Evaluation of feral hog (*Sus scrofa*) diet in the Great Smoky Mountains National Park through the use of DNA metabarcoding

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Introduction

Feral hogs (*Sus scrofa*), hybrids of Eurasian wild boar and feral domestic pigs, are one of the most invasive vertebrates globally[1,2], posing a significant threat to hundreds of taxa and serving as the primary factor in the extinction of several species[3]. Feral hogs are omnivores; their diet consists primarily of plants but also includes a number of animal taxa[3,4].



Figure 1. A wild hog (*Sus scrofa*). Photo courtesy of Discover Wildlife (www.discoverparksandwildlife.com).

The introduction of feral hogs in the Southern Appalachians predates the establishment of the Great Smoky Mountains National Park (GSMNP) in 1934[4], and by the 1940s, feral hogs had colonized the newly established park[5–8]. Numerous studies have demonstrated the threat which feral hogs pose to the flora[5] and fauna[4] of the park, including impacts on salamander populations[5,9,10]. Feral hog control programs were initiated in the park in 1959[11]; however, the extensive size of the park and vast areas of rugged topography complicated these efforts. Both feral hogs and ongoing control efforts exist in the park today[11].



Figure 2. Hog trap on the North Carolina side of the Great Smoky Mountains National Park. Photo courtesy of Hikewithgravity.com.

The recent emergence of DNA metabarcoding, including both second-[12] and third-generation sequencing[13], has transformed approaches to wildlife dietary studies[14–16], providing an effective tool cable of high-resolution diet composition evaluation[17–19]. Numerous studies have demonstrated the efficacy of fecal metabarcoding studies in the evaluation of feral hog diets[20–23]. Although several park studies have focused on the impact of feral hogs to GSMNP ecosystems[5,9], including diet composition[10], none have yet applied DNA metabarcoding to evaluate diet composition of park feral hogs.

Methods

Swine fecal sample collection

Feral swine were captured in drop traps in two locations in the GSMNP between August 3 and August 30, 2025. GSMNP wildlife officials determined the age, sex, and condition of captured hogs. Feces was collected directly from the rectum and stored at -20°C until analysis.

DNA extraction

DNA was extracted using an Omega Bio-Tek E.Z.N.A. Tissue Kit according to protocol. Proteinase K incubation was conducted for 12 hours and final elutions were conducted twice in 200 µl of elution buffer (400 µl total). Extracted DNA concentration was analyzed using a Qubit 2.0.

Inhibitor removal

Each sample was treated with a OneStep Inhibitor Removal Kit (Zymo Research).

NGS library preparation

The primers “ITS2-2For” and “ITS2-3Rev” were used to amplify plant DNA found in fecal samples in a manner similar to recent studies in other species[26]. These primers amplify approximately 475 nucleotides of the ITS2 region of nuclear ribosomal DNA, and their efficacy in plant identification is well established[27,28]. Primers were modified with adaptors including binding sites for a round two primer. Site-specific barcodes were added in a second PCR to enable the identification of individual hog fecal samples.

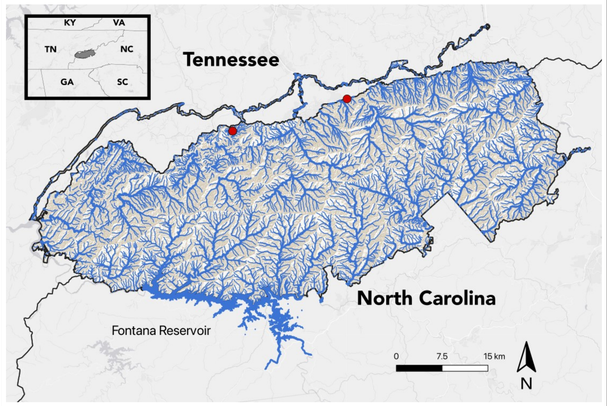


Figure 3. Location of feral hog collection sites in the GSMNP.

Sequencing

Samples were quantified (Qubit) and then pooled in an equimolar fashion prior to sequencing. Samples were sequenced utilizing Oxford Nanopore Technologies PromethION; sequencing was completed by Eurofins Genomics.

Bioinformatics

Unmodified reads were command-line BLASTed (BLASTn) against the NCBI non-redundant nucleotide database using the remote flag (-remote) and –max_hps to limit the results to only the highest scoring alignments. All other parameters, including word size, were default BLASTn settings. The highest scoring result for each amplicon was manually checked for presence in the GSMNP, matches with no park records were disregarded, and later results considered until a match was found. Alignments with similarities greater than 90% were utilized for genus level assignment, and alignments with 97% or greater similarity were used for species level assignment.

Results

A total of 1,726 reads were obtained, 1,524 (88.2%) of which yielded matches exceeding 90% in BLASTn searches. A total of 39 plant genera were identified in hog fecal extracts (Fig. 4 and Table 1). Ten genera comprised 94% of the identifiable reads (Fig. 4). Three genera, *Quercus* (oak), *Juglans* (walnut), and *Carya* (hickory) made up 67% of the total reads (Fig. 4).

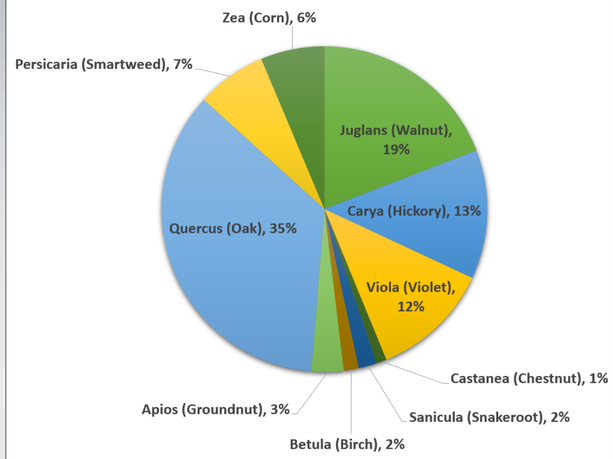


Figure 4. Percent composition of the ten plant genera occurring most frequently in wild hog fecal samples (5) from the Great Smoky Mountains National Park.

Twenty-nine additional species from 17 families comprised the remaining six percent of the identifiable reads (Table 1). With the exception of *Zea* (corn used in baiting), the top ten genera observed in hog diets were all species native to the park (Fig. 4). Invasive exotics such as *Pueraria* (Kudzu) and *Lonicera* (Honeysuckle) were observed as minor components of hog diet (Table 1).

Table 1. Percent composition of plant genera comprising less than 1% of the total reads in wild hog fecal samples (5) from the Great Smoky Mountains National Park.

Family	Genus	Percent
Acanthaceae	*Justicia*	0.1
Apiaceae	*Osmorhiza*	0.5
	Oxypolis	0.3
	Cicuta	0.2
	Cryptotaenia	0.2
Betulaceae	*Carpinus*	0.5
	Alnus	0.1
	Corylus	0.1
Bignoniaceae	*Bignonia*	0.4
	Fridericia	0.1
Boraginaceae	*Phacelia*	0.1
Caprifoliaceae	*Lonicera*	0.1
Celastraceae	*Euonymus*	0.1
Cucurbitaceae	*Cucumis*	0.1
Ericaceae	*Leucothoe*	0.5
	Vaccinium	0.3
	Rhododendron	0.1
Fabaceae	*Pueraria*	0.4
	Trifolium	0.4
	Lespedeza	0.3
	Vicia	0.1
	Glycine	0.1
Malvaceae	*Tilia*	0.1
Myricaceae	*Myrica*	0.1
Nyssaceae	*Nyssa*	0.2
Poaceae	*Eragrostis*	0.1
Ranunculaceae	*Actaea*	0.3
Rosaceae	*Amelanchier*	0.4
Urticaceae	*Pilea*	0.1

Results

A total of 27 species level identifications (≥ 97% similarity) from 17 genera were completed (Table 2). These included 19 native species as well as seven non-native species in addition to *Zea mays* used in the capture process (Table 2).

Table 2. Species level identifications (≥ 97%) from wild hog fecal samples (5) from the Great Smoky Mountains National Park.

Genus	Species	Common Name	Native
Actaea	*Actaea racemosa*	Black Bugbane	Y
Apios	*Apios americana*	Groundnut	Y
Betula	*Betula pendula*	European white birch	N
Bignonia	*Bignonia capreolata*	Crossvine	Y
Carya	*Carya cordiformis*	Bitternut hickory	Y
	Carya glabra	Pignut hickory	Y
	Carya illinoinensis	Pecan	N
	Carya tomentosa	Mockernut hickory	Y
Juglans	*Juglans nigra*	Black walnut	Y
Leucothoe	*Leucothoe fontanesiana*	Mountain doghobble	Y
Oxypolis	*Oxypolis rigidior*	Stiff cowbane	Y
Persicaria	*Persicaria hydropiper*	Marshpepper Smartweed	Y
	Persicaria japonica	Japanese knotweed	N
Persicaria	*Persicaria maculosa*	Spotted Ladysthumb	N
	Persicaria virginiana	Jumpseed	Y
Pilea	*Pilea pumila*	Canadian Clearweed	Y
Pueraria	*Pueraria montana*	Kudzu	N
Quercus	*Quercus alba*	White oak	Y
	Quercus falcata	Southern red oak	Y
	Quercus montana	Chestnut oak	Y
	Quercus muehlenbergii	Chinkapin oak	Y
	Quercus rubra	Red oak	Y
Sanicula	*Sanicula canadensis*	Canadian Blacksnakeroot	Y
	Sanicula marilandica	Maryland Sanicle	Y
Trifolium	*Trifolium pratense*	Red clover	N
Vicia	*Vicia sativa*	Garden Vetch	N
Zea	*Zea mays*	Corn or maize	N

Conclusions

- Our results are consistent with a previous study reporting DNA reads from deciduous, mast-producing trees as the most abundant sequences in feral hog diet in July and August in South Carolina[22].
- Scott and Pelton[12] reported fruits and seeds as the dominant food item during summer in GSMNP feral hogs in a stomach content study; while our technique is unable to discern which plant part has been consumed, these results appear consistent with our results.
- The appearance of *Castanea* (Chestnut) is interesting. American Chestnuts persist in the park in form of roots and sprouts, which are perhaps consumed by feral hogs.
- Ongoing work will now focus on vertebrate and invertebrate taxa found in hog diets.

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