

**Table S1.** Summarized information about Cuban fly (Diptera) hosts and their trypanosomatids, including intensity of infection (**Int.**), tissue localization (**Tissue**) of the infection in the host intestine (HG, hindgut; MG, midgut) and the list of detected trypanosomatid species based on cultivation (**Culture**) and **Sanger** and/or **Nanopore** sequencing following the (nested) PCR of the homogenized host intestine.

Specimen	Locality	Family	Int.	Tissue	Culture	Sanger sequencing	Nanopor sp.#1	Nanopor sp.#2	Nanopor sp.#3	Nanopor sp.#4	Nanopor sp.#5	Nanopor sp.#6	Nanopor sp.#7	Nanopor sp.#8
MCu-01	Vinales	Ulidiidae	2	HG		<i>Kentomonas</i> sp. 1a								
MCu-02	Vinales	Muscidae	2	HG+MG	<i>Herpetomonas</i> sp. 2		<i>Vickermania</i> sp. 8	<i>Vickermania</i> sp. 13	"Muscomonas"	"Newbiana"	<i>Herpetomonas</i> sp. 1	<i>Herpetomonas</i> sp. 2	<i>Herpetomonas</i> sp. 3	<i>Angomonas</i> sp. 1
MCu-03	Vinales	Muscidae	2	MG (?)			<i>Vickermania</i> sp. 8	<i>Vickermania</i> sp. 11	<i>Vickermania</i> sp. 14	<i>Vickermania</i> sp. 15	"Muscomonas"			
MCu-04	Vinales	Muscidae	2	MG (?)	<i>Wallacemonas</i> sp. A		<i>Vickermania</i> sp. 8	<i>Vickermania</i> sp. 11	<i>Vickermania</i> sp. 15	"Muscomonas"				
MCu-05	Vinales	Muscidae	3	MG	<i>Wallacemonas</i> sp. A		<i>Vickermania</i> sp. 8	<i>Vickermania</i> sp. 9	<i>Vickermania</i> sp. 14	<i>Vickermania</i> sp. 15	"Muscomonas"			
MCu-06	Vinales	Ulidiidae	2	HG	<i>Wallacemonas</i> sp. A	<i>Vickermania</i> sp. 2a								
MCu-07	Vinales	Sepsidae	3	MG			<i>Vickermania</i> sp. 10	<i>Parabodo caudatus</i>						
MCu-08	Vinales	Sepsidae	3	MG			<i>Vickermania</i> sp. 2b	<i>Vickermania</i> sp. 7	<i>Vickermania</i> sp. 10					
MCu-09	Vinales	Sepsidae	3	MG		<i>Vickermania</i> sp. 1								
MCu-10	Vinales	Sepsidae	3	MG			<i>Vickermania</i> sp. 10	<i>Parabodo</i> sp. 1	<i>Parabodo</i> sp. 2	<i>Parabodo</i> sp. 3				
MCu-11	Vinales	Sepsidae	3	MG										
MCu-12	Palma Rubia	Muscidae	2	MG			<i>Vickermania</i> sp. 6	<i>Vickermania</i> sp. 8	<i>Vickermania</i> sp. 9	<i>Vickermania</i> sp. 11	<i>Vickermania</i> sp. 12	<i>Vickermania</i> sp. 15	"Muscomonas"	"Newbiana"
MCu-13	Palma Rubia	Muscidae (cf.)	2	HG+MG	<i>Herpetomonas modestus</i>		<i>Vickermania</i> sp. 16	<i>Herpetomonas modestus</i>						
MCu-14	Palma Rubia	Muscidae	4	MG			<i>Vickermania</i> sp. 6	<i>Vickermania</i> sp. 9	<i>Vickermania</i> sp. 12	<i>Vickermania</i> sp. 15	"Muscomonas"			
MCu-15	Cienfuegos	Lauxaniidae (cf.)	3	HG+MG		<i>Vickermania</i> sp. 4								
MCu-16	Trinidad	Calliphoridae	3	HG+MG	<i>Angomonas</i> sp. 1	<i>Vickermania</i> sp. 3								
MCu-17	Varaderos	Muscidae	3	MG		<i>Vickermania</i> sp. 5								
MCu-18	Varaderos	Muscidae	3	HG		<i>Kentomonas</i> sp. 1b								
MCu-19	Varaderos	Muscidae	2	HG	<i>Kentomonas</i> sp. 1a		<i>Kentomonas</i> sp. 1a	<i>Herpetomonas samuelpeessoai</i>						
MCu-KV	Varaderos	Drosophilidae	1	?		<i>Wallacemonas</i> -like								

\* Intensity of infection (1-4)

\*\* Localization in host: HG, hindgut; MG, midgut