Schilling, E.E. and A. Floden. 2013. Barcoding the Asteraceae of Tennessee, tribes Helenieae and Polymnieae. Phytoneuron 2013-81: 1–6. Published 14 October 2013. ISSN 2153 733X

BARCODING THE ASTERACEAE OF TENNESSEE, TRIBES HELENIEAE AND POLYMNIEAE

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ABSTRACT

Results from barcoding studies of tribes Helenieae and Polymnieae for the Tennessee flora using data from the nuclear ribosomal ITS marker region are presented and include first complete reports of this marker for 5 of the 11 species of these tribes that occur in the state. Sequence data from the ITS region separated all species of *Helenium* from Tennessee from one another, as well as from other, non-Tennessee species. Species of *Marshallia* were similarly distinct from one another for this marker. In contrast, species of *Polymnia* were much less divergent for the ITS region, and two of the four species had basically identical ITS sequences. The striking difference in amounts and patterns of interspecific variation for the ITS marker in Helenieae compared to Polymnieae and to other groups of Asteraceae adds to evidence that there has been considerable variability within the family in the timing and modes of divergence in southeastern North America.

KEY WORDS: *Helenium*, *Marshallia*, *Polymnia*, Helenieae, Polymnieae, molecular barcoding

The genera included in groups formerly recognized as tribes Heliantheae and Helenieae and now referred to as the "Heliantheae alliance" (Baldwin 2009) present some of the major remaining taxonomic problems in Tennessee Asteraceae. The presence of the distinctive Eupatorieae within the Heliantheae alliance has necessitated the recognition of several other lineages as tribes, including Helenieae and Polymnieae (Anderberg et al. 2007; Baldwin 2009). Helenieae now includes only a subset of the larger group of epaleate genera that formerly were considered to be a tribe of the same name. Polymnieae is a recently recognized tribe containing only *Polymnia*, the genus itself much reduced by recognition of *Smallanthus* Mack. as distinct (Robinson 1978; Rauscher 2002). The current study of these two tribes continues the effort to characterize the levels and patterns of molecular diversity found in species of Asteraceae in Tennessee and southeastern North America (Schilling & Floden 2012; Schilling 2013) and to assess the potential of the nuclear ribosomal ITS region as a molecular barcode to identify species.

Helenieae is represented in Tennessee by three genera and eight species (Chester et al 2009). The single species of *Gaillardia* is considered to be introduced as a garden escape that is native further to the south and west in North America (Marlowe & Hufford 2007), but the Tennessee species of *Helenium* and *Marshallia* are considered to be natives. *Helenium* is a widespread genus, reaching South America, with the Tennessee species part of its easternmost distribution (Bierner 1972a, b). One species, *H. brevifolium*, is known from only two counties in Tennessee and is listed as endangered for the state (Crabtree 2012). *Marshallia* is endemic to the southeastern USA. with its species extending from Texas to as far north as Pennsylvania (Watson & Estes 1990). The species of *Marshallia* are somewhat sporadic in occurrence, and all of the Tennessee species are considered rare in the state (Crabtree 2012) with one, *M. grandiflora*, considered to be globally imperiled (G2 listing in NatureServe).

Polymnia has been found to represent a highly distinctive lineage that is sister to a large clade containing several tribes including Heliantheae sensu stricto and Eupatorieae (Anderberg et al. 2007). The genus is entirely North American in geographic distribution and currently comprises four species (Estes & Beck 2011). The recently described *P. johnbeckii* is known only from Tennessee and is considered to be globally rare (G1 listing in NatureServe). The genus exhibits considerable plasticity at several levels (e.g., Bender et al. 2000) and the species level taxonomy is still under investigation.

The goal of this study was to conduct a survey of variation for the ITS marker for all species of Helenieae and Polymnieae that occur in Tennessee. The presence of genera endemic to the southeastern USA made the tribes of particular interest and, for both, sampling was extended to include other species of the southeastern USA to assess the pattern of variability across this region.

Table 1. Plant material used for ITS barcoding studies of Helenieae and Polymnieae. All voucher specimens at TENN. *sequence submitted to GenBank, unprocessed because of U.S. government shutdown.

Species	DNA#	Genbank	Voucher info
HELENIEAE			
GAILLARDIA Foug.			
G. pulchella Foug.	3850	KF607074	*Schilling 13-03*, Knox Co., TN
HELENIUM L.			
H. amarum (Raf.) H. Rock	3096	KF607067	*Estes 3706*, Giles Co., TN
H. autumnale L.	2569	KF607068	*Schilling CF8*, Unicoi Co., TN
H. brevifolium Wood	3097	KF607069	*Bailey & Shaw s.n*, 6/21/2001, Cumberland Co., TN
H. flexuosum Raf	3098	KF607070	*DeSelm 06-03*, Monroe Co., TN
Non-Tennessee samples			
H. pinnatifidum Rydb.	3907	KF607071	*McNeilus 90-21*, Clinch Co., GA
H. quadridentatum Labill.	3909	KF607072	*Thomas 117510*, Assumption Par., LA
H. vernale Walter	3908	KF607073	*McNeilus 01-44*, Camden Co., GA
MARSHALLIA Schreb.			
M. grandiflora Beadle & Boynt.	3303	KF607075	*Floden s.n.*, Garden grown specimen
M. obovata (Walt.) Beadle & Boynt.	3066	KF607076	*Rothberger s.n.* 6/7/87, Polk Co., TN
M. trinervia (Walt.) Trel.	3067	KF607077	*Horn 2006-7*, Lawrence Co., TN
POLYMNIEAE			
POLYMNIA L.			
P. canadensis L.	723	KF607079	*Schilling 2017*, Knox Co., TN
	3529	KF607078	*Floden & Estes 1331*, Hickman Co., TN
P. johnbeckii D.Estes	3527	KF607080	*Floden et al. 1044*, Marion Co., TN
P. laevigata Beadle	1133	KF607082	*Schilling PL-1*, Polk Co., TN
	1134	KF607081	*Schilling PL-4*, Polk Co., TN
Non-Tennessee sample			
P. cossatotensis Pittman & Bates	3948	*	*Pittman & Bates 7222*, Montgomery Co., AR

Materials and Methods

DNA was extracted from leaf samples either collected fresh or taken from herbarium specimens (Table 1). DNA extraction, PCR amplification, and sequencing protocols followed Schilling and Floden (2012). Samples that had a length polymorphism in the ITS region were sequenced with multiple primers to allow "clean" sequence to be obtained from each direction up to the site of the polymorphism. GenBank accession numbers are provided in Table 1. Although this study was not designed to undertake a rigorous phylogenetic analysis, a neighbor joining tree was generated using PAUP * 4.0b10 (Swofford 2003) to provide a convenient way to make a comparative visualization of the sequence results and rooted using an ITS sequence from GenBank for *Athroisma hastifolia*, an early diverging member of the Heliantheae alliance (Anderberg et al. 2007). The analysis also utilized sequences deposited at GenBank of conspecific samples or closely related species.

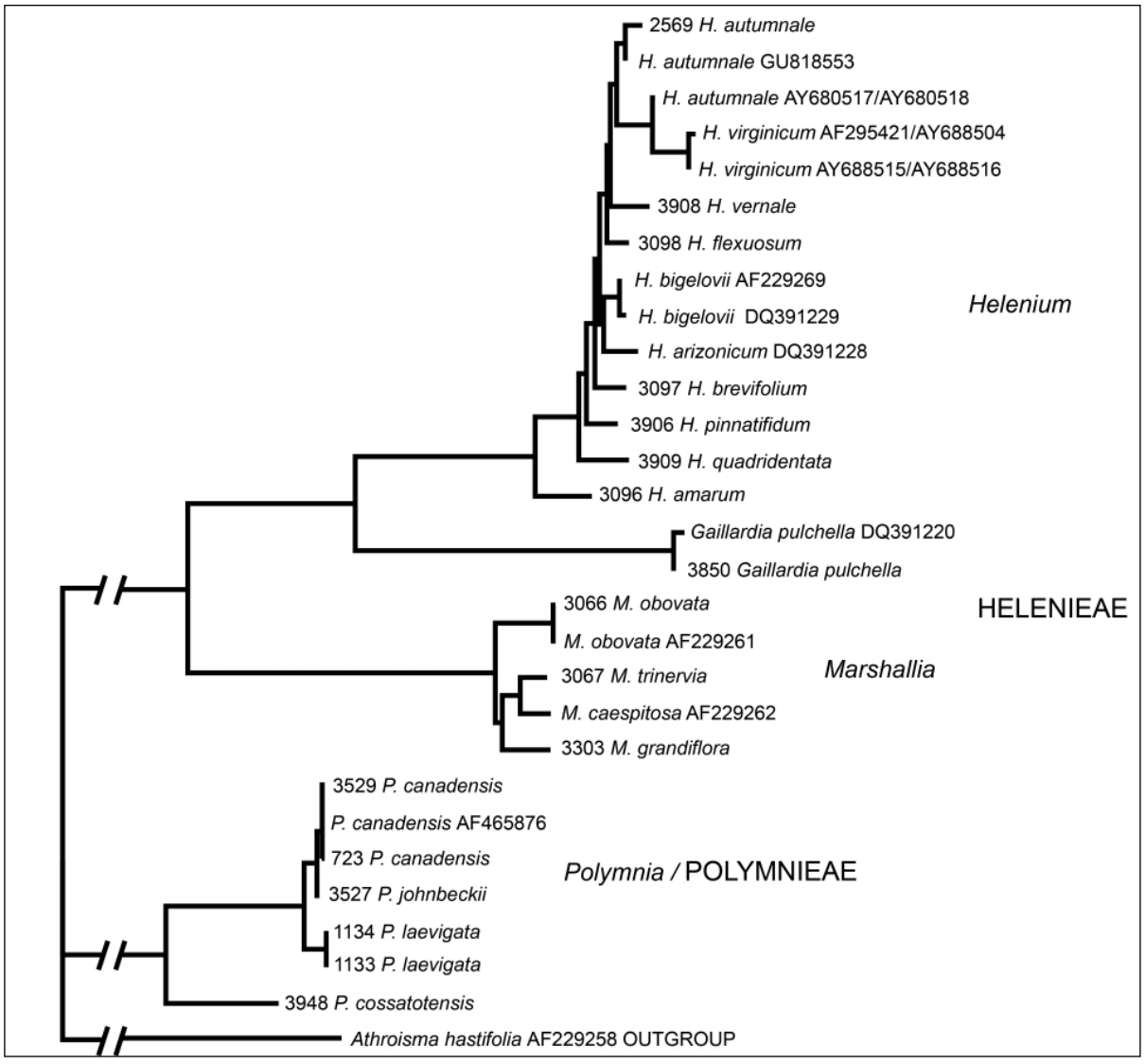

Figure 1. Neighbor-joining tree showing relationships of species of Helenieae and Polymniae based on ITS sequence data, using *Athroisma hastifolia* as the outgroup. Newly obtained sequences designated by DNA number preceding species name (Table 1); GenBank numbers for other sequences follow species name.

Results and Discussion

Newly obtained ITS sequences for Helenieae, including first reports for four species, ranged in length from 643-650 bp. Sequences of *Helenium* were 646-650 bp in length whereas those of *Marshallia* were 643-646 bp. The sequences for Helenieae had no length polymorphisms and relatively few positional polymorphisms (i.e., a double peak at a single position). This suggests that there is little or no interspecific hybridization within the tribe, in contrast to other groups of Asteraceae from the southeastern USA.

The species of Helenieae from Tennessee were well differentiated based on ITS sequence results (Fig. 1). The divergence between sampled species within genera was relatively high, ranging from about 2% to 5% (a minimum of 10 differences between species of *Helenium* and 15 between species of *Marshallia*). The newly obtained sequences for Helenieae also matched almost exactly their conspecific records in GenBank where available. The newly obtained sequence for *Helenium autumnale* differed by 3 bp from that in GenBank; the newly obtained and GenBank sequences for *Marshallia obovata* were essentially identical. Thus, ITS sequence data provide a molecular barcode that would uniquely identify species of *Helenium* and *Marshallia* from Tennessee.

The species-level differentiation for the ITS region within *Helenium* is particularly notable. The most divergent Tennessee species was *H. amarum* (Fig. 1), but this species is part of distinctive section of the genus and is probably a recent introduction from western North America that has spread along roads (Bierner 1972b, 1989). There is little phylogenetic structure among the remaining species of the southeastern USA (Fig. 1), including three endemic to the coastal plain, *H. pinnatifidum*, *H. quadridentatum*, and *H. vernale*, but all are distinctive in ITS sequence. These data add further perspective to the distinctiveness of *H. virginicum*, whose rarity and unusual disjunct distribution in Virginia and Missouri have made it the subject of detailed study (Simurda & Knox 2000; Simurda et al. 2005; Rimer & Summers 2006) and suggest that other morphologically distinct populations of *H. autumnale* may deserve additional analysis. There was no clear correspondence of the ITS data with the sectional classification proposed by Rock (1957) or Bierner (1972a, 2006). There were also no indications, such as positional polymorphisms, from the sequence data to support a hybrid origin for *H. flexuosum* (Bierner 2006).

Newly obtained sequences for *Polymnia*, including one new species report, were uniformly 640 bp in length, matching previous reports except for *P. cossatotensis* which is 641 bp. A length polymorphism was present, however, in ITS sequences for three individual samples, two of *P. canadensis* and one of *P. laevigatus*, all at a common location and different from the position of the extra base insertion in *P. cossatotensis*. Inference from the direct sequence results suggested that a second ITS copy with a 2 bp deletion accounted for the length polymorphism; there were only 1-2 positional polymorphisms in these samples, so they do not appear to be of interspecific hybrid origin. The sample of *P. johnbeckii* had eight positional polymorphisms, but only two were in locations where there was any variability among other samples.

The species of *Polymnia* from Tennessee were not well differentiated from one another based on the ITS sequence results (Fig. 1). Sequences of *P. laevigata* consistently had five bp differences (two in ITS-1 and three in ITS-2) compared to those of *P. canadensis*. The ITS sequence of *P. johnbeckii* was identical, other than for positional polymorphisms, to those from *P. canadensis*. The ITS sequence for *P. cossatotensis*, which was rerun for this study, was conspicuously distinctive compared to its congeners, with differences at 45-50 positions (7.5-8.2%). Thus, this species is distinctive both at the molecular as well as at the morphological level (Pittman & Bates 1989; Hardcastle et al. 2007), and its continued protection should be a high priority.

The results of this study highlight two problematic aspects of GenBank as a database for species identification using a barcoding approach. One is simply the incompleteness of the dataset; the data presented here provide the first reports of the ITS region for almost half (5/11) of the Tennessee species of Helenieae and Polymniae. The second is the apparent error in the GenBank sequence reports for *Polymnia laevigata* (AF465878) and *P. cossatotensis* (AF465878) that led us to resample the latter species. It appears that the Genbank sequences for these two species were inadvertently mixed, such that the one for *P. laevigata* contains the correct ITS-1 region, but the ITS-2 region of *P. cossatotensis*, and vice versa. According to the paper that reports these (Rauscher 2002), the ITS-1 and ITS-2 regions were amplified separately for these samples, making it conceivable that they were reassembled incorrectly. This reflects the limitations of older technology and also the lack of scrutiny of outgroup samples that can occur when they are not the main focus of a study.

The striking difference in amounts and patterns of interspecific variation for the ITS marker in Helenieae compared to Polymnieae and to other groups of Asteraceae adds to evidence that there has been considerable variability within the family in the timing and modes of divergence in southeastern North America. Helenieae is similar to Cardueae in having differentiation at the species level for ITS sequences (Schilling 2013). Both Helenieae and Cardueae appear to have arrived in southeastern North America from further west and either differentiated before arriving or have been in the region long enough for differentiation to occur. Polymnieae is more similar to Vernonieae (Schilling 2013) in exhibiting little differentiation among species. The presence of both length and positional polymorphisms in the ITS region of samples of *Polymnia* suggest that there may be additional variability to be uncovered by further sampling of the genus.

ACKNOWLEDGEMENTS

Thanks to N. Mattson and J. Schilling for laboratory assistance, J. May and V. Brown of the University of Tennessee MBRF for help with DNA sequencing, C. Hansen (Auburn University), J. Beck (Wichita State University), and M. Bierner (University of Texas) for helpful comments, and the numerous collectors who have helped to assemble a broad and useful herbarium at TENN. Financial support provided by the Hesler Fund of the University of Tennessee Herbarium.

LITERATURE CITED

Anderberg, A.A., B.G. Baldwin, R.G. Bayer, J. Breitwieser, C. Jeffrey, M.O. Dillon, P. Eldenäs, V. Funk, N. Garcia-Jacas, D.J.N. Hind, P.O. Karis, H.W. Lack, G. Nesom, B. Nordenstam, Ch. Oberprieler, J.L. Panero, C. Puttock, H. Robinson, T.F. Stuessy, A. Susanna, E. Urtubey, R. Vogt, J. Ward, and L.E. Watson. 2007. Compositae. Pp. 61–588, in J.W. Kadereit and C. Jeffrey (eds). The Families and Genera of Vascular Plants. VIII. Flowering plants, eudicots, asterales. Berlin: Springer..

Baldwin, B. 2009. Heliantheae alliance. Pp. 689–711, in V.A. Funk, A. Susanna, T.F. Stuessy, and R.J. Bayer (eds.). Systematics, Evolution, and Biogeography of the Compositae. International Association for Plant Taxonomy, Vienna.

Bender, J.H., J.M. Baskin, and C.C. Baskin. 2000. Ecological life history of *Polymnia canadensis*, a monocarpic species of the North American temperate deciduous forest: demography. Pl. Ecol. 147: 117–136.

Bierner, M.W. 1972a. Taxonomy of *Helenium* sect. *Tetrodus* and a conspectus of North American *Helenium* (Compositae). Brittonia 24: 331–355.

Bierner, M.W. 1972b. The taxa of *Helenium* in Tennessee. Castanea 39: 346–349.

Bierner, M.W. 1989. Taxonomy of *Helenium* sect. *Amarum* (Asteraceae). Sida 13: 453–459.

Bierner, M.W. 2006. *Helenium*. Pp. 426–435 in Flora of North America Editorial Committee (eds.). Flora of North America North of Mexico, Vol. 21. Oxford University Press, New York.

Chester, E.W., B.E. Wofford, D. Estes, and C.J. Bailey Jr. 2009. A Fifth Checklist of Tennessee Vascular Plants. Sida Bot. Misc. No. 31. Bot. Res. Inst. of Texas, Fort Worth.

Crabtree, T. 2012. Tennessee Natural Heritage Program rare plant list. <http://www.tn.gov/environment/na/pdf/plant_list.pdf> Accessed 22 Aug 2013.

Estes, D. and J. Beck. 2011. A new species of *Polymnia* (Asteraceae: tribe Polymnieae) from Tennessee. Syst. Bot. 36: 481–486.

Hardcastle, E.L., R.M. McElderry, and J.L. Gentry. 2007. Distribution, habitat, and current population size estimates for *Polymnia cossatotensis* (Cossatot Leafcup) [Asteraceae], a rare Arkansas endemic. Castanea 72: 194–204.

Marlowe, K. and L. Hufford. 2007. Taxonomy and biogeography of *Gaillardia* (Asteraceae): A phylogenetic analysis. Syst. Bot. 32: 208–226.

Pittman, A.B. and V. Bates. 1989. A new species of *Polymnia* (Compositae: Heliantheae) from the Ouachita Mountain region of Arkansas. Sida 13: 481–486.

Rauscher, J.T. 2002. Molecular phylogenetics of the *Espeletia* complex (Asteraceae): evidence from nrDNA ITS sequences no the closest relatives of an Andean adaptive radiation. Amer. J. Bot. 89: 1074–1084.

Rimer, R.L. and J.W. Summers. 2006. Range and ecology of *Helenium virginicum* in the Missouri Ozarks. Southeast. Nat. 5: 515–522.

Robinson, H. 1978. Studies in the Heliantheae (Asteraceae). XII. Re-establishment of the genus *Smallanthus.* Phytologia 39: 47–53.

Rock, H.F.L. 1957. A revision of the vernal species of *Helenium* (Compositae). Rhodora 59: 101–116, 128–158, 168–178, 203–216.

Schilling, E.E. 2013. Barcoding the Asteraceae of Tennessee, tribes Cardueae and Vernonieae. Phytoneuron 2012-99: 1–6.

Schilling, E.E. and A. Floden. 2012. Barcoding the Asteraceae of Tennessee, tribes Gnaphalieae and Inuleae. Phytoneuron 2012-99: 1–6.

Simurda, M.C. and J.S. Knox. 2000. Phylogeography of the narrow endemic *Helenium virginicum* (Asteraceae), based upon ITS sequence comparisons. Syst. Bot. 30: 887–898.

Simurda, M.C., D.C. Marshall, and J.S. Knox. 2005. ITS sequence evidence for the disjunct distribution of the narrow endemic *Helenium virginicum* (Asteraceae). J. Torr. Bot. Soc. 127: 316–323.

Swofford, D.L. 2003. PAUP* – Phylogenetic Analysis Using Parsimony (*and other methods), version 4.0b10. Sunderland, Massachusetts: Sinauer.

Watson, L.E. and J.R. Estes. 1990. Biosystematic and phenetic analysis of *Marshallia* (Asteraceae). Syst. Bot. 15: 403–415.