Identification of Long Island Mosquitoes Collected in a Wetlands Remediation Sump

Authors: Marisa Calderone[1], Bethany Fanning[1]

Mentors: Mary Kroll[1]; Cristina Fernández- Marco[2]; Sharon Pepenella[3]

[1]West Islip High School; [2]Cold Spring Harbor Laboratory's DNA Learning Center

Abstract

Approximately 176 species of mosquitoes are found in the United States. It is important to identify if there is a dangerous local species. Recently on Long Island, mosquitoes have been transmitting diseases like West Nile Virus. A BG-Sentinel trap provided by Suffolk County Department of Health was used to collect our mosquitoes. Traps were placed in a wetlands remediation sump for 24 hours. DNA was extracted from each mosquito, the CO1 gene was amplified using PCR, and verified using gel electrophoresis. Each positive result was sent to a lab for Sanger sequencing. DNA Subway Blue Line was used to analyze DNA sequences of samples collected. Results showed 50% were identified as *Aedes albopictus*, 10% were *Sciara kitakamiensis*, and 10% were *Sympycininae*. After barcoding, it was discovered not all species were classified as mosquitoes. 30% were *Rhipidia chenwenyoungi* which differentiates at the family level. Identifying these organisms can help researchers in the future learn more, and determine if they threaten human health.

Introduction and Hypothesis

- The goal in studying the biodiversity of mosquitoes is to identify known and potentially unknown mosquitoes that could pose a risk to human health by spreading diseases that can result in sickness or even death.
- "West Nile virus is the most common mosquito-borne disease in the U.S. It can be spread by the *Culex pipiens* mosquito, also known as the northern house mosquito, which is common in Suffolk County" ("Suffolk County Government", 2016, para. 3).
- Barcoding mosquitoes is necessary as the differences between some species cannot be distinguished anatomically after trapping.
- Research Question: How does the biodiversity of mosquitoes on Long Island pose a risk to human health?
- Hypothesis: There will be a small range of diversity regarding the type of mosquitoes collected.

Figure 1: This red pin on the map of Long Island indicates where the mosquitoes were caught, Wetlands Remediation Sump (Google Maps, 2018).
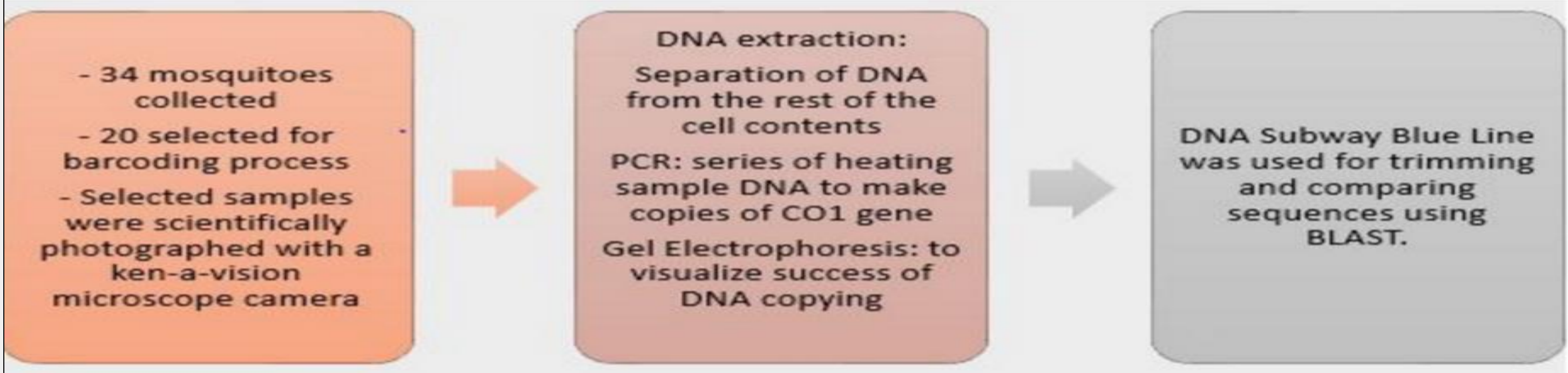
Methods

- 34 mosquitoes collected
- 20 selected for barcoding process
- Selected samples were scientifically photographed with a ken-a-vision microscope camera

DNA extraction: Separation of DNA from the rest of the cell contents
PCR: series of heating sample DNA to make copies of CO1 gene
Gel Electrophoresis: to visualize success of DNA copying

DNA Subway Blue Line was used for trimming and comparing sequences using BLAST.

Figure 2: BG-Sentinel trap provided by the Suffolk County Department of Health. Photo taken by authors.

Figure 3: Sample caught in BG-Sentinel trap. Photo taken by Marisa Calderone using Ken-a-vision microscope with digital camera.

Figure 4: Extracting DNA from mosquitoes in the lab. Photo courtesy of Mrs. Kroll.

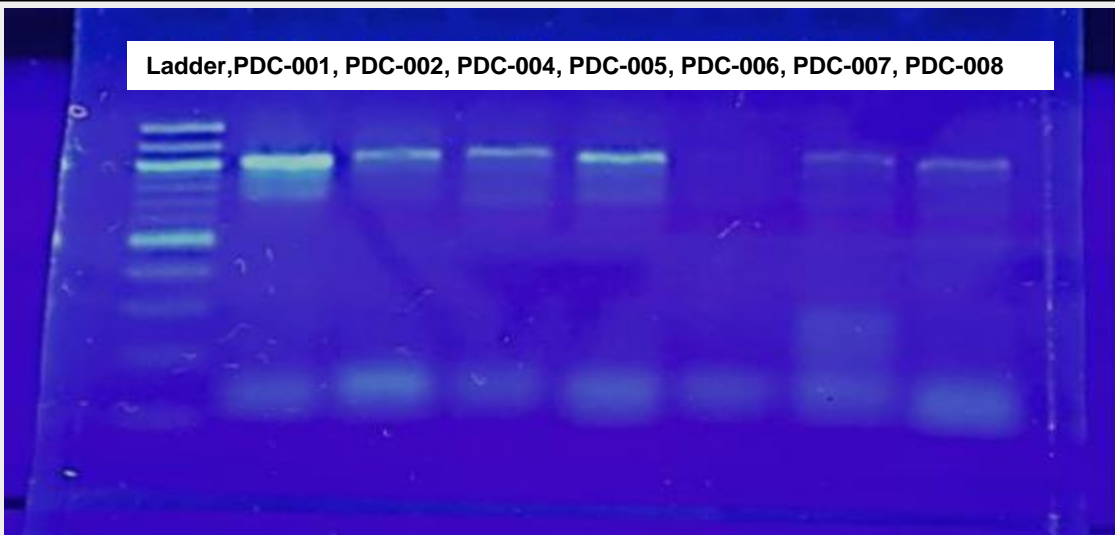Ladder,PDC-001, PDC-002, PDC-004, PDC-005, PDC-006, PDC-007, PDC-008

Figure 5: Gel electrophoresis results of samples PDC-001-008. All results were sent for sequencing except for PDC-006 which showed no DNA amplification. Photo courtesy of Mrs. Kroll.

Results

Table 1: Metadata and DNA subway data for samples sequenced

Sample ID	Trap Name	Aln. Length	Bit Score	e value	# mismatches	Scientific Name
PDC-001	BG Sentinel	542	765	0	47	*Rhipidia chenwenyoungi*
PDC-002	BG Sentinel	516	931	0	0	*Sciara kitakamiensis*
PDC-003	BG Sentinel	552	783	0	47	*Rhipidia chenwenyoungi*
PDC-004	BG Sentinel	511	922	0	0	*Sympycninae sp.*
PDC-005	BG Sentinel	529	762	0	49	*Rhipidia chenwenyoungi*
PDC-007	BG Sentinel	542	962	0	0	*Aedes albopictus*
PDC-008	BG Sentinel	505	902	0	0	*Aedes sp.*
PDC-010	BG Sentinel	609	1099	0	0	*Aedes albopictus*
PDC-013	BG Sentinel	521	940	0	0	*Aedes albopictus*
PDC-017	BG Sentinel	508	917	0	0	*Aedes albopictus*

Table 2: percent similarities between collected samples, BLAST samples, and reference samples

	Consensus	PDC-010	PDC-002	PDC-008	Aedes albopictus	PDC-007	PDC-013	PDC-017	PDC-004	Sympycninae	Rhipidia	PDC-001	PDC-004	PDC-003	PDC-005
Consensus	-	64.83	89.73	89.73	92.28	92.26	86.03	89.25	88.19	91.98	91.98	90.77	91.33	91.3	90.74
PDC-010	64.83	-	54.1	54.1	55.04	55.04	50.21	53.75	53.67	53.62	53.62	55.16	55.36	55.36	55
PDC-002	89.73	54.1	-	100	84.46	84.46	77.58	81.4	80.45	85.52	85.52	85.08	83.53	83.53	83.11
PDC-008	89.73	54.1	100	-	84.46	84.46	77.84	81.4	80.45	85.52	85.52	85.08	83.53	83.53	83.11
Aedes albo	92.28	55.04	84.46	84.46	-	100	77.84	89.88	89.94	86.77	86.77	85.52	84.92	84.92	84.43
PDC-007	92.26	55.04	84.36	84.46	100	-	85.22	89.88	89.94	86.77	86.77	85.52	84.92	84.92	84.43
PDC-013	86.03	50.21	77.58	77.58	85.22	85.4	85.4	93.32	92.96	80.76	80.76	78.27	79.23	79.28	78.5
PDC-017	89.25	53.75	81.4	82.4	89.88	89.88	-	-	99.8	85.13	85.13	81.38	81.77	81.77	81.47
PDC-004	88.19	53.67	80.45	80.45	89.94	89.94	93.32	99.8	-	84.46	84.46	80.4	80.99	80.99	80.56
Sympycninae	91.98	53.62	85.52	85.52	86.77	86.77	92.97	85.13	86.5	-	100	86.69	87.67	87.67	87.28
Rhipidia	91.98	53.62	85.52	85.52	86.77	86.77	80.76	85.13	86.5	100	-	86.69	87.67	87.67	87.28
PDC-001	90.77	55.16	85.08	85.08	85.52	85.52	80.76	81.38	85.61	86.69	86.69	-	91.33	91.33	90.72
PDC-004	91.33	55.36	83.53	83.53	84.92	84.93	78.27	81.77	87.08	87.67	87.67	91.33	-	100	99.62
PDC-003	91.3	55.36	83.53	83.53	84.92	84.93	79.23	81.77	87.14	87.67	87.67	91.33	100	-	99.62
PDC-005	90.74	55	83.11	83.11	84.43	84.43	79.28	81.47	80.56	87.28	87.28	90.72	99.62	99.62	-

Figure 8: Phylogenetic tree above shows similarities between barcoded samples (PDC), BLAST samples, and reference samples. Figure created in DNA Subway.

Figure 9: Sample PDC-013 *Aedes albopictus*

Figure 10: Sample PDC-002 *Sciara kitakamiensis*

Figure 11: Sample PDC- 001 *Rhipidia chenwenyoungi*

Figure 12: Sample PDC-004 *Sympycninae sp.*

Sequence Conservation
Sequence Variation
Consensus
1. PDC-002
2. PDC-008
3. BLAST- Aedes_sp.
4. PDC-013
5. PDC-017
6. Fruit_Fly-Diptera
7. PDC-004
8. BLAST- Rhipidia_chenwenyoungi
9. PDC-001
10. PDC-003
11. PDC-005

Figure 13: Barcode of samples PDC-001 – PDC-017 compared to BLAST samples and a consensus Figure created in DNA Subway.

Figure 6: Sample PDC-001 electropherogram Photo produced in DNA Subway.

Sample Collection Percentages

- Aedes albopictus — 50%
- Rhipidia chenwenyoungi — 30%
- Sympicninae sp. — 10%
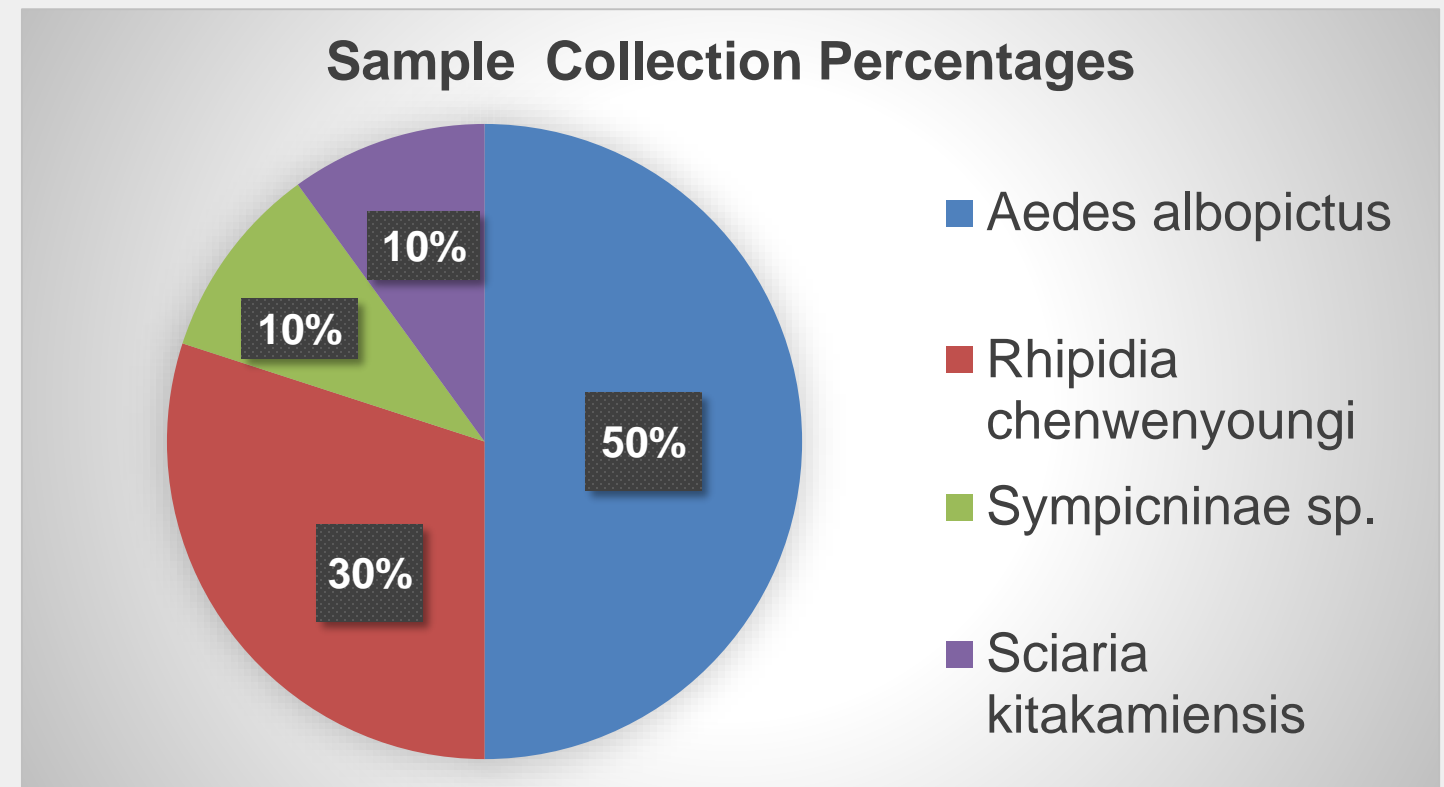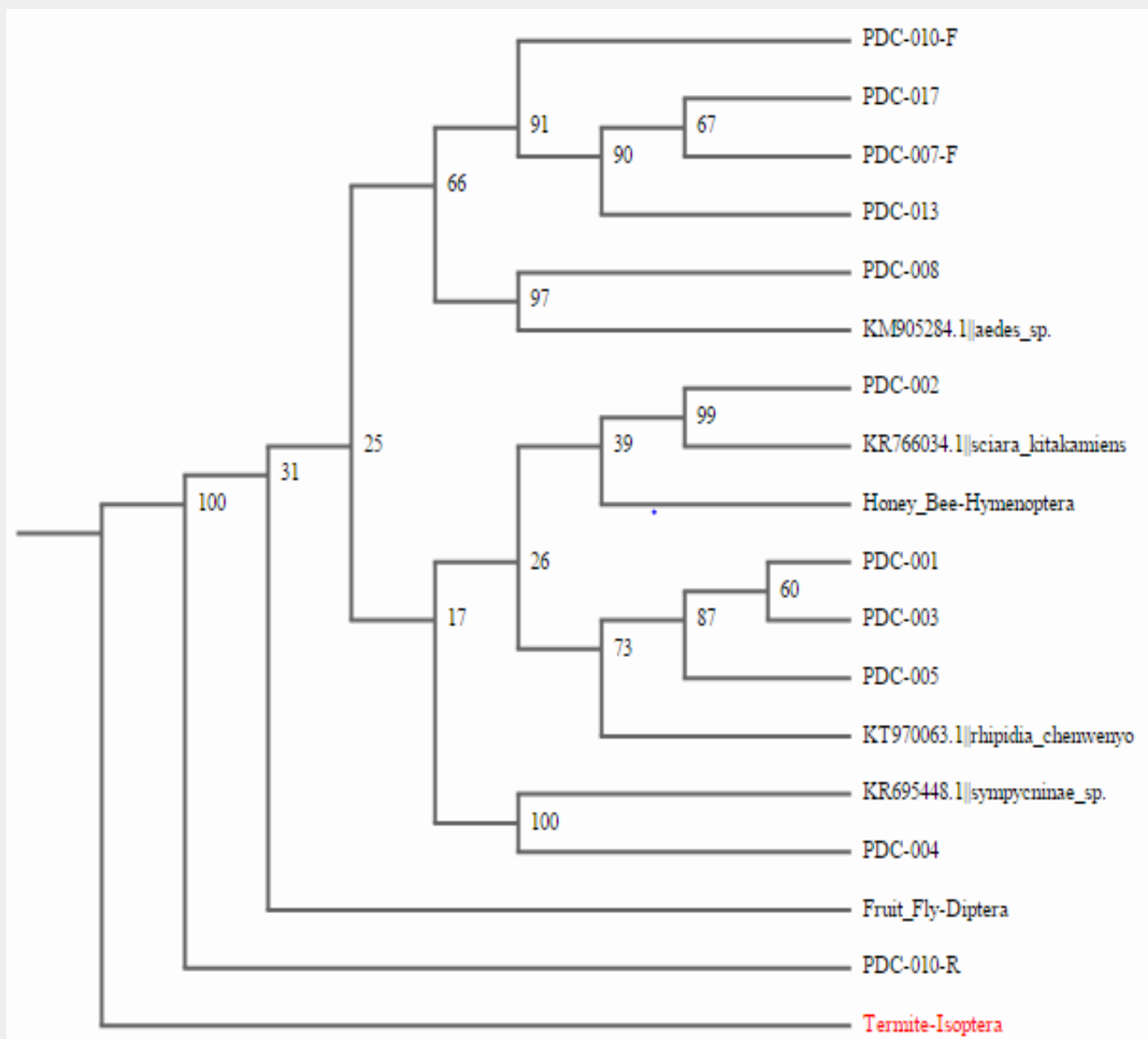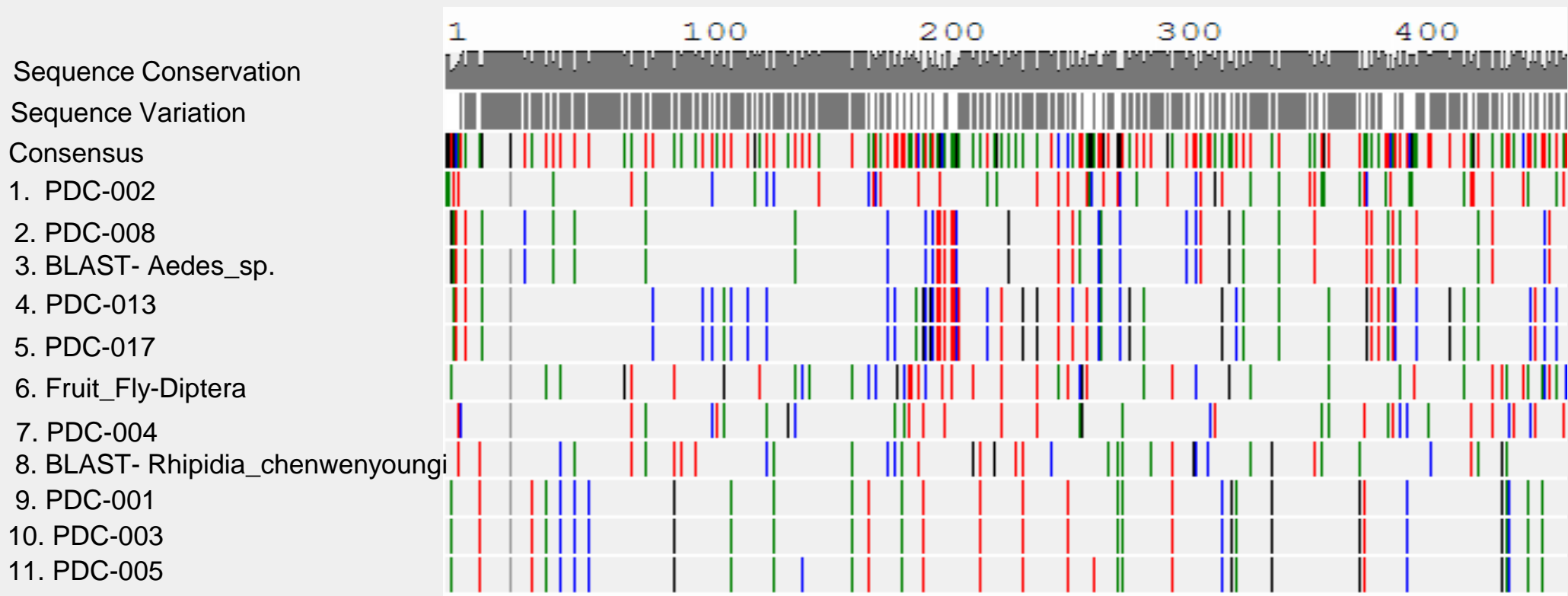- Sciaria kitakamiensis — 10%

Figure 7: percentages of the collected organisms by taxonomic classification; 50% of the collected samples were the mosquito *Aedes albopictus*. Graph created by researchers.

Conclusions/ Future Research

- DNA Subway Blue Line was used to analyze the DNA sequence of each mosquito collected. The samples were then compared to other already published species.
- 50 percent of the mosquitoes sequenced were identified as *Aedes albopictus*, 30 percent were *Rhipidia chenwenyoungi*, 10 percent were *Sciara kitakamiensis*, and 10 percent were *Sympycninae*.
- It was discovered that not all species were classified as mosquitoes. The *Rhipidia chenwenyoungi* is differentiated from a mosquito at the family level.
- Knowing the identification of these organisms can help researchers in the future to learn more about them and if they are a possible threat to human health.

References

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Acknowledgements