed by pressing.

All morphometric analyses were conducted using Microsoft Excel 2007 (Excel 2007), MINITAB Version 15 (Minitab 15 Statistical Software 2010) and PAST version 2.05 (Hammer et al. 2009). Both exploratory and hypothesis-testing methods were employed. Multiple methods and analyses were performed when looking at the data as the goal was to obtain a robust picture of morphological variability in Monotropis (discussed below).

**PCA:** Principle Components Analyses were carried out first in order to see if any clustering of the Operational Taxonomic Units (OTUs) was occurring and, more importantly, if this clustering mirrored described taxa. In this case, an OTU was an individual herbarium specimen. All PCA analyses were carried out in PAST because of the ability of this program to incorporate OTUs into the analysis for which the information for a small number of characters is missing. Three separate data sets were used in the PCA: a large data set of floral and vegetative characters (characters 2-4, 8-11, 14-26, and 31 in Table 3); a data set using only characters measured in the same units (characters 4, 8-11, 14-23, 31); and one using only floral characters (characters 10,11, 14-23, 31). For each of the data sets, data were also log$_{10}$ transformed and analyzed for a total of six separate PCA analyses (three data sets, two “treatments”). Data measured in the same
units were used exclusively for some PCAs because this allowed for the use of a more robust variance-covariance matrix as opposed to the typical correlation matrix which normalizes variables by dividing by their standard deviations. The data for the length of the exposed corolla (character 31) had to be linearly transformed by adding 5 for the log_{10} transformed data set since the values of some data points was zero or negative, which occurred if the length of the sepals was equal to or greater than the length of the petals.

The fit of the data to the assumptions of PCA was examined. Data normality was assessed using histograms and normal probability plots for individual characters, as well as a multivariate normality test investigating skewness and kurtosis. Linear relationships between variables were assessed using a matrix of plots with regression lines drawn through the cloud of data. The results of each PCA analysis were visualized in a scatter plot of the first two principle components. Each OTU was given a different symbol corresponding to the described species it was identifiable to in order to aid in interpretation of the results, although PCA analysis does not in itself assign any a priori groupings to OTUs. These symbol codes corresponded to Monotropsis odorata (northern specimens, north of the Coastal Plain of Georgia; 32° N), M. reynoldsiae (southern specimens, south of 32° N), or Cryptophila pudica (Cullman, Alabama locality).

The taxonomic significance of each variable was assessed by looking at a plot of the PC coefficients for each variable. Variables considered to have a high
taxonomic significance were those with a coefficient of ± 0.30. Note that this is an arbitrary assessment, but this number was chosen in order to focus on the variables which had the greatest effect on the scatter.

*Phenetic Clustering:* For each of the six PCA matrices, UPGMA trees using Euclidean distances and their associated co-phenetic values/coefficients of correlation ($r$) were generated. UPGMA was selected instead of neighbor joining because of expected homogeneity in rates of evolution at this low of a taxonomic level. Individuals were expected to have a relatively low phenetic distance to other OTUs from the same “group”.

*Plots:* Given the result of the PCA and, to a lesser extent, the UPGMA analyses above, scatter plots of informative characters plotted against were generated in order to see if there was true separation between any distinct clusters observed. The use of scatter plots is a similar approach to that used by Case and Case (1976) for the *Sarracenia rubra* complex.

In addition to scatter plots, box plots were also generated. Box plots were used in order to better assess any differences between distinct clusters by visualizing the range of observed values. This allowed for any true quantitative differences to be observed while avoiding confusion that may arise when comparing quantities in a formal statistical framework where assumptions may be (and likely are) violated. When comparing box plots for individual characters, a
lack of overlap in the interquartile range (IQR) between clusters was sought to identify characters that may be used to differentiate them. This criterion likely underestimates the number of true character differences since characters which have some overlap in the IQR also have means or medians which are quite different from one another. This assumption was checked by performing two sample t-tests (α=0.05) for those characters which exhibited a distribution (normal) and variance (equal) suitable for the assumptions of these tests as assessed by normal probability plots and Shapiro-Wilk tests. Only the untransformed data were examined when using plots to ensure that the results would be the most applicable in a practical sense since means would be comparable instead of medians.

Phenology

As sheets were examined for quantitative morphological characters, specimens were also scored for phenological condition. Each specimen was assigned to one of three categories: flower, fruit, or bud. If specimens on a particular sheet were in multiple developmental stages, they were assigned to a half (or a third) of each stage, depending on how many they were in. For example, if a specimen was in both fruit and bud, a value of one half was assigned to both the fruit and bud categories. The percentage of specimens in a
particular stage was then calculated and plotted against collection month on a histogram.

**Anatomy**

Preserved flowers from a subset of the total number of populations examined in the field were embedded, sectioned, and stained to assess differences at the cellular level between northern and southern individuals of *Monotropsis*. Flowers from collections Rose #10-54, #10-63, #11-16, and *Lickey 11* (Table 2) which had been preserved in FAA in the field were taken through an ethanol/tertiary butyl alcohol dehydration series and embedded in paraffin. Sections including both longitudinal and transverse views of the flower were made using a microtome. These sections varied in thickness from 10 to 12 µm. Sections were mounted on slides and stained in Alcian Blue (1%) and Safranin-O (0.2%). Sections were examined and putative differences between individuals were photographed using a compound light microscope. Individual photographs were taken at 50, 100, or 400 times magnification.

**Species Concept**

The species concept used in this monograph, particularly in this chapter, relies heavily on the phylogenetic species concept (PSC) *sensu* Nixon and
Wheeler (1990) in that species are recognized based on characters which are fixed among all populations of a recognized species. The large number of duplicate collections made for populations in parts of Tennessee, North Carolina, Florida, and Alabama, as well as examination of populations in the field, allow for a sufficient assessment of variation within populations and reinforcement that the character differences are fixed within a recognized species. Recognizing multiple fixed characters within a population (both morphological and molecular) which are systematically significant was emphasized whenever possible and increases confidence that the recognized species actually exist in nature.

RESULTS

Morphometrics

PCA: For histograms of the entire, untransformed data set, few characters exhibited an approximately normal distribution pattern (see Table 7 for those that did). This lack of normality (assessed using the Anderson-Darling statistic, 95% CI) was confirmed in normal probability plots in which no characters exhibited an approximately normal distribution and only two characters had a probability of being normally distributed above 0.02. Log_{10} transformation resulted in normal distributions for two characters (the same two), but many characters exhibited a less normal distribution than with the untransformed data, suggesting the
potential for polymodality in the data set (this was also suggested by the histograms). However, violation of normality in this case is less critical since the interest was in the clustering of the data, rather than testing hypotheses.

Matrix plots (also mentioned when discussing the scatter plot results below) also allowed for cursory examination of linear relationships among variables. All variables showed a linear relationship to one another (although the slope of the line may be close to zero). A matrix of correlation coefficients showed no significant correlation among variables (greater than 0.90).

The scatter for all PCA permutations was essentially identical, showing two distinct clusters which exhibited little or no overlap as circumscribed by convex hulls and 95% confidence ellipses. These clusters corresponded to geographic and taxonomic groupings. One cluster, representing northern individuals, corresponded to Monotropsis odorata. The other cluster, representing southern individuals corresponded to Monotropsis reynoldsiae.

Conversely, those specimens from northern Alabama (Cryptophila pudica) were embedded within the scatter of the northern individuals. The range in the scatter for the PCA permutations is well represented by the untransformed floral variance-covariance matrix and the $\log_{10}$ transformed correlation matrix of the same data set (Figures 1, 2).

For the untransformed data set, all three PCAs gave similar scatter with minimal to no overlap between specimens corresponding to northern and southern individuals (Table 4). For the $\log_{10}$-transformed data, all PCA plots
showed no overlap between groups (Table 4). The low eigenvalues for the log	extsubscript{10} transformed data are a result of using a variance-covariance matrix instead of the correlation matrix since all of the variables were measured in the same units, making standardization unnecessary. When the same data were run through a correlation matrix, high eigenvalues similar to the other PCA iterations were returned. The variables which returned the highest correlation values (greater than 0.30) were quite consistent between PCA iterations. Those which were returned as significant in all iterations were sepal length and sepal width. Petal length was significant for five of the six iterations, length of exposed corolla for two, and inflorescence diameter for a single iteration.

*Phenetic Clustering*: In general, the six phenograms generated using the same six data matrices as the PCA iterations grouped specimens into two clear groups corresponding to northern and southern individuals. There were only a few exceptions for any particular matrix (Table 5). Correlation Coefficients were nearly identical, ranging from 0.68 to 0.75. The correlation matrix for the untransformed vegetative and floral data performed exceedingly poorly however, with 24 OTUs assigned to a different cluster than was thought based on *a priori* “assignment”. The other clustering analyses performed much better however, assigning between one and four OTUs to the “wrong” group. Interestingly, all analyses on the log	extsubscript{10} transformed data left some OTUs unassigned (sister to the two major groups of the phenogram). Phenograms are not illustrated because of
poor resolution with 166 OTUs. The specimens “incorrectly” are from throughout the range of *Monotropis*: North Carolina, Tennessee, Virginia, Florida, and Alabama. Several of these specimens were noted by the author upon time of examination as having “strange” phenotypes. In these cases, individuals were intentionally scored because they exhibited potentially intermediate or extreme phenotypes.

*Scatter plots:* A matrix plot of scatterplots (not shown) comprised of characters 2-4, 8-11, 14-26, and 31 revealed that plots of most characters against one another showed at least some morphological differentiation between the northern and southern clusters. However, some of these differences may be due to correlation of characters as in the case a derived measure graphed against one of the measures that go into the quantity. Additionally, some differences may be due to random chance (i.e., when the characters are not ontogenetically related). Some of the measures that seemed to be ontogenetically related (i.e., measures from the same or similar structures) showed little overlap between clusters. These plots were investigated at a more practicable resolution. They included (but were not limited to) plots of sepal length versus sepal width (Figure 3), sepal length versus petal length (Figure 4), and sepal width versus petal width (Figure 5). These three plots were selected because they depicted unambiguous differences between clusters. The plots of sepal length versus sepal width and sepal length versus petal length showed that for southern individuals, both values
tended to be smaller. In the plot of sepal width versus petal width, southern individuals tended to have less wide sepals but petal width was similar to that of northern individuals.

**Box Plots:** For most characters, box plots showed little or no difference between the northern and southern clusters, particularly for vegetative characters (figures excluded). This is not to say that the box plots were identical and each character had the same median. In most cases, the median for all characters was different between the northern and southern clusters. However, these differences were largely ignored because there was significant overlap in the interquartile range. Box plots were chosen to evaluate differences between clusters since few characters exhibited approximately normal distributions (for both untransformed and transformed data). Characters which showed a lack of IQR overlap (or little overlap) were sepal length (Figure 6), sepal width (Figure 7), length of exposed corolla (Figure 9), anther length (Figure 10), stigma width (Figure 11), bract length (Figure 12) and the ratio of sepal length to sepal width (Figure 8). Descriptive statistics for all of these measures within northern and southern clusters are presented in Table 6. Southern individuals have a smaller median sepal length, sepal width, anther length, stigma width, and bract width, while northern individuals have a smaller median sepal length to sepal width ratio and length of exposed corolla.
Shapiro-Wiki tests showed an approximately normal distribution for bract length, sepal length, and length of exposed corolla. T-tests (α=0.05) conducted on the untransformed data for these normally distributed variables confirmed that little or no overlap in the IQR range of box plots was indeed a conservative estimate of which characters differ significantly between the clusters (Table 7). A good example of this phenomenon is bract length where there is a considerable amount of box plot overlap, but t-tests show that the two clusters have significantly different means (Figure 13). Therefore, the characters listed in Table 7 can be interpreted as those which differ most significantly between the northern and southern clusters.

**Phenology**

A histogram of month of collection versus developmental state shows that in the Appalachian Mountains, *Monotropsis* flowers mostly during the spring (February to June) and also much more rarely in the fall during September (Figure 14). The histogram also shows that plants are in fruit from April to July and also in November. In the southern part of its range, *Monotropsis* flowers almost exclusively between October and February, except for one historical collection from May at NY (not illustrated). In the south, plants begin to develop fruit in January and February but strangely, plants on herbarium specimens were
also noted to be developing fruit in collections made in November and December.

**Anatomy**

Flowers corresponding to northern individuals were larger in both length and width than the flowers from the southern individual. However, as noted in the morphological results, flower length and width were not distinct between clusters, so the difference in width may be due to chance. The nectar lobes are larger in northern individuals of *Monotropis* than in the southern individual, but the variability of this character was not assessed in the morphometric study. There are several differences between the clusters at the cellular level. In northern individuals, the length of the cells comprising the nectary is smaller in their longest dimension than in southern individuals, with the median cells averaging about 24 µm in the longest dimension as opposed to 33 µm. An interesting and seemingly consistent character exhibited by the northern individuals (three sampled) is an epidermal layer on most tissues that responds very positively to staining by Safranin (Figure 15), indicating the presence of lignin, cutin, or suberin (Ruzin 1999). This layer is present in plants collected in both autumn and spring and is not found in the southern individual.

Sections of northern material collected in autumn show relatively underdeveloped flowers with imbricate petals and anthers in various stages of
development, from apparently fully-formed pollen cells to containing tissue which will presumably form mother cells and pollen grains (tapetum). In contrast to spring-collected material, anther locules are not fully formed, and there is no pore development (Figure 16). In terms of gynoecium development, the ovules of autumn-collected material appear to be in a state of rapid cell division, with cells in the egg sacs apparently not fully differentiated (Figure 16).

DISCUSSION

Is Monotropsis lehmaniae a distinct taxon?

From the evidence presented in this chapter, it does not appear that Monotropsis lehmaniae is a distinct taxon, and it should be reduced to synonymy under M. odorata. Burnham (1906) distinguished this species from M. odorata on the basis of a fall flowering phenology (September onward), scentless flowers, fleshy, pink floral parts throughout, small petals one half or less the length of the sepals, and more deeply divided corolla lobes. However, based on an examination of herbarium specimens and the literature, these characters clearly vary with ontogeny as stated by Ahles (1967). Indeed, it appears that inflorescences emerge from the ground in bud during the fall and spend the winter growing at a slow rate before flowering in spring. The appearance of buds in fall and subsequent spring flowering have been noted by Baldwin (1957) who
cited a series of specimens from the same or close localities near Williamsburg, Virginia, which he claimed intergrade between the two species. Most of these specimens were examined for this study. Unfortunately, of the series of specimens listed in Baldwin, the earliest specimens examined are from February (GH, NY). Even on these specimens, however, the sepals are clearly much longer than the petals, a feature not seen on specimens collected later in April. Baldwin concludes that *Monotropsis lehmaniae* represents immature individuals of *M. odorata*, but this point is refuted somewhat by Ahles (1967) who claims that some individuals of *M. odorata* do flower in the fall, forced into bloom by exceptionally warm weather. Appearance in fall and subsequent spring flowering has also been noted by Plitt (1909), and noted in great detail by Wolf (1922) and Klooster and Culley (2009).

Nearly all specimens collected during the fall which I have observed are far from being close to flowering (Figure 14). Specimens collected up to mid January (including the isotype and several topotypes of *Monotropsis lehmaniae*) are consistently characterized by having imbricate petals which may still be fused at the tip or along the eventual corolla lobes, and sepals which may be fused at the tips. In addition, the androecium is immature (Figure 16). The filaments of the stamen are short and the anthers are white with clearly undeveloped, easily compressed pores and pollen grains which are still in tetrads instead of in mature monads (Nowicke 1966, Takahashi 1987). The single herbarium specimen which exhibits fall flowering was collected by Asa Gray in September, 1843 at the
base of Table Mountain in North Carolina (GH). However, the flowering material in the collection is representative of typical *M. odorata* and is mixed with specimens in bud which are representative of *M. lehmaniae*. Therefore, it is probable that this is either a mixed collection from different places and from different times or, less likely, a collection containing abnormal fall-flowering *M. odorata* s.s. (*sensu* Ahles). Duplicates of this collection at NY only show typical fall inflorescences, confirming the idea that this is a mixed collection as the specimen at GH was likely from Gray’s personal collection and may have become mixed in with this collection if the specimen was used to illustrate part of the plate accompanying Gray’s *Chloris Boreali-Americana* (1846).

In addition to the collections of Baldwin mentioned above, other specimens have been examined which indicate that *Monotropsis lehmaniae* and *M. odorata* are conspecific. Two collections from the same population in Madison County, Virginia; *Rose, Sinn, & Cox 10-87* (OS) and *Dierauf, Cox, & Morgan s.n.* (VPI), show both typical *odorata* (from the former collection) and typical *lehmaniae* (from the latter collection). In addition, collections at PH and AUA from plants gathered in July and November, respectively, show infructescences as well as new, emerging inflorescences in bud. Also, a series of collections made near Cullman, Alabama (AUA) show a clear ontogenetic transition from what has been called *M. lehmaniae* to *M. odorata* from plants collected between November and May. Lastly, field observations of plants have shown flowering inflorescences mixed with dead, secund inflorescences which
were not much rotted, indicating that they may have been killed by frost the previous fall.

Anatomical evidence further justifies the reduction of *Monotropis lehmaniae* to a synonym of *M. odorata*. Bud sections from Tennessee material collected in late October confirm the observations made from herbarium specimens regarding the immaturity of floral reproductive parts. The stamens exhibit locules that were not fully formed, undifferentiated pollen-forming tissue, a well-differentiated tapetum, no discernible pore development, and anthers not yet in the mature position (Figure 16). In addition, cells comprising the ovules appeared to be in a very active stage of development with cells not yet differentiated into the eight cells comprising a mature egg sac (Figure 16). These observations are inconsistent with those made on decidedly mature Appalachian and Florida material (from March and January, respectively), suggesting that autumnal material of *Monotropis* is in no way close to flowering before harsh winter weather sets in.

In summary, what has been described as *Monotropis lehmaniae* actually represents immature inflorescences of *Monotropis odorata*. *Monotropis odorata* appears in fall and develops over the winter. The overwintering development involves the elongation of filaments, growth of ovary and petals, as well as a change in texture of the bracts and sepals from purple and succulent to brown and chaffy.
Is *Monotropsis reynoldsiae* a distinct taxon?

The morphological, phenological, and accompanying geographic data clearly support the recognition of *Monotropsis reynoldsiae* as a distinct taxon. Gray (1885) distinguished *Monotropsis reynoldsiae* from *M. odorata* using several characters as follow in the original description: “*Gracilior; squamis parvulis (lin. 1-3 longis) haud imbricatis; spica angusta secundiflora nuda e floribus sat numerosis mox nutantibus; sepalis ovatis seu ovato-lanceolatis corolla alba (vix lin. 3 longa) dimidio brevioribus.*” (p. 301). In the above description, Gray noted the smaller size of the bracts which are not at all imbricate and more narrow and floriferous inflorescence which is white and less wide relative to *Monotropsis odorata*. He also noted ovate to ovate-lanceolate sepals which are about half the length of the corolla or less. Wallace (1975b) dismissed the notion that there were any differences between the two taxa, stating that there is a considerable degree of overlap in all of these characters.

The morphometric analyses do support Wallace’s assertion that there is considerable overlap in all the characteristics that Gray notes (except flower color). Most floral characters exhibit a wide range of variability and these characters are by no means wholly distinct among described taxa (c.f. Figure 8). This suggests that *Monotropsis odorata* and *M. reynoldsiae* diverged very recently.
In spite of this overall floral similarity, the morphometric analyses reveal several differences which Gray failed to state and which Wallace may have failed to note. The most dramatic difference (which Gray may have hinted at) is that the sepals of *Monotropis reynoldiae* are much shorter than those of *M. odorata*, in addition to being much narrower. Also, the length of exposed corolla and stigma width are useful in distinguishing the two species. Anther length is also a useful character in a biological sense, but the differences in length are relatively minute and therefore not useful for purposes of identification. The significance of these characters in differentiating *Monotropis reynoldiae* and *M. odorata* is confirmed in scatter plots, box plots, t-tests, and all Principle Component Analyses. Morphological differences between these species are as great, if not greater than, differences between other closely related groups of species which exhibit “cryptic” morphologies (Compton and Hedderson 1997, Saarela et al. 2003, Sewall and Vincent 2006), particularly when the lack of population overlap on the PCA is considered given the large number of specimens and floral characters used in this study. Those *Monotropis* specimens which do show some overlap in the PCAs and the cluster analyses are few in number (about four specimens) and do not show a geographic cline in variation. They are scattered throughout the range of *Monotropis*, and include both Florida (one) and Appalachian (three) accessions (Tennessee and North Carolina). The reasons for the similarity between these four specimens of *Monotropis odorata* and *M. reynoldiae* may be twofold. First, particular
specimens were selected because they represented extremes in variation from a
given population; individuals of which were present on multiple sheets. The
Tennessee collection and Florida collection represent such extreme individuals.
The other two Appalachian accessions are restricted to a (presumably) single
population near Tryon, North Carolina and seem to be clustering close to
*Monotropis reynoldsiae* because they have abnormally short sepals. However,
this is the only character for these accessions which shows some overlap, and
these accessions are easily separated from *M. reynoldsiae* based on qualitative
characters.

Two other specimens, one from Appalachian Virginia and another from
Florida, do not seem to group with any geographically delimited populations
insofar as recognized by 95% confidence ellipses around the centroid of each of
the populations. The Appalachian collection segregates out based on smaller
sepal length, while the Florida collection is grouped outside based on a larger
flower width. Each is placed into the northern or southern clusters, respectively,
based on the plurality of characters.

The anatomical results, though few, also support this segregation due to
the presence of vacuoles in the epidermal cells of northern individuals
(*Monotropis odorata*) whose inclusions stain red in Safranin (Figure 15). This
layer is seen in ontogenetically young individuals as well, and so is likely not an
environmental response to a stress such as cold. Though the identity of the
substance taking up the stain is unknown, it may be lignin, cutin or suberin
The presence of these substances has been proposed as a way to prevent ice crystallization (Chalker-Scott 1992, Jones et al. 2000), which would be presumably selectively advantageous for the overwintering inflorescences of northern individuals.

The biological reality of these morphological differences is supported by both phenology and geography. All specimens in the northern cluster flower from late February to June while those of the southern cluster flower from November to late January or sometimes into early February or possibly (but very rarely) May. The differing flowering periods therefore provide little room for gene flow between the two populations, even if geographic range is not taken into consideration, although variation of flowering time in Monotropsis reynoldsiae merits further investigation to determine whether this is an artifact of historical collection and curation practices or not (see taxonomic treatment below). The geographic range of the specimens examined in this study (Figures 25, 27) is consistent with the distribution maps of Spawn (1940) and Wallace (1975b). Of important note is the clear geographic disjunction of several hundred miles between northern Georgia and Florida which lends further evidence to the idea that there has been genetic isolation between the northern and southern populations.
Is *Cryptophila pudica* a distinct taxon?

As with *Monotropsis lehmaniae*, *Cryptophila pudica* is not a distinct species (or genus) and is clearly a synonym of *Monotropsis odorata*. This is confirmed in the PCA analyses in which all collections corresponding to *Cryptophila pudica* are embedded within the cluster of specimens corresponding to *Monotropsis odorata* s.s. This synonymy has essentially been recognized ever since the original publication of Wolf (1922), as Small (1933) treated it as a synonym of *M. odorata* without further comment. It appears from a letter at NY that Wolf was in contact with Small during the 1910’s (apparently Wolf initially believed his Alabama specimens to be *Monotropsis reynoldsiae*!). This contact included sending specimens to Small. Slides included with the specimens at NY indicate that the flowers were sectioned at one time, and stamps on the specimens show that they were examined for Small’s Flora of North America treatment (1914).

Wolf distinguished *Cryptophila pudica* from *Monotropsis* based on locule number (one as opposed to five), placentation type (parietal as opposed to axile), and fruit type (berry instead of capsule). It is clear from the illustrations in the original publication, as well as toptypes from the Herbarium of St. Bernard College in Cullman, Alabama, where Wolf worked as a monk (the collection is now housed at AUA) that *Cryptophila pudica* is identical to *Monotropsis* in gross morphology.
Differences in less readily noticeable characteristics were due to misunderstandings by previous authors, as well as misunderstandings by Wolf himself. Plate III of Wolf’s publication shows several cross sections of the ovary, notably sections from both the base and tip. At the tip the ovary is distinctly five-celled (Plate III figure 18), but as one moves further toward the base the septa disappear (Plate III figure 19). While unilocular at the base, the ovary is clearly composed of five carpels. Therefore, depending on where one sections the ovary it may appear to be one-celled or five-celled. The number of cells in the ovary is confirmed by Olson (1994) who conducted a detailed study of the gynoecium of *Monotropsis odorata* s.s. Olson states that the ovary is “essentially unilocular. . . with parietal placentae borne on incomplete, intruding ovarian septa” (p. 720). Olson’s observations also confirm that *M. odorata* has parietal placentation. The observations of Olson have been confirmed by free-hand sections made by the author on fresh collections made in the Appalachians, as well as by the anatomical sections.

*Monotropsis* fruits were apparently never seen by authors prior to Wolf. These authors apparently assumed that the fruit was a capsule, as it is in many other monotropoids including *Monotropa hypopitys*, a species with which *Monotropsis odorata* often co-occurs. Indeed, several collections represent mixed collections of both *Monotropa* in fruit and *Monotropsis* in flower.

However, it is clear that the fruit is baccate (e.g. *Rose 10-380, OS*), as there are no lines of dehiscence on the mature (or maturing) fruit as there are on
species with capsular fruit such as *Monotropa uniflora*. Plitt (1909) refutes the notion that the fruit of *Monotropsis* is a berry and asserts that the fruit is a capsule, while at the same time acknowledging that *Monotropsis* is a close associate of *Monotropa hypopitys*, whose fruit he is likely confusing with that of *Monotropsis*. Since all of the distinguishing characters that Wolf applied to *Cryptophila pudica* have been found upon closer examination also been found in *Monotropsis odorata*, it is clear that the two names are synonyms.

**CONCLUSIONS**

Morphometric, anatomical, phenological, and geographical evidence strongly supports the recognition of two species within *Monotropsis*: *M. odorata* and *M. reynoldsiae*. These two taxa are readily distinguishable based on a unique combination of characters as specified by the PSC. *Monotropsis odorata* is distinguished by a maroon, thick, fleshy corolla, chaffy bracts at maturity, sepals about as wide and as long as the petals, a distinctly angled stigma, an epidermal cell layer which stains red in Safranin, and an overwintering inflorescence. *Monotropsis reynoldsiae* is distinguishable by a white, relatively thin-petaled corolla, fleshy bracts at maturity, sepals about half as wide and as long as the petals, an obscurely angled stigma, and inflorescences which flower soon after emerging from the soil. These taxonomic units are further isolated by
allopatric geographic ranges and non-overlapping flowering period which indicate that gene flow between the two species is highly improbable, if not impossible.
Table 2: Field collections used in this study. These collections were used for both the molecular and morphological components. Number of individuals sampled per population is also indicated.

<table>
<thead>
<tr>
<th>Collection Number</th>
<th>Location</th>
<th>Date</th>
<th>Individuals Sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rose 10-41</td>
<td>Sevier Co. TN</td>
<td>22 March 2010</td>
<td>1</td>
</tr>
<tr>
<td>Rose 10-47</td>
<td>Blount Co. TN</td>
<td>22 March 2010</td>
<td>3 (2 DNA)</td>
</tr>
<tr>
<td>Rose 10-54</td>
<td>Monroe Co. TN</td>
<td>22 March 2010</td>
<td>1</td>
</tr>
<tr>
<td>Rose 10-61</td>
<td>Rabun Co. GA</td>
<td>24 March 2010</td>
<td>2</td>
</tr>
<tr>
<td>Rose 10-62</td>
<td>Oconee Co. SC</td>
<td>24 March 2010</td>
<td>1</td>
</tr>
<tr>
<td>Rose 10-63</td>
<td>Oconee Co. SC</td>
<td>24 March 2010</td>
<td>1</td>
</tr>
<tr>
<td>Rose 10-65</td>
<td>Oconee Co. SC</td>
<td>24 March 2010</td>
<td>2</td>
</tr>
<tr>
<td>Rose 10-78</td>
<td>Caldwell Co. NC</td>
<td>25 March 2010</td>
<td>1</td>
</tr>
<tr>
<td>Rose 10-79</td>
<td>Orange Co. NC</td>
<td>25 March 2010</td>
<td>1</td>
</tr>
<tr>
<td>Rose 10-87</td>
<td>Madison Co. VA</td>
<td>26 March 2010</td>
<td>1</td>
</tr>
<tr>
<td>Rose 10-380</td>
<td>Polk Co. TN</td>
<td>22 May 2010</td>
<td>1</td>
</tr>
<tr>
<td>Rose 11-1</td>
<td>Citrus Co. FL</td>
<td>25 January 2011</td>
<td>1</td>
</tr>
<tr>
<td>Rose 11-16</td>
<td>Citrus Co. FL</td>
<td>27 January 2011</td>
<td>1</td>
</tr>
<tr>
<td>Lickey 05-11</td>
<td>Blount Co. TN</td>
<td>24 October 2005</td>
<td>1</td>
</tr>
<tr>
<td>Townsend s.n.</td>
<td>Williamsburg VA</td>
<td>22 March 2010</td>
<td>1</td>
</tr>
<tr>
<td>Huber s.n.</td>
<td>Western VA</td>
<td>24 March 2010</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 3: List of characters used in morphological analyses. Characters 1-23 were sampled directly from herbarium sheets while characters 24-31 were calculated from the directly measured characters.

<table>
<thead>
<tr>
<th>CHARACTER</th>
<th>NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Measures</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Inflorescence height (cm)</td>
</tr>
<tr>
<td>2</td>
<td>Inflorescence below flowers (cm)</td>
</tr>
<tr>
<td>3</td>
<td>Number of flowers</td>
</tr>
<tr>
<td>4</td>
<td>Rachis diameter (mm)</td>
</tr>
<tr>
<td>5</td>
<td>Number of bracts below inflorescence</td>
</tr>
<tr>
<td>6</td>
<td>Bract margin (entire=0, dentate=1)</td>
</tr>
<tr>
<td>7</td>
<td>Bract apex (obtuse=0, acute=1)</td>
</tr>
<tr>
<td>8</td>
<td>Bract length (mm)</td>
</tr>
<tr>
<td>9</td>
<td>Bract width (mm)</td>
</tr>
<tr>
<td>10</td>
<td>Sepal length (mm)</td>
</tr>
<tr>
<td>11</td>
<td>Sepal width (mm)</td>
</tr>
<tr>
<td>12</td>
<td>Sepal margin (entire=0, dentate=1)</td>
</tr>
<tr>
<td>13</td>
<td>Sepal apex (obtuse=0, acute=1)</td>
</tr>
<tr>
<td>14</td>
<td>Petal length (mm)</td>
</tr>
<tr>
<td>15</td>
<td>Petal width (mm)</td>
</tr>
<tr>
<td>16</td>
<td>Length of corolla lobe (mm)</td>
</tr>
<tr>
<td>17</td>
<td>Corolla width (mm)</td>
</tr>
<tr>
<td>18</td>
<td>Filament length (mm)</td>
</tr>
<tr>
<td>19</td>
<td>Anther length (mm)</td>
</tr>
<tr>
<td>20</td>
<td>Ovary height (mm)</td>
</tr>
<tr>
<td>21</td>
<td>Ovary width (mm)</td>
</tr>
<tr>
<td>22</td>
<td>Style height (mm)</td>
</tr>
<tr>
<td>23</td>
<td>Stigma width (mm)</td>
</tr>
<tr>
<td>Derived Measures</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Fertile inflorescence length (cm)</td>
</tr>
<tr>
<td>25</td>
<td>Flower density(number/cm)</td>
</tr>
<tr>
<td>26</td>
<td>Bract density (number/cm of sterile infl.)</td>
</tr>
<tr>
<td>27</td>
<td>Bract length: bract width</td>
</tr>
<tr>
<td>28</td>
<td>Sepal length: sepal width</td>
</tr>
<tr>
<td>29</td>
<td>Petal length: petal width</td>
</tr>
<tr>
<td>30</td>
<td>Corolla length: width</td>
</tr>
<tr>
<td>31</td>
<td>Length of exposed corolla (ch. 14 – ch. 10)</td>
</tr>
</tbody>
</table>
Figure 1: PCA of untransformed floral data. Southern individuals are indicated by open squares, northern individuals are noted by closed circles, and specimens from northern Alabama are marked by diamonds. The isotype of *Monotropsis reynoldsiae* is indicated by an asterisk. In addition, no topotypes of *M. reynoldsiae* overlap with *M. odorata* s.s. Note the small amount of overlap between the northern and southern individuals, and the complete overlap of Alabama and northern specimens.
**Figure 2:** PCA of log_{10} transformed floral data. Southern individuals are indicated by open squares, northern individuals are noted by closed circles, and specimens from northern Alabama are marked by diamonds. Note the lack of overlap between the northern and southern individuals, and the complete overlap of Alabama and northern specimens.
**Table 4**: Summary of the six PCA iterations. This table shows the percent of variance explained by the first two principle components, as well as the associated eigenvalues and the occurrence, if any, of overlap between the northern and southern individuals of *Monotropsis* (the most distinct clusters).

<table>
<thead>
<tr>
<th>PCA</th>
<th>PC1 %</th>
<th>PC 1 Eigenvalue</th>
<th>PC2 %</th>
<th>PC2 Eigenvalue</th>
<th>PC3 %</th>
<th>PC3 Eigenvalue</th>
<th>Overlap?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw correlation matrix</td>
<td>30.1</td>
<td>6.3</td>
<td>12.8</td>
<td>2.7</td>
<td>8.5</td>
<td>1.8</td>
<td>No</td>
</tr>
<tr>
<td>Raw full var-covar matrix</td>
<td>49.3</td>
<td>6.0</td>
<td>14.5</td>
<td>1.8</td>
<td>9.3</td>
<td>1.1</td>
<td>Yes</td>
</tr>
<tr>
<td>Raw floral data var-covar</td>
<td>55.3</td>
<td>5.3</td>
<td>18.6</td>
<td>1.8</td>
<td>7.0</td>
<td>0.67</td>
<td>Yes</td>
</tr>
<tr>
<td>Log correlation matrix</td>
<td>29.8</td>
<td>6.6</td>
<td>12.1</td>
<td>2.7</td>
<td>10.2</td>
<td>2.2</td>
<td>No</td>
</tr>
<tr>
<td>Log full var-covar matrix</td>
<td>45.6</td>
<td>0.1</td>
<td>12.4</td>
<td>0.02</td>
<td>7.6</td>
<td>0.02</td>
<td>No</td>
</tr>
<tr>
<td>Log floral var-covar</td>
<td>46.2</td>
<td>0.1</td>
<td>15.6</td>
<td>0.01</td>
<td>9.0</td>
<td>0.01</td>
<td>No</td>
</tr>
</tbody>
</table>
Table 5: Summary of the UPGMA trees. These trees were generated using the same data matrices as for the six PCA analyses.

<table>
<thead>
<tr>
<th>Phenogram</th>
<th>Number of OTUs not grouping with pre-defined groups</th>
<th>Number of OTUs Unassigned</th>
<th>Correlation Coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw correlation matrix</td>
<td>24</td>
<td>0</td>
<td>0.68</td>
</tr>
<tr>
<td>Raw full var-covar matrix</td>
<td>3</td>
<td>0</td>
<td>0.67</td>
</tr>
<tr>
<td>Raw floral data var-covar matrix</td>
<td>4</td>
<td>0</td>
<td>0.70</td>
</tr>
<tr>
<td>Log correlation matrix</td>
<td>2</td>
<td>2</td>
<td>0.75</td>
</tr>
<tr>
<td>Log full var-covar matrix</td>
<td>2</td>
<td>1</td>
<td>0.74</td>
</tr>
<tr>
<td>Log floral var-covar</td>
<td>1</td>
<td>1</td>
<td>0.75</td>
</tr>
</tbody>
</table>
Figure 3: Scatter plot of sepal length verses sepal width. Northern (circles) and southern (squares) individuals of Monotropis are marked. Note the minimal overlap between the clusters (n=166).
Figure 4: Scatter plot of sepal length versus petal length. Northern (circles) and southern (squares) individuals of *Monotropsis* are marked. The overlap is not as minimal as in Figure 3 but clusters are clearly differentiable based on these characters (n=166).
Figure 5: Scatter plot of sepal width versus petal width. Northern (circles) and southern (squares) individuals of *Monotropsis* are marked. The overlap between clusters is minimal as in Figure 3 (n=166).
Figure 6: Box plots of sepal length. Pictured are northern (left) and southern (right) individuals of *Monotropsis*. Outliers are marked as points. Note that southern cluster has a decidedly smaller median sepal length as the median value for this cluster does not overlap the distribution of values for the northern individuals at all. Outliers are marked as single points.
Figure 7: Box plots of sepal width. Pictured are northern (left) and southern (right) clusters of *Monotropsis*. Outliers are marked as points. Note that the southern cluster has a decidedly smaller median sepal width as the median value for this cluster does not overlap the distribution of values for the northern individuals at all. Outliers are marked as single points.
**Figure 8**: Box plots of the ratio of sepal length to sepal width. Pictured are northern (left) and southern (right) clusters of *Monotropsis*. Note that the southern cluster has a decidedly larger median ratio of sepal length to sepal width as the median value for this cluster does not overlap the distribution of values for the northern individuals at all. Outliers are marked as single points.
Figure 9: Box plots of length of exposed corolla. Pictured are northern (left) and southern (right) clusters of *Monotropsis*. Note that the southern cluster has a decidedly larger median length of exposed corolla as the median value for this cluster does not overlap the distribution of values for the northern individuals at all. Outliers are marked as single points.
Figure 10: Box plots of anther length. Pictured are northern (left) and southern (right) clusters of *Monotropsis*. Note that the southern cluster has a decidedly smaller median anther length as the median value for this cluster does not overlap the distribution of values for the northern individuals at all. Outliers are marked as single points.
Figure 11: Box plots of stigma width. Pictured are northern (left) and southern (right) clusters of *Monotropis*. Note that the southern cluster has a smaller median stigma width as the median value for this cluster does not overlap the IQR of the northern individuals at all. Outliers are marked as single points.
Figure 12: Box plots of bract width. Pictured are the northern (left) and southern (right) clusters of *Monotropsis*. Note that the southern cluster has a smaller median stigma width as the median value for this cluster does not overlap the IQR of the northern individuals at all. Outliers are marked as single points.
Table 6: Averages ± one standard deviation for characters whose box plots showed little to no IQR overlap. Associated box plots are shown in Figures 6-12. Measurements (where applicable) are in mm.

<table>
<thead>
<tr>
<th>Character</th>
<th>Northern Cluster</th>
<th>Southern Cluster</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepal length</td>
<td>7.5±1.0</td>
<td>4.2±1.0</td>
</tr>
<tr>
<td>Sepal width</td>
<td>3.1±0.7</td>
<td>1.1±0.3</td>
</tr>
<tr>
<td>Length of exposed corolla</td>
<td>0.6±0.9</td>
<td>2.6±0.6</td>
</tr>
<tr>
<td>Anther length</td>
<td>1.1±0.14</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>Stigma width</td>
<td>1.4±0.4</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td>Bract width</td>
<td>3.6±0.9</td>
<td>2.5±0.5</td>
</tr>
<tr>
<td>Sepal length: Sepal width</td>
<td>3.6±0.7</td>
<td>2.3±0.5</td>
</tr>
</tbody>
</table>
Figure 13: Box plots of bract length. Pictured are northern (left) and southern (right) clusters of *Monotropsis*. Note extensive overlap in the box plots.
Table 7: T-tests for characters with normal distributions. This is for characters which showed thus distribution when the data were not log_{10} transformed. N denotes the northern cluster, S denotes the southern cluster. Note that bract length shows considerable overlap in the IQR range when the data are viewed as box plots.

<table>
<thead>
<tr>
<th>Character</th>
<th>$n$ (N)</th>
<th>$n$ (S)</th>
<th>$\mu_N$ ($S^2$)</th>
<th>$\mu_S$ ($S^2$)</th>
<th>$t$</th>
<th>$p$ ((\mu_N = \mu_S))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bract Length (mm)</td>
<td>134</td>
<td>32</td>
<td>6.1 (1.3)</td>
<td>5.2 (0.9)</td>
<td>4.1</td>
<td>$6.8 \times 10^{-5}$</td>
</tr>
<tr>
<td>Sepal Length (mm)</td>
<td>133</td>
<td>32</td>
<td>7.5 (1.0)</td>
<td>4.2 (0.9)</td>
<td>17.2</td>
<td>$2.2 \times 10^{-36}$</td>
</tr>
<tr>
<td>Length of Exposed Corolla (mm)</td>
<td>131</td>
<td>32</td>
<td>0.5 (0.8)</td>
<td>2.6 (0.3)</td>
<td>-12.9</td>
<td>$1.3 \times 10^{-26}$</td>
</tr>
</tbody>
</table>
Figure 14: Phenological condition of herbarium specimens of *Monotropsis* collected in the Appalachian Mountains. Month 1 is January, month 2 is February etc. None of the examined specimens were collected during the month of August. Note the presence a flowering specimen during the month of September, as well as a fruiting specimen in November.
Figure 15: Anatomical differentiation between northern and southern populations of *Monotropis*. Representative sections of northern populations on the left and southern populations on the right illustrating the positive Safranin staining of inclusions in the epidermal layer of organs. The top two panels depict nectary lobes at 50X magnification and the bottom two panels depict the style margin at 200X magnification.
Figure 16: Anatomical differences between spring and fall material of Monotropis. Spring material is pictured at left, fall material at right. These images demonstrate the immaturity of anthers (top) and ovules (bottom) in fall material. Note the immature orientation, lack of pore development, and no tapetum layer on the anther section at left (magnified 50X). Note also that there are fewer cells comprising the ovules on left; indicating continued cellular
Chapter 3: Molecular Phylogeny of *Monotropsis*.

In producing this monograph of *Monotropsis*, the genetic relationships of the described species within the genus were examined in keeping with the desire for a thorough, modern, and holistic approach to species delimitation.

Methods and Materials

Sampling

An attempt was made to sample populations of *Monotropsis* from within the entirety of its known range. This included the ranges of three of the four previously described taxa with the exception of material representing *Cryptophila pudica*. Sampling was also included from biogeographically interesting localities such as the Piedmont and Cumberland Plateau, and the Coastal Plain of Virginia. The results of Chapter 2 demonstrate that there are two species within *Monotropsis*, so the aim of this chapter was to independently verify the conclusions by obtaining samples from taxa reduced to synonymy in Chapter 2 (such as *M. lehmaniae*). A list of all fresh collections and any population subsampling made is included in Table 2. In addition to these accessions, DNA was
obtained from a collection of *Monotropis reynoldsiae* made as part of the “barcoding of Florida project” at the University of Florida. This specimen is vouchered at FLAS (*L. C. Majure 2825*). As noted in Chapter 2, sampling of multiple individuals per population was, in most cases, impractical because most populations were restricted to a single cluster of several dozen stems, likely from a single, clonal individual. Apart from *Monotropis odorata*, the sampling of other taxa was minimal because *Monotropis reynoldsiae* and “*M. lehmaniae*” are rare. Indeed, records of “*Monotropis lehmaniae*” are virtually nonexistent, especially records from the last 20 years. *Monotropis reynoldsiae* is state endangered in Florida (*Ward et al. 2003*). During January 2011 only two populations of *Monotropis reynoldsiae* were found to supplement the specimen of *M. reynoldsiae* from the Barcoding of Florida project. The Lickey collection (Table 2) is assignable to “*Monotropis lehmaniae*” since it was collected during the fall. In most cases, fungal tissue was also collected by digging up an inflorescence to collect some root material. Once back in the lab, root masses were carefully dissected and washed in distilled water and finer roots were set aside for DNA isolation. Fungal hyphae penetrate the roots, so extractions on root material would presumably also contain DNA from the host fungus. In all cases, DNA isolated in the laboratory from fresh material.
DNA from plants and fungi were extracted from fresh floral tissue or root tissue using the CTAB protocol of Doyle and Doyle (1987). Three plant loci and one fungal locus were examined in this study. For plants, two loci were nuclear and one locus was on the chloroplast. The ITS/26S region was selected because it may be informative at lower phylogenetic levels due to the rapid evolution of some segments (Kuzoff et al. 1998) and because it has elucidated relationships among species complexes in the monotropoids (Broe and Freudenstein unpubl., Bidartondo and Bruns 2001). The second nuclear locus examined was a portion of the xanthine dehydrogenase (Xdh) locus using the 1296F and 1869R primer pair (Morton 2011). This also has shown some intraspecific resolution within the monotropoids (Broe and Freudenstein unpubl.). The last locus examined was the chloroplast locus rpl32-trnL using the primer pair rpl32F and trnLR (Shaw et al. 2007). Chloroplast loci within the monotropoids have been shown to have issues with paralogous copies. For example, Broe and Freudenstein (unpubl.) show that in Monotropa hypopitys, there are several paralogous copies of rps2 which may explain why individuals of M. hypopitys do not form a group in the rps2 tree presented in Bidartondo and Bruns (2001). In addition, chloroplast loci in achlorophyllous plants may be pseudogenized or lost completely (Krause 2008, Wolfe et al. 1992). Attempts to amplify matK within the monotropoids have met with difficulty. However, there
does not seem to be the same problem with this section of *rpl32*, which amplifies very easily in the monotropoids.

For the fungal DNA, the fungal barcoding locus ITS (a nuclear region) was used using the primer pair ITS1/ITS4 (Gardes and Bruns 1993). In this way, sequences could be compared to accessions already in GenBank to determine fungal host identity and level of specificity (*sensu* Bidartondo and Bruns 2002, Kretzer *et al.* 2000). Because plant and fungal DNA were present together the fungal-specific forward primer ITS1F and the general reverse primer ITS4 were used. Gardes and Bruns (1993) have shown that this primer pair does not amplify monotropoid DNA (using *Monotropa hypopitys*) and the protocol in this publication was followed for amplifications.

**Alignment and Phylogenetic Analyses**

DNA sequences were aligned using the MUSCLE and/or MAFFT alignment option in Geneious version 5.4 (Drummond *et al.* 2011). Alignments were then adjusted by hand. Based on results of both Bidartondo and Bruns (2001) and Broe and Freudenstein (unpubl.), *Monotropa uniflora, Monotropastrum humile, Hemitomes congestum, North American Monotropa hypopitys,* and *Allotropa virgata* were selected as outgroups. In some cases, sequence data for some loci were not available for all outgroups. In these cases, only *Allotropa, Monotropa*
hypopitys, and Hemitomes were used, but this assemblage of outgroups is likely very closely related to Monotropis (see Chapter 1).

In order to obtain a sense of how varied ingroup topologies were, two methods for reconstructing phylogenies were employed. Maximum parsimony analysis was conducted using TNT (Goloboff et al. 2008) with jackknife values used as measures of support. Maximum likelihood was carried out in Garli (Zwickl 2006) using the GTR + I + model with bootstrap resampling values. Clades which had a jackknife support value greater than 63 were considered to be well-supported since this jackknife value tends to correspond to one uncontroverted synapomorphy for a clade (Farris et al. 1996). Resampling was replicated 10,000 times and the resulting frequencies that a particular clade appeared plotted on the strict consensus tree. Trees were generated for each locus separately, as well as for a concatenated data set formed using the sequence concatenation option in Geneious.

Species Concept

The species concept employed in interpreting the meaning of well-supported clades in the resulting cladograms was based on the Phylogenetic Species Concept (Nixon and Wheeler 1990). It was hoped that the clades recognized as species would be consistent with the Genealogical Species Concept of Baum and Shaw (1995) in that species must be exclusive groupings
(i.e. the individuals must be “monophyletic” in that they share a common ancestor). The two species concepts would recover similar species groupings if all species were supported by apomorphic states.

RESULTS

For all accessions and loci, sequence quality as returned by the sequencing facility was very high (85 percent or above high quality DNA reads). In most cases (except as mentioned) alignment was straightforward and little adjustment had to be made to the algorithm-generated alignment. In the cases where adjustments had to be made, adjustments should not have affected the position of ingroup taxa. In all cases, the topology of the most likely tree in maximum likelihood mirrored the topology of the ingroup taxa for maximum parsimony results. This may not be surprising given a lack of homoplasy and long branches among the ingroup taxa. Therefore, only the results of the maximum parsimony results are figured and explicitly discussed.

ITS/26S

For the ITS/26S analysis, the matrix of 1715 characters and 28 taxa (seven outgroups) recovered four most parsimonious trees, each with a length of 349. In the strict consensus tree, two-well supported clades of Monotropis were recovered with no or weakly supported internal resolution (Figure 17). Both clades were supported by jackknife values of 85 or above and these clades
corresponded to the geographic distribution of the individuals: either Florida (2 apomorphies) or the Appalachian Mountains (2 apomorphies). There was one weakly supported clade within the Appalachian Mountain accessions (bootstrap and jackknife values in the 60’s) composed of the two North Carolina accessions (from the Blue Ridge and Piedmont).

**Xdh 1296-1869**

The parsimony analysis of the Xdh locus for the matrix of 409 characters and 28 taxa (10 outgroups) generated two most parsimonious trees of length 51. The strict consensus tree showed a well-supported polytomy (jackknife value of 93) for all Monotropsis accessions (Figure 18). In the alignment, many accessions showed many autapomorphies. Two accessions (one from Virginia and another from Kentucky) were removed from the analysis because of a feared paralogy issue at this locus. These two accessions had nearly identical sequences which had many more apomorphies (25) relative to all other ingroup taxa. All other accessions showed very little sequence divergence among each other (1-2 autapomorphies per accession). Additionally, the sequences from these two taxa were relatively similar to the sequence of Monotropa hypopitys used in the analysis. Contamination during sequencing can be ruled out given the position of the wells containing these Monotropsis accessions on the plate relative to wells containing *M. hypopitys*. Indeed, when these two taxa were included in an analysis, the two taxa appeared on a branch sister to the rest of the Monotropsis accessions (not pictured).
The parsimony analysis of the matrix of 528 characters and 24 taxa (three outgroups) recovered a single most parsimonious tree of length 91. Alignment of the matrix was particularly challenging due to the insertion of many gaps and apparent tandem repeats in the outgroups, but the alignment of this portion of the sequence should not have had any effect on the placement of the ingroup taxa since they were all identical at these ambiguous sites. In this single most parsimonious tree only a single clade of *Monotropis* supported by an apomorphic state was recovered with jackknife support of 61 (Figure 19). This clade corresponded to the Appalachian Mountain accessions of *Monotropis*. The weak support was not surprising given that there was only one difference between the ingroup taxa for this locus (a synapomorphy for the Appalachian material).

**Concatenated Analysis**

The parsimony analysis of the concatenated data set for all three loci from a matrix of 2651 characters and 25 taxa (three outgroups) resulted in five most parsimonious trees of length 423 (Figure 20). The data matrix had missing data for some accessions of Appalachian *Monotropis*, particularly for Xdh (the problems with this locus are discussed above). Within *Monotropis* there were two moderately supported or weakly supported clades (jackknife values of 71 and 62, respectively). The more strongly supported clade corresponded to the
Florida accessions, while the more weakly supported clade corresponded to Appalachian Mountain material.

Missing data for one accession appears to be responsible for the lower support values. Removing this individual resulted in three most parsimonious trees of length 423. Jackknife support values for the Florida and Appalachian accessions went up as a result of removing the problematic accession. Values for both clades were in the mid 90’s once the accession was removed (Figure 21). Both clades had a branch length of 3.

**Fungal ITS1/ITS4**

GenBank BLAST searches for three fungal ITS sequences showed that sequences with the highest overall match were limited to two closely related genera: *Hydnellum* and *Sarcodon*, both in the basidiomycete family Bankeraceae. Sequences were only obtained from Appalachian material.

**DISCUSSION**

**Plant Cladograms**

The molecular data lends support to the notion that there are two species in *Monotropsis*: *M. odorata* and *M. reynoldsiæ*. The fall collection of *Monotropsis* (*Lickey 05-11*) does not show any molecular variability relative to the spring flowering individuals of *Monotropsis odorata*. Therefore, from the molecular evidence alone, “*Monotropsis lehmaniae*” is not a distinct species.

In most cases, one or both groups were diagnosable. The ITS/26S data
show support for two diagnosable and exclusive clades corresponding to *Monotropis reynoldsiae* and *Monotropis odorata*. The support is strong and both clades are diagnosable by the presence of two or three synapomorphies, respectively. In the case of *rpl32-trnL*, there is support for two clades within *Monotropis* based on the diagnosability criterion, with *M. odorata* possessing the apomorphic state. This conclusion is inconsistent with the Genealogical Species Concept as *Monotropis reynoldsiae* forms a “metaspecies.” However, this grouping is not surprising given the fact that there is only a single difference between the ingroup taxa. By contrast, the *Xdh* results are uninformative in terms of grouping information.

Therefore, the combined analysis reinforced the conclusions from the ITS/26S data set with stronger support values. There is more geographic distance within the Appalachian range of *Monotropis* than between the parts of the disjunct range, yet the variability between the units is greater than that within them.

**Fungi**

The results from a GenBank search show continued support for host specificity within the monotropoids as concluded by Bidartondo and Bruns (2002) and Kretzer *et al.* (2000). The most closely matching sequences for all three accessions were from two closely related genera; *Hydnellum* and *Sarcodon*. Furthermore, the results are congruent with Bidartondo and Bruns (2001) who indicated that the host for *Monotropis* was *Hydnellum*. Since no fungal DNA
was amplified for the root isolation of *Monotropsis reynoldsiae*, speciation as a result of host shift could not be hypothesized.

**CONCLUSIONS**

The molecular data support recognizing *Monotropsis reynoldsiae* as distinct from *M. odorata*, while at the same time support recognizing *Monotropsis lehmaniae* as a synonym of *M. odorata*. Support values are generally strong, and several uncontroverted synapomorphies support each grouping. The molecular results, taken in conjunction with the morphological and phenological data, as well as the geographical data discussed briefly in Chapter 2 and in more detail in Chapter 4, provides very strong evidence for the recognition of two species within *Monotropsis* and the monograph presented in Chapter 4 is written from this point of view. Based on morphological and molecular data *Monotropsis odorata* and *M. reynoldsiae* appear to be recently diverged taxa. This is evidenced through highly similar internal floral morphology, as well as relatively little molecular change relative to that between other species in the Monotropoideae. In terms of morphology, *Monotropsis reynoldsiae* possesses more plesiomorphic character states as compared with *Monotropa uniflora*.  

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Figure 17: ITS/26S cladogram. Jackknife values above 50 percent (of 10,000) replicates are labeled above branches. Branch lengths are in parentheses next to the jackknife values.
Figure 18: $Xdh$ 1296-1869 cladogram. Jackknife values above 50 percent (of 10,000) replicates are labeled above branches. Branch lengths are in parentheses next to the jackknife values.
Figure 19: *rpl32-trnL* cladogram. Jackknife values above 50 percent (of 10,000) replicates are labeled above branches. Branch lengths are in parentheses next to the jackknife values.
Figure 20: cladogram from the analysis of the concatenated data set. This includes the accession with much missing data. Jackknife values above 50 percent (of 10,000) replicates are labeled above branches. Branch lengths are in parentheses next to the jackknife values.
Figure 21: cladogram resulting from the analysis of the concatenated data set when the problematic accession is removed. Jackknife values above 50 percent (of 10,000) replicates are labeled above branches. Branch lengths are in parentheses next to the jackknife values.
Chapter 4: Taxonomic Treatment of *Monotropsis*

In addition to the specimens examined in Chapter 2, additional specimens were seen. In all, specimens in the form of herbarium sheets or photographs were examined from the following institutions: A, AUA, BH, CLEMS, CHARL, CONN, DUKE, EKU, FLAS, FSU, GA, GH, MO, NCSC, NY, NYS, OS, P, PH, TENN, UNA, US, USF, USCH, VPI, WIS, and WVA. The descriptions and key below rely heavily on the conclusions derived from the analyses performed in Chapter 2 and Chapter 3, and these results should be consulted if further details are desired. In summarizing those two chapters, there are two species of *Monotropsis* recognized: *M. odorata* and *M. reynoldsiae*.

TAXONOMIC TREATMENT

*Monotropsis* Schweinitz in S. Elliott, *A Sketch of the Botany of South Carolina and Georgia* 1: 479. 1817. Type: *Monotropsis odorata* Schweinitz

*Schweinitzia* Elliott, *A Sketch of the Botany of South Carolina and Georgia* 1: 479. 1817.

Terrestrial, solitary or colonial herbs. Glabrous throughout. Plants spreading from a wiry, fleshy, somewhat brittle (particularly when older) rhizome. Roots adventitious, whitish when young, becoming dark purple with age, generally free of any hyphal covering except when branched to form finer roots, these roots densely covered with mats of fungal mycelia. Adventitious buds produced at intervals, these being whitish when fresh and young, densely covered with imbricate scales, maturing to form inflorescences (Figure 22C). Mature inflorescences much thicker than and abruptly differentiated from parent root at maturity (Figure 22B). Inflorescence racemose, 4.0-21.5 cm tall, rachis varying from deep purple to maroon, fertile only in the upper portion, densely or sparsely covered with scale-like bracts throughout. Bracts broadly to narrowly ovate to deltoid, sessile, obtuse to acute, brown and chaffy or somewhat fleshy and + transparent when exposed above ground, fleshy and white when still below ground. Racemes 2-16 flowered, often somewhat secund at anthesis, upright in fruit; flowers nodding, each flower subtended by a bract and usually two additional bracteoles at the base. Bracteoles subulate, brown and chaffy, often with a distinct midrib. Flowers complete, hypogynous, with a distinctive nectary disc below the gynoecium (Figure 22A). Sepals normally 5, oblong, somewhat decurrent at the base, alternating with the corolla lobes. Corolla white to maroon, sympetalous, campanulate to slightly urceolate, corolla lobes 5, connate for most of their length, distinctly saccate at the base. Stamen 10, included, nearly reaching the base of the stigma, in two apparently alternating lengths but really of
equal lengths; 5 stamens alternating with the corolla lobes with a point of attachment at the base of the ovary, 5 stamens inserted between the lobes of the nectar disc (Figure 22A). Filaments terete at the base, flattened above, usually pinkish. Anthers oblong, yellowish, unawned. Thecae 2, dehiscing from apical slits. Pollen shed in monads. Gynoecium flask-shaped, pinkish to whitish when fresh, darkening to purplish when dry. Ovary globose, prominently to indistinctly 10-ridged, each stamen filament nestled within a rib, inflating to a smooth surface when in fruit. Ovary abruptly demarked from or confluent with the style, placentation intruded-parietal. Stigma abruptly differentiated from the style, capitate, distinctly or indistinctly five-lobed, with a fluid-filled depression in the center leading down into the style. Nectary disc 5-lobed, these lobes further divided to give the impression of a 10-lobed disc, the disc narrower than the ovary. Fruit baccate, without any lines of dehiscence, purplish, juice sticky, stigma and style persistent on the fruit, seeds small, numerous.

*Monotropsis* can be distinguished from all other monotropoids by a combination of a sympetalous corolla, baccate fruit, and the presence of bracteoles (bracts subtending the pedicels). It often grows in close proximity with and is occasionally confused with *Monotropa hypopitys* L. (particularly when the latter is in fruit), but a winter/spring flowering period, glabrous as opposed to pubescent inflorescence, sympetalous corolla, and curved pedicels even in fruit distinguish it at once. From the morphological and molecular evidence presented above in Chapter 2 and Chapter 3 there is no evidence to support recognizing
the two species in this genus as two monotypic genera instead. The position of *Monotropastrum sciaphalum* (H. Andres) G. Wallace (Andres 1953, Wallace 1987) relative to *Monotropsis* is of interest as it seems to potentially be intermediate between *Monotropsis* and *Monotropastrum humile* with a racemose inflorescence, bracteoles, unfused corolla, distinct sepals, short nectar lobes, and a baccate fruit.

**Etymology**

*Monotropsis* was named based on a resemblance to *Monotropa*. The generic name *Schweinitzia* was proposed by Elliot in honor of Schweinitz. Rafinesque (1818) in a diatribe outlines why this name is preferable to *Monotropsis* based on the Linnaean principle that names should not be confusing, in this case the confusion of the name with *Monotropa*.

**KEY TO THE SPECIES OF MONOTROPSIS**

1a. Sepals nearly as long as or slightly longer than the corolla lobes and nearly as wide, 5.0-10.0 mm long, 0.7-2 mm wide; corollas fleshy, with a distinct maroon coloration at some point along their length; fresh specimens with brown chaffy bracts.................................................................1. *M. odorata*
1b. Sepals much shorter and narrower than the corolla lobes, 2.4-6.0 mm long, 1.7-5.2 mm wide; corollas not fleshy, white or slightly pink-tinged, fresh specimens with fleshy bracts identical in color to the rachis of the inflorescence

2. *M. reynoldsiae*


*Schweinitzia odorata* (Schweinitz) Elliot, A Sketch of the Botany of South Carolina and Georgia 1: 479, 1817.


*Cryptophila pudica* W. Wolf Am. Midl. Nat. 8: 117 1922 TYPE: St. Bernard,
Cullman, Alabama, (holotype listed by Wolf as Herb. St. Bernard College no. 1071, not seen)


TYPE: St. Bernard, Cullman, Alabama, (holotype listed by Wolf as Herb. St. Bernard College no. 1452, not seen)


TYPE: St. Bernard, Cullman, Alabama, (holotype: Plate II, Illustration 3!)

Plant glabrous throughout. Inflorescence racemose, secund, nodding when first emerging from soil, becoming erect through anthesis, to fully erect in fruit. Inflorescence fertile in the upper $\frac{1}{10}$ to $\frac{1}{4}$, 4.0-21.3 mm (9.2) long, 1.1-6.0 (2.5) mm wide, 2-12 (6) flowered, maroon to dark purple, sparsely to densely covered with 14-66 (34) bracts throughout, these bracts broadly to less often narrowly ovate to deltoid, sessile, obtuse, margins entire to dentate, variable on the same plant, 3.9-9.9 (6.1) mm long, 2.0-7.0 (3.6) mm wide. Bracts brown and chaffy at maturity, lavender and fleshy when young. Flowers subtended at the base by two subulate bracteoles at the base of the pedicel in addition to a broadly rhombic bract. Bracteoles and bract similar in color, texture, and margins to the lower bracts, but these bracts and bracteoles often with a prominent midvein sometimes excurrent as a blunt apiculus. Flowers nodding, on bent pedicels. Calyx 5-merous (rarely 4- or even 7-merous), sepals free, oblong, brown and chaffy at maturity, margins entire or dentate, often with a blunt mucro,
margins and apex variable even on the same flower, lavender and fleshy when young, varying from as long as to longer than the corolla lobes, 5.0-10.0 (7.6) mm long, 1.7-5.2 (3.1) mm wide, the ratio of the sepal length to width 1.4-4.1 (2.5). Corolla actinomorphic or ± zygomorphic due to uneven splitting of the lobes, sympetalous, campanulate, 5 (-7) -lobed, fleshy (due to accumulation of waxes in the epidermis), distinctly ridged internally along the lines of fusion of the petals, solid maroon in the upper 2/3 of the corolla, whitish at the base and around the tip (reported to be entirely white occasionally, but no specimens seen), 2.4-9.0 (4.4) mm wide, the lobes erect to distinctly spreading at anthesis, the lobes sometimes slightly mucronate or cucullate. Lobes 6.0-10.7 (8.0) mm long, 1.1-3.3 (8.0) mm wide, free for 1.7-5.1 (3.0) mm, the length of the free portion varying somewhat between lobes on the same flower. Stamen 10, filaments maroon, 0.3-6.8 (4.1) mm long, anthers yellow, 0.9-2.6 (1.1) mm long. Gynoecium flask-shaped, bright pink to maroon, darkening to purple at maturity, especially in the proximal portion. Ovary distinctly 10-ridged when young, 2.0-6.0 (3.6) mm high, 1.9-6.5 (3.3) mm long, ridges disappearing upon maturity, style 0.4-3.5 (1.6) mm long, sometimes quite confluent with the ovary. Stigma broadly capitate, conspicuously angled, distinctly deeper and wider than the style at the point of its attachment, whitish to purplish, distinctly or indistinctly 5-lobed, 0.8-3.0 (1.4) mm wide. Fruit baccate, mucilaginous, containing many seeds; seeds small and brown, unornamented, broadly spindle-shaped in outline, 0.4 mm long, 0.2 mm wide at their widest point.
Common Names

Pygmy pipes, Sweet pinesap, Carolina beech drops

Illustrations

Figures 23, 24, Wolf 1922, Plate III

Distribution

*Monotropis odorata* is endemic to the southeastern United States occurring in the Appalachian Mountains, including the Blue Ridge, Piedmont, the Cumberland Plateau, and the Coastal Plain in Virginia, Maryland, and Delaware. It occurs from coastal Maryland and central West Virginia south to central Alabama and northern Georgia. Specimens have been examined from the following states: Alabama, Delaware, Georgia, Kentucky, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia (see Figure 26). Being mycoheterotrophic, it should not be expected to appear above ground every year. In the case of *M. odorata*, its rarity is no doubt artificially inflated by the fact that it often does not emerge very far above the leaf litter layer, its brown bracts and sepals blend in with the dead leaves, and it conducts most of its reproductive activity when botanical activity is generally at a minimum. Indeed, as Wallace
(1975b) notes it is “infrequently collected so range poorly known” (p. 51). My own field experiences suggest that while it may be infrequent in the northern part of its range, it appears to be quite common in some parts of the southern portion (e.g. Oconee Co. South Carolina), and is said to be common on the Coastal Plain in Virginia (Plitt 1909, Baldwin 1957).

Ecology

*Monotropsis odorata* grows between 570-4000 feet elevation (175-1220m). It is found in rich, upland, open well-drained forests where there is sandy soil underlying a thick humus layer (Figure 25). Habitat includes slopes, ridge tops, bluffs, and rarely sandy banks of water courses. The canopy layer of these forests can be almost completely coniferous or composed solely of hardwoods. In most cases the canopy seems to be mixed conifer-hardwood with the most common tree species being pines (*Pinus* sp.) and oaks (*Quercus* sp.). The understory layer is commonly made up of evergreen heaths (*Kalmia latifolia* and *Rhododendron* spp.). There are usually very few herbaceous or sub-shrub associates (Figure 25). Noted tree associates from herbarium labels include *Pinus rigida* Miller, *P. pungens* Lambert, *P. virginiana* Miller, *P. strobus* Linnaeus, *Tsuga candensis* (Linnaeus) Carriere, *Quercus montana* Willdenow, *Q. alba* Linnaeus, *Q. velutina* Lamarck, *Fagus grandifolia* Ehrhart, *Acer rubrum* Linnaeus, *Nyssa sylvatica* Marshall, *Prunus serotina* Ehrhart, *Oxydendrum arboreum*

From my own detailed field notes at two specific sites I have recorded the following community compositions: *Ilex opaca*, *Kalmia latifolia*, *Pinus strobus*, *Pinus virginiana*, *Quercus velutina*, *Quercus* spp. (white oak group), *Epigaea repens*, *Smilax* sp., *Gaultheria procumbens*, *Vaccinium* sp., *Acer rubrum*, *Tsuga canadensis*, *Mitchella repens*, and *Oxydendrum arboreum* (Powell County Kentucky) and *Cornus florida*, *Kalmia latifolia*, *Pinus virginiana*, *Nyssa sylvatica*,
Sassafras albidum, Viola sp., Magnolia macrophylla, Acer rubrum, Quercus spp. (white oak group), and Oxydendrum arboreum (Oconee Co. South Carolina).

**Reproductive Biology**

*Monotropsis odorata* flowers from February to May (sometimes in June). Inflorescences appear from underneath the leaf litter beginning in September or October and gradually mature over the winter. Wolf (1922) suggests that most of the maturation occurs in late winter (February-March). Immature inflorescences are lavender colored (described as “purple vinaceous” on a herbarium specimen using an early color chart), and the bracts and sepals turn chaffy and brown over the winter. Based on herbarium specimens, the sepals appear in fully formed on the fall inflorescences, but the corolla and sexual organs are very underdeveloped. The corolla appears to expand in both length and girth throughout the winter. Klooster and Culley (2009) provide a thorough summary of the reproduction of *M. odorata* based on observational and experimental data from two populations: one in Kentucky and another in Tennessee. Their detailed observations confirm that inflorescences of *M. odorata* are above ground from October to mid-June, with flowering occurring in a short period from April to May, consistent with data from herbarium specimens (Figure 14) and the observations of Wolf (1922). In addition, they found that variation in the timing of anthesis between years is only +/- 7-10 days. Klooster and Culley characterize *M.*
*odorata* as having a herkogamous floral morphology, with stamen and gynoecium receptive at the same time. In addition, *M. odorata* shows a high frequency of visits by pollinators and crossing experiments reveal little fruit set when there is pollination within an individual (autogamy or self compatibility). Pollination is mostly carried out by bumble bees (*Bombus* sp.), which “buzz pollinate” the plants. Therefore, *M. odorata* appears to be an obligate outcrossing species. Pollinators are attracted to the flowers by a fragrance (described as smelling like cloves or violets), and rewarded by nectar (for more details on the anatomy of the nectary see Wallace, 1977). The fragrance appears to be emitted only when the flowers are in full anthesis. In addition, personal observations in the field (late March 2010) suggest that the chemical which emits the fragrance may require a minimum temperature to become volatilized, as it was only detected on warm days (60 degrees Fahrenheit or more).

The brown chaffy bracts and sepals, which are of similar color and reflectance to the leaf litter aid in ensuring reproduction by way of predator avoidance, as suggested by Klooster *et al.* (2009). Experimental removal of bracts and sepals result in increased herbivory of inflorescences and decreased fruit set. The reflectance of the bracts is similar to that of the leaf litter layer, while the reflectance of the rachis and petals was found to be different. It appears that these bracts aid in camouflaging the plants from herbivores. Klooster *et al.* also note that the bracts may provide an insulating role for the
flowers. The method by which the seeds are dispersed is presently unknown, although Klooster et al. (2009) suggest mammalian dispersers, while Wood (1961) proposed an ant dispersal mechanism. In any event, the short dispersal potential of the fruit type and obligate outcrossing make for interesting questions surrounding the population genetics of this species (and genus), particularly in light of the mycoheterotrophic habit and disjunction of the genus.

A Note on Typification

Insofar as possible given the Code of Botanical Nomenclature (2006) and the information regarding the disposition of specimens provided in the original descriptions, I have tried to follow the decisions of Wallace (1975b) if only for the sake of avoiding future confusion. However, a few things must be said on the subject.

Selecting a type specimen for Monotropsis odorata is made difficult by the fact that the history of the Schweinitz herbarium is clouded. Stuckey (1979) provided a summary of these problems. The herbarium of Schweinitz was apparently acquired by PH in 1834, at which time many of the labels were remade and the originals discarded. In addition, specimens were apparently remounted in the late 19th century. However, during the re-labeling process all text on the original labels was placed in parentheses. The specimens at PH indicate that “Schweinitzia odorata Salem” was the text present on the original
labels. The herbarium at the Charleston Museum of Natural History in Charleston, South Carolina (CHARL) apparently contains the personal collection of Stephen Elliott. Among this collection is a specimen of *M. odorata* with early 19th century paper and labels. This label reads "*Monotropsis. Schweinitz Hab: juxta Salem NoC: Flor. Feb. Mar. W. Schweinitz*". Spawn (1940) also shed some light on the typification issue by citing four specimens as potential types: those at PH, one at GH, one at NY, and one at P. Spawn dismissed the one at NY as being a potential type since it was sent to that institution in 1819, two years after *Monotropsis* was first described by Schweinitz. Indeed, this specimen at NY does not have very much information accompanying it, merely the name "Monotropsis" in script and a rubber stamp reading "Schweinitz." The specimen at GH was not located for this study (it may be lost), but Spawn believed that it is a duplicate of the specimen at P. An image of the specimen at P on the website of that institution shows a label which reads "Schweinitzia odorata N. Carolina" in a handwriting that does not match that of Schweinitz (see Fig. 1 in Stuckey). Based on the fact that in the protologue of *A Sketch of the Botany of South Carolina and Georgia* Schweinitz refers to *Monotropsis* and not *Schweinitzia*, the specimen is preserved in its original state, the label information is most consistent with the protologue, and that it would reduce confusion to maintain the precedent set by Wallace (1975b), I have chosen to continue to recognize the specimen housed at CHARL as the type specimen of *Monotropsis odorata*. However, Wallace designated this as a holotype. This specimen should instead
be considered a lectotype because of the more or less certain history of the specimen at CHARL and the uncertain history of the specimens at PH, which may or may not have come from the type collection, and is here designated as a lectotype.

The type specimen of *Monotropis lehmaniae* is listed in the protologue as being housed at the New York State Museum (NYS). However, Spawn (1940) and Wallace (1975b) cite a specimen at BH as the holotype. Specimens, each labeled as types, exist at both institutions. The specimen at BH bears a note indicating that a portion of the type is at NYS. The personal collection of Stewart Burnham was deposited at BH, and so this sheet may indeed be part of the type collection. However, the sheet at NYS is not cross-labeled. Therefore, I am recognizing the specimen at NYS as the holotype of *Monotropis lehmaniae* and that at BH as an isotype, as opposed to identifying both as syntypes.

The collections of *Cryptophila pudica* and its sub-specific taxa were originally housed at the herbarium of St. Bernard College in Cullman, Alabama. Based on communication with the staff there, the entirety of the remaining herbarium was transferred to AUA in 1995, although it appears that some specimens were sent to J.K. Small by Wolfe around 1911 (NY), to Dr. Gary Wallace at RSA (personal communication) and to WIS via the acquisition of the Monotropaceae collection at LCU (Tucker *et al.* 1989). Of the material at AUA, NY, RSA, and WIS, none corresponds to any specimens designated by Wolf (1922). Interestingly, all original labels bear the name “*Monotropis*” instead of
“Cryptophila.” At this point, because of the haphazard dispersal of material from St. Bernard College, I am disinclined to recognize any material as a neotype. If any material were to be so designated, I would encourage neotypes to be sought at AUA. However, it should be noted that none of the extant material corresponds to the description of *C. pudica* forma *maxima*, nor are any figures presented by Wolf that could be designated as the neotype of this taxon as specified by the Code.

**Specimens Examined**

What follows is a state-by-state list of all specimens of *Monotropsis odorata* examined in this study. See also the distribution map of this taxon (Figure 26).

DELAWARE: *Durand, s.n.* (US, PH).


MARYLAND:  Anne Arundel Co., Conway, 10 Apr 1938, Spawn 1081 (BH, US); Conway Station on the WBA electric RR, 27 Apr 1924, Freeman s.n. (US); Severn River, near Robinson Station, Apr 1904, Plitt s.n. (US); Near Benfield, 28 Mar 1903, Plitt s.n. (US); Kalmia Farm, Woodwardville, 21 Apr 1912, Waite s.n. (US); Benfield, 28 Mar 1903, Plitt 652 (GH); Conway, 27 Jul 1938, Spawn 1085 (PH); Near Benfield, 20 Apr 1938, Egerton s.n. (PH); Near Benfield, 22 Apr 1939, Fisher s.n. (PH). Baltimore Co. Lutherville, Baltimore, 24 Apr 1902, Johnson s.n. (US); About 8 miles from Baltimore on the Philadelphia Road, Griffeth s.n. (NY); vicinity of Baltimore, Griffith s.n. (P, photograph!). Baltimore City Co., Near Baltimore, LeRoy s.n. (BH); Near Baltimore, May 1866, LeRoy s.n. (GH); Near Baltimore, 1867, LeRoy s.n. (GH, NY); Near Baltimore, 1866, LeRoy (NY, PH); Near Baltimore, May 1867, LeRoy s.n. (PH); Near Baltimore, Apr 1942, Johnson s.n. (NY). Other specimens not assignable to a county: Herb. Durnad s.n. (NY).

NORTH CAROLINA:  Buncombe Co., Craggy Mtn., near Biltmore, 28 Sep 1900, Herb. Biltmore 4543b (US); 28 Mar 1894, Roehbling s.n. (US). Burke Co., South Mountain State Park, Jacob Fork River, 0.25 mi above High Shoals, 27 Mar 1975, Smith 235 (NCSC); Base of Table Mountain, Sep 1843, Gray s.n. (GH, NY), Table Mountain, Sep 1843, Gray s.n. (NY); Salem, 1904, Lehman s.n. (NY). Caldwell Co., Wilson Creek area of Pisgah National Forest, at end of FS 4096, 25 Mar 2010, Rose, Sinn, & Kauffman 10-78 (OS); Lenoir, 8 Apr 1914, Harper
s.n. (NY). Catawba Co., North end of Hickory within the city limits, 22 Mar 1968, 
*Daggy & Moye 4667* (CLEMS); North Hickory near end of Co. Rd. 1321, 22 Apr 
1969, *Daggy & Moye 5257* (AUA, CLEMS, FLAS, GH, MO, NY, PH, TENN, UNA, 
(NY). Durham Co., Hill Forest, 12 May 1948, *Jones s.n.* (NCSC); Little River, 5 
Dec 1931, *Jenkins s.n.* (DUKE). Henderson Co., Holly Mountain about 2 miles 
west of Hendersonville, 1898, *Huger s.n.* (NY); Hendersonville, Summer 1898, 
*Huger s.n.* (NY); Flat Rock, 15 Oct 1885 (NY). Macon Co., Highlands, 9 Sep 
1949, *Hesler s.n.* (TENN); Near Harbison Lake, Highlands, 10 Sep 1949 *Hesler* 
s.n. (NCSC). McDowell Co., About ¼ mi W of Kistler Memorial Hwy., opposite 
Conley Cove Trail, 4 May 1968, *Selley, Moye, Daggy s.n.* (TENN). Orange Co., 
West of Laurel Hill, 2 Apr 1933, *Bloomquist 4447* (DUKE); Rhododendron Bluff 
off Piney Mtn. Rd. along the New Hope Creek, 14 Mar 1973, *Cohen 1* (DUKE); 
Apr 1942, *Boyd s.n.* (MO); Tryon, 30 Mar 1919, *Day s.n.* (BH); Round Top, near 
Columbus, 4 Mar 1897, *Townsend 249* (BH, US); Tryon, Apr 1896, *Herb. 
Biltmore 4543a* (US); Tryon, 6 Feb 1897, *Case s.n.* (US); Tryon, Apr 1901, 
*Wilson s.n.* (NY); Tryon, Mar 1896, *Huger s.n.* (NY). Stokes Co., Near Salem, 
Feb-Mar, *Schweinitz s.n.* (CHARL, NY (photograph), PH); Cascades, Hanging 
Rock State Park, 8 Apr 1956, *Schuster 36998* (DUKE, TENN, GA, NCSC, GH); 
Hanging Rock State Park, Wolf Rock Loop Trail, 5 Nov 2005, *Burge 345* (DUKE); 

Huper s.n. (MO). Other specimens not assignable to a county: Yadkin Valley, 26 Apr 1899, Pickney s.n. (PH).

near Wartburg, 1893, *Percival s.n.* (NY). Polk Co., Cherokee National Forest, N
side of FS 11512 near its terminus, 11 May 1993, *Pistrang s.n.* (TENN);
Cherokee National Forest, off FS 1176-1 approx 0.5 mi from intersection with Rd.
23, 3 June 1993. *Pistrang s.n.* (TENN); Cherokee National Forest, approx. 0.5
(TENN); SE of Rogers Branch, ca. 0.25 mi ENE of Hwy. 64, 12 May 1991,
*Wofford & Clebsch 91-11* (TENN); Along McFarland Rd./FS 23, 22 May 2010,
*Rose 10-380* (OS). Scott Co. Big South Fork National River and Recreation
Area, Gentleman’s Swimming Hole, 24 May 2000, *Shaw, Beck, & Shaw 633*
(TENN). Sevier Co., Chilhowee Mtn, 21 Apr 1971, *Thomas s.n.* (TENN);
Bearwallow Mtn., Chilhowee Mtn, 20 Apr 1971, *Thomas et al. 22861* (TENN);
Bullhead Mtn., 10 May 1969, *Sharp & Meijer 43160* (TENN); Meigs Creek, The
Sinks, 4 Apr 1937, *Sharp & Jennison 3952* (TENN); Bullhead Mtn., 13 May
1961, *Sharp, Jones, Pringle, & Schneider 28526* (GA, TENN, AUA); High on
Bullhead Trail, 5 May 1950, *Herb. Braun s.n.* (US); Great Smoky Mountains
National Park, connecting trail between Ashopper Branch and Sugarlands

VIRGINIA: Alleghany Co., Near Longdale exit off Interstate Hwy. 64, 8 Apr 1992,
*Havelack s.n.* (VPI). Amherst Co., near Snowden, 5 May 1940, *Massey s.n.*
(VPI). Bedford Co., 20 Mar 1871, *Curtiss s.n.* (MO, NY); Mar, *Curtiss s.n.* (US);
25 Mar 1873, *Curtiss s.n.* (NY, PH); 1 Mar 1873, Curtiss s.n. (NY). Botetourt Co.,
s.n. (OS). York Co., Scimmino Millpond, 100 ft W of Rt. 604, 7 Apr 1963, Baldwin 16061 (A); E of Williamsburg, beside Colonial Pkwy., 20 yds from parking area at Jones Pond, 8 May 1968, Noake s.n. (USF, FLAS).


Additional specimens without any geographic information: a Specimen labeled “Schweinitz” with a rubber stamp without any other information (NY).

2. **Monotropsis reynoldsiae** (A. Gray) A. A. Heller, Catalog of North American Plants North of Mexico, 5, 1898.

Schweinitzia reynoldsiae A. Gray *Proceedings of the American Academy of Arts and Sciences* 20: 300-301, 1885. TYPE: “E. Florida, near St. Augustine and on Indian River” (holotype: GH [photograph!], isotypes: GH!, P [photograph!]).

Plant glabrous throughout. Inflorescence racemose, secund, erect when emerging from soil, nodding at anthesis, becoming fully erect in fruit. Inflorescence fertile in the upper $\frac{1}{5}$ to $\frac{3}{5}$, 6.0-15.0 (8.5) mm long, 0.8-3.2 (1.6) mm wide, 4-16 (9) flowered, rachis dark purple-black, pinkish to even white below (where covered with humus), sparsely to densely covered with 20-57 (35)
bracts throughout, these bracts broadly to less often narrowly ovate to deltoid, sessile, obtuse or acute, margins entire or very rarely dentate, 3.3-7.2 (5.2) mm long, 1.7-3.6 (2.5) mm wide. Bracts blackish purple when young, fleshy, identical in color to the rachis of the inflorescence, becoming slightly chaffy when old (on fruiting specimens), this being particularly noticeable on dried specimens. Flowers subtended at the base by two subulate bracteoles at the base of the pedicel in addition to a broadly rhombic bract. Flowers nodding, on bent pedicels. Calyx 5-merous, sepals free, oblong, brown and chaffy at maturity, margins entire or dentate, much shorter than the corolla lobes, 2.4-6.0 (4.2) mm long, 0.7-2.4 (1.2) mm wide, the ratio of the sepal length to width 1.8-6.7 (3.9). Corolla actinomorphic, sympetalous, campanulate, 5-lobed, not fleshy, white or tinged with pink toward the base, 2.0-6.0 (3.7) mm wide, the lobes distinctly spreading at anthesis, 5.3-9.0 (6.8) mm long, 0.8-2.9 (1.9) mm wide, free for 1.3-3.7 (2.4) mm. Stamens 10, filaments pinkish, 2.2-5.1 (3.7) mm long, anthers yellow to more often covered in a network of reddish reticulations, 0.6-1.0 (0.8) mm long. Gynoecium flask-shaped, bright pink. Ovary indistinctly 10-ridged, 2.0-5.0 (3.1) mm high, 1.5-5.2 (2.8) mm wide, style 1.0-2.4 (1.6) mm high, confluent with the ovary. Stigma capitate, not conspicuously angled, whitish, indistinctly 5-lobed, 0.6-1.9 (1.0) mm wide.

Gray (1885) distinguished *M. reynoldiae* based on a slender inflorescence, smaller, more numerous, and more widely spaced white flowers, and the ratio of sepal length to width. As noted above, many of these characters
are variable, but the species is easily distinguished morphologically from *Monotropis odorata* by its small, narrow sepals, concolorous corolla, and bracts which are similar in color to the rachis of the inflorescence at anthesis and become more or less translucent and curled upon drying. This species does not seem to undergo any sort of aboveground dormancy, as observed in a population in Citrus Co. FL (*Rose 11-16, OS*) while conducting field work. In this population small plants in bud were observed intermixed with some in full anthesis. These smaller inflorescences were in an upright as opposed to nodding position. This suggests a lack of above ground dormancy and development similar to that of other monotropoids. The apparent lack of above-ground dormancy reduces long time exposure to herbivores. In addition, a winter flowering period when herbivore activity is reduced along with the non-freezing winter temperatures in Florida may make the presence of these chaffy bracts irrelevant (see discussion under *M. odorata*). Several atypical specimens have been noted. These are particularly robust individuals which represent extreme forms of *M. reynoldsiae* and scarcely overlap with the variation of *M. odorata* (i.e. *Kunzer 2655, USF; Rose 11-16 [in part], OS*). They are easily distinguished from the latter taxon based on a suite of other characters. Part of a collection made by Mary Reynolds (USCH) shows a specimen of *M. reynoldsiae* in bud which may, at first glance, be confused with *M. odorata*. However, this confusion results from two factors. Firstly, the inflorescence is not yet fully expanded so it is much more clustered than usual and hard to determine exactly the
arrangement and shape of organs. However, close examination reveals that this USCH specimen has atypically large bracts which are concealing the linear sepals typical for this species. Secondly, because it is in bud and the petals are not yet fully expanded, the sepals look more similar in length to the petals than they would on a more fully developed specimen. This inflorescence is mixed with a typical individual of the species in full anthesis.

**Common Names**

None, although Florida or Reynolds’ pygmy pipes is suggested here.

**Illustrations**

Figures 26, 27.

**Distribution**

*Monotropsis reynoldsiae* is highly endemic, restricted to scrub-oak areas in the northern peninsular region of Florida. The known range of this species has increased dramatically since the publication of Wallace (1975b). Prior to about 1977, the species was only known from three localities along the Atlantic Coast of Florida in the region around St. Augustine and Daytona Beach. Since then, it
has been found throughout the northern peninsular region and now extends in
distribution along a narrow band from the St. Augustine region to the Gulf Coast
(see distribution map, Figure 28). Within this geographic range, *Monotropis
reynoldsiae* appears to be restricted to the turkey oak and coastal hammock
areas of the state (*sensu* Moore 1968).

Ecology

*Monotropis reynoldsiae* grows at low elevations from 0-137 feet (0-42 m)
in moist, well-drained oak hammocks (Figure 25). Based on field experience,
these hammocks vary from rather dense to more open, but like *M. odorata*, *M.
reynoldsiae* prefers a deep layer of leaf litter. Dominant tree species are mostly
tree oaks and scrub oaks including *Quercus laurifolia* Michaux, *Q. chapmannii*
Small, and *Q. myrtifolia* Willdenow. Other associated tree species include *Pinus
palustris* Miller, *P. taeda* Linnaeus, *P. clausa* (Chapman) Sargent, *Carya glabra*
(Miller) Sweet, and *Magnolia grandiflora* Linnaeus. Understory associates
include *Cornus florida* Linnaeus, *Ilex opaca* Aiton, *Persea borbonia* (Linnaeus)
virginiana* Linnaeus, *Sideroxylon tenax* Linnaeus, and *Prunus* spp. Herbaceous
associates include *Opuntia pusilla* (Haworth) Haworth, *O. humifusa* (Rafinesque)

**Reproductive Biology**

*Monotropis reynoldsiae* flowers from late fall into winter (November-February). The fruit, as in *M. odorata*, is baccate. Fruiting specimens have been observed on several sheets from material collected in December (many collections from the 1880’s) and November (*Kunzer 2655, USF*). The collections from the 1880’s could represent the fruit of specimens that flowered in November, but the fruit on these specimens may also represent fruit set as a response to being picked as a result of ethylene production from damaged tissue and accompanying delay in pressing (see Yang and Hoffman 1984). Indeed, these specimens may have traveled un-pressed for a long period of time as Gray (1885) states that he (presumably in Cambridge, Massachusetts) received from Mary Reynolds in St. Augustine, “a full series of freshly gathered specimens” (p. 301). The history behind the Kunzer specimen, collected in 2008, is unknown, and this may or may not represent a legitimately fruiting specimen. At any rate, it appears that there may be two different but overlapping flowering periods which are dependent on habitat. All specimens from eastern Florida which grow in coastal hammocks were collected between November (possibly October) and January, although the details on some of the early labels are suspect. Those
collected in January appear to be from late in the flowering season. Plants from western Florida grow in moist scrub oak hammocks interspersed in sand hills. These populations apparently flower from January to February based on herbarium specimens. This phenomenon requires further investigation.

Conservation

*Monotropsis reynoldsiae* is currently known from only a handful of sites. These sites may only be known from vague herbarium labels or, based on personal observations, are in areas which are heavily used by the public or are in close proximity to construction projects. According to Ward *et al.* (2003) this species is endangered in Florida. Because of the relatively few records known, this species would be a good candidate for federal protection at some level. Certainly some of the rarity may be attributed to the cryptic nature of the species in terms of size, coloration, flowering period and its ephemeral nature. Extensive floristic studies certainly should be conducted to obtain more information on the distribution of *Monotropsis reynoldsiae*.

A Note on Typification

Wallace (1975b) designated the holotype as a specimen at GH. He also designated an isotype at that institution and at P. In the protologue, Gray never
cited a specific specimen. However, the holotype specimen designated by Wallace at GH has written on it in Gray’s own handwriting “Schweinitzia reynoldsiae, n. sp. Miss Reynolds Dec 1884.” The label for the isotype contains essentially the same information but is written in a hand other than that of Gray. The isotype specimen, as designated by Wallace, was also collected during December 1884. Reynolds apparently made many collections in 1883 and disseminated these widely, but seems to have made few collections during 1884. Some of the collections made prior to December 1884 have been designated as types by people other than Wallace (i.e. collections at NY), but these are in no way type specimens as currently recognized by the Code and are at best “topotypes.” Some Reynolds collections from 1884 exist at other institutions, but the month of collection seems at best uncertain and these specimens were probably not seen by Gray. The specimen at P was collected in December 1884 (no day given) and on a label accompanying it is a printed label reading “Ex Herb Gray” identical to those on specimens at GH. Therefore, it was likely seen by Gray when diagnosing Schweinitzia reynoldsiae. One specimen at NY collected on 1 December 1884 clearly states on the accompanying label that Gray did not see that specimen. No specific specimens are listed in the protologue, but Gray seems to have clearly designated this currently accepted holotype at GH as the type by placing the words “n. sp.” after the name. Although the time of collection is unambiguous, I am retaining this as a holotype instead of a lectotype. I am
also, with some reservation, retaining Wallace’s designation of the second specimen at GH an isotype, and the one at P as an isotype as well.

Specimens Examined

What follows is a list of all specimens of *Monotropsis reynoldsiae* examined for the morphometric analyses conducted in this study. See also the distribution map of this species (Figure 28).

Lassiter & Lassiter 614 (USF); Withlacoochee State Forest, McKethan Park, 29 Jan 1977, Wunderlin 5735 (USF); Lake Lindsey area N of Brooksville, 5 Jan 1978, Van Hoek s.n. (USF). Marion Co., Ocala National Forest, off Salt Springs Trail 1 mi S of intersection of SR 19 and CR 314, 7 Feb 1995, Miller & Lindler s.n. (FLAS); Ocklawah River Bridge near Eureka, 29 Jan 1996, Miller s.n. (FLAS). Pasco Co., on S side of Fla. 578 at Mission Rd. in Dade City, 19 Dec 1983, Demaree & Hall s.n. (FLAS). St. Johns Co., St. Augustine, 1884, Reynolds s.n. (MO); St. Augustine, 1 Dec 1884, Reynolds s.n. (BH, NY); St. Augustine, Oct Dec 1884, Reynolds s.n. (BH); St. Augustine, Reynolds s.n. (USCH); St. Augustine, Ex Herb. Gray, Dec 1884 (P, photograph); St. Augustine, collected also on Indian River, Nov 1883, Reynolds s.n. (P, photograph); near St. Augustine, Nov 1883, Reynolds s.n. (NY, US); 2 localities near St. Augustine, Oct Dec 1884, Reynolds s.n. (PH, US); 2 localities near St. Augustine, Oct 1874 Oct Dec 1883 and 1884, Reynolds s.n. (NY); near St. Augustine, Reynolds s.n. (US); St. Augustine Dec 1884, Reynolds s.n. (GH); E Florida on Indian River, May 1889, Reynolds s.n. (NY); Near St. Augustine Oct-Dec 1883, Reynolds s.n. (NY-2); Guana River State Park, Guana River Dam, along Tolomato River, 12 Jan 1992, Harrison, Harrison & Miller 416 (FLAS); near St. Augustine along the St. Sebastian River, Oct to Dec 1884, Reynolds s.n. (PH); St. Augustine, winter, Reynolds s.n. (PH). Volusia Co., E coast of Florida, 30 mi. S of St. Augustine, 28 Feb 1881, Curtiss s.n. (GH); Bulow Creek State Park, ca. 5.9 mi NW of the Old Dixie Hwy. bridge, 15 Nov 2008, Kunzer 2655 (USF).
**Figure 22:** General features of *Monotropsis* illustrated in *M. reynoldsiae*. A: Stamen (arrows) origination when alternate or when opposite nectary lobes (N) and petals (P). B: Base of inflorescence. Fungal hyphae are marked with an arrow. C: Next year’s inflorescence bud. (Black bar represents 1 mm).
Figure 23: Habit of *Monotropsis odorata*. A: Inflorescence, showing distinctly chaffy, light brown bracts and sepals. B: Same inflorescence slightly magnified. C: In situ in fruit, showing a distinctly baccate fruit with no lines of dehiscence (May 2011). D: A cluster of inflorescences *in situ*, just about to flower. Note how they blend in with the surrounding leaf litter (March 2010).
Figure 24: Floral morphology of *Monotropsis odorata*. A: Complete flower, sepals attached. B: sepals removed. Note lighter patches at base and apex of petals and campanulate corolla. C: Flower with 3 petals removed. Note the broadly capitate stigma. D: Gynoecium. (Bar = 1 mm).
Figure 25: Habitats of *Monotropsis*. That of *M. reynoldiaea* pictured above, that of *M. odorata* below. Note the relative openness of the habitat of *M. odorata*. 
Figure 26: Known distribution of *Monotropsis odorata*. This species occurs in the US states of Alabama, Delaware, Georgia, Kentucky, Maryland, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia. All dots represent an estimate of the exact location of the population, excluding the dot in Delaware and several in Virginia. For a state and county listing of specimens, see text.
Figure 28: Floral morphology of *Monotropis reynoldsiae*. A: Complete flower with sepals attached. Note the concolorous, slightly urceolate corolla. B: Flower with petals and sepals removed (two stamens have also been removed). C: Gynoecium. Note that the stigma is not as broadly capitate and distinctly angled as in *M. odorata*. (Bar represents 1 mm).
Figure 29: Known distribution of *Monotropsis reynoldsiiae*. This species is endemic to the state of Florida, USA. For a county listing of specimens, see text.
References


