

Report for New Mexico Department of Game and Fish Share with Wildlife projects:

“An eDNA-based inventory of the distribution and abundance of Chihuahua chub and Rio Grande sucker in the Mimbres River basin” (final report)

“An eDNA-based survey of the distributions of Rio Grande Sucker and Rio Grande Chub in the Upper Rio Grande and Rio Chama basins”

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I. Background

Due to the COVID-19 pandemic, the originally planned sampling dates for both projects were postponed until spring of 2022 in order to ensure safety for the residents of New Mexico and field personnel. As such, the environmental DNA (eDNA) samples collected in spring of 2022 are still being processed and final results are not yet available. We will prepare the final reports as noted in our Share with Wildlife proposal following the completion of sample processing. This report will highlight the work completed to date: 1) eDNA assay development for the Chihuahua Chub (*Gila nigrescens*), 2) eDNA sampling in the Mimbres basin, and 3) eDNA sampling in the Upper Rio Grande and Rio Chama basins.

III. An eDNA-based inventory of the distribution and abundance of Chihuahua chub and Rio Grande sucker in the Mimbres River basin: eDNA sampling effort 2022

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METHODS

Sampling materials, field equipment, and site locations were prepared by the National Genomics Center for Wildlife and Fish Conservation (NGC) in Missoula, MT. In total, we visited 106 sites in the Mimbres basin to collect eDNA samples (Tables 5 and 6, Figure 1) between 10 April 2022 and 19 April 2022. Of the 106 sites visited, 76 eDNA samples were collected, six sites were not publicly accessible, and 24 sites were dry (no flowing water within 200m of the site; Tables 5 and 6, Figure 1). Seventy of the eDNA samples were collected to determine the current distribution of the species and the remaining six eDNA samples collected were paired with electrofishing depletion surveys. The electrofishing surveys were carried out by partners with New Mexico Department of Game and Fish and U.S. Forest Service – Gila National Forest. We will use these six eDNA samples to relate copy numbers of Chihuahua chub (*Gila nigrescens*) DNA to known electrofishing abundance counts (Table 6). For each sample collected, we pumped up to 5 L of stream water through a 1.5 µm glass filter (GE HealthCare) using a peristaltic pump (GeoTech Environmental Equipment, Inc.) following the protocol developed by Carim et al. (2016). We placed collected filters in individual plastic bags with silica desiccant and packaged in individual envelopes labeled with field information (e.g., date and sampling location). We kept the filters cool and out of direct light until they could be mailed to the NGC.

Upon receipt of samples at the NGC, we catalogued the sampling data and stored the samples at -20°C until analysis. **We are currently in the process of extracting and analyzing these eDNA samples.** For each sample, eDNA is extracted from half of the sample filter using the Qiagen DNEasy® Blood and Tissue Kit following a modified protocol described in Franklin et al. (2019). The other half of the filter is retained and stored at -20 °C. If more than one filter was used to collect the sample, DNA from all extracted filter halves for a given sample is combined during DNA extraction.

All 76 samples will be analyzed for Chihuahua chub and Rio Grande sucker (*Pantosteus plebeius*) DNA using species-specific assays designed by the NGC. Each sample will be analyzed in triplicate on a QuantStudio 3 qPCR System (Life Technologies) alongside a standard curve to estimate the number of DNA copies from the target species, if present. A sample will be considered positive for the presence of the target species if one or more of the three qPCR reactions amplifies DNA of that species. After all samples are analyzed, we will investigate the relationship between Chihuahua chub DNA copy numbers and the abundance estimates from the electrofishing surveys. If the relationship is strong enough, we will expand the abundance estimates to all Chihuahua chub positive eDNA samples.

All reactions include an internal positive control (IPC) to ensure that the reaction is effective and sensitive to the presence of the target species' DNA. If the IPC appears inhibited (i.e., reduced amplification of the IPC DNA due to chemical compounds in the sample), we will treat the sample with a PCR inhibitor removal kit (Zymo Research) and re-analyze the sample in triplicate. Removal

of inhibitors may result in loss of DNA in a sample. With elution volumes of 100–200 μ l, loss of DNA during inhibitor removal is on average less than 10% (see <http://www.zymoresearch.com> for more details). Thus, to minimize potential DNA loss, we extract the second half of the sample filter and combine all extracted DNA from a given sample to obtain ~200 μ l of extracted DNA. All laboratory experiments are conducted with negative controls to ensure there is no contamination during DNA extraction or qPCR setup.

CONCLUSIONS

We will follow up with a final report once all of the eDNA samples have been analyzed and pass QAQC standards. If you have any questions about the project details to date, please do not hesitate to contact us.

Table 5. Location and collection details of eDNA samples collected by the National Genomics Center in the Mimbres basin for the delineation of Chihuahua chub and Rio Grande sucker distributions. “Y” in denotes eDNA sample was collected, “N” denotes site was visited but no eDNA could be collected.

Site	Drainage	Stream	Sampled	Latitude	Longitude	Date	No.	Filter	Field Notes
						Collected	Filters	Volume (L)	
1269-1	Mimbres	Allie Canyon	Y	32.923412	-108.045476	4/11/2022	1	5	
1269-2	Mimbres	Allie Canyon	Y	32.920002	-108.054846	4/11/2022	1	5	
1273-1	Mimbres	Allie Canyon	Y	32.91883	-108.068563	4/11/2022	1	5	
1278-1	Mimbres	Allie Canyon	Y	32.920038	-108.073283	4/11/2022	1	5	
1306-1	Mimbres	East Canyon	Y	32.990019	-107.88113	4/10/2022	1	5	
1321-1	Mimbres	East Canyon	Y	32.991144	-107.897983	4/10/2022	1	5	
1321-2	Mimbres	East Canyon	Y	32.990575	-107.887333	4/10/2022	1	5	
1324-1	Mimbres	East Canyon	Y	32.993471	-107.902548	4/10/2022	1	5	
1327-1	Mimbres	East Canyon	Y	32.99579	-107.907501	4/10/2022	1	5	
1328-3	Mimbres	East Canyon	Y	32.99127	-107.928172	4/10/2022	1	5	
1328-4	Mimbres	East Canyon	Y	32.996204	-107.919544	4/10/2022	1	5	
1347-1	Mimbres	East Fork Mimbres River	Y	33.037581	-107.894577	4/15/2022	1	5	
1347-2	Mimbres	East Fork Mimbres River	Y	33.0326	-107.886305	4/15/2022	1	5	
1352-1	Mimbres	East Fork Mimbres River	Y	33.024143	-107.882873	4/15/2022	1	5	
1352-2	Mimbres	East Fork Mimbres River	Y	33.019761	-107.874042	4/15/2022	1	5	
1352-3	Mimbres	East Fork Mimbres River	Y	33.01586	-107.865266	4/15/2022	1	5	
1356-1	Mimbres	East Fork Mimbres River	Y	32.996923	-107.94975	4/15/2022	1	5	Sampled 50M downstream to avoid stagnant pool
1356-2	Mimbres	East Fork Mimbres River	Y	33.00445	-107.945929	4/15/2022	1	5	
1356-3	Mimbres	East Fork Mimbres River	Y	33.012141	-107.942392	4/15/2022	1	5	
1356-4	Mimbres	East Fork Mimbres River	Y	33.019807	-107.93806	4/15/2022	1	5	
1356-5	Mimbres	East Fork Mimbres River	Y	33.02715	-107.932156	4/15/2022	1	5	
1356-6	Mimbres	East Fork Mimbres River	Y	33.031532	-107.924128	4/15/2022	1	5	
1356-7	Mimbres	East Fork Mimbres River	Y	33.0356	-107.914523	4/15/2022	1	5	
1356-8	Mimbres	East Fork Mimbres River	Y	33.037464	-107.903986	4/15/2022	1	5	
1243-1	Mimbres	Gallinas Canyon	Y	32.907617	-107.817243	4/11/2022	1	5	
1190-5	Mimbres	Iron Creek	Y	32.882163	-107.853676	4/11/2022	1	5	
1190-6	Mimbres	Iron Creek	Y	32.886736	-107.845526	4/11/2022	1	5	
1220-1	Mimbres	Iron Creek	Y	32.892047	-107.840766	4/11/2022	1	5	
1220-2	Mimbres	Iron Creek	Y	32.892298	-107.83346	4/11/2022	1	5	
1220-3	Mimbres	Iron Creek	Y	32.896753	-107.826054	4/11/2022	1	5	
1234-1	Mimbres	Iron Creek	Y	32.907362	-107.816799	4/11/2022	1	5	
959-1	Mimbres	Mimbres River	Y	32.73535	-107.87581	4/16/2022	2	a:3.5, b:1.5	
000-01	Mimbres	Mimbres River	Y	32.775704	-107.911173	4/16/2022	1	5	
000-02	Mimbres	Mimbres River	Y	32.76721	-107.90751	4/16/2022	1	5	
000-03	Mimbres	Mimbres River	Y	32.71951	-107.85545	4/16/2022	2	a:2, b:3	
1119-4	Mimbres	Mimbres River	Y	32.842756	-107.958451	4/16/2022	1	5	Dog in stream
1241-2	Mimbres	Mimbres River	Y	32.894082	-107.993697	4/10/2022	1	5	
1259-1	Mimbres	Mimbres River	Y	32.908745	-108.002392	4/11/2022	1	5	
1355-1	Mimbres	Mimbres River	Y	33.01729	-107.996266	4/10/2022	1	5	
1358-1	Mimbres	Mimbres River	Y	33.025269	-107.992462	4/10/2022	1	5	
1366-1	Mimbres	Mimbres River	Y	33.032503	-107.984848	4/15/2022	1	5	
1366-2	Mimbres	Mimbres River	Y	33.041768	-107.979958	4/15/2022	1	5	Moved upstream 200M due to hunters
1381-2	Mimbres	Mimbres River	Y	33.049695	-107.972565	4/15/2022	1	5	
1382-1	Mimbres	Mimbres River	Y	33.057664	-107.964657	4/12/2022	1	5	
1389-1	Mimbres	Mimbres River	Y	33.06236	-107.959026	4/12/2022	1	5	
1389-2	Mimbres	Mimbres River	Y	33.068233	-107.953588	4/12/2022	1	5	
1389-3	Mimbres	Mimbres River	Y	33.072834	-107.945587	4/12/2022	1	5	
1391-1	Mimbres	Mimbres River	Y	33.077157	-107.938958	4/12/2022	1	5	
1391-2	Mimbres	Mimbres River	Y	33.081756	-107.931332	4/12/2022	1	5	
1395-2	Mimbres	Mimbres River	Y	33.086279	-107.917626	4/12/2022	1	5	

1395-3	Mimbres	Mimbres River	Y	33.088219	-107.908165	4/12/2022	1	5	
1395-4	Mimbres	Mimbres River	Y	33.092081	-107.899616	4/12/2022	1	5	
1397-1	Mimbres	Mimbres River	Y	33.084505	-107.926652	4/12/2022	1	5	
1398-1	Mimbres	NF Mimbres	Y	33.094289	-107.889845	4/12/2022	1	5	
1402-1	Mimbres	NF Mimbres	Y	33.127861	-107.870269	4/13/2022	1	5	
1407-1	Mimbres	NF Mimbres	Y	33.103158	-107.88469	4/12/2022	1	5	
1407-2	Mimbres	NF Mimbres	Y	33.111485	-107.885767	4/13/2022	1	5	
1407-3	Mimbres	NF Mimbres	Y	33.119651	-107.885309	4/13/2022	1	5	
1407-4	Mimbres	NF Mimbres	Y	33.126427	-107.878898	4/13/2022	1	5	
1227-2	Mimbres	Noonday Creek	Y	32.88228	-107.875147	4/11/2022	1	5	
1227-4	Mimbres	Noonday Creek	Y	32.894542	-107.862699	4/11/2022	1	5	
1227-5	Mimbres	Noonday Creek	Y	32.902684	-107.860573	4/11/2022	1	5	
1267-1	Mimbres	Noonday Creek	Y	32.906764	-107.859649	4/11/2022	1	5	
1387-1	Mimbres	SF Mimbres	Y	33.091557	-107.865512	4/13/2022	1	5	
1393-1	Mimbres	SF Mimbres	Y	33.091817	-107.88219	4/13/2022	1	5	
1393-2	Mimbres	SF Mimbres	Y	33.090639	-107.872392	4/13/2022	1	5	
1394-1	Mimbres	SF Mimbres	Y	33.093468	-107.889295	4/13/2022	1	5	
1349-1	Mimbres	Tributary to EF Mimbres River	Y	33.024148	-107.882884	4/15/2022	1	4	Wetted width ~0.5 M, no second filter
1363-1	Mimbres	Tributary to EF Mimbres River	Y	33.037967	-107.89515	4/15/2022	1	5	
1406-1	Mimbres	Tributary to NF Mimbres	Y	33.128139	-107.870271	4/13/2022	1	5	
1274-2	Mimbres	Allee Canyon	N	32.9193	-108.03295				Dry
1278-2	Mimbres	Allee Canyon	N	32.91763	-108.08245				Dry
1312-1	Mimbres	East Canyon	N	32.96568	-107.93856				Dry
1314-1	Mimbres	East Canyon	N	32.96667	-107.93948				Dry
1328-1	Mimbres	East Canyon	N	32.97543	-107.93414				Dry
1328-2	Mimbres	East Canyon	N	32.98369	-107.93087				Dry
1331-2	Mimbres	East Fork Mimbres River	N	32.99121	-107.95345				Dry
1338-1	Mimbres	Little McKnight Canyon	N	32.99677	-107.94889				Dry
1338-2	Mimbres	Little McKnight Canyon	N	33.00205	-107.94098				Dry
1348-1	Mimbres	Little McKnight Canyon	N	33.00914	-107.93851				Dry
1348-2	Mimbres	Little McKnight Canyon	N	33.0171	-107.9348				Dry
1152-1	Mimbres	Mimbres River	N	32.84856	-107.97092				No public access
1266-1	Mimbres	Mimbres River	N	32.91734	-108.00584				No public access
1276-1	Mimbres	Mimbres River	N	32.92265	-108.00939				Dry
1286-1	Mimbres	Mimbres River	N	32.9319	-108.01112				Dry
1286-2	Mimbres	Mimbres River	N	32.93898	-108.01697				Dry
1311-1	Mimbres	Mimbres River	N	32.94949	-108.01718				Dry
1311-2	Mimbres	Mimbres River	N	32.95702	-108.01298				Dry
1323-1	Mimbres	Mimbres River	N	32.966	-108.00864				Dry
1323-2	Mimbres	Mimbres River	N	32.97395	-108.00704				Dry
1330-1	Mimbres	Mimbres River	N	32.9802	-108.00603				Dry
1343-1	Mimbres	Mimbres River	N	32.98865	-108.00401				Dry
1343-2	Mimbres	Mimbres River	N	32.99643	-108.00152				Dry
1350-1	Mimbres	Mimbres River	N	33.00865	-107.99831				Dry
939-1	Mimbres	Mimbres River	N	32.72971	-107.86117				No public access
947-1	Mimbres	Mimbres River	N	32.73152	-107.868				No public access
995-1	Mimbres	Mimbres River	N	32.7439	-107.88075				No public access
995-2	Mimbres	Mimbres River	N	32.74864	-107.88927				No public access
1227-3	Mimbres	Noonday Creek	N	32.886636	-107.866574				Dry
1262-1	Mimbres	Tributary to Noonday Creek	N	32.906122	-107.859134				Dry

Table 6. Location and collection details of eDNA samples collected with paired electrofishing (E-fishing) surveys. “Y” in denotes eDNA sample was collected, “N” denotes site was visited but no eDNA could be collected.

Site ID	Site	Drainage	Stream	Sampled	Latitude	Longitude	Date Collected	No. Filters	Filter Volume	Field Notes
E1-L	Mimbres at NMDGF perm site	Mimbres	Mimbres River	Y	32.84362059	-107.9619274	4/19/2022	1	5	Collected at lower end of E-fishing depletion site.
E1-U	Mimbres at NMDGF perm site	Mimbres	Mimbres River	Y	32.84412023	-107.9636635	4/19/2022	1	5	Collected at upper end of E-fishing depletion site.
E2-L	TNC Property at East Canyon Confluence MR2-034	Mimbres	Mimbres River	Y	32.90669929	-108.002178	4/19/2022	1	5	Collected at lower end of E-fishing depletion site.
E2-U	TNC Property at East Canyon Confluence MR2-034	Mimbres	Mimbres River	Y	32.9082667	-108.0024992	4/19/2022	1	5	Collected at upper end of E-fishing depletion site.
E3-L	Mimbres River at TNC McAnaly	Mimbres	Mimbres River	Y	32.89684824	-107.9954594	4/19/2022	1	5	Collected at lower end of E-fishing depletion site.
E3-U	Mimbres River at TNC McAnaly	Mimbres	Mimbres River	Y	32.89832071	-107.9964601	4/19/2022	1	5	Collected at upper end of E-fishing depletion site.

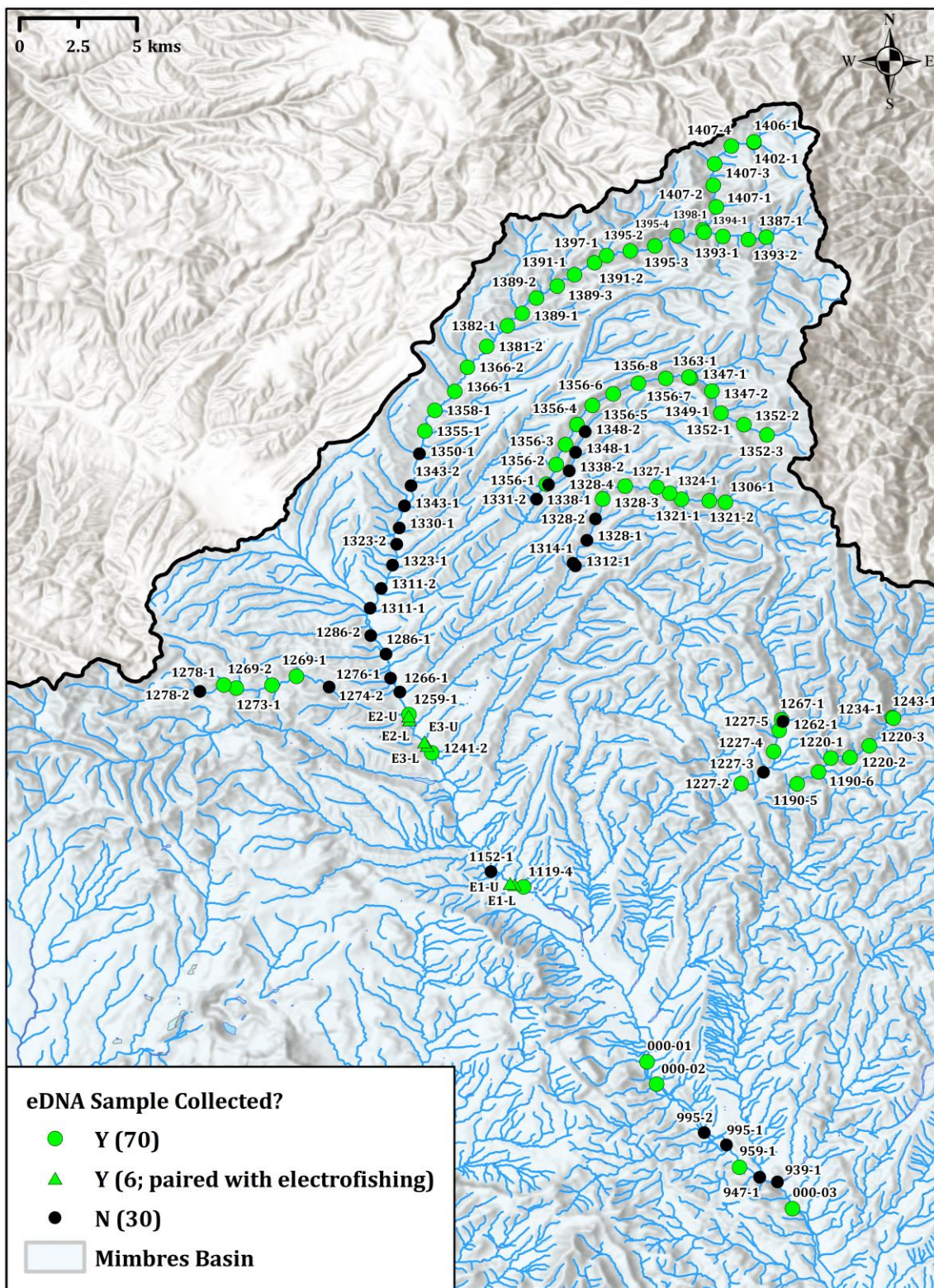


Figure 1. Locations of eDNA sites visited in the Mimbres basin by the National Genomics Center in 2022. A green circle denotes eDNA sample collected, green triangle denotes eDNA sample collected in conjunction with electrofishing depletion estimates, and a black circle denotes the eDNA site was visited but no sample could be collected. The blue hydrography lines with a thick black border represents the Mimbres basin.

IV. An eDNA-based survey of the distributions of Rio Grande Sucker and Rio Grande Chub in the Upper Rio Grande and Rio Chama basins: eDNA sampling effort 2022

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METHODS

Sampling materials, field equipment, and site locations were prepared by the National Genomics Center for Wildlife and Fish Conservation (NGC) in Missoula, MT. In total, we visited 62 sites in the Rio Chama basin and 60 sites in the Upper Rio Grande basin to collect eDNA samples (Tables 7 and 8, Figures 2 and 3) between 29 March 2022 and 7 April 2022 and 17 April 2022 and 21 April 2022. Of the 122 sites visited, 107 eDNA samples were collected and 15 sites were visited but eDNA samples could not be collected (stream channel dry, no public access, or inaccessible due to snow; Tables 7 and 8, Figures 2 and 3). For each sample collected, we pumped up to 5 L of stream water through a 1.5 μ m glass filter (GE HealthCare) using a peristaltic pump (GeoTech Environmental Equipment, Inc.) following the protocol developed by Carim et al. (2016). We placed collected filters in individual plastic bags with silica desiccant and packaged in individual envelopes labeled with field information (e.g., date and sampling location). We kept the filters cool and out of direct light until they could be mailed to the NGC.

Upon receipt of samples at the NGC, we catalogued the sampling data and stored the samples at -20°C until analysis. **We are currently in the process of extracting and analyzing these eDNA samples.** For each sample, eDNA is extracted from half of the sample filter using the Qiagen DNEasy® Blood and Tissue Kit following a modified protocol described in Franklin et al. (2019). The other half of the filter is retained and stored at -20 °C. If more than one filter was used to collect the sample, DNA from all extracted filter halves for a given sample is combined during DNA extraction.

All 107 samples will be analyzed for Rio Grande chub (*Gila pandora*) and Rio Grande sucker (*Pantosteus plebeius*) DNA using species-specific assays designed by the NGC. Each sample will be analyzed in triplicate on a QuantStudio 3 qPCR System (Life Technologies). A sample will be considered positive for the presence of the target species if one or more of the three qPCR reactions amplifies DNA of that species.

All reactions include an internal positive control (IPC) to ensure that the reaction is effective and sensitive to the presence of the target species' DNA. If the IPC appears inhibited (i.e., reduced amplification of the IPC DNA due to chemical compounds in the sample), we will treat the sample with a PCR inhibitor removal kit (Zymo Research) and re-analyze the sample in triplicate. Removal of inhibitors may result in loss of DNA in a sample. With elution volumes of 100–200 μ l, loss of DNA during inhibitor removal is on average less than 10% (see <http://www.zymoresearch.com> for more details). Thus, to minimize potential DNA loss, we extract the second half of the sample filter and combine all extracted DNA from a given sample to obtain ~200 μ l of extracted DNA. All laboratory experiments are conducted with negative controls to ensure there is no contamination during DNA extraction or qPCR setup.

CONCLUSIONS

We will follow up with a final report once all of the eDNA samples have been analyzed and pass QAQC standards. If you have any questions about the project details to date, please do not hesitate to contact us.

Table 7. Location and collection details of eDNA samples collected by the National Genomics Center in the Rio Chama basin for the delineation of Rio Grande sucker and Rio Grande chub distributions. “Y” in denotes eDNA sample was collected, “N” denotes site was visited but no eDNA could be collected.

Site	Drainage	Stream	Sampled	Latitude	Longitude	Date Collected	No. Filters	Filter Volume (L)	Field Notes
100-1	Rio Chama	Polvadera Creek	Y	36.085885	-106.443873	4/20/2022	3	a:2, b:2.1, c:2	
1071-2	Rio Chama	Rio Chama	Y	36.421312	-106.70357	4/6/2022	3	a:0.89, b:0.8, c:0.9	
109-1	Rio Chama	Polvadera Creek	Y	36.101076	-106.445966	4/20/2022	3	a:1.6, b:1.6, c:1.7	
1145-1	Rio Chama	Rio Cebolla	Y	36.461353	-106.70355	4/6/2022	1	5	
1156-2	Rio Chama	Rio Cebolla	Y	36.466473	-106.685732	4/19/2022	1	5	possible crayfish observed ~1km upstream
1160-1	Rio Chama	Rio Chama	Y	36.461933	-106.705779	4/6/2022	3	a:0.8, b:0.8, c:0.8	
1162-1	Rio Chama	Rio Cebolla	Y	36.170557	-106.664774	4/19/2022	1	5	Fish present ~30 M upstream, crayfish present ~270 M upstream
1167-2	Rio Chama	Rio Cebolla	Y	36.482276	-106.622487	4/19/2022	3	a:1, b:1.5, c:2	crayfish present
1171-1	Rio Chama	Rio Cebolla	Y	36.480305	-106.605796	4/19/2022	3	a:1.5, b:2, c:1.5	no flow
1187-3	Rio Chama	Canjilon Creek	Y	36.508376	-106.397826	4/17/2022	3	a:0.5, b:0.5, c:0.5	
1194-2	Rio Chama	Canjilon Creek	Y	36.509705	-106.372819	4/17/2022	3	a:0.3, b:0.3, c:0.3	
1236-1	Rio Chama	Rio Chama	Y	36.484059	-106.723964	4/6/2022	3	a:0.8, b:0.85, c:0.85	
125-2	Rio Chama	Coyote Creek	Y	36.09234	-106.618711	4/20/2022	1	5	
1253-3	Rio Chama	Canjilon Creek	Y	36.533657	-106.326695	4/17/2022	3	a:1, b:0.5, c:0.5	
1268-3	Rio Chama	Rio Chama	Y	36.516437	-106.722645	4/6/2022	3	a:0.8, b:0.8, c:0.8	
128-2	Rio Chama	Polvadera Creek	Y	36.119886	-106.437636	4/20/2022	3	a:1.2, b:1.2, c:1.2	
1311-1	Rio Chama	Rio Chama	Y	36.53951	-106.736523	4/6/2022	3	a:0.8, b:0.8, c:0.8	Muddy/silty
1319-1	Rio Chama	Rio Nutrias	Y	36.552991	-106.715254	4/6/2022	3	a:1.75, b:2, c:1.75	
1319-3	Rio Chama	Rio Nutrias	Y	36.554272	-106.696108	4/18/2022	3	a:0.01, b:0.01, c:0.01	No water moving through filters, estimated volume in tubing
1324-10	Rio Chama	Terrero Creek	Y	36.595298	-106.424829	4/20/2022	3	a:0.5, b:0.5, c:0.5	Water stained
1324-13	Rio Chama	Terrero Creek	Y	36.59814	-106.39545	4/20/2022	3	a:1.5, b:1.3, c:1.2	Incised channel below culvert, stained water
1352-1	Rio Chama	Rio Chama	Y	36.553096	-106.716464	4/6/2022	3	a:1.5, b:1.4, c:1.5	
1370-1	Rio Chama	Rio Nutrias	Y	36.61492	-106.41884	4/20/2022	3	a:0.4, b:0.4, c:0.3	
1377-1	Rio Chama	Rio Nutrias	Y	36.616442	-106.395639	4/20/2022	3	a:0.5, b:0.5, c:0.5	Frogs in pond on intermittent tributary, bear track in snow
155-1	Rio Chama	Coyote Creek	Y	36.102244	-106.616931	4/20/2022	3	a:1.7, b:1.9, c:1.4	
160-1	Rio Chama	Coyote Creek	Y	36.117649	-106.614175	4/20/2022	2	a:2.5, b:2.5	
186-2	Rio Chama	Polvadera Creek	Y	36.136122	-106.425848	4/20/2022	1	5	
192-1	Rio Chama	Canones Creek	Y	36.138184	-106.45368	4/18/2022	2	a:2.5, b:2.5	Moved downstream 114M? For access
209-4	Rio Chama	Polvadera Creek	Y	36.166463	-106.425776	4/18/2022	1	5	
216-1	Rio Chama	Coyote Creek	Y	36.138908	-106.616752	4/20/2022	1	5	
225-1	Rio Chama	Canones Creek	Y	36.174847	-106.443489	4/17/2022	3	a:2, b:2, c:1	
225-3	Rio Chama	Canones Creek	Y	36.158254	-106.445708	4/18/2022	2	a:2.5, b:2.5	
268-2	Rio Chama	Rio Capulin - Corrected	Y	36.195569	-106.811359	4/18/2022	3	a:0.9, b:0.75, c:0.8	
29-3	Rio Chama	Rio del Oso	Y	36.049748	-106.348183	4/21/2022	1	5	
313-1	Rio Chama	Canones Creek	Y	36.199044	-106.450895	4/17/2022	3	a:1.1, b:1.1, c:1	Moved upstream 300M to get on BLM land
348-1	Rio Chama	Rio Chama	Y	36.209014	-106.325155	4/4/2022	2	a:2.5, b:2.5	
354-1	Rio Chama	Rio Chama	Y	36.21605065	-106.2469835	4/4/2022	2	a:2.5, b:2.5	
372-1	Rio Chama	Rio Gallina	Y	36.202913	-106.855958	4/18/2022	3	a:0.5, b:0.55, c:0.52	
372-3	Rio Chama	Rio Gallina	Y	36.194374	-106.844122	4/18/2022	3	a:1, b:1, c:1	
413-2	Rio Chama	Rio Capulin	Y	36.229991	-106.810377	4/19/2022	3	a:0.35, b:0.37, c:0.36	Site moved to road
413-4	Rio Chama	Rio Capulin	Y	36.21462	-106.787905	4/18/2022	3	a:0.4, b:0.4, c:0.5	Snow melt/runoff. Very turbid
413-5	Rio Chama	Rio Capulin	Y	36.20836	-106.796749	4/18/2022	3	a:0.3, b:0.3, c:0.3	

456-2	Rio Chama	Rio Gallina	Y	36.212058	-106.861197	4/18/2022	3	a:0.5, b:0.5, c:0.5	Extremely turbid, likely clays from runoff
541-3	Rio Chama	Rio Capulin	Y	36.240542	-106.829396	4/19/2022	3	a:0.12, b:0.13, c:0.13	
545-1	Rio Chama	Rio Gallina	Y	36.248716	-106.859091	4/19/2022	3	a:0.1, b:0.13, c:0.1	
56-1	Rio Chama	Coyote Creek	Y	36.072226	-106.627484	4/20/2022	1	5	
56-3	Rio Chama	Coyote Creek	Y	36.059631	-106.614908	4/20/2022	1	5	
56-5	Rio Chama	Coyote Creek	Y	36.046704	-106.601353	4/20/2022	1	5	
60-1	Rio Chama	Rio del Oso	Y	36.083536	-106.207951	4/18/2022	1	5	
645-1	Rio Chama	Cañada de la Fuertes	Y	36.329677	-106.624627	4/17/2022	3	a:0.24, b:0.24, c:0.24	
837-2	Rio Chama	Rio Chama	Y	36.351989	-106.662246	4/17/2022	3	a:.29, b:0.28, c:0.32	
897-1	Rio Chama	Rio Gallina	Y	36.372107	-106.681866	4/7/2022	3	a:0.1, b:0.1, c:0.1	Extremely muddy. Mink (?) in mainstream Chama upstream
958-1	Rio Chama	Rio Gallina	Y	36.390953	-106.811957	4/19/2022	3	a:0.07, b:0.07, c:0.07	Extremely turbid
961-1	Rio Chama	Rio Chama	Y	36.373229	-106.681497	4/7/2022	3	a:0.6, b:0.6, c:0.6	Upstream of Rio Gallina. Very low flow
961-3	Rio Chama	Rio Chama	Y	36.387847	-106.689621	4/7/2022	3	a:1, b:1, c:1	
984-1	Rio Chama	Canjilon Creek	Y	36.411978	-106.483856	4/17/2022	3	a:0.5, b:0.5, c:0.2	
993-3	Rio Chama	Rio Gallina	Y	36.395237	-106.781382	4/19/2022	3	a:0.05, b:0.05, c:0.05	Extremely turbid
994-1	Rio Chama	Rio Gallina	Y	36.391938	-106.800084	4/19/2022	3	a:0.075, b:0.075, c:0.075	Extremely silty, lots of sediment on filters
001-1	Rio Chama	Cañones Creek	Y	36.22163	-106.451096	4/17/2022	3	a:0.53, b:0.56	
001-1	Rio Chama	Rio Chama	Y	36.311804	-106.582645	4/17/2022	3	a:0.2, b:0.2, c:0.2	Weekend high flows, added site at big eddy takeout, all volumes between 100-200mL (estimated)
001-1	Rio Chama	Rio Chama	Y	36.233112	-106.389359	4/20/2022	3	a:1.4, b:1.4, c:1.45	
500-1	Rio Chama	Rio Capulin	Y	36.237572	-106.826159	4/19/2022	3	a:0.17, b:0.18, c:0.15	

Table 8. Location and collection details of eDNA samples collected by the National Genomics Center in the Upper Rio Grande basin for the delineation of Rio Grande sucker and Rio Grande chub distributions. “Y” in denotes eDNA sample was collected, “N” denotes site was visited but no eDNA could be collected.

Site	Drainage	Stream	Sampled	Latitude	Longitude	Date Collected	No. Filters	Filter Volume (L)	Field Notes
1013-1	Upper Rio Grande	Rio Grande	Y	36.648405	-105.693996	3/29/2022	3	a:1, b:1, c:1	
1013-6	Upper Rio Grande	Rio Grande	Y	36.682274	-105.690162	4/1/2022	3	a:0.55, b:0.525, c:0.5	Moved downstream due to access
1085-2	Upper Rio Grande	Rio Grande	Y	36.721122	-105.691274	3/29/2022	3	a:1.2, b:1.2, c:1.2	Moved downstream ~1km due to canyon wall
1166-1	Upper Rio Grande	Rio Grande	Y	36.764109	-105.670452	4/1/2022	3	a:0.65, b:0.65, c:0.625	
1255-1	Upper Rio Grande	Rio Grande	Y	36.81485378	-105.6963296	3/31/2022	3	a:0.7, b:0.7, c:0.6	
1321-5	Upper Rio Grande	Rio Grande	Y	36.84402861	-105.6987363	3/31/2022	3	a:0.6, b:0.6, c:0.55	
1361-1	Upper Rio Grande	Rio Grande	Y	36.88275634	-105.703039	3/31/2022	3	a:0.45, b:1.1, c:0.5	filter "b" has two small holes that developed towards end of filtering
1390-2	Upper Rio Grande	Rio Grande	Y	36.90516818	-105.7310709	3/31/2022	3	a:0.7, b:0.675, c:0.725	
1424-1	Upper Rio Grande	Rio Grande	Y	36.9429486	-105.7337788	3/30/2022	3	a:0.75, b:0.7, c:0.65	~600m downstream of site
1439-1	Upper Rio Grande	Rio Grande	Y	36.98108186	-105.7170887	3/30/2022	3	a:0.65, b:0.75, c:0.68	
1439-3	Upper Rio Grande	Rio Grande	Y	36.9954545	-105.7179316	3/30/2022	3	a:0.65, b:0.55, c:0.55	State line
221-1	Upper Rio Grande	Rio Medio	Y	35.965354	-105.904062	4/3/2022	2	a:3.5, b:1.5	
221-2	Upper Rio Grande	Rio Medio	Y	35.972227	-105.897124	4/4/2022	2	a:4, b:1	
344-1	Upper Rio Grande	Rio Santa Barbara	Y	36.09023742	-105.6110723	4/4/2022	1	5	
353-11	Upper Rio Grande	Rio Santa Barbara	Y	36.11067635	-105.6352408	4/4/2022	1	5	
353-13	Upper Rio Grande	Rio Santa Barbara	Y	36.10122485	-105.6186117	4/4/2022	1	5	
474-1	Upper Rio Grande	Rio Grande del Rancho	Y	36.25326028	-105.5794586	3/29/2022	1	5	Beaver activity
502-3	Upper Rio Grande	Rio Grande	Y	36.19494852	-105.9749896	4/3/2022	3	a:0.5, b:0.5, c:0.3	
502-7	Upper Rio Grande	Rio Grande	Y	36.21087879	-105.9400334	4/3/2022	3	a:0.5, b:0.5, c:0.5	
516-9	Upper Rio Grande	Rio Grande	Y	36.204146	-105.837648	4/3/2022	3	a:0.6, b:0.6, c:0.7	Moved upstream due to private land
530-1	Upper Rio Grande	Rio Grande del Rancho	Y	36.29782722	-105.5823719	3/29/2022	2	a:3, b:2	
533-2	Upper Rio Grande	Rio Grande	Y	36.258982	-105.8102	4/3/2022	3	a:0.6, b:0.625, c:0.6	
544-1	Upper Rio Grande	Rio Grande del Rancho	Y	36.30996897	-105.584612	3/29/2022	2	a:4, b:1	Beaver activity
549-3	Upper Rio Grande	Rio Grande del Rancho	Y	36.32484563	-105.5861714	3/29/2022	2	a:3.75, b:1.25	

555-3	Upper Rio Grande	Rio Grande	Y	36.292379	-105.782494	4/3/2022	3	a:0.7, b:0.8, c:0.8	
557-1	Upper Rio Grande	Rio Chiquito	Y	36.32938423	-105.5413934	3/29/2022	1	5	
559-4	Upper Rio Grande	Rio Chiquito	Y	36.33333687	-105.5827252	3/29/2022	1	5	Beaver activity
559-6	Upper Rio Grande	Rio Chiquito	Y	36.33285	-105.56189	3/29/2022	1	5	Beaver activity
565-2	Upper Rio Grande	Rio Grande	Y	36.311904	-105.764694	4/3/2022	3	a:0.7, b:0.85, c:0.625	
584-3	Upper Rio Grande	Rio Fernando de Taos	Y	36.37219682	-105.3860654	4/1/2022	3	a:0.75, b:0.75, c:1.25	Beaver activity
587-1	Upper Rio Grande	Rio Grande	Y	36.34988	-105.72994	4/3/2022	3	a:0.65, b:0.65, c:0.65	Moved upstream due to access
588-2	Upper Rio Grande	Rio Fernando de Taos	Y	36.36829143	-105.4741882	4/3/2022	3	a:1, b:1, c:1	
590-2	Upper Rio Grande	Rio Fernando de Taos	Y	36.36767929	-105.4434403	4/1/2022	2	a:3.5, b:1.5	Beaver activity
605-4	Upper Rio Grande	Rio Fernando de Taos	Y	36.39952077	-105.5894751	4/2/2022	3	a:2, b:2, c:1	No flow, fish observed
605-9	Upper Rio Grande	Rio Fernando de Taos	Y	36.37563834	-105.5480572	3/29/2022	3	a:1.5, b:1, c:1.5	
645-3	Upper Rio Grande	Rio Fernando de Taos	Y	36.3887714	-105.3555955	4/1/2022	1	5	
645-5	Upper Rio Grande	Rio Fernando de Taos	Y	36.40330593	-105.3457473	4/1/2022	2	a:2.5, b:2.5	
645-7	Upper Rio Grande	Rio Fernando de Taos	Y	36.42019051	-105.3429776	4/1/2022	1	5	
742-1	Upper Rio Grande	Rio Grande	Y	36.51444161	-105.7184106	4/3/2022	3	a:1, b:1, b:0.75	Angler reports "chub" in upstream reach
752-1	Upper Rio Grande	Rio Hondo	Y	36.534263	-105.706771	3/29/2022	2	a:3, b:2	
859-1	Upper Rio Grande	Rio Grande	Y	36.603441	-105.688334	4/3/2022	3	a:0.9, b:0.9, c:0.825	
850-3	Upper Rio Grande	San Cristobal Creek	Y	36.61451713	-105.6133385	4/2/2022	1	5	Move onto stream, coordinates off
603-1	Upper Rio Grande	Rio Fernando de Taos	Y	36.38183446	-105.5233494	4/3/2022	2	a:2, b:3	Shifted because private. May share coordinates with 605-11
001-1	Upper Rio Grande	Red River	Y	36.648236	-105.69322	3/29/2022	2	a:3, b:2	Added site. Upstream of confluence with Rio Grande
001-1	Upper Rio Grande	Rio Pueblo de Taos	Y	36.337817	-105.727474	4/3/2022	3	a:1.05, b:1.05, c:1	No access
1426-1	Upper Rio Grande	Costilla Creek	N	36.980599	-105.716545				Dry
305-2	Upper Rio Grande	Rio Santa Barbara	N	36.072054	-105.610909				No access, snow
474-11	Upper Rio Grande	Rio Grande del Rancho	N	36.215027	-105.534641				No access, snow
474-3	Upper Rio Grande	Rio Grande del Rancho	N	36.237451	-105.586008				No access, snow
474-5	Upper Rio Grande	Rio Grande del Rancho	N	36.222302	-105.588643				No access, snow
474-7	Upper Rio Grande	Rio Grande del Rancho	N	36.211557	-105.575324				No access, snow

474-9	Upper Rio Grande	Rio Grande del Rancho	N	36.218503	-105.555415	No access, snow
478-2	Upper Rio Grande	Rio Grande del Rancho	N	36.213772	-105.512493	No access, snow
478-4	Upper Rio Grande	Rio Grande del Rancho	N	36.218409	-105.494522	No access, snow
605-11	Upper Rio Grande	Rio Fernando de Taos	N	36.380658	-105.528503	No access
645-9	Upper Rio Grande	Rio Fernando de Taos	N	36.436206	-105.341598	No access, snow
797-3	Upper Rio Grande	Rio Grande	N	36.552468	-105.703153	No access
832-1	Upper Rio Grande	San Cristobal Creek	N	36.583897	-105.694831	Dry
832-3	Upper Rio Grande	San Cristobal Creek	N	36.583608	-105.674607	Dry
850-1	Upper Rio Grande	San Cristobal Creek	N	36.599851	-105.625253	No access

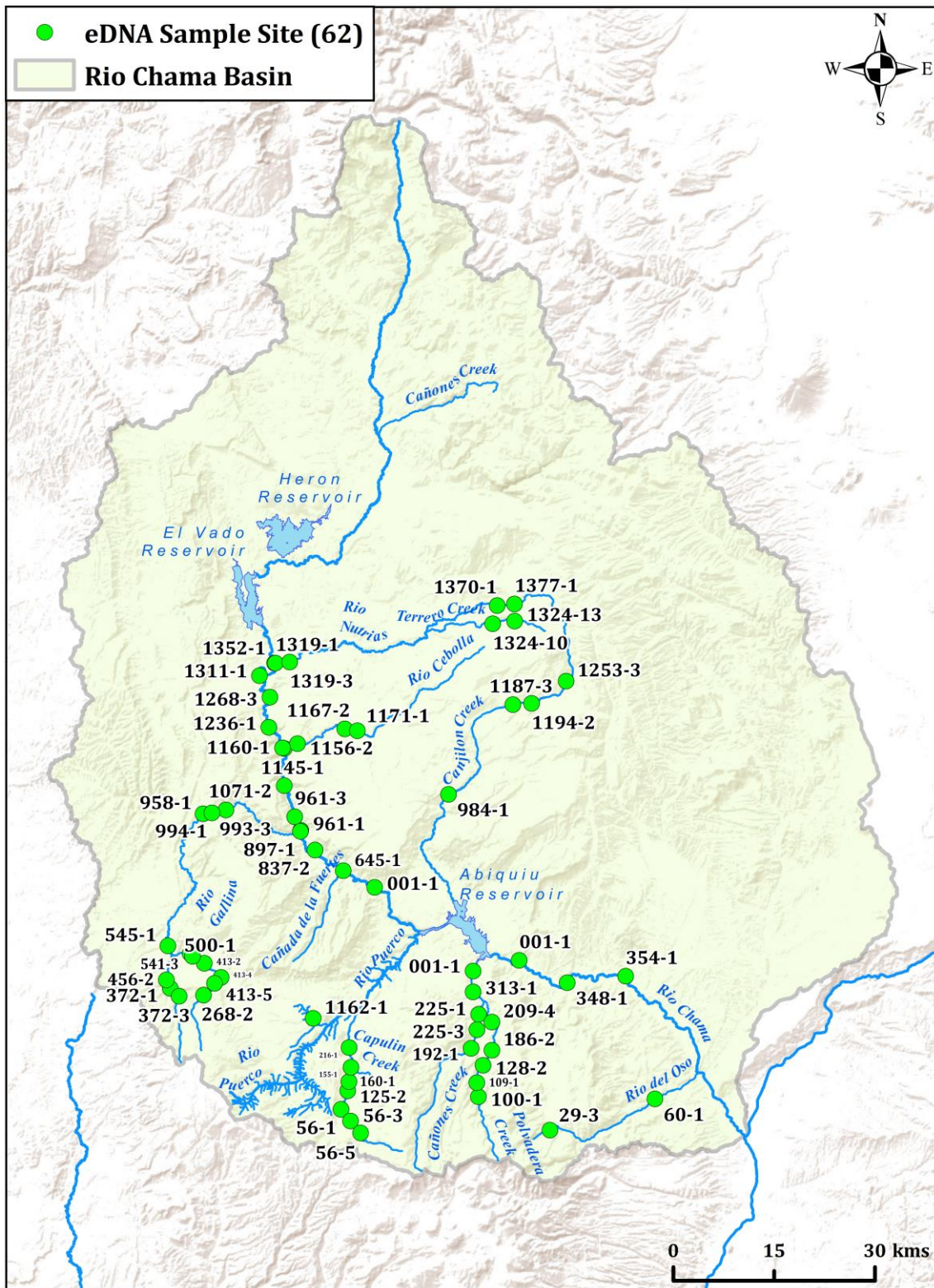


Figure 2. Locations of eDNA sites visited in the Rio Chama basin by the National Genomics Center in 2022. A green circle denotes eDNA sample collected. The transparent light yellow background with a thick gray border represents the Rio Chama basin.

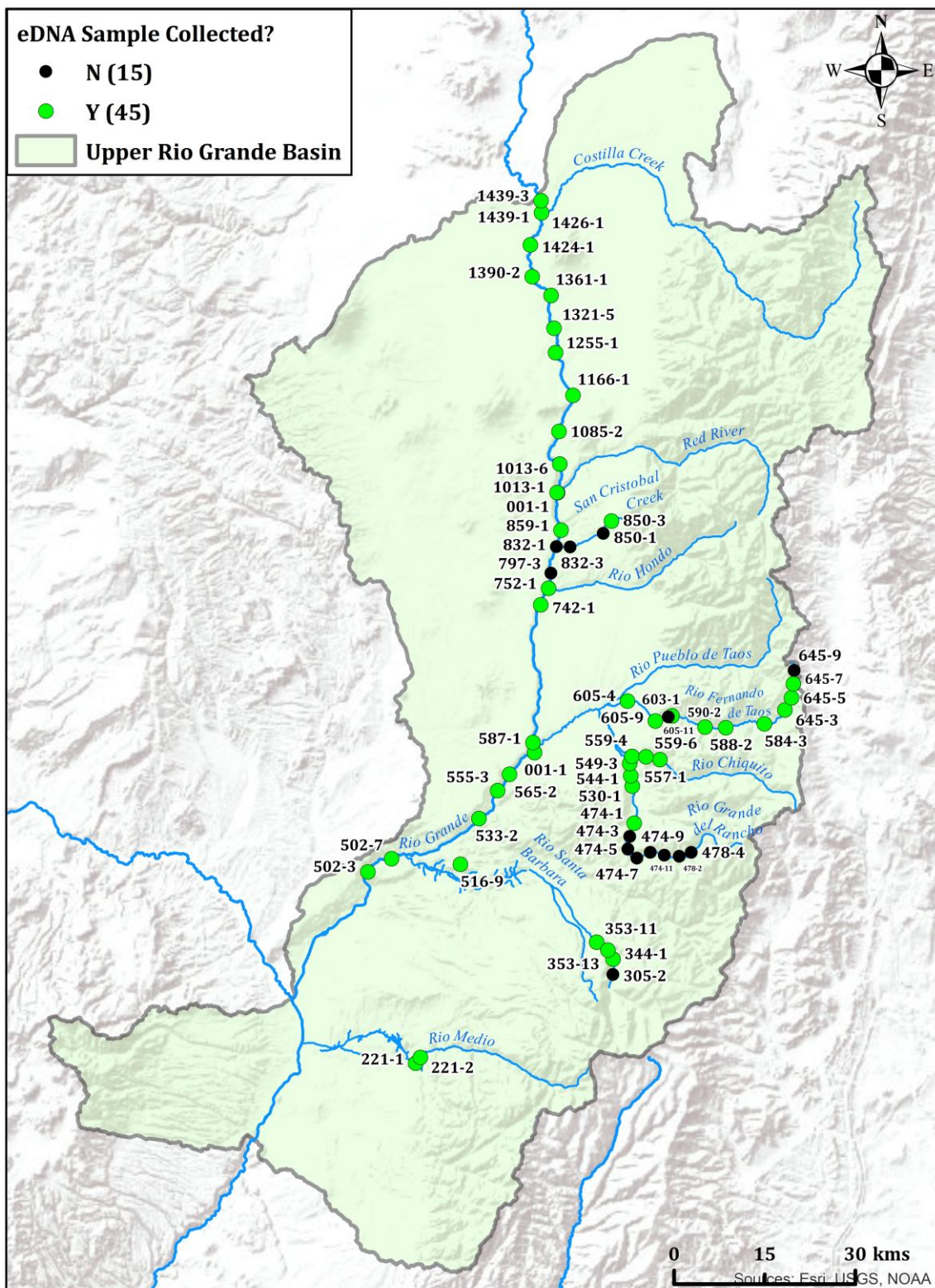


Figure 3. Locations of eDNA sites visited in the Upper Rio Grande basin by the National Genomics Center in 2022. A green circle denotes eDNA sample collected and a black circle denotes the eDNA site was visited but no sample could be collected. The transparent light green background with a thick gray border represents the Upper Rio Grande basin.

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