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Phylogeny and Morphological Variability of Trypanosomes from African Pelomedusid Turtles with Redescription of *Trypanosoma mocambicum* Pienaar, 1962

Nela Dvořáková[a,b,1], **Ivan Čepička**[c], **Moneeb A. Qablan**[b,d], **Wendy Gibson**[e], **Radim Blažek**[f], and **Pavel Široký**[a,b]

[a]Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1/3, 612 42 Brno, Czech Republic
[b]CEITEC-Central European Institute of Technology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1/3, 612 42 Brno, Czech Republic
[c]Department of Zoology, Faculty of Science, Charles University in Prague, Viničná 7, 120 44 Prague 2, Czech Republic
[d]Department of Pathology and Parasitology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Palackého tř. 1/3, 612 42 Brno, Czech Republic
[e]School of Biological Sciences, University of Bristol, Bristol BS8 1TQ, United Kingdom
[f]Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, v. v. i., Květná 8, 603 65 Brno, Czech Republic

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Little is known about host specificity, genetic diversity and phylogenetic relationships of African turtle trypanosomes. Using PCR targeting the SSU rRNA gene, we detected trypanosomes in 24 of 134 (17.9%) wild caught African pelomedusid turtles: *Pelusios upembae* (n = 14), *P. bechuanicus* (n = 1), *P. rhodesianus* (n = 3) and *P. subniger* (n = 6). Mixed infection of *Trypanosoma* species was confirmed by PCR in three specimens of *P. upembae*, and in one specimen each of *P. bechuanicus*, *P. rhodesianus*, and *P. subniger*. Microscopic examination of stained blood smears revealed two distinct forms (broad and slender) of trypomastigotes. The broad form coincided in morphology with *T. mocambicum* Pienaar, 1962. Accordingly, we have designated this form as the neotype of *T. mocambicum*. In phylogenetic analysis of the SSU rRNA gene, all the new turtle trypanosome sequences grouped in a single clade within the strongly supported "aquatic" clade of *Trypanosoma* species. The turtle trypanosome clade was further subdivided into two subclades, which did not correlate with host turtle species or

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[1]Corresponding author; fax +420 541 562 631
e-mail nelka@volny.cz (N. Dvořáková).

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trypanosome morphology. This study provides the first sequence data of *Trypanosoma* species isolated from freshwater turtles from tropical Africa and extends knowledge on diversity of trypanosomes in the Afrotropical zoogeographical realm.

Key words: *Trypanosoma*; turtle; *Pelusios*; polymorphism; phylogeny; SSU rRNA gene.

Introduction

Genus *Trypanosoma* Gruby, 1843 (Euglenozoa: Kinetoplastea) infects all classes of vertebrate hosts, but most attention is directed to species that cause serious forms of human and animal diseases and heavy economic losses (Hoare 1972). Trypanosomes are transmitted mostly by bloodsucking arthropods and leeches (Hamilton et al. 2005). Morphological diagnosis and identification of trypanosomes based on microscopic examination of stained blood films has many pitfalls due to overlap of characteristics, species polymorphism and false negative results associated with low parasitaemia (Austen et al. 2009; Gu et al. 2007; Thompson et al. 2013). Diagnostic sensitivity increases with the use of specific PCR-based methods, which can also help to classify a parasite into species using genetic barcodes, and also distinguish mixed infections, depending on the marker used. Small subunit (SSU) rRNA and glycosomal glyceraldehyde 3-phosphate dehydrogenase (gGAPDH) genes are currently the most widely used markers to identify *Trypanosoma* species and to characterize their genetic variability and phylogeny (Ferreira et al. 2008; McInnes et al. 2010; Paparini et al. 2014), ensuring that many sequences are available for comparison from public databases.

Based on morphology, 14 *Trypanosoma* species from 19 chelonian species have been described, although some of them may be synonymous (Telford 2009). The first trypanosome reported in turtles was *T. damoniae* Laveran et Mesnil, 1902 in the Chinese pond turtle (*Mauremys reevesii*). However, a complete life cycle is known only for *T. balithaensis* Ray, 1987, *T. chrysemydis* Roudabush et Coatney, 1937 and *T. vittata* Robertson, 1908; aquatic leeches of genera *Glossiphonia*, *Helobdella* and *Placobdella* were confirmed as their vectors (Jefferson 1965; Ray 1987; Siddall and Desser 1992). Five *Trypanosoma* species parasitizing chelonians were reported in the Afrotropical realm - *T. pontyi* Bouet, 1909, *T. leroyi* Commes, 1919, *T. neitzi* Travassos Santos Dias, 1952*, T. sheppardi* Travassos Santos Dias, 1952 and *T. mocambicum* Pienaar, 1962. These species have not been reported since their original descriptions were published. Paperna (1989) found a trypanosome in the peripheral blood of *Pelusios sinuatus* and in the foregut of the leech *Placobdella multistrigata*, but did not provide full morphological data. The original descriptions of turtle trypanosomes often include host species, locality, prevalence, and drawings of bloodstream forms with inadequate morphometric data. Without using molecular genetic methods, estimation of prevalence could be misleading because of the frequently low parasitaemia (Gibson 2003). To date, sequence data of turtle trypanosomes are available only for *T. chelodinae* Johnston, 1907 from Australia (Jakes et al. 2001).

African side-neck turtles of the family Pelomedusidae Cope, 1868 include more than 20 currently recognized species of two genera – *Pelomedusa* Wagler, 1830 and *Pelusios* Wagler, 1830 (for recent taxonomy of the family Pelomedusidae used in this paper see Fritz and Havaš 2007, 2013; Petzold et al. 2014). Members of both genera are freshwater turtles living in a variety of water habitats from rainforest to open savannah of the Afrotropic zoogeographic realm (Fritz et al. 2011; Fritz and Havaš 2007).

In this paper, we examined samples from twelve African side-neck turtle species originating from sub-Saharan Africa for the presence of *Trypanosoma* species. We evaluated their host specificity, genetic diversity and phylogenetic relationships with other *Trypanosoma* species on the basis of SSU rRNA gene sequences. Additionally, morphological characteristics were compared with published data of known species from the Afrotropic zoogeographic region.

Results

Morphology of Endogenous Stages

A total of 49 hemoflagellates were detected by microscopic examination in 11 out of 94 (11.7%) peripheral blood films. Nine adult specimens of *Pelusios upembae*, one of *P. bechuanicus* and one