

# Undergraduate Biology Research, Cochise Community College, Sierra Vista and Douglas, AZ.

## Introduction:

The San Pedro River (Fig.1), located in the southeastern part of Arizona, is home to hundreds of migrating and residential birds, as well as many species of mammals, amphibians, and reptiles who live in the riparian area. The aquatic insects emerging from the river are a food source for the terrestrial consumers in the San Pedro. Insects and invertebrates play a big role as consumers in the San Pedro River riparian food web. The larvae of Simuliidae, a species of blackfly in the order Diptera, serve as a major role in stream ecosystems. By doing this study it can help us determine the heath and water quality of the San Pedro River, as well as to be used in a long-term study in the determination of genetic changes within populations and communities occurring due to seasonal and intermittent stream flow. Simuliidae larvae grow in large numbers under favorable conditions in streams, and because larval densities are often so high, considerable amounts of sedimenting faecal pellets increase the local retention of organic material, thus providing nutrition for invertebrates and micro-organisms, as wells as potentially fertilizing river margins.(Bjorn,2004)

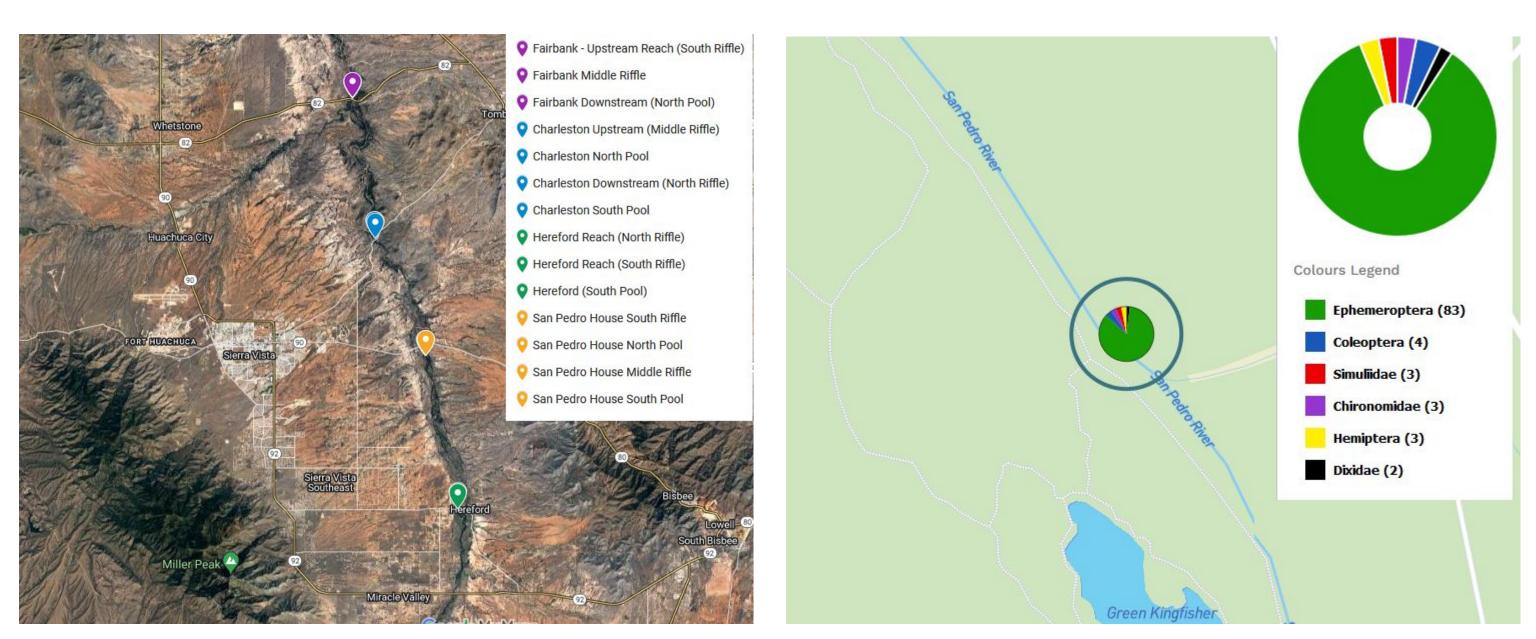


Fig. 1: San Pedro River collection sites with legend

#### **Procedure:**

Aquatic invertebrate samples were collected from both riffles and pools from a site in the San Pedro River (Fig.2). In riffles, we used a Surber sampler (30 x 30cm, 1mm mesh) to collect aquatic macroinvertebrates. For pools, we used a D-net (~500µm mesh) with an effort of 10 s/m2. The invertebrate samples were stored and transferred to the lab in a 70% ethanol solution for identification. We used a dichotomous key to identify the samples to the family level. Due to the number of Simuliidae collected, we decided to use them to do DNA sequencing.

The PCR protocol was as follows:

1 min at 96°C; 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min 30 s at 72°C; and finally, 7 min at 72°C

The melt curve protocol:

30 sec at 90°C, 1 min at 60°C, then slowly reach 95°C and hold for 30 sec. A point is taken at every degree change to create the melt curve.

We created a 50-microliter working solution by taking 5 microliters of stock primers and 45 microliters of TE buffer, creating a 10 microMolar concentration of each of the primers. Each PCR reaction was 25 microliters total: consisting of 1 microliter of each primer, 1 microliter of the DNA sample, and 22 microliters of CHAI PCR master mix (What Is a PCR Master Mix?).

Quantification of amplicon concentrations were conducted using a Qubit 4 Fluorometer. To clean the PCR product, we used Exo-SAP-IT following the protocols listed for this product. Samples of 10.5 microliters of each product were sent to Eurofins Genomics for sequencing along with primers.

# USE OF GENETIC SEQUENCING TO DETERMINE THE IDENTITY OF BLACK FLIES (FAMILY: SIMULIIDAE) IN THE SAN PEDRO RIVER (AZ, USA) **Kylee Pereda and Frank Emanuele**

Fig. 2: San Pedro House collection site

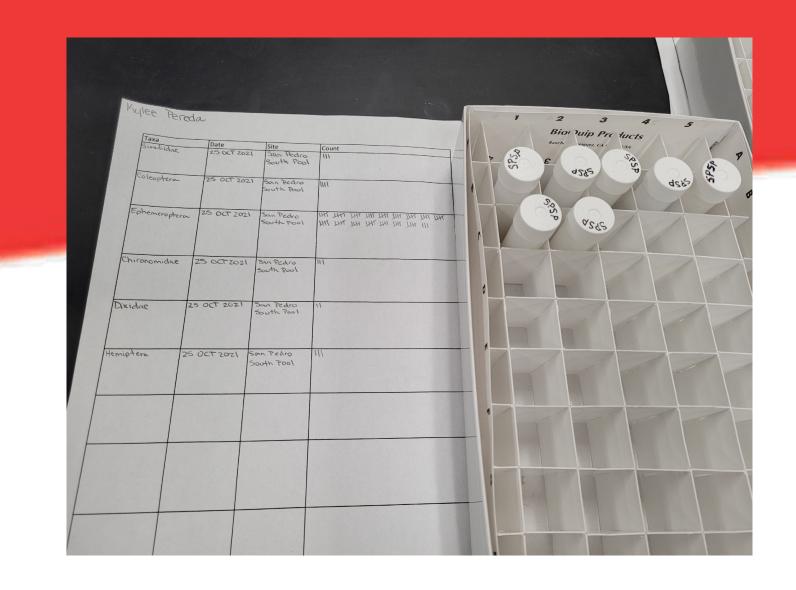




Fig. 3: Microscopic view of the Simuliidae larvae collected at the San Pedro River site

### **Results:**

Our initial BLAST search of the best sequences we obtained showed a species identification of Simulium paynei (Fig.5,6,7). The predicted and observed melt curve comparison also confirmed this finding. The predicted melt curve (Fig.8) has a peak at 82°C and a secondary peak at 85°C. Our observed melt curve (Fig.9) showed similar peaks and therefore provided more evidence of close relationship of our unknown to the S. paynei. A phylogenetic tree (Fig. 10) was created with the samples we collected to show the relationship in DNA between them and other Simuliidae species. The tree showed a difference in data compared to our findings with the initial BLAST search. This could be due to the issue of using 3 larvae in order to get enough DNA to do the sequencing, with the possibility of the larva being two different species.

Selection of the se		Description	Scientific Name	Max Score	Total Score	Query Cover		Per. Ident	Acc.	Accession
<ul> <li></li></ul>	1	<u>\$imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4884 cytochrome oxidase subunit 1 (COI) gene, partial cds:</u>	Simulium sp. BO	. 1077	1686	89%	0.0	98.06%	658	HQ941270.
	100	Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-5736 cytochrome oxidase subunit 1 (COI) gene, partial cds;	Simulium sp. BO	. 1072	1675	<mark>89%</mark>	0.0	97.90%	6 <mark>5</mark> 8	JF870504.1
Image and point and poi	-	Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-5506 cytochrome oxidase subunit 1 (COI) gene, partial cds;	Simulium sp. BO.	. 1072	1675	89%	0.0	97.90%	658	JF870277.1
Sama 2000/2000 solution 1000000 solution 1000000000000000000000000000000000000	10.00	Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-1833 cytochrome oxidase subunit 1 (COI) gene, partial cds:	Simulium sp. BO	. 1072	1675	<mark>89%</mark>	0.0	97.90%	658	HQ939888.
Image 2014 2439 manue 12000 2430 manue and an and an 100 map of a 10 mm of a 1	14.00	Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-2454 cytochrome oxidase subunit 1 (COI) gene, partial cds:	Simulium sp. BO	. 1066	1669	89%	0.0	97.74%	658	HQ940459.
<section-header>Image: 0.100000000000000000000000000000000000</section-header>	100	imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-2436 cytochrome oxidase subunit 1 (COI) gene, partial cds:	Simulium sp. BO	. 1033	1642	89%	0.0	96.77%	658	HQ940442.
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Lengtion         Sourd Control         Sourd Contr         Sourd Control         Sourd Control </td <td></td> <td>Fig. 5: Blast search for E</td> <td>xo1 s</td> <td>am</td> <td>ple</td> <td>9</td> <td></td> <td></td> <td></td> <td></td>		Fig. 5: Blast search for E	xo1 s	am	ple	9				
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Simulum is, BOLD AAC4424 worker 10 SCCWRP 0157 rytechrome avidese subart 1 (COI) gene, partial cist, Simulum as, BO.       106       106       00       97.4%       64       H02355211         Simulum as, BOLD AAC4424 worker 10 SCCWRP 0157 rytechrome avidese subart 1 (COI) gene, partial cist, Simulum as, BO.       1064       80%       0.0       97.3%       681       H0210521         Simulum as, BOLD AAC4424 worker 10 SCCWRP 0151 rytechrome avidese subart 1 (COI) gene, partial cist, Simulum as, BO.       1062       1062       90%       0.0       97.3%       681       H02355211         Simulum as, BOLD AAC4424 worker 10 SCCWRP 0151 rytechrome avidese subart 1 (COI) gene, partial cist, Simulum as, BO.       1062       90%       0.0       97.5%       681       H02355211         Simulum as, BOLD AAC4424 worker 10 SCCWRP 0153 rytechrome avidese subart 1 (COI) gene, partial cist, Simulum as, BO.       1067       107       0.0       97.5%       681       H0245221         Simulum as, BOLD AAC4424 worker 10 SCCWRP 0153 rytechrome avidese subart 1 (COI) gene, partial cist, Simulum as, BO.       107       107       0.0       97.4%       681       H0241221         Simulum as, BOLD AAC4424 worker 10 SCCWRP 0553 rytechrome avidese subart 1 (COI) gene, partial cist, Simulum as, B.O.       107       107       0.0       97.4%       681       H0241221         Simulum as, BOLD AAC4424 worker 10 SCCWRP 0552 rytechrome		Simulium paynei voucher 01ME1444_1444 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial §	Simulium paynei	1070	1070	85%	0.0	99.49%	598	MW346207.1
Simular normal works with the second and search works		imulium sp. BOLD:AAC4424 voucher 10-SCCWRP-5578 cytochrome oxidase subunit 1 (COI) gene, partial cds; §	<u>Simulium sp. BO</u>	1068	1068	90%	0.0	97.74%	658	JF870349.1
Simulum 36       BOLD AAC4424 voucher 10-SCCWRP-4776 sytechrome oxideas subunit 1 (COI) gene, eartial cds,       Simulum 39, CFW 640-10 voucher 10-SCCWRP-1476 sytechrome oxideas subunit 1 (COI) gene, eartial cds,       Simulum 39, CFW 640-10 voucher 10-SCCWRP-1167 sytechrome oxideas subunit 1 (COI) gene, eartial cds,       Simulum 39, CFW 640-10 voucher 10-SCCWRP-1163 sytechrome oxideas subunit 1 (COI) gene, eartial cds,       Simulum 39, DOI DAAC4424 voucher 10-SCCWRP-1163 sytechrome oxideas subunit 1 (COI) gene, eartial cds,       Simulum 39, DOI DAAC4424 voucher 10-SCCWRP-1163 sytechrome oxideas subunit 1 (COI) gene, eartial cds,       Simulum 39, DOI DAAC4424 voucher 10-SCCWRP-1416 sytechrome oxideas subunit 1 (COI) gene, eartial cds,       Simulum 39, DOI DAAC4424 voucher 10-SCCWRP-1416 sytechrome oxideas subunit 1 (COI) gene, eartial cds,       Simulum 39, DOI DAAC4424 voucher 10-SCCWRP-1416 sytechrome oxideas subunit 1 (COI) gene, eartial cds,       Simulum 39, DOI DAAC4424 voucher 10-SCCWRP-4176 sytechrome oxideas subunit 1 (COI) gene, eartial cds,       Simulum 39, DOI DAAC4424 voucher 10-SCCWRP-4176 sytechrome oxideas subunit 1 (COI) gene, eartial cds,       Simulum 39, DOI DAAC4424 voucher 10-SCCWRP-4176 sytechrome oxideas subunit 1 (COI) gene, eartial cds,       Simulum 39, DOI DAAC4424 voucher 10-SCCWRP-4176 sytechrome oxideas subunit 1 (COI) gene, eartial cds,       Simulum 39, DOI DAAC4424 voucher 10-SCCWRP-4176 sytechrome oxideas subunit 1 (COI) gene, eartial cds,       Simulum 39, DOI DAAC4424 voucher 10-SCCWRP-4176 sytechrome oxideas subunit 1 (COI) gene, eartial cds,       Simulum 39, DOI DAAC4424 voucher 10-SCCWRP-4176 sytechrome oxideas subunit 1 (COI) gene, antial cds,       Simulum 39, DOI DAAC4424 voucher 10-SCCWRP-4176 sytechrome oxideas subun		imulium sp. BOLD:AAC4424 voucher 10-SCCWRP-0157 cytochrome oxidase subunit 1 (COI) gene_partial cds: §	<u>Simulium sp. BO</u>	1068	1068	90%	0.0	97.74%	646	HQ938521.1
Simulam of CM/E484 (worker 10 SCC/MPE 3175 cytochrome oxidase suburil 1 (CO) gene, partial cite in Simulam see, E in origination of the set of		imulium paynei voucher 02ME1433_1433 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	<u>Simulium paynei</u>	1064	1064	85%	0.0	99.32%	598 <u> </u>	MW346208.1
Simulum 36. B01. DAAC 4424 vuocher 10. SCCWRP-0141 cytochmon oxidase subunt 1 (COI) gene, partial cds Simulum 36. B0.       102       102       102       00       97.5%       658       H033385211         Simulum 36. B01. DAAC 4424 vuocher 10. SCCWRP-0145 cytochmon oxidase subunt 1 (COI) gene, partial cds Simulum 36. B0.       102       102       102       00       97.5%       658       H033385211         Simulum 36. B01. DAAC 4424 vuocher 10. SCCWRP-0145 cytochmon oxidase subunt 1 (COI) gene, partial cds Simulum 36. B0.       1057       1057       00       07.5%       658       H033385211         Simulum 36. B01. DAAC 4424 vuocher 10. SCCWRP-0145 cytochmon oxidase subunt 1 (COI) gene, partial cds Simulum 36. B0       1057       1057       00       07.5%       658       H03314221         Simulum 36. B01. DAAC 4424 vuocher 10. SCCWRP-0147 cytochmon oxidase subunt 1 (COI) gene, partial cds Simulum 36. B0       107       107       00       07.4%       658       H0341221         Simulum 36. B01. DAAC 4424 vuocher 10. SCCWRP-1481 cytochmon oxidase subunt 1 (COI) gene, partial cds Simulum 36. B0       107       107       07       00       97.4%       658       H03412211         Simulum 36. B0. DAAC 4424 vuocher 10. SCCWRP-1481 cytochmon oxidase subunt 1 (COI) gene, partial cds Simulum 36. B0       102       257       676       00       97.4%       658       H03412211         Simulum		imulium sp. BOLD:AAC4424 voucher 10-SCCWRP-4706 cytochrome oxidase subunit 1 (COI) gene, partial cds; §	<u>Simulium sp. BO</u>	1064	1064	89%	0.0	97.73%	658	HQ941092.1
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Simulum sp. B01D AAC4242 vaucher 10: SCCWRP-0143 cytochrome oxidase subunit 1(C01) gene, partial cds, Simulum sp. B0.       1062       010       97.5%       688       H03380421         Simulum sp. B01D AAC4242 vaucher 10: SCCWRP-0125 cytochrome oxidase subunit 1(C01) gene, partial cds, Simulum sp. B0.       1067       915       915       0.0       97.5%       688       H03380421         Simulum sp. B01D AAC4242 vaucher 10: SCCWRP-0125 cytochrome oxidase subunit 1(C01) gene, partial cds, Simulum sp. B0.       1067       915       915       0.0       97.5%       688       H03380421         Simulum sp. B01D AAC4242 vaucher 10: SCCWRP-4719 cytochrome oxidase subunit 1(C01) gene, partial cds, Simulum sp. B0.       1067       915       916       0.0       97.5%       688       H0341051         Simulum sp. B01D AAC42424 vaucher 10: SCCWRP-4719 cytochrome oxidase subunit 1(C01) gene, partial cds, Simulum sp. B0.       1067       916       916       668       H03412721         Simulum sp. B01D AAC4244 vaucher 10: SCCWRP-4884 cytochrome oxidase subunit 1(C01) gene, partial cds, Simulum sp. B0.       1024       2577       675       0.0       97.4%       658       H03412721         Simulum sp. B01D AAE8840 vaucher 10: SCCWRP-4884 cytochrome oxidase subunit 1(C01) gene, partial cds, Simulum sp. B0.       1024       277       676       0.0       97.4%       658       H03412721         Simulum sp. B01D AAE8840 vaucher 1		imulium sp. BOLD:AAC4424 voucher 10-SCCWRP-0141 cytochrome oxidase subunit 1 (COI) gene, partial cds; §	<u>Simulium sp. BO</u>	1062	1062	90%	0.0	97.58%	658	HQ938506.1
Simulum 3p. B01D AAC4424 voucher 10-SCCWRP-125 sytuchrome oxidase subunt 1 (C01) gene, partial cds.       Simulum 3p. B01D AAC4424 voucher 10-SCCWRP-4996 sytuchrome oxidase subunt 1 (C01) gene, partial cds.       Simulum 3p. B01D AAC4424 voucher 10-SCCWRP-4996 sytuchrome oxidase subunt 1 (C01) gene, partial cds.       Simulum 3p. B01D AAC4424 voucher 10-SCCWRP-4996 sytuchrome oxidase subunt 1 (C01) gene, partial cds.       Simulum 3p. B01D AAC4424 voucher 10-SCCWRP-4795 sytuchrome oxidase subunt 1 (C01) gene, partial cds.       Simulum 3p. B01D AAC4424 voucher 10-SCCWRP-4791 sytuchrome oxidase subunt 1 (C01) gene, partial cds.       Simulum 3p. B01D AAC4424 voucher 10-SCCWRP-4791 sytuchrome oxidase subunt 1 (C01) gene, partial cds.       Simulum 3p. B01D AAC4424 voucher 10-SCCWRP-4791 sytuchrome oxidase subunt 1 (C01) gene, partial cds.       Simulum 3p. B01D AAC4424 voucher 10-SCCWRP-4791 sytuchrome oxidase subunt 1 (C01) gene, partial cds.       Simulum 3p. B01D AAC4424 voucher 10-SCCWRP-4894 cytuchrome oxidase subunt 1 (C01) gene, partial cds.       Simulum 3p. B01D AAC4840 voucher 10-SCCWRP-4894 cytuchrome oxidase subunt 1 (C01) gene, partial cds.       Simulum 3p. B01D AAE840 voucher 10-SCCWRP-536 cytuchrome oxidase subunt 1 (C01) gene, partial cds.       Simulum 3p. B01D AAE840 voucher 10-SCCWRP-536 cytuchrome oxidase subunt 1 (C01) gene, partial cds.       Simulum 3p. B01D AAE840 voucher 10-SCCWRP-536 cytuchrome oxidase subunt 1 (C01) gene, partial cds.       Simulum 3p. B01 102       Z07       67%       0.0       97.49%       68       H09412721         imulum 3p. B01D AAE840 voucher 10-SCCWRP-536 cytuchrome oxidase subunt 1 (C01) gene, partial cds.       Simulum 3p. B01 102       Z07       7%       0.0       97.49%		imulium sp. BOLD:AAC4424 voucher 10-SCCWRP-0160 cytochrome oxidase subunit 1 (COI) gene, partial cds; §	Simulium sp. BO	1062	1062	90%	0.0	97.58%	658	HQ938524.1
Simulium sp. BOLD AAC4424 voucher 10-SCCWRP-4906 cytochrome oxidase subunit 1 (COI) gene, partial cds.       Simulium sp. BOL       1057       1057       010       97.4%       658       HQ941122.1         Simulium sp. BOLD AAC4424 voucher 10-SCCWRP-4765 cytochrome oxidase subunit 1 (COI) gene, partial cds.       Simulium sp. BOL       1057       1057       010       97.4%       658       HQ941151.1         Simulium sp. BOLD AAC4424 voucher 10-SCCWRP-4719 cytochrome oxidase subunit 1 (COI) gene, partial cds.       Simulium sp. BOL       1057       1057       010       97.4%       658       HQ941105.1         Simulium sp. BOLD AAC4424 voucher 10-SCCWRP-4719 cytochrome oxidase subunit 1 (COI) gene, partial cds.       Simulium sp. BOL       Max       Total       Query       E       Mex       Mex       Mex       Total       Query       E       Mex		imulium sp. BOLD:AAC4424 voucher 10-SCCWRP-0143 cytochrome oxidase subunit 1 (COI) gene, partial cds; §	<u>Simulium sp. BO</u>	1062	1062	90%	0.0	97.58%	658	HQ938507.1
Simulium sp. BOLDAAC4424 voucher 10-SCCWRP-4765 cytochrome oxidase subunit 1 (COI) gene, eartial cds Simulium sp. BOL       1057       1057       1057       00       97.42%       658       HQ941151.1         Simulium sp. BOLDAAC4424 voucher 10-SCCWRP-4765 cytochrome oxidase subunit 1 (COI) gene, eartial cds Simulium sp. BOL       1057       1057       1057       00       97.42%       658       HQ941151.1         Simulium sp. BOLDAAC4424 voucher 10-SCCWRP-4765 cytochrome oxidase subunit 1 (COI) gene, eartial cds Simulium sp. BOL       Max       Tial       Verter       Per.       Acc         Simulium sp. BOLDAAE8840 voucher 10-SCCWRP-4884 cytochrome oxidase subunit 1 (COI) gene, eartial cds Simulium sp. BOL       1029       257       87%       0.0       97.49%       658       HQ9412121         simulium sp. BOLDAAE8840 voucher 10-SCCWRP-5505 cytochrome oxidase subunit 1 (COI) gene, eartial cds Simulium sp. BOL       1029       257       87%       0.0       97.49%       658       HE9042121         simulium sp. BOLDAAE8840 voucher 10-SCCWRP-5505 cytochrome oxidase subunit 1 (COI) gene, eartial cds Simulium sp. BOL       1024       2507       87%       0.0       97.49%       658       HE9042121         simulium sp. BOLDAAE8840 voucher 10-SCCWRP-5450 cytochrome oxidase subunit 1 (COI) gene, eartial cds Simulium sp. BOL       1024       2507       87%       0.0       97.49%       658       HQ940451 </td <td></td>										
Binulum sp. BOLD AAC4424 voucher 10.SCCWRP.4719 cytochrome oxidases subunit 1 (CO) gene, partial cds         Binulum sp. BOL         Other         Other         Per         Accession           Immulum sp. BOLD AAC4424 voucher 10.SCCWRP.4789 cytochrome oxidases subunit 1 (CO) gene, partial cds         Simulum sp. BOL         Not         V.V.V.V.V.V.V.V.V.V.V.V.V.V.V.V.V.V.V.			1. 10 YEAR						ADDROVE S	
Fig. 6: Blast search for Exc2 surplic         Scientific Num       Nax       Total       Curv       E       Per       Acc       Icentific         insultum sp. BOLDAAE8840 voucher 10-SCCWRP-4884 cytochrome oxidase subunit 1 (C0) gene, partial cds       Simultum sp. Bol       202       87%       0.0       97.6%       658       H0241271         insultum sp. BOLDAAE8840 voucher 10-SCCWRP-4884 cytochrome oxidase subunit 1 (C0) gene, partial cds       Simultum sp. Bol       202       87%       0.0       97.6%       658       H0241271         insultum sp. BOLDAAE8840 voucher 10-SCCWRP-1833 cytochrome oxidase subunit 1 (C0) gene, partial cds       Simultum sp. Bol       216       87%       0.0       97.4%       658       H0241271         insultum sp. BOLDAAE8840 voucher 10-SCCWRP-1833 cytochrome oxidase subunit 1 (C0) gene, partial cds       Simultum sp. Bol       102       257       87%       0.0       97.4%       658       H0240251         imultum sp. BOLDAAE8840 voucher 10-SCCWRP-2456 cytochrome oxidase subunit 1 (C0) gene, partial cds       Simultum sp. Bol       108       246       87%       0.0       97.4%       658       H02404251         imultum sp. BOLDAAE8840 voucher 10-SCCWRP-2456 cytochrome oxidase subunit 1 (C0) gene, partial cds       Simultum sp. Bol       74       244       87%       0.0       95.9%       658										A MARK AND AND A MARK
Description         Scientific Name         Score         Score         Cover         Value         Ident         Len         Accession           imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4884 cytochrome oxidase subunit 1 (COI) gene, partial cds         Simulium sp. BO         102         2524         87%         0.0         97.66%         658         HQ9412701           imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-5736 cytochrome oxidase subunit 1 (COI) gene, partial cds         Simulium sp. BO         1024         2507         87%         0.0         97.49%         658         JE870277.1           imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-5506 cytochrome oxidase subunit 1 (COI) gene, partial cds         Simulium sp. BO         1024         2507         87%         0.0         97.49%         658         JE870277.1           imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-1833 cytochrome oxidase subunit 1 (COI) gene, partial cds         Simulium sp. BO         1018         246         87%         0.0         97.32%         658         HQ940459.1           imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-2456 cytochrome oxidase subunit 1 (COI) gene, partial cds         Simulium sp. BO         974         242         87%         0.0         95.99%         658         HQ940452.1           imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4456 cytochrome oxidase subunit 1 (COI) gene, partial cds <th></th> <th>Fig. 6: Blast search for E</th> <th>xo2 sa</th> <th>am</th> <th>ple</th> <th>9</th> <th></th> <th></th> <th></th> <th></th>		Fig. 6: Blast search for E	xo2 sa	am	ple	9				
Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-5736 cytochrome oxidase subunit 1 (COI) gene, partial cds Simulium sp. BO.       1024       2507       87%       0.0       97.49%       658       JE870504.1         Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-5306 cytochrome oxidase subunit 1 (COI) gene, partial cds Simulium sp. BO.       1024       2507       87%       0.0       97.49%       658       JE870504.1         Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-1833 cytochrome oxidase subunit 1 (COI) gene, partial cds Simulium sp. BO.       1014       2407       87%       0.0       97.49%       658       HQ930888.1         Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-2456 cytochrome oxidase subunit 1 (COI) gene, partial cds Simulium sp. BO.       1018       2406       87%       0.0       97.49%       658       HQ940452.1         Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-2456 cytochrome oxidase subunit 1 (COI) gene, partial cds Simulium sp. BO.       979       240       87%       0.0       96.15%       658       HQ940452.1         Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-3173 cytochrome oxidase subunit 1 (COI) gene, partial cds Simulium sp. BO.       974       2424       87%       0.0       95.99%       658       HQ940452.1         Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4730 cytochrome oxidase subunit 1 (COI) gene, partial cds Simulium sp. BO.       974       243       87%       0.0 <td< td=""><td></td><td>÷</td><td>*</td><td>Score</td><td>Score</td><td>Cove</td><td>value</td><td>e Ident</td><td>Ler</td><td>Accession</td></td<>		÷	*	Score	Score	Cove	value	e Ident	Ler	Accession
imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-5506 cytochrome oxidase subunit 1 (COI) gene, partial cds.Simulium sp. BO.1024250787%0.097.49%658JE870277.1imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-1833 cytochrome oxidase subunit 1 (COI) gene, partial cds.Simulium sp. BO.1018240687%0.097.49%658HQ9308881imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-2454 cytochrome oxidase subunit 1 (COI) gene, partial cds.Simulium sp. BO.1018249687%0.097.32%658HQ9404521imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-2456 cytochrome oxidase subunit 1 (COI) gene, partial cds.Simulium sp. BO.975244087%0.096.15%658HQ9404521imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-2450 cytochrome oxidase subunit 1 (COI) gene, partial cds.Simulium sp. CF.974242487%0.095.99%658HQ9404521imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-3173 cytochrome oxidase subunit 1 (COI) gene, partial cds.Simulium sp. BO.974242887%0.095.99%658HQ9412401imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4780 cytochrome oxidase subunit 1 (COI) gene, partial cds.Simulium sp. BO.974242887%0.095.99%658HQ9411461imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4780 cytochrome oxidase subunit 1 (COI) gene, partial cds.Simulium sp. BO.974242887%0.095.99%658HQ9411751imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4798 cytochrome oxidase subunit 1 (COI) gene, partial cds.Simulium sp.			100 00 0000				1 10000			
imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-1833 cytochrome oxidase subunit 1 (COI) gene, partial cds;       Simulium sp. BOL       Simulium sp. BOL       AE8840 voucher 10-SCCWRP-2454 cytochrome oxidase subunit 1 (COI) gene, partial cds;       Simulium sp. BOL       B       No.       97.49%       658       HQ930888.1         imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-2456 cytochrome oxidase subunit 1 (COI) gene, partial cds;       Simulium sp. BOL       B       446       87%       0.0       96.32%       658       HQ940459.1         imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-2450 cytochrome oxidase subunit 1 (COI) gene, partial cds;       Simulium sp. BO.       979       2440       87%       0.0       96.32%       658       HQ940455.1         imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-3173 cytochrome oxidase subunit 1 (COI) gene, partial cds;       Simulium sp. BO.       974       242       87%       0.0       95.99%       658       HQ940455.1         imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4730 cytochrome oxidase subunit 1 (COI) gene, partial cds;       Simulium sp. BO.       974       2424       87%       0.0       95.99%       658       HQ941240.1         imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4730 cytochrome oxidase subunit 1 (COI) gene, partial cds;       Simulium sp. BO.       974       2424       87%       0.0       95.99%       658       HQ941116.1         imulium sp. BOLD:AAE8840										
Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-2454 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.       1018       2496       87%       0.0       97.32%       658       HQ940459.1         Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-2436 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.       975       2446       87%       0.0       96.32%       658       HQ940455.1         Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-2450 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.       977       2440       87%       0.0       96.15%       658       HQ940455.1         Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-3173 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.       974       2435       87%       0.0       95.99%       658       HQ940451.1         Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4730 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.       974       2435       87%       0.0       95.99%       658       HQ941161.1         Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4730 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.       974       2424       87%       0.0       95.99%       658       HQ941161.1         Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4730 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.       974       2424       87%       0.0										
imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-2436 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO. 985 2446 87% 0.0 96.32% 658 HQ94042.1 imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-2450 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO. 974 2424 87% 0.0 95.99% 658 HQ940967.1 imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4854 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO. 974 2424 87% 0.0 95.99% 658 HQ940967.1 imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4730 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO. 974 2424 87% 0.0 95.99% 658 HQ941240.1 imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4739 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO. 974 2424 87% 0.0 95.99% 658 HQ941116.1 imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4789 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO. 974 2429 87% 0.0 95.99% 658 HQ941175.1 imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4789 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO. 974 2429 87% 0.0 95.99% 658 HQ941175.1 imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-1817 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO. 974 2429 87% 0.0 95.99% 658 HQ941175.1 imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4789 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO. 974 2435 87% 0.0 95.99% 658 HQ941175.1 imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4798 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO. 974 2435 87% 0.0 95.99% 658 HQ941175.1 imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4798 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO. 974 2435 87% 0.0 95.99% 658 HQ941184.1 imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4798 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO. 968 2407 87% 0.0 95.82% 658 HQ941184.1										
Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-2450 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.979244087%0.096.15%658HQ940455.1Simulium sp. CFWIE038-10 voucher 10-SCCWRP-3173 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. CF.974242487%0.095.99%658HQ940451.1Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4854 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.974242487%0.095.99%658HQ941140.1Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4730 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.974242487%0.095.99%658HQ941175.1Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4789 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.974243587%0.095.99%658HQ941175.1Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4789 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.974243587%0.095.99%658HQ941175.1Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4798 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.974243587%0.095.99%658HQ940455.1Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4798 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.974243587%0.095.82%658HQ941145.1Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4798 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.968 <td< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></td<>										
Simulium sp. CFWIE038-10 voucher 10-SCCWRP-3173 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. EC.974242487%0.095.99%658HQ940967.1Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4854 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.974243587%0.095.99%658HQ9411240.1Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4730 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.974242487%0.095.99%658HQ941175.1Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4789 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.974243587%0.095.99%658HQ941175.1Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4789 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.974243587%0.095.99%658HQ941175.1Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4798 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.974243587%0.095.99%658HQ941175.1Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4798 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.974243587%0.095.82%658HQ941184.1Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4863 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO.968240787%0.095.82%658HQ941249.1										
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Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-1817 cytochrome oxidase subunit 1 (COI) gene, partial cds;       Simulium sp. BO       974       2435       87%       0.0       95.99%       658       HQ939872.1         Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4798 cytochrome oxidase subunit 1 (COI) gene, partial cds;       Simulium sp. BO       968       2396       87%       0.0       95.82%       658       HQ941184.1         Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4863 cytochrome oxidase subunit 1 (COI) gene, partial cds;       Simulium sp. BO       968       2407       87%       0.0       95.82%       658       HQ941249.1						87%	0.0	95.99%	658	B HQ941116.1
Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4798 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO 968 2396 87% 0.0 95.82% 658 HQ941184.1 Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4863 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO 968 2407 87% 0.0 95.82% 658 HQ941249.1		mulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4789 cytochrome oxidase subunit 1 (COI) gene, partial cds;	Simulium sp. BO.	. 974	2429	87%	0.0	95.99%	658	B HQ941175.1
Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4863 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO 968 2407 87% 0.0 95.82% 658 HQ941249.1	2	mulium sp. BOLD:AAE8840 voucher 10-SCCWRP-1817 cytochrome oxidase subunit 1 (COI) gene, partial cds;	Simulium sp. BO.	. 974	2435	87%	0.0	95.99%	658	B HQ939872.1
	2	imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4798 cytochrome oxidase subunit 1 (COI) gene, partial cds;	Simulium sp. BO.	. 968	2396	87%	0.0	95.82%	658	B <u>HQ941184.1</u>
Simulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4834 cytochrome oxidase subunit 1 (COI) gene, partial cds; Simulium sp. BO 968 2418 87% 0.0 95.82% 658 HQ941220.1		imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4863 cytochrome oxidase subunit 1 (COI) gene, partial cds:	Simulium sp. BO.	. 968	2407	87 <mark>%</mark>	0.0	9 <mark>5.82</mark> %	658	B <u>HQ941249.1</u>
	ò	imulium sp. BOLD:AAE8840 voucher 10-SCCWRP-4834 cytochrome oxidase subunit 1 (COI) gene, partial cds;	Simulium sp. BO.	. 968	2418	87%	0.0	9 <mark>5.8</mark> 2%	658	B HQ941220.1

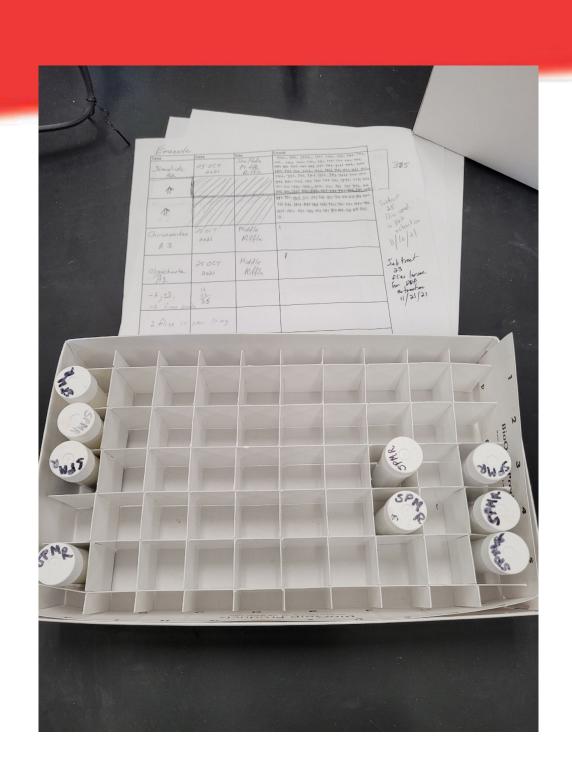
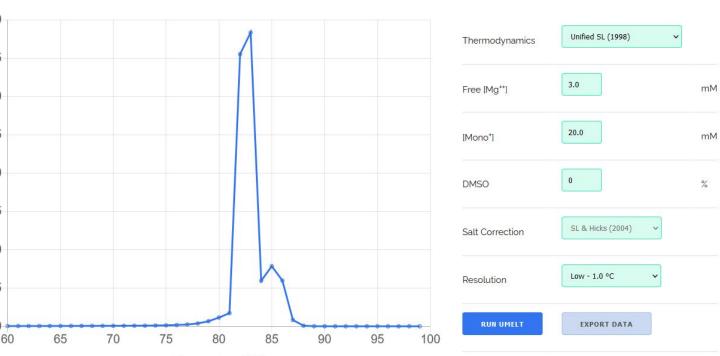


Fig. 4: Samples and counts of the different invertebrates collected from pools and riffles in the San **Pedro River** 



GC Content = 32%, Length = 680b

Fig. 8: Predicted melt curve of Simulium paynei (Simulium Paynei Sensu Lato)

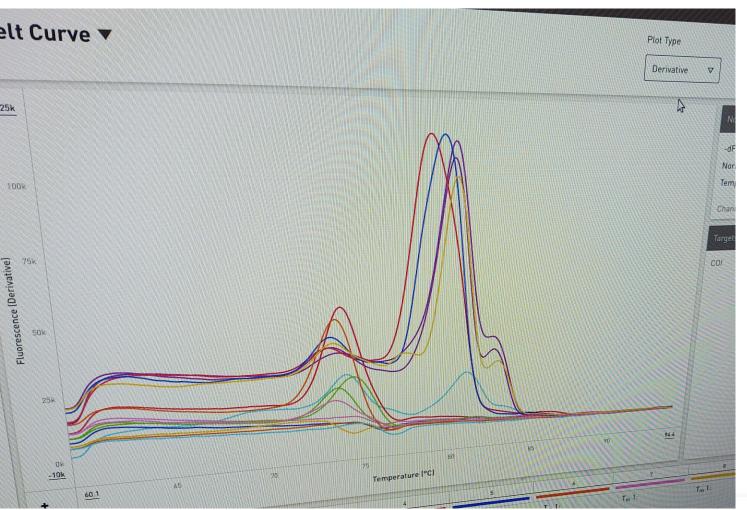
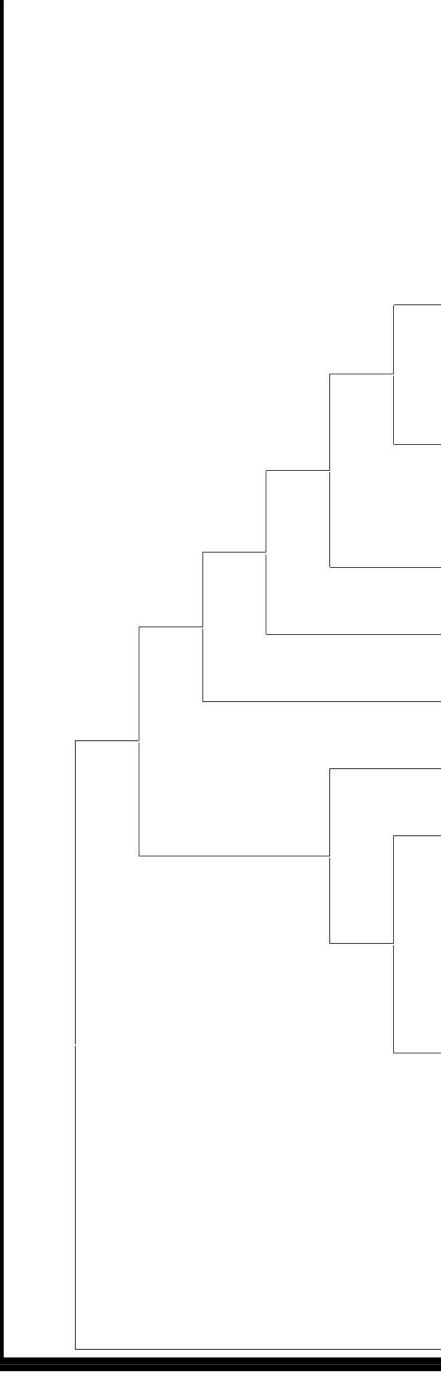


Fig. 9: Observed melt curve of our unknown samples

### **Future:**

accurate and specific DNA sequence

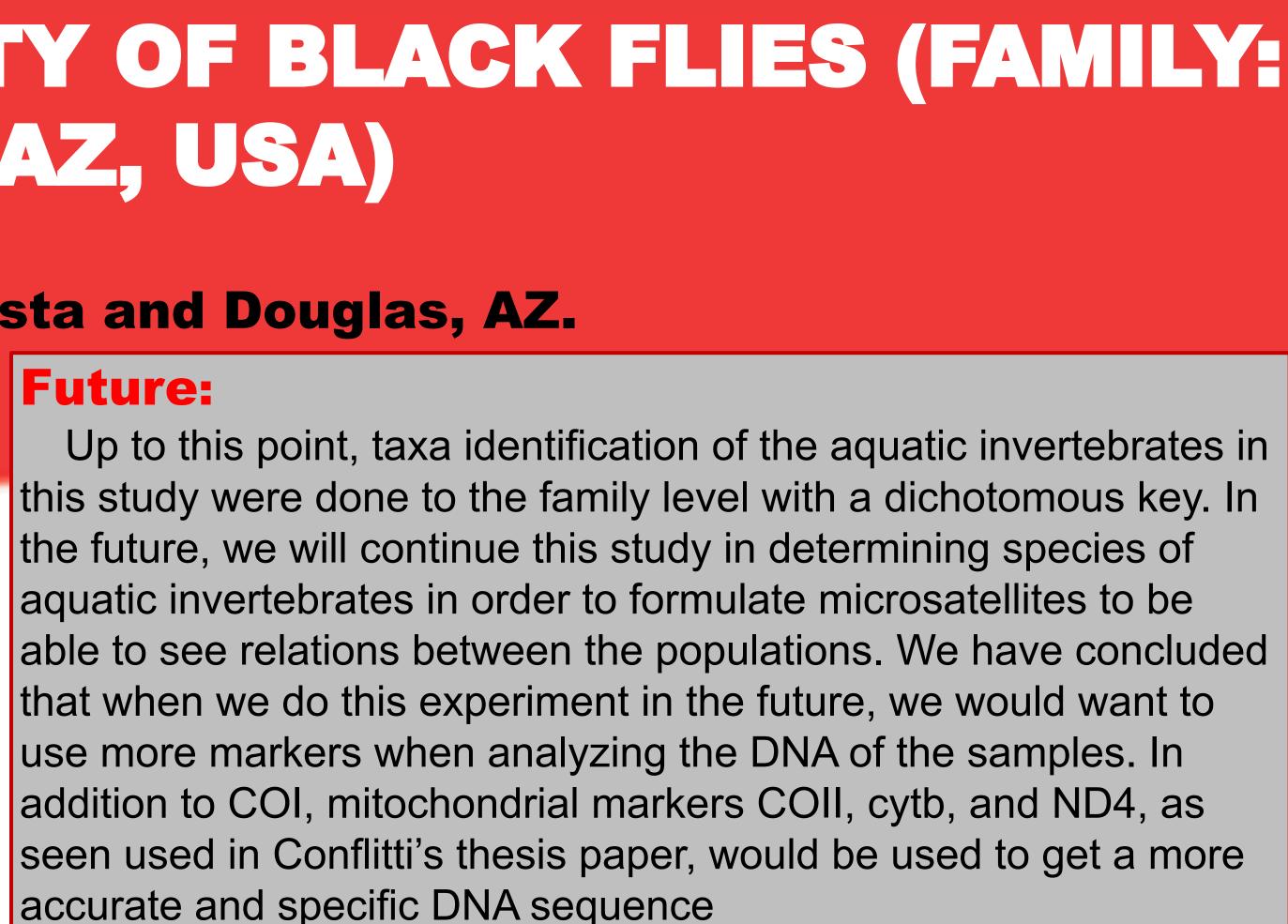


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<ul> <li>Simulium venator</li> </ul>
<ul> <li>Simulium argus</li> </ul>
- COISample4 COILCO1490
<ul> <li>Simulium canadense</li> </ul>
<ul> <li>Simulium virgatum</li> </ul>
<ul> <li>Simulium paynei voucher VE64 191</li> </ul>
- Simulium hunteri
<ul> <li>Simulium pugetense</li> </ul>
<ul> <li>Prosimulium flaviantennus</li> </ul>
– Prosimulium travisi
– Prosimulium frohnei
<ul> <li>Helodon decemarticulatus</li> </ul>
– Drosophila melanogaster
– Simulium johannseni
<ul> <li>Stegopterna mutata</li> </ul>
<ul> <li>Simulium vittatum</li> </ul>
- COIUnExo 1 COILCO1490
– Simulium encisoi
- COIUnExo 2 COILCO1490
– Simulium vernum
<ul> <li>Simulium solarii</li> </ul>
<ul> <li>Prosimulium exigens</li> </ul>
<ul> <li>Helodon onychodactylus complex sp.</li> </ul>
– Simulium piperi
<ul> <li>Simulium tuberosum</li> </ul>
<ul> <li>Simulium decorum</li> </ul>
- Simulium verecundum
<ul> <li>Simulium venustum</li> </ul>
<ul> <li>Simulium defoliarti</li> </ul>
 <ul> <li>Simulium arcticum</li> </ul>

Fig. 10: Phylogeny tree created using the three best DNA sequences we obtained

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