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Bartonella spp. in Phlebotomine Sand Flies, Brazil

Appendix

Appendix Table 1. Description of molecular assays (target gene, primers/probe identification and sequence, thermal conditions, amplicon size and reference) used for sand flies' endogenous gene screening and characterization of *Bartonella* spp. in the present study.

Molecular Marker	Primers/Probe	Thermal conditions	Amplicon size (bp)	Reference
<i>cox-1</i>	LCO1490 (5' -GGTCAACAAATCATAAAGATATTGG- 3') HCO2198 (5' -TAAACTTCAGGGTGACCAAAAAATCA- 3')	95°C for 1min; 35 cycles of 95°C for 1min, 40°C for 1min and 72°C for 1.5min; 72°C for 10min	710	(1)
16S-23S ITS	BsppITS325S Forward (5' – CTTCAGATGATGATCCAAGCCTTCTGGCG – 3') 543as Reverse (5' – AATTGGTGGGCCTGGGAGGACTTG – 3') BsppITS500 (5' – [6FAM]GTTAGAGCGCGCGCTTGATAAG[BHQ1] – 3')	95°C for 3min; 45 cycles of 94°C for 10s, 68°C for 10s, 72°C for 10s and plate read; 72°C for 30s	243	(2,3)
<i>gltA</i>	BhCS.781p (gltA F) (5' GGGGACCAGCTCATGGTGG- 3') BhCS.1137n (gltA R) (5' AATGCAAAAAGAACAGTAAACA- 3')	95°C for 5min; 35 cycles of 95°C for 30s, 51°C for 30s and 72°C for 2min; 72°C for 5min	380–400	(4)
	CS443f (5' -GCTATGTCTGCATTCTATCA- 3') CS1210r (5' -GATCYTCAATCATTCTTTCCA- 3')	94°C for 2min; 45 cycles of 94°C for 30s, 48°C for 1min and 72°C for 1min; 72°C for 7min	767	(5)
<i>ftsZ</i>	ftsZ F (5' -CATATGGTTTTTCATTACTGCYGGTATGG- 3') ftsZ R (5' -TTCTTCGCGAATACGATTAGCAGCTTC- 3')	94°C for 2min; 40 cycles of 94°C for 2min, 61°C for 45s and 72°C for 45s; 72°C for 7min	515	(6)
<i>groEL</i>	GroEL F (5' -GGAAAAAGTGGGCAATGAAG- 3') GroEL R (5' -TCCTTTAACGGTCAACGCATT- 3')	94°C for 2min; 40 cycles of 94°C for 2min, 47°C for 45s and 72°C for 45s; 72°C for 7min	752	(6)
<i>nuoG</i>	nuoG F (5' -GGCGTGATTGTTCTCGTTA- 3')	94°C for 5min; 35 cycles of 94°C for 30s, 53°C for 30s and 72°C for 5min; 72°C for 5min	346	(7)

Molecular Marker	Primers/Probe	Thermal conditions	Amplicon size (bp)	Reference
	nuoG R (5' -CACGACCACGGCTATCAAT -3')			
<i>Pap31</i>	165s (5' -GACTTCTGTTATCGCTTTGATTT- 3') 688as (5' -CACCACCAGCAAMATAAGGCAT- 3')	95°C for 5min; 45 cycles of 94°C for 30s, 56°C for 30s and 72°C for 45s; 72°C for 5min	564	(8)
<i>rpoB</i>	1400F (5' -CGCATTGGCTTACTTCGTATG- 3') 2300R (5' -GTAGACTGATTAGAACGCTG- 3')	94°C for 2min; 35 cycles of 94°C for 30s, 53°C for 30s and 72°C for 1min; 72°C for 2min	825	(9)
<i>ribC</i>	Barton-1 (5' -TAACCGATATTGGTTGTGTTGAAG- 3') Barton-2 (5' -TAAAGCTAGAAAGTCTGGCAACATAACG- 3')	95°C for 10min; 37 cycles of 95°C for 1min, 51°C for 1min and 72°C for 1min; 72°C for 3min	585–588	(10)
16S-23S ITS	321s (5' -AGATGATGATCCCAAGCCTTCTGGCG- 3') 938as (5' -TGTTCTYACAACAATGATGATG- 3')	95°C for 5min; 45 cycles of 94°C for 30s, 54°C for 30s and 72°C for 45s; 72°C for 5min	453–717	(11)

Appendix Table 2. Sequence identity of the 16S-23S ITS positive samples to the most closely related *Bartonella* species.

ID	Sand fly species	Sequence size (bp)	BLASTn result	Host	Query Cover	E value	Identity	Country	Accession Number
AB121	<i>Trichophoromyia</i> sp.	222	<i>Bartonella ancashensis</i>	<i>Homo sapiens</i>	98%	9e-63	87.56%	Peru	KC886740.1
AB410	<i>Nyssomyia antunesi</i>	197	<i>Bartonella</i> sp.	<i>Mimon cozumelae</i>	81%	4e-71	98.16%	Guatemala	MN504740.1
			<i>Bartonella</i> sp.	<i>Micronycteris microtis</i>	81%	4e-71	98.16%	Guatemala	MN258132.1
AB594	<i>Psychodopygus davisi</i>	189	<i>Bartonella</i> sp.	<i>Mimon cozumelae</i>	87%	2e-78	100%	Guatemala	MN504740.1
			<i>Bartonella</i> sp.	<i>Micronycteris microtis</i>	87%	2e-78	100%	Guatemala	MN258132.1
AB164	<i>Psychodopygus llanosmartinsi</i>	189	<i>Bartonella</i> sp.	<i>Apodemus sylvaticus</i>	87%	1e-60	93.49%	United Kingdom	AJ269793.1
AB537	<i>Evandromyia walker</i>	179	<i>Bartonella</i> sp.	<i>Mimon cozumelae</i>	89%	4e-75	99.38%	Guatemala	MN504740.1
			<i>Bartonella</i> sp.	<i>Micronycteris microtis</i>	89%	4e-75	99.38%	Guatemala	MN258132.1
AB540	<i>Pintomyia serrana</i>	175	<i>Bartonella</i> sp.	<i>Apodemus sylvaticus</i>	100%	1e-60	92.09%	United Kingdom	AJ269793.1
AB12	<i>Nyssomyia antunesi</i>	197	<i>Bartonella</i> sp.	<i>Apodemus sylvaticus</i>	89%	1e-65	93.3%	United Kingdom	AJ269793.1

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