



Endangered monoxenous trypanosomatid parasites: a lesson from island biogeography

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Abstract

Most remote and oceanic islands are important, yet highly vulnerable biodiversity hotspots, which host a significant proportion of endemic species. Along with iconic endangered or extinct animals and plants, the disappearance of their co-inhabitants, including protist parasites, gets usually unnoticed from the conservation perspective. Here, we examined insects from Madagascar, Reunion, and Mauritius for the presence of trypanosomatid parasites (Kinetoplastea). Out of 660 specimens of the true bugs (Heteroptera) belonging to 87 species and 18 families, 95 individuals of 30 species were found to be infected (14% prevalence) by at least one trypanosomatid species, here referred to as typing units (TUs). Out of 141 flies (Diptera), 19 (13%) were infected. High diversity of the host species correlated with a high diversity of detected TUs belonging to 11 trypanosomatid genera, and representatives of 7 genera (*Angomonas*, *Blastocrithidia*, *Herpetomonas*, ‘*jaculum*’, *Leptomonas*, *Wallacemonas*, and *Zelonia*) yielded axenic cultures. Of 39 detected TUs, more than half have not been encountered in other geographical regions and appear to be endemic. Altogether, 27 TUs, including 15 newly detected ones, were found exclusively in bugs, while flies hosted 11 TUs, out of which five were found exclusively on the studied islands. Only a single species, *Leptomonas moramango*, was found in both insect groups. Several new isolates have significantly extended the diversity of the plant-pathogenic *Phytomonas*. Geographically widespread as well as endemic TUs were detected in both widely distributed and (sub)endemic insects. The high proportion of endemic TUs suggests that the prominent role of islands in the global diversity of macroscopic organisms likely extends also to their protistan parasites and that the protection of macro-organisms in biodiversity hot spots can also protect the vast, yet mainly invisible, diversity of their parasitic companions.

Keywords Biodiversity loss · Conservation · Endemic · Host specificity · Coevolution · *Phytomonas* · Heteroptera

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Extended author information available on the last page of the article

Introduction

The overwhelming majority of conservation efforts is focused on vertebrate animals and/or “higher” plants (see IUCN Red Data List, <https://www.iucnredlist.org/>), despite the already indisputable fact that from the extant eukaryotic groups, insects and protists are the most diverse ones (Novotný et al. 2006; Pawlowski et al. 2012). Eukaryotes with the parasitic lifestyle account for a large proportion of the overall diversity and play a major regulatory role in most ecosystems (Poulin 2014; Rocha et al. 2016). When a broader definition of this lifestyle is considered, parasites, somewhat counterintuitively, are a dominant group in terms of biodiversity, actually representing over half of all the species inhabiting our planet (Windsor 1998; Okamura et al. 2018). Still, they remain unjustifiably neglected by conservation biologists.

However, the ubiquity of parasites and their overwhelming diversity does not inform us about the vanishing species and those threatened with extinction. Unlike free-living organisms, most of them live in hiding, as the ecosystem for parasites is the body of their hosts. While the host specificity is, when trypanosomatids are concerned, not as high as previously believed (Grybchuk-Ieremenko et al. 2014; Kozminsky et al. 2015; Votýpka et al. 2015), extinction of a host species still poses a serious danger to its parasites. Although the term coextinction was introduced to describe this situation (Stork and Lyal 1993), very little data is available to assess its extent (Gómez and Nichols 2013). For example, it is estimated that a significant number of feather mites, lice, and ticks, which are highly host-specific parasites, have disappeared as a result of the extinction of their mammalian and bird hosts (Mihalca et al. 2011; Buckley et al. 2012; Rózsa and Vas 2015), and that along with extinct fishes and plants, their specific ectoparasites, primarily helminths and gall-forming or mining insects, have vanished (Dobson et al. 2008; Gómez and Nichols 2013). In a similar vein, one can safely assume that also many endoparasites, such as the highly host-specific monoxenous coccidia of the genera *Eimeria* and *Isospora*, were exterminated along with their hosts.

Due to specific features of the parasitic lifestyle, complex transmission modes and epidemiological “regularity”, parasites may get extinct when the population density of their hosts is reduced below a certain threshold. Indeed, since parasitic species tend to decline at a faster rate than their hosts, even when a limited population of the latter survives, their parasites may already be gone (Dougherty et al. 2015; Strona 2015). Similarly, yet as an even more extreme example—if a host species disappears (albeit temporarily) from natural habitat and is maintained in rescue centers or zoos, its parasites may already be exterminated by drugs and/or breeding conditions (Gómez and Nichols 2013; Dougherty et al. 2015; Rózsa and Vas 2015).

Are parasites threatened or threatening? This is an unusual but completely legitimate issue that we should be addressing in more detail than previously. Although parasitic species may appear to be innumerable, many of them are (critically) endangered or extinct. Hence, the unique parasitofauna together with various commensals and endo/ectosymbionts (Windsor 1995; Yurchenko and Lukes 2018; Dheilly et al. 2019) should be protected along with their hosts. As such, parasites represent neglected wildlife and their diversity should be perceived as a conservation target (Gómez and Nichols 2013).

General appreciation of parasite diversity is inevitably biased by research on macroscopic parasites, such as helminths that infect terrestrial vertebrate hosts (Strona and Fattorini 2014; Carlson et al. 2020). Since protists parasitizing hosts with limited geographic occurrence remained largely neglected, in this study we have focused on the

occurrence of members of the family Trypanosomatidae infecting insects on the remote and oceanic islands. This was done to assess their geographic occurrence and host specificity and, consequently, their rarity and potential risk of extinction.

Since the formulation of the theory of island biogeography (MacArthur and Wilson 1967), the extreme biological diversity of remote islands has long stimulated research in ecology and evolution, with the islands being widely viewed as model systems for studying fundamental principles in ecology, evolutionary biology and biogeography (Emerson 2002; Warren et al. 2015; Graham et al. 2017). Remote islands host very vulnerable ecosystems that often lose a large part of their wildlife. Long isolation from mainland terrestrial ecosystems produced fauna and flora that display a high degree of endemism; however, island wildlife is generally badly affected by human activities including agriculture, destruction of ecosystems, and the introduction of alien invasive species. A rapid decline of many native species has led to numerous extinctions, including the most famous one—that of the flightless dodo of Mauritius (Turvey and Cheke 2008).

The majority of trypanosomatid flagellates is represented by monoxenous species restricted to the insect hosts (Maslov et al. 2013; Votýpka et al. 2015; Lukeš et al. 2018). Within the last decade, many new species have been described and several new genera and subfamilies of monoxenous trypanosomatids have been erected, overhauling the taxonomy of this speciose group, and substantially increasing our knowledge about their prevalence, diversity, pathogenicity, geographic distribution, as well as their intracellular symbionts (Kostygov et al. 2016; Barratt et al. 2017a, b; Grybchuk et al. 2018; Lukeš et al. 2018; Maslov et al. 2019). Examination of environmental samples from primarily heteropteran but also dipteran hosts collected worldwide seems to show initial signs of saturation (d’Avila-Levy et al. 2015). As a corollary, the available information allows us to characterize, for the first time, general rules regarding the distribution of trypanosomatids. Several studies focusing on their occurrence on islands have recently been published (Králová et al. 2019; Votýpka et al. 2019), allowing to address the island biogeography theory from the perspective of these protistan parasites. The current study analyzes their occurrence on the large remote island of Madagascar and two smaller oceanic islands in its distant neighborhood, Reunion and Mauritius.

Materials and methods

Localities, collection of insects and cultivation

The study was conducted on three remote islands of the southwestern Indian Ocean, namely Madagascar and the two largest of the Mascarene Islands, Reunion and Mauritius (Fig. S1). Madagascar, the fourth largest island in the world, is approximately 400 km off the southeast coast of Africa. Reunion is 700 km east of Madagascar and 175 km southwest of Mauritius. The habitats of these islands significantly differ in size, topography, rainfalls, geographic history, human settlements, and proximity to the nearest major landmass. Insects, predominantly true bugs (the suborder Heteroptera) and flies (Diptera: Brachycera), were captured in March 2010 in Madagascar and in March 2017 in Reunion and Mauritius. In Madagascar, seven different localities (Morondava village 20°18'2.82"S, 44°16'17.93"E; Ambatolampy 19°22'58.38"S, 47°25'57.86"E; Ambatofosty 19°16'46.79"S, 47°28'44.47"E; Moramango/Andasibe 18°55'33.89"S, 48°25'4.33"E; Mahambo 17°28'38.49"S, 49°27'50.84"E; Foulpointe (Mahavelona) 17°40'54.15"S, 49°30'9.80"E; Ivoloina Zoological Park (Toamasina) 18°3'32.95"S, 49°21'31.65"E), in

Reunion five localities (Cilaos 21°9′23.59″S, 55°28′15.07″E; Takamaka 21°4′40.98″S, 55°37′41.11″E; Salazie 21°0′44.97″S, 55°33′35.50″E; Saint-Denis 20°56′38.31″S, 55°28′49.52″E; Mafate Cirque—Dos d’Ane 20°59′21.13″S, 55°22′52.69″E), and one site in Mauritius (Vanille 20°30′15.10″S, 57°34′19.80″E) were inspected (Fig. S1).

Insects were collected predominantly by net sweeping from the vegetation, but also by manual picking or by light attraction to local light sources. Within 24 h after capture, the insects were processed as described in detail elsewhere (Lukeš and Votýpka 2020).

Host insect identification

To facilitate host identification, most specimens were dry-mounted and deposited to the collections of the Department of Entomology, National Museum, Prague, Czech Republic. When appropriate, the material was sent for identification to specialists on particular groups (see “[Acknowledgements](#)” section). The remaining specimens were compared with available taxonomic revisions and/or the collections of Muséum nationale d’Histoire naturelle, Paris, France, and The Natural History Museum, London, UK. Based on the available catalogs and revisions, the distribution of species and genera of the insect host was sorted into the following three categories: (i) endemic for the particular island; (ii) sub-endemic, distributed in Madagascar, and the Mascarene Islands, the Comoro Islands, and Seychelles (or at least some of the islands); (iii) widely distributed taxa, including other biogeographic realms, such as the studied island(s) plus continental Africa, and/or beyond.

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from the insect gut tissue, or in the case of successful cultivations, from 1 ml of axenically grown cultures, as described previously (Kostygov et al. 2014; Votýpka et al. 2014), using either a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) or a DNA tissue isolation kit (Roche Diagnostics, Indianapolis, USA) according to the manufacturers’ protocols.

The extracted DNA was used for amplification of the 18S rRNA gene with primers S762 and S763, producing an almost full-length gene (~ 2.1 kb), as described previously (Maslov et al. 1996). When amplification with the first primer pair resulted in a weak or invisible PCR product, which occurs frequently for DNA samples extracted from the insect tissues, the second round of PCR was performed with nested primers TRnSSU-F2 and TRn-SSU-R2 (Seward et al. 2017). The obtained amplicons were directly sequenced, the sequences were aligned using Geneious software (version 10.0.6), and phylogenetic trees were inferred using related sequences available in GenBank. Alignments for phylogenetic analyses were generated by MAFFT v7, with ambiguously aligned positions in the trimmed alignment being removed manually in Geneious. The final dataset contained 398 taxa (Table S2) and 2484 characters. Analyses were performed in PhyML v3.0.1 and MrBayes v3.2.2 with model optimization in ModelTest v3.06. A general time-reversible substitution model with a mixed model for among site rate variation (GTR + Γ + I) was chosen as the best fitting model of sequence evolution. Bootstrap analyses involved heuristic searches with 1000 replicates (Maximum likelihood). Bayesian inference analysis was run for five million generations with covarion and sampling every 100 generations. All other parameters were left in their default states.

Results and discussion

Field examination of insect hosts for trypanosomatid infection

Altogether, 660 specimens of heteropteran bugs belonging to 87 species and 18 families were captured and inspected for the presence of trypanosomatid flagellates in their gut (Table 1). Out of them, 95 (overall prevalence 14%) specimens belonging to 30 species (35%) and 8 (45%) families were infected by these parasites (Tables 1, 2 and S1; Fig. S1). Out of 57 trypanosomatid-free species, a significant fraction (52 species; 91%) was represented by only a very small number of dissected individuals, mostly less than five (Table 1). More than 10 specimens were inspected for the following trypanosomatid-free species: *Deraeocoris howanus* (14 individuals), *Pachygrontha angularis* (23), *Pachygrontha* sp. (18), *Coptosoma depulsa* (19), and *Diniella marginata* (28) (Table 1). The overall prevalence of heteropteran bug infection on the studied islands (14%) was similar to that in China (16%) and Papua New Guinea (15%), but significantly lower when compared to 26–38% prevalence documented in Africa and the Neotropics (Maslov et al. 2007; Votýpka et al. 2010, 2019; Jirků et al. 2012; Kozminsky et al. 2015; Lukeš et al. 2018; Králová et al. 2019). However, these differences may be explained not only by different geographic origin but also by varying number of dissected bugs within various heteropteran and dipteran families.

Most individuals were caught and subsequently examined in the island of Madagascar. Out of 438 heteropterans, which were determined to belong to 68 species and 16 families, 52 specimens belonging to 21 species were infected. In Reunion, out of 173 dissected specimens (22 species; 9 families), 33 individuals (10 species) were infected, and in Mauritius out of 49 bugs (10 species; 7 families), 10 (6 species) were found to be parasitized (Tables 1 and S1; Fig. S1). On the higher taxonomic level, the prevalence of trypanosomatids in heteropteran families is similar to the situation observed on the species level—out of 18 inspected families, only 8 were trypanosomatid-positive: Alydidae (10 positive specimens out of 52 examined, 19%), Coreidae (25/76, 33%), Lygaeidae (18/95, 19%), Miridae (15/82, 18%), Pachygronthidae (1/57, 2%), Pentatomidae (18/133, 16%), Rhopalidae (7/66, 11%), and Rhyparochromidae (2/43, 5%).

This is in good correlation with studies from other geographic areas, where Alydidae, Coreidae, Lygaeidae, Pentatomidae, and Rhopalidae showed a prominently high prevalence of infection (Votýpka et al. 2010, 2012a, b, 2019; Králová et al. 2019). In the current study, however, two true bug families, the Reduviidae and Pyrrhocoridae, elsewhere found to be heavily infected by trypanosomatids (Votýpka et al. 2010, 2012a, b; Kozminsky et al. 2015; Králová et al. 2019), are missing from the list of parasite-positive taxa. Still, this may reflect the fact that reduviids and pyrrhocorids were underrepresented in the present study.

The behavior of many species of the families Pyrrhocoridae, Lygaeidae, and Rhyparochromidae are gregarious and due to their feeding on seeds on the ground, there is a higher probability of them being contaminated by infective stages present in insect feces, acquired during co-feeding. These families are also well-known for accidental coprophagy and/or necrophagy. In contrast to the phytophagous seed-feeding or sap-sucking species, predatory bugs such as Reduviidae, Gerridae, Hydrometridae, and Nabidae may acquire, in addition to their own parasites, less specific monoxenous trypanosomatids from their prey and thus may represent a kind of “collection container” for parasites occurring in their vicinity. Indeed, this is what was observed when the heteropteran-trypanosomatid relationship was inspected on several continents (Votýpka et al. 2010, 2012a, b; Kozminsky

Table 1 Summarized information about true bug (Heteroptera) hosts from Madagascar, Reunion, and Mauritius and their trypanosomatids (including the insect species distribution, the prevalence of parasites (number of dissected/infected specimens), and the list of detected trypanosomatids (typing units (TUs) and genera/species)

Family	Species	Genus	Species	MG	REU	MU	TUs	Genus/species
Alydidae	<i>Nariscus</i> sp.	WIDE	?		1/1		*TU217	' <i>jaculum</i> ' sp.
	<i>Stenocoris annulicornis</i>	WIDE	WIDE	1/28		1/2	*TU6/7AB2	' <i>jaculum</i> ' sp.
	Alydinae gen. sp.	WIDE	?	3/12			*TU218	' <i>jaculum</i> ' sp.
							*TU216	' <i>jaculum</i> ' sp.
							*TU6/7D2	' <i>jaculum</i> ' sp.
	<i>Mirperus jaculus</i>	WIDE	WIDE	1/2			TU88b	' <i>jaculum</i> ' sp.
							TU6/7D	' <i>jaculum</i> ' sp.
	<i>Riptortus fabricii</i>	WIDE	ENDE	3/5			TU6/7E	' <i>jaculum</i> ' sp.
							TU6/7C	' <i>jaculum</i> ' sp.
Belostomatidae	<i>Tenosius proletarius</i>	WIDE	WIDE	0/2				
	<i>Appasus quadrivittatus</i>	WIDE	ENDE	0/2				
Coreidae	<i>Clavigralla madagascariensis</i>	WIDE	ENDE	4/6			TU6/7C	' <i>jaculum</i> ' sp.
							*TU248	<i>Phytomonas</i> sp.
	<i>Cletoscellus spinijugis</i>	ENDE	ENDE	0/2				
	<i>Cletus capensis</i>	WIDE	WIDE		5/14	1/4	TU72	' <i>jaculum</i> ' sp.
	<i>Cletus clavatus</i>	WIDE	SUBE	0/1				
	<i>Cletus ochraceus</i>	WIDE	WIDE	0/1	7/18	0/3	TU32	<i>L. spiculata</i>
							TU72	' <i>jaculum</i> ' sp.
							TU48A	<i>L. podlipaevi</i>
	<i>Cletus poikilus</i>	WIDE	ENDE	0/1				
	<i>Cletus presignus</i>	WIDE	ENDE	2/2			*TU218	' <i>jaculum</i> ' sp.
	<i>Hydara tenuicornis</i>	WIDE	WIDE	6/11	0/1		TU6/7C	' <i>jaculum</i> ' sp.
	<i>Mevanidea spiniceps</i>	WIDE	ENDE	0/2				

Table 1 continued

Family	Species	Genus	Species	MG	REU	MU	TUs	Genus/species
Gerridae	<i>Limnogonus guttatus</i>	WIDE	ENDE	0/6				
	<i>Limnogonus cereiventris</i>	WIDE	WIDE	0/6				
	<i>Tenagometra lanuginea</i>	WIDE	ENDE	0/2				
	<i>Geocoris</i> sp. nov	WIDE	ENDE		0/1			
Geocoridae	<i>Hydrometra bifurcata</i>	WIDE	SUBE	0/6			TU88	'jaculum' sp.
Hydrometridae	<i>Aspillocoryphus unimaculatus</i>	WIDE	WIDE	2/2			TU88b	'jaculum' sp.
	<i>Caenocoris croceosignatus</i>	WIDE	WIDE	0/1				
Lygaeidae	<i>Nysius albipennis/euphorbiae</i>	WIDE	SUBE	0/15	6/41	1/21	*TU251 TU32	<i>Phytomonas</i> sp. <i>L. spiculata</i>
	<i>Spilostethus furculus</i>	WIDE	WIDE	1/6			TU72	'jaculum' sp.
	<i>Spilostethus panduratus</i>	WIDE	WIDE	7/7			TU88b	'jaculum' sp.
	<i>Stalagmosethus furcatus</i>	WIDE	WIDE	1/2			TU88b	'jaculum' sp.
							*TU214	<i>Zeltonia</i> sp.
							TU88b	'jaculum' sp.

Table 1 continued

Family	Species	Genus	Species	MG	REU	MU	TUs	Genus/species
Miridae	<i>Collaria bourbonica</i>	WIDE	ENDE		0/1			
	<i>Corizidolon notaticolle</i>	WIDE	SUBE		0/5			
	<i>Creontiades pallidus</i>	WIDE	WIDE			0/1		
	<i>Creontiades cf. subpellucidus</i>	WIDE	WIDE	0/1				
	<i>Creontiades cf. suturalis</i>	WIDE	WIDE	0/1				
	<i>Deraeocoris howanus</i>	WIDE	SUBE		0/14			
	<i>Deraeocoris ostentans</i>	WIDE	WIDE		0/5			
	<i>Dolichomiris linearis</i>	WIDE	WIDE	0/2				
	<i>Gutrida gabonia</i>	WIDE	WIDE	0/3				
	<i>Hyalopeplus madagascariensis</i>	WIDE	ENDE	0/1				
	<i>Charagochilus</i> sp.	WIDE	?	0/1				
	<i>Probosciodocoris cf. intermedius</i>	WIDE	WIDE	1/1			*TU211	<i>Leptomonas</i> sp.
	<i>Probosciodocoris fuliginosus</i>	WIDE	WIDE	1/2	1/1		*TU212	<i>Leptomonas</i> sp.
Nabidae							*TU213	<i>Leptomonas</i> sp.
	<i>Taylorlygus apicalis</i>	WIDE	WIDE	0/5	7/28	5/8	TU32	<i>L. spiculata</i>
							*TU251	<i>Phytomonas</i> sp.
Naucoridae	<i>Taylorlygus simonyi</i>	WIDE	WIDE	0/1	0/1			
	<i>Arbela elegantula elegantula</i>	WIDE	WIDE		0/4			
	<i>Nabis capsiformis</i>	WIDE	WIDE			0/1		
Nepidae	gen. sp.	WIDE	?	0/6				
	<i>Heleocoris humeralis</i>	WIDE	ENDE	0/2				
	<i>Macrocoris distinctus</i>	WIDE	ENDE	0/5				
	<i>Naucoris madagascariensis</i>	WIDE	ENDE	0/3				
	<i>Laccotrephes pseudoampliatius</i>	WIDE	ENDE	0/1				

Table 1 continued

Family	Species	Genus	Species	MG	REU	MU	TUs	Genus/species
Pachygronthidae	<i>Pachygrontha angularis</i>	WIDE	ENDE	0/23				
	<i>Pachygrontha bipunctata</i>	WIDE	WIDE	0/6				
	<i>Pachygrontha quadripunctata</i>	WIDE	ENDE	0/3				
	<i>Pachygrontha</i> sp.	WIDE	?	0/18				
Pentatomidae	<i>Teracrius namaquensis</i>	WIDE	WIDE	1/1			TU88b	'jaculum' sp.
	<i>Aelionomorpha viridis</i>	WIDE	ENDE	0/1				
	<i>Afrius flavirostrum</i>	WIDE	ENDE	0/2				
	<i>Antestia variabilis</i>	WIDE	ENDE	0/8				
	<i>Antestiella mauritii</i>	WIDE	SUBE		1/7		TU99	<i>B. raabei</i>
	<i>Aspavia albidomaculata</i>	WIDE	WIDE	1/38			TU44	<i>Blastocrithidia</i> sp.
	<i>Aspavia longispina</i>	WIDE	ENDE	3/11			TU6/7D	'jaculum' sp.
							TU77/177-1	<i>Phytomonas</i> sp.
Plataspidae	<i>Bathycœlia</i> cf. <i>conferenda</i>	WIDE	ENDE	0/1				
	<i>Carbula frappai</i>	WIDE	ENDE	0/1				
	<i>Diploxys fallax</i>	WIDE	WIDE	0/1				
	<i>Dorycoris pavonius</i>	WIDE	WIDE	0/6				
	<i>Eysarcoris v-flavum</i>	WIDE	ENDE	3/6			TU44	<i>Blastocrithidia</i> sp.
	<i>Nezara viridula</i>	WIDE	WIDE		0/1			
	<i>Phricodus hystrix</i>	WIDE	WIDE	7/9			*TU252	<i>Phytomonas</i> sp.
	<i>Sciocoris wolffi</i>	WIDE	ENDE	1/24			TU77/177-2	<i>Phytomonas</i> sp.
	<i>Scotinophara tibialis</i>	WIDE	SUBE		0/1			
	<i>Veterna nigromarginata</i>	WIDE	ENDE	2/16			TU62	<i>Blastocrithidia</i> sp.
	<i>Coptosoma depulsa</i>	WIDE	ENDE	0/19				
	<i>Oncocephalus angulatus</i>	WIDE	SUBE	0/1				
	<i>Sphedanolestes</i> sp.	WIDE	?	0/2				
Reduviidae								

Table 1 continued

Family	Species	Genus	Species	MG	REU	MU	TUs	Genus/species
Rhopalidae	<i>Agraphopus brevicollis</i>	WIDE	ENDE	0/5				
	<i>Leptocoris hexophthalma lateralis</i>	WIDE	SUBE		0/1			
	<i>Leptocoris mutilata</i>	WIDE	WIDE		4/18		TU48A	<i>L. podlipaevi</i>
							*TU249	<i>Phytomonas</i> sp.
	<i>Liorhyssus hyalinus</i>	WIDE	WIDE		0/6	1/1	TU73	'jaculum' sp.
	<i>Stictopleurus scutellaris coquerelii</i>	WIDE	SUBE	1/32	0/3		TU105	<i>L. moramango</i>
Rhyparochromidae							TU73	'jaculum' sp.
	<i>Dieuches placidus</i>	WIDE	SUBE	0/2				
	<i>Diniella marginata</i>	WIDE	ENDE	0/28				
	<i>Gyndes capito</i>	WIDE	ENDE	0/2				
	<i>Horridipamera</i> sp.	WIDE	?		1/1		*TU250	<i>Phytomonas</i> sp.
	<i>Paromius gracilis</i>	WIDE	WIDE			0/7		
	<i>Perimeda dimidiata</i>	WIDE	ENDE	0/1				
	<i>Pseudopachybrachius reductus</i>	WIDE	WIDE	0/1				
	<i>Stigmatonotum geniculatum</i>	WIDE	WIDE					
	<i>Dicranocephalus punctipes</i>	WIDE	SUBE	0/1		1/1	*TU215	<i>Blastocrithidia</i> sp.
Stenocephalidae								

Distribution of species and genus of the insect host was sorted into the following categories: ENDE = taxon endemic for the particular island; SUBE = taxon sub-endemic, distributed in Madagascar, the Mascarene Islands, the Comoro Islands, and Seychelles; and WIDE = widely distributed taxa, including other biogeographic realms, such as the studied island(s) plus continental Africa, and/or beyond; ? = undeterminable distribution. MG, Madagascar; REU, Reunion; MU, Mauritius

Families and specimens positive for trypanosomatids are in bold; * TU newly detected in this study

Table 2 Summarized information about the detected trypanosomatids (species and/or typing unit; TUs), insect host species (including the number of infected specimens and species distribution), localization of the infection in the host intestine, the GenBank™ accession numbers of the 18S rRNA sequences determined in this study, isolate designation, and availability in culture

Trypanosomatid species	TUs	Madagascar	Reunion	Mauritius	Family
<i>Leptomonas moramango</i>	TU105	<i>Pachycerina</i> cf. <i>vaga</i>			DIPTERA
<i>Leptomonas podlipaevi</i>	TU48A	<i>Stictopleurus scutellaris coquerellii</i>	<i>Leptocoris mutilata</i> (2)		Rhopalidae
			<i>Cletus ochraceus</i>		Rhopalidae
<i>Leptomonas spiculata</i>	TU32		<i>Tayloriylgus apicalis</i> (7)	<i>Tayloriylgus apicalis</i> (5)	Coreidae
			<i>Nysius albipennis/euphorbiae</i>	<i>Nysius albipennis/euphorbiae</i>	Miridae
			<i>Cletus ochraceus</i>		Lygaeidae
<i>Leptomonas</i> sp. 1	*TU211	<i>Proboscidocoris</i> cf. <i>intermedius</i>			Coreidae
<i>Leptomonas</i> sp. 2	*TU212	<i>Proboscidocoris fuliginosus</i>			Miridae
<i>Leptomonas</i> sp. 3	*TU213	<i>Proboscidocoris fuliginosus</i>			Miridae
<i>Zelonia</i> sp. 1	*TU214	<i>Stalagmostethus furcatus</i>			Miridae
<i>Blastocrithidia raabeii</i>	TU99				Lygaeidae
<i>Blastocrithidia</i> sp.	TU44	<i>Eysarcoris v-flavum</i> (3)	<i>Antesiella mauritii</i>		Pentatomidae
		<i>Aspavia albidomaculata</i>			Pentatomidae
<i>Blastocrithidia</i> sp.	TU62	<i>Veterna nigromarginata</i> (2)			Pentatomidae
<i>Blastocrithidia</i> sp. A	*TU215				Pentatomidae
'jaculum' sp.	*TU67AB2			<i>Stigmatonotum geniculatum</i>	Pentatomidae
'jaculum' sp.	TU67C	<i>Hydara tenuicornis</i> (6)		<i>Stenocoris annulicornis</i>	Rhyparochromidae
		<i>Clavigralla madagascariensis</i>			Alydidae
'jaculum' sp.	TU67D	<i>Mirperus jaculus</i>			Coreidae
		<i>Riptortus fabricii</i>			Coreidae
		<i>Aspavia longispina</i>			Alydidae
'jaculum' sp.	*TU67D2	<i>Alydinae</i> gen. sp.			Alydidae
'jaculum' sp.	TU67E	<i>Riptortus fabricii</i>			Pentatomidae
					Alydidae
					Alydidae

Table 2 continued

Trypanosomatid species	TUs	Madagascar	Reunion	Mauritius	Family
' <i>jaculum</i> ' sp.	TU73	<i>Stictopleurus scutellaris coquerellii</i>			Rhopalidae
' <i>jaculum</i> ' sp.	TU72			<i>Liorhyssus hyalinus</i>	Rhopalidae
' <i>jaculum</i> ' sp.	TU88	<i>Aspilocoryphus unimaculatus</i>	<i>Cletus ochraceus</i> (10)	<i>Cletus ochraceus</i>	Coreidae
' <i>jaculum</i> ' sp.	TU88b	<i>Spilostethus pandurus</i> (7)	<i>Nysius albipennis/euphorbiae</i>		Lygaeidae
		<i>Spilostethus furculus</i>			Lygaeidae
		<i>Stalagmostethus furcatus</i>			Lygaeidae
		<i>Aspilocoryphus unimaculatus</i>			Lygaeidae
		<i>Teracrius namaquensis</i>			Pachygronthidae
' <i>jaculum</i> ' sp. A	*TU216	Alydinae gen. sp. (2)			Alydidae
' <i>jaculum</i> ' sp. B	*TU217				Alydidae
' <i>jaculum</i> ' sp. C	*TU218	<i>Cletus presignus</i> (2)	<i>Nariscus</i> sp.		Coreidae
		Alydinae gen. sp. (2)			Alydidae
<i>Phytomonas</i> sp.	TU77/1177-1	<i>Aspavia longispina</i> (2)			Pentatomidae
<i>Phytomonas</i> sp.	TU77/1177-2	<i>Sciocoris wolffi</i>			Pentatomidae
<i>Phytomonas</i> sp. 1	*TU248	<i>Clavigralla madagascariensis</i> (4)			Coreidae
<i>Phytomonas</i> sp. 2	*TU249		<i>Leptocoris mutilata</i>		Rhopalidae
<i>Phytomonas</i> sp. 3	*TU250		<i>Horridipamera</i> sp.		Rhyparochromidae
<i>Phytomonas</i> sp. 4	*TU251		<i>Nysius albipennis/euphorbiae</i> (6)		Lygaeidae
			<i>Tayloriylgus apicalis</i>		Miridae
<i>Phytomonas</i> sp. 5	*TU252	<i>Phricodus hystrix</i> (7)			Pentatomidae
<i>Herpetomonas muscarum</i>	N/A	<i>Chrysomya putoria</i>			DIPTERA
<i>Herpetomonas isaaci</i>	TU107	<i>Chrysomya putoria</i>			DIPTERA
<i>Herpetomonas puellarum</i>	N/A		fly (3)		DIPTERA
<i>Herpetomonas</i> sp. A	TU108	<i>Chrysomya putoria</i> + <i>Musca</i> sp.			DIPTERA

Table 2 continued

Trypanosomatid species	TUs	Madagascar	Reunion	Mauritius	Family	
<i>Herpetomonas</i> sp. B <i>Lafontella marideanei</i> <i>Wallacemonas raviniae</i> <i>Wallacemonas</i> sp. 1 'new clade II' sp. A <i>Angomonas deanei</i> <i>Kentomonas</i> sp. A	*TU221	<i>Musca</i> sp.			DIPTERA	
	N/A	<i>Chrysomya putoria</i>			DIPTERA	
	TU110	fly	fly (3)		DIPTERA	
	*TU220				DIPTERA	
	*TU253	<i>Chrysomya putoria</i> (2)	fly		DIPTERA	
	N/A	fly (2)			DIPTERA	
	*TU219		fly (3)		DIPTERA	
Trypanosomatid species	Genus	Species	Localization	Isolate for SSU	GB Acc.No.	Cult.
<i>Leptomonas moramango</i>						
<i>Leptomonas podlipaevi</i>	WIDE	ENDE	RA	MMO-09_cult	KC205990	Y
	WIDE	WIDE	MG, HG, MT	M05_cult	MT174471	Y
	WIDE	WIDE	MG	Re23_cult	MT174503	Y
<i>Leptomonas spiculata</i>	WIDE	WIDE	MG			
	WIDE	SUBE	MG	Re31_cult	MT174505	Y
	WIDE	WIDE	MG			
<i>Leptomonas</i> sp. 1 <i>Leptomonas</i> sp. 2 <i>Leptomonas</i> sp. 3	WIDE	WIDE	MG			
	WIDE	WIDE	MT	M31_envi	MT174482	
	WIDE	WIDE	MG	M32_envi	MT174483	Y
<i>Zelonis</i> sp. 1 <i>Blastocrithidia raabei</i> <i>Blastocrithidia</i> sp.	WIDE	WIDE	MG	Re13_envi	MT174502	
	WIDE	WIDE	MG	M08_cult	MT174474	Y
	WIDE	SUBE	MG	Re12_envi	MT174501	
<i>Blastocrithidia</i> sp. <i>Blastocrithidia</i> sp. A 'jaculum' sp.	WIDE	ENDE	MG	M37_envi	MT174484	
	WIDE	WIDE	MG			
	WIDE	ENDE	TMG	M07_cult	MT174473	Y
<i>Blastocrithidia</i> sp. A 'jaculum' sp.	WIDE	WIDE	MG (?)	Re36_envi	MT174507	
	WIDE	WIDE	MG/HG	Re43A_envi	MT174508	

Table 2 continued

Trypanosomatid species	Genus	Species	Localization	Isolate for SSU	GB Acc.No.	Cult.
' <i>jaculum</i> ' sp.	WIDE	WIDE	MG, (HG, MT)	M47_envi	MT174487	
	WIDE	ENDE	MG	M22_cult		Y
' <i>jaculum</i> ' sp.	WIDE	WIDE	HG	M04_cult/envi	MT174470	Y
	WIDE	ENDE	MG			
	WIDE	ENDE	MG (?)			
' <i>jaculum</i> ' sp.	WIDE	?	AMG	M21_cult	MT174478	Y
' <i>jaculum</i> ' sp.	WIDE	ENDE	MG	M26_envi	MT174480	
' <i>jaculum</i> ' sp.	WIDE	ENDE	MG, HG, MT	M05_envi	MT174472	
	WIDE	WIDE	MG	Re35_envi	MT174506	
' <i>jaculum</i> ' sp.	WIDE	WIDE	MG, (HG)	Re02_envi	MT174498	
	WIDE	SUBE	MG			
' <i>jaculum</i> ' sp.	WIDE	WIDE	MG	M09_cult	MT174475	Y
' <i>jaculum</i> ' sp.	WIDE	WIDE	AMG	M12_cult	MT174476	Y
	WIDE	WIDE	MG			
	WIDE	WIDE	MG			
	WIDE	WIDE	AMG			
	WIDE	WIDE	AMG			
' <i>jaculum</i> ' sp. A	WIDE	?	AMG	M20_cult	MT174477	Y
' <i>jaculum</i> ' sp. B	WIDE	?	MG (?)	Re01_envi	MT174497	
' <i>jaculum</i> ' sp. C	WIDE	ENDE	HG	M30_envi	MT174481	
	WIDE	?	AMG	M19/M20_cult		Y
<i>Phytomonas</i> sp.	WIDE	ENDE	AMG	M03_envi	MT174469	
<i>Phytomonas</i> sp.	WIDE	ENDE	MG	M41_envi	MT174485	
<i>Phytomonas</i> sp. 1	WIDE	ENDE	MG	M23_envi	MT174479	
<i>Phytomonas</i> sp. 2	WIDE	WIDE	MG	Re25A_envi	MT174504	
<i>Phytomonas</i> sp. 3	WIDE	?	MG	Re06_envi	MT174500	

Table 2 continued

Trypanosomatid species	Genus	Species	Localization	Isolate for SSU	GB Acc.No.	Cult.
<i>Phytomonas</i> sp. 4	WIDE	SUBE	MG	Re03_envi	MT174499	
<i>Phytomonas</i> sp. 5	WIDE	WIDE	MG			
<i>Herpetomonas muscarum</i>	WIDE	WIDE	MG	M42_envi	MT174486	
<i>Herpetomonas isaaci</i>			HG, MT	MMO-01_cult	KC205982	Y
<i>Herpetomonas puellarum</i>			?	MMO-02_cult	KC205992	Y
<i>Herpetomonas</i> sp. A			RA	MRe-01_envi	MT174494	
<i>Herpetomonas</i> sp. B			MG (?)	MMO-03_envi	MT174488	
<i>Lafontella marideanei</i>			HG	MMO-06_envi	MT174490	
<i>Wallacemonas raviniiae</i>			MG	MMO-08_envi	MT174491	
<i>Wallacemonas</i> sp. 1			HG	MMO-12_envi	MT174493	
new clade II sp. A			RA	MRe-04_cult	MT174496	Y
<i>Angomonas deanei</i>			MG	MMO-05_envi	MT174489	
<i>Kentomonas</i> sp. A			RA, HG	MMO-10_cult	MT174492	Y
			HG, RA	MRe-03_envi	MT174495	

Localization of the infection in the host intestine: *AMG* abdominal midgut, *HG* hindgut, *MG* midgut, *MT* Malpighian tubules, *RA* Rectal ampulla, *TMG* thoracic midgut
 * TU newly detected in this study. Near full-length 18S rRNA gene (SSU) obtained from cells available in axenic culture (cult) or from DNA parasites originated from insect gut (envi). Distribution of species and genus of the insect host was sorted into the following categories: ENDE = taxon endemic for the particular island; SUBE = taxon sub-endemic, distributed in Madagascar, the Mascarene Islands, the Comoro Islands, and Seychelles; and WIDE = widely distributed taxa, including other biogeographic realms, such as the studied island(s) plus continental Africa, and/or beyond; ? = unknown distribution

et al. 2015; Králová et al. 2019), yet among the captured island bug families, along with Reduviidae, other six families (Belostomatidae, Geocoridae, Hydrometridae, Nabidae, Nepidae, and Stenocephalidae) were examined only scarcely and found to be negative. The same applies to 14, 16 and 19 parasite-free specimens belonging to Gerridae, Naucoridae, and Plataspidae, respectively.

In addition to the heteropteran hosts, 141 brachyceran flies were captured and examined. Unfortunately, due to logistic problems, a detailed taxonomic determination could have been carried out only on a limited number of specimens. Dissection and microscopic inspection revealed 13 and six trypanosomatid-infected individuals out of 85 and 56 flies captured in Madagascar and Reunion, respectively.

Determination and phylogenetic analysis of trypanosomatids

Out of 114 DNA samples extracted from the intestine of infected bugs (95 specimens) and flies (19 specimens) detected under light microscope, the 18S rRNA gene was successfully amplified from 111 individuals (98% efficiency) and in all but one case, we were able to obtain nearly full-length sequences. Based on these data, 39 different TUs, including 19 TUs not seen before, belonging to 11 trypanosomatid genera of three subfamilies were documented (Table 2; Fig. 1 and S1). Since in the current classification system, six

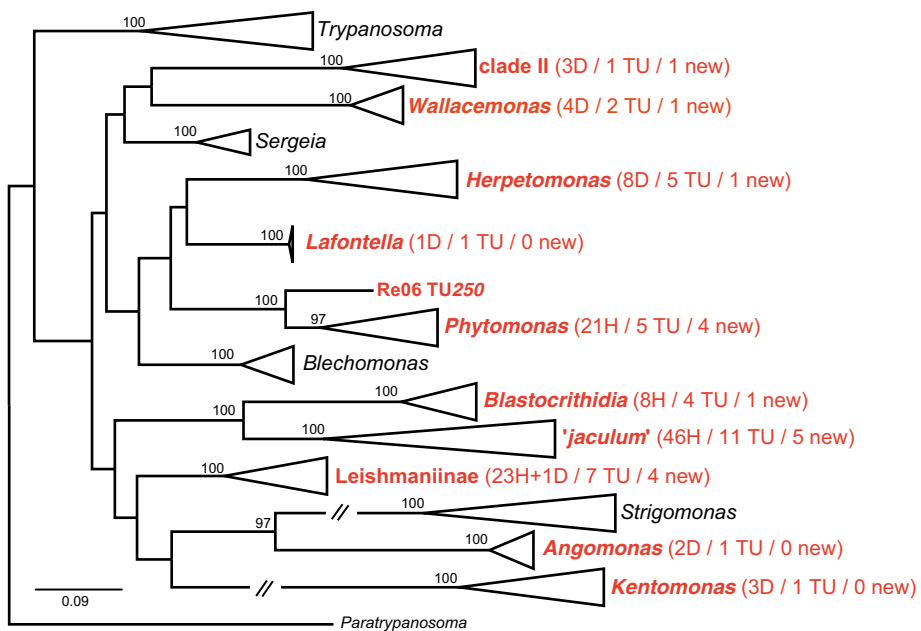


Fig. 1 Phylogenetic tree of trypanosomatids (the family Trypanosomatidae) based on the 18S rRNA gene sequences and reconstructed using the Maximum likelihood method. All genera are collapsed and their contents are shown in individual subtrees (Figs. 2, 3, 4, 5, 6). Numbers in parentheses show the total number of infected heteropterans (H) and dipterans (D), the total number of Typing Units (TU) detected in the insects captured in Madagascar, Reunion, and Mauritius, and the number of “endemic” TUs for a respective clade. The tree was rooted with the sequence of *Paratrypanosoma confusum*; the bootstrap values over 50% (1000 replicates) are shown at the nodes; the scale bar denotes the number of substitutions per site

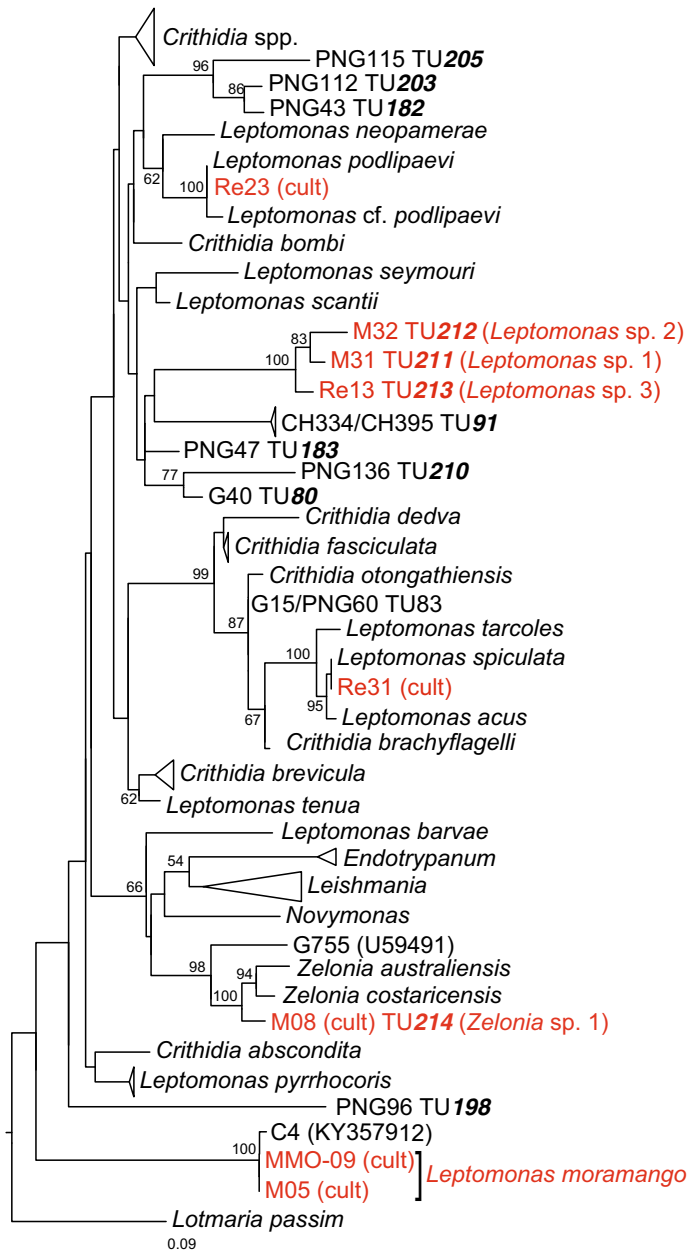


Fig. 2 Expanded subtree of the subfamily Leishmaniinae

formally recognized subfamilies and 22 genera are included in the family Trypanosomatidae (Maslov et al. 2019), this study covers half of the known trypanosomatid diversity.

In 28 specimens (18 true bugs and 10 flies) a simultaneous infection by two different trypanosomatid species was documented based on the sequences obtained from either dissected gut samples or axenic cultures derived from the same specimens (Table S1 and

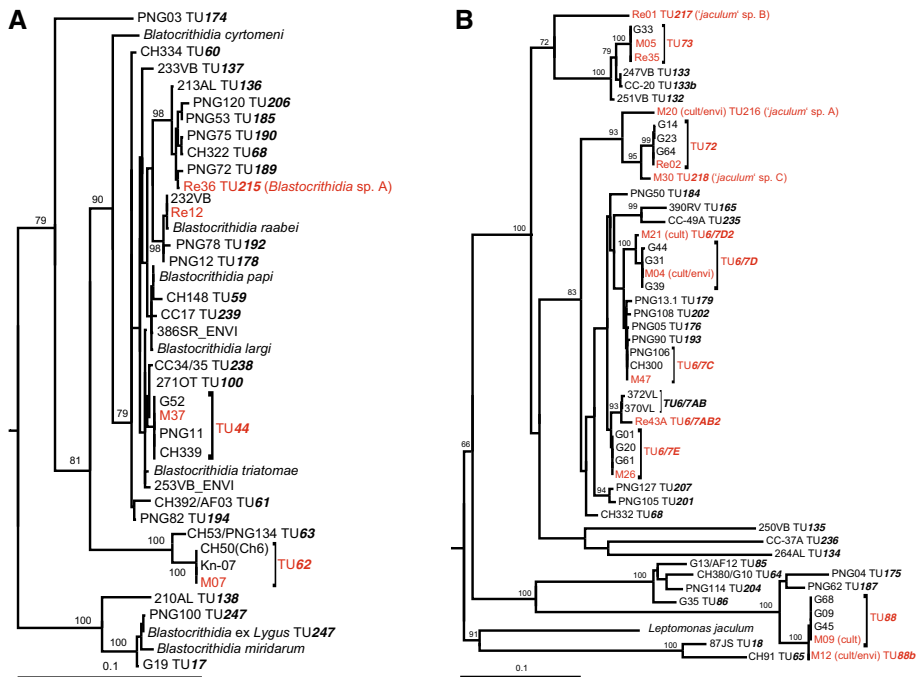


Fig. 3 Expanded subtree of the genus *Blastocrithidia* (A) and 'jaculum' phylogroup (B)

see below). However, because PCR products were not cloned in the case of mixed infections detected in DNA samples extracted from the infected guts, the analysis of chromatograms resulted in most cases in the identification of only one species.

The increasing number of monoxenous trypanosomatid species (TUs) described to date allowed us to compare their phylogeny and distribution over a wide range of host taxa and geographic areas. It was obvious that several clades are associated with particular host groups, such as the “fly” genera (*Herpetomonas*, *Lafontella*, *Wallacemonas*, *Angomonas*, *Kentomonas*, and clade II. sensu Týč et al. 2013) or the “bug” genera (*Blastocrithidia* and ‘*jaculum*’). Reference sequences were selected for all major trypanosomatid clades; for the clades/genera comprising TUs found in the current dataset, all available 18S rRNA gene sequences were used. The resulting simplified tree topology (Fig. 1) is congruent with those published previously (Týč et al. 2013; Votýpka et al. 2013, 2019; Kostygov et al. 2016; Yurchenko et al. 2016; Ishemgulova et al. 2017; Králová et al. 2019). The phylogenetic analysis did not reveal any novel taxonomic group on the generic or family levels (Fig. 1; but see below).

Although due to the logistic reasons our collections of trypanosomatids from the studied islands significantly differed when the number of captured specimens and inspected localities are considered, there was a correlation between the amount of dissected insects and their trypanosomatid diversity. In Madagascar, 26 TUs were recorded in 438 dissected bugs belonging to 68 species, supplemented by 85 examined flies. Smaller collections from Reunion yielded 12 TUs from 56 flies and 173 bugs ranked into 22 species, while five TUs were derived from 49 bugs representing 10 species originating from Mauritius (Tables 1, 2).

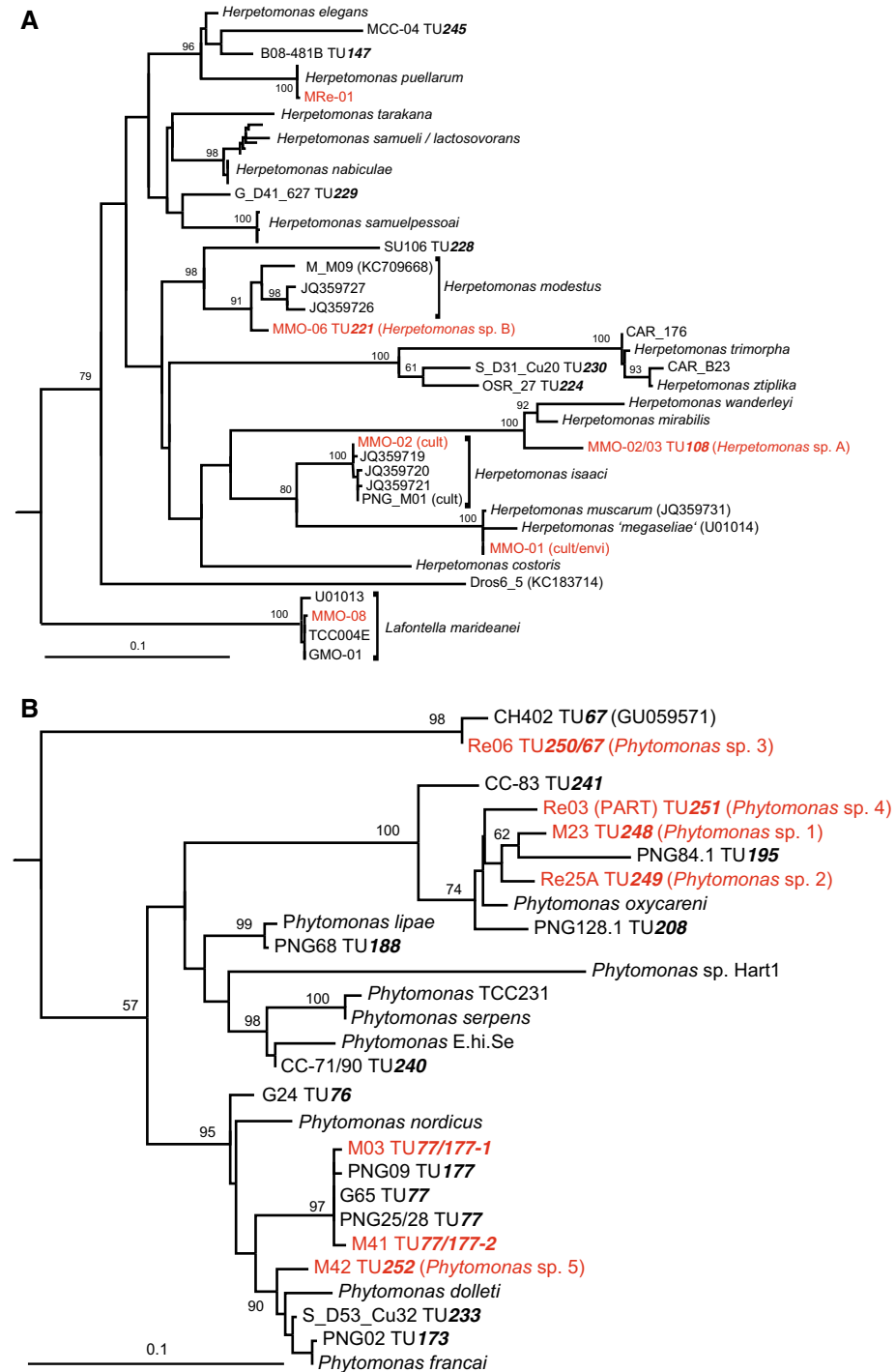


Fig. 4 Expanded subtree of the subfamily Phytomonadinae: *Herpetomonas* (A) and *Phytomonas* (B)

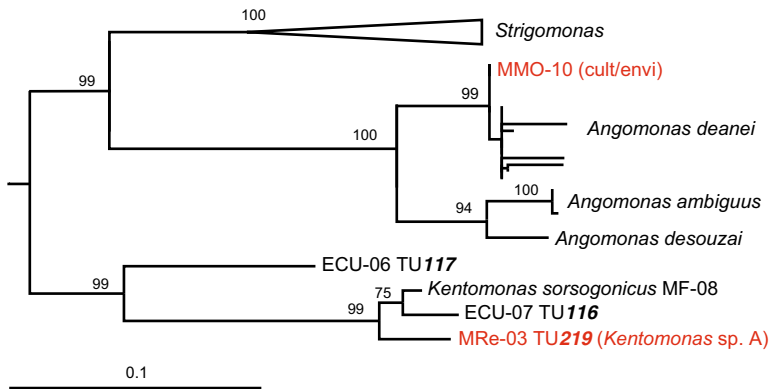


Fig. 5 Expanded subtree of the subfamily Strigomonadinae (*Angomonas*, *Strigomonas*, and *Kentomonas*)

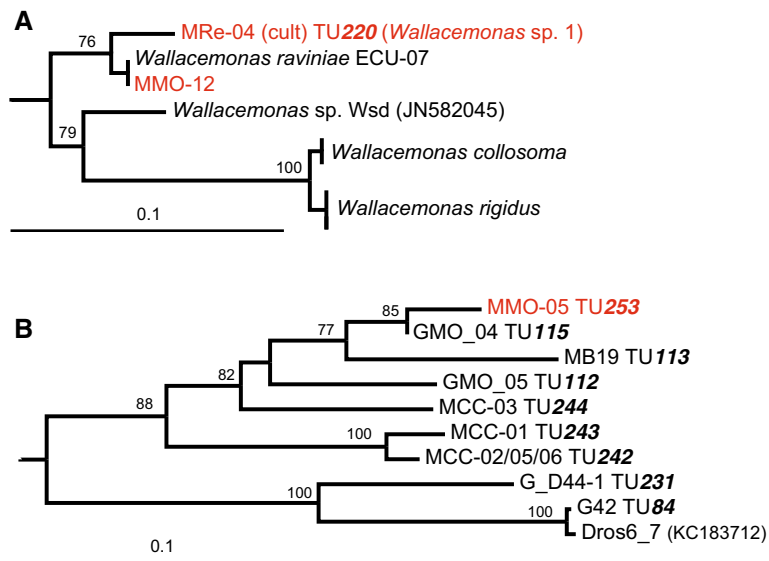


Fig. 6 Expanded subtree of the genus *Wallacemonas* (A) and clade II sensu Týč et al. 2013 (B)

Most of the 39 recorded TUs were found only in a single host species and only on one island (Table 2). However, several exceptions deserve to be mentioned, as they testify of a wider distribution of trypanosomatids across the studied islands and insect host species. Lower host specificity is clearly a common feature of the following 9 species—*Leptomonas moramango* [TU105], *Leptomonas podlipaevi* [TU48A], TU6/7C, TU44, TU72, TU77/177, TU108, and novel TU218 and TU251—captured in two host species on the same island. In Madagascar, three heteropteran host species were found to be infected by TU6/7D, and five host species accommodate TU88/TU88b. *Leptomonas spiculata* (TU32) was found in three and two host species in Reunion and Mauritius, respectively. Different insect hosts captured on different islands were detected also for already described TU73

(Madagascar and Mauritius) and the previously unknown TU253 (Madagascar and Reunion). Finally, TU72 parasitized the same host species on Reunion and Mauritius (Table 2).

Trypanosomatids from heteropteran bugs

Out of the 39 documented TUs, heteropteran bugs exclusively hosted 27 TUs of five genera including 15 (56%) new ones, while 11 TUs of seven genera were confined to dipteran flies, of which four (36%) were new (Table 2). Only a single species, *L. moramango* (TU105), was found in both unrelated groups of insects. Phylogenetic analysis based on the PCR-amplified 18S rRNA sequences from the microscopically-positive heteropteran bugs revealed the presence of a high number of trypanosomatid species and genera (Table 2; Fig. 1).

In our dataset, the genus *Leptomonas* is represented by three novel species (TU211, TU212, and TU213) and three already known ones (Table 2; Fig. 2). While *L. podlipaevi* (TU48A) (Kozminsky et al. 2015) and *L. spiculata* (TU32) (Maslov et al. 2007; Kozminsky et al. 2015) were previously identified in the Neotropics, *L. moramango* (TU105) is a special case. It was already encountered previously (Týč et al. 2013; Yurchenko et al. 2014); however, until now it was found exclusively in Madagascar and, unlike the previous two American leptomonads, represents a species with a limited geographical range. However, the 18S rRNA gene (KY357912) of the trypanosomatid strain C4 isolated in 1987 from the water strider *Limnopus* (formerly *Gerris*) *rufoscutellatus* (Gerridae) captured in Leningrad region, Russia (Podlipaev et al. 2004) differs in only three nucleotides from *L. moramango*.

The newly detected TU214 fell into the genus *Zelonia*, which so far accommodates only two species, namely *Z. costaricensis* from the reduviid bugs captured in Costa Rica, Brazil, and Panama (Yurchenko et al. 2006; Espinosa et al. 2018) and *Z. australiensis* isolated from the dipteran blackfly *Simulium dycei* in Australia (Barratt et al. 2017a, b). Both recently erected genera *Novymonas* and *Zelonia* are basal to all dixerous Leishmaniinae (*Leishmania* s.l. plus *Paraleishmania*) (Yurchenko et al. 2006; Kostygov et al. 2016; Barratt et al. 2017a, b; Kostygov and Yurchenko 2017; Espinosa et al. 2018), and presumably represent the closest ancestors of parasites that transitioned from a monoxenous to a dixerous life cycle (Jirku et al. 2012; Lukeš et al. 2014; Kostygov and Yurchenko 2017). Phylogenetic analyses suggested that the South American *Z. costaricensis* and the Australian *Z. australiensis* diverged when these continents completely separated (Barratt et al. 2017a, b). As a new member of the genus *Zelonia*, the Madagascan TU214 nicely fills the gap in the distribution of these flagellates over the former Gondwana, a supercontinent that existed from the Neoproterozoic (about 550 MYR) until the Jurassic (about 180 MYR). Indeed, our finding further supports the Gondwanan origin of the dixerous parasitism in Leishmaniinae (Barratt et al. 2017a, b).

The genus *Blastocrithidia*, characterized by its unique non-canonical genetic code (Záhonová et al. 2016; Bianchi et al. 2019), is represented by a single new typing unit, TU215 and three previously encountered species (Table 2; Fig. 3A). *Blastocrithidia raabei*, detected here in the sub-endemic pentatomid bug *Antestiella mauritii* collected in Reunion, was originally described from the intestine and hemolymph of the dock bug *Coreus marginatus* (Heteroptera: Coreidae) in Poland (Lipa 1966) and later from the same host in Russia, Kazakhstan, Tajikistan, and Armenia as the subspecies *B. raabei rostrata* (Podlipaev 1988). However, the previously mentioned possibility of a hemolymph infection (Lipa 1966) was recently refuted, as the parasite was repeatedly found only in the host

midgut (Frolov et al. 2020), which also corresponds with our findings of the elongated cells having a morphotype described previously (Lipa 1966; Podlipaev 1988; Frolov et al. 2020). It is worth noting that the digestive tract of phytophagous bugs, such as *C. marginatus*, has a very unusual organization, with its anterior and posterior parts being effectively isolated from each other by two intermediate segments impassable for food fluids and microorganisms (Kikuchi et al. 2011; Ohbayashi et al. 2015). Consequently, it was speculated that because of this unusual organization of the intestinal tract, the flagellates must overcome barriers, which are otherwise refractory to microbial infections (Frolov et al. 2020). However, *A. mauritii* probably lacks this anatomic peculiarity (Miyamoto 1961).

Another member of the genus *Blastocrithidia*, TU44, was found in a wide range of heteropteran families collected in the Neotropics (Maslov et al. 2007; Kozminsky et al. 2015), China (Votýpka et al. 2010), and Ghana (Votýpka et al. 2012a), so its current documentation in two pentatomid species from Madagascar further supports its cosmopolitan distribution. The detection of TU62, also clearly affiliated with the genus *Blastocrithidia*, in the pentatomid bugs nicely correlates with its host's occurrence in China (Votýpka et al. 2010) and Kenya (Votýpka et al. 2012a).

The ‘*jaculum*’ group (Fig. 3B), which is a well-defined, although not yet formally described genus closely related to *Blastocrithidia*, contained 11 TUs from the studied islands, out of which six were described previously (TU6/7C, TU6/7D, TU6/7E, TU72, TU73, and TU88 [split into two different genotypes TU88 and TU88b]), and three that can be justifiably considered as novel (TU216, TU217, and TU218). With respect to the two remaining detected genotypes (TU6/7D2 and TU6/7AB2) we cannot currently distinguish between them being members of the existing variable species groups TU6/7D and TU6/7AB or new species (Fig. 3B). Indeed, the TU6/7 species complex is composed of several closely related taxa poorly resolved by the 18S rRNA marker. However, an alternative marker, the spliced leader (SL) RNA sequence (Maslov et al. 2013), provides a finer resolution. TU6/7AB was previously recorded in two families, Alydidae and Reduviidae, in the Neotropics (Westenberger et al. 2004; Kozminsky et al. 2015) and Ghana (Votýpka et al. 2012a), while other two members of the TU6/7 complex (TU6/7D and TU6/7E) were detected in Alydidae from Ghana (Votýpka et al. 2012a) and TU6/7E was also found in the families Gerridae and Miridae from Europe (our unpubl. data; Maslov et al. 2013). Previously, TU72 was detected in Ghana in several bug families (Votýpka et al. 2012a, b), and in a sarcophagid fly (Týč et al. 2013), while TU73 was found in a pyrrhocorid bug captured in France (Votýpka et al. 2012b) and bugs from Ghana, with the latter country also hosting TU88 (Votýpka et al. 2012a).

The only dixenous trypanosomatids in the current islands-derived dataset belong to the genus *Phytomonas*, which is capable of parasitizing over 20 plant families (Jaskowska et al. 2015). It is interesting to note that the type species of this genus, *Phytomonas davidi*, was described more than 100 years ago from the latex of *Euphorbia* spp. in Mauritius (Lafont 1909, 1910). Later on, Lafont (1911) and França (1920) demonstrated its transmission by the phytophagous bugs *Nysius euphorbiae* (Lygaeidae) and *Stenocephalus agilis* (Stenocephalidae), respectively. While none of the detected *Phytomonas* spp. is from Mauritius, several TUs were found in Reunion and Madagascar. Whereas the family Lygaeidae is represented in our collection by 95 specimens parasitized by several TUs, including one new *Phytomonas* species (TU251), the family Stenocephalidae was represented by a single parasite-free specimen.

While only one TU (TU77/177) has a sequence virtually identical to 18S rRNA published previously, the other five detected phytomonads (TU248, TU249, TU250, TU251,

and TU252) clearly qualify as new species (Table 2; Fig. 4B). Despite their economic importance as serious pathogens of plants (Schwelm et al. 2018), our knowledge of the diversity and biogeography of phytomonads remains rather fragmented. Since only about one-quarter of all described species comes from Europe, Asia, Africa, and Australia/Oceania, while the majority is of American origin (Jankowska et al. 2015; Frolov et al. 2019), five new *Phytomonas* TUs encountered herein represent a significant contribution.

One of them, *Phytomonas* sp. 3 (TU250; environmental isolate Re06) from a bug belonging to the genus *Horridipamera* (Rhyparochromidae) captured in Reunion deserves special attention. Despite significant efforts, only a partial 18S rRNA sequence of an almost identical TU67 (isolate CH402; Acc. No. GU059571) from Chinese *Metochus* sp. (belonging to the same family Rhyparochromidae) is available (Votýpka et al. 2010) and forms an unstable branch within the *Phytomonas* clade (Votýpka et al. 2010, 2012a). However, the newly obtained full-size 18S rRNA sequence of TU250 (Re06_envi) provides sufficient information for a thorough phylogenetic analysis (Fig. 4B). In both maximum likelihood (Fig. 1) and Bayesian analyses (data not shown), TU250/67 appeared as the basal branch within the genus *Phytomonas* and replaced *P. lipae* and *P. oxycareni* in this position, supported by phylogenies based on the 18S rRNA, glycosomal glyceraldehyde dehydrogenase and heat shock protein 83 sequences (Seward et al. 2017; Frolov et al. 2019). Although our attempts to amplify protein-coding genes of TU250 from the intestine of *Horridipamera* sp. failed, we consider our data robust enough to claim that this flagellate is so distant from all known members of the genus *Phytomonas* (Figs. 1, 4B) that it may qualify as a member of a new genus, although more sequence data will be needed for such an assignment.

The only previously recorded *Phytomonas* (TU77/177) in our dataset comes in two slightly different genotypes: genotype 1 was found in two specimens of the pentatomid *Aspavia longispina*, while genotype 2 originated from another pentatomid, *Sciocoris wolffi* (Fig. 4B). TU77 was found in several bug families (Reduviidae, Alydinae, Pentatomidae, Coreidae, and Lygaeidae) in Ghana (Votýpka et al. 2012a) and both TU77 and TU177 parasitize pentatomids in Papua New Guinea (Králová et al. 2019). Due to only two to three nucleotide differences in the 18S rRNA gene, we assume that TU77, TU177, and two new Madagascan genotypes represent a single *Phytomonas* species (TU77/177) distributed in Africa, the adjacent islands, Papua New Guinea and, likely, beyond.

Trypanosomatids from dipteran flies

Except for the above-mentioned *L. moramango* (TU105), in the dissected flies we have recorded only trypanosomatid genera typical for dipteran hosts (Table 2; Fig. 1). From the endosymbiont-containing subfamily Strigomonadinae (Votýpka et al. 2014; Maslov et al. 2019), two genera were encountered (Table 2). The well-studied *Angomonas deanei* with cosmopolitan distribution (Teixeira et al. 2011; Týč et al. 2013) was detected in two flies from Madagascar, while the closely related genus *Kentomonas* (Votýpka et al. 2014) was represented by new TU219 (*Kentomonas* sp. A), which is closely related to *Kentomonas sorsogonicus* (Fig. 5). The finding of *Wallacemonas raviniae* (TU110), a representative of the genus *Wallacemonas* (Kostygov et al. 2014; Yurchenko et al. 2014), makes it a cosmopolitan flagellate, since it was previously detected in a sarcophagid fly from Ecuador (Týč et al. 2013). Moreover, TU220 (*Wallacemonas* sp. 1) also clearly falls into this genus (Table 2; Fig. 6A). The ‘clade II sensu Týč et al. 2013’, which clearly qualifies for a new genus, is represented by a novel TU253 (Table 2, Fig. 6B). This group appears to be

restricted to flies (Diptera) (Týč et al. 2013; Chandler and James 2013; Votýpka et al. 2018, 2019), although it was at least once found in a weakly mixed infection in the Ghanaian reduviid bug *Nagusta* cf. *punctaticollis* (Votýpka et al. 2012a), which might have acquired it from its fly prey.

The genus *Herpetomonas*, also typically found in flies, appeared in our dataset as five species, with three of them previously described and probably having a cosmopolitan distribution (Table 2; Fig. 4A). The well-known *Herpetomonas muscarum*, the type species of the genus *Herpetomonas*, has been confirmed in the USA and Brazil using sequence data (Teixeira et al. 1997; Borghesan et al. 2013), while based on morphology, it has been encountered all over the world (Podlipaev 1990). *Herpetomonas puellarum* was previously detected in Ghana, Czechia, Brazil, and Guinea Bissau (Borghesan et al. 2013; Týč et al. 2013) and *Herpetomonas isaaci* (TU107) is known from Papua New Guinea, Brazil, and Guinea Bissau (Borghesan et al. 2013; Týč et al. 2013). *Herpetomonas* sp. A (TU108; Týč et al. 2013) was found exclusively on Madagascar and, unlike the previous three cosmopolitan herpetomonads, has a limited geographic range. The novel *Herpetomonas* sp. B (TU221) is closely related to *Herpetomonas modestus*, so far confirmed from Brazil (Borghesan et al. 2013) and Mongolia (Týč et al. 2013). Finally, the monotypic *Lafontella*, formerly part of the genus *Herpetomonas* (Yurchenko et al. 2016), is represented by *Lafontella mariadeanei* (Table 2; Fig. 4A) found in the fly *Chrysomya putoria* (Calliphoridae) captured in Madagascar. This genetically slightly polymorphic species was reported from Brazil (Borghesan et al. 2013) and Ghana (Týč et al. 2013).

Endemic versus wide distribution

Based on the distribution of studied trypanosomatids in heteropterans, the hosts were sorted into the following three categories: (i) endemic (ENDE) to either Madagascar, Reunion, or Mauritius; (ii) sub-endemically (SUBE) distributed in Madagascar, the Mascarenes, Comoro, and Seychelles (usually only some of them); (iii) and widespread (WIDE), which means found at least on one island plus continental Africa and (possibly) beyond (Tables 1, 2, and S1; Fig. S1). Since none of the genera of true bugs that were found to be infected is (sub)endemic to the studied islands, the mutual comparison could not be made at the generic level. However, the analysis was informative at the host species level, with the following ratio based on the known geographic distribution: (i) endemic 32/8(25%)/8/2(25%) meaning that out of 32 endemic host species, 8 (= 25%) were infected by 8 different TUs, and out of these two (= 25%) were detected for the first time. Consequently, they are labeled as endemic for the particular island; (ii) sub-endemic 12/3(25%)/6/1(17%), and (iii) widespread 36/18(50%)/16/8(50%). Since it is unclear whether TU6/7AB2 from the widely distributed *Stenocoris annulicornis* is novel or not (Fig. 3B), it has been excluded from the analysis.

At first glance, widespread host species are more frequently infected by trypanosomatids and also more often host endemic (i.e. newly detected) TUs. Altogether, only two newly detected TUs were found exclusively in the (sub)endemic hosts: TU218 in *Cletus presignus* and TU248 in *Clavigralla madagascariensis*; TU251 was found not only in the sub-endemic *Nysius albipennis/euphorbiae*, but also in the widely distributed *Taylorilygus apicalis* (Tables 2 and S1). However, it is necessary to take into consideration that, at least in some cases, the widespread true bug species are more common in the inspected biotopes and therefore possibly over-represented in our collection. Due to the odds of a given parasite being detected in a particular host significantly increasing with the number of the

dissected specimens, it is not surprising that the more the host species is encountered, the higher the frequency of its detected parasites.

Out of 39 identified TUs, 21 were exclusive to the studied islands and are, at least provisionally, labeled as endemic. Out of them, 19 TUs were detected for the first time, the remaining two TUs, namely *L. moramango* (TU105) and *Herpetomonas* sp. A (TU108) have been mentioned previously (Týč et al. 2013; Yurchenko et al. 2014); however, they were found only in Madagascar in the dipterid flies *Chrysomya putoria*, *Musca* sp. (TU108), and *Pachycerina* cf. *vaga* and the heteropteran bug *Stictopleurus scutellaris coquerelii* (TU105).

Insects captured in Madagascar were found to host 12 endemic TUs. In true bugs, it was three species of the ‘*jaculum*’ group (TU6/7D2, TU216, and TU218), three *Leptomonas* species (*L. moramango* (TU105), TU211, and TU212), and one and two members of the genera *Zelonia* (TU214) and *Phytomonas* (TU248 and TU252), respectively. Additional three likely endemic TUs, two from the genus *Herpetomonas* (TU108 and new TU221) and the other new typing unit (TU253) assigned to the unnamed genus ‘clade II. sensu Týč et al. 2013’ were found in the dissected flies (Table 2). In Reunion, 8 newly detected TUs were found; five in heteropterans and three in dipterans. True bugs carried one new ‘*jaculum*’ (TU217), one *Leptomonas* (TU213), and three *Phytomonas* species (TU249, TU250, and TU251). This collection was complemented with one new species each from the genera *Wallacemonas* (TU220) and *Kentomonas* (TU219), both detected in flies. The above-mentioned (sub)endemic TU253 found in flies represents the only newly described TU occurring on more than one studied island. For comparison, only three previously described TUs (TU32, TU72, and TU73) were detected on more than one studied island (see above). Finally, only two new TUs affiliated with the provisional genus ‘*jaculum*’ (TU6/7AB2) and the genus *Blastocrithidia* (TU215) originated from heteropteran hosts captured in Mauritius.

The finding of 21 novel *a.k.a.* endemic TUs represent more than half (56%) of all the identified trypanosomatids found in heteropterans and dipterans, demonstrating very high endemism of these parasites in the island ecosystem, almost equal for both host insect groups. This clearly shows that endemism affects not only animals including insect hosts, but also their parasites. However, there is a considerable discrepancy between the incidence of endemic TUs in (sub)endemic vs. widespread heteropterans. Intuitively, one would assume that an endemic TU will preferentially parasitize an endemic host species, yet this is not the case, as the majority (17 out of 21) of novel TUs were found in the geographically widely distributed hosts. This intriguing observation may be best explained by the fact that these trypanosomatids have lower host specificity. Hence, they are endemic for a specific island(s), but in addition to its original, although still unknown endemic host(s), they also infect other host species. Alternatively, these TUs are *de facto* not endemic to the islands and share a wide geographic distribution with their hosts. We think that the real picture covers both possibilities, namely that some of the newly discovered TUs are genuinely endemic, occurring only on islands of the south-western Indian Ocean, whereas the other newly detected TUs may also occupy other geographic areas.

Madagascar is a continental island and thus the Madagascar Plate (Madagascar block) was once attached to the Gondwana supercontinent (breaking apart from Africa about 115–120 million years ago), and later the Indo-Australian Plate (split from India between 84 and 95 million years ago). The fauna of Madagascar is quite different from the one of the adjacent continents. Madagascar hosts a species-rich and highly endemic fauna of Heteroptera, but due to the lack of any comprehensive monograph or catalog, more precise estimates could be provided only for a few taxonomic groups, such as Tingidae (with 135

species of which 61% are endemic; Guilbert 2020), Rhyparochromidae (62/66%; Kment et al. 2016), Reduviidae: Ectrichodiinae (73/100%), and Pentatomidae: Halyini (40/100%). Most of the Madagascan taxa have apparent relationships with the fauna of continental Africa, but there are also taxa with Oriental affinities (Kment 2013; Forthman et al. 2016); for example the still undescribed representatives of the otherwise south-east Asian family Urostylididae (Zhou and Rédei 2018). On the other hand, Mauritius and Reunion are oceanic islands of volcanic origin not older than 10 and 2 million years, respectively. Their fauna of Heteroptera is not very rich, mostly shared with the African continent and Madagascar, and with the considerably lower endemism than in Madagascar; more detailed data are available only for Reunion, hosting 108 species of which 11 (10.2%) are endemic (Legros et al. 2016).

Axenic cultures, environmental samples, and mixed infections

Under the laboratory conditions, axenic cultures from 37 true bugs and dipteran flies were successfully established, and the cryopreserved cells are available for further studies (Tables 2 and S1). The success rate of establishing axenic flagellates was relatively high; out of 114 dissected trypanosomatid-positive specimens, we have tried to obtain the culture from 96 individuals and were successful in 37 cases (114/96(82%)/37(39%)). Being applied on all three islands, this approach yielded the following consistent results: Madagascan true bugs 52/42(81%)/16(38%) and flies 13/10(78%)/4(40%), Reunion true bugs 33/25(76%)/9(36%) and flies 6/6(100%)/3(50%), and Mauritius true bugs 10/10(100%)/5(50%).

Subsequent 18S rRNA-based sequence analysis (Figs. 1, 2, 3, 4, 5, 6; Tables 2 and S1) assigned 37 axenic cultures to 15 species: one *Angomonas*, *Blastocrithidia*, *Wallacemonas*, and *Zelonia* each, two *Herpetomonas*, four *Leptomonas* and five ‘*jaculum*’. Out of them, five (33%) are new species: *Leptomonas* sp. 2 (TU212), ‘*jaculum*’ sp. C (TU218), ‘*jaculum*’ TU6/7D2, *Wallacemonas* sp. 1 (TU220), and *Zelonia* sp. 1 (TU214). Out of 37 well-established cultures, dissection of true bugs originating from Madagascar resulted in 16 axenic cultures representing a single *Blastocrithidia* and *Zelonia*, two *Leptomonas*, and five ‘*jaculum*’. Flies from the same island yielded four cultures represented by one *Leptomonas* and *Angomonas* each and two *Herpetomonas* spp. All 9 stabilized cultures obtained from the dissected true bugs captured in Reunion are just two *Leptomonas* species, while three cultures isolated there from flies belong to a single *Wallacemonas* species. Finally, all five cultures established from bugs captured in Mauritius were identified as a single *Leptomonas* species.

In any case, a comparison of trypanosomatids that propagated in the axenic cultures with those detected only by their 18S rRNA sequences amplified from the primary material, which was almost exclusively the insect intestine, is quite informative. In 8 cases a difference between the culture and PCR-detected parasites was noticed (Table S1). In five of them, the species detected in DNA extracted from the true bug intestine and belonging to the genus ‘*jaculum*’ (TU73, TU88, and TU216) or *Phytomonas* (TU248) were in the cultures overgrown by *L. moramango* (TU105), *Zelonia* sp. 1 (TU214), and ‘*jaculum*’ (TU218 and TU6/7 complex). In three samples from flies captured in Reunion, *H. pullarum* and TU253 (clade II sensu Týč et al. 2013) were under the axenic conditions suppressed by *Wallacemonas* sp. 1 (TU220). This comparison of primary material from the insect hosts with the established cultures clearly demonstrates that: i) mixed infections are relatively common, ii) trypanosomatids obtained by axenic cultivation may not be the dominant

species within the mixed infection in vivo, and iii) only a subset has the capacity to flourish in media and under the conditions provided in our experimental setup (Lukeš and Votýpka 2020).

Tissue localization and host-parasite specificity

Out of 11 trypanosomatid genera detected in this study, four (*Blastocrithidia*, ‘*jaculum*’, *Phytomonas*, and *Zelonia*) were exclusive for bugs and six (*Herpetomonas*, *Lafontella*, *Wallacemonas*, *Angomonas*, *Kentomonas*, and clade II. sensu Týč et al. 2013) for flies. These host preferences correspond nicely with similar studies performed in a range of geographic locations (Wallace 1966; Podlipaev 1990; Texeira et al. 1997, 2011; Votýpka et al. 2010, 2012a, b, 2019; Wilfert et al. 2011; Borghesan et al. 2013; Týč et al. 2013; Kozminsky et al. 2015; Schoener et al. 2018; Králová et al. 2019) and further confirm very high host specificity of the trypanosomatid genera. Only the genus *Leptomonas* was found in both host groups.

The observed intensity of infection ranged from very mild (in some cases only solitary flagellates were observed and consequently PCR has occasionally failed) to very heavy (Tables 2 and S1). In the latter cases, the infected part of the intestine was literally packed with the parasites. Although in a small fraction of dissected specimens it was not possible to unambiguously determine the exact location of the infection within the digestive tract, the flagellates were most frequently found in the midgut (79%), followed by the hindgut (29%), and the Malpighian tubules (5%) (note that in some cases multiple infections of midgut and hindgut occur).

Most detected members of the subfamily Leishmaniinae (*L. moramango*, *L. spiculata*, *L. podlipaevi*, *Leptomonas* sp. 2 [TU212], *Leptomonas* sp. 3 [TU213], and *Zelonia* sp. 1 [TU214]) were confined to the middle of the intestine (mesenteron) of their heteropteran hosts (Tables 2 and S1), which is characteristic for most leptomonads. *Leptomonas* sp. 1 (TU211) was an exception, as it infects exclusively the Malpighian tubules, which is the preferred organ of only about 2% of monoxenous trypanosomatids (Lukeš et al. 2018). The situation related to tissue localization and host specificity is somewhat unusual in the case of *L. moramango*. In its heteropteran host it occupies simultaneously midgut, hindgut, and the Malpighian tubules while in dipterans, the flagellates were observed exclusively in the hindgut. The axenic culture of *L. moramango* was previously obtained from the fly *Pachycerina* cf. *vaga* (Týč et al. 2013), which is therefore listed as its type host (Yurchenko et al. 2014). However, the heteropteran *Stictopleurus scutellaris* may as well be the primary host that might have acquired this trypanosomatid through its accidental necrophagous and/or coprophagous behavior, and one may speculate that *L. moramango* has a very broad ecological valence and can parasitize a wide range of hosts. Although it is difficult to discriminate between the specific and non-specific infections in trypanosomatids with a wide range of known hosts, based on their localization, very low prevalence, and host specificity of other *Leptomonas* species, we believe that the heteropteran bug is the primary host.

A good correlation with the available data is also apparent when members of the genera *Blastocrithidia* and ‘*jaculum*’ are concerned (Tables 2 and S1). They usually parasitize the midgut of true bugs (Votýpka et al. 2010, 2012a, b; Králová et al. 2019), but some species are predominantly found in the hindgut (Votýpka et al. 2019). All detected TUs belonging to the “fly” genera (*Herpetomonas*, *Lafontella*, *Wallacemonas*, *Angomonas*, *Kentomonas*, and clade II. sensu Týč et al. 2013) were usually found in the hindgut, more specifically in

the rectal ampulla, and quite rarely also in the midgut (Tables 2 and S1), which is in agreement with previous studies (Teixeira et al. 1997, 2011; Borghesan et al. 2013; Týč et al. 2013; Votýpka et al. 2019).

The only encountered genus with a dixenous life cycle is *Phytomonas*, here well represented by 7 different TUs (Tables 2; Fig. 4). We have found them in six families of sap-sucking bugs (Pentatomidae, Coreidae, Rhopalidae, Rhyparochromidae, Lygaeidae, and Miridae), which obviously became infected from plants (Camargo et al. 1990). Although we have found *Phytomonas* spp. invariably in the midgut (Tables 2 and S1), based on the available data, this location shall only be transient, as they are transmitted to plants throughout the bite and are therefore typically located in the salivary glands of their insect vectors (Jaskowska et al. 2015; Frolov et al. 2016; Seward et al. 2017). We are fully aware that the dissection and examination focused exclusively on the intestinal tract can be a source of bias, yet the field conditions do not allow satisfactory inspection of the salivary glands. In addition to this, despite all our efforts, the vast majority of *Phytomonas* TUs could not be introduced into culture (our unpubl. data; Seward et al. 2017).

Conclusions

In this work, we surveyed trypanosomatids from the heteropteran and dipteran hosts collected in Madagascar, Reunion, and Mauritius. These islands are famous as biodiversity hotspots with many endemic macro-organisms. Based on two short-term field expeditions we documented a very high diversity of insect trypanosomatids. Although the proportion of endemic TUs (56%) was not as high as in our previous study in Papua New Guinea (83%), the newly detected species certainly represent a significant contribution to the increasing biodiversity of trypanosomatids known so far and demonstrate that our knowledge is still far from being comprehensive. In line with previous reports (Teixeira et al. 1997, 2011; Votýpka et al. 2010, 2012a, 2019; Borghesan et al. 2013; Týč et al. 2013; Kozminsky et al. 2015; Králová et al. 2019), our study highlights the association between the insect host families and parasites and supports the cosmopolitan distribution of a subset of species. On the other hand, it demonstrates for the first time that the high endemism of macro-organisms on the remote islands is inherent to parasitic protists as well. Based on these findings we conclude that any conservation activities should take into account not only the protection of animals themselves, but also consider the protection of their symbionts, including parasites and, thus, protect a chiefly neglected segment of biodiversity, which is potentially comparable with the "visible" biodiversity of their hosts. Although our analysis has not revealed any new clades on the generic level, indicating possible saturation in this respect, the newly detected *Phytomonas* sp. 3 (TU250; isolate Re06-envi) is remarkable in that it constitutes the basal and highly diverged branch. Moreover, five newly detected TUs (including two belonging to the enigmatic '*jaculum*' clade) are available in axenic cultures and are worthy of further investigation.

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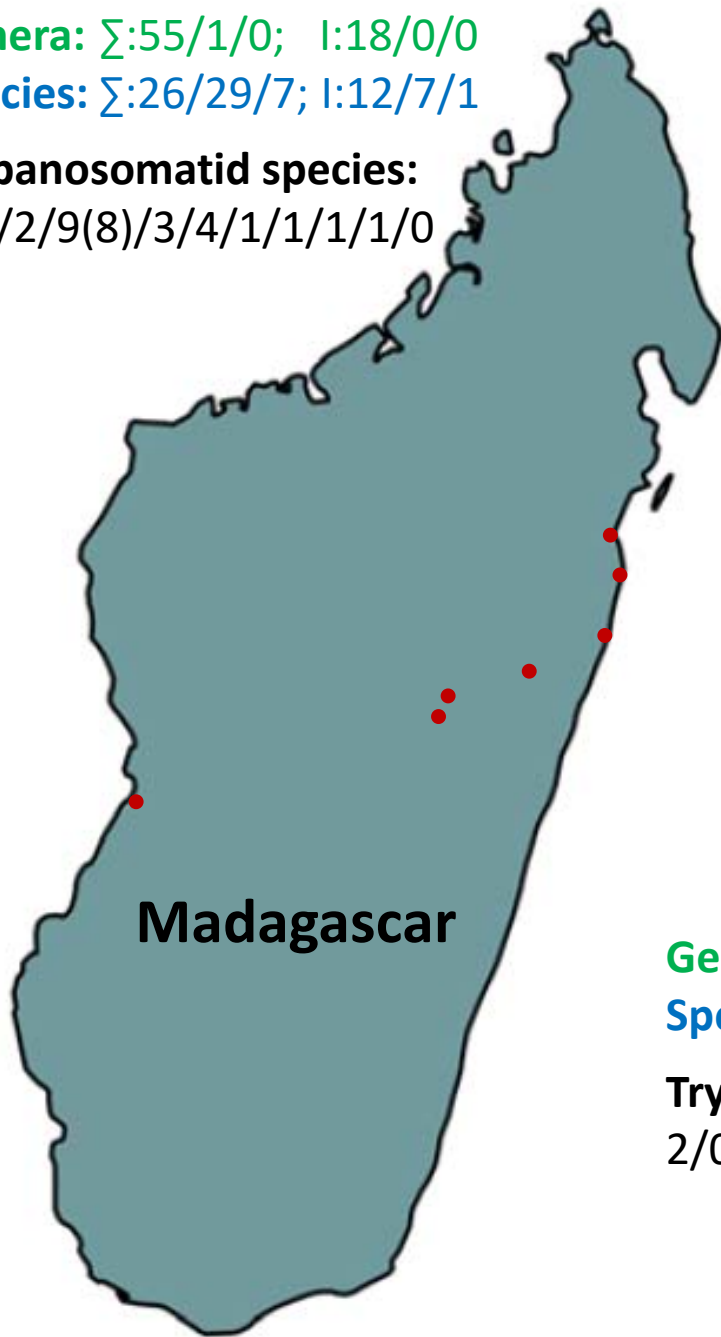
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Genera: Σ :55/1/0; I:18/0/0

Species: Σ :26/29/7; I:12/7/1

Trypanosomatid species:

4/1/2/9(8)/3/4/1/1/1/1/0



Madagascar

• collection sites

Genera: Σ :8/0/0; I:5/0/0

Species: Σ :8/0/1; I:5/0/1

Trypanosomatid species:

1/0/1/3/0/0/0/0/0/0/0

Mauritius

Reunion



Genera: Σ :18/0/0; I:9/0/0

Species: Σ :11/2/7; I:6/0/2

Trypanosomatid species:

2/0/1/2/3/1/0/1/1/0/1

Island	Locatity	Code	Int	Localization	Type	Family	Species	Genus	Species	Source	TU	MIX
Madagascar	Morondava	M01	0.1	MG (?)	L	Pentatomidae	<i>Aspavia longispina</i>	WIDE	ENDE	envi	TU6/7D	
Madagascar	Morondava	M02	1	MG (?)	L	Pentatomidae	<i>Aspavia longispina</i>	WIDE	ENDE	envi	TU77/177-1	
Madagascar	Morondava	M03	3	AMG	L	Pentatomidae	<i>Aspavia longispina</i>	WIDE	ENDE	envi	TU77/177-1	
Madagascar	Morondava	M04	2	HG	M	Alydidae	<i>Mirperus jaculus</i>	WIDE	WIDE	envi cult	TU6/7D	
Madagascar	Ambatolampy	M05	3	MG, HG, MT	M	Rhopalidae	<i>Stictopleurus scutellaris coquerelii</i>	WIDE	SUBE	envi cult	TU73 TU105	YES
Madagascar	Ambatolampy	M06	3	TMG	M	Pentatomidae	<i>Veterna nigromarginata</i>	WIDE	ENDE	envi	TU62	
Madagascar	Ambatolampy	M07	3	TMG	M	Pentatomidae	<i>Veterna nigromarginata</i>	WIDE	ENDE	envi cult	TU62	
Madagascar	Ambatolampy	M08	3	MG	L	Lygaeidae	<i>Stalagmostethus furcatus</i>	WIDE	WIDE	envi cult	TU88b *TU214	YES
Madagascar	Ambatofosty	M09	3	AMG, TMG	L	Lygaeidae	<i>Aspilocoryphus unimaculatus</i>	WIDE	WIDE	envi cult	TU88	
Madagascar	Ambatofosty	M10	1	AMG	L	Lygaeidae	<i>Aspilocoryphus unimaculatus</i>	WIDE	WIDE	envi	TU88b	
Madagascar	Ambatofosty	M11	3	AMG, HG	M+S	Lygaeidae	<i>Spilostethus pandurus</i>	WIDE	WIDE	envi cult	TU88b	YES
Madagascar	Ambatofosty	M12	3	MG	M+S	Lygaeidae	<i>Spilostethus pandurus</i>	WIDE	WIDE	envi cult	TU88b	
Madagascar	Ambatofosty	M13	3	MG	M+S	Lygaeidae	<i>Spilostethus pandurus</i>	WIDE	WIDE	envi cult	TU88b	
Madagascar	Ambatofosty	M14	3	MG	M+S	Lygaeidae	<i>Spilostethus pandurus</i>	WIDE	WIDE	envi cult	TU88b	YES
Madagascar	Ambatofosty	M15	3	MG	M+S	Lygaeidae	<i>Spilostethus pandurus</i>	WIDE	WIDE	envi cult	TU88b	YES
Madagascar	Ambatofosty	M16	3	MG	M+S	Lygaeidae	<i>Spilostethus pandurus</i>	WIDE	WIDE	envi	TU88b	
Madagascar	Ambatofosty	M17	3	MG	M+S	Lygaeidae	<i>Spilostethus pandurus</i>	WIDE	WIDE	envi	TU88b	
Madagascar	Ambatofosty	M18	1	MG (?)	L	Pachygronthidae	<i>Teracrius namaquensis</i>	WIDE	WIDE	envi	TU88b	
Madagascar	Moramango	M19	2	HG or AMG	S	Alydidae	<i>Alydinae</i> gen. sp.	WIDE	?	envi cult	*TU216 *TU218	YES
Madagascar	Moramango	M20	3	AMG	S	Alydidae	<i>Alydinae</i> gen. sp.	WIDE	?	envi cult	*TU216 *TU218	YES
Madagascar	Moramango	M21	2	AMG	S	Alydidae	<i>Alydinae</i> gen. sp.	WIDE	?	envi cult	*TU6/7D2	
Madagascar	Moramango	M22	2	MG	M	Coreidae	<i>Clavigralla madagascariensis</i>	WIDE	ENDE	envi cult	*TU248 TU6/7C	YES
Madagascar	Moramango	M23	1	MG	M	Coreidae	<i>Clavigralla madagascariensis</i>	WIDE	ENDE	envi	*TU248	
Madagascar	Moramango	M24	1	MG	M	Coreidae	<i>Clavigralla madagascariensis</i>	WIDE	ENDE	envi	*TU248	

Madagascar	Moramango	M25	1	MG	M	Coreidae	<i>Clavigralla madagascariensis</i>	WIDE	ENDE	envi	*TU248	
Madagascar	Moramango	M26	2	MG	M	Alydidae	<i>Riptortus fabricii</i>	WIDE	ENDE	envi	TU6/7E	
Madagascar	Moramango	M27	2	HG	M	Alydidae	<i>Riptortus fabricii</i>	WIDE	ENDE	envi cult	TU6/7C	
Madagascar	Moramango	M28	2	MG	L	Pentatomidae	<i>Aspavia albidomaculata</i>	WIDE	WIDE	envi	TU44	
Madagascar	Moramango	M29	3	HG	M	Coreidae	<i>Cletus presignus</i>	WIDE	ENDE	envi	*TU218	
Madagascar	Moramango	M30	3	HG	M	Coreidae	<i>Cletus presignus</i>	WIDE	ENDE	envi	*TU218	
Madagascar	Moramango	M31	3	MT	L	Miridae	<i>Proboscidoecoris cf. intermedius</i>	WIDE	WIDE	envi	*TU211	
Madagascar	Moramango	M32	2	MG	M+L	Miridae	<i>Proboscidoecoris fuliginosus</i>	WIDE	WIDE	envi cult	*TU212	
Madagascar	Mahambo	M33	3	MG	L	Coreidae	<i>Hydara tenuicornis</i>	WIDE	WIDE	envi	TU6/7C	
Madagascar	Mahambo	M34	2	MG	L	Pentatomidae	<i>Phricodus hystrix</i>	WIDE	WIDE	envi	*TU252	
Madagascar	Mahambo	M35	2	MG	M+L	Pentatomidae	<i>Phricodus hystrix</i>	WIDE	WIDE	envi	*TU252	
Madagascar	Mahambo	M36	3	MG	M	Pentatomidae	<i>Eysarcoris v-flavum</i>	WIDE	ENDE	envi	TU44	
Madagascar	Mahambo	M37	2	MG	M	Pentatomidae	<i>Eysarcoris v-flavum</i>	WIDE	ENDE	envi	TU44	
Madagascar	Mahambo	M38	3	MG	M	Pentatomidae	<i>Eysarcoris v-flavum</i>	WIDE	ENDE	envi	TU44	
Madagascar	Mahambo	M39	0.1	MG	L	Alydidae	<i>Stenocoris annulicornis</i>	WIDE	ENDE	envi	PCR failed	
Madagascar	Mahambo	M40	2	MG	M	Alydidae	<i>Riptortus fabricii</i>	WIDE	ENDE	envi	TU6/7D	
Madagascar	Foulpointe	M41	2	MG	L	Pentatomidae	<i>Sciocoris wolffi</i>	WIDE	ENDE	envi	TU77/177-2	
Madagascar	Foulpointe	M42	2	MG	L	Pentatomidae	<i>Phricodus hystrix</i>	WIDE	WIDE	envi	*TU252	
Madagascar	Foulpointe	M43	2	MG	L	Pentatomidae	<i>Phricodus hystrix</i>	WIDE	WIDE	envi	*TU252	
Madagascar	Foulpointe	M44	1	MG	L	Pentatomidae	<i>Phricodus hystrix</i>	WIDE	WIDE	envi	*TU252	
Madagascar	Foulpointe	M45	2	MG	L	Pentatomidae	<i>Phricodus hystrix</i>	WIDE	WIDE	envi	*TU252	
Madagascar	Foulpointe	M46	1	MG	L	Pentatomidae	<i>Phricodus hystrix</i>	WIDE	WIDE	envi	*TU252	
Madagascar	Foulpointe	M47	3	MG	L	Coreidae	<i>Hydara tenuicornis</i>	WIDE	WIDE	envi	TU6/7C	
Madagascar	Foulpointe	M48	1	MG	M	Lygaeidae	<i>Spilostethus furculus</i>	WIDE	WIDE	envi	TU88b	
Madagascar	Ivoloina (Zoo)	M49	2	HG	M	Coreidae	<i>Hydara tenuicornis</i>	WIDE	WIDE	envi	TU6/7C	
Madagascar	Ivoloina (Zoo)	M50	2	MG	M+L	Coreidae	<i>Hydara tenuicornis</i>	WIDE	WIDE	envi	TU6/7C	
Madagascar	Ivoloina (Zoo)	M51	2	MG	M	Coreidae	<i>Hydara tenuicornis</i>	WIDE	WIDE	envi	TU6/7C	
Madagascar	Ivoloina (Zoo)	M52	2	AMG, HG, MT	M	Coreidae	<i>Hydara tenuicornis</i>	WIDE	WIDE	envi	TU6/7C	
Madagascar	Ambatolampy	MMO-01	3	MG (?), HG, MT	M	Calliphoridae	<i>Chrysomya putoria</i>			envi cult	<i>H. muscarum</i>	
Madagascar	Ambatolampy	MMO-02	3	?	L	Calliphoridae	<i>Chrysomya putoria</i>			envi cult	TU107	YES
Madagascar	Ambatolampy	MMO-03	3	?	L	Calliphoridae	<i>Chrysomya putoria</i>			envi	TU108	
Madagascar	Ambatolampy	MMO-04	3	?	M	Calliphoridae	<i>Chrysomya putoria</i>			envi	*TU253	YES
Madagascar	Ambatolampy	MMO-05	3	?	M	Calliphoridae	<i>Chrysomya putoria</i>			envi	*TU253	
Madagascar	Ambatolampy	MMO-06	3	HG	S	Muscidae	<i>Musca</i> sp.			envi	*TU221	
Madagascar	Ambatofosty	MMO-07	1	MG (?)	L	Muscidae	<i>Musca</i> sp.			envi	TU108	YES

Madagascar	Ambatofosty	MMO-08	1	MG (?)	L	Calliphoridae	<i>Chrysomya putoria</i>			envi	<i>La. marideanei</i>	
Madagascar	Moramango	MMO-09	3	RA	S	Lauxaniidae	<i>Pachycerina cf. vaga</i>			envi cult	TU 105	
Madagascar	Moramango	MMO-10	3	RA, HG	S	Muscidae	<i>Acritochaeta orientalis</i>			envi cult	<i>A. deanei</i>	
Madagascar	Moramango	MMO-11	0.1	HG (?)	S	DIPTERA				envi	<i>A. deanei</i>	YES
Madagascar	Ivoloina	MMO-12	1	HG	S	DIPTERA				envi	TU 110	
Madagascar	Ivoloina	MMO-13	1	MG	M	DIPTERA				envi	PCR failed	YES
Reunion	Salazie	MRe-01	3	RA	S	DIPTERA				envi cult	<i>H. puellarum</i> *TU 220	YES
Reunion	Salazie	MRe-02	2	RA	S	DIPTERA				envi	*TU 219	YES
Reunion	Salazie	MRe-03	3	RA	S	DIPTERA				envi	*TU 219	
Reunion	Salazie	MRe-04	2	RA	S	DIPTERA				envi cult	<i>H. puellarum</i> *TU 220	YES
Reunion	Saint-Denis	MRe-05	2	HG	S	DIPTERA				envi	*TU 219	YES
Reunion	Mafate cirque	MRe-06	3	MG	M	DIPTERA				envi cult	*TU 253 *TU 220	YES
Reunion	Cilaos	Re01	2	MG (?)	M	Alydidae	<i>Nariscus sp.</i>	WIDE	?	envi	*TU 217	
Reunion	Cilaos	Re02	0.1	?	M	Coreidae	<i>Cletus ochraceus</i>	WIDE	WIDE	envi	TU 72	
Reunion	Cilaos	Re03	1	?	M	Lygaeidae	<i>Nysius albipennis/euphorbiae</i>	WIDE	SUBE	envi	*TU 251	
Reunion	Cilaos	Re04	3	MG (?)	M	Coreidae	<i>Cletus ochraceus</i>	WIDE	WIDE	envi	TU 72	
Reunion	Cilaos	Re05	2	MG (?)	M	Coreidae	<i>Cletus ochraceus</i>	WIDE	WIDE	envi	TU 72	
Reunion	Takamaka	Re06	3	MG	M+S	Rhyparochromidae	<i>Horridipamera sp.</i>	WIDE	?	envi	*TU 250	
Reunion	Salazie	Re07	4	MG, HG (?)	L	Miridae	<i>Taylorilygus apicalis</i>	WIDE	WIDE	envi cult	TU 32	YES
Reunion	Salazie	Re08	4	MG, HG (?)	L	Miridae	<i>Taylorilygus apicalis</i>	WIDE	WIDE	envi cult	TU 32	
Reunion	Salazie	Re09	4	MG, HG (?)	L	Miridae	<i>Taylorilygus apicalis</i>	WIDE	WIDE	envi cult	TU 32	
Reunion	Salazie	Re10	4	MG or HG	L+M+S	Miridae	<i>Taylorilygus apicalis</i>	WIDE	WIDE	envi cult	TU 32	
Reunion	Salazie	Re11	4	MG	L+M+S	Miridae	<i>Taylorilygus apicalis</i>	WIDE	WIDE	envi cult	TU 32	
Reunion	Salazie	Re12	4	MG	L	Pentatomidae	<i>Antestiella mauritii</i>	WIDE	SUBE	envi	TU 99	
Reunion	Salazie	Re13	1	?	L	Miridae	<i>Probosciodocoris fuliginosus</i>	WIDE	WIDE	envi	*TU 213	YES
Reunion	Salazie	Re14	1	MG	L	Coreidae	<i>Cletus ochraceus</i>	WIDE	WIDE	envi	TU 32	
Reunion	Salazie	Re15	1	MG	L	Coreidae	<i>Cletus ochraceus</i>	WIDE	WIDE	envi	TU 72	
Reunion	Salazie	Re16	1	MG	L	Coreidae	<i>Cletus ochraceus</i>	WIDE	WIDE	envi	TU 72	
Reunion	Salazie	Re17	2	MG	L	Coreidae	<i>Cletus capensis</i>	WIDE	WIDE	envi	TU 72	

Reunion	Salazie	Re18	2	MG	L	Coreidae	<i>Cletus ochraceus</i>	WIDE	WIDE	envi	TU 48A	
Reunion	Salazie	Re19	2	MG	L	Coreidae	<i>Cletus capensis</i>	WIDE	WIDE	envi	TU 72	
Reunion	Salazie	Re20	3	MG	L	Coreidae	<i>Cletus capensis</i>	WIDE	WIDE	envi	TU 72	
Reunion	Salazie	Re21	3	MG	L	Coreidae	<i>Cletus capensis</i>	WIDE	WIDE	envi	TU 72	YES
Reunion	Salazie	Re22	1	MG (?)	M+L	Rhopalidae	<i>Liorhyssus hyalinus</i>	WIDE	WIDE	envi	PCR failed	
Reunion	Saint-Denis	Re23	3	MG	L	Rhopalidae	<i>Leptocoris mutilata</i>	WIDE	WIDE	envi cult	TU 48A	
Reunion	Saint-Denis	Re24	4	MG	L	Rhopalidae	<i>Leptocoris mutilata</i>	WIDE	WIDE	envi cult	TU 48A	
Reunion	Saint-Denis	Re25A Re25B	3	MG	L	Rhopalidae	<i>Leptocoris mutilata</i>	WIDE	WIDE	envi envi	*TU 249 TU 48A	YES
Reunion	Saint-Denis	Re26	2	MG (?)	L	Lygaeidae	<i>Nysius albipennis/euphorbiae</i>	WIDE	SUBE	envi	TU 72	YES
Reunion	Saint-Denis	Re27	2	MG (?)	L	Lygaeidae	<i>Nysius albipennis/euphorbiae</i>	WIDE	SUBE	envi	*TU 251	
Reunion	Saint-Denis	Re28	1	MG (?)	L	Lygaeidae	<i>Nysius albipennis/euphorbiae</i>	WIDE	SUBE	envi	*TU 251	
Reunion	Saint-Denis	Re29	1	MG (?)	M+L	Lygaeidae	<i>Nysius albipennis/euphorbiae</i>	WIDE	SUBE	envi	*TU 251	
Reunion	Saint-Denis	Re30	2	MG (?)	L	Lygaeidae	<i>Nysius albipennis/euphorbiae</i>	WIDE	SUBE	envi	TU 32	
Reunion	Saint-Denis	Re31	3	MG	L	Miridae	<i>Taylorilygus apicalis</i>	WIDE	WIDE	envi cult	TU 32	YES
Reunion	Saint-Denis	Re32	4	MG	L	Miridae	<i>Taylorilygus apicalis</i>	WIDE	WIDE	envi cult	TU 32	
Reunion	Mafate cirque	Re33	2	MG	M	Coreidae	<i>Cletus capensis</i>	WIDE	WIDE	envi	TU 72	YES
Mauritius	Vanille south	Re34	2	MG	M	Lygaeidae	<i>Nysius albipennis/euphorbiae</i>	WIDE	SUBE	envi	TU 32	YES
Mauritius	Vanille south	Re35	2	MG	L	Rhopalidae	<i>Liorhyssus hyalinus</i>	WIDE	WIDE	envi	TU 73	YES
Mauritius	Vanille south	Re36	3	MG (?)	M	Rhyparochromidae	<i>Stigmatonotum geniculatum</i>	WIDE	WIDE	envi	*TU 215	
Mauritius	Vanille south	Re37	2	HG	M	Coreidae	<i>Cletus capensis</i>	WIDE	WIDE	envi	TU 72	
Mauritius	Vanille south	Re38	3	MG	L	Miridae	<i>Taylorilygus apicalis</i>	WIDE	WIDE	envi cult	TU 32	
Mauritius	Vanille south	Re39	2	MG	L	Miridae	<i>Taylorilygus apicalis</i>	WIDE	WIDE	envi cult	TU 32	
Mauritius	Vanille south	Re40	2	MG	S	Miridae	<i>Taylorilygus apicalis</i>	WIDE	WIDE	envi cult	TU 32	
Mauritius	Vanille south	Re41	2	MG	M	Miridae	<i>Taylorilygus apicalis</i>	WIDE	WIDE	envi cult	TU 32	
Mauritius	Vanille south	Re42	2	MG (?)	M	Miridae	<i>Taylorilygus apicalis</i>	WIDE	WIDE	envi cult	TU 32	
Mauritius	Vanille south	Re43	2	MG or HG	L+M	Alydidae	<i>Stenocoris annulicornis</i>	WIDE	WIDE	envi	*TU 6/7AB2	YES

Name (Strain, Isolate, Typing Unit etc.)	GenBank Acc. No.
Angomonas_ambiguus	HM593015
Angomonas_ambiguus	KC205973
Angomonas_deanei	EU099538
Angomonas_deanei	HM593012
Angomonas_deanei	KC205974
Angomonas_deanei	KC205975
Angomonas_deanei	KC205977
Angomonas_deanei	KC205978
Angomonas_deanei	KC205979
Angomonas_deanei	MT174492
Angomonas_denaiei	KC205976
Angomonas_desouzai	HM593016
Angomonas_desouzai	KC205980
Angomonas_oncopelti	EU099539
Angomonas_oncopelti	L29264
Angomonas_TCC263	AF038024
Blastocrithidia_aff_triatomae_CC34_TU238	MK056188
Blastocrithidia_cyrtomeni	FJ916992
Blastocrithidia_frustrata(PNG134_TU63)	MK929465
Blastocrithidia_largi	FJ968531
Blastocrithidia_miridarum	EU079128
Blastocrithidia_miridarum	KX138600
Blastocrithidia_papi	KX641338
Blastocrithidia_raabei	MN366348
Blastocrithidia_raabei	MT174501
Blastocrithidia_triatomae	KX138599
Blastocrithidia_CC17_TU239	MK056189
Blastocrithidia_ex_Lygus_393M8_TU247	KX138601
Blastocrithidia_HF49_TU247	MK395374
Blechomonas_ayalai	KF054116
Blechomonas_ayalai	KF054117
Blechomonas_campbelli	KF054133
Blechomonas_danrayi	KF054135
Blechomonas_danrayi	KF054137
Blechomonas_englundi	KF054118
Blechomonas_englundi	KF054119
Blechomonas_keelingi	KF054130
Blechomonas_keelingi	KF054131
Blechomonas_lauriereadi	KF054127
Blechomonas_luni	KF054115
Blechomonas_maslovi	KF054122
Blechomonas_maslovi	KF054123
Blechomonas_maslovi	KF054124
Blechomonas_pulexsimulantis	KF054128
Blechomonas_pulexsimulantis	KF054129
Blechomonas_wendygibsoni	KF054126
Borovskiya_barvae	FJ968532
Crithidia_abscondita	EU079126
Crithidia_bombi	FN546181

Crithidia_brachyflagelli	JF717840
Crithidia_brevicula	AF153036
Crithidia_brevicula	KJ443344
Crithidia_brevicula	KJ443345
Crithidia_confusa	JF717837
Crithidia_deanei	EU079129
Crithidia_dedva	JN624299
Crithidia_expoeki	KM980187
Crithidia_fasciculata	Y00055
Crithidia_insperata	EU079125
Crithidia_mellificae	KM980183
Crithidia_mellificae	MK395375
Crithidia_mellificae	MK395375
Crithidia_otongatchiensis	JQ658813
Crithidia_otongatchiensis	KC205989
Crithidia_pachysarca	JF717836
Crithidia_permixta	EU079127
Crithidia_pragensis	KC205988
Crithidia_thermophila	KY264937
Crithidia_C4_TU105	KY357912
Endotrypanum_monterogeii	JQ863389
Endotrypanum_monterogeii	X53911
Herpetomonas_aff_modestus_MMO_06_TU221	MT174490
Herpetomonas_cf_roitmani	AF267738
Herpetomonas_costoris	JQ359728
Herpetomonas_elegans	JQ359725
Herpetomonas_isaaci	JQ359719
Herpetomonas_isaaci	JQ359720
Herpetomonas_isaaci	JQ359721
Herpetomonas_isaaci	KC205992
Herpetomonas_isaaci	KC709667
Herpetomonas_megaseliae	U01014
Herpetomonas_mirabilis	JQ359729
Herpetomonas_modestus	JQ359726
Herpetomonas_modestus	JQ359727
Herpetomonas_modestus	KC709668
Herpetomonas_muscarum	JQ359715
Herpetomonas_muscarum	JQ359731
Herpetomonas_muscarum	KC205982
Herpetomonas_muscarum	KX901631
Herpetomonas_muscarum	L18872
Herpetomonas_muscarum	U01015
Herpetomonas_nabicalae	JN624300
Herpetomonas_nabicalae	KF054113
Herpetomonas_puellarum	JQ359723
Herpetomonas_puellarum	JQ359724
Herpetomonas_puellarum	KC205994
Herpetomonas_puellarum	KC205995
Herpetomonas_puellarum	MT174494
Herpetomonas_roitmani	AF038023

Herpetomonas_samueli	JQ359722
Herpetomonas_samuelpessoai	JQ359716
Herpetomonas_samuelpessoai	JQ359717
Herpetomonas_samuelpessoai	JQ359718
Herpetomonas_samuelpessoai	KC205983
Herpetomonas_samuelpessoai	KC205984
Herpetomonas_samuelpessoai	KC205985
Herpetomonas_samuelpessoai	U01016
Herpetomonas_tarakana	KR868690
Herpetomonas_trimorpha	EU179326
Herpetomonas_wanderleyi	JQ359730
Herpetomonas_ztiplika	AF416560
Herpetomonas_B08-481B_TU147	KF054112
Herpetomonas_D31_Cu20_TU230	MG845928
Herpetomonas_D41_627_TU229	MG845925
Herpetomonas_M_MMO_02_TU108	KC205993
Herpetomonas_MCC04_TU245	MK056190
Herpetomonas_MMO_03_TU108	MT174488
Herpetomonas_sp_OSR_27_TU224	KR868691
Herpetomonas_TRSU106_TU228	MG255960
Jaenimonas_drosophilae	KP260534
Kentomonas_sorsogonicus	KM242075
Kentomonas_MRe_03_TU219	MT174495
Lafontella_mariadeaneii	JQ359714
Lafontella_mariadeaneii	KC205981
Lafontella_mariadeaneii	MT174491
Lafontella_mariadeaneii	U01013
Leishmania_braziliensis	JX030135
Leishmania_donovani	X07773
Leishmania_major	FR796423
Leishmania_mexicana	GQ332360
Leishmania_panamensis	JN003595
Leishmania_tarentolae	KC205986
Leishmania_tarentolae	M84225
Leishmania_tropica	GQ332363
Leptomonas_acus	DQ910923
Leptomonas_bifurcata	DQ910925
Leptomonas_brevicula(peterhoffi)	AF153039
Leptomonas_cf_podlipaevi	EU079124
Leptomonas_jaderae	EU079123
Leptomonas_lactosovorans	EU079122
Leptomonas_moramango	KC205990
Leptomonas_moramango	MT174471
Leptomonas_neopamerae	DQ910924
Leptomonas_podlipaevi	DQ383649
Leptomonas_podlipaevi	MT174503
Leptomonas_pyrrhocoris	JN036653
Leptomonas_pyrrhocoris	JQ658808
Leptomonas_pyrrhocoris	JQ658837
Leptomonas_pyrrhocoris	MK056191

Leptomonas_scantii	JN036654
Leptomonas_seymouri	MK056192
Leptomonas_seymouri	X53914
Leptomonas_spiculata	JF717838
Leptomonas_spiculata	MT174505
Leptomonas_tarcoles	EF546786
Leptomonas_tenua	JF717839
Leptomonas_tenua	KF054114
Leptomonas_pyrrhocoris	KY593785
Leptomonas_CFM	AF153041
Leptomonas_F6	AF153042
Leptomonas_M31_TU211	MT174482
Leptomonas_M32_TU212	MT174483
Leptomonas_Re13_TU213	MT174502
Lotmaria_passim	KM980188
Novymonas_esmeraldas	KT944309
Phytomonas_dolleti	KX219754
Phytomonas_francai	KU659041
Phytomonas_lipae	MK036047
Phytomonas_nordicus	KT223609
Phytomonas_oxycareni	KX257483
Phytomonas_serpens	AF016320
Phytomonas_CC71_TU240	MK056193
Phytomonas_CC83_TU241	MK056194
Phytomonas_D53_Cu32_TU233	MG845926
Phytomonas_EhiSe	L35077
Phytomonas_G24_TU76	JQ658819
Phytomonas_G65_TU77	JQ658841
Phytomonas_HART1	L35076
Phytomonas_Kn01	JQ658849
Phytomonas_M03_TU77/177-1	MT174469
Phytomonas_M23_TU248	MT174479
Phytomonas_M41_TU77/TU177-2	MT174485
Phytomonas_M42_TU252	MT174486
Phytomonas_Re03_TU251	MT174499
Phytomonas_Re06_TU250	MT174500
Phytomonas_Re25A_TU249	MT174504
Phytomonas_TCC231	AF016321
Sergeia_podlipaevi	DQ394362
Sergeia_EVA	AF071866
Strigomonas_culicis	HM593009
Strigomonas_culicis	L29266
Strigomonas_culicis	U05679
Strigomonas_galati	HM593010
Strigomonas_oncopelti	AF038025
Wallacemonas_collosoma	AF153038
Wallacemonas_collosoma	JN582046
Wallacemonas_raviniae	KC205996
Wallacemonas_raviniae	MT174493
Wallacemonas_rigidus	JN582047

Wallacemonas_rigidus	JN582048
Wallacemonas_rigidus	JN582049
Wallacemonas_Wsd	JN582045
Wallacemonas_MRe_04_TU220	MT174496
Zelonia_australiensis	KY273498
Zelonia_costaricensis	DQ383648
Zelonia_M08_TU214	MT174474
„Leptomonas_jaculum_Nep1“	EF184218
AF03_Et03_TU61	JQ658845
AF04_E04	JQ658846
AF07_Et07	JQ658847
AF12_E12_TU85	JQ658848
CC20_TU133b	MK056200
CC37A_TU236	MK056199
CC49A_TU235	MK056198
D44_1_TU231	MG845924
D51_Cu30_TU232	MG845927
Dros21_1	KC183711
Dros4_4	KC183713
Dros4_8	KC183715
Dros6_5	KC183714
Dros6_7	KC183712
G01_TU6/7E	JQ658807
G09/G45/G68_TU88	JQ658809
G10_TU64	JQ658810
G13_TU85	JQ658811
G14_TU72	JQ658812
G19_TU17	JQ658814
G20_TU6/7E	JQ658815
G21_TU89	JQ658816
G22_TU89	JQ658817
G23_TU72	JQ658818
G30_TU53	JQ658820
G31_TU6/7D	JQ658821
G33_TU73	JQ658823
G34_TU53	JQ658824
G35_TU86	JQ658825
G36/G52/G55/G63_TU44	JQ658826
G38_TU53	JQ658828
G39_TU6/7D	JQ658829
G40_TU80	JQ658830
G42_TU84	JQ658831
G44_TU6/7D	JQ658832
G61_TU6/7E	JQ658838
G64_TU72	JQ658840
G66/G67_TU81	JQ658842
CH148Ch2Env_TU59	GU059561
CH231Ch15Env_TU69	GU059562
CH300Ch14Env_TU6/7C	GU059563
CH322Ch5Env_TU68	GU059564

CH332Ch13_TU68	GU059565
CH334Ch16_TU91	JQ658851
CH334Ch3_TU60	GU059566
CH339Ch1_TU44	GU059567
CH380Ch8_TU64	GU059568
CH391Ch10_TU66	GU059569
CH392Ch4_TU61	GU059570
CH395Ch16_TU91	JQ658852
CH402Ch12_TU67	GU059571
CH404Ch15_TU69	GU059572
Ch50Ch6_TU62	GU059558
CH53Ch7_TU63	GU059559
CH91Ch9_TU65	GU059560
Kn_07_PL7_TU62	JQ658850
M_ECU_07_TU116	KC206002
M_ECU6_1a2_TU117	KC206003
M_GMO_05_TU112	KC205998
M_GMO04_TU115	KC206001
M_Ke22_TU114	KC206000
M_MB19_TU113	KC205999
M_MB22_TU17	KC205987
M_MCZ_09_TU111	KC205997
M_MCZ_10_TU106	KC205991
M_MCZ_13_TU119	KC206005
M_MCZ_14_TU118	KC206004
M04_TU6/7D	MT174470
M05_TU73-1	MT174472
M07_TU62	MT174473
M09_TU88	MT174475
M12_TU88b	MT174476
M20_TU216	MT174477
M21_TU6/7D2	MT174478
M26_TU6/7E	MT174480
M30_TU218	MT174481
M37_TU44	MT174484
M47_TU6/7C	MT174487
MCC01_TU243	MK056196
MCC02_TU242	MK056195
MCC03_TU244	MK056197
MMO_05_envi_TU253	MT174489
PNG02_TU173	KY593709
PNG03_TU174	MF969016
PNG04_TU175	MK929397
PNG05_TU176	MK929398
PNG09_TU177	MK929400
PNG100_TU247	KY593769
PNG102_TU199	MF969033
PNG105_TU201	MF969036
PNG106_TU6/7C	MF969037
PNG108_TU202	KY593781

PNG11_TU44	MK929402
PNG112_TU203	KY593784
PNG114_TU204	MK929459
PNG115_TU205	MF969039
PNG12_TU178	KY593715
PNG120_TU206	MK929462
PNG127_TU207	MF969043
PNG128-1_TU208	MF969044
PNG13-1_TU179	KY593716
PNG13-2_TU180	KY593717
PNG135_TU209	MF969046
PNG136_TU210	KY593813
PNG138_TU181	MF969047
PNG21_TU89	MK929408
PNG25_TU77	MK929412
PNG43_TU182	KY593729
PNG47_TU183	MK929430
PNG50_TU184	KY593731
PNG53_TU185	MK929435
PNG57_TU186	KY593733
PNG60_TU83	KY593734
PNG62_TU187	KY593735
PNG68_TU188	MK929442
PNG72_TU189	MK929446
PNG75_TU190	MK929449
PNG77_TU191	KY593740
PNG78_TU192	MK929451
PNG82_TU194	MF969021
PNG84_1_TU195	MN215472
PNG90_TU193	MF969025
PNG95_2_TU197	KY593764
PNG96_TU198	MF969029
PNG98_TU200	MF969031
Re01_TU217	MT174497
Re02_TU72	MT174498
Re35_TU73	MT174506
Re36_TU215	MT174507
Re43A_TU6/7AB2	MT174508
Paratrypanosoma_confusum	KF963538
Neobodo_designis	AY998650
Bodo_saltans	AF208887
Bodo_saltans	AY490224
Bodo_saltans	DQ207575
Bodo_uncinatus	AF208884