



Detection of *Leishmania martiniquensis* and *Trypanosoma* sp. in biting midges (Diptera: Ceratopogonidae) collected from an endemic area of leishmaniasis in northern Thailand

การตรวจหาเชื้อ *Leishmania martiniquensis* และ *Trypanosoma* sp. ในริ้นน้ำเค็มที่เก็บจากพื้นที่ระบาดของโรคไลชมาเนียในภาคเหนือของประเทศไทย

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Abstract

Biting midges are small blood sucking insects belonging to the Ceratopogonidae family. These are mainly known as the potential vectors of pathogens including viruses, protozoa, and filarial worm, which are transmitted pathogenic organisms to humans and livestock. In Thailand, biting midges can be found around the leishmaniasis patient's home, the high abundance of *Culicoides* in areas with active leishmaniasis transmission points to the possibility of *Culicoides* infection by these protozoa. However, the detection of *Leishmania* spp. and *Trypanosoma* spp. in biting midges have never been reported in Thailand. In this study, we aim to detect the presence of *Leishmania* sp. and *Trypanosoma* sp. in biting midges collected from active leishmaniasis from Lamphun province of northern, Thailand. A total of 233 female biting midges were collected with ultraviolet (UV) CDC traps from 6:00 pm- 6:00 am. Head, wings, and genitalia dissection of female biting midges were done for morphology identification, and the remaining parts of *Culicoides* were then used for detecting *Leishmania* and *Trypanosoma* DNA by using PCR based on *ITS1* and *SSU rRNA* gene, respectively. Morphological identification revealed 7 species: *Culicoides arakawae* (47.53%), *C. guttifer* (22.87%), *C. innoxius* (12.56%), *C. sumatrae* (0.9%), *C. huffi* (9.42%), *C. oxystoma* (2.24%) and *C. palpifer* (4.48%), respectively. The PCR and sequence analysis were able to detect *Leishmania* DNA, which identified as *L. martiniquensis* in 3 specimens of *C. arakawae*. Moreover, one sample was detected *Trypanosoma* DNA in *C. huffi*. A phylogenetic of *Trypanosoma* was closely related to avian *Trypanosoma*. The finding of the study indicates that biting midges suspected to be the potential vector of leishmaniasis or trypanosomiasis, whereas vector competence and transmission capability must be confirmed in future investigation.

Keywords (Biting midges, *Leishmania martiniquensis*, *Trypanosoma* sp. Thailand)

Introduction

- Biting midges are minute hematophagous insect belonging to the genus *Culicoides* Latreille (Diptera: Ceratopogonidae). Several biting midges species play an important role in the transmission of arboviruses in domestic and wild ruminants.
- Several studies have demonstrated the capability of *Leishmania*, and *Trypanosoma* parasites development in biting midges.
- Leishmaniasis* is a vector-borne disease that are caused by flagellated protozoan parasites of the *Leishmania* genus. In Thailand, the causative agents of autochthonous leishmaniasis are *Leishmania martiniquensis* and *L. orientalis* (previously reported as "*L. siamensis*").
- However, the potential of biting midges as a potential vector for *Leishmania* or *Trypanosoma* parasites transmission in Thailand are limited.
- We aim to determine the presence of *Leishmania* and *Trypanosoma* parasites in biting midges collected from an endemic area of leishmaniasis in northern Thailand.

Results

- Seven species of the genus *Culicoides* were identified as *Culicoides arakawae* (47.53%), *C. guttifer* (22.87%), *C. innoxius* (12.56%), *C. huffi* (9.42%), *C. palpifer* (4.48%), *C. oxystoma* (2.24%) and *C. sumatrae* (0.9%) (Fig1). In this study, *C. arakawae* was found to be the most abundant species.
- ITS1*-PCR were positive for *Leishmania* DNA detected in three samples of *C. arakawae*.
- The phylogenetic tree of all positive *L. martiniquensis* *ITS1* sequences was clearly classified into the *L. martiniquensis* group with bootstrap support value of 99% (Fig 2).
- SSU rRNA*-PCR was detected of *Trypanosoma* sp. DNA in one samples of *C. huffi*.
- Our *Trypanosoma* sequence was classified within *Trypanosoma* parasites from avian *Trypanosoma* clade (Fig 3).

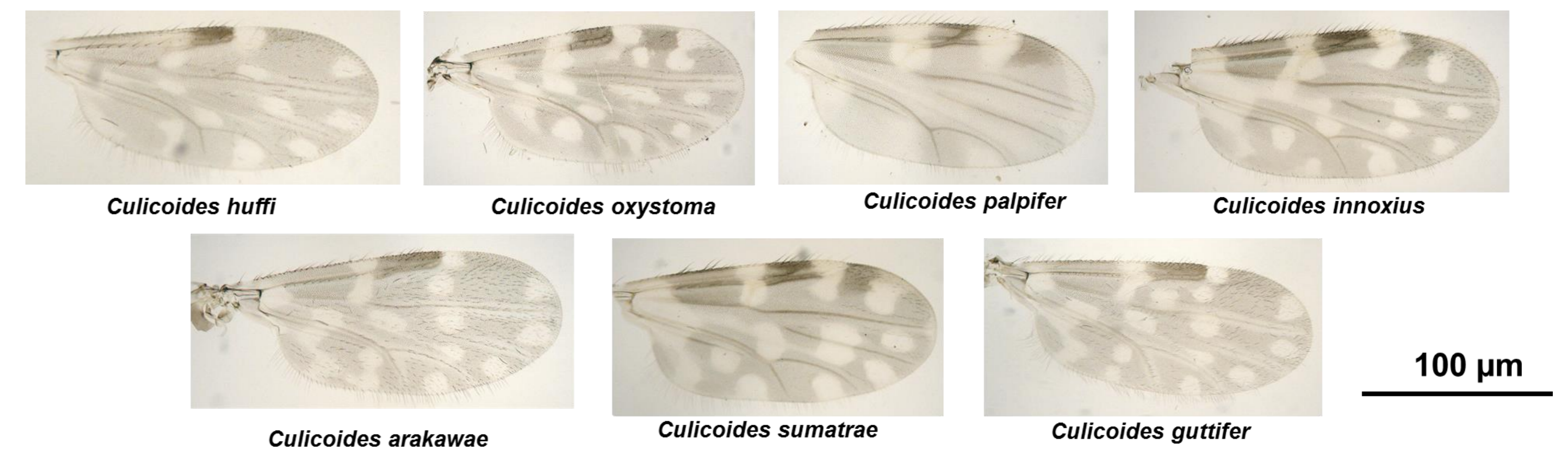


Fig 1. Wings morphological pattern of the of *Culicoides* found in this study

Methods

Samples collection

- Female biting midges were collected around a leishmaniasis patient's house in Ban Hong District, Lamphun province, northern Thailand (18°19'1"N 98°48'51"E).

- The UV-trap operated for over 12 hours from 6.00 pm to 6.00 am.
- The samples were kept in liquid nitrogen and sent to the Vector Biology and Vector Borne Disease Research Unit, Department of Parasitology, Faculty of Medicine, Chulalongkorn University.

Detection of *Leishmania* and *Trypanosome*

Morphological identification

Head, wing and genitalia of female biting midges

Wings pattern

Thorax, abdomen DNA extraction

PCR amplification

- Leishmania* (*ITS1* gene)
- Trypanosoma* (*SSU rRNA* gene)

DNA sequencing Phylogenetic analysis

Discussion and Conclusion

- Of 223 samples of biting midges, we demonstrated that *Leishmania martiniquensis* DNA was detected in biting midges by using *ITS1*-PCR, for the first time, which belong to *Culicoides arakawae*.
- In this study, we reported the presence only *L. martiniquensis* DNA in the sample. However, to confirm the role of *C. arakawae* is a vector of *Leishmania* transmission, studies should be required by isolation and cultivation of *Leishmania* parasite.
- This is the first detection of *Trypanosoma* sp. in *C. huffi* from Thailand. The phylogenetic analysis of *Trypanosoma* from our positive sample was classified into the *Trypanosoma* parasites from avian clade.
- The detected of both *L. martiniquensis* and *Trypanosoma* parasite in biting midges suggests that it might be a potential vector of leishmaniasis or trypanosomiasis in Thailand.

Acknowledgement

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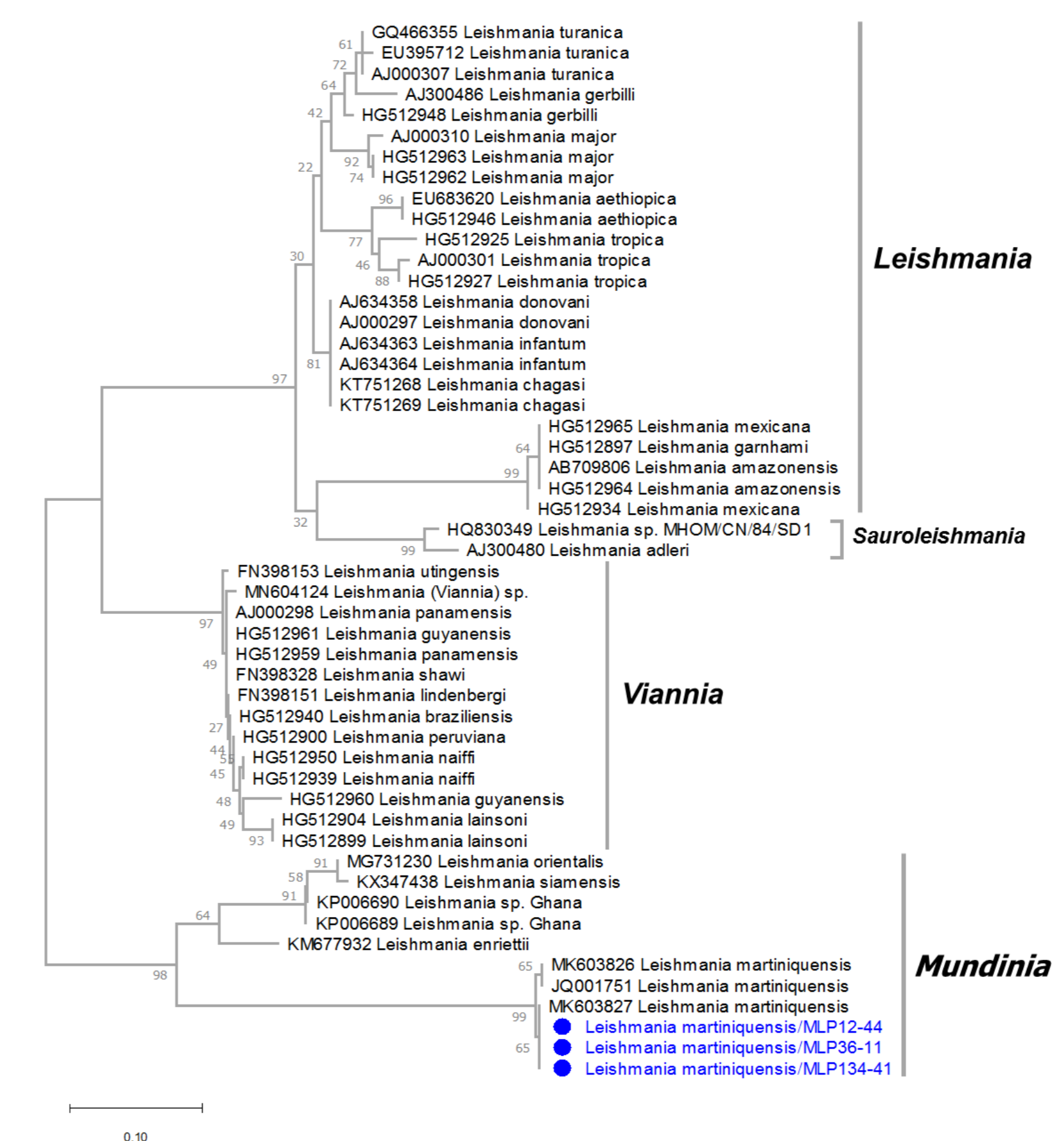


Fig 2. Maximum likelihood tree of the *Leishmania* spp. was constructed from partial *ITS1* sequences. Blue circles indicated the positive samples that were obtained from this study.

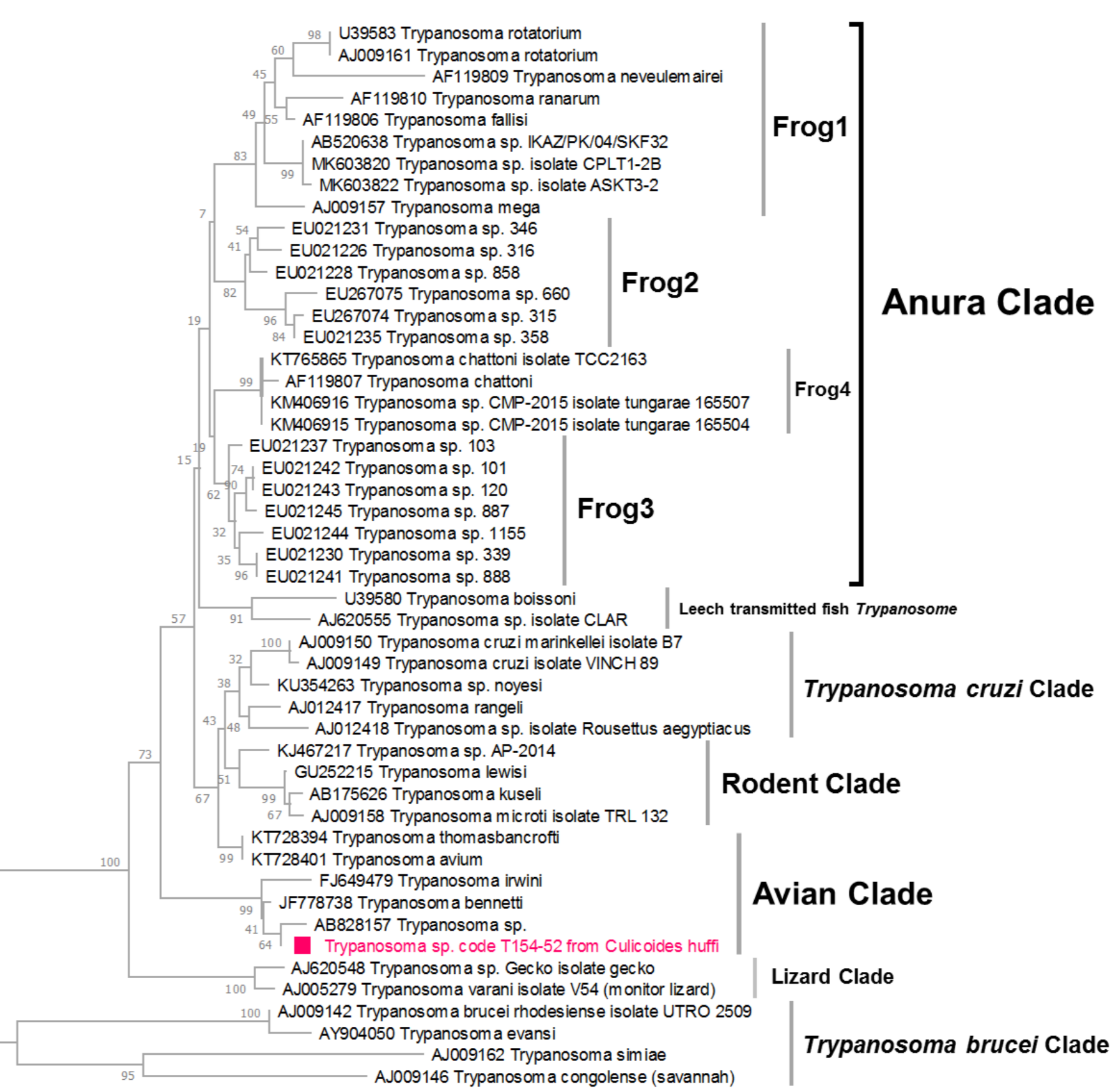


Fig 3. Maximum likelihood tree was constructed from the partial *SSU rRNA* sequences of *Trypanosoma* parasites. The *Trypanosoma* *SSU rRNA* sequence from this study is indicated with pink squares.