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BARCODING THE ASTERACEAE OF TENNESSEE, TRIBE SENECIONEAE

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ABSTRACT

Results from barcoding studies of tribe Senecioneae for the Tennessee flora using data from the nuclear ribosomal ITS marker region are presented and include first complete reports of this marker for 3 of the 15 species of these tribes that occur in the state. Sequence data from the ITS region separated all Tennessee species of *Arnoglossum*, *Erechtites*, *Hasteola*, and *Rugelia* (all of which are native) from one another and from other, non-Tennessee congeners. In contrast, many of the species of *Packera*, both from the state and from other parts of the southeastern USA, had basically identical ITS sequences. The contrast in the distinctiveness of *Arnoglossum* species compared to those of *Packera* suggests the two genera have had different histories of introduction and diversification in southeastern North America.

Tribe Senecioneae is one of the largest in Asteraceae and with a worldwide distribution has had the opportunity to diversify in many different regions. The boundaries and circumscription of the tribe have, however, changed over the past few decades, and its generic level circumscription is still being settled (Nordenstam et al. 2009; Pelser et al. 2007, 2010). Notable is the problem of the circumscription of the huge *Senecio* (ca. 1000 species), but changes have also affected other genera from the southeastern USA, most notably the recognition of *Arnoglossum* and *Hasteola* as distinct from *Cacalia* (Anderson 1974). The nuclear ribosomal ITS region has been surveyed widely in studies of the tribe (Bain & Golden 2000; Pelser et al. 2007, 2010), but there has never been a focus on species that occur in the southeastern USA, and some species remain unsampled. The current study continues the effort to survey the molecular diversity found in species of Asteraceae in Tennessee (Schilling & Floden 2012, 2013; Schilling 2013), with an increasing emphasis on revealing patterns in levels of interspecific differentiation in the ITS marker, in addition to its potential use as a barcoding region.

Senecioneae is represented in Tennessee by 7 genera and 15 species (Chester et al. 2009), of which all but two species are considered to be native members of the flora. The non-natives are *Senecio vulgaris* and *Tussilago farfara*, both widespread elsewhere as weeds and considered potential threats as invasives. Other species formerly recognized within *Senecio* in Tennessee are now placed in *Packera* (Löve & Löve 1976; Bain & Golden 2000). Several species of the tribe are listed as rare in Tennessee (Crabtree 2012), including *Arnoglossum plantagineum*, *Hasteola suaveolens*, *Packera schweinitziana*, and *Rugelia nudicaulis*, but of these only the monotypic *Rugelia* is rare globally. Also listed as rare in Tennessee is *Packera plattensis*, although it has been proposed that Tennessee populations previously assigned to this species be recognized as *P. paupercula* var. *appalachiana* (Mahoney & Kral 2008).

The goal of this study was to complete the sampling for the ITS marker for all species of Senecioneae that occur in Tennessee. Particular emphasis was placed on the two genera *Arnoglossum* and *Packera*, which exhibit radiations in southeastern North America, and sampling of additional species of both genera from areas of southeastern North America outside of Tennessee was done to allow evaluation of the overall patterns of diversification and to compare them to other Asteraceae genera of the region.

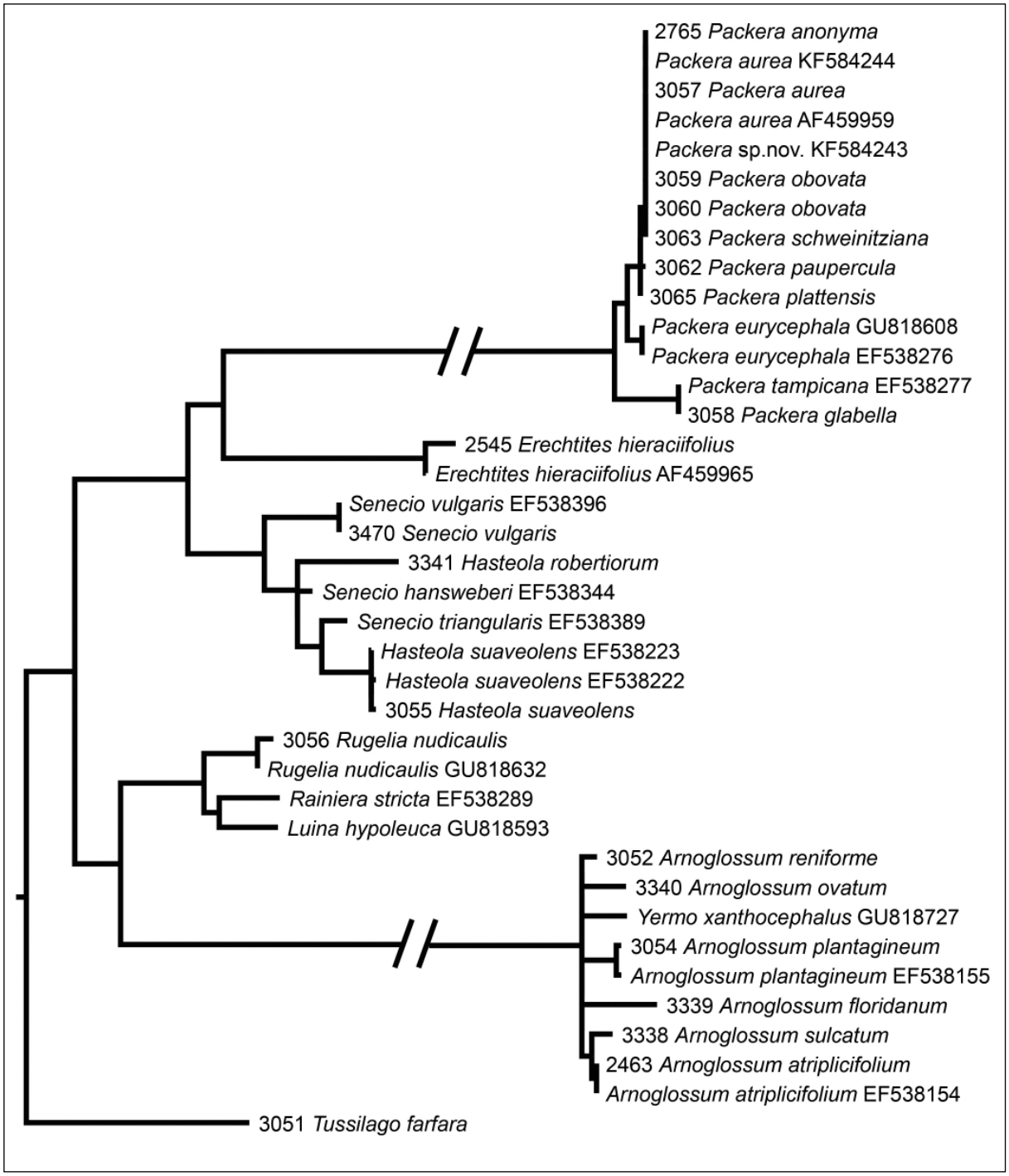

Figure 1. Maximum likelihood bootstrap tree (500 replicates) showing relationships of species of Senecioneae based on ITS sequence data, using *Tussilago farfara* as the root. Newly obtained sequences designated by DNA number preceding species name (Table 1); GenBank numbers for other sequences follow species name.

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). A sample that had a length polymorphism in the ITS region was

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, maximum likelihood analyses using the MEGA5 program (Tamura et al. 2011) were utilized to provide comparative visualization of the sequence results. The resulting tree was rooted using the sample of the introduced *Tussilago farfara*, based on results of Pelser et al. (2007, 2010). The analysis also incorporated sequences deposited at GenBank of conspecific samples or closely related species.

Results and Discussion

Newly obtained ITS sequences for Senecioneae ranged in length from 623-643 bp. Sequences of *Packera* were mostly 625 bp, but *P. glabella* was 628; sequences of *Arnoglossum* were 637-644 bp; those of *Hasteola* were 640 or 644 bp. Only a single species was observed to have a length polymorphism in the ITS region, *H. suaveolens*, in which there was a 2 bp indel that varied between copies. The number of positional polymorphisms (inferred from a double peak on the sequencing electropherogram) was relatively low for all samples, varying from 0 to 5 in individual samples.

The ITS sequences of the sampled genera of Senecioneae were quite different from one another (Fig. 1). Relative to the designated outgroup, *Tussilago farfara*, the species were placed into about five clades in the consensus ML tree (Fig. 1). The species of *Arnoglossum* formed one clade, within which the rare, monotypic western North American *Yermo* was placed as well as the non-Tennessee species *A. floridanum*, *A. ovatum*, and *A. sulcatum*. Sister to the *Arnoglossum* group was a clade that included *Rugelia nudicaulis*, which was quite distinctive from the other Tennessee species of Senecioneae. *Rugelia* was placed in a clade with two small genera of western North America, *Rainiera* Greene and *Luina* Benth., in agreement with the results of Pelser et al. (2010). A third clade included species of *Senecio* and *Hasteola* (Fig. 1). Consistent with results reported by Pelser et al. (2007, 2010), *Hasteola* is phylogenetically embedded within *Senecio*, and its current two species likely should be included within *Senecio*. Besides *H. suaveolens*, which occurs in Tennessee, the only other member of *Hasteola* is the Florida panhandle endemic *H. robertiorum*, and not only was there a significant difference between the two species for ITS sequence, they were not even placed as monophyletic in the ML tree (Fig. 1). A fourth group was formed by the two included samples of *Erechtites*, which was placed as sister to *Packera*, albeit with weak support. All of the species of *Packera* were grouped into a single clade within which *P. aurea* and similar species formed a large polytomy and *P. glabella* was placed with (and almost identical to) *P. tampicana* from western North America.

Within genera with more than one species, there was a conspicuous difference among genera in the amount of interspecific variability. Within *Arnoglossum*, for example, all of the species differed from one another by at least 10 bp (3%). In contrast, the Tennessee species of *Packera*, except for *P. glabella*, were essentially identical to one another for ITS sequence. The lack of differentiation in ITS sequence extended to a sample of *P. plattensis* from Oklahoma, which made it impossible to evaluate whether or not the Appalachian populations formerly assigned to this species are distinct from it. A barcoding approach using ITS sequence data could thus only verify identifications of members of *Packera* to genus.

The striking differences in the amounts of interspecific divergence among the different genera of Senecioneae in the southeastern USA suggest that they have undergone divergence at different time intervals. The large amount of divergence within *Arnoglossum* species, and their apparent close relationship to *Yermo* Dorn from western North America, would be consistent with a relatively long history in southeastern North America; Pelser et al. (2010) dated the divergence of the two genera at ca 1.2–1.3 ma (million years ago). The divergence between *Rugelia* and its sister group from western North America is even older, at about 2.3–2.5 ma (Pelser et al. 2010), suggesting that it occurred at a

different time and possibly route than for *Arnoglossum*. Similarly, the large number of differences in ITS sequence between the two species of *Hasteola* also would be consistent with a relatively old separation, and their relationships within *Senecio* may point to separate arrivals in eastern North America. In contrast the lack of divergence among morphologically distinct species of *Packera* suggests a relatively recent arrival in eastern North America.

The results of BLAST searches in GenBank for members of Senecioneae generally gave a top match to a conspecific sample, if the species had been sampled, and sequences already deposited for species represented in Tennessee generally matched closely if not identically the newly sampled ones. The exceptions included a sample labeled *Erechtites hieraciifolius* (EF107652), which differed improbably at almost 30 positions compared to other samples of this species, and a sample labeled *Petasites japonicus* (FJ980332) which gave a nearly identical match to sequences from samples of *Tussilago farfara*; both of these apparently erroneous records stemmed from reports described as studies of Chinese medicinal plants. These results provide further examples to show that GenBank cannot be used uncritically as a reference for comparison of molecular barcoding data.

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Table 1. Plant material used for ITS barcoding studies of Senecioneae. All voucher specimens at TENN.

Species	DNA#	Genbank	Voucher info
ARNOGLOSSUM Raf.			
A. atriplicifolium (L.) H. Rob.	2563	KJ418356	*Schilling CF-11*, Unicoi Co., TN
A. plantagineum Raf.	3054	KJ418354	*Estes 3384*, Marshall Co., TN
A. reniforme (Hook.) H. Rob.	3052	KJ418353	*Clements 224*, Franklin Co., TN
Non-Tennessee samples			
A. floridanum (A.Gray) H. Rob.	3339	KJ418358	*Beck 9096*, Putnam Co., FL
A. ovatum (Walter) H. Rob.	3340	KJ418355	*Thomas 107296*, Natchitoches Par., LA
A. sulcatum (Fernald) H. Rob.	3338	KJ418357	*McNeilus 01-349*, Camden Co., GA
ERECHTITES Raf.			
E. hieraciifolius (L.) Raf. ex DC.	2545	KJ418341	*Schilling 07-DNA2545*, Knox Co., TN
HASTEOLA Raf.			
H. suaveolens (L.) Pojark.	3055	KJ418351	*Estes 9196*, Wayne Co., TN
Non-Tennessee sample			
H. robertiorum L.C. Anderson	3341	KJ418352	*Kral 64504*, Levy Co., FL
PACKERA Á. Löve & D. Löve			
P. anonyma (Wood) Weber & Á. Löve	2765	KJ418348	*Schilling 08-DNA2765*, Knox Co., TN
P. aurea (L.) Á. Löve & D. Löve	3057	KJ418347	*Floden 866*, Campbell Co., TN
P. glabella (Poir.) C.Jeffrey	3058	KJ418349	*Deselm 06-04*, Bradley Co., TN
P. obovata (Willd.) Weber & Á. Löve	3059	KJ418345	*Rhinehart s.n. 5/2/2005*, Campbell Co., TN
P. obovata (Willd.) Weber & Á. Löve	3060	KJ418346	*Estes 8742*, Cumberland Co., TN
P. paupercula (Michx.) Weber & Á. Löve	3461	KJ418343	*Shaw et al. 682*, Scott Co., TN
P. schweinitziana (Nutt.) Weber & Á. Löve	3063	KJ418344	*DeSelm 01-067*, Unicoi Co., TN
Non-Tennessee sample			
P. plattensis (Nutt.) Weber & Á. Löve	3065	KJ418342	*Taylor 31314*, Taylor Co., OK
RUGELIA Shuttlew. ex Chapm.			
R. nudicaulis Shuttlew. ex Chapm.	3056	KJ418340	*Phillippe 40488*, Sevier Co., TN
SENECIO L.			
S. vulgaris L.	3502	KJ418350	*Schilling 12-DNA3502*, Knox Co., TN
TUSSILAGO L.			
T. farfara L.	3051	KJ418339	*Floden 250*, Campbell Co., TN