

Biogeography of *Leptospira* in wild animal communities inhabiting the insular ecosystem of the western Indian Ocean islands and neighboring Africa

Dietrich *et al.* – Supporting Information

Text S1. Animal capture methodology

Animals were captured, manipulated and dispatched in accordance with guidelines accepted by the scientific community and professional mammalogists for the handling of wild mammals¹. For rodents, tenrecs and shrews, animals were trapped using pitfall buckets and baited-live traps put in place during the night and inspected the following morning. Only baited-live traps were used for sampling on La Réunion and in the Seychelles.

Bats were captured at dusk or during the night using mist nets or harp traps placed in proximity to cave entrances, across presumed flight pathways, or near water sources. On several occasions, a butterfly net was used to obtain bats inside day roost sites.

Captured animals were identified based on different external and crano-dental characters or using genetic barcoding². Euthanasia of terrestrial small mammals was performed using cervical dislocation, and for bats with the injection of ketamine or inhalation of isoflurane, followed by cardiac puncture. Dissections were performed using sterile utensils. All tissue samples were immediately conserved in liquid nitrogen, and stored at -80°C after transportation to the laboratory.

Table S1. List and details of samples used in this study. A black cross indicates failed PCR.

Sample ID	Museum or field collection number	Island/Country	Location	Host family or sub-family	Host species	Year	icdA	secY	lipL41	adk	rrs2	Included in the multilocus analysis
6	FMNH 220036	Comoros	Fassi (Grande Comore)	Pteropodidae	<i>Rousettus obliviosus</i>	2011	X	X	X	X	This study	NO
30	FMNH 220056	Comoros	Bazimini (Anjouan)	Miniopteridae	<i>Miniopterus griveaudi</i>	2011	This study	This study	This study	This study	This study	YES
16704	FMNH 220030	Comoros	Fassi (Grande Comore)	Pteropodidae	<i>Rousettus obliviosus</i>	2011	This study	This study	This study	This study	This study	YES
122	FMNH 209278	Madagascar	Toliara	Miniopteridae	<i>Miniopterus mahafaliensis</i>	2009	This study	Dietrich et al. 2014	YES			
135	FMNH 209260	Madagascar	Toliara	Rhinonycteridae	<i>Triaenops menamena</i>	2009	This study	This study	This study	This study	This study	YES
136	UADBA 43229	Madagascar	Toliara	Miniopteridae	<i>Miniopterus griffithsi</i>	2009	This study	Dietrich et al. 2014	YES			
265	FMNH 209265	Madagascar	Toliara	Molossidae	<i>Otomops madagascariensis</i>	2009	This study	This study	This study	This study	This study	YES
266	FMNH 209258	Madagascar	Toliara	Rhinonycteridae	<i>Triaenops menamena</i>	2009	X	This study	X	X	This study	NO
271	FMNH 209276	Madagascar	Toliara	Miniopteridae	<i>Miniopterus mahafaliensis</i>	2009	This study	Dietrich et al. 2014	YES			
272	UADBA SMG-16886	Madagascar	Toliara	Vespertilionidae	<i>Myotis goudotii</i>	2009	This study	Dietrich et al. 2014	YES			
575	UADBA 30869	Madagascar	Ankazomivady	Tenrecidae	<i>Microgale dobsoni</i>	2010	This study	Dietrich et al. 2014	YES			
588	UADBA 30289	Madagascar	Ankazomivady	Tenrecidae	<i>Microgale majori</i>	2010	This study	Dietrich et al. 2014	YES			
590	UADBA 30291	Madagascar	Ankazomivady	Tenrecidae	<i>Microgale longicaudata</i>	2010	This study	Dietrich et al. 2014	YES			
597	UADBA 30850	Madagascar	Ankazomivady	Tenrecidae	<i>Hemicentetes nigriceps</i>	2010	This study	Dietrich et al. 2014	This study	This study	This study	YES
599	UADBA 30296	Madagascar	Ankazomivady	Nesomyinae	<i>Eliurus minor</i>	2010	This study	Dietrich et al. 2014	YES			
610	UADBA 31054	Madagascar	Ankazomivady	Nesomyinae	<i>Eliurus minor</i>	2010	X	X	X	X	This study	NO
620	UADBA 31056	Madagascar	Ankazomivady	Nesomyinae	<i>Eliurus minor</i>	2010	This study	Dietrich et al. 2014	YES			
623	UADBA 31057	Madagascar	Ankazomivady	Nesomyinae	<i>Eliurus minor</i>	2010	This study	Dietrich et al. 2014	YES			
624	UADBA 31058	Madagascar	Ankazomivady	Nesomyinae	<i>Eliurus minor</i>	2010	X	X	X	X	This study	NO
625	UADBA 31059	Madagascar	Ankazomivady	Nesomyinae	<i>Eliurus minor</i>	2010	This study	Dietrich et al. 2014	YES			

Sample ID	Museum or field collection number	Island/Country	Location	Host family or sub-family	Host species	Year	icdA	secY	lipL41	adk	rrs2	Included in the multilocus analysis
626	UADBA 30859	Madagascar	Ankazomivady	Nesomyinae	<i>Eliurus minor</i>	2010	This study	Dietrich <i>et al.</i> 2014	YES			
628	UADBA 30861	Madagascar	Ankazomivady	Nesomyinae	<i>Eliurus minor</i>	2010	This study	Dietrich <i>et al.</i> 2014	YES			
635	UADBA 43253	Madagascar	Fianarantsoa	Miniopteridae	<i>Miniopterus majori</i>	2010	X	Dietrich <i>et al.</i> 2014	NO			
653	UADBA 43265	Madagascar	Fianarantsoa	Miniopteridae	<i>Miniopterus sororculus</i>	2010	X	Dietrich <i>et al.</i> 2014	NO			
685	FMNH 209271	Madagascar	Toliara	Miniopteridae	<i>Miniopterus gleni</i>	2009	This study	Dietrich <i>et al.</i> 2014	YES			
692	UADBA 43211	Madagascar	Toliara	Molossidae	<i>Mormopterus jugularis</i>	2009	X	This study	X	X	This study	NO
693	UADBA 43213	Madagascar	Toliara	Molossidae	<i>Mormopterus jugularis</i>	2009	This study	This study	This study	This study	This study	YES
696	UADBA 43220	Madagascar	Toliara	Miniopteridae	<i>Miniopterus mahafaliensis</i>	2009	This study	Dietrich <i>et al.</i> 2014	YES			
700	UADBA 43225	Madagascar	Toliara	Rhinonycteridae	<i>Triaenops menamena</i>	2009	X	This study	X	X	This study	NO
701	UADBA 43227	Madagascar	Toliara	Rhinonycteridae	<i>Triaenops menamena</i>	2009	This study	This study	This study	This study	This study	YES
702	FMNH 209259	Madagascar	Toliara	Rhinonycteridae	<i>Triaenops menamena</i>	2009	This study	This study	This study	This study	This study	YES
770	UADBA 31595	Madagascar	Lakato	Tenrecidae	<i>Hemicentetes semispinosus</i>	2011	X	Dietrich <i>et al.</i> 2014	X	X	This study	NO
1309	UADBA 50057	Madagascar	Ankazomivady	Tenrecidae	<i>Microgale principula</i>	2011	This study	Dietrich <i>et al.</i> 2014	YES			
1310	UADBA 32095	Madagascar	Ankazomivady	Tenrecidae	<i>Microgale cowani</i>	2011	X	Dietrich <i>et al.</i> 2014	X	X	This study	NO
1326	UADBA 32057	Madagascar	Ankazomivady	Nesomyinae	<i>Eliurus minor</i>	2011	This study	Dietrich <i>et al.</i> 2014	YES			
1329	UADBA 32062	Madagascar	Ankazomivady	Nesomyinae	<i>Eliurus minor</i>	2011	This study	Dietrich <i>et al.</i> 2014	YES			
1335	UADBA 32122	Madagascar	Ankazomivady	Tenrecidae	<i>Microgale majori</i>	2011	This study	Dietrich <i>et al.</i> 2014	YES			
1387	UADBA 50028	Madagascar	Ankazomivady	Nesomyinae	<i>Eliurus minor</i>	2011	This study	Dietrich <i>et al.</i> 2014	YES			
1443	UADBA 32399	Madagascar	Ambovondramanesy	Pteropodidae	<i>Pteropus rufus</i>	2012	X	X	X	X	This study	NO
1453	UADBA 32125	Madagascar	Ankazomivady	Tenrecidae	<i>Microgale principula</i>	2011	This study	Dietrich <i>et al.</i> 2014	YES			
1467	UADBA 32101	Madagascar	Ankazomivady	Tenrecidae	<i>Microgale cowani</i>	2011	This study	Dietrich <i>et al.</i> 2014	YES			
1485	UADBA 32069	Madagascar	Ankazomivady	Nesomyinae	<i>Eliurus minor</i>	2011	This study	Dietrich <i>et al.</i> 2014	YES			

Sample ID	Museum or field collection number	Island/Country	Location	Host family or sub-family	Host species	Year	icdA	secY	lipL41	adk	rrs2	Included in the multilocus analysis
1491	UADBA 50050	Madagascar	Ankazomivady	Nesomyinae	<i>Eliurus minor</i>	2011	This study	Dietrich <i>et al.</i> 2014	YES			
1493	UADBA 32105	Madagascar	Ankazomivady	Nesomyinae	<i>Eliurus minor</i>	2011	This study	Dietrich <i>et al.</i> 2014	YES			
1501	UADBA 32086	Madagascar	Ankazomivady	Nesomyinae	<i>Eliurus minor</i>	2011	This study	Dietrich <i>et al.</i> 2014	YES			
1572	UADBA 50018	Madagascar	Lakato	Tenrecidae	<i>Microgale dobsoni</i>	2012	This study	X	X	X	This study	NO
FMNH 229014	FMNH 229014	Madagascar	Ambohitantely	Tenrecidae	<i>Microgale dobsoni</i>	2014	This study	Lagadec <i>et al.</i> 2016	YES			
UADBA 50367	UADBA 50367	Madagascar	Ambohitantely	Tenrecidae	<i>Microgale dobsoni</i>	2014	This study	Lagadec <i>et al.</i> 2016	YES			
UADBA VS-2474	UADBA VS-2474	Madagascar	Ambohitantely	Tenrecidae	<i>Microgale dobsoni</i>	2014	This study	Lagadec <i>et al.</i> 2016	YES			
152	FMNH 213456	Mauritius	Palma	Molossidae	<i>Mormopterus acetabulosus</i>	2010	This study	This study	This study	This study	This study	YES
160	FMNH 213464	Mauritius	Palma	Molossidae	<i>Mormopterus acetabulosus</i>	2010	This study	This study	This study	This study	This study	YES
161	FMNH 213465	Mauritius	Palma	Molossidae	<i>Mormopterus acetabulosus</i>	2010	This study	This study	This study	This study	This study	YES
162	FMNH 213466	Mauritius	Palma	Molossidae	<i>Mormopterus acetabulosus</i>	2010	This study	This study	This study	This study	This study	YES
165	FMNH 213469	Mauritius	Palma	Molossidae	<i>Mormopterus acetabulosus</i>	2010	This study	This study	This study	This study	This study	YES
183	FMNH 213487	Mauritius	Trois Bras	Molossidae	<i>Mormopterus acetabulosus</i>	2010	This study	This study	This study	This study	This study	YES
188	FMNH 213492	Mauritius	Trois Bras	Molossidae	<i>Mormopterus acetabulosus</i>	2010	This study	This study	This study	This study	This study	YES
195	FMNH 213499	Mauritius	Camp Thorel	Molossidae	<i>Mormopterus acetabulosus</i>	2010	X	This study	X	X	This study	NO
MDI219	MDI 219	Mayotte	Coconi	Muridae	<i>Rattus rattus</i>	2014	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	YES
MDI222	MDI 222	Mayotte	Coconi	Tenrecidae	<i>Tenrec ecaudatus</i>	2014	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	YES
MDI224	MDI 224	Mayotte	Coconi	Tenrecidae	<i>Tenrec ecaudatus</i>	2014	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	YES
MDI247	MDI 247	Mayotte	Kwalé	Muridae	<i>Rattus rattus</i>	2014	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	YES
MDI250	MDI 250	Mayotte	Kwalé	Muridae	<i>Rattus rattus</i>	2014	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	Lagadec <i>et al.</i> 2016	YES

Sample ID	Museum or field collection number	Island/Country	Location	Host family or sub-family	Host species	Year	icdA	secY	lipL41	adk	rrs2	Included in the multilocus analysis
MDI251	MDI 251	Mayotte	Kwalé	Muridae	<i>Rattus rattus</i>	2014	Lagadec et al. 2016	YES				
MDI259	MDI 259	Mayotte	Kwalé	Muridae	<i>Rattus rattus</i>	2014	Lagadec et al. 2016	YES				
MDI260	MDI 260	Mayotte	Kwalé	Muridae	<i>Rattus rattus</i>	2014	Lagadec et al. 2016	YES				
MDI272	MDI 272	Mayotte	Tsoundzou	Tenrecidae	<i>Tenrec ecaudatus</i>	2014	Lagadec et al. 2016	YES				
MDI284	MDI 284	Mayotte	Tsoundzou	Muridae	<i>Rattus rattus</i>	2014	Lagadec et al. 2016	YES				
MDI291	MDI 291	Mayotte	Tsoundzou	Muridae	<i>Rattus rattus</i>	2014	Lagadec et al. 2016	YES				
MDI294	MDI 294	Mayotte	Kwalé	Tenrecidae	<i>Tenrec ecaudatus</i>	2014	Lagadec et al. 2016	YES				
MDI295	MDI 295	Mayotte	Kwalé	Tenrecidae	<i>Tenrec ecaudatus</i>	2014	Lagadec et al. 2016	YES				
MDI306	MDI 306	Mayotte	Vahibe	Tenrecidae	<i>Tenrec ecaudatus</i>	2014	Lagadec et al. 2016	YES				
MDI308	MDI 308	Mayotte	Vahibe	Tenrecidae	<i>Tenrec ecaudatus</i>	2014	Lagadec et al. 2016	YES				
MDI321	MDI 321	Mayotte	Vahibe	Tenrecidae	<i>Tenrec ecaudatus</i>	2014	Lagadec et al. 2016	YES				
1113	FMNH 213651	Mozambique	Nampula	Miniopteridae	<i>Miniopterus mossambicus</i>	2010	This study	YES				
494	JCH 001	La Réunion	Sainte Clotilde	Muridae	<i>Rattus rattus</i>	2011	X	X	X	X	This study	NO
1712	GLM003	La Réunion	Trois Bassins	Muridae	<i>Rattus rattus</i>	2013	This study	YES				
1714	GLM005	La Réunion	Trois Bassins	Muridae	<i>Rattus rattus</i>	2013	This study	YES				
MG354	MG 354	La Réunion	Trois Bassins	Molossidae	<i>Mormopterus francoismoutoui</i>	2013	This study	YES				
MG372	MG 372	La Réunion	Trois Bassins	Molossidae	<i>Mormopterus francoismoutoui</i>	2013	This study	YES				
MG384	MG 384	La Réunion	Trois Bassins	Molossidae	<i>Mormopterus francoismoutoui</i>	2013	This study	YES				
MG386	MG 386	La Réunion	Trois Bassins	Molossidae	<i>Mormopterus francoismoutoui</i>	2013	This study	YES				
337	GR080	Seychelles	Mahé	Muridae	<i>Rattus rattus</i>	2011	This study	YES				
YGO42	YGO42	Seychelles	Fairyland	Pteropodidae	<i>Pteropus seychellensis</i>	2014	X	X	X	X	This study	NO
744	TM 48522	South Africa	Pafuri, Kruger National Park	Pteropodidae	<i>Rousettus aegyptiacus</i>	2010	This study	YES				

Sample ID	Museum or field collection number	Island/Country	Location	Host family or sub-family	Host species	Year	icdA	secY	lipL41	adk	rrs2	Included in the multilocus analysis
846	TM 48630	South Africa	Rocktail Bay, St. Lucia	Vespertilionidae	<i>Glauconycteris variegata</i>	2010	X	X	X	X	This study	NO
927	TM 48574	South Africa	Pafuri, Kruger National Park	Pteropodidae	<i>Rousettus aegyptiacus</i>	2010	X	X	X	X	This study	NO
1019	TM 48652	South Africa	Kgaswane Nature Reserve, Rustenburg	Vespertilionidae	<i>Scotophilus dinganii</i>	2010	This study	YES				
1385	UP 1385	South Africa	Irene Caves, Centurion	Miniopteridae	<i>Miniopterus natalensis</i>	2011	This study	YES				
1388	UP 1388	South Africa	Irene Caves, Centurion	Miniopteridae	<i>Miniopterus natalensis</i>	2011	This study	YES				
1389	UP 1389	South Africa	Irene Caves, Centurion	Miniopteridae	<i>Miniopterus natalensis</i>	2011	X	X	X	X	This study	NO
1471	UP 1471	South Africa	Mahune Cave, Mafefe	Pteropodidae	<i>Rousettus aegyptiacus</i>	2012	X	X	X	X	This study	NO
1483	UP 1483	South Africa	Mahune Cave, Mafefe	Pteropodidae	<i>Rousettus aegyptiacus</i>	2012	X	This study	X	X	This study	NO
1504	UP 1504	South Africa	Mahune Cave, Mafefe	Pteropodidae	<i>Rousettus aegyptiacus</i>	2012	X	This study	X	X	This study	NO
1541	UP 1541	South Africa	Rooiberg, Monate	Nycteridae	<i>Nycterus thebaica</i>	2012	This study	YES				
1542	UP 1542	South Africa	Rooiberg, Monate	Nycteridae	<i>Nycterus thebaica</i>	2012	This study	YES				
1564	UP 1564	South Africa	Mahune Cave, Mafefe	Pteropodidae	<i>Rousettus aegyptiacus</i>	2012	X	X	X	X	This study	NO
1574	UP 1574	South Africa	Mahune Cave, Mafefe	Pteropodidae	<i>Rousettus aegyptiacus</i>	2012	X	This study	X	X	This study	NO
1577	UP 1577	South Africa	Mahune Cave, Mafefe	Pteropodidae	<i>Rousettus aegyptiacus</i>	2012	X	This study	X	X	This study	NO
1717	UP 1717	South Africa	Meletse, Thabazimbi	Miniopteridae	<i>Miniopterus natalensis</i>	2013	This study	YES				
1718	UP 1718	South Africa	Meletse, Thabazimbi	Miniopteridae	<i>Miniopterus natalensis</i>	2013	X	This study	X	X	This study	NO
1755	UP 1755	South Africa	Meletse, Thabazimbi	Miniopteridae	<i>Miniopterus natalensis</i>	2013	This study	YES				
1786	UP 1786	South Africa	Meletse, Thabazimbi	Miniopteridae	<i>Miniopterus natalensis</i>	2013	This study	YES				
1491.2	UP 1491	South Africa	Mahune Cave, Mafefe	Miniopteridae	<i>Miniopterus natalensis</i>	2012	This study	YES				
373	UP 373	Swaziland	Mlawula Nature Reserve	Nycteridae	<i>Nycterus thebaica</i>	2008	X	This study	X	X	X	NO
76	76	Tanzania	not available	Muridae	<i>Mastomys</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				

Sample ID	Museum or field collection number	Island/Country	Location	Host family or sub-family	Host species	Year	icdA	secY	lipL41	adk	rrs2	Included in the multilocus analysis
77	77	Tanzania	not available	Soricidae	<i>Crocidura</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
78	78	Tanzania	not available	Muridae	<i>Mastomys</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
79	79	Tanzania	not available	Muridae	<i>Mastomys</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
80	80	Tanzania	not available	Soricidae	<i>Crocidura</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
81	81	Tanzania	not available	Soricidae	<i>Crocidura</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
155	155	Tanzania	not available	Cricetomyinae	<i>Cricetomys</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
156	156	Tanzania	not available	Cricetomyinae	<i>Cricetomys</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
157	157	Tanzania	not available	Cricetomyinae	<i>Cricetomys</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
159	159	Tanzania	not available	Cricetomyinae	<i>Cricetomys</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
158	158	Tanzania	not available	Cricetomyinae	<i>Cricetomys</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
230	230	Tanzania	not available	Muridae	<i>Mastomys</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
231	231	Tanzania	not available	Soricidae	<i>Crocidura</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
232	232	Tanzania	not available	Soricidae	<i>Crocidura</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
233	233	Tanzania	not available	Cricetomyinae	<i>Cricetomys</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
234	234	Tanzania	not available	Soricidae	<i>Crocidura</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
235	235	Tanzania	not available	Soricidae	<i>Crocidura</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
236	236	Tanzania	not available	Soricidae	<i>Crocidura</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
237	237	Tanzania	not available	Soricidae	<i>Crocidura</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
238	238	Tanzania	not available	Soricidae	<i>Crocidura</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
239	239	Tanzania	not available	Muridae	<i>Mastomys</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
240	240	Tanzania	not available	Soricidae	<i>Crocidura</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				

160.2	160	Tanzania	not available	Cricetomyinae	<i>Cricetomys</i> sp.	not available	Nalam <i>et al.</i> 2010	YES				
-------	-----	----------	---------------	---------------	-----------------------	---------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	-----

Table S2. List of primers and conditions used for the amplification of the five *Leptospira* loci. Primers previously published by Ahmed *et al.* (2006) and Dietrich *et al.* (2014) are marked with an asterisk *¹ and *², respectively.

Gene	Primers	Sequence (5' – 3')	T° annealing
<i>rrs2</i>	rrs2F* ¹	CATGCAAGTCAAGCGGAGTA	58°C
	rrs2R* ¹	AGTTGAGCCCGCAGTTTC	
	rrs2Fd* ²	CATGCAAGTCRAGCGRAGTA	54°C
	rrs2Rd* ²	AGTTRAGCYCRCAGTTTC	
<i>lipL41</i>	lipL41F* ¹	TAGGAAATTGCGCAGCTACA	58°C
	lipL41R* ¹	GCATCGAGAGGAATTAAACATCA	
	or lipL41Rd* ²	GCATCGAGAGGRATYARCATCA	or 54°C
	lipL41F3* ²	GTCGATGTAGAATATCCGGTATT	
	lipL41R3* ²	ACGATTCTCGTAACCTTCTTG	52°C
<i>icdA</i>	lipL41F4d* ²	GATGTAGRATATCCRGTATT	
	lipL41R5d* ²	GGAGAAAGTCTRCCYTTIGATATA	50°C
	icdAF* ¹	ATCTCCGTTGCACTTTGC	58°C
	icdAR* ¹	ACCATCATCATCGTCCA	
<i>icdA</i>	icdAF3d	TGGAYTATTATGATTAGGCGTGG	
	icdAR2	CTTTTTGAGATCTCCGGCTTT	54°C
	or icdAR3d	GTGTCYTTGTMGCRAACCAA	
<i>adk</i>	adkF* ¹	GGGCTGGAAAAGGTACACAA	58°C
	adkR* ¹	ACGCAAGCTCCTTTGAATC	
	adkFd* ²	GGGCTGGAAAAGGYACRCAA	56°C
	or adkF3d* ²	CCTCAGATTCTYACRGGGMATA	or 58°C
	adkR3d* ²	GCCACTCTACRCAAGCTCC	
<i>secY</i>	adkF6d* ²	GGYTTTCTATYCCTCAGATTTC	50°C
	adkR5d* ²	CCGACGCCRTTYACTTBAGA	
	secYF* ¹	ATGCCGATCATTGGCTTC	58°C
	secYR* ¹	CCGTCCCTTAATTAGACTTCTTC	
<i>secY</i>	secYFd* ²	ATGCCGATCATYTTYGCTTC	52°C
	secYR3* ²	TTCATGAAGCCTTCATAATTCTCA	
	secYF4* ²	GCTTCTCCTTGATCCTGTTTC	52°C
	secR4d* ²	TTCATRAAGCCTTCRTAATTCTCA	

Amplifications were performed with 2 µl of cDNA in a 24 µl reaction mixture containing 12.5 µl of GoTaq Hot Start Green Master Mix 2X (Promega, Madison, WI), 0.5 µl (10 µM) of each primer and 10.5 µl of RNAase free water. The PCR conditions consisted of an initial denaturation step at 95°C for 5 min, followed by 45 cycles of denaturation at 94°C for 30 s, annealing between 50-58°C for 30 s, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min. For the *adk* gene, we performed a second semi-nested PCR (primers adkF3d and adkR3d) for samples exhibiting weak detection signals.

Table S3. Details of the 47 reference strains used in the phylogenetic analyses. All these strains were used in the *secY* (Figure S1) and *rrs2* (Figure S2) phylogenies. The strains

from *L. santarosai* and *L. noguchi* were not included in the multilocus phylogeny (Figure 3), as these *Leptospira* species were not identified in our samples. In addition, four samples (denoted with an asterisk) were not included in the multilocus phylogeny, as the *icdA* gene was not available for these samples.

Tree ID	Label	Geographic origin	Host species	Reference
<i>Leptospira borgpetersenii</i>				
Lb2	208-Ref8	Italy	Unknown	3
Lb6	212-UK22	Indonesia	Unknown	3
Lb10	221-QSL91	Kenya	Human	3
Lb11	222-QSL104	Bulgaria	Human	3
Lb12	223-QSL108	Kenya	Human	3
Lb15	226-QSL131	Russia	Human	3
Lb16	227-QSL132	Tunisia	Pig	3
Lb17	228-QSL134	China	Pig	3
Lb28	241-R54	Ireland	Dog	3
Lb29	242-R55	Ireland	Pygmy shrew	3
Lb30	243-R56	Ireland	Gemsbok	3
Lb32	246-R61	Portugal	<i>Mus musculus</i>	3
Lb38*	st. 200701204	Mayotte	Human	4
<i>Leptospira mayottensis</i>				
Lm1*	st. 200901116	Mayotte	Human	4
<i>Leptospira interrogans</i>				
Li2*	st. 200901482	Mayotte	Human	4
Li3	31-QSL27	Philippines	Rat	3
Li4	42-QSL51	Malaysia	Rat	3
Li5	44-QSL55	Korea	Mouse	3
Li6	62-R7	Brazil	Rat	3
Li7	68-R17	Brazil	Cattle	3
Li8	69-R17	United Kingdom	Mouse	3
Li9	40-QSL47	Peru	Opossum	3
Li10	39-QSL46	Malaysia	Human	3
Li11	56-QSL105	Sri Lanka	Human	3
Li13	22-QSL3	New Guinea	Bandicoot	3
Li14	54-QSL95	Australia	Human	3
<i>Leptospira santarosai</i>				
Ls1	182-QSL93	Trinidad	Human	3
Ls2	184-QSL100	Brazil	Rat	3
Ls3	183-QSL99	Peru	Opossum	3
Ls4	202-R21	Costa Rica	Human	3
Ls5	197-R1	Brazil	Opossum	3
Ls6	200-R14	Brazil	Capybara	3
Ls7	189-QSL117	USA	Opossum	3
Ls8	192-QSL121	Panama	Spiny Rat	3
<i>Leptospira kirschneri</i>				
Lk1	146-QSL14	Kenya	Rat	3
Lk2	145-QSL12	Russia	Hedgehog	3
Lk3	143-QSL9	Bulgaria	Human	3
Lk4	152-QSL59	Zimbabwe	Cattle	3
Lk5	151-QSL53	Jamaica	Rat	3
Lk6	150-QSL44	Ghana	Human	3
Lk7	162-R58	Ireland	Mouse	3
Lk9*	st. 200801925	Mayotte	Human	4
<i>Leptospira noguchi</i>				
Ln1	257-QSL82	Panama	Opossum	3
Ln2	254-QSL20	Argentina	Armadillo	3

Ln3	255-QSL24	Panama	Rat	3
Ln4	256-QSL74	USA	Nutria	3
Ln5	253-QSL13	USA	Human	3

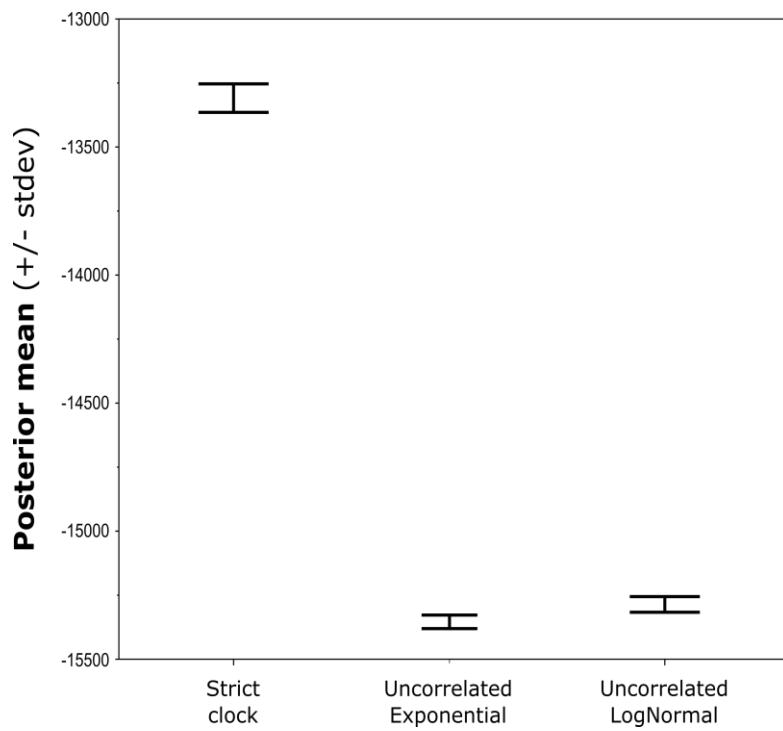
Table S4. Results of the hierarchical AMOVA analysis. * p < 0.05, *** p < 0.001.

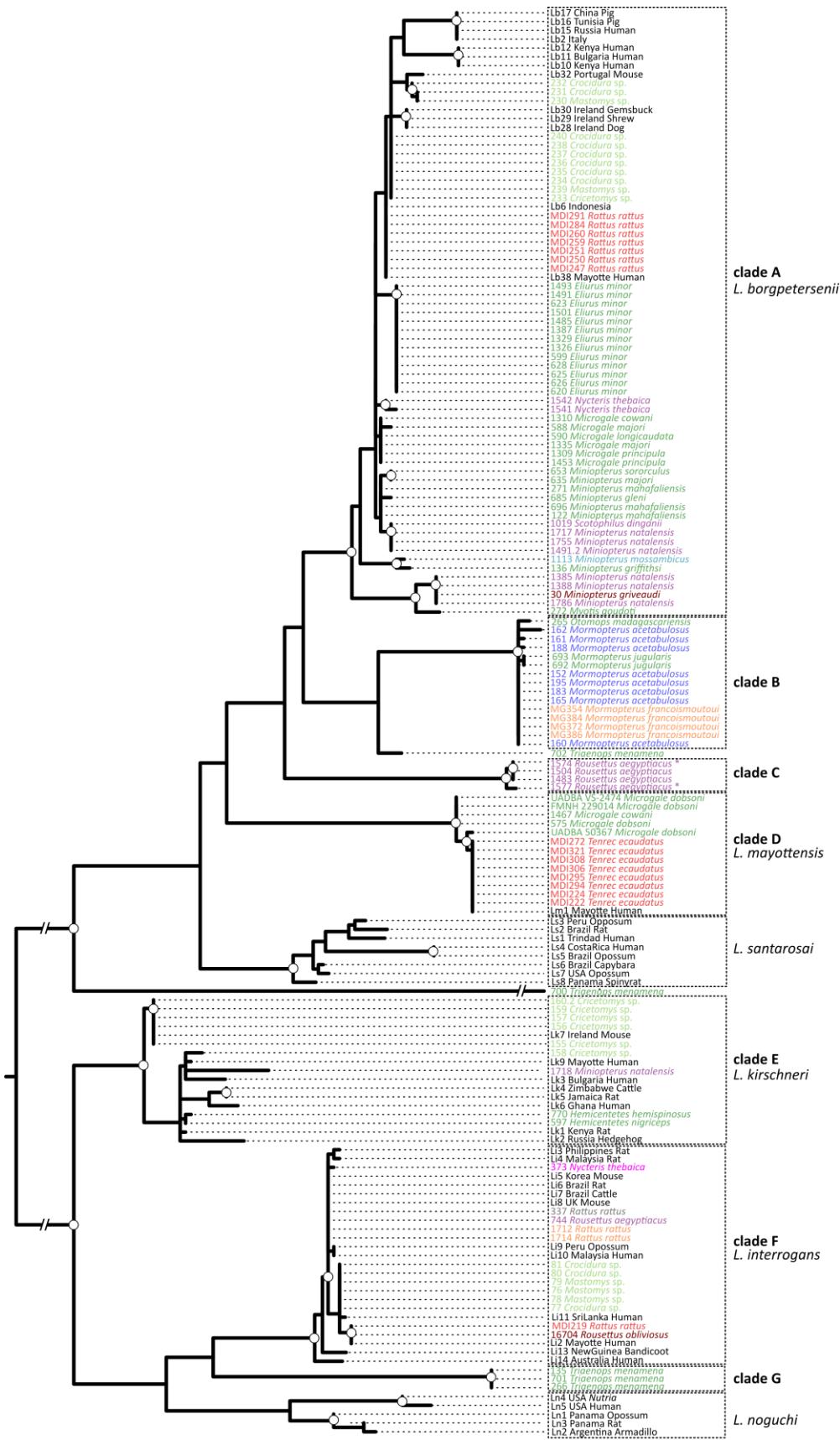
	d.f.	Φ statistics	% total variation
<i>Geographic structure (bats only)</i>			
Among islands/countries (South Africa, Madagascar, Mauritius, Comoros, La Réunion)	4	$\Phi_{CT} = 0.067$	6.69
Among host species within islands/countries	8	$\Phi_{SC} = 0.636^*$	59.38
Within populations	21	$\Phi_{ST} = 0.661^{***}$	33.93
<i>Geographic structure (terrestrial small mammals only)</i>			
Among islands/countries (Madagascar, La Réunion, Tanzania, Mayotte)	3	$\Phi_{CT} = 0.062$	6.22
Among host species within islands/countries	5	$\Phi_{SC} = 0.580^{***}$	54.33
Within populations	56	$\Phi_{ST} = 0.610^{***}$	39.45
<i>Structure by host groups (bats vs. terrestrial small mammals) in Madagascar only</i>			
Among host groups	1	$\Phi_{CT} = -0.087$	8.67
Among host species within host groups	6	$\Phi_{SC} = 0.577^{***}$	62.71
Within populations	27	$\Phi_{ST} = 0.540^{***}$	45.97

Table S5. Haplotype diversity of *Leptospira* in the western Indian Ocean islands and neighboring Africa. Haplotype diversity was not calculated for Mozambique, Swaziland and Seychelles, as either no sample or only one sample was available for these locations. “tsm” refer to terrestrial small mammals.

	adk	icdA	lipL41	rrs2	secY
<u>Locations</u>					
South Africa	0.778	0.533	0.756	0.733	0.867
Madagascar	0.851	0.794	0.815	0.530	0.845
Mauritius	0	0	0.905	0	0.952
Comoros	1	1	1	1	1
La Réunion	0.533	0.533	0.533	0.533	0.533
Tanzania	0.798	0.897	0.877	0.826	0.787
<u>Host groups</u>					
bats	0.889	0.854	0.938	0.822	0.953
tsm	0.871	0.863	0.882	0.693	0.880

Figure S1. Mean posterior values obtained with BEAST for the strict clock, the uncorrelated relaxed lognormal and exponential clocks.





0.2

Figure S2. Maximum likelihood phylogenetic tree based on the analysis of *secY* gene fragment. Taxa are colored according to the location (see Figure 1). Taxa with an asterisk (*) were suspected of co-infection. Reference sequences are indicated in black. Bootstrap values higher than 75% are represented by white circles. The boxes with dashed lines correspond to the major genetic clades. The tree was constructed with a TIM3+I+G model.

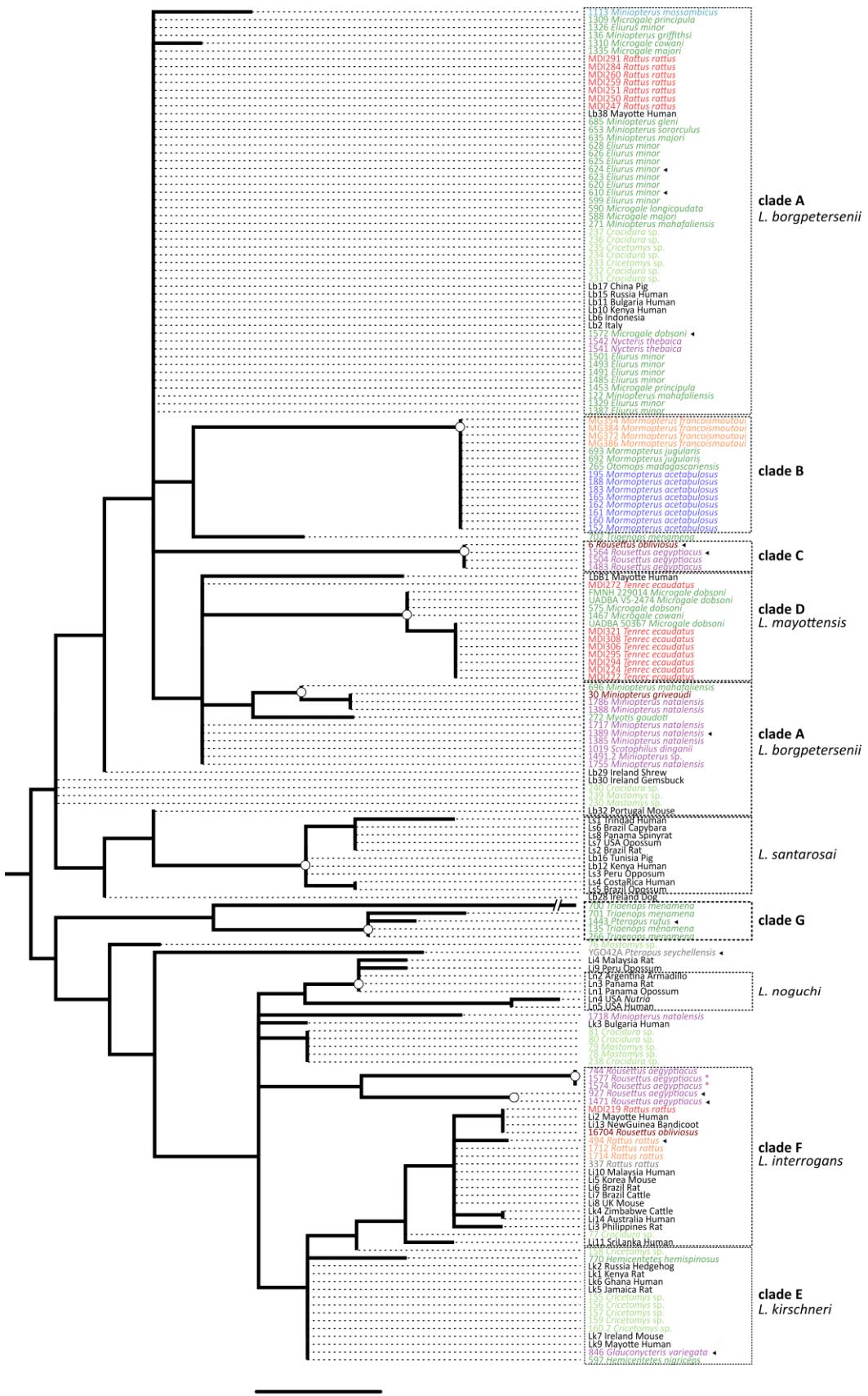


Figure S3. Maximum likelihood phylogenetic tree based on the analysis of *rrs2* gene fragment. Taxa are colored according to the location (see Figure 1). The 12 sequences added to the *rrs2* phylogeny, compared to the *secY* phylogeny, are denoted by a black triangle (♦). Taxa with an asterisk (*) were suspected of co-infection. Reference sequences are indicated in black. Clades identified with the *secY* phylogeny (figure S1) are indicated on the tree. Bootstrap values higher than 75% are represented by white circles. The tree was constructed with a TIM3+I+G model.

References

- 1 Sikes RS, The Animal Care and use C of the AS of M. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *J Mammal* 2016; **97**: 663–688.
- 2 Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol* 1994; **3**: 294–299.
- 3 Ahmed N, Devi SM, Valverde MDLÁ, Vijayachari P, Machang RS, Ellis WA *et al.* Multilocus sequence typing method for identification and genotypic classification of pathogenic *Leptospira* species. *Ann Clin Microbiol Antimicrob* 2006; **5**: 28.
- 4 Bourhy P, Collet L, Lernout T, Zinini F, Hartskeerl RA, van der Linden H *et al.* Human *Leptospira* isolates circulating in Mayotte (Indian Ocean) have unique serological and molecular features. *J Clinical Microbiol* 2012; **50**: 307–11.