

a.

	<i>S. meridionale</i>		<i>S. mediovittatum</i>		<i>S. robynae</i>		<i>S. griseum/notatum</i>	
	To Voucher Sequence	Between Samples	To Voucher Sequence	Between Samples	To Voucher Sequence	Between Samples	To Voucher Sequence	Between Samples
Min	95.0%	95.1%	95.0%	96.1%	96.7%	95.3%	98.3%	NA
Max	99.4%	99.8%	97.2%	99.8%	98.4%	99.7%	98.3%	NA
Avg	97.6%	98.0%	96.4%	99.1%	97.8%	98.2%	98.3%	NA
± SD	0.7%	0.8%	0.4%	0.5%	0.4%	0.9%	N/A	NA

b.

	<i>S. meridionale</i>		<i>S. mediovittatum</i>		<i>S. robynae</i>	
	To Voucher Sequence	Between Samples	To Voucher Sequence	Between Samples	To Voucher Sequence	Between Samples
Min	94.90%	95.20%	95.10%	96.10%	96.80%	96.80%
Max	99.20%	100%	97.60%	99.80%	98.40%	99%
Avg	97.70%	97.90%	96.20%	99.00%	97.90%	98.10%
± SD	0.70%	0.90%	0.70%	0.80%	0.50%	0.50%

c.

	<i>S. argus</i>		<i>S. encisoii</i>		<i>S. vittatum</i>	
	To Voucher Sequence	Between Samples	To Voucher Sequence	Between Samples	To Voucher Sequence	Between Samples
Min	98.10%	97.314	99.842	NA	97.91%	97.30%
Max	99.84%	100%	99.842	NA	99.842	100%
Avg	98.79%	98.90%	99.842	NA	99.38%	99.13%
± SD	0.54%	0.74%	NA	NA	0.48%	0.61

Additional File 5: a) Minimum, maximum, and average sequence identity % ± standard deviation of adult black fly sequences sampled along Rio Grande River in 2020. b) Minimum, maximum, and average sequence identity % ± standard deviation of adult black fly sequences sampled along Rio Grande River, stable and lateral sites in 2022. c) Minimum, maximum, and average sequence identity % ± standard deviation of black fly larvae sequences sampled in Rio Grande River in 2023.