***Leishmania* (*Sauroleishmania*) (reptiles)**
(protist: flagellate)

Overview

Protists are single-celled organisms with membrane-bound nuclei (eukaryotes). Flagellates are protists that swim using one or more flagella (undulipodia); each arising from a small centriole (basal body, kinetosome) and having a microtubular axoneme core (2+9 configuration). Rather than forming a monophyletic group, flagellates are divided into several disparate groups: metamonads (amitochondriate flagellates), heteroloboseans (amoeboflagellates), euglenozoans (euglenids and kinetoplastids), stramenopiles (heterokonts), alveolates (dinoflagellates) and cercozoans (biflagellates). Most kinetoplastids are parasitic in vertebrate or invertebrate hosts (some in plants) whereas the remainder are free-living aquatic organisms. All species are characterized by the possession of extranuclear DNA in the form of a kinetoplast, a unique structure formed by massed DNA (circles or lattice) within the single large mitochondrion near the flagellar basal body. The flagellates reproduce by longitudinal binary fission and parasitic species may have simple monoxenous (one-host) or more complicated heteroxenous (two-host) life cycles involving different developmental stages. Trypanosomes have a single flagellum and they form four main developmental stages: trypomastigotes (with a posterior kinetoplast and an emergent flagellum forming a long undulating membrane); epimastigotes (with an anterior kinetoplast and an emergent flagellum forming a short undulating membrane); promastigotes (with an anterior kinetoplast and a short emergent flagellum, but no undulating membrane); and amastigotes (with a kinetoplast but no emergent flagellum or undulating membrane). Many trypanosome species are parasitic only in insects whereas others are transmitted by insect vectors to a wide range of vertebrate hosts. Three main groups infect the blood and/or tissues of humans and animals causing severe clinical diseases: including the leishmanias which develop in the foregut of insect vectors and are transmitted via bite to the tissues of vertebrate hosts (e.g. sand flies transmit *L. Sauroleishmania* spp. to lizards).

Classification:

Domain:	Eukaryota (membrane-bound nucleus)
Supergroup:	Excavata (with conspicuous ventral feeding groove)
Group:	Discoba (diverse group supported robustly by molecular studies)
Phylum:	Euglenozoa (flagella inserted in anterior pocket, some heterotrophs, some autotrophs (with chloroplasts))
Class:	Kinetoplastea (heterotrophs, with extranuclear DNA (= kinetoplast) associated with mitochondrion)
Subclass:	Metakinetoplastina (large polyphyletic group supported by molecular studies)
Order:	Trypanosomatida (parasitic, single anterior flagellum, often forming undulating membrane)
Family:	Trypanosomatidae (monogenetic forms in insects/plants, digenetic forms in vertebrates & arthropods)
Genus:	*Leishmania* (*Sauroleishmania*) (vector-borne tissue parasites)
Species:	various species infect lizards

Parasite biodiversity and host range: Protists are unicellular eukaryotes that move using undulipodia (flagella or cilia), pseudopodia (false-feet) or a unique gliding motion. Flagellated species have one or more flagella with an internal microtubular core (in a characteristic 2+9 configuration comprising 2 single central microtubules and 9 peripheral doublets) anchored to a submembranous protein structure (known variously as a centriole, basal body, kinetosome or blepharoplast). Many types of flagellated cells have been described and recent phylogenetic studies have classified them into several disparate groups: including the metamonads (amitochondriate flagellates), heteroloboseans (amoeboflagellates), euglenozoans (euglenids and kinetoplastids), stramenopiles (heterokonts), alveolates (dinoflagellates) and cercozoans (biflagellates). While most flagellated protists are free-living organisms swimming and feeding in aquatic environments, representatives of several groups have developed symbiotic relationships with various hosts; some being endoparasitic in vertebrates (notably anaerobic metamonads in tubular organs, and heterotrophic euglenozoans occurring in blood or tissues), and some being parasitic in invertebrates (alveolates in crustacean tissues) (representatives tabulated below).

Higher taxonomy	Class or order	Family	Genera	Hosts (tissues)	Trans-mission*
Supergroup: Excavata (with conspicuous ventral feeding groove)					
Group: Metamonad (amitochondriate flagellates with karyomastigonts)					
Phylum: Fornicata (diplomonads)	Order: Diplomonadida (1-2 karyomastigonts)	Hexamitidae (2 karyomastigonts with binary axial symmetry)	*Giardia*	vertebrates (gut)	direct (f-o)
			Hexamita Spironucleus	vertebrates (tissues)	direct (f-o, w)
Phylum: Parabasalia (with parabasal body)	Order: Trichomonadida (3-5 anterior flagella plus recurrent flagellum)	Monocercomonadidae (costa absent, most without undulating membrane)	*Histomonas*	birds (gut, liver)	direct (f-o)
			Dientamoeba	vertebrates (gut)	direct (f-o)
		Trichomonadidae (stout axostyle, costa, undulating membrane)	*Trichomonas*	vertebrates (urogenital tract, gut)	direct (f-o, v)
		Cochlosomatidae (anterior adhesive disc)	*Cochlosoma*	birds (gut)	direct (f-o)
Group: Discoba (diverse group supported robustly by molecular studies)					
Phylum: Euglenozoa (flagella inserted in anterior pocket, heterotrophs, autotrophs)	Class: Kinetoplastea (heterotrophs, with extranuclear DNA (= kinetoplast) associated with mitochondrion)	Ichthyobodonidae (flagellar pocket continues as groove)	*Ichthyobodo* (= *Costia*)	fish (gills, skin)	direct (w)
		Parabodonidae (epizoic or endozoic)	*Cryptobia*	fish (gills, skin)	direct (w)
			Trypanoplasma	fish (blood)	indirect (v-b)
		Trypanosomatidae (monogenetic forms in insects/plants, digenetic forms in vertebrates & arthropods)	*Trypanosoma*	vertebrates (blood, tissues)	indirect (v-b)
			Leishmania	vertebrates (blood, tissues)	indirect (v-b)
Supergroup: SAR (Stramenopiles + Alveolata + Rhizaria) (3 groups unified by molecular studies)					
Group: Alveolata (with cortical alveoli)					
Phylum: Dinoflagellata (with unique mesokaryotic nuclei)	Order: Blastodiniales (uninucleate trophonts with chloroplasts)	Oodiniaceae (trophont with rhizoid-like invasive organelle)	*Amyloodinium Crepidoodinium Piscinoodinium*	fish (skin)	direct (w)
	Order: Syndiniales (multinucleate plasmodial trophonts)	Syndiniaceae (without chloroplasts)	*Haematodinium Ichthyodinium*	crustaceans, fish (tissues)	direct (w)
Phylum: Perkinsozoa (parasitic)	Order: Perkinsorida (released trophonts form biflagellated zoospores)	Perkinsidae (incomplete conoid)	*Perkinsus*	gastropods, bivalves (tissues)	direct (w)

*f-o = faecal-oral transmission; v-b = vector-borne transmission, w = water-borne transmission; v = venereal transmission

Euglenozoans comprise a large group of excavates (with ventral feeding groove), most with 1-2 flagella inserted into an anterior pocket. Many species are free-living aquatic autotrophs possessing chloroplasts while others are free-living or symbiotic heterotrophs feeding on solutes, particles and even other organisms. Kinetoplastids are characterised by the possession of a kinetoplast (containing mitochondrial DNA separate from nuclear DNA), a flagellar pocket, basal bodies with three microtubular roots and paraxonemal (paraxial or paraflagellar) rods, and asexual multiplication by longitudinal binary fission. The unique kinetoplast is formed by massed DNA (circles or lattice) usually closely associated with the flagellar basal body (eukinetoplastic) although some species may be polykinetoplastic (with several kinetoplasts) or pankinetoplastic (irregular kDNA) and some mutants even dyskinetoplastic (without a kinetoplast). Two major kinetoplastid groups are recognized: bodonids with two flagella (most being free-living bactivores in aquatic/terrestrial habitats); and trypanosomes with a single flagellum (most being parasites of animals or plants with monoxenous or dixenous life-cycles). Different kinetoplastid assemblages exhibit increasing morphological/ultrastructural complexity in their cellular organization thought to reflect evolutionary grades or clines. Amastigotes are simple non-flagellated cells, choano-, pro- and opistho-mastigotes are flagellated cells with elongate flagella, while epi- and trypo-mastigotes are flagellated cells with undulating membranes. Most kinetoplastids have amastigote and promastigote developmental stages but monoxenous parasites of insects (e.g. *Crithidia*, *Herpetomonas*) do not have more elaborate forms whereas dixenous parasites of plants or animals with invertebrate vectors (e.g. *Trypanosoma, Leishmania*) do have more morphologically complex forms such as epimastigotes and trypomastigotes.

Traditional classification	Molecular classification	Genera	No. spp.	Vertebrate hosts	Transmission (vectors)
F: Trypanosomatidae	SC: Metakinetoplastina F: Trypanosomatidae	*Trypanosoma*	537	mammals, reptiles, frogs, birds, fish	indirect (arthropods, leeches)
		Leishmania	53	mammals, lizards	indirect (sand flies)
F: Bodonidae	SC: Metakinetoplastina F: Parabodonidae	*Cryptobia, Trypanoplasma*	79	fish	direct or indirect (leeches)
	SC: Prokinetoplastina F: Ichthyobodonidae	*Ichthyobodo* (*Costia*)	5	fish	direct

Conventional taxonomic classification systems divide the kinetoplastids into 2 groups: the free-living bi-flagellated Bodonina; and the parasitic uni-flagellated Trypanosomatina. Over 600 species have been described on the basis of multiple phenotypic characters (host occurrence, geographic distribution, vectors, transmission cycles, morphology, development, pathogenicity, culture requirements, etc.). Modern molecular characterization studies, however, have shown that many traditional groups are polyphyletic and composed of numerous clades. Contemporary phylogenetic classifications recognize 2 main lineages: the Prokinetoplastina represented by 2 diverse genera (*Ichthyobodo* biflagellates ectoparasitic on freshwater and marine fishes, and *Perkinsela* (= *Perkinsiella*) aflagellates endosymbiotic (as parasomes or parasome-like organisms (PLOs)) in amoeba *Paramoeba* and *Neoparamoeba*); and the Metakinetoplastina containing 4 groups, including free-living aquatic eu-bodonids (with one genus *Bodo*), free-living neo-bodonids (with 10 genera, including *Rhynchomonas*), free-living or commensal/parasitic para-bodonids (with 5 genera, including *Cryptobia, Trypanoplasma*), and the parasitic trypanosomatids (containing some 39 genera, including *Trypanosoma* and *Leishmania*).

Trypanosomatids are dixenous (2-host) parasites with indirect transmission cycles between vertebrates and invertebrate vectors. *Leishmania* spp. form amastigote stages in the tissues of their vertebrate hosts, and promastigote stages in invertebrate haematophagous vectors. Species of *Leishmania* in lizards appear to be restricted to the Old World, with the exception of *L. henrici* in the Caribbean. The genus *Sauroleishmania* was originally proposed to include *Leishmania* species infecting reptiles (lizards) rather than mammals, but molecular phylogenetic studies have indicated that it be ranked at the subgeneric rather than the generic level. Nonetheless, there appear to be many differences between leishmanial parasites of mammals and reptiles, including variations in host range and specificity (involving both vertebrate and invertebrate hosts), developmental cycles, modes of transmission, tissue tropism, parasite antigens, isoenzymes and nucleic acids.

***Leishmania* species**	**Hosts**	**Location**	**Vectors**	**Distribution**
Subgenus: *Sauroleishmania*				
L. (*S.*) *adleri*	lacertid (fringe-toed lizard, green lizard, southern long-tailed lizard); Rodentia: cricetid (golden hamster)	blood, skin	Diptera: psychodid (*Sergentomyia clydei*)	Africa
L. (*S.*) *agamae*	agamid (steppe agama, roughtail rock agama)	blood	Diptera: psychodid (*Phlebotomus papatasi, caucasicus, Sergentomyia sintoni*)	Mediterranean
L. (*S.*) *ceramodactyli*	gekkonid (Doria's comb-fingered gecko)	blood	Diptera: psychodid (*Phlebotomus papatasi, caucasicus, Sergentomyia sintoni*)	Mediterranean
L. (*S.*) *gymnodactyli*	gekkonid (comb-toed gecko, Caspian bent-toed gecko, grey thin-toed gecko, frog-eyed gecko), agamid (steppe agama, Caucasian agama, secret toadhead agama, sunwatcher toadhead agama, Lichtenstein's toadhead agama, Radde's toadhead agama), anguid (sheltopusik), lacertid (small-spotted lizard, sand racerunner, striped racerunner, reticulate racerunner, rapid racerunner, Aralo-Caspian racerunner), scincid (ribbon-sided skink, Schneider's skink, golden grass skink); Serpentes: colubrid (common cliff racer, spotted desert racer, dwarf racer), psammophiid (Schokari sand racer), viperid (saw-scaled viper)	blood	Diptera: psychodid (*Phlebotomus papatasi, caucasicus, Sergentomyia sintoni, arpaciensis, clydei, grecovi*)	Russia

L. (*S.*) *hemidactyli*	gekkonid (Gleadow's house gecko, Brooke's house gecko)	blood		India
L. (*S.*) *hoogstraali*	gekkonid (Mediterranean house gecko)	blood	Diptera: psychodid (*Sergentomyia clydei*)	Sudan
L. (*S.*) *platycephala*	gekkonids (flathead leaf-toed gecko, Barbour's gecko)	red and white blood cells	Diptera: psychodid (*Sergentomyia* sp., *Lutzomyia vexator*)	Tanzania
L. (*S.*) *tarentolae*	gekkonids (Moorish gecko, white-spotted wall gecko, Kotschy's gecko)	red and white blood cells, spleen	Diptera: psychodid (*Phlebotomus papatasi, Sergentomyia antennata* (*minuta*), *parroti*)	Mediterranean
L. (*S.*) *zuckermani*	gekkonids (Turner's thick-toed gecko)	red blood cells -		South Africa
Incertae sedis (uncertain subgeneric placement)				
L. chamaeleonis	chamaeleonid (common chameleon, Cape dwarf chameleon)	cloaca, intestines		Middle-East, Africa
L. davidi	teiid (rainbow whiptail)	cloaca, large intestines		Americas
L. gulikae	agamid (Caucasian agama)	blood		Middle-East
L. henrici	dactyloid (leopard anole, green anole)	blood, cloaca		Caribbean
L. nicollei (syn. *L. helioscopi, phrynocephali*)	agamid (steppe agama, sunwatcher toadhead agama, secret toadhead agama)	blood		Middle-East
L. senegalensis	phyllodactylid (ringed wall gecko)	blood		Africa
L. sofieffi	agamid (spotted toadhead agama, secret toadhead agama)	blood		Middle-East
L. zmeevi	lacertid (reticulate racerunner, Aralo-Caspian racerunner)	blood	Diptera: psychodid (*Phlebotomus papatasi, Sergentomyia sintoni*)	Russia

Parasite morphology: Sauroleishmanias form 2 different types of developmental stages: amastigotes and promastigotes. Amastigotes are small spherical cells ranging from 1-5 µm in diameter (they are among the smallest eukaryotic (nucleated) cells known). They are located intracellularly in vacuoles within the cytoplasm of host cells, sometimes occurring in groups of up to 3-10 cells. Amastigotes are not flagellated but they contain a flagellar sac and a non-emergent vestigial flagellum. They have a rounded central nucleus and a smaller adjacent kinetoplast (containing mitochondrial DNA) surrounded by a small ring of vacuolated cytoplasm. Promastigotes are elongate slender cells ranging in size from 5-20 x 1.5-3.5 µm. They occur as extracellular stages and have a single emergent flagellum that undulates freely from the anterior cell surface (it is not recurrent and does not form an undulating membrane). The flagellum arises from a small dense kinetoplast located anterior to the central cell nucleus. Promastigotes develop from short procyclic forms to long thin metacyclic forms, sometimes involving intermediary forms described as thin nectomonads with short flagella, small leptomonads with long flagellum and short stout haptomonads with short leaf-shaped flagella attached to the vector gut wall.

Site of infection: In reptiles, promastigotes may be found extracellularly in the bloodstream while amastigotes occur as intracellular stages in circulating blood cells (erythrocytes, thrombocytes, monocytes) or phagocytes (macrophages, histiocytes and precursors) within host tissues (skin or viscera). A total of 17 parasites species have been described in reptilian hosts, including 42 lizard species from 9 families (agamids, lacertids, gekkonids, teiids, chamaeleonids, dactyloids, phyllodactylids, scincids) and 5 snake species from 3 families (colubrid, psammophiid, viperid). In vectors, promastigotes develop within the gut either in the posterior station (hypopylarian) or the anterior station (peripylarian). Some 9 species of psychodid sandflies have been found to act as vectors for reptilian leishmanias, including 2 *Phlebotomus* spp., 6 *Sergentomyia* spp. and one *Lutzomyia* sp.

Pathogenesis: Metacyclic infective promastigotes that invade vertebrate host tissues have numerous lipophosphoglycan molecules on their membranes which enables them to resist lysis as well as to adhere to macrophage membranes facilitating their phagocytosis. They transform into amastigotes within parasitophorous vacuoles formed by fusion of phagosomes with lysosomes where they are resistant to digestion by lysozymal enzyme. The amastigotes feed, grow and multiply by asexual division, ultimately rupturing the host cell to release new infective stages**.** Despite their eventual destructive effects on host cells, they do not cause enough cumulative damage to manifest as clinical disease and infections in reptiles are asymptomatic (subclinical at the very least).

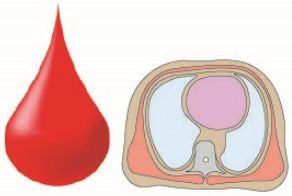
Experimental transmission studies have shown that the parasites often persist in small numbers and/or in cryptic locations as latent or chronic infections.

Developmental cycle and mode of transmission: *Sauroleishmania* spp. have heteroxenous (2-host) life-cycles involving blood and tissue stages in reptiles being taken up by haematophagous (blood-feeding) sandfly vectors where infective stages develop in the gut. Vector-borne transmission to reptiles occurs by the ingestion of vectors (consumptive transmission), via contamination by vector faeces (stercorarian transmission) or by vector bite (inoculative transmission). All vectors for reptilian leishmanias belong to the subfamily Phlebotominae in the dipteran family Psychodidae. These sand flies are small (2.0-3.5 mm long) and have lanceolate wings and tiny hairs covering their bodies. Adult female flies feed on vertebrate blood to obtain the nutrients required to complete their reproductive cycles. Amastigotes ingested during feeding transform in the gut into promastigotes which multiply by binary fission (development from procyclic to metacyclic forms sometimes proceeding through several intermediary stages). Development in the vector gut occurs either in the posterior station (hypopylarian) or anterior station (peripylarian). Promastigotes of hypopylarian species multiply predominantly in the abdominal hindgut, thus implicating transmission by vector faeces or by ingestion of the vector. Promastigotes of peripylarian species multiply primarily in the abdominal midgut and then move forward to the thoracic midgut, oesophagus, pharynx and head, thus implicating transmission by vector bite. Metacyclic promastigotes invading reptiles are taken up by phagocytic cells where they transform into amastigotes and undergo asexual proliferation.

Differential diagnosis: Most infections in reptiles have been detected by isolating promastigotes in culture, indeed many species are only known from cultural forms (which are very similar). Promastigotes grow well *in vitro* in cultures containing enriched media (particularly nutrient agar-blood mixtures). Cultures may be seeded with samples of heart blood, peripheral blood or lacerated tissues from reptiles, as well as gut contents of sandfly vectors. Amastigotes may sometimes be detected by the microscopic examination of blood smears, tissue impression smears or histological sections after staining with Giemsa or Leishman's stains. Several immunoserological tests (fluorescent-antibody tests) have been developed to detect host antibodies against infection in some reptiles, but host responses to cutaneous infections are often poor and most tests could not distinguish between recent and chronic infections. Parasite isolates have been analysed using various protein characterization techniques, including isoenzyme analyses, polyacrylamide gel electrophoresis and immunoblotting, but they have not been adapted for diagnostic use. Modern molecular characterization techniques have used the polymerase chain reaction (PCR) to amplify specific gene sequences (parasite DNA) from host samples; including small subunit (18S) ribosomal RNA (rRNA), internal transcribed spacer regions, and RNA polymerase II large subunit genes.

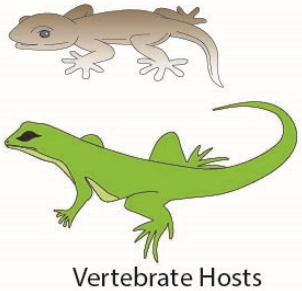
Treatment and control: Infections in reptiles are asymptomatic and do not require treatment. Various preventive measures may be used in captive reptile colonies to minimize the risks of spreading infections by protecting animals from sandflies using physical barriers, chemical insecticides or biological control programs.

Leishmania (*Sauroleishmania*) (reptilian species)

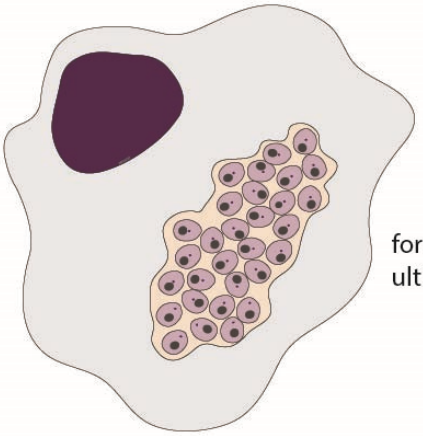


blood, tissues
(nonpathogenic)

division by binary fission
in blood cells or tissue
phagocytes



Vertebrate Hosts
(lizards)

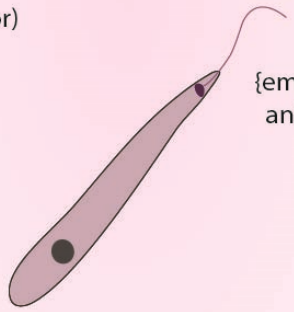


form intracellular colonies
ultimately lysing host cell

amastigotes (1-5 µm)
(no emergent flagellum)

transmission either
inoculative (via vector bite),
consumptive (via ingestion of vector)
or contaminative (via bite site)

ingested with
bloodmeal

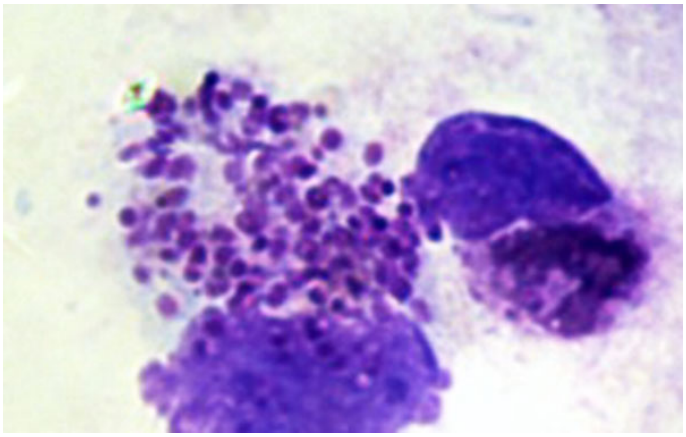


{emergent flagellum,
anterior kinetoplast]

promastigotes
(5-20 µm)

Invertebrate Hosts
(sandfly vectors)
(anterior or posterior gut)

vector-borne transmission

	
Sauroleishmania amastigotes in lizard tissues	*Sauroleishmania* promastigote in sandfly gut