



INTERLOCAL COOPERATION CONTRACT NO. 25-025-TES
BETWEEN THE
EDWARDS AQUIFER AUTHORITY
AND
TEXAS STATE UNIVERSITY
FOR COMAL SPRINGS RIFFLE BEETLE GENETICS STUDY

This INTERLOCAL COOPERATION CONTRACT (Contract) is made and entered into under the Texas Interlocal Cooperation Act (ICA), Chapter 791, Texas Government Code, by and between the EDWARDS AQUIFER AUTHORITY (EAA), a conservation and reclamation district and a political subdivision of the State of Texas, with its principal place of business located at 900 E. Quincy Street, San Antonio, Texas 78215, and TEXAS STATE UNIVERSITY (TEXAS STATE), an institution of higher learning, with its principal place of business located at 601 University Drive, San Marcos, Texas, 78666. Each of these entities is, at times, referred to in this Contract individually as a “Party,” and both are referred to collectively as “Parties.”

RECITALS

WHEREAS, the EAA was created by the Edwards Aquifer Authority Act, Act of May 30, 1993, 73rd Leg., R.S., ch. 626, 1993 Tex. Gen. Laws 2350, as amended (Act); and

WHEREAS, under Section 1.02 of the Act, the EAA was created by virtue of Article XVI, Section 59 of the Texas Constitution, and is a governmental agency and body politic and corporate vested with the full authority to exercise the powers and to perform the functions specified in the Act; and

WHEREAS, under Section 1.08(a) of the Act, the EAA has all the powers, rights and privileges necessary to manage, conserve, preserve, and protect the Edwards Aquifer (Aquifer) and to increase the recharge of, and prevent the waste or pollution of water in, the Aquifer; and

WHEREAS, under Section 1.11(d)(2) of the Act, and other applicable law, the EAA may enter into contracts; and

WHEREAS, this Contract is an interlocal contract entered into under the authority of the ICA, Chapter 791, Texas Government Code; and

WHEREAS, under Section 791.003(4)(A) and (5) of the ICA, the EAA is a “local government”; and

WHEREAS, under Section 771.002(1)(B) of the Texas Government Code, TEXAS STATE is an “agency” of the State of Texas; and

WHEREAS, under Section 791.011 of the ICA, a local government may contract with a state agency, as that term is defined in 771.002 of the Texas Government Code; and

WHEREAS, under Section 791.011(g), a state agency “that makes purchases or provides purchasing services under an interlocal contract for a state agency, as that term is defined under Section 771.002, must comply with Chapter 2161 [relating to historically underutilized businesses] in making the purchase or providing the services”; and

WHEREAS, under Section 791.003(3) of the ICA, the activities that are the subject of this Contract are governmental functions and services in that they involve the public health and welfare, planning, administrative functions, and/or other governmental functions in which the Parties are mutually interested; and

WHEREAS, the EAA Board of Directors approved this Contract on November 12, 2025 and authorized the EAA’s General Manager to execute the Contract; and

WHEREAS, the EAA’s Board of Directors has elected to pursue a genetics study of the Comal Springs Riffle Beetle; and

WHEREAS, the EAA and TEXAS STATE have been authorized by their respective governing bodies to enter into this Contract; and

WHEREAS, it is in the public interest that the EAA enter into this Contract.

AGREEMENT

NOW THEREFORE, for and in consideration of the mutual promises and agreements set forth in this Contract, the EAA and TEXAS STATE agree as follows:

ARTICLE I – TERM; DESCRIPTION OF WORK

Section 1.1. Term. This Contract is effective and commences on the date that has been executed by both Parties (Effective Date) and terminates on December 31, 2027 (Expiration Date).

Section 1.2. Services. Subject to the terms and conditions of this Contract, the EAA engages TEXAS STATE to perform, for the benefit of the EAA, the research work set forth and described in this Contract and in the following documents (Services), which are attached hereto: (1) the Scope of Work, which is attached hereto as Exhibit A; (2) the Project Schedule, which is attached hereto as Exhibit B; and (3) the Budget Estimate, which is attached hereto as Exhibit C. TEXAS STATE accepts such engagement and agrees to devote its best efforts and abilities, and furnish all necessary labor, machinery, equipment, tools, and transportation necessary in furtherance thereof.

Section 1.3. Commencement and Completion of Services. TEXAS STATE will commence performing the Services consistent with the Effective Date. All Services will be completed and delivered to the EAA by the Expiration Date and shall be completed in compliance with the

schedules, budgets, descriptions and specifications contained herein and in the Exhibits attached hereto. It shall be TEXAS STATE's responsibility to ensure that the completion times for the tasks required under this Contract are met. Best efforts shall be used in the performance and completion of this project with appropriate and frequent communication relating to unforeseen circumstances.

ARTICLE II – AMENDMENTS

Section 2.1. Amendments. This Contract, including Exhibits, may be amended only by written agreement of the Parties.

Section 2.2. Delegation to the General Manager. The Board of Directors of the EAA delegates the authority to the General Manager to enter into amendments to this Contract without further authorization by the Board consistent with the General Manager's authority to enter into contracts under Section 5.01 of the EAA's Bylaws.

ARTICLE III – COMPENSATION

Section 3.1. Fees and Expenses. The EAA agrees to pay TEXAS STATE for the Services rendered in accordance with the Scope of Work and Budget Estimate, but in no event shall payments to TEXAS STATE exceed \$73,790. TEXAS STATE may not exceed this amount and will be responsible for the payment of all of its other additional costs and expenses. TEXAS STATE is not authorized to expend any additional funds in excess of this amount without the prior written approval from the EAA. The EAA will not be held accountable for any unauthorized work performed or funds expended by TEXAS STATE in providing the Services. TEXAS STATE agrees to be directly responsible for the payment of any invoices, costs or expenses incurred by it or its Subcontractor in the performance of the Services.

Section 3.2. Payment. All "invoice packets" from the Contractor to the EAA for the Services for each previous month's activities shall be sent no later than the thirtieth (30th) day of each month beginning January 15, 2026, and shall provide an itemization of the Services rendered, costs and expenses incurred during the billing cycle. Final invoice will be sent no later than ninety (90) days after the end of the project either due to termination or project expiration.

As applicable, documentation in the invoice packet must be sufficiently itemized and provide detail for all purchases and expenses to allow the EAA to clearly discern purchases made. Purchases and expenses documented without required receipts are not eligible for reimbursement. Receipts without purchase details or that are not legible are not acceptable at any time. Alcohol purchases will NOT be reimbursed under any circumstances. Mileage will be reimbursed at the then-current IRS approved rate, if submitted. In no event will the EAA pay per diem-related expenses; expenses will be paid at cost with appropriate documentation. Each invoice packet shall contain, at a minimum, a progress report containing: (1) a description of the work completed in each task in the Scope of Work during the billing cycle, (2) a monthly update of the work schedule as it relates to achievement of the deliverables, (3) an estimate of the percent completion of each task in the Scope of Work, and (4) a discussion of any issues or problems that may result in a change in the deliverable due date; and a certified invoice summary sheet. The monthly invoice

packet will be submitted electronically in Adobe Acrobat (pdf) format via email to Damon Childs, Sr. Contract Administrator at dchilds@edwardsaquifer.org and accounting@edwardsaquifer.org. The terms of each invoice shall be net thirty (30) days upon EAA receipt and approval of that invoice.

ARTICLE IV – INDEPENDENT CONTRACTOR

Section 4.1. No Employment Relationship. The Parties understand and agree that this Contract does not create a fiduciary relationship between them, that they are separate entities, that TEXAS STATE is an independent Contractor with respect to the performance of the Services and is not subject to the direct or continuous control and supervision of the EAA, and that nothing in this Contract is intended to make either Party a subsidiary, joint venturer, partner, employee, agent, servant or representative of the other Party for any purpose whatsoever. TEXAS STATE shall provide any and all equipment and materials necessary for the performance of the Services. The EAA shall have no right of direction or control of TEXAS STATE, or its employees and agents, except in the results to be obtained, and in a general right to order the performance of the Services to start or stop as agreed to herein, to inspect the progress of the Services, and to receive reports. TEXAS STATE shall accommodate reasonable requests from the EAA to allow EAA employees, agents or representatives to accompany and observe TEXAS STATE's personnel in carrying out the Services under this Contract.

ARTICLE V – TEXAS STATE PERSONNEL AND SUBCONTRACTORS

Section 5.1. Personnel. TEXAS STATE will provide any and all personnel necessary for its performance of the Services. To the extent permitted by the laws and Constitution of the State of Texas, TEXAS STATE will be responsible for its employees and agents in all respects, including, without limitation, their compliance with applicable laws and their safety. To the extent authorized by the laws and Constitution of the State of Texas, TEXAS STATE indemnifies and holds harmless the EAA, its officers, employees, and directors, from and against any claims brought by any employee, subcontractor, or other agent of TEXAS STATE relating in any way to the Services performed under this Contract. Notwithstanding any provision of this Contract to the contrary, nothing herein shall be construed as a waiver by TEXAS STATE of its constitutional, statutory, or common law rights, privileges, immunities, or defenses.

Section 5.2. Subcontractors. In performing the Services under this Contract, TEXAS STATE may retain and utilize as its subcontractors, to the extent that they are not already employees of TEXAS STATE, those individuals identified to and approved in writing by the EAA, in advance. The EAA, in consultation with TEXAS STATE, shall have the right to terminate, limit, or alter, at any time, the participation of any subcontractor utilized by TEXAS STATE. No additional subcontractors may be retained by TEXAS STATE to perform any Services under this Contract without the prior written consent of the EAA, provided that no such consent shall be necessary for the retention of any subcontractor previously approved by the EAA and identified by TEXAS STATE on the Effective Date. TEXAS STATE will be responsible for its subcontractors in all respects, including their compliance with applicable laws and their safety, including without limitation, all OSHA standards, requirements, and regulations.

Subcontracting services under this contract may be provided by:

- University of California at Berkeley

ARTICLE VI – TERMINATION

Section 6.1. Termination for Convenience. Either Party may terminate this Contract at any time, including at the expiration of each budget or payment period during the term of this Contract, with or without cause, upon ten (10) days' prior written notice to the other Party.

Section 6.2. Termination for Cause. If the Services are not being carried out in a manner satisfactory to the EAA, the EAA may terminate this Contract upon the failure of TEXAS STATE to cure the unsatisfactory condition within ten (10) days after receipt of written notice from the EAA.

Section 6.3. Actions in the Event of Early Termination. Upon receipt of a notice of termination under this Article, TEXAS STATE shall immediately stop all work in progress, including all work performed by its employees, agents, or subcontractors. Insofar as possible, all work in progress will be brought to a logical termination point. Within thirty (30) days of the final invoice following termination, the EAA shall pay TEXAS STATE all moneys then due and owing for the Services rendered, and costs and expenses reasonably incurred up to the time of termination. TEXAS STATE will deliver to the EAA copies of all finished and unfinished documents, data, studies, surveys, drawings, maps, reports, photographs, and other materials prepared by TEXAS STATE, and each Party shall be free to utilize such documents, data, studies, surveys, drawings, maps, reports, photographs, and other materials as it sees fit.

ARTICLE VII – OWNERSHIP OF MATERIALS

Section 7.1. Ownership. All information, documents, property, or materials produced, created, or supplied under this Contract by TEXAS STATE, its employees, agents or subcontractors or anyone else, and whether finished or unfinished or in draft or final form, will be the property of the EAA, although each Party shall be free to utilize such documents, data, studies, surveys, drawings, maps, reports, photographs, and other materials as it sees fit. The EAA shall have unlimited rights to technical and other data from the performance of TEXAS STATE's under this Contract. The EAA hereby grants to TEXAS STATE a non-revocable, perpetual, royalty-free, fully paid license to use project intellectual property as needed for furthering research, academic purposes, and other needs.

Section 7.2. Delivery of Documents upon Termination. Upon termination of this Contract under Sections 1.3 or 6.1, all such information, documents, property and materials not already in the possession of the EAA will be promptly delivered to the EAA.

Section 7.3. Nondisclosure of Documents. To the extent permitted by the laws and Constitution of the State of Texas, including but not limited to the Texas Public Information Act, the information, documents, property, or materials produced, created or supplied under this Contract by TEXAS STATE, including preliminary technical reports and studies, shall not be disclosed by TEXAS STATE to any third party without the prior written consent of the EAA.

TEXAS STATE shall advise the EAA of any requests by a third party for any such information, document, property, or materials.

Section 7.4. Record Copies. TEXAS STATE shall retain a record copy of all information, documents, property, or materials developed in the course of performing the Services. Upon request of the EAA, such information, documents, property, or materials will be promptly supplied to the EAA, including after the Expiration Date or the termination of this Contract under Section 6.1. The EAA will reimburse TEXAS STATE for actual cost of time and expenses of reproduction of such materials when requested.

ARTICLE VIII – PERFORMANCE

Section 8.1. Performance. TEXAS STATE represents that it will perform this project in a good and workmanlike manner, strictly in accordance with the standards of the representatives of TEXAS STATE's profession, the Scope of Work, and as otherwise provided in this Contract and the Exhibits attached hereto. Best efforts shall be used in the performance and completion of this project with appropriate and frequent communication relating to unforeseen circumstances.

ARTICLE IX – LIQUIDATED DAMAGES

THIS ARTICLE LEFT BLANK

ARTICLE X – BOND COVERAGE

THIS ARTICLE LEFT BLANK

ARTICLE XI – INSURANCE

Section 11.1. Insurance Coverages. EAA acknowledges that, because TEXAS STATE is an agency of the State of Texas, liability for the tortious conduct of the agents and employees of TEXAS STATE is governed by Chapters 101 and 104, Texas Civil Practice and Remedies Code and Chapter 2259, Texas Government Code, and that Workers' Compensation Insurance coverage for employees of TEXAS STATE is provided by TEXAS STATE as mandated by the provisions of Chapter 501, Texas Labor Code. The liability of TEXAS STATE and its representatives for personal injury and property damage is controlled by the Texas Tort Claims Act, Texas Civil Practice and Remedies Code, Chapter 101, Section 101.021. Per these statutes, the limits of liability are \$250,000 for each person, \$500,000 for each single occurrence for bodily injury or death and \$100,000 for each single occurrence for injury to or destruction of property.

Section 11.2. Intentionally Omitted

Section 11.3. Intentionally Omitted

ARTICLE XII – ASSUMPTION OF RISK AND INDEMNIFICATION

Section 12.1. Risk. To the extent authorized by the laws and Constitution of the State of

Texas each Party shall assume all risks associated with TEXAS STATE's or its subcontractors' performance under this Contract and shall waive any claim against the EAA and other participants for damages arising out of the performance of the Services under this Contract.

Section 12.2. Indemnification. To the extent authorized by the laws and Constitution of the State of Texas, each Party shall defend, indemnify, and hold harmless the other Party, its directors, officers, and employees from any and all damages, loss, or liability arising from (a) other contracts or arrangements between the indemnifying Party and any third parties that are entered into to perform the Services of this Contract, (b) any claims brought by any person relating to this Contract or the Services, or (c) the quality or the performance of the Services. The foregoing indemnification is limited to the extent that such liability, loss, or damage was caused by the negligent act, error, or omission of the indemnifying Party or any person or entity for whom the indemnifying Party is legally liable.

Notwithstanding any provision of this Contract, nothing herein shall be construed as a waiver by TEXAS STATE or the EAA of its constitutional, statutory or common law rights, privileges, immunities or defenses, including, but not limited to, waiver of TEXAS STATE's or the EAA's (i) sovereign immunity to suit; (ii) sovereign immunity against the recovery of money damages; or (iii) right to a jury trial for any issue arising under this Contract. To the extent the terms of this paragraph conflict with any other provision in the Contract, the terms of this paragraph shall control.

ARTICLE XIII – NOTICES

Section 13.1. Notices to the EAA. All notices or communications under this Contract to be mailed or delivered to the EAA shall be in writing and shall be sent to the EAA's principal place of business as follows, unless and until TEXAS STATE is otherwise notified:

EDWARDS AQUIFER AUTHORITY
ATTENTION: Shelly Hendrix, Controller/Sr. Director of Finance
900 E. Quincy Street
San Antonio, Texas 78215
Phone: (210) 222-2204
contracting@edwardsaquifer.org

Section 13.2. Notices to TEXAS STATE. All notices or communications under this Contract to be mailed or delivered to TEXAS STATE shall be in writing and shall be sent to the address of TEXAS STATE as follows, unless and until the EAA is otherwise notified:

TEXAS STATE UNIVERSITY
Attn: Department of Biology
601 University Drive
San Marcos, TX 78666
ccnice@txstate.edu

with a copy to:

TEXAS STATE UNIVERSITY
Attn: Office of Sponsored Programs
601 University Drive, JCK 420
San Marcos, TX 78666-4684
Phone: 512-245-2101
grants@txstate.edu

Section 13.3. Effective Date of Notice. Any notices or communications required to be given in writing by one Party to the other shall be considered as having been given to the addressee on the date the notice of communication is posted by the sending Party. Electronic notices shall be deemed delivered as of the time received as indicated by the receiving Party's system.

ARTICLE XIV – MISCELLANEOUS

Section 14.1. Entire Agreement. This Contract and the attached Exhibits constitute the entire agreement between the Parties regarding the Services to be performed by TEXAS STATE and there are no representations, warranties, agreements or commitments between the Parties except as set forth herein. Unless otherwise authorized herein, no amendments or additions to this Contract shall be binding on the Parties unless in writing and signed by the Parties.

Section 14.2. Non-Waiver. No delay or failure by either Party to exercise any right under this Contract, nor any partial or single exercise of that right, shall constitute a waiver of that or any other right, unless otherwise expressly provided herein.

Section 14.3. Headings. Headings in this Contract are for convenience only and shall not be used to interpret or construe its provisions.

Section 14.4. Governing Law. This Contract shall be deemed to have been executed and performed in the State of Texas and shall be construed in accordance with and governed by the laws of the State of Texas.

Section 14.5. Counterparts. This Contract may be executed in two or more counterparts, each of which shall be deemed an original but all of which together shall constitute one and the same instrument.

Section 14.6. Binding Effect. The provisions of this Contract shall be binding upon and inure to the benefit of the Parties and their respective successors and assigns, provided, however, that TEXAS STATE may not assign any of its rights nor delegate any of its duties hereunder without the EAA's prior written consent.

Section 14.7. Validity. The invalidity of any provision or provisions of this Contract shall not affect any other provision of this Contract, which shall remain in full force and effect, nor shall the invalidity of a portion of any provision of this Contract affect the balance of such provision.

Section 14.8. Non-Waiver of Immunity. Nothing in this Contract is intended as any waiver by either Party of any immunity from suit to which it is entitled under Texas law.

Section 14.9. Survival. Termination of this Contract for breach shall not constitute a waiver of any rights or remedies available at law or in equity to a Party to redress such breach. All remedies, either under this Contract or at law or in equity or otherwise available to a Party, are cumulative and not alternative and may be exercised or pursued separately or collectively in any order, sequence or combination. In addition, to these provisions, applicable provisions of this Contract shall survive any termination of this Contract.

Section 14.10. Attachments. The Exhibits, schedules and/or other documents attached, referred to, are incorporated in and made a part of this Contract for all purposes. As used herein, the expression "Contract" means the body of this Contract and such attachments, Exhibits, schedules and/or other documents, and the expressions "herein," "hereof," and "hereunder" and other words of similar import refer to this Contract and such attachments, exhibits, schedules and/or other documents as a whole and not to any particular part or subdivision thereof.

Section 14.11. Intentionally Omitted.

Section 14.12. Authority to Contract.

(a) Each Party represents for the benefit of the other Party that: (1) it has the legal authority to enter into this Contract under Section 791.011(a), Texas Government Code, and/or other applicable law; (2) it has the legal authority to provide the Services contracted for under this Contract; (3) this Contract has been duly approved and executed; (4) no other authorizations or approvals are or will be necessary in order to approve this Contract and to enable that Party to enter into and comply with the terms and conditions of this Contract; (5) the person executing this Contract on behalf of each Party has the authority to bind that Party; and (6) the Party is empowered by law to execute any other agreement or documents and to give such other approvals, in writing or otherwise, as are or may hereafter be required to implement and comply with this Contract.

(b) TEXAS STATE represents that it believes that the contractual payment to be made under this Contract is in an amount that fairly compensates it for the Services performed under the Contract as required by Section 791.011(e), Texas Government Code.

Section 14.13. Officers or Agents. No officer or agent of the Parties is authorized to waive or modify any provision of this Contract. No amendment to or rescission of this Contract may be made except by a written document signed by the Parties' authorized representatives.

Section 14.14. Payment from Current Revenues. As required by Section 791.011(d)(3), Texas Government Code, the EAA is making all payments required under this Contract from current revenues available to the EAA.

Section 14.15. Mediation. (a) Either Party may request informal consultation with the other Party at any time to resolve a current or anticipated controversy arising under this Contract. Any such request will not be unreasonably refused by the other Party. The consultation shall be

undertaken by both Parties in good faith and with due diligence to effect the purpose of this Contract. (b) In the event any current or anticipated controversy arising under this Contract is not resolved by informal consultations between the Parties within thirty (30) business days after any Party requests consultations, then, upon the request of any Party, the controversy will be referred, to the extent applicable, to the dispute resolution process provided for in Chapter 2260 of the Texas Government Code. If it is determined that Chapter 2260 of the Texas Government Code is not applicable, the parties agree to utilize the process provided by the Texas Civil Practice and Remedies Code, Chapter 154, to the extent applicable, as may be amended, or its successor statute.

Section 14.16. Force Majeure. If by reason of Force Majeure either Party will be rendered unable wholly or in part to carry out its obligations under this Contract, such Party will give notice and full particulars of such Force Majeure in writing to the other Party within a reasonable time after the occurrence of the event or cause relied on. After providing such notice, the obligation of the Party giving such notice, so far as its performance is prevented by such Force Majeure, will be suspended during the continuance of the inability then claimed, but for no longer period, and any such Party will endeavor to remove or overcome such inability with reasonable dispatch. The term "Force Majeure" as used herein will mean those situations or conditions which are beyond the control of the Party and which, after the exercise of due diligence to remedy such situation or condition, render the Party unable, wholly or in part, to carry out the covenants in this Contract. Such Force Majeure events are limited to: Acts of God, strikes, lockouts or other industrial disturbances, acts of public enemy, orders of any kind of the government of the United States or the State of Texas, regulatory restrictions imposed on TEXAS STATE by the Texas Legislature, any civil or military authority, insurrection, war, terrorism, riots, epidemics, landslides, lightning, earthquake, fires, hurricanes, tornados, storms, floods, washouts, restraint of government and people, civil disturbances, explosions, extraordinary breakage or accidents to machinery, or on account of any other causes insofar as any of the foregoing are beyond the reasonable control of the Party claiming such inability, or its employees, agents, contractors or subcontractors. Neither Party shall be deemed in default hereunder for any failure to perform due to any Force Majeure event.

IN WITNESS WHEREOF, this Contract is executed as of the day and date first written above, in Section 1.1.

EDWARDS AQUIFER AUTHORITY

TEXAS STATE UNIVERSITY

By: _____
Roland Ruiz
General Manager

By: _____
Shreek Mandayam
Vice President - Research

ATTEST:

By: _____
Jennifer Wong-Esparza
Assistant to the Board Secretary

APPROVED AS TO FORM:



Deborah Trejo
General Counsel
Edwards Aquifer Authority

EXHIBIT A SCOPE OF WORK

Background

The limestone aquifer systems of the Edwards Plateau in south-central Texas support a large number of endemic species, including the Comal Springs riffle beetle. Found in the headwaters of both the Comal Springs and the San Marcos Springs, the Comal Springs riffle beetle was listed as federally endangered in 1997. This entirely aquatic species has limited dispersal ability and completes its life cycle in close association with the thermally stable spring habitats it occupies. Due to these ecological constraints, the primary threats to Comal Springs riffle beetle are the quantity and quality of water in the aquifer.

Due to the inaccessibility of some portions of the aquifer system inhabited by the Comal Springs riffle beetle, key questions remain about their use of deeper habitats, responses to drought, and the degree of population connectivity. The project will aim to leverage population genetic analyses of existing temporal samples from the Comal Springs system to assess the status of Comal Springs riffle beetle populations and their resilience to drought conditions.

Summary of Objectives

The primary objective of this Contract is to examine the population genetics of the Comal Springs riffle beetle based on collected specimens from 2007-2024 at various locations in the Comal Springs system

In general, the objectives for this Contract are to:

1. Estimate genetic diversity for each locality for each temporal sample and assess if genetic diversity has changed over time.
2. Explore patterns of gene flow and population structure among localities.
3. Estimate the fixation index between localities.
4. Estimate effective population sizes at each locality and explore if there is evidence that any populations have gone through a genetic bottleneck.

Methodologies

Existing Collections Methods

In collaborative efforts between the U.S. Fish and Wildlife Service (USFWS) and the EAA, Comal Springs riffle beetles have been collected from five locations in the Comal Springs and one in the San Marcos Springs. At each location (excluding Hotel Springs), samples were collected in 2007, 2016, 2020 and 2023-24. These sampling dates span a range of ecological conditions including the severe drought which occurred between 2010 and 2014 and resulted in a total of 824 specimens collected across sites and years. For sampling events prior to 2023-24, specimens were collected using drift nets and cotton lures by USFWS personnel. In 2023-24, samples were collected by USFWS and EAA. Table 1 below illustrates the year and number of specimens collected at each

location.

Table 1 - Sampling information for Comal Springs riffle beetle

Locality	Year Collected	Number of Individuals
Spring Run One	2007	20
	2016	31
	2020	45
	2023/24	10
Spring Run Two	2007	21
	2016	32
	2020	42
	2023/24	17
Spring Run Three	2007	21
	2016	30
	2020	45
	2023/24	55
Spring Island	2007	21
	2016	26
	2020	42
	2023/24	97
Western Shore	2007	29
	2016	33
	2020	42
	2023/24	63
Hotel Springs San Marcos	2007	28
	2016	29
	2020	45
TOTAL		824

Existing Molecular Methods

For all previous samples listed, DNA was extracted and reduced representation genetic libraries were produced. TEXAS STATE will use this approach by randomly fragmenting the genome and attaching custom 8-10 base pair (bp) sequences to fragments, which allows for the identification of individuals. TEXAS STATE will amplify these fragments using polymerase chain reaction (PCR), pooling the resulting fragments, and selecting only fragments between 250-350 bp for sequencing using Illumina technology. Sequence reads for all 824 individuals listed in Table 1 are currently stored at Texas State, and they will be processed following a custom pipeline that has been used successfully for several other invertebrate species.

In addition to using DNA samples from previous collections, a reference genome for the Comal Springs riffle beetle will be generated at the University of California-Berkeley genomics lab. Sequencing the entire genome for the Comal Springs riffle beetle enables the use of powerful demographic analyses, including inferring changes in effective population size over time using coalescent-based approaches. These analyses provide insight into long-term evolutionary trends in populations.

Sequencing a reference genome for a second species only slightly increases the lab processing costs. Therefore, developing a reference genome for a second EAHCP covered species, the Comal Springs dryopid beetle (*Stygoparnus comalensis*) will also be attempted in this study. Since the two beetles have similar genome sizes, their genomes should be able to be sequenced together. Similar to the Comal Springs riffle beetle, there is little information about the Comal Springs dryopid beetle, so having a reference genome would be beneficial in detecting long-term changes in its population.

Population Genetic Analyses

Following sequence alignment, filtering, and variant calling, TEXAS STATE will estimate the genotype probabilities using the program ENTROPY. For each locality and temporal sample, TEXAS STATE will estimate three genetic diversity parameters. To visualize patterns of population connectivity, TEXAS STATE will use principal component analyses on individual genotype probabilities using R software for statistical computing. To further explore population structure, TEXAS STATE will conduct a clustering analysis that estimates admixture proportions (the proportion of an individual's genome that belongs to a particular population or group) using ENTROPY. To further quantify genetic differentiation between sites and between sampling years within sites, TEXAS STATE will calculate pairwise the fixation index in R. TEXAS STATE will estimate the effective population size of each sample in two ways, first using model-based Approximate Bayesian Computation (ABC) and then using a linkage disequilibrium-based method. Additional methods of estimating effective population size will also be considered.

EXHIBIT B
PROJECT SCHEDULE

Work will be completed and results delivered to EAA by December 31, 2027.

2025: Genome sequencing and sample collection

2026: Sequence alignment and analysis, report writing

Meetings with EAA staff will occur at the end of 2025 and 2026 to provide updates on the status of the project. Interim reports will be submitted in 2025 and 2026 that outlines work completed in that calendar year.

Results will be presented to the EAA upon completion of the work as a final report and submitted as a peer-reviewed paper within a year of completion. A draft of the final report will be submitted to EAA by December 1, 2027 with a final report completed by December 31, 2027.

EXHIBIT C
BUDGET ESTIMATE

Expense	Cost
Salary/Fringe	\$ 45,440.00
Sequencing (University of California-Berkeley)	\$ 3,088.00
Miscellaneous non-capital equipment	\$ 500.00
Indirect Costs (50.5%)	\$ 24,762.00
Total	\$ 73,790.00