An appraisal of the taxonomy and nomenclature of trypanosomatids presently classified as *Leishmania* and *Endotrypanum*

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SUMMARY

We propose a taxonomic revision of the dixenous trypanosomatids currently classified as *Endotrypanum* and *Leishmania*, including parasites that do not fall within the subgenera *L.* (*Leishmania*) and *L.* (*Viannia*) related to human leishmaniasis or *L.* (*Sauroleishmania*) formed by leishmanias of lizards: *L. colombiensis, L. equatorensis, L. herreri, L. hertigi, L. deanei, L. enriettii* and *L. martiniquensis*. The comparison of these species with newly characterized isolates from sloths, porcupines and phlebotomines from central and South America unveiled new genera and subgenera supported by past (RNA PolII gene) and present (V7V8 SSU rRNA, Hsp70 and gGAPDH) phylogenetic analyses of the organisms. The genus *Endotrypanum* is restricted to Central and South America, comprising isolates from sloths and transmitted by phlebotomines that sporadically infect humans. This genus is the closest to the new genus *Porcisia* proposed to accommodate the Neotropical porcupine parasites originally described as *L. hertigi* and *L. deanei*. A new subgenus *Leishmania* (*Mundinia*) is created for the *L. enriettii* complex that includes *L. martiniquensis*. The new genus *Zelonia* harbours trypanosomatids from Neotropical hemipterans placed at the edge of the *Leishmania–Endotrypanum-Porcisia* clade. Finally, attention is drawn to the status of *L. siamensis* and *L. australiensis* as nomem nudums.

Key words: Leishmaniinae, *Leishmania*, *Endotrypanum*, new genera, new subgenera, SSU rRNA, HSP70, gGAPDH, phylogeny, molecular taxonomy.

INTRODUCTION

The classification of *Leishmania* was initially based on the clinical symptoms of the disease that they generated. Parasites causing cutaneous leishmaniasis throughout the world were considered as being *L. tropica* and those causing visceral leishmaniasis as being *L. donovani*. The first move away from this position was by Nicolle (1908) who considered that Mediterranean visceral leishmaniasis was clinically and epidemiologically different from that of India, naming it *L. infantum*. Three years later Vianna (1911) gave the name *L. braziliensis* to the parasite responsible for a case of disseminated leishmaniasis from Brazil. Following this, Yakimoff and Schokhor (1914) considered that the parasites causing urban and rural cutaneous leishmaniasis in Asia were different varieties of *L. tropica*, denominating the urban form as var. *minor* and the rural form as var. *major*.

Over the years more parasites from different parts of the world were examined and in the 1980s Lainson and Shaw (1979, 1987) concluded that there were three very different groups of parasites that warranted sub-generic status: *L.* (*Leishmania*), *L.* (*Viannia*) and *L.* (*Sauroleishmania*). However, the taxonomic position of some *Leishmania*-like parasites remained uncertain such as *L. colombiensis* and *L. martiniquensis* isolated from patients and *L. enriettii, L. equatorensis, L. herreri* and *L. hertigi* isolated from wild animals. Another dixenous parasite that like the *Leishmania* also produces promastigotes in its vector and in culture is *Endotrypanum*. This genus was created to accommodate endoerythrocytic sloth trypanosomatids described in French Guyana (Mesnil and Brimont, 1908).

Molecular phylogeny has helped to clarify the taxonomy of the trypanosomatids, including many pathogens. In 2012, a group formed by dixenous and monoxenous trypanosomatids originally called 'slow evolving', due to the high conservation of SSU rRNA sequences, was given subfamily status under the name Leishmaniinae (Jirků *et al*. 2012). The dixenous members of this subfamily included parasites of wild animals that may accidentally infect man, causing diseases generically known as leishmaniasis. The depth of the taxonomic complexity of the parasites originally described as

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Leishmania is presently being disclosed by molecular studies. The question is: should they all be classified as belonging to the same genus? The mere suggestion that organisms other than *Leishmania* species cause leishmaniasis is immediately boycotted or met with scepticism. But is this view acceptable in the light of our present day knowledge of their genetic diversity and phylogenetic relationships?

Renewed interests on trypanosomatids from wild animals have unveiled a diversified genetic repertoire within the genera *Trypanosoma* and *Leishmania*. Recent studies have provided relevant insights into the broader genetic diversity and new reservoirs of human- and non-human infective species of *Leishmania* (Cupolillo *et al*. 1998; Asato *et al*. 2009; Cassia-Pires *et al*. 2014; Pothirat *et al*. 2014). Consequently, there is an increasing number of 'leishmanias' from non-human hosts that cannot be classified in any of the existing subgenera of *Leishmania*, and are herein refereed as enigmatic or *Leishmania*-like trypanosomatids. The focus of this paper is to molecularly characterize a large number of new isolates from Amazonian wild mammals (sloth and porcupine) and sand flies from Central and South America, and to compare the data obtained with data available from other enigmatic leishmanias and reference species of all accepted subgenera of *Leishmania*, and their closest related monoxenous species. For this, we inferred taxon-rich phylogenetic trees based on gGAPDH and HSP70 genes, and used the data to critically discuss the generic status and nomenclature of the dixenous parasites that produce promastigotes in their vectors and cultures, some of which are the aetiological agents of the cohort of diseases known as leishmaniasis.

MATERIAL AND METHODS

Organisms and cultures

The organisms characterized in this study are routinely grown at 23–25 °C in TC100 medium (=Grace medium) supplemented with 10% FBS, and deposited in the Trypanosomatid Culture Collection of the University of São Paulo (TCC-USP). Routinely, upon inclusion in the TCC-USP, the predominant morphotype of each new isolate is recorded, while the isolate itself is barcoded by V7V8 SSU rRNA sequencing (Teixeira *et al*. 2011). This procedure serves only as preliminary information for future phylogenetic analyses of selected samples based on gGAPDH sequences. The samples selected for this study comprised cultures of trypanosomatids isolated from phlebotomines, sloths and porcupines from different areas of Central and South America (Table 1). For comparison, the analyses included DNA sequences determined in this study or recovered from GenBank and genome data banks of all *Leishmania*-like and *Endotrypanum* species, and reference-species of all subgenera of *Leishmania*.

PCR amplification, sequencing and phylogenetic analysis

Total DNA was extracted from cultured flagellates using the traditional phenol–chloroform method. The V7V8 SSU rRNA and gGAPDH gene were PCR-amplified as previously described (Hamilton *et al*. 2004; Teixeira *et al*. 2011). The amplification of HSP70 gene was done under the same reaction conditions adopted for gGAPDH and using the following primers: Hspf 5′-TGC GCA TCA TCA ACG AGC C-3′ and Hspr 5′-ATC TTG GTC ATGA TCG GGT TGC-3′.

PCR-amplified sequences were cloned using the TA Cloning Kit (Carlsbad, CA, USA), and from one to five clones were sequenced for each gene of each trypanosomatid isolate. Sequences were aligned using CLUSTALX (Thompson *et al*. 1997), and manually refined to obtain the following alignments: 1, V7V8 SSU rRNA sequences (~850 bp = barcodes); 2, gGAPDH sequences (768 bp); 3, HSP70 sequences (611 bp); 4, consisting of concatenated gGAPDH and HSP70 sequences (1·378 characters). Sequences determined in this study were all deposited in GenBank (Table S1). Sequences from *Leptomonas costaricensis* (Costa Rica), the isolate TCC169 (*L. costaricensis*-like from Brazil), *Leptomonas seymouri, Leptomonas pyrrhocoris* and *Crithidia fasciculata* were included in all analyses (Supplementary Table S1). Sequences from *Angomonas deanei, Angomonas desouzai* and species of other genera of trypanosomatids were used as outgroups of Leishmaniinae (Jirků *et al*. 2012). The dendrogram inferred using V7V8 SSU rRNA sequences of the Leishmaniinae subfamily and closely related trypanosomatids was done using the method of Maximum Parsimony (MP). The alignments of gGAPDH and HSP70 sequences were employed for phylogenetic inferences based on MP, Maximum Likelihood (ML) and Bayesian inference (BI) analyses. The MP and bootstrap analyses used the PAUP* version 4.0b10 software (Swofford, 2002), with 500 random sequence addition replicates followed by branch swapping (RAS-TBR), and the ML analyses used RAxML v.2.2.3 (Stamatakis, 2006). The general time reversible (GTR) model of nucleotide substitution with proportion of invariable sites and gamma distribution was selected for the datasets. We used the GTR model in individual analyses of each gene as well as in the combined analyses, which was run for 1 000 000 generations with trees sampled every 100 generations using four chains, and 25% of the early sample trees were discarded as 'burn-in'.

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Table 1. Details of trypanosomatids used in the V7V8 SSU rRNA and HSP70/gGAPDH phylogenetic analyses

TCC	WHO Code	Species	Order	Host	State	Country
255	MCOE/BR/75/M4059	*P. deanei*	Rodentia	*Coendou* sp.	PA	Brazil
256	MCOE/BR/91/M13291	*P. deanei*	Rodentia	*Coendou* sp.	PA	Brazil
258	MCOE/BR/91/M13451	*P. deanei*	Rodentia	*Coendou* sp.	PA	Brazil
260	MCOE/PA/80/C8	*P. hertigi*	Rodentia	*Co. rothsechildi*		Panama
586	MHOM/VE/96/PM-H230	*E. colombiensis*	Primata	*Homo sapiens*	LA	Venezuela
259	MCHO/EC/82/Lsp-1	*E. equatorensis*	Pilosa	*Ch. hoffmanni*		Ecuador
252	MSCI/EC/82/Lsp-2	*E. equatorensis*	Rodentia	*S. granatensis*		Ecuador
251	MCHO/CR/75/Ch-29	*E. herreri*	Pilosa	*Ch. hoffmanni*		Costa Rica
1063	MMAC/AU/2004/Roo1	*L.* (*M.*) sp. Roo1	Diprotodontia	*Macropus* sp.	NT	Australia
2114	MCAV/BR/45/L88	*L.* (*M.*) *enriettii*	Rodentia	*Cavia porcelus*	PR	Brazil
224	MCHO/PA/62/M907	*E. schaudinni*	Pilosa	*Ch. hoffmanni*	CO	Panama
222	MCHO/CR/62/A9	*E. monterogeii*	Pilosa	*Ch. hoffmanni*	CA	Costa Rica
102	IDEN/BR/98/M17035	*Endotrypanum* sp.	Diptera	*Ps.dendrophyla*	RO	Brazil
889	IPHL/BR/??/Fleb.341	*Endotrypanum* sp.	Diptera	*Phlebotominae*	AM	Brazil
890	IPHL/BR/??/Fleb.28	*Endotrypanum* sp.	Diptera	*Phlebotominae*	AM	Brazil
250	MCHO/BR/89/M12629	[a]*Endotrypanum* sp.	Pilosa	*Ch. didactylus*	PA	Brazil
225	MCHO/BR/80/M6159	*Endotrypanum* sp.	Pilosa	*Ch. didactylus*	PA	Brazil
226	MBRA/PA/??/415P01	*Endotrypanum sp.*	Pilosa	*Br. variagatus*		Panamá
286	MBRA/GF/??/LE 2954	*Endotrypanum* sp.	Pilosa	*Ch. didactylus*		Fr. Guyana
230	MCHO/BR/79/M5725	*Endotrypanum* sp.	Pilosa	*Ch. didactylus*	PA	Brazil
239	MCHO/BR/67/M595	*Endotrypanum* sp.	Pilosa	*Ch. didactylus*	PA	Brazil
231	MCHO/BR/82/M6862	*Endotrypanum* sp.	Pilosa	*Ch. didactylus*	PA	Brazil
017E	RTAR/DZ/49/G10	*L.* (*S.*) *tarentolae*	Reptilia	*T.mauritanica*		Algeria
717	RLAT/KE/57/SKINK-7	*L.* (*S.*) *adleri*	Squamata	*Lt. longicaudata*		Kenya
722	RGYM/TM/40/	*L.* (*S.*) *gymnodactali*	Squamata	*G.caspius*		Turkmenistan
723	RHEM/SD/63/NG26	*L.* (*S.*) *hoogastrali*	Squamata	*H. turcicus*		Sudan Rep.
	MHOM/MQ/92/MAR1	*L.* (*M.*) *martiniquensis*	Primata	*Homo sapiens*		Martinique
	MDAS/BR/81/M6246	*L.* (*V.*) *naiffi*	Cingulata	*Dasypus* sp.	PA	Brazil
	MHOM/BO/??/CUM 180	*L.* (*V.*) *braziliensis*	Primata	*Homo sapiens*		Bolivia
	MHOM/CO/81/L13	*L.* (*V.*) *panamensis*	Primata	*Homo sapiens*	CH	Colombia
	MNYC/BZ/62/M379	*L.* (*L.*) *mexicana*	Rodentia	*N. sumichrasti*		Belize
	MHOM/MT/85/Buck	*L.* (*L.*) *infantum*	Primata	*Homo sapiens*		Malta
	MHOM/SD/68/1S	*L.* (*L.*) *donovani*	Primata	*Homo sapiens*		India
2165	IRIC/CR/2003/15EC	*Z. costaricensis*	Reduviidae	*Ri. simillina*		Costa Rica
169E	IZEL/BR/89/169E	*Z. costaricensis*-like	Reduviidae	*Zelus* sp.	AM	Brazil
504	IRIC/BR/88/504	*Z. costaricensis*-like	Reduviidae	*Ri. quadrispinosa*	RO	Brazil
2696	IRIC/PA/2014/2696	*Z. costaricensis*-like	Reduviidae	*Ricolla* sp.		Panama
079E	IORN/BR/89/079E	*A. desouzai*	Syrphidae	*Ornidia obesa*	MG	Brazil
036E	IZEL/BR/73/036E	*A. deanei*	Reduvidae	*Z. leucogrammus*	GO	Brazil
	ICUL/US/42/Wallace	*C. fasciculata*	Culicidae	*Culex pipiens*	MN	USA
	IDYS/US/59/ATC30220	*L. seymouri*	Pyrrhocoridae	*D. suturellus*	FL	USA
	IPYR/CZ/78/H10	*L. pyrrhocoris*	Pyrrhocoridae	*Py. apterus*		Czech Rep.

TCC, Trypanosomatid Culture Collection of the University of São Paulo;
P., Porcisia; E., Endotrypanum; L. (M.), L (Mundinia); Z., Zelonia; A., Angomonas; C., Crihtidia; Lp., Leptomonas. Br., Bradypus; Ch., Choloepus; Co., Coendou; D., Dysdercus; G., Gymnodactylus; H., Hemidactylus; Lt., Latastia. N., Nyctomys; S., Sciurus; T., Tarentola; Z., Zelus; Lu., Lutzomyia; Ps., Psathyromia; Py., Pyrrhocoris; Ri., Ricolla. State Abbreviations: Australia: NT, Northern Territory; Brazil: AM, Amazonas, GO, Goiás, MG, Minas Gerais, PA, Pará, PR, Paraná, RO, Rondônia; Colombia: CH, Choco Department; Costa Rica: CA, Cartago Province, HE, Heredia Province; Panama: CO, Colón Province; USA: MN, Minnesota; Venezuela: LA, Lara.
[a] This strain was considered initially to be *Endotrypanum* but was identified as *L. deanei*.

RESULTS

Barcoding of Endotrypanum *and* Leishmania*-like isolates and comparison with other species of trypanosomatids*

In this study, we barcoded through V7V8 SSU rRNA sequences the reference isolates of *Endotrypanum schaudinni* and *Endotrypanum monterogeii*, and isolates previously classified in this genus by isoenzyme patterns: six from sloths (*Choloepus hoffmanni, C. didactylus* and *Bradypus infuscatus*) and three from sand flies (*Lutzomyia gomezi* and *Psathyromyia dendrophyla*). In addition, we also barcoded all isolates of *Leishmania*-like that are not positioned within the recognized subgenera of *Leishmania: L. equatorensis, L. colombiensis, L. herreri, L. deanei* and *L. hertigi*. All these organisms were found to be closely related to *Endotrypanum* species, sharing highly conserved V7V8 SSU rRNA sequences (average of 98·7%) with members of this genus. However, despite the

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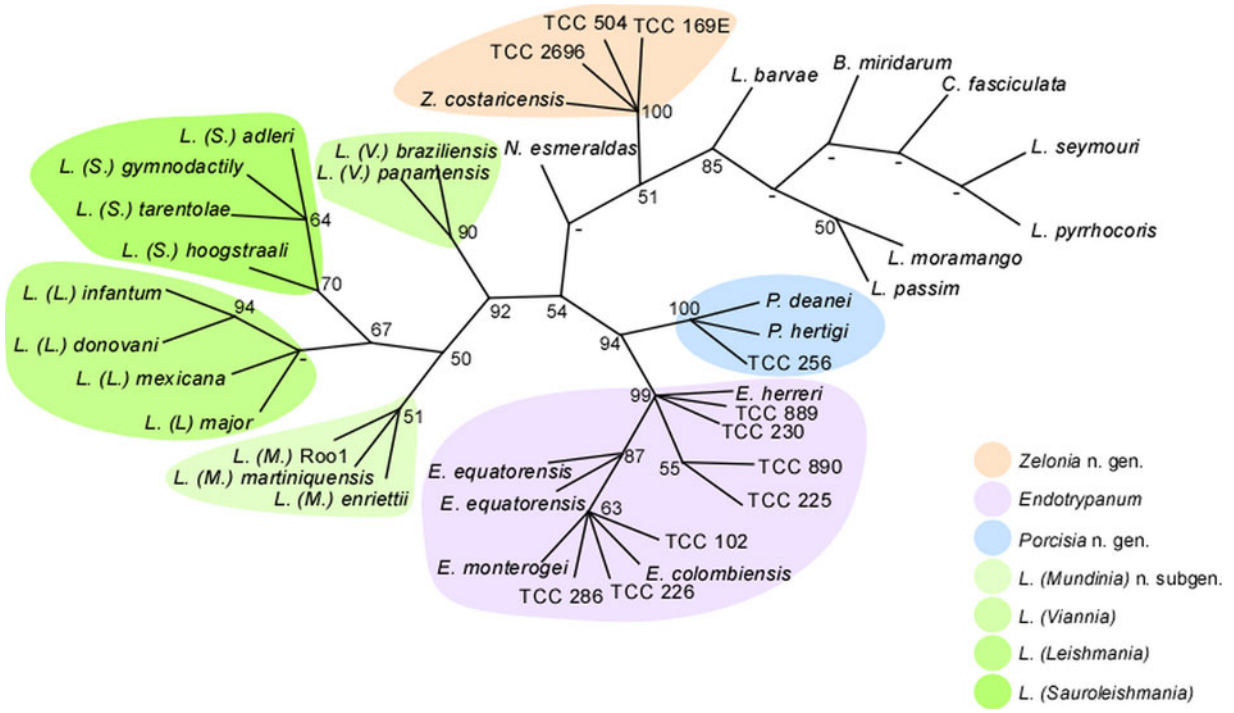

Fig. 1. A dendrogram inferred by MP analysis using 33 sequences from V7V8 SSU rRNA (alignment A1, 820 characters) from references species of Heteroxenous trypanosomatids and new isolates from sand flies, sloth and porcupine. The numbers at nodes correspond to percentage of bootstrap values derived from 100 replicates (– support value <50%). The accession numbers of sequences in GenBank are in Supplementary Table S1. All new isolates grouped with *Endotrypanum* and *Leishmania*-like species.

high conservation, inferred dendrogram supported a branch formed by two main groups of isolates respectively headed by *E. schaudinni* and *L. hertigi* (Fig. 1) and separated by ~2·7% of sequence divergence. In addition, the analysis of V7V8 SSU rRNA sequences allowed the clustering of most species of *Leishmania* spp. in the three currently recognized subgenera, and uncovered two additional clusters headed by *L. deanei* and *L. enriettii*, respectively. A new group was formed by monoxenous flagellates of hemipterans, including *L. costaricensis* (Yurchenko *et al.* 2006) from Costa Rica and three new isolates characterized in the present study: TCC169E and TCC504 from Brazilian Amazonia and TCC2696 from Panama, all sharing highly similar V7V8 SSU rRNA barcodes and placed into the subfamily Leishmaniinae (Fig. 1, Table S1).

Although the comparison of V7V8 SSU rRNA sequences is valuable to place new isolates in clusters representing genus/subgenera, more polymorphic sequences are required to distinguish the species within genera/subgenera, and to infer well-resolved phylogenetic relationships within and among them.

Phylogenetic relationships among isolates of Endotrypanum *and* Leishmania*-like*

In order to resolve the relationship between *Endotrypanum* and *Leishmania*-like isolates we inferred their phylogenetic relationships based on gGAPDH and HSP70 gene sequences. The analyses also included sequences from species of the complex *L. enriettii*, and the subgenera *L.* (*Viannia*), *L.* (*Leishmania*) and *L.* (*Sauroleishmania*). In the inferred phylogenies, all *Leishmania* spp. and *Endotrypanum* spp. formed a strongly supported monophyletic group (100% support) (Fig. 2). In addition, all analyses (P, ML and BI) using a single gene or concatenated gene sequences underscored (100% bootstrap support) three major monophyletic lineages (clades): *L. hertigi, L. herreri* and *Endotrypanum* spp.

The monophyletic group containing *L. herreri, L. equatorensis, L. colombiensis* and *Endotrypanum* species comprises two clades (100% support), headed by *Endotrypanun* spp. and *L. herreri*, separated by 2·0 and 4·2% of HSP70 and gGAPDH sequence divergences, respectively. The clade grouping the reference species *E. monterogeii* (Costa Rica, *C. hoffmanni*) and *E. schaudinni* (Panama, *C. hoffmanni*) also harboured *L. colombiensis* (isolated from a patient from Venezuela), TCC 226 (from the sloth, *B. infuscatus* of Panama), TCC 102 (from the sand fly *Psanthyromya dendrophyla*, from the state of Rondônia, Brazilian Amazonia) with strongly support values of 100% for P, ML and BI. The clade harbouring *L. herreri* (Costa Rica) clustered six new isolates: TCC889 and TCC890 from sand flies from the State of Amazonas, Brazil, TCC225 from *Choloepus didactylus*, and TCC230, TCC231 and TCC239 from *C. hoffmanni* from the state of Pará,

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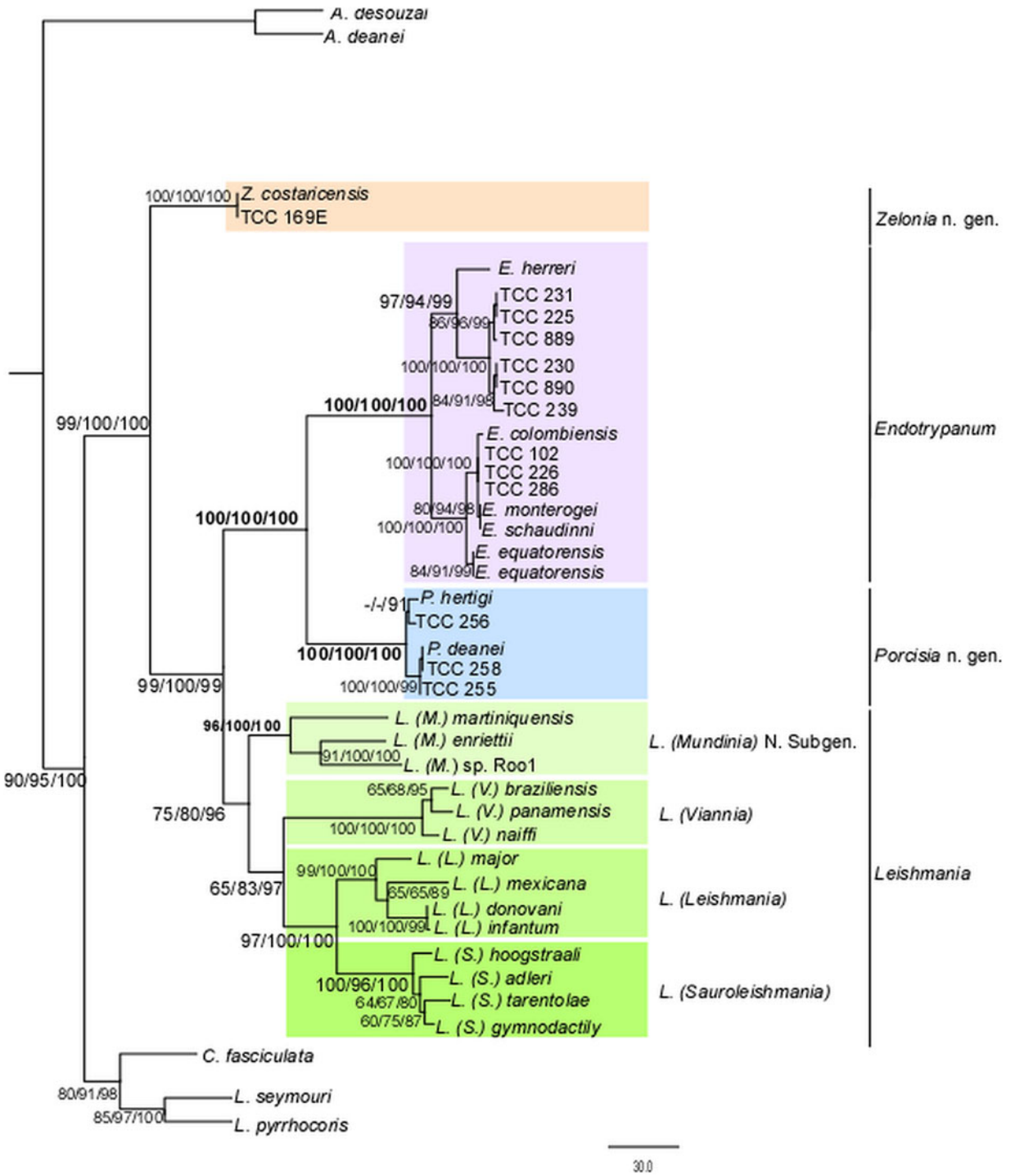

Fig. 2. Phylogenetic tree of heteroxenous trypanosomatid genera of the subfamily Leishmaninae inferred by MP, ML and Bayesian analysis, based on concatenated heat shock protein 70 and glycosomal glyceraldehyde-3-phosphate dehydrogenase (gGAPDH) genes sequences (alignment A2, 1354 characters). The accession numbers of HSP70 and gGAPDH sequences of these trypanosomatids in GenBank are in Table X. Numbers at the major nodes correspond respectively to MP, ML and BI support values (– support value <50%). The four heteroxenous genera *Endotrypanum, Porcisia, Zelonia* and *Leishmania* are strongly supported and the new subgenus *L.* (*Mundina*), is the most basal taxonomic group of the genus *Leishmania*.

Brazil. The trypanosomatids of *L. herreri* group share similarity of ~96 and 99% of gGAPDH and HSP70 gene sequences, respectively. The similarity between the sequences of *L. equatorensis* from *Sciurus granatensis* (squirrel) and *C. hoffmanni* (sloth) from Ecuador was 100% for both HSP70 and gGAPDH genes.

Basal to the major clade comprising *Endotrypanum* and *L. herreri* subclades was the clade composed of porcupine parasites: *L. hertigi* from Panama and *L. deanei* from Brazil, with support of 100% in all analyses. The isolates forming the complex *hertigi-deanei* share great similarity in the sequences of gGAPDH

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and HSP70 (99 and 100% respectively). However, this is a very distant clade, separated by ~12 and 5·0% of divergences in gGAPDH and HSP70 sequences respectively from *Endotrypanum* and other *Leishmania*-like.

Leishmania enriettii complex

In the inferred phylogenies based on gGAPDH and HSP70 sequences, *L. enriettii*, the Australian *Leishmania* sp. from the red kangaroo and *L. martiniquensis* compose a clade positioned basal (support values of 75, 80 and 96% for P, ML and BI, respectively) to the major clade comprising *L.* (*Viannia*), *L.* (*Leishmania*) and *L.* (*Sauroleishmania*) species. The *L. enriettii* clade was well-supported (96, 100 and 100% for P, ML and BI, respectively) as the more distant clade using concatenated genes, whereas the analyses based on single genes showed lower support values. This clade comprises species separated by large genetic distances, sharing 5·0 and 4·0% of gGAPDH and HSP70 sequence divergences, respectively. Even though, in all analyses this was the most basal clade of the genus *Leishmania*. The results further demonstrate that parasites belonging to the *L. enriettii* complex shares the greatest similarity of gGAPDH and HSP70 sequences with the species of the subgenus *L.* (*Leishmania*) (92 and 96%, respectively) than with the species of the subgenus *L.* (*Viannia*) (87 and 94%, respectively).

DISCUSSION

The creation of the subgenus *L.* (*Viannia*) and the validation of the subgenera *L.* (*Leishmania*) and *L.* (*Sauroleishmania*) (Lainson and Shaw, 1987) was a major step forward to organizing the taxonomy of the leishmaniasis parasites. However, in the 1990s biochemical and molecular data began to show that some flagellates classified as *Leishmania* did not fall comfortably within any of the three subgenera (Croan *et al*. 1997). The parasite that spearheaded this group, but was not immediately linked with leishmaniasis, was *Endotrypanum*, an enigmatic endoerythrocytic sloth parasite described in French Guyana (Mesnil and Brimont, 1908) that develops as promastigotes in culture and sand flies (Shaw, 1963, 1969, 1981).

Phylogenetic analysis (Croan *et al*. 1997) based on the genes encoding the DNA polymerase alpha catalytic polypeptide (POLA) and the RNA polymerase II largest subunit (RPOIILS) showed that *L. hertigi* and *Endotrypanum* formed two groups that were distinct from the *Leishmania* subgenera *L.* (*Leishmania*), *L.* (*Viannia*) and *L.* (*Sauroleishmania*). Multilocus enzyme electrophoresis (MLEE), sialidase activity, ITS rRNA restriction profiles and minicircle kDNA sequences (Cupolillo *et al*. 1998) showed that two leishmania, *L. colombiensis* and *L. equatorensis* grouped within the *Endotrypanum* clade. Following these results a revised classification was proposed (Cupolillo *et al*. 2000) that created two informal groups. One, the Paraleishmania, contained the *L. hertigi* and *Endotrypanum* clades and the other, the Euleishmania, contained the subgenera *L.* (*Leishmania*) and *L.* (*Viannia*).

Taxonomy: amendments to the subfamily Leishmaniinae and the genus Endotrypanum *and the creation of the new genera* Porcisia, *Zelonia and the new subgenus* Leishmania (Mundinia)

Phylum Euglenozoa (Cavalier-Smith, 1981); Class Kinetoplastea Honigberg 1963; Order Trypanosomatida (Kent 1880; Hollande 1982), Family Trypanosomatidae (Doflein 1901), Leishmaniinae (Maslov and Lükes, 2012 emend Espinosa *et al*. 2016)

Subfamily Leishmaniinae Maslov and Lukeš (Jirků et al. *2012*) *emend* Shaw, Camargo and Teixeira 2016.

The subfamily Leishmaniinae (Jirků *et al*. 2012) was erected for a group of monoxenous and dixenous trypanosomatid parasites based on a phylogenetic analysis of a concatenated dataset of SSU rRNA and gGAPDH gene sequences. This previous study included more monoxenous than dixenous trypanosomatids and did not compare sequences from the *Leishmania*-like herein taxonomically revised. Our phylogenetic analyses (MP, ML and BI) based on combined gGAPDH and HSP70 sequences and using trypanosomatids not included in the subfamily Leishmaniinae as outgroup (Jirků *et al*. 2012) strongly support two major clades, one comprising *Leishmania–Endotrypanum* and the other containing of monoxenous trypanosomatids herein represented by *C. fasciculata, L. seymouri* and *L. pyrrrochoris* (Fig. 2). Phylogenetic analyses based on concatenated V7V8 SSU rRNA and gGAPDH support congruent branching patterns (data not shown). Our findings are consistent with published phylogenetic trees inferred using SSU rRNA and gGAPDH sequences supporting the subdivision of the subfamily Leishmaniinae into two clades (Jirků *et al*. 2012; Kostygov *et al*. 2016). In face of the data gathered in the present study on well-resolved and taxon-rich phylogenies, including *Leishmania* and *Leishmania*-like, *Leptomonas* and *Crithidia* species, we suggest that the subfamily Leishmaniinae is limited to the strongly supported clade that includes the dixenous trypanosomatids and, in addition, also harbours some trypanosomatids that are thought to be monoxenous positioned into the new genus *Novymonas* (Kostygov *et al*. 2016) and into the clade headed by *L. costaricensis* (Figs 1 and 2), which was erected to a new genus in the present study (section below). New subfamilies may eventually be further created to accommodate the monoxenous trypanosomatids that nested into the other major clade (*Crithidia, Lotmaria* and *Leptomonas*) of the originally proposed subfamily

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Leishmaniinae when their phylogenetic relationships are more clearly defined.

The distinction between monoxenous and heteroxenous trypanosomatids as a taxonomic criterion must be viewed with caution. Presently, molecular characters support this general division, but it is conceivable that as more trypanosomatids are analysed mono- and heteroxenous trypanosomatids may cluster together in several trypanosomatid taxa. The fact cannot be ignored that the apparently monoxenous trypanosomatids *L. costaricensis* (Yurchenko *et al*. 2006) and *Novymonas* sp. (Kostygov *et al*. 2016) are more closely related to the heteroxenous *Leishmania* and *Endotrypanum* spp. than to the monoxenous *Crithidia* and *Leptomona*s. Another trypanosomatid (G755) isolated from Guatemala (Noyes *et al*. 1997) is also a member of the *L. costaricensis* clade. Knowing so little about the life history of these parasites it is difficult to say whether they are in fact truly monoxenous. A parasite isolated from diffuse lesions of a HIV patient from Martinique was considered to be a monoxenous trypanosomatid (Dedet and Pratlong, 2000). Further analysis showed that it was a leishmanine parasite, but it did not belong to the subgenera previously associated with human leishmaniasis (Noyes *et al*. 2002). Other parasites also considered to be monoxenous parasites have been isolated from HIV patients from Spain (Jimenez *et al*. 1996) and Brazil (Pacheco *et al*. 1998). Their phylogenetic position was not determined. The 18S rRNA sequence of a parasite isolated from the blood of a HIV patient living in France (Morio *et al*. 2008) showed a 99·8% similarity to *Herpetomonas samuelpessoai*. Of 33 strains from Indian cases of visceral leishmaniasis, the ITS1 rDNA of 21 matched that of *L. seymouri* (Ghosh *et al*. 2012). These monoxenous organisms are from HIV patients or patients with infections, such as *L.* (*L.*) *donovani* that also depress the immune system. These observations show that the line between monoxenous and heteroxenous is a very fine one. It also suggests that infections of monoxenous organisms may occur in immunological competent individuals, but since they are resolved quickly they are not detected.

Endotrypanum and some enigmatic *Leishmania*, parasites of Neotropical animals, have previously been grouped together under the name paraleishmania (Cupolillo *et al*. 2000; Pothirat *et al*. 2014). Actually, they form two natural clades. One is composed of flagellates previously classified as either *Leishmania* or *Endotrypanum*, and the other of parasites from American porcupines.

Porcisia *n. gen. Shaw, Camargo and Teixeira*

Type species: Porcisia hertigi (Herrer, 1971) (Synonym: *Leishmania hertigi*).

Diagnosis: Our phylogenetic analyses of five isolates of the complex *L. hertigi*/*L. deanei* confirm their uniqueness and clustering in a clade strongly supported in all phylogenetic analyses and separated from its sister clade formed by *Leishmania*-like and *Endotrypanum* by relevant genetic distance. With the above points in mind we consider that the *L. hertigi* complex warrants generic status and propose the name *Porcisia* for the new genus.

Etymology: The genus name is based on first four letters of porcupine; this animal is the principal host.

Historical comments: Leishmania hertigi was discovered by Aristides Herrer (Herrer, 1971) in porcupines, *Coendou rothschildi*, from Central Panama and was later found in Costa Rica (Zeledon *et al*. 1977). Similar parasites were found in porcupines in the Brazilian states of Piaui and Pará (Deane *et al*. 1974; Lainson and Shaw, 1977). The Brazilian parasites were biochemically and morphologically distinct from *P. hertigi* and were named *L. hertigi deanei* (Lainson and Shaw, 1977). In 1987, this subspecies was given specific status as *L. deanei* (Lainson and Shaw, 1987). Later, a parasite was identified by partial 18S rDNA sequence as *L. hertigi* in a porcupine from the city of Brasília, which is located in centre-west region of Brazil (Silva *et al*. 2013). This was most probably *P. deanei* and not *P. hertigi*, but more detailed studies of the Brasilia parasite are needed to confirm this. The 18S rDNA sequences are too conserved and consequently may not distinguish between these closely related species. These two parasites occur in different species of porcupines, distinct geographical regions and in our study *P. hertigi* and *P. deanei* are clearly separated. The porcupine parasites are biochemically, molecularly and morphologically different from the other species of Leishmaniinae. Their vectors are unknown.

Molecularly validated species: Porcisia hertigi and *Porcisia deanei* SSU rRNA, gGAPDH and HSP70 gene sequences were deposited in GenBank under the accession numbers listed in Table S1.

So far all parasites of the genus *Porcisia* have been found in porcupines. In the present study, one isolate (TCC 250), whose preliminary identification as *Endotrypanum* was based on it being isolated from the blood of a two-toed sloth, was molecularly identified as *P. deanei*. Porcupines and sloths are arboreal animals and it is possible that this represents a rare infection of a *P. deanei* in a sloth. However, we could not discard the possibility of misleading cultures.

Zelonia n. gen Shaw, Camargo and Teixeira

Type species: Zelonia costaricensis (Yurchenko *et al*. 2006) syn: *L. costaricensis*

Type host: Ricolla simillima (Heteroptera, Reduviidae).

Type locality: El Ceibo (10 × 20 kN, 84 × 05 kW), La Virgen, Province Heredia, Costa Rica

Etymology: The name is based on the surname of the famous Costarican protozoologist, Professor

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Rodrigo Zeledon, whose pioneering studies contributed enormously to trypanosomatid research in Central America and especially in Costa Rica.

The type species of the new genus was isolated from a predator hemipteran from Costa Rica and originally described as *L. costarricensis* by Yurchenko *et al.* (2006). Previous phylogenetic analyses (Jirkú *et al.* 2012; Kostygov *et al.* 2016) also have shown that it is distant from *L. pyrrhocoris* and *L. seymouri*. It is therefore unreasonable to continue to consider it to be a species of *Leptomonas* and so we have created the genus *Zelonia* to accommodate the clade headed by this parasite. The barcoding of our trypanosomatid collection revealed three additional isolates sharing highly similar V7V8 SSU rRNA sequences with *Z. costaricensis*. Interestingly, all isolates of the genus *Zelonia* were obtained from predatory hemipterans (Reduviidae) in Equatorial regions of Costa Rica, Brazil (Amazonia) and Panamá (Fig. 1, Table 1). The isolate TCC169 exhibits promastigote and opistomatigote-like forms in log- and stationary axenic cultures, and produced a few intracellular amastigotes in macrophage cultures; however, these forms disappeared in 3–4 days (unpublished results). The trypanosomatid from the digestive tract of a hemipteran (Rhopalidae) from Ecuador and recently described under the new genus *Novymonas* was the most closely phylogenetically related to *Zelonia costarricensis* in a tree based on 18S SSU rRNA, 28S LSU rRNA and Hsp83. The authors (Kostygov *et al.* 2016) considered that 'It does not cluster within either the *Leishmania* clade or the *Leptomonas–Lotmaria–Crithidia* group'. In agreement with these previous studies, in our phylogenetic analyses the new genus *Zelonia* is one basal group of the re-defined Leishmaniinae subfamily, therefore, more phylogenetically related to *Endotrypanum–Leishmania* than to *Leptomonas–Lotmaria–Crithidia*. Jirkú *et al.* (2012) clearly show that *Z. costaricensis* and *Leptomonas barvae* belong to a separate group they designated as Le, which coincides with our redefinition of the subfamily Leishmaniinae. Further analyses are still required regarding the apparently most basal species of this subfamily, *L. barvae, Leptomonas moramango* and *Blastocrithidia miridarum* (Kostygov *et al.* 2014; Maslov *et al.* 2010), all unquestionably requiring a taxonomic reappraisal at subfamily and genus levels.

Amendment to the genus Endotrypanum (Mesnil and Brimont, *1908*) *emend Shaw*, Camargo and Teixeira 2016.

Type species: Endotrypanum schaudinni Mesnil and Brimont, 1908. Type material not available.

Host Choloepus didactylus;

Type Locality: French Guyana.

Diagnosis: Phylogenetic positioning within the family Trypanosomatidae was based on gGAPDH and HSP70 gene sequences deposited in GenBank (Supplementary Table S1).

Genus composition and historical comments: There are two strongly supported groups within the genus. One contains a species previously placed within the genus *Leishmania* – *E. herreri*, and the second group contains two other species previously considered to be *Leishmania* – *E. colombiensis* and *E. equatorensis*. These two groups also contain parasites of sloths from Central and South America identified as *Endotrypanum* species.

Our analysis and past ones (Croan *et al.* 1997; Cupolillo *et al.* 2000; Noyes *et al.* 2002; Asato *et al.* 2009; Pothirat *et al.* 2014) using a variety of different markers consistently show a strongly supported monophyletic group that includes the following parasites: *E. schaudinni, E. monterogeii, L. colombiensis, L. equatorensis* and *L. herreri*. Based on RNA Pol II sequences (Pothirat *et al.* 2014) *Endotrypanum* and *L. colombiensis* group together and are separate from both *L. equatorensis* and *L. herreri*. Our results follow this pattern (Fig. 2). Previous analyses of this group were hampered by the fact that *Endotrypanum* was not compared with isolates considered to be *Leishmania*. *Endotrypanum* was not included in the differential diagnosis in the original descriptions of *L. colombiensis* (Kreutzer *et al.* 1991) and *L. equatorensis* (Grimaldi Junior *et al.* 1992). It was also absent in subsequent publications in which four visceral isolates of *L. colombiensis* from Venezuelan patients differed from four cutaneous isolates and from a standard of this species that was isolated from *L. gomezi* (Rodriguez-Bonfante *et al.* 2003).

Karyotype analyses of 17 isolates labelled as *Endotrypanum* from Brazilian, Panamanian and Colombian sloths and Brazilian sand flies unveiled four groups (Lopes *et al.* 1990). The first and second groups were closely related, the first being composed of isolates from Colombia, Brazil and Panama and the second of strains from Panama. Isolates in the third and fourth groups were all from Brazil. Parasites of the first two groups have different levels of virus-like-particles that are absent in the last two groups. The fourth group, consisting of two strains, was so different that the authors suggested that it was in fact not *Endotrypanum*, echoing the opinion of the group who isolated and first characterized them (Arias *et al.* 1985). Both strains failed to react with *Endotrypanum*-specific monoclonals (Lopes and McMahon-Pratt, 1989) confirming that they were not *Endotrypanum*. Our results reflect a similar situation. The strains in the group headed by TC889 are all from the Brazilian Amazon, while the second group contains *E. colombiensis* (TCC583, TCC586) from Venezuela, *Endotrypanum* isolates from Panama (TCC 226), French Guyana (TCC286), Rondônia, Brazil (TCC102), *E. schaudinni* (TCC224) from Panama and *E. monterogeii* (TCC222) from Costa Rica.

The nomenclature of the genus *Endotrypanum* remains extremely complex. The type species

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E. schaudinni does not exist, so a neotype will have to be designated for one parasite from French Guiana where it was originally described. If we choose the isolate TCC286 (MBRA/GF/??/LE 2954), reportedly isolated from French Guiana, as the neotype of *E. schaudinni* then it suggests that *E. colombiensis* is a synonym of *E. schaudinni* since the two are identical in our analysis. Of the two endotrypanums from Central America the one from Panama was named *E. schaudinni* (MCHO/PA/62/M907) and the one from Costa Rica *E. monterogeii* (MCHO/CR/62/A9). In our analysis, both are identical, but differ from the French Guiana isolate, then strain M907 should be called *E. monterogeii* and not *E. schaudinni*. From this it follows that the name that has priority for Central American *Endotrypanum* spp., is *E. monterogeii*. *E. herreri* from Costa Rica and *E. equatorensis* from Ecuador are distinct and appear to be valid species. It is possible that the TC889 group represents a new taxon within the genus *Endotrypanum*. We recommend that more data are gathered before proposing to the ICZN a neotype for the amended genus *Endotrypanum* and to define species within this genus.

The vectors of *Endotrypanum* species appear to be phlebotomine sand flies in which they have a peripylarian development (Shaw, 1963, 1981; Christensen and Herrer, 1976; Franco *et al*. 1997). Different *Endotrypanum* isolates have been obtained from wild sand flies in Brazil, Panama, Costa Rica, Ecuador and Venezuela. There is some evidence of vectorial specificity of endotrypanums. After three days flagellates were found from the cardia to the rectal ampulla in experimental infections of *E. colombiensis* in *L. trapidoi* but in *P. papatasi* parasites were limited to the blood meal (Kreutzer *et al*. 1991).

The *E. colombiensis* is the only species that has been associated with leishmaniasis. So far, none of the endotrypanums examined to date produce lesions or lasting infections in experimental animals, such as hamsters or mice. *E. colombiensis* produced intracellular amastigotes in cultures of J774 macrophages, but from 72 h onwards the parasites began to die and by 120 h they had disappeared (Kreutzer *et al*. 1991). In their original description of *E. herreri* Zeledon *et al*. (1977) reported that it produced amastigotes in tissue culture and hamster but they did not give details of the cells used nor if lesions were produced in hamsters.

Our phylogenetic analysis shows deep rooted and well-supported differences within the heteroxenous parasites confirming that *Endotrypanum* is a valid genus within the emended family Leishmaniinae. It follows that it should not be synonymized with the genus *Leishmania*.

Leishmania (Mundinia) *n. subgenus* Shaw, Camargo and Teixeira 2016

Type species: Leishmania (*Mundinia*) *enriettii* Muniz and Medina, 1948

Host: Domestic Guinea-pig (*Cavia porcellus*); Type Locality: Curitiba, Parana State, Brazil

Diagnosis: gGAPDH and HSP70 gene sequences deposited in GenBank (Table S1).

Molecularly validated species: *L.* (*M.*) *enriettii*, *L.* (*M.*) *martiniquensis*, a parasite of the red Kangaroo

Etymology: The name *Mundinia* is in honour of Muniz (Mun) and Medina (din) who discovered and named this parasite.

Comments on nomenclature: In 1995, a paper was published (Dedet *et al*. 1995) reporting the presence of a very unusual trypanosomatid isolated from a case of diffuse cutaneous leishmaniasis in an HIV positive patient. Initially, it was thought to be a monoxenous trypanosomatid, but a phylogenetic analysis (Noyes *et al*. 2002) showed that it belonged to the *L. enriettii* clade. This parasite received the name *Leishmania martiniquensis* (Desbois *et al*. 2014). Subsequently cytochrome b studies (Asato *et al*. 2009) confirmed the uniqueness of the *L. enriettii* clade.

More enigmatic parasites associated with leishmaniasis in cows and horses were discovered in Europe and the United States (Müller *et al*. 2009; Lobsiger *et al*. 2010; Reuss *et al*. 2012). Based on ITS1 rDNA analyses the aetiological agents of these infections were identified as *L. siamensis* but no parasites were isolated. Unfortunately, the specific name *siamensis* is a **nomem nudum** as it was not described in accordance with Article 13 of the International Code of Zoological Nomenclature. As such this name becomes unavailable. The authors (Sukmee *et al*. 2008) used the wording 'suspected new species' and did not include the name *siamensis* in their 2008 publication. The name first appears as *Leishmania* sp. *siamensis* in GenBank (entries EF200012 and EF200011), and the next in a publication a year later when material from horses was considered to be close to *Leishmania* sp. *siamensis* (Müller *et al*. 2009). Since then the name has been extensively used in the literature and at the time of writing 18 citations were found in a PubMed search using *Leishmania siamensis*. In 2012, a parasite from the Trang region of Thailand was found to be phylogenetically closest to *L. enriettii* (Bualert *et al*. 2012). A recent ITS1 analysis (Pothirat *et al*. 2014) showed that sequences previously identified as *L. siamensis* were identical to those of *L. martiniquensis*. So besides being a **nomem nudum** *L. siamensis* becomes a synonym of *L. martiniquensis*. This ITS1 rDNA analysis also showed that a yet unnamed kangaroo *Leishmania* (Rose *et al*. 2004) grouped with *L. martiniquensis*, and that an isolate previously identified as *L. siamensis* from Trang Thailand grouped with *L. enriettii*. These same workers (Pothirat *et al*. 2014) showed that these two groups formed a monophyletic *L. enriettii* complex based on RNA pol II sequences (Pothirat *et al*. 2014). The main difference between the ITS1 (the most polymorphic sequences) and RNA Pol II analyses was

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that in the latter both the kangaroo *Leishmania* and Trang isolate (MHOM/TH/2010/PCM2) grouped with *L. enriettii*. This indicates that at least two different parasites are responsible for leishmaniasis in Thailand. One is *L. martiniquensis* and the other remains to be named. Recently another species of this subgenus has been described from Ghana (Kwakye-Nuako *et al*. 2015) that is phylogenetically closer to *L. enriettii* than to *L. martiniquensis*.

Unfortunately, the specific name *australiensis* is not available for the Australian red kangaroo parasite. The name was used in a recent review paper (Akhoundi *et al*. 2016) without any formal description. The name was written as '*L. australiensis*'. The use of inverted commas does not change the validity of a name. So *L. australiensis* is a **nomem nudum.**

The growing reluctance to formally name parasites in accordance with the International Code of Zoological Nomenclature that are obviously distinct or considered to be distinct by their authors is frustrating. The cases of *L. siamensis* and *L. australiensis* have different motives, but clearly the use of the specific name helps authors communicate. We have refrained from correctly naming these two parasites as clearly this should be done by the researchers involved in their discovery. But it is their apparent indisposition to name them that has led to the present nomenclature problems. A species name is essential in following a parasite in the literature and draws attention to the parasite in question. If later studies show that it is the same as another previously described parasite the name will become a subspecies or synonym. A classical case was the discussion as to whether *L.* (*L.*) *chagasi* should be called *L.* (*L.*) *infantum*. The general conclusion was the *L.* (*L.*) *chagasi* should no longer be considered as a species but as a subspecies of *L.* (*L.*) *infantum*. However, the work that led up to this generated data that was important in understanding the epidemiology of visceral leishmaniasis in the Americas.

Our Hsp70 and gGPDH analyses (Fig. 2) also show that *L. martiniquensis, L. enriettii* and the kangaroo *Leishmania* are distinct species that form a monophyletic group within the genus *Leishmania*. The above data strongly supports our proposal that this group previously called the *L. enriettii* complex should be given sub generic status within the genus *Leishmania*.

The type species of the subgenus is *Leishmania* (*Mundinia*) *enriettii* Muniz and Medina, 1948. Its vector is unknown but biting midges (Diptera, Ceratopogonidae) have been incriminated as vectors of the kangaroo parasite and *L.* (*M.*) *enriettii* (Dougall *et al*. 2011; Seblova *et al*. 2015). It is possible that biting midges may also prove to be vectors of the subgenus *L.* (*Mundinia*). A monoxenous trypanosomatid isolated from the midgut of naturally infected biting midges was classified as *Sergeia podlipaevi*, which is closely related to the Trypanosomatidae EVA from the sand fly *Lutzomyia evansi* from South America (Svobodová *et al*. 2007). In addition, the monoxenous trypanosomatids *Herpetomonas trimorpha* and *H. zitplika* were also isolated from biting midges (Podlipaev *et al*. 2004; Zidkova *et al*. 2010). Both *Sergeia* and *Herpetomonas* exhibited promastigote in cultures and were placed in phylogenetic trees distant from Leishmaniinae. *Novymonas esmeraldas* was also detected in biting midges (Kostygov *et al*. 2016). Finally, a trypanosomatid was reported in a fossil ceratopogonid from the early cretaceous (Poinar, 2008).

The lack of genetic variation within isolates identified by comparison of their ITS1 sequences as *L.* (*M.*) *martiniquensis* form very different geographical regions of the world is intriguing (Pothirat *et al*. 2014) and needs to be investigated in greater detail.

Taxonomic summary

The revised classification and nomenclature of Leishmaniinae parasites, some of which are associated with leishmaniasis in man.*

Phylum Euglenozoa Cavalier-Smith, 1981

Class Kinetoplastea Honigberg, 1963 emend., Vickerman, 1976

Subclass Metakinetoplastina Vickerman, 2004

Order Trypanosomatida Kent, 1880 stat. nov. Hollande, 1952

Family Trypanosomatidae Doflein, 1951

Subfamily Leishmaniinae, Maslov & Lükes 2012 emend Shaw, Teixeira and Camargo 2016

Type species of the genus *Leishmania donovani* (Laveran & Mesnil 1903)

Genus Leishmania *Ross 1908*

Subgenus L. (Leishmania) *Safjanova 1982*

Type species: *Leishmania* (*Leishmania*) *donovani* (Laveran Mesnil 1903)

L. (*L.*) *donovani* * (Laveran Mesnil 1903); *L.* (*L.*) *infantum* (Nicolle 1908); *L.* (*L.*) *archibald* * Castellani and Chalmers 1919; *L.* (*L.*) *tropica* * (Wright, 1903) Lühe, 1906; *L.* (*L.*) *aethiopica* *Bray, Ashford Bray, 1983; *L.* (*L.*) *major* * Yakimoff & Schkolor; *L.* (*L.*) *arabica* * Peters *et al*. 1986; L. (L.) *turanica* Strelkova *et al*. 1990; *L.* (*L.*) *gerbilli* Wang *et al*. 1964; L. (*L.*) *mexicana* Biagi, 1953 emend Garnham 1962; *L.* (*L.*) *amazonensis* * Lainson & Shaw 1982; *L. (L.) aristidesi* Lainson and Shaw, 1979; *L.* (*L.*) *venzuelensis* * Bonfante-Garrido 1980; *L.* (*L.*) *pifanoi* * Medina & Romero 1959, emend Medina & Romero, 1962; *L.* (*L.*) *waltoni* * Shaw, Pratlong & Dedet 2015; specific status requires confirmation *L.* (*L.*) *garnhami* * Scroza *et al*. 1979; *L.* (*L.*) *forattinii* Yoshida *et al*. 1993.

Subgenus L. (Viannia) Lainson and Shaw, 1987

Type species: *Leishmania* (*Viannia*) *braziliensis* * Vianna, 1911, emend Matta 1916

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L. (*V.*) *braziliensis* * Vianna, 1911; *L.* (*V.*) *peruviana* * Velez, 1913; *L.* (*V.*) *guyanensis* * Floch, 1954; *L.* (*V.*) *panamensis* * Lainson & Shaw, 1972; *L.* (*V.*) *lainsoni* * Silveira *et al.* 1987; *L.* (*V.*) *shawi* * Lainson *et al.* 1989; *L.* (*V.*) *naiffi* * Lainson & Shaw, 1989; *L.* (*V.*) *lindenbergi* * Silveira *et al.* 2002; *L.* (*V.*) *utingensis* Braga *et al.* 2003.

Subgenus L. (Sauroleishmania) Ranque 1973 *emend.* Safyanova 1982

Type species: *L.* (*Sauroleishmania*) *L. (S.) tarentolae* Wenyon 1921

L. (*S.*) *adleri* Heisch 1954; *L.* (*S.*) *agamae* David 1929; *L. (S.) ceramodactyli* Adler & Thoedor 1929; *L. (S.) davidi* Strong 1924; L. (*S.*) *gulikae* Ovezmuchammedov & Safjanova 1987; *L. (S.) gymnodactyli* Khodukin & Sofiev 1929; L. (S.) *helioscopi* Chodukin & Sofieff 1940; *L.* (*S.*) *hemidactyli* Mackie *et al.* 1923; *L.* (*S.*) hoogstraali McMillan 1965; *L.* (*S.*) *nicollei* Chodukin & Sofieff 1940; *L.* (*S.*) *phrynocephali* Chodukin & Sofieff 1940; *L.* (*S.*) *platycephala* Telford 2008; *L.* (*S.*) *senegalensis* Ranque 1973; *L.* (*S.*) *sofieffi* Markov *et al.* 1964; *L.* (*S.*) *tarentolae* Wenyon 1921; *L.* (*S.*) *zmeevi* Andruchko & Markov 1955; *L.* (*S.*) *zuckermani* Paperna *et al.* 2011; *L.* (*S.*) sp., I Telford 1979; *L.* (*S.*) sp., II Telford 1979.

L. (*S.*) *henrici* † Leger 1918; *L.* (*S.*) *chamaeleonesis* † Wenyon 1921: † Most probably intestinal flagellates as parasites were in the cloaca

Subgenus L. (Mundinia) Shaw, Camargo and Teixeira 2016

Type species: *L.* (*Mundinia*) *enriettii* (Muniz and Medina, 1948)

L. (*M.*) enriettii (Muniz and Medina, 1948) *L.* (*M.*) *martiniquensis* * (Desbois *et al.* 2014; Syn. *L. siamensis*), *L.* (*M.*) spp. Kangaroo [MMAC/AU/2004/Roo1]; *L.* (*M.*) spp. * Ghana [MHOM/GH/2012/GH5 (LV757)]; *L.* (*M.*) spp. * Trang, Thailand [MHOM/TH/2010/PVM2].

Genus Porcisia Shaw, Camargo and Teixeira 2016

Type species: *Porcisia hertigi* (Herrer, 1971)

P. hertigi (Herrer, 1971); *P. deanei* (Lainson and Shaw, 1977 emend., Lainson and Shaw, 1987)

Genus Endotrypanum Mesnil and Brimont, 1908

Type species: *Endotrypanum schaudinni* Mesnil and Brimont, *1908*

E. schaudinni Mesnil and Brimont, 1908; *E. monterogeii* Shaw, 1969; *E. colombiensis* * (Kreutzer *et al.*1991); *E. equatorensis* (Grimaldi Junior *et al.*1992); *E. herreri* (Zeledon, Ponce, Murillo,1979)

Genus Zelonia Shaw, Camargo and Teixeira 2016

Type species: *Z. costaricensis* (Yurchenko *et al.* 2006)

Z. costaricensis, strain G755 (Noyes *et al.* 2002), TCC169E, 504 and 2696 (present study)

Genus Novymonas Kostygov and Yurchenko 2016

Type species: *Novymonas esmeraldas* Votýpka, Kostygov, Maslov and Lukeš 2016

N. esmeraldas

Concluding remarks

Up to now the phylogenetic robustness of the Leishmaniinae has been based on the use of a limited number of gene sequences. Recently Harkins *et al.* (2016) used a bioinformatics' method employing 200 000 informative sites across the genomes of 13 species of Leishmaniinae that included three *L.* (*Leishmania*) species, 5 *L.* (*Viannia*) species, *L.* (*S.*) *adleri, L.* (*M.*) *enriettii*, two *Porcisia* species and *Endotrypanum*. The validity of the genera *Porcisia* and *Endotrypanum* is supported by Harkins *et al.* (2016) who showed that their origin predated that of the four *Leishmania* subgenera. These same authors showed that using a relaxed molecular clock that dixenous leishmanine radiation began around 80 mya. This agrees with a previous hypothesis (Shaw, 1997) that had suggested that a monoxenous insect flagellate established itself in mammals some 90 mya and that after this the now dixenous line radiated in the evolving mammalian orders. In essence, this means that the leishmanine trypanosomatids evolved in mammals from an insect parasite and not from a reptilian parasite, in agreement with phylogenetic inferences (Croan *et al.* 1997; Noyes *et al.* 2002; Pothirat *et al.* 2014; Harkins *et al.* 2016).

For many years, leishmaniasis' vectors worldwide were considered to be *Phlebotomus* sand flies. This position became untenable as the true genetic diversity of the subfamily Phlebotominae became evident. It is now accepted that, with perhaps the exception of *L.* (*Mundinia*) parasites, the leishmaniasis' vectors are phlebotomine sand flies and not *Phlebotomus* sand flies. Similarly, our greater understanding of the genetic complexity of the parasites causing leishmaniasis means that it is more correct to say that the leishmaniases are caused by leishmanine parasites rather than *Leishmania* parasites.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at https://doi.org/10.1017/S0031182016002092

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