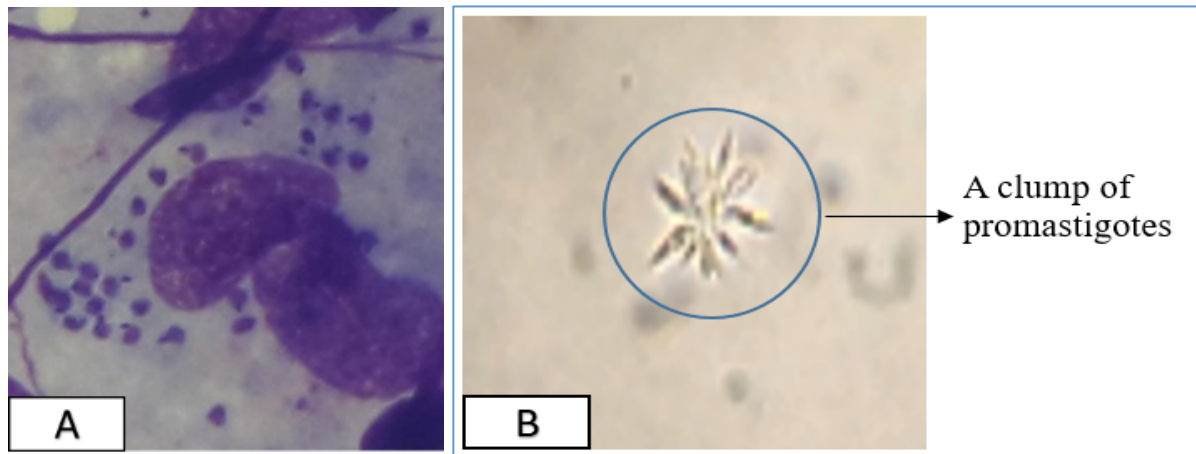


Supplementary Figures and Tables



Supplementary Figure S-I: Whole parasite visualization as microscopic and culture diagnosis. Giemsa stained parasite (A) and culture of parasite in NNN media (B)

Supplementary Table S-II: Clinical manifestations of the cutaneous lesions

Geographic Region	Province no. (Name)	District	VL Endemicity	GPS coordinates of district's center**		CL positive cases ***	General information of patient		CL etiology		National VL elimination program launched district	Travel history of patient outside Nepal		Travel history of patient inside Nepal		Medication used before sampling
				Latitude	Longitude		Age (year)	Gender	<i>L. major</i>	<i>L. donovani</i>		Country	Status of endemicity	Inside country	Nepal's Kalaazar endemic district* ***	
Hill	1 (Koshi)	Bhojpur	Endemic	27°21'32.868"	87°8'51.36"	1	43	M		CL27	Yes	UAE Dubai	No cases	No		Antifungal
		Okhaldhunga	Endemic	27°18'35.748"	86°24'31.608"	1	58	M	CL29		Yes	No		No		No medication
	3 (Bagmati)	Ramechhap	Doubtful endemic	27°29'11.868"	85°51'6.912"	1	15	M	CL12		No	No		No		Antibiotic and Antifungal
	4 (Gandaki)	Tanahun	Doubtful endemic	27°53'18.348"	83°58'28.992"	1	85	M	CL8		No	No		No		Antibiotic
		Syangja	Doubtful endemic	27°53'32.82"	83°48'45.612"	1	67	F		CL10	No	UK	No	No		NA
		Gorkha*	Doubtful endemic	27°59'9.276"	84°37'34.392"	2	54	F		CL17	No	No		No		NA
							66	F		CL23	No	No		Yes, Chitwan	Doubtful endemic	NA
		Baglung	Non-endemic	28°24'36.648"	83°0'7.956"	1	48	M	CL36		No	Saudi Arabia	Yes (CL, VL)	No		NA
	5 (Lumbini)	Palpa	Endemic	27°43'13.548"	83°38'21.66"	1	23	M		CL15	Yes	India	Yes (CL, VL)	No		Antibiotic, Antifungal and Antileishmanial
		Rukum*	Doubtful endemic	28°45'39.312"	82°27'39.456"	1	20	M		CL35	No	No		Yes, Dang	Doubtful endemic	NA
	6 (Karnali)	Jajarkot*	Doubtful endemic	28°43'51.6"	82°12'10.26"	1	35	M		CL22	No	No		No		Antifungal
	7 (Sudur Pashchim)	Baitadi	Doubtful endemic	29°33'54.072"	80°23'55.752"	2	54	F		CL14	No	No		No		Antituberculosis
							60	F		CL25	No	No		No		Antifungal
Mountain	6. (Karnali)	Humla	Doubtful endemic	29°59'12.192"	81°49'59.34"	1	33	M		CL16	No	No		No		Antibiotic and Antifungal
		Kalikot*	Doubtful endemic	29°12'49.896"	81°35'23.532"	3	19	F		CL2	No	No		Yes, Pyuthan	Doubtful endemic	NA
							9	F	CL5		No	No		Yes, Surkhet	Yes	Antifungal
							61	M		CL32	No	No		No		NA
	7 (Sudur)	Bajura	Doubtful endemic	29°31'47.568"	81°46'59.268"	3	16	F		CL18	No	No		No		NA
							15	F		CL19	No	No		No		NA
							11	M		CL40	No	No		No		No medication
Terai	1	Jhapa	Endemic	26°36'25.308"	87°44'41.748"	1	55	M	CL39		Yes	Saudi Arabia	Yes (CL, VL)	No		NA
	2 (Madhesh Pradesh)	Rautahat	Endemic	26°58'9.156"	85°15'37.296"	1	26	M	CL13		Yes	No		Yes, Darchula	Doubtful endemic	Antifungal

*District spread with hilly and mountainous areas. **GPS coordinates the center of the districts. ***Number of cases with positive parasitological and molecular diagnosis or only molecular diagnosis. ****Visceral leishmaniasis (VL) endemic district of Nepal described in National Guideline on Kala-azar Elimination Program 2019 (<http://www.edcd.gov.np/resource-detail/national-guideline-on-kala-azar-elimination-program-2019>); Endemicity status of foreign country defined by WHO according to the reported

cases (https://apps.who.int/neglected_diseases/ntddata/leishmaniasis/leishmaniasis.html). Male (M); Female (F); NA: Not Available; Shaded cells: No travel history.

Supplementary Table S-III: Clinical manifestations of the cutaneous lesions

Site of Lesion	Frequency	Number of lesion			Type of lesion		Dispersion of the lesion	
		1	2	>3	Dry	Moist	Localized	Dispersed
Face	19	13	3	3	16	3	16	3
Neck	5	5			2	3	4	1
Hand	5	3	1	1	4	1	3	2
Leg	3	2		1		3	3	
Abdominal part or body	3	1		2	3			3
Face and Hand	2		1	1		2	2	
Hand and Leg	1			1		1	1	
Hand and abdomen	1		1		1		1	
Face, Hand and Leg	1			1		1	1	
Total	40	24	6	10	26	14	31	9

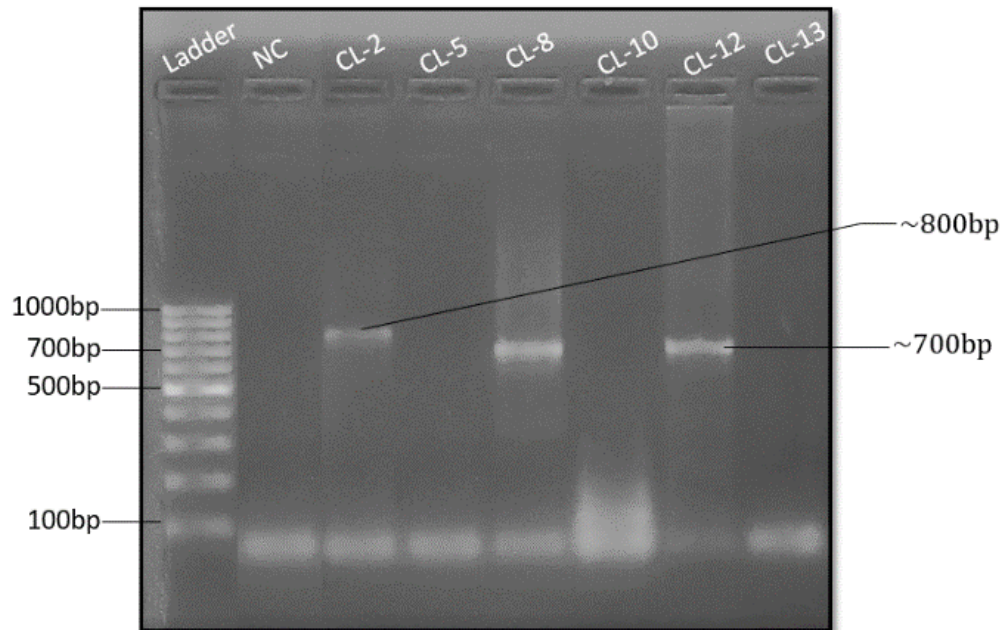
Supplementary data S-IV: Multiple sequence alignment of ITS1 of representative parasite isolates from patients with those of *L. donovani* complex and *L. major* reference strains.

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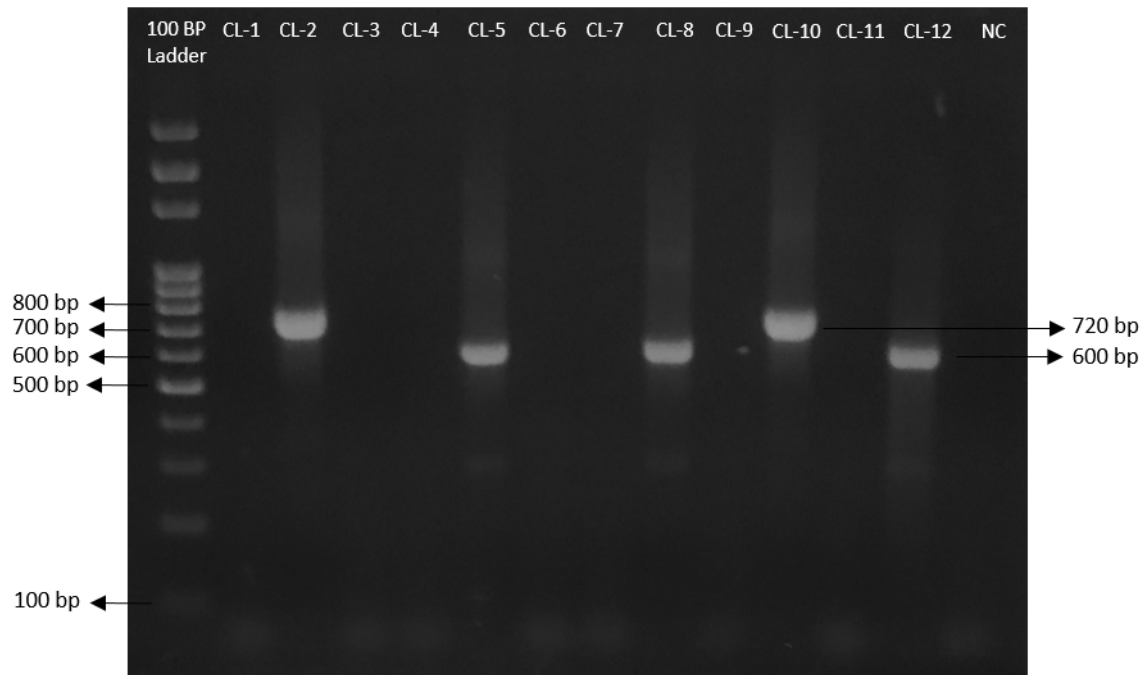
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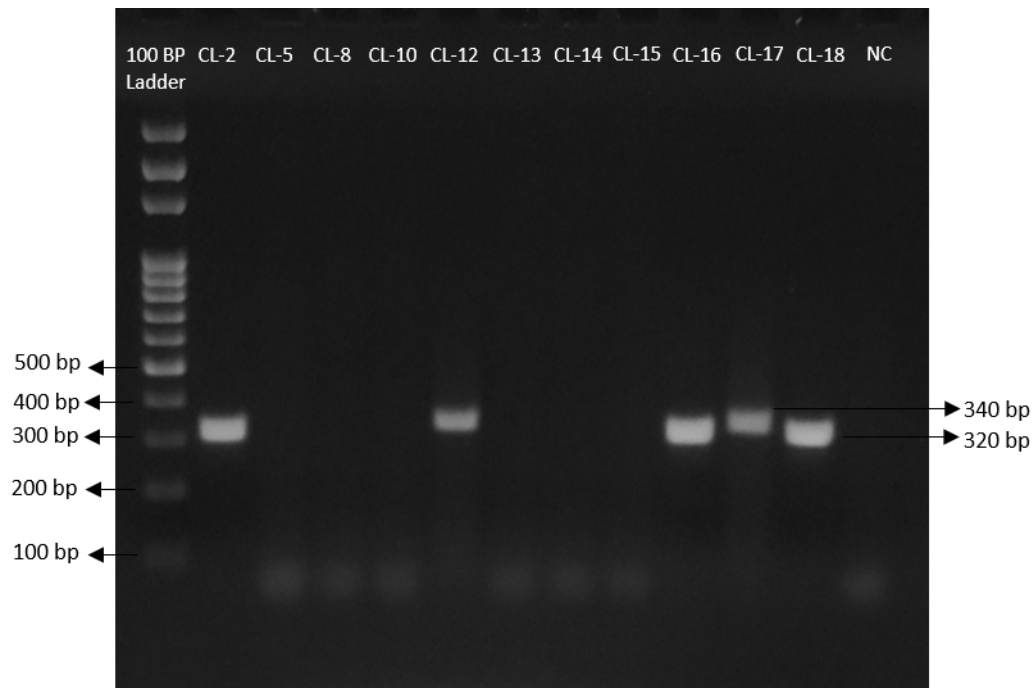
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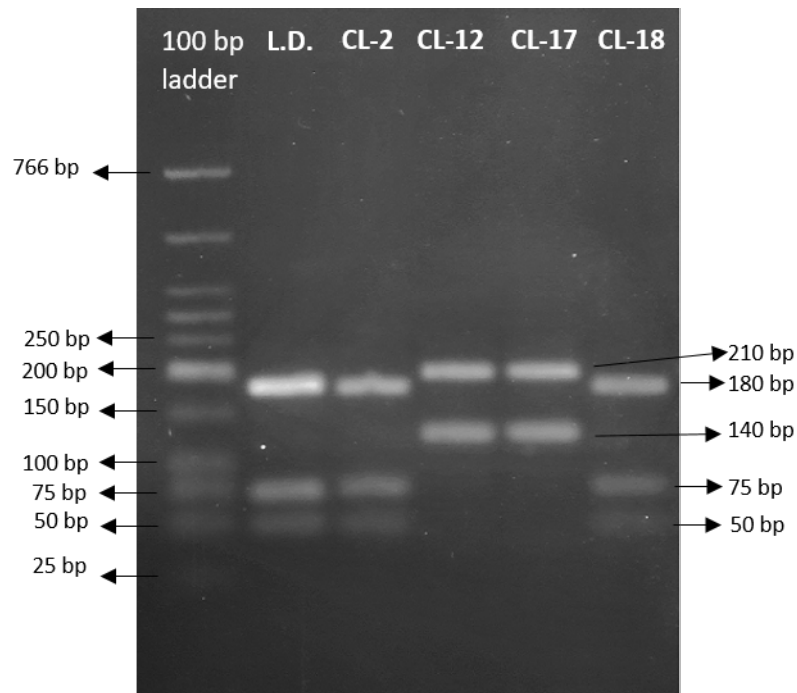
Supplementary Figure S-V: Minicircle kDNA PCR-1 assay of clinical samples. DNA isolated from the skin biopsies run for gel electrophoresis on 1.5% agarose gel and analysis. Lane1- 100 bp ladder, Lane 2- Negative control (NC), Lane 3-8 cutaneous leishmaniasis (CL) samples, Lane-3 (~800bp), Lane- 5&7, (~700bp), Lane 4,6,8- Not visualized



Supplementary Figure S-VI: Minicircle kDNA PCR-2 assay of clinical samples. PCR 1 amplicons of DNA isolated from the skin biopsies run for gel electrophoresis on 1.5% agarose gel and analysis. Lane1- 100 bp ladder, Lane 2- Lane 13, amplified product of skin biopsies of cutaneous leishmaniasis patients. NC, Negative control (No DNA). Lane 6, Lane 9 and Lane 13 (~600 bp of *L. major*), L3 and L11 (~720 bp *L. donovani*)



Supplementary Figure S-VII: ITS1 PCR assay of clinical samples. DNA isolated from the skin biopsies from the lesion was amplified and analyzed on 1.5% agarose gel. Lane1- 100 bp ladder, Lane2- Lane12- amplified product of skin biopsies of cutaneous leishmaniasis patients. NC, Negative control. Lane2, Lane10 and Lane12 (~320 bp of *L. donovani*), Lane6 and Lane11 (~340 bp of *L. major*).



Supplementary Figure S-VIII: ITS1 RFLP assay of ITS1 PCR positive samples. Lane1- 100 bp ladder, Lane2, *L. donovani* (LEM 138), Lane3 and Lane6 (180bp, 75bp and 50bp of *L. donovani*), Lane4 and Lane5 (210 bp and 140 bp of *L. major*).