

Supplemental Information

Table S1. List of collection sites of *Reticulitermes* specimens used as a “test panel” when assessing performance of the new PCR-RFLP assay. Spatial coordinates are in decimal degrees, and elevation is reported in meters above sea level. State abbreviations are: Mississippi, MS; Alabama, AL; Georgia, GA; Tennessee, TN; South Carolina, SC; North Carolina, NC; Virginia, VA; and West Virginia, WV. Region abbreviations are: National Forest, NF; National Park, NP; State Forest, SF; State Park, SP; and Wildlife Management Area, WMA.

Site No.	Latitude	Longitude	Elevation	State	Region
1	38.82585	-79.38506	548	WV	Monongahela NF
2	38.82374	-79.38618	528	WV	Monongahela NF
3	38.80508	-78.18149	755	VA	Shenandoah NP
4	38.72694	-79.48494	936	WV	Monongahela NF
5	38.62592	-78.34060	1032	VA	Shenandoah NP
6	38.29123	-78.64308	761	VA	Shenandoah NP
7	38.12902	-78.78368	814	VA	Shenandoah NP
8	38.04052	-79.34980	784	VA	Washington NF
9	37.34757	-80.54509	1121	VA	Jefferson NF
10	36.31100	-82.07211	648	TN	Cherokee NF
11	36.13606	-84.48829	496	TN	Frozen Head SP
12	36.12452	-84.74478	378	TN	Clear Crk
13	35.77140	-83.21343	575	TN	Great Smoky Mtn NP
14	35.70232	-83.35717	653	TN	Great Smoky Mtn NP
15	35.65682	-83.51849	780	TN	Great Smoky Mtn NP
16	35.61933	-83.66993	593	TN	Great Smoky Mtn NP
17	35.59535	-82.48742	722	NC	Blue Ridge Pkwy
18	35.52117	-83.31077	666	TN	Great Smoky Mtn NP
19	35.39384	-87.52677	304	TN	Natchez Trace Pkwy
20	35.34883	-84.24760	327	TN	Cherokee NF
21	35.34534	-84.19383	425	TN	Cherokee NF
22	35.32969	-83.59187	593	NC	Nantahala NF
23	35.10896	-84.62477	530	TN	Cherokee NF
24	35.01376	-83.05563	887	SC	Sumter NF
25	34.94523	-83.08929	744	SC	Sumter NF
26	34.93135	-84.65486	485	GA	Chattahoochee NF
27	34.87866	-84.71137	354	GA	Chattahoochee NF
28	34.86200	-83.10755	536	SC	Oconee SP
29	34.84695	-85.49971	315	GA	Cloudland Canyon SP
30	34.77972	-84.63805	764	GA	Chattahoochee NF
31	34.77755	-83.31242	469	SC	Sumter NF
32	34.77507	-84.33880	730	GA	Chattahoochee NF
33	34.75931	-84.69140	804	GA	Chattahoochee NF
34	34.74192	-83.73265	766	GA	Chattahoochee NF
35	34.72782	-83.22783	394	SC	Sumter NF
36	34.68311	-84.25093	810	GA	Chattahoochee NF
37	34.64336	-85.21630	386	GA	Johns Mtn WMA

Table S1. Cont.

Site No.	Latitude	Longitude	Elevation	State	Region
38	34.60502	−88.19299	177	MS	Tishomingo SP
39	34.57297	−85.06536	450	GA	Johns Mtn WMA
40	34.56515	−85.24268	408	GA	Johns Mtn WMA
41	34.56416	−85.24043	427	GA	Johns Mtn WMA
42	34.54107	−85.25067	341	GA	Johns Mtn WMA
43	34.45540	−85.58357	395	AL	Little River Canyon
44	34.41979	−87.33273	321	AL	Bankhead NF
45	34.17659	−87.27680	248	AL	Bankhead NF
46	34.14676	−85.84679	188	AL	Shinbone Ridge
47	34.12260	−85.26428	232	GA	Lyons Bridge
48	33.96340	−85.45730	300	AL	Talladega NF
49	33.91858	−85.49764	257	AL	Talladega NF
50	33.57157	−85.69391	328	AL	Talladega NF
51	33.56059	−85.70074	425	AL	Talladega NF
52	33.47105	−85.80658	621	AL	Talladega NF
53	33.46215	−85.81731	485	AL	Talladega NF
54	33.40451	−85.87318	460	AL	Talladega NF
55	33.20150	−86.07201	291	AL	Talladega NF

Table S2. List of GenBank accessions of mtCOII used for initial design of a PCR-RFLP assay for identification of eastern United States *Reticulitermes* termite species.

Species	State	GenBank accession(s)	Original reference
<i>R. flavipes</i>	DE	JQ280721–JQ280727	[1]
	FL	AF525321	[2]
	FL	AY808077, AY808082–AY808086	[3]
	FL	JQ280662–JQ280694, JQ280745–JQ280746	[1]
	GA	AF107479–AF107482, AF107484	[4]
	GA	AY027477	[5]
	GA	EF206316–EF206317	[6]
	GA	JQ280701	[1]
	IN	AY168203, AY168210–AY168211	[7]
	IN	JQ280705	[1]
	MA	DQ493725	[8]
	MS	JQ280622–JQ280626, JQ280741–JQ280744	[1]
	NC	DQ493728	[8]
	NC	JQ280716–JQ280720	[1]
	OH	JQ280702	[1]
	SC	JQ280711–JQ280715	[1]
	TN	JQ280703	[1]
	VA	JQ280728–JQ280736	[1]
	GA	JF796216	[9]

Table S2. Cont.

Species	State	GenBank accession(s)	Original reference
<i>R. flavipes</i>	GA	JF796217	[9]
	?	JF796218	[9]
	FL	JF796220	[9]
	GA	JN207492	[9]
<i>R. hageni</i>	GA	AF107486	[4]
	GA	AY027478	[5]
	GA	AF525328	[2]
	GA	EU689026	[10]
	NC	DQ493729	[8]
	GA	JF796224	[9]
	GA	JF796225	[9]
<i>R. mallei</i>	GA	GU550074	[10]
	GA	JF796226	[9]
	GA	JF796227	[9]
	NC	JF796228	[9]
<i>R. nelsonae</i>	GA	EU689013	[9]
	NC	JF796229	[9]
	GA	JF796230	[9]
	FL	JF796231	[9]
	GA	JF796232	[9]
	FL	JF796233	[9]
	GA	JF796235	[9]
	GA	JF796236	[9]
<i>R. virginicus</i>	FL	AF525356	[2]
	FL	AY808096	[3]
	FL	AY808098	[3]
	FL	EF206319	[6]
	GA	AF107487	[4]
	GA	AY027479	[5]
	GA	EF206318	[6]
	GA	EU689027	[10]
	NC	DQ493743	[8]
	VA	AF525357	[2]
	GA	JF796221	[9]
	GA	JF796222	[9]
	GA	JF796223	[9]
	FL	JF796234	[9]

Table S3. Consensus mt*COII* sequences for each five *Reticulitermes* termite species, showing polymorphic nucleotide positions within a 658-bp alignment. IUPAC ambiguity codes reflect intraspecific variability, based on the GenBank accessions from which consensus sequences were generated (Table S2). Polymorphisms that could potentially be used for species identification are color-coded according to whether only two alternative nucleotide states exist (red = one state is unique to a particular species, or green = one state is characteristic of a subset of species) vs. three alternative nucleotide states (blue). In each case, underlined nucleotides indicate potentially diagnostic character(s). The ✓ and ✕ symbols indicate whether DNA sequence polymorphisms do or do not generate restriction site polymorphisms, respectively. Dark grey shading indicates locations of PCR primers that we designed (*i.e.*, RetCo2-F and RetCo2-R) to amplify the most information-rich region (pale grey).

Position (bp)	6	12	18	19	22	23	24	27	28	32	36	39	42	58	60	72	75	78	82	90	93	94	105	107	111	114	117	123	129							
<i>R. flavipes</i>	R	A	H	M	A	S	R	W	C	R	A	A	Y	Y	R	Y	Y	T	H	Y	R	T	Y	Y	C	T	A	R	Y							
<i>R. hageni</i>	A	A	C	A	A	G	A	A	C	A	A	A	T	C	G	Y	C	Y	T	C	A	Y	C	C	C	A	R	A	T							
<i>R. mallei</i>	A	R	C	A	A	G	A	A	C	A	R	G	T	C	G	T	C	T	T	Y	A	C	C	C	C	R	R	A	C							
<i>R. nelsonae</i>	A	A	Y	A	A	G	A	A	C	A	W	A	Y	C	R	C	C	Y	T	T	A	C	C	C	C	A	R	A	Y							
<i>R. virginicus</i>	A	A	C	A	R	G	A	W	S	A	A	A	C	C	R	Y	C	T	T	Y	A	T	Y	C	Y	A	G	A	C							
Cut site													x												x											
	135	137	138	141	144	147	150	152	153	162	163	165	166	171	177	178	186	189	195	201	202	207	213	217	219	228	230	231	234							
<i>R. flavipes</i>	R	Y	T	R	T	A	Y	Y	Y	Y	R	C	C	R	A	H	A	Y	R	T	R	A	Y	Y	R	T	C	A	A							
<i>R. hageni</i>	A	T	Y	R	T	A	C	C	T	T	A	T	T	A	R	C	R	Y	A	T	G	A	C	C	A	T	C	A	A							
<i>R. mallei</i>	A	T	T	A	T	A	C	C	C	C	A	T	T	A	A	M	A	C	A	T	G	A	C	C	A	T	C	G	A							
<i>R. nelsonae</i>	A	T	T	A	Y	R	Y	C	T	Y	A	Y	Y	A	R	Y	R	C	A	Y	G	A	C	C	A	Y	C	R	R							
<i>R. virginicus</i>	A	T	T	A	T	R	C	C	T	T	A	T	T	A	A	C	A	Y	A	T	G	R	C	C	A	T	S	R	A							
Cut site																																				
	240	249	250	252	255	256	258	264	268	270	273	279	280	288	289	291	294	297	300	303	306	309	312	318	323	324	327	330	333							
<i>R. flavipes</i>	Y	Y	C	R	Y	Y	A	Y	A	Y	Y	A	A	Y	C	R	R	D	D	R	Y	R	A	A	D	T	A	Y	M							
<i>R. hageni</i>	C	C	C	A	Y	C	A	C	A	C	C	R	A	T	C	A	A	A	A	A	C	A	G	R	A	T	A	Y	A							
<i>R. mallei</i>	C	T	C	A	T	C	A	C	A	C	C	A	A	C	C	A	A	A	A	A	C	A	A	G	A	T	A	C	A							
<i>R. nelsonae</i>	C	T	Y	A	T	C	A	C	W	Y	Y	A	R	C	Y	A	A	A	A	A	C	R	A	A	A	T	R	T	A							
<i>R. virginicus</i>	Y	T	C	A	T	C	W	C	A	C	Y	A	A	C	C	A	A	A	R	A	C	A	A	R	A	Y	A	C	A							
Cut site	✓																																			

Tale S3. Cont.

Position (bp)	339	348	354	360	363	369	372	375	381	382	388	389	394	396	399	406	408	417	420	421	426	430	432	441	444	450	454	456	458
<i>R. flavipes</i>	Y	A	Y	Y	Y	<u>C</u>	R	R	W	W	S	C	R	Y	Y	Y	A	Y	<u>T</u>	C	T	H	A	Y	H	Y	C	R	Y
<i>R. hageni</i>	C	A	T	T	C	T	A	R	A	A	A	C	A	C	C	Y	A	C	C	C	Y	C	A	C	A	C	Y	A	T
<i>R. mallei</i>	C	A	T	T	C	T	A	A	A	A	G	C	A	C	C	T	A	C	C	C	T	C	A	T	A	C	T	A	T
<i>R. nelsonae</i>	C	R	T	T	C	T	R	A	A	A	R	C	R	C	C	T	R	Y	C	C	T	C	A	Y	A	Y	Y	A	T
<i>R. virginicus</i>	C	A	T	T	C	T	A	R	A	A	A	Y	A	C	C	C	A	C	C	S	T	C	<u>S</u>	T	A	C	T	A	T
Cut site	✓										✓										✗								
	462	474	477	478	480	483	484	486	495	504	507	510	513	519	522	525	528	531	534	535	540	544	545	546	551	552	554	556	561
<i>R. flavipes</i>	R	Y	R	T	R	C	T	D	Y	D	D	R	R	Y	Y	R	A	R	A	C	Y	G	Y	A	Y	C	Y	W	Y
<i>R. hageni</i>	A	C	A	T	A	C	T	A	T	R	G	G	A	C	C	A	G	R	R	Y	T	W	C	A	T	C	C	A	T
<i>R. mallei</i>	A	C	A	<u>C</u>	A	C	T	A	T	A	R	G	A	C	C	A	R	A	A	T	T	G	C	A	T	C	C	A	T
<i>R. nelsonae</i>	A	C	R	<u>C</u>	A	C	Y	W	Y	R	R	G	A	C	Y	A	G	R	A	Y	T	A	C	A	T	Y	C	A	T
<i>R. virginicus</i>	A	C	A	T	A	Y	T	A	T	A	R	G	R	C	C	A	A	A	A	Y	T	A	M	M	T	C	C	A	T
Cut site	✓																												
	564	567	571	573	574	578	579	585	597	600	604	606	609	612	618	620	621	622	624	627	630	646	648	652					
<i>R. flavipes</i>	Y	R	R	Y	C	M	C	R	M	C	G	H	T	<u>C</u>	Y	K	S	S	Y	T	M	R	A	M					
<i>R. hageni</i>	C	A	A	S	C	A	Y	A	C	T	G	A	Y	T	T	T	G	C	T	<u>C</u>	A	G	A	C					
<i>R. mallei</i>	C	<u>C</u>	A	T	C	A	C	A	C	T	G	A	T	T	T	T	A	C	T	T	A	G	A	C					
<i>R. nelsonae</i>	C	A	R	Y	C	A	C	A	Y	Y	G	A	T	T	T	T	G	C	T	T	A	G	A	C					
<i>R. virginicus</i>	C	A	A	C	Y	A	C	A	C	T	R	A	T	T	T	T	R	C	Y	T	A	G	R	Y					
Cut site	✓										✓										✗								

Table S4. Original interpretive framework for identifying eastern United States *Reticulitermes* termite species using PCR-RFLP applied to the mtCOII gene, based on “training data” from NCBI’s GenBank database. Expected fragment sizes produced by three restriction enzyme digests (performed one-at-a-time) for each of five recognized taxa are represented by check marks. Following application to a “test panel” of Southern Appalachian samples, the interpretive framework was refined to accommodate new polymorphisms (see Table 1 in the main text).

Restriction Enzyme	Fragment Sizes (bp)	Species				
		<i>R. flavipes</i>	<i>R. hageni</i>	<i>R. mallei</i>	<i>R. nelsonae</i>	<i>R. virginicus</i>
<i>Rsa I</i>	175, 201	✓	×	×	✓	✓
	48, 127, 201	×	×	✓	✓	×
	86, 115, 175	×	✓	×	×	×
<i>Taq I</i>	376	×	✓	✓	✓	✓
	153, 223	×	×	×	✓	×
	183, 193	×	×	×	×	×
	67, 126, 183	✓	×	×	×	×
	30, 67, 126, 153	✓	×	×	×	×
<i>Msp I</i>	376	✓	×	×	×	✓
	37, 339 *	✓	×	✓	×	×
	77, 299	×	✓	×	✓	×
	37, 40, 299	×	×	✓	×	×

* For *R. flavipes*, fragment sizes may instead be 38-bp and 338-bp, but since the 1-bp differences compared to those reported in the table are indistinguishable, only the shorter fragments are listed.

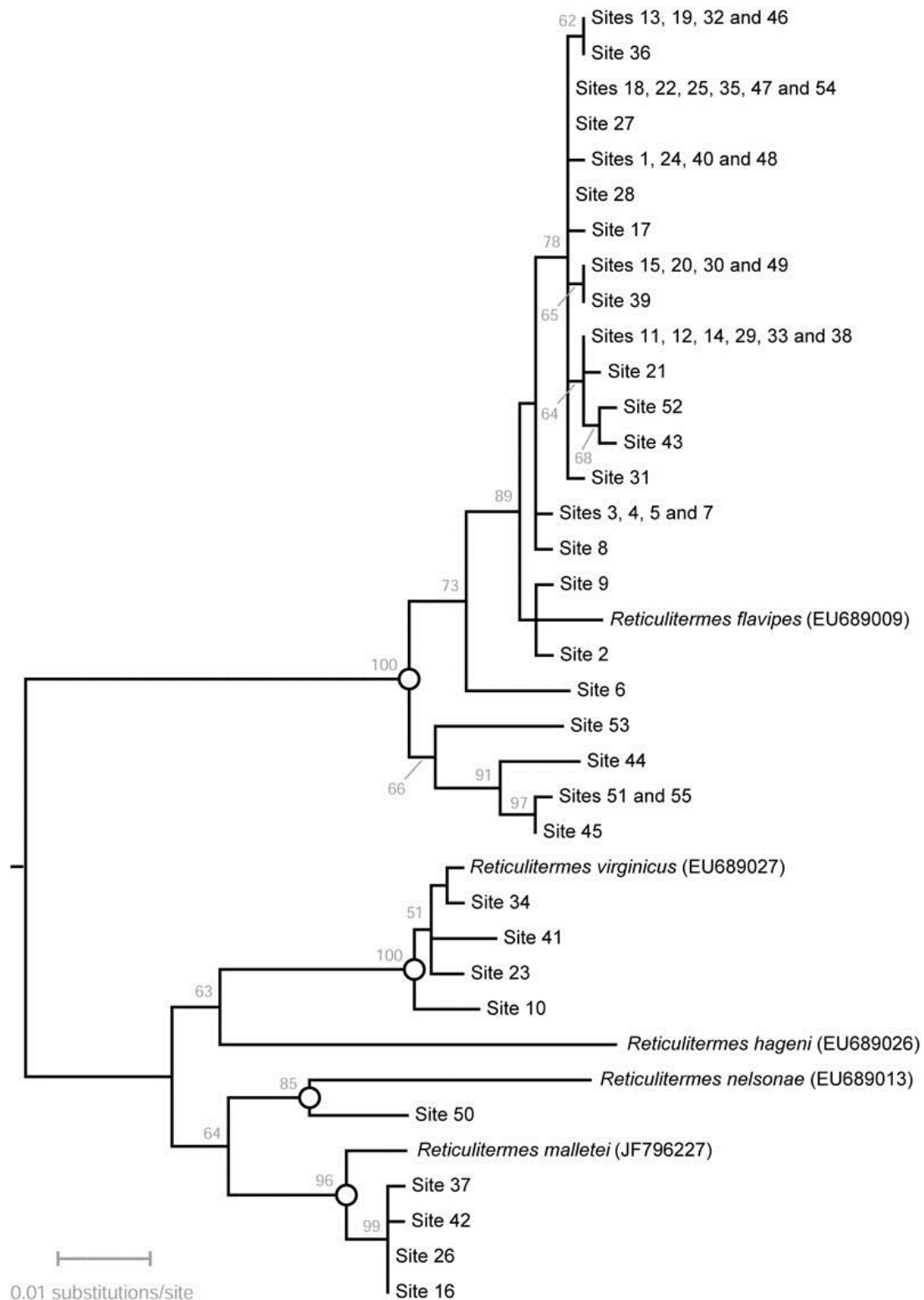


Figure S1. Phylogenetic relationships among *Reticulitermes* mtCOII sequences from 55 new and five reference specimens (n = 37 non-redundant haplotypes, 607-bp each), estimated using Maximum Likelihood and the HKY+I model of nucleotide substitution. Shown here is the tree with the highest log likelihood. Numbers above nodes are bootstrap support values. Open circles on nodes mark the most inclusive well-support clades that contain only one reference taxon (the same groups were recovered using Maximum Parsimony; see Figure 2 of the main text).

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