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DESCRIPTION, BIOLOGY, AND MEDICAL SIGNIFICANCE OF *LEISHMANIA* (*MUNDINIA*) *CHANCEI* N. SP. (KINETOPLASTEA: TRYPANOSOMATIDAE) FROM GHANA AND *LEISHMANIA* (*MUNDINIA*) *PROCAVIENSIS* N. SP. (KINETOPLASTEA: TRYPANOSOMATIDAE) FROM NAMIBIA

Godwin Kwakye-Nuako[1], Mba-Tihssommah Mosore[2], Daniel Boakye[3], and Paul A. Bates[4]

[1] Department of Biomedical Sciences, University of Cape Coast, Cape Coast, Ghana.
[2] U.S. Naval Medical Research Unit No. 3, Ghana Detachment, Accra, Ghana.
[3] Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana.
[4] Division of Biomedical and Life Sciences, Lancaster University, Lancaster, LA1 4YW, U.K.
Correspondence should be sent to Paul Bates (https://orcid.org/0000-0001-6861-5421) at: *p.bates@lancaster.ac.uk*

KEY WORDS

Leishmania (*Mundinia*) *chancei* n. sp.
Leishmania (*Mundinia*) *procaviensis* n. sp.
Ghana
Namibia
Kinetoplastea
Trypanosomatidae

ABSTRACT

Genetic and phylogenetic analysis was performed on 2 isolates of *Leishmania* using DNA sequence data from the RNA polymerase II large subunit gene and the ribosomal protein L23a intergenic sequence. This showed the isolates to represent 2 new species within the subgenus *Leishmania* (*Mundinia*). The addition of *Leishmania* (*Mundinia*) *chancei* and *Leishmania* (*Mundinia*) *procaviensis* creates a total of 6 named species to date within this recently described subgenus of parasitic protozoa, containing both human pathogens and nonpathogens. Their widespread geographical distribution, basal phylogenetic position within the genus *Leishmania,* and probable non–sand fly vectors make these *L.* (*Mundinia*) species of significant medical and biological interest.

Leishmaniasis is a vector-borne disease caused by parasitic protozoa of the genus *Leishmania*. These include various species of medical significance, responsible for approximately 1 million new cases and tens of thousands of deaths per annum (Burza et al., 2018). Ongoing research on the genus *Leishmania* has recently seen the emergence of a new subgenus of parasites, the *Leishmania* (*Mundinia*) (Espinosa et al., 2018; Cotton, 2017) to sit alongside the other 3 established subgenera, *Leishmania* (*Leishmania*), *Leishmania* (*Viannia*), and *Leishmania* (*Sauroleishmania*) (Lainson and Shaw, 1987; Akhoundi et al., 2016). The parasites in the subgenus *L.* (*Sauroleishmania*) infect reptiles and, although they are not of direct medical importance, they are of comparative interest, as they have been secondarily derived from mammal-infecting species (Croan et al., 1997; Noyes et al., 1998; Raymond et al., 2012; Coughlan et al., 2017). The majority of reported human infections arise from parasites in the subgenera *L.* (*Leishmania*) and *L.* (*Viannia*). However, the new subgenus *L.* (*Mundinia*) also contains human pathogens, *Leishmania* (*Mundinia*) *martiniquensis* (Desbois et al., 2014) and *Leishmania* (*Mundinia*) *orientalis* (Jariyapan et al., 2018), and another human pathogen is 1 of the new species described here, *Leishmania* (*Mundinia*) *chancei*. Species nonpathogenic to humans in subgenus *Mundinia* include *Leishmania* (*Mundinia*) *enriettii* (Muniz and Medina, 1948) found in guinea pigs and *Leishmania* (*Mundinia*) *macropodum* (Barratt et al., 2017) found in macropods, and the other new species described here, *Leishmania* (*Mundinia*) *procaviensis*. Phylogenetic analysis indicates that *L.* (*Mundinia*) sit at the base of the *Leishmania* clade and are the earliest branching subgenus (Butenko et al., 2019). This may partially explain their wide geographical distribution and variation in mammalian hosts, and possibly their transmission by non–sand fly vectors (Chanmol et al., 2019b, Bečvár et al., 2021). The subgenus *L.* (*Mundinia*) is therefore of both biological and medical significance within the genus *Leishmania*. Here we describe 2 new species in the subgenus, *Leishmania* (*Mundinia*) *chancei* n. sp. from Ghana and *Leishmania* (*Mundinia*) *procaviensis* n. sp. from Namibia.

MATERIALS AND METHODS

Isolation of organisms

Leishmania strain MHOM/GH/2012/GH5;LV757, here named as the type strain of *L. chancei*, was isolated from a single cutaneous lesion on the arm of a 35-yr-old female farmer in

Version of Record, first published online with fixed content and layout, in compliance with ICZN Arts. 8.1.3.2, 8.5, and 21.8.2 as amended, 2012. ZooBank publication registration: urn:lsid:zoobank.org:pub: FD2326DC-8E64-45D3-988B-4F2B19DE66C8.

Dodome-Doglome, Ho District, Ghana in 2012 as previously described (Kwakye-Nuako et al., 2015). *Leishmania* strain MPRV/NA/1975/253;LV425, here named as the type strain of *L. procaviensis*, was isolated from the nose of a rock hyrax captured near Keetmanshoop, Namibia by Grové and colleagues in 1975, as previously described (Grové and Ledger, 1975). Promastigotes derived from the latter isolate were kept in a cryobank at the Liverpool School of Tropical Medicine maintained by Dr. Michael Chance, then transferred to Lancaster University in 2009 by Professor Paul Bates.

Culture and morphology

Promastigote forms of *L. chancei* and *L. procaviensis* were cultured in vitro and their morphology was assessed by light microscopy, as previously described (Jariyapan et al., 2018; Chanmol et al., 2019a).

DNA isolation, polymerase chain reaction (PCR), and sequencing

Parasite DNA was isolated as previously described (Dougall et al., 2011). PCR amplification of the RNA polymerase II large subunit gene (RNA PolII) with S1/S2 and S3/S4 primers was performed as described (Pothirat et al., 2014) and of the ribosomal protein L23a intergenic sequence (RPL23a) with BN1/BN2 primers as described (Chiewchanvit et al., 2015). Products were directly sequenced using commercial services (Source Bioscience, Nottingham, U.K.) and quality assurance of chromatograms performed with Chromas Lite (http://technelysium.com.au/) (Jariyapan et al., 2018). Forward and reverse complement sequences were aligned with each other and existing data using Clustal Omega (https://www.ebi.ac.uk/tools/msa/clustalo/) (Jariyapan et al., 2018) to derive finalized sequence data.

Phylogenetic analysis

Leishmania species sequence data were analyzed using *Porcisia hertigi* (formerly *Leishmania hertigi*) as a closely related outgroup. Alignment and tree building was performed using version 11 of the Molecular Evolutionary Genetics Analysis (MEGA) software (Tamura et al., 2021). Sequences were aligned using Clustal W and the best model of sequence evolution for each data set was determined using maximum likelihood. The models with the lowest Bayesian information criteria were used in each case. For the RNA PolII data set, this was the Tamura-Nei model with invariant sites; for the RPL23a data set, this was the Hasegawa-Kishino-Yano model with a Gamma distribution for nonuniformity of evolutionary rates. Maximum likelihood, neighbor-joining, minimum evolution, and maximum parsimony trees were generated for each data set, using bootstrapping with 1,000 replicates to test the statistical robustness of the optimum tree in each case.

RESULTS

Sequences of 1,200 nucleotides for RNA PolII and 488 nucleotides for RPL23a from *L. chancei* and *L. procaviensis* were analyzed by BLAST searches, which showed the sequences differed from those for all other previously named *Leishmania* species deposited within the DDBJ/ENA/GenBank and TriTrypDB databases. The new RNA PolII sequences were most similar to those from *Leishmania enriettii*, with 98.4% and 98.5% identity for *L. chancei* and *L. procaviensis*, respectively, whereas the new RPL23a sequences were most similar to those from *Leishmania orientalis*, with 98.5% and 95.5% identity for *L. chancei* and *L. procaviensis*, respectively. These results indicated a probable classification within the subgenus *L.* (*Mundinia*).

The genetic distances revealed by the new sequence data were examined by making various pairwise comparisons. Regarding RNA PolII, the differences of 1.6% between *L. chancei* and *L. enriettii*, 1.5% between *L. procaviensis* and *L. enriettii*, and 1.6% between *L. chancei* and *L. procaviensis* are all greater than the differences of 0.6% between *Leishmania donovani* and *Leishmania infantum* and 0.6% between *Leishmania amazonensis* and *Leishmania mexicana*. The 4 species in these latter 2 pairwise comparisons are well-established existing species of *Leishmania*. Similarly, for RPL23a, the differences of 1.5% between *L. chancei* and *L. orientalis*, 4.7% between *L. procaviensis* and *L. orientalis*, and 4.7% between *L. chancei* and *L. procaviensis* are all greater than the differences of 0.4% between *L. donovani* and *L. infantum* and 1.1% between *L. amazonensis* and *L. mexicana*. These analyses support the designation of the isolates of *L. chancei* and *L. procaviensis* as new species.

To investigate this further, 4 different types of phylogenetic trees were generated, maximum likelihood (ML), neighbor-joining (NJ), minimum evolution (ME), and maximum parsimony (MP) trees, with both RNA PolII and RPL23a data sets, and in all 8 combinations the 2 new species clustered with the others in the subgenus *L.* (*Mundinia*) (Figs. 1, 2). In each case *L. chancei* and *L. procaviensis* clustered with *L. enriettii* and *L. orientalis* in a subgroup of these 4 species, in most trees with the other 2 *L.* (*Mundinia*) species, *L. martiniquensis* and *L. macropodum*, as a sister group, but always these 6 species were within a well-defined *L.* (*Mundinia*) clade with 91–100% bootstrap support (depending on the tree method used). The other 3 subgenera of *Leishmania*, *L.* (*Leishmania*), *L.* (*Viannia*), and *L.* (*Sauroleishmania*) were also resolved with high bootstrap support (87–100%), consistent with previous analyses (Butenko et al., 2019).

DESCRIPTIONS

Leishmania (*Mundinia*) *chancei* n. sp.

Morphology: Characteristic morphotypes of the genus *Leishmania*; amastigotes 1–2 μm in width and 2–4 μm in length; promastigotes of various sizes with body length ranging between 5 and 15 μm and motile with a free anterior flagellum of variable length.

Taxonomic summary

Class Kinetoplastea Honigberg, 1963 emend., Vickerman, 1976
Order Trypanosomatida Kent, 1880 stat. nov. Hollande, 1952
Family Trypanosomatidae Doflein, 1951
Genus Leishmania Ross, 1903
Subgenus *L.* (*Mundinia*) Shaw, Camargo and Teixeira 2016

Type host: Homo sapiens.

Type locality: Ho Municipal District (6°36′42.9984″N, 0°28′13.0008″E), Volta Region, Ghana.

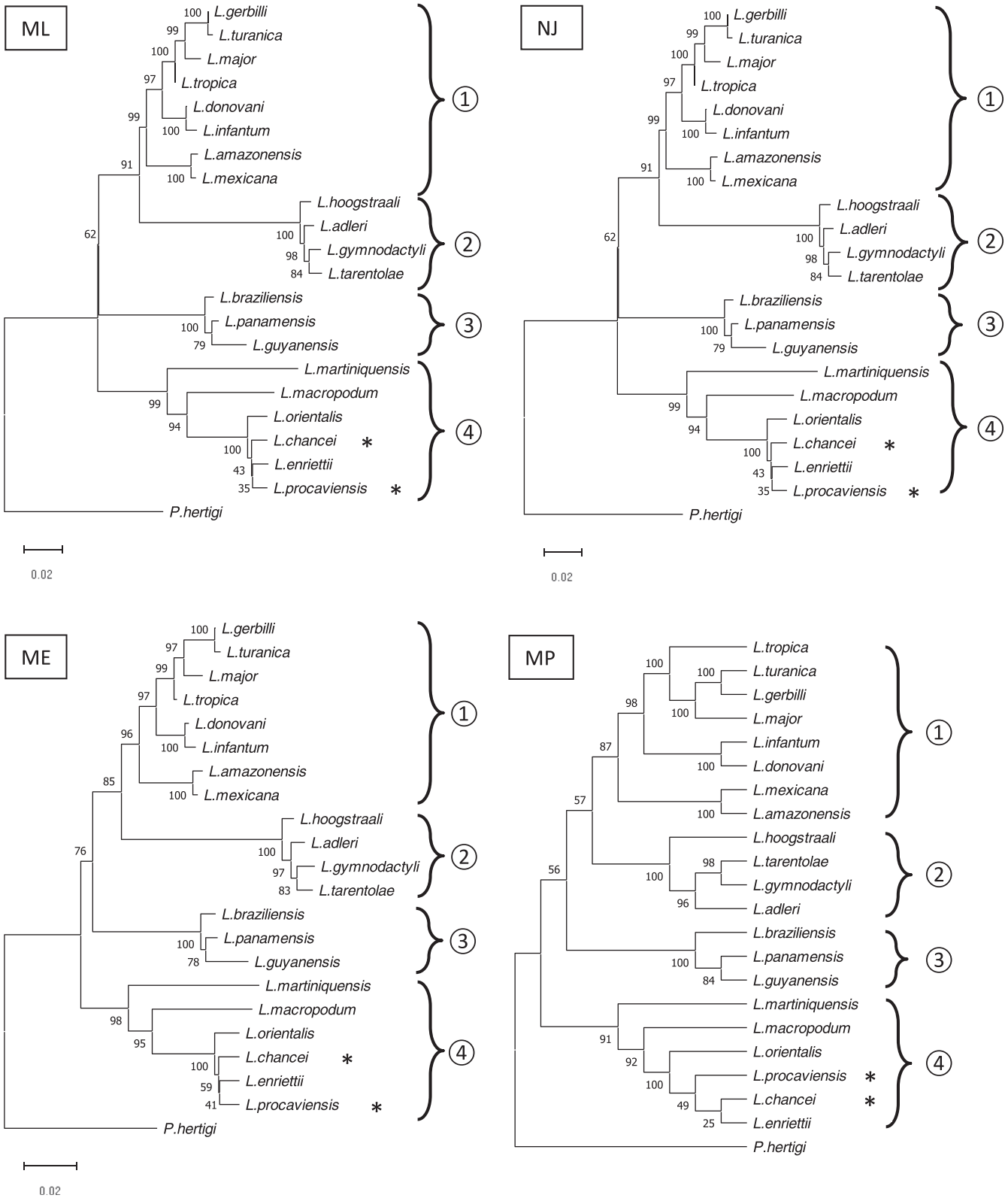

Figure 1. Phylogenetic analysis of RNA PolII sequences from *Leishmania* species using different algorithms: maximum likelihood (ML), neighbor-joining (NJ), minimum evolution (ME), or maximum parsimony (MP). Numbers at nodes were derived by bootstrapping with 1,000 replicates. The 4 subgenera of *Leishmania* are indicated with numbered brackets: 1. *L.* (*Leishmania*); 2. *Leishmania* (*Sauroleishmania);* 3. *Leishmania* (*Viannia*); and 4. *L.* (*Mundinia*). The positions of the 2 new species are indicated with asterisks. Accession numbers for each species are given in Table I.

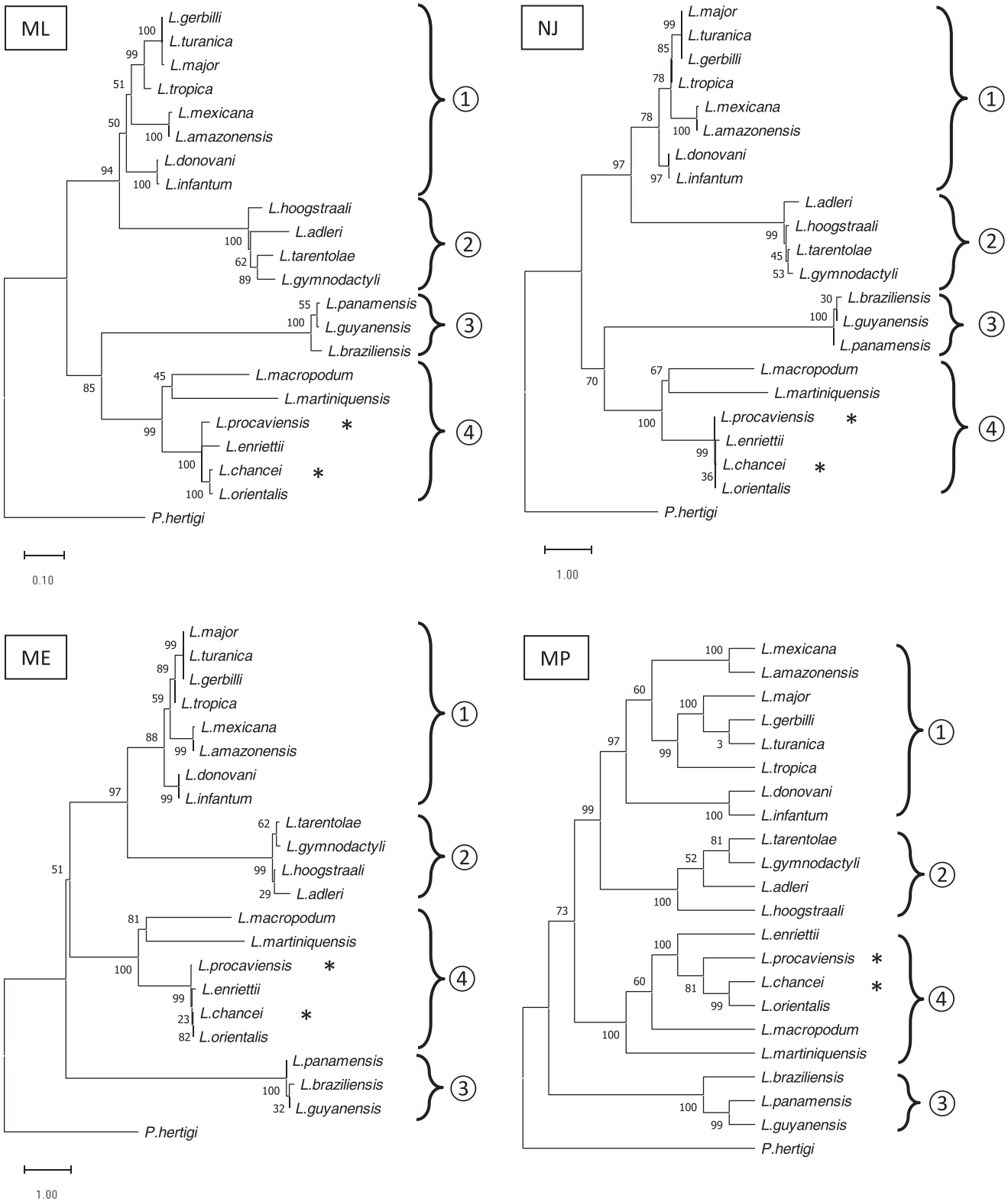

Figure 2. Phylogenetic analysis of RPL23a sequences from *Leishmania* species using different algorithms: maximum likelihood (ML), neighbor-joining (NJ), minimum evolution (ME), or maximum parsimony (MP). Numbers at nodes were derived by bootstrapping with 1,000 replicates. The 4 subgenera of *Leishmania* are indicated with numbered brackets: 1. *L.* (*Leishmania*); 2. *L.* (*Sauroleishmania*); 3. *L.* (*Viannia*); and 4. *L.* (*Mundinia*). The positions of the 2 new species are indicated with asterisks. Accession numbers for each species are given in Table I.

Table I. List of species used for phylogenetic analysis together with the World Health Organization codes of the isolates and their Genbank/ENA/DDJB accession numbers.

Species	World Health Organization code	Accession number for RPL23a IGS	World Health Organization code	Accession number for RNAPolII
Leishmania adleri	RLIZ/KE/XXXX/LV30	KP025941	RLIZ/KE/XXXX/LV30	AF009153
Leishmania amazonensis	MHOM/BR/1997/M2269	FR693777	MHOM/BR/1973/LV78	AF009154
Leishmania braziliensis	MHOM/BR/83/LTB300	FR693776	MHOM/VE/XXXX/LBV	AF009155
Leishmania chancei	MHOM/GH/2012/GH5;LV757	KP006691	MHOM/GH/2012/GH5;LV757	KP054394
Leishmania donovani	MHOM/ET/67/HU3;L82;LV9	FR693771	MHOM/IN/1980/DD8	AF009157
Leishmania enriettii	MCAV/BR/1945/LV90	FR693773	MCAV/BR/1945/LV90	AF151727
Leishmania gerbilli	MRHO/CN/1960/Gerbilli;LON-25	FR693785	MRHO/CN/1960/Gerbilli;LON-25	AJ304947
Leishmania guyanensis	MHOM/BR/75/M4147	FR693784	MHOM/SR/1987/TRUUSI	AJ304949
Leishmania gymnodactyli	RGEC/SU/XXXX/LV247	FR693781	RGEC/SU/XXXX/LV247	AF009159
Leishmania hoogstraali	RLIZ/SD/XXXX/LV31	FR693782	RLIZ/SD/XXXX/LV31	AF009162
Leishmania infantum	MCAN/ES/1998/LEM-935;JPCM5	FR796438	MCAN/ES/1998/LEM-935;JPCM5	XM_001467548
Leishmania macropodum	MMAC/AU/2004/AM-2004;Roo1	FR693774	MMAC/AU/2004/AM-2004;Roo1	HM775497
Leishmania major	MHOM/IL/1980/Friedlin;FV1	FR796402	MHOM/IL/1980/Friedlin;FV1	XM_001685196
Leishmania martiniquensis	MHOM/MQ/1992/MAR1; LEM2494	KP025945	MHOM/MQ/1992/MAR1; LEM2494	KM820663
Leishmania mexicana	MNYC/BZ/1962/M379	FR693769	MNYC/BZ/1962/M379	AF009164
Leishmania orientalis	MHOM/TH/2014/LSCM4	MG731231	MHOM/TH/2014/LSCM4	MG731232
Leishmania panamensis	MHOM/PA/XX/53A;LV145	FR693783	MHOM/PA/XXXX/CIDEP004	AF009165
Leishmania procaviensis	MPRV/NA/1975/253;LV425	OP715862	MPRV/NA/1975/253;LV425	OP715860
Leishmania tarentolae	RTAR/SE/67/G10/LV108	FR693780	RTAR/DZ/1939/LV414	AF009166
Leishmania tropica	MHOM/IR/60/LV357	FR693778	MHOM/SU/1958/Strain-OD	AF009167
Leishmania turanica	MRHO/SU/83/MARZ-051	FR693786	IRAI/TR/1991/M87	AJ304946
Porcisia hertigi	MCOE/PA/1965/C-8;LV42	FR693775	MCOE/PA/1965/C-8;LV42	AF009161

Type material: Hapantotypes, cryopreserved promastigotes stored in liquid nitrogen at the Division of Biomedical and Life Sciences, Lancaster University, U.K. (accession LV757).

Strain designation: MHOM/GH/2012/GH5;LV757.

Homologous strains: MHOM/GH/2012/GH10;LV758, MHOM/GH/2012/GH11;LV759 (Kwakye-Nuako et al., 2015).

Vector: Unknown.

Reservoir(s): Unknown.

Representative DNA sequences: A chromosome-scale full genome assembly and annotations are available under GenBank GCA_017918215.1 (Almutairi et al., 2021). The master record for the whole genome-sequencing project is available at JAFJZN000000000. The new species was characterized using molecular techniques revealing a genetically distinct parasite within *Leishmania* (*Mundinia*). Diagnostic sequences and their accession numbers include RPL23a intergenic sequence (KP006691), RNA polymerase II large subunit (KP054394), ribosomal RNA internal transcribed spacer-1 (ITS-1) (KP006688), and heat shock protein-70 (HSP-70) (MG731234).

ZooBank registration: urn:lsid:zoobank.org:act:8DCB5295-508F-49CB-A2B2-3364BF1A2441.

Etymology: The species is named in honor of Dr. Michael Chance, distinguished leishmaniasis researcher and pioneer of multilocus isoenzyme electrophoresis for the taxonomy of *Leishmania* species.

Growth in vitro: Clinical isolates initially cultured in Sloppy Evans (Kwakye-Nuako et al., 2015), thereafter grown at 26 C as promastigotes in Schneider's insect medium supplemented with 20% (v/v) fetal bovine serum and 25 μg/ml gentamicin sulfate, or in M199 medium with Hank's balanced salt solution supplemented with 10% (v/v) fetal bovine serum, 2% (v/v) healthy human urine, 1% (v/v) Basal Medium Eagle vitamins and 25 μg/ml gentamicin sulfate. Promastigotes were subpassaged to fresh medium every 4–7 days to maintain the growth and viability of the parasites.

Pathology: Cutaneous lesions in human patients present as typical cutaneous leishmaniasis (Kwakye-Nuako et al., 2015).

Remarks

Leishmania chancei is distinguished from other *Leishmania* species, including *L. procaviensis,* based on genetic differences in marker DNA sequences, these being greater than those between specified existing valid species of *Leishmania*. *Leishmania chancei* is classified within subgenus *L.* (*Mundinia*) based on phylogenetic analysis using DNA sequences that have previously been used for this purpose.

Leishmania (*Mundinia*) *procaviensis* n. sp.

Morphology: Characteristic morphotypes of the genus *Leishmania*; amastigotes 1–2 μm in width and 2–4 μm in length; promastigotes of various sizes with body length ranging between 5 and15 μm and motile with a free anterior flagellum of variable length.

Taxonomic summary

Type host: Procavia capensis.

Type locality: Keetmanshoop (–26°34′59.99″S, 18°07′59.99″E), Karas Region, Namibia.

Type material: Hapantotypes, cryopreserved promastigotes stored in liquid nitrogen at the Division of Biomedical and Life Sciences, Lancaster University, U.K. (accession LV425).

Strain designation: MPRV/NA/1975/253;LV425.

Homologous strains: None known.

Vector: Unknown.

Reservoir(s): Unknown.

Representative DNA sequences: A chromosome-scale full genome assembly and annotations are available under GenBank accession GCA_017918225.1. The master record for the whole genome-sequencing project is available at JAFNID000000000. The new species was characterized using molecular techniques revealing a genetically distinct parasite within *Leishmania* (*Mundinia*). Diagnostic sequences and their accession numbers include RPL23a intergenic sequence (OP715862), RNA polymerase II large subunit (OP715860), rRNA ITS-1 (OP723334) and HSP-70 (OP715861).

ZooBank registration: urn:lsid:zoobank.org:act:A96FA812-9B34-47BD-B92D-28F60BD797E7.

Etymology: The species is named after the mammalian host, the rock hyrax *Procavia capensis*.

Growth in vitro: Cryopreserved promastigotes were revived from long-term storage in liquid nitrogen (over 30 yr) in Sloppy Evans (Kwakye-Nuako et al., 2015), thereafter grown at 26 C as promastigotes in Schneider's insect medium supplemented with 20% (v/v) fetal bovine serum and 25 µg/ml gentamicin sulfate, or in M199 medium with Hank's balanced salt solution supplemented with 10% (v/v) fetal bovine serum, 2% (v/v) healthy human urine, 1% (v/v) Basal Medium Eagle vitamins and 25 µg/ml gentamicin sulfate. Promastigotes were subpassaged to fresh medium every 4–7 days to maintain the growth and viability of the parasites.

Pathology: Original isolate was derived from tissue taken from the tip of the nose of 1 *Procavia capensis* (Grové and Ledger, 1975; Grové, 1989). No mention is made of any obvious pathology in infected rock hyraxes.

Remarks

Leishmania procaviensis is distinguished from other *Leishmania* species, including *L. chancei*, based on genetic differences in marker DNA sequences, these being greater than those between specified existing valid species of *Leishmania*. *Leishmania procaviensis* is classified within subgenus *L.* (*Mundinia*) based on phylogenetic analysis using DNA sequences that have previously been used for this purpose.

DISCUSSION

Based on the genetic and phylogenetic analyses described here we conclude that the 2 new species described are both members of the subgenus *L.* (*Mundinia*). Ghana has been long suspected as endemic for leishmaniasis as the north of the country is close to the semiarid Sahel belt, an ecotope in which *Leishmania major* cases have been reported in other parts of West Africa (Boakye et al., 2005). However, when the first Ghanaian cases were reported in 1999 they were found in the Ho District of the Volta region in the south of Ghana (Kweku et al., 2011), in areas with luxuriant vegetation that were originally primary rain forest. Biopsy samples from this new focus of leishmaniasis were initially identified as *L. major* (Fryauff et al., 2006), but a follow-up study instead indicated a new species (Villinski et al., 2008). This conclusion was confirmed by the first isolation of parasites in culture and their initial characterization (Kwakye-Nuako et al., 2015), and here we name this parasite *Leishmania* (*Mundinia*) *chancei*.

There have been limited studies to determine the prevalence of leishmaniasis in Ghana, but where these have been conducted clinical signs have indicated 3–4% of the general population and 12–30% of schoolchildren to have ulcers and/or scars suggestive of leishmaniasis (Kweku et al., 2011). More recent studies in 3 communities in the Oti region (formerly part of the Volta region) showed that 32% of the skin ulcers examined were *Leishmania* positive by PCR (Akuffo et al., 2021a). In a parallel study *Leishmania* skin test positivity indicated that 42% of individuals in these communities had been exposed to *Leishmania* (Akuffo et al., 2021b). Therefore, although the exact prevalence and incidence in the population remain uncertain, it is certain that cutaneous leishmaniasis is a frequent infection in the Oti and Volta regions of Ghana. From the existing data, it is not possible to conclude that all of these are due to infection with *L. chancei* but given the normally focal nature of the disease this may well be the case, and this remains the only parasite conclusively identified from these regions.

Animal reservoirs of *L.* (*M.*) *chancei* are presumed to exist based on the zoonotic nature of most of the leishmaniases, but remain unknown to date. Experimental studies have shown that *L.* (*M.*) *chancei* is unable to infect guinea pigs (Bečvár et al., 2020) and that the African rodents *Arvicanthus niloticus* and *Mastomys natalensis* are unlikely to be reservoir hosts (Sadlova et al., 2020). The vectors are also unknown and, whilst these would normally be expected to be phlebotomine sand flies, there is increasing evidence for midges acting as vectors for *Leishmania* (*Mundinia*) species (Dougall et al., 2011; Seblova et al., 2015; Chanmol et al., 2019a; Bečvár et al., 2021). This has not been conclusively proven, but if it was confirmed that some or even all of the subgenus *L.* (*Mundinia*) had midge vectors, this would then require consideration of the definition of the genus *Leishmania* itself. In this circumstance, an option would be to take the *Leishmania* (*Mundinia*) into a new genus based on their different vectors. However, given the clinical and molecular similarity of the *L.* (*Mundinia*) species to other *Leishmania*, a preferable option would be to revise the definition of the genus *Leishmania*, allowing for both sand fly and midge vectors. Either way, this awaits definitive conclusions regarding the natural vectors of *Leishmania* (*Mundinia*) species.

There are only a few reports of leishmaniasis from southern Africa, and this region is not usually considered a high risk for the acquisition of infection by either travelers or residents. Nevertheless, Grové and colleagues conducted some investigations in the 1970s in the area surrounding Keetmanshoop in Namibia (Grové, 1989). Several isolates of *Leishmania* were collected, some from human cases, some from sand flies, and some from rock hyraxes. These were characterized using the methods available at the time, including multilocus isoenzyme analysis, and it is interesting to note that some of the rock hyrax isolates were recognized as "distinctly different" to those from the human cases and sand flies (Grové, 1989). One of these isolates survived in the Lancaster cryobank, LV425, and upon DNA sequence analysis was found to represent a new species, here named *Leishmania* (*Mundinia*) *procaviensis*. Further work is required to characterize the biological properties of this parasite, including its pathogenicity. Based on its difference from all other currently known human-infective parasites, its presence in rock hyraxes but an apparent absence of human infection, our working assumption is this new species is nonpathogenic to humans, but this has not

been conclusively proven. If true, in this respect it appears to be like *L.* (*M.*) *enriettii* and *L.* (*M.*) *macropodum* within *L.* (*Mundinia*).

The results described here now bring the total number of named species in *L.* (*Mundinia*) to 6. One interesting feature of the *L.* (*Mundinia*) is their wide geographical distribution compared to other subgenera. Various analyses (Noyes et al., 2002; Dougall et al., 2011; Pothirat et al., 2014; Kwakye-Nuako et al., 2015; Harkins et al., 2016; Butenko et al., 2019) have shown them to typically be the earliest branching clade within the genus *Leishmania*, including the analyses described here. The explanation for this may be that they evolved when the continents were together in Gondwana, with the other subgenera evolving after the breakup of the supercontinent. Thus, further study of the *L.* (*Mundinia*) will help us to understand the evolution of the genus *Leishmania* and how some of these species came to be important human pathogens.

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