

## Budget Justification

*Note: all personnel include at 2% annual increase starting in Year 2 unless otherwise noted. The University of Idaho has determined a salary year to be based on a calendar year.*

### **A. Senior Personnel (Year 1 \$10,351, Year 2 \$10,558, Year 3 \$10,769, Year 4 \$10,983, Year 5 \$11,203, Cumulative \$53,864 Total)**

**Chris Hamilton (Principal Investigator):** Chris A. Hamilton, PhD, Assistant Professor at the University of Idaho, will serve as the Principal Investigator on this project. PI Hamilton will oversee the project, contribute to collecting field data, designing experiments, analyzing data, disseminating results, mentoring students and leading the educational component of this proposal. PI Hamilton will be responsible for maintaining and revising as necessary the field research permits to work in Arizona, New Mexico, and Mexico. Salary support is requested for 1 month of summer salary in Years 1-5 to cover field work and the education component. Field work will consist of specimen collecting trips in Years 1-5. Base salary is (\$71,994) with a fringe benefit rate of (29.4%). Summer salary is requested in Year 1 (\$7,999), Year 2 (\$8,159), Year 3 (\$8,322), Year 4 (\$8,488), and Year 5 (\$8,658), with fringe benefit requested for Year 1 (\$2,352), Year 2 (\$2,399), Year 3 (\$2,447), Year 4 (\$2,495), Year 5 (\$2,545).

### **B. Other Personnel (Year 1 \$53,319, Year 2 \$53,581, Year 3 \$53,848, Year 4 \$54,121, Year 5 \$54,399, Cumulative \$269,268 Total)**

**Research Technician (supervised by Hamilton):** Salary support is requested for 0.3 FTE for a Research Technician in Years 1-5. Research Technician will aid in management of the project by coordinating lab and field work, conducting field work, recording morphological measurements, specimen imaging, maintaining data archives, and helping guide the graduate and undergraduate students. Base pay is (\$19.86/hour) with a fringe benefit rate of (40.8%). For a 0.3 FTE employee, this equates to (\$9,294) in Year 1 for 0.3 FTE, (\$9,480) in Year 2, (\$9,670) in Year 3, (\$9,863) in Year 4, and (\$10,061) in Year 5. Fringe benefits in Year 1 are (\$3,792), Year 2 (\$3,868), Year 3 (\$3,945), Year 4 (\$4,024), and Year 5 (\$4,105).

**Graduate Student (PhD, supervised by Hamilton):** Salary support is requested for a full-time PhD Graduate Student to work on the project in Years 1-5 (12 Calendar Months effort each year). The Graduate Student will work on all aspects of this project: phylogenomics, population genetics, taxonomic work, genome evolution work, and will experience the education component with PI Hamilton. The Graduate Student stipend will follow the NSF equivalent to the GRFP stipend, (\$34,000) with a fringe benefit rate of (3.0%) and no 2% annual increase. Years 1-5 stipend request is (\$34,000/year) with fringe benefits of (\$1,020/year).

**Undergraduate Assistant (supervised by Hamilton):** Salary support is requested for an Undergraduate Assistant during the summers of Years 1-5. Undergraduate assistant will be involved with field work, molecular work, morphological work, and will experience the education component with PI Hamilton. Base rate of pay is (\$15/hr) with a fringe benefit rate of (8.6%) and no 2% annual increase. The Undergraduate Assistant will devote 640 hours in Years 1-5 (20 hours/week for 16 weeks). Salary request is (\$4,800/year) with fringe benefits of (\$413/year).

### **C. Equipment (Year 1 \$16,799, Year 2-5 \$0, Cumulative \$16,799 Total)**

PI Hamilton is requesting (\$16,799) for a Polaris Ranger Crew 1000 utility terrain vehicle, The UTV will be used to carry personnel and gear deep into the Madrean sky islands in a more efficient and safe manner. The trails can be narrow and rocky, limiting how far a regular field vehicle is able to travel.

### **D. Travel (Year 1 \$7,780, Year 2 \$13,105, Year 3 \$12,280, Year 4 \$12,280, Year 5 \$12,280, Cumulative \$57,725 Total)**

**Research site travel:** Funds are requested for travel from UI's Moscow, Idaho, campus to the

Madrean sky island region of SE Arizona, SW New Mexico, and northern Mexico each year of the project. Based upon PI Hamilton's previous collecting experience, we are budgeting 4,000 miles/trip/year (@\$0.56/mile) for two trips/year (1/summer and 1/fall). The fall is the most important time of the year for these species because it is the mating season, when mature males wander looking for females; the summer monsoons bring increased activity due to the abundance of prey items (i.e., easier to find burrows). Mileage is for traveling to different collecting sites in each of the Madrean sky islands. Total mileage budget for each year from Years 1-5 (\$4,480/yr). Per diem is requested for each year (60 days/year @ \$55/day); the 60 days are for 15 days/trip for 2 people, for 2 trips/year. Total out of state per diem is (\$3,300) each year for Years 1-5.

**Conference Travel:** Funds are requested in Years 2-5 for PD Hamilton, the PhD student, and the undergraduate student to attend the Society for the Study of Evolution/Society of Systematic Biologists annual conference. During these trips, PI Hamilton and the students will present their research progress. Trips will be from Moscow, ID, to Albuquerque, NM (in 2022); Montreal, Canada (2023); Athens, GA (2024); and Cleveland, OH (2025). Total trip request is (\$3,825/year) and includes: airfare and hotel (\$1,000/per person/per trip) and per diem (5 days @ \$55/day x 3 individuals (\$825/trip). Total yearly request is (\$3,825).

**F. Other Direct Costs (Year 1 \$23,454, Year 2 \$81,658, Year 3 \$119,576, Year 4 \$10,064, Year 5 \$12,184, Cumulative Total \$237,876)**

**1. Materials and Supplies** – We are requesting funds for materials and supplies to be used in field and lab work, as well as computational work. Field and lab supplies for Years 1-5 are for tips, gloves, reagents, and other lab consumables, as well as collecting gear, etc. (\$500/year). Computational work (HPC access and storage) in Years 1-5 is (\$1,500/year) to access UI's Institute for Interdisciplinary Data Sciences (IIDS) Genomics and Bioinformatics Resources Core (GBRC) high-performance computing cluster. DNA extraction kits (250 samples x 2) will be purchased (\$760) in Year 1. We will also purchase 10 Lacie Mobile Drive 5TB (\$165 x10) for external genomic data storage (\$1,650) in Year 1. Funds are requested in Years 3 and 5 for Dryad Data Repository access for housing project data (sequence data, scripts, etc.) in an open access depository (\$120/year). We will also purchase and build 2 additional LEGO sequencers for use in the education component (\$200) in Year 1. Total materials and supplies (**\$12,850**).

## **2. Publication Costs**

We are requesting open access fees to publish research results in Years 3 and 5 (\$2,000/year = **\$4,000**).

## **8. Tuition/Fees/Insurance**

Graduate student tuition and fees are based on the 2021/22 rate of (\$9,876/year) for Years 1-5, for a total of (**\$49,380**). Graduate student health insurance is (\$1,902/year) for Years 1-5, for a total of (**\$9,510**).

**9. Other Direct Costs (Year 1 \$35,232, Year 2 \$94,936, Year 3 \$132,854, Year 4 \$23,342, Year 5 \$25,462, Cumulative \$311,826 Total)**

**Conference registration costs** – Funds are requested for the Society for the Study of Evolution/Society of Systematic Biologists annual conference registration in Years 2-5. The estimated cost is \$500/person (PhD student, undergraduate student, and PI Hamilton = \$1,500/year in Years 2-5 = **\$6,000**).

**Utility/ATV Trailer for UTV field work** – Funds are requested in Year 1 for a Mission 83" x 14' Aluminum Utility/ATV Trailer (**\$4,000**). The trailer will be used to transport the UTV from Idaho to the sky islands region. When not being used, the UTV will be available for usage to others in the UI Entomology, Plant Pathology & Nematology department.

**Sequencing Fees** – We are requesting funds for UCE phylogenomics and whole genome sequencing (WGS). Ultraconserved Element sequencing (UCE) – for phylogenetics and population genomics. In Years 1-5, library prep and Illumina sequencing of 96 specimens (1 plate) will be carried out by RAPiD Genomics (Gainesville, FL), which PI Hamilton has an established working relationship (Letter of Collaboration). The

current negotiated fee is \$7,200/plate (96 x 5 = 480 samples). We are targeting 5 males and 5 females of each species, with >40 undescribed species predicted to be discovered (400 samples + redos and additional samples of interest). Total UCE sequencing is (\$7,200/year) in Years 1-5 = **(\$36,000)**.

PacBio HiFi sequencing – We are requesting funds for whole genome sequencing (WGS) of 9 species in the Marxi species group. This objective will sequence and compare genomes of a desert species (*A. vorhiesi*) with six mid-elevation replicates of lineages that moved into different sky islands (*A. chiricahua*, *A. catalina*, *A. madera*, *A. sp.* Galiuro, *A. sp.* Dagoon, *A. sp.* Pinaleño), and versus a novel, undescribed high-elevation species (*A. sp. nov.* high elevation Chiricahua) (Fig. 3). We will also compare *A. vorhiesi* with the Colorado Plateau species (*A. marxi*), as well as compare a mid-elevation species (*A. chiricahua*) versus a novel, undescribed high-elevation species (*A. sp. nov.* Chiricahua) whose distributions overlap in the Chiricahua mountains. Genome sequencing will begin in Year 2 **(\$71,594)** and finish in Year 3 **(\$107,392)**, followed by analysis in Years 4 & 5. We are targeting ≥10x coverage, but because of the very large estimated genome sizes of tarantulas, we will need 5 SMRT cells per species. Library prep and sequencing will be carried out by UI's Institute for Interdisciplinary Data Sciences (IIDS) Genomics and Bioinformatics Resources Core (GBRC). The quoted cost for Years 1-5 is **(\$178,986)**.

Oxford Nanopore sequencing – We are requesting funds for additional whole genome sequencing, using Oxford Nanopore long-read technology. These supplies will primarily be for the education component, where students sequence Marxi species group genomes on a MinION, during a summer course on the San Carlos Apache reservation. The sequences they generate will be used to pull CO1 from, for comparison against my previously sequenced >1,000 CO1 barcodes for *Aphonopelma*. The entirety of the sequencing runs will be added to the PacBio reads to complement the genome assemblies and annotations. In Year 1, we will purchase an Mk1C (the MinION sequencing and compute module; \$5,500) and the Flongle starter pack (comes with Flongle adapter and 12 flow cells; \$1,460). The Flongle flow cell is a more cost-effective and easier to prep flow cell for the students but will still generate effective sequences of the same 9 species used in the PacBio sequencing. Each year we will need to purchase a Flow Cell Wash Kit (\$85x5) and Field Sequencing Kit (\$599x5). In Years 2-5, we will purchase 2 Flongle flow cells for use in the education component (\$180x4). We will be purchasing more flow cells and kits than is required for the education component, but this is due to their expiration each year. Because of this, we will perform additional sequencing in PI Hamilton's lab, so that flow cells are not wasted and so that we can increase sequencing coverage. Year 1 \$7,644, Years 2-5 \$864/year = **(\$11,100)**.

**G. Total Direct Costs (Year 1 \$123,482, Year 2 \$170,681, Year 3 \$208,251, Year 4 \$99,226, Year 5 \$101,844, Cumulative \$703,483 Total)**

**H. Total Indirect Costs (Year 1 \$46,385, Year 2 \$79,451, Year 3 \$98,236, Year 4 \$43,274, Year 5 \$45,033, Cumulative \$312,829 Total)**

Indirect costs have been calculated using the University of Idaho Federally Negotiated on campus research rate applied to a Modified Total Direct Cost base. The current agreement is comprised of a stepped rate. Indirect costs for the first year are calculated using the 48.5% FY22 rate for nine months (\$34,522) and the 50% FY23-24 rate for 3 months (\$11,863), with the remainder of the project using the 50% rate.

**I. Total Direct and Indirect Costs (Year 1 \$169,866, Year 2 \$250,132, Year 3 \$306,487, Year 4 \$142,950, Year 5 \$146,877, Cumulative \$1,016,312 Total)**