

SUPPLEMENTAL INFORMATION

**DETECTION OF STREAMSIDE PLETHODONTID SALAMANDERS
USING TRADITIONAL AND ENVIRONMENTAL DNA SURVEYS
IN AN URBAN SETTING**

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TABLE S1. Site number; drainage area (km²), percentage impervious surface cover (ISC), and percentage developed land of the drainage basin; the number of streamside plethodontid species detected by eDNA, traditional manual surveys, and in total.

Site number	Drainage area (km ²)	ISC (%)	Developed (%)	Detected species: eDNA	Detected species: Traditional	Detected species: Total
1	0.41	10.8	58.6	2	1	2
2	0.31	8.3	68.0	4	4	4
3	2.62	21.6	88.3	3	3	4
4	0.44	31.3	92.9	2	1	2
5	0.31	2.9	15.3	5	4	5
6	0.23	61.7	97.7	2	2	2
7	0.24	14.7	88.0	0	2	2
8	50.50	28.7	80.8	0	3	3
9	2.38	39.8	92.1	2	1	2
10	1.50	51.6	89.4	0	1	1
11	0.49	21.9	93.8	2	2	2
12	10.72	57.1	99.5	1	2	2
13	3.37	37.3	87.9	2	2	2
14	5.93	36.8	87.2	1	1	1
15	0.44	18.6	62.0	1	1	1
16	0.14	19.4	87.4	2	2	2
17	0.47	38.4	91.1	2	2	2
18	0.18	18.1	51.1	0	3	3
19	0.83	32.8	84.3	1	1	1

TABLE S2. Species, collection location, and additional information (GenBank accession number, museum record, and/or literature source) of 12S sequences used for primer evaluation and eDNA reference database.

Species	Location	Source
Two-lined Salamander (<i>Eurycea cirrigera</i>)	Mississippi, USA	JAWWEH010000013; GSU 27312; Pyron et al. (2024)
Three-lined Salamander (<i>Eurycea guttolineata</i>)	Mississippi, USA	JAXCLM010000021; GSU 27313; Pyron et al. (2024)
Chattooga Dusky Salamander (<i>Desmognathus perlapsus</i>)	Georgia, USA	JAUEJI010100984; MNHN-RA 2021.0118; Pyron et al. (2024)
Talladega Seal Salamander (<i>Desmognathus cheaha</i>)	Alabama, USA	AY549668; Rissler et al. (2004)
Red Salamander (<i>Pseudotriton ruber</i>)	New Jersey, USA	PV034389
Spring Salamander (<i>Gyrinophilus porphyriticus</i>)	Tennessee, USA	EU336432; Niemiller et al. (2008)

Niemiller, M.L., B.M. Fitzpatrick, and B.T. Miller. 2008. Recent divergence with gene flow in Tennessee cave salamanders (Plethodontidae: *Gyrinophilus*) inferred from gene genealogies.

Molecular Ecology 17:2258–2275.

Pyron, R.A., S. Pirro, T. Hains, T.J. Colston, E.A. Myers, K.A. O’Connell, and D.A. Beamer.

2024. The draft genome sequences of 50 salamander species (Caudata, Amphibia).

Biodiversity Genomes 2024:1–4. <https://doi.org/10.56179/001c.116891>.

Rissler, L.J., H.M. Wilbur, and D.R. Taylor. 2004. The influence of ecology and genetics on

behavioral variation in salamander populations across the Eastern Continental Divide. The

American Naturalist 164:201–213.

TABLE S3. Sample ID and type, number of raw reads, number of reads passing quality-filtering, number of reads after denoising, and the number of reads for each of the six reference species. The abbreviation ntc = no template control and nc = negative control.

Sample	Type	Raw	Quality	Denoise	<i>E. cirrigera</i>	<i>E. guttolineata</i>	<i>D. perlapsus</i>	<i>D. cheaha</i>	<i>P. ruber</i>	<i>G. porphyriticus</i>
ntc_01	lab_nc	0	0	0	0	0	0	0	0	0
ntc_02	lab_nc	0	0	0	0	0	0	0	0	0
ntc_03	lab_nc	1	1	1	0	0	0	0	0	0
ntc_04	lab_nc	0	0	0	0	0	0	0	0	0
ntc_05	lab_nc	0	0	0	0	0	0	0	0	0
ntc_06	lab_nc	0	0	0	0	0	0	0	0	0
ntc_07	lab_nc	1	1	0	0	0	0	0	0	0
site01_1	sample	114437	113920	69613	10384	0	92	0	0	0
site01_2	sample	58212	57887	35233	6455	0	15	0	0	0
site01_3	sample	148686	147913	88057	8879	0	228	0	0	0
site01_N	field_nc	69701	69437	5	0	0	0	0	0	0
site02_1	sample	36960	36701	4859	2729	0	38	0	392	0
site02_2	sample	22543	22405	2511	1559	0	0	17	153	0
site02_3	sample	53016	52602	6540	3622	0	21	0	797	0
site02_N	field_nc	62566	60825	5	0	0	0	0	0	0
site03_1	sample	56605	56189	47914	2109	0	0	0	0	0

site03_2	sample	66928	66404	58993	2977	0	7	0	54	0
site03_3	sample	172213	170940	145529	2967	0	44	0	0	0
site03_N	field_nc	5207	5181	3	0	0	0	0	0	0
site04_1	sample	4688	4639	1961	102	0	5	0	0	0
site04_2	sample	8897	8791	4838	542	0	0	0	0	0
site04_3	sample	4162	4090	2025	45	0	0	0	0	0
site04_N	field_nc	9694	9617	0	0	0	0	0	0	0
site05_1	sample	37808	37505	7232	3789	0	14	43	85	404
site05_2	sample	53663	53314	6782	3192	0	5	14	180	171
site05_3	sample	72970	72505	7993	3528	0	0	99	160	345
site05_N	field_nc	122872	122226	0	0	0	0	0	0	0
site06_1	sample	75051	74104	20703	10043	0	338	0	0	0
site06_2	sample	126544	125281	29982	14743	0	583	0	0	0
site06_3	sample	83830	83206	32077	16633	0	1580	0	0	0
site06_N	field_nc	6974	6948	0	0	0	0	0	0	0
site07_1	sample	29	29	0	0	0	0	0	0	0
site07_2	sample	0	0	0	0	0	0	0	0	0
site07_3	sample	0	0	0	0	0	0	0	0	0
site07_N	field_nc	184342	183595	8	0	0	0	0	0	0

site08_1	sample	3	3	0	0	0	0	0	0	0
site08_2	sample	14	14	0	0	0	0	0	0	0
site08_3	sample	6	5	0	0	0	0	0	0	0
site08_N	field_nc	81992	81729	0	0	0	0	0	0	0
site09_1	sample	133375	132318	67334	20576	0	0	0	179	0
site09_2	sample	116515	115585	56546	15828	0	0	0	77	0
site09_3	sample	163422	162164	82501	21301	0	0	0	244	0
site09_N	field_nc	47104	46980	20	0	0	0	0	0	0
site10_1	sample	6998	6966	5713	0	0	0	0	0	0
site10_2	sample	17933	17826	15463	0	0	0	0	0	0
site10_3	sample	158	158	36	0	0	0	0	0	0
site10_N	field_nc	10958	10915	0	0	0	0	0	0	0
site11_1	sample	24612	24440	16355	13403	0	0	0	0	0
site11_2	sample	46693	46232	20077	8738	0	27	0	0	0
site11_3	sample	11343	11225	8839	7825	0	214	0	0	0
site11_N	field_nc	15087	15021	6	0	0	0	0	0	0
site12_1	sample	313	311	0	0	0	0	0	0	0
site12_2	sample	5070	5039	670	0	0	8	0	0	0
site12_3	sample	2	2	0	0	0	0	0	0	0

site12_N	field_nc	26880	26732	0	0	0	0	0	0	0
site13_1	sample	7521	7494	4354	785	0	11	0	0	0
site13_2	sample	670	668	2	0	0	0	0	0	0
site13_3	sample	4186	4174	1805	337	0	0	0	0	0
site13_N	field_nc	0	0	0	0	0	0	0	0	0
site14_1	sample	5574	5507	1585	0	0	0	0	0	0
site14_2	sample	24596	24433	16746	205	0	0	0	0	0
site14_3	sample	27	27	0	0	0	0	0	0	0
site14_N	field_nc	3134	3121	0	0	0	0	0	0	0
site15_1	sample	576003	572434	538251	13206	0	0	0	0	0
site15_2	sample	663453	659335	619277	16841	0	0	0	0	0
site15_3	sample	665675	661594	625069	23142	0	0	0	0	0
site15_N	field_nc	109589	109013	7	0	0	0	0	0	0
site16_1	sample	27726	27593	19813	13261	0	24	0	0	0
site16_2	sample	77823	77018	47796	25606	0	53	0	0	0
site16_3	sample	83275	82459	48197	12551	0	310	0	0	0
site16_N	field_nc	86772	86398	54	0	0	0	0	0	0
site17_1	sample	87272	86505	34657	7054	0	654	0	0	0
site17_2	sample	184531	182669	86995	30829	0	690	0	0	0

site17_3	sample	160704	159256	78204	17673	0	1135	0	0	0
site17_N	field_nc	71348	71102	14	0	0	0	0	0	0
site18_1	sample	0	0	0	0	0	0	0	0	0
site18_2	sample	0	0	0	0	0	0	0	0	0
site18_3	sample	1362	1353	0	0	0	0	0	0	0
site18_N	field_nc	0	0	0	0	0	0	0	0	0
site19_1	sample	3217	3185	1961	33	0	0	0	0	0
site19_2	sample	37364	37171	28602	5951	0	0	0	0	0
site19_3	sample	77457	77031	57634	8971	0	0	0	0	0
site19_N	field_nc	3685	3666	7	0	0	0	0	0	0
