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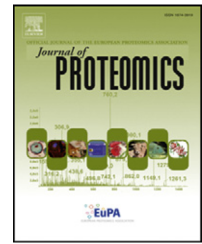

The *Trypanosoma rangeli* trypomastigote surfaceome reveals novel proteins and targets for specific diagnosis

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Sympatric distribution and sharing of hosts and antigens by *Trypanosoma rangeli* and *Trypanosoma cruzi*, the etiological agent of Chagas' disease, often incur in misdiagnosis and improper epidemiological inferences. Many secreted and surface proteins (SP) have been described as important antigens shared by these species. This work describes the *T. rangeli* surfaceome obtained by gel-free (LC–ESI-MS/MS) and gel-based (GeLC–ESI-MS/MS) proteomic approaches, and immunoblotting analyses and the comparison of these SP with *T. cruzi*. A total of 138 *T. rangeli* proteins and 343 *T. cruzi* proteins were obtained, among which, 42 and 157 proteins were exclusively identified in *T. rangeli* or *T. cruzi* trypomastigotes, respectively. Immunoblotting assays using sera from experimentally infected mice revealed a distinct band pattern for each species. MS/MS analysis of *T. rangeli* exclusive bands revealed two unique GP63-related proteins and flagellar calcium-binding protein. Also, a ~32 kDa band composed of 12 distinct proteins was exclusively recognized by anti-*T. cruzi* serum. This highly sensitive proteomic assessment of surface proteins characterized the *T. rangeli* surfaceome, revealing several differences and similarities between these two parasites. The study reports new *T. rangeli*-specific proteins with promising use in differential diagnosis from *T. cruzi*.

Biological significance
In this manuscript, we report the first proteomic analysis of the *T. rangeli* surface (surfaceome), a non-pathogenic parasite occurring in sympatry with *T. cruzi*, the etiological agent of Chagas disease. This comparative proteomic analysis was performed using high-throughput in-gel and gel-free proteomic approaches combined with immunoblotting, allowing us to identify new *T. rangeli*-specific proteins with promising use in differential serodiagnosis, among several other protein not previously reported for this taxon. Additionally, cross-recognition assays showed that *T. cruzi* surface proteins were recognized by heterologous serum (anti-*T. rangeli*) that strengthens the possibility of misdiagnosis of Chagas disease in humans and other mammals. Thus, this work provides new insights to understand the serological cross-reactivity between

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T. cruzi and *T. rangeli*, as well as, the identification of targets for specific *T. rangeli* diagnosis as revealed by the comparative surfaceome analysis. We strongly believe that this research is of importance to the readers of *Journal of Proteomics* since it provides new potential markers for diagnosis of both *T. cruzi* and *T. rangeli* parasites increasing the spectrum of specific targets for unambiguous diagnosis of *T. rangeli* and *T. cruzi* infections, besides describing new approaches to assess the trypanosomatids proteome.

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1. Introduction

Trypanosoma (*Herpetosoma*) *rangeli* Tejera 1920 is a non-pathogenic hemoflagellate parasite widely distributed throughout Central and South America [1]. *T. rangeli* shares triatomine vectors and mammalian hosts, including humans, with *Trypanosoma* (*Schyzotrypanum*) *cruzi*, the etiological agent of Chagas' Disease [2,3]. Chagas' disease, or American trypanosomiasis, is a major public health problem in Central and South America, where ~10 million people are infected with *T. cruzi* and approximately 25 million people are living at risk for infection [4]. Human infections caused by *T. rangeli* have been described in endemic areas where Chagas' disease exists [5]. The infection patterns indicate the possibility of single and/or mixed infections with *T. cruzi* [6], a situation that could potentially lead to misdiagnosis.

As a result of the overlapping geographical distribution and the sharing of vectors, hosts, and antigens [1], researchers have proposed specific DNA markers to differentiate *T. rangeli* and *T. cruzi* [7,8]. However, the majority of the tested DNA markers were unable to detect the parasites in chronic infections. Therefore, researchers have suggested the use of species-specific proteins as potential biomarkers for differential diagnosis [9,10]. Consequently, the occurrence of serological cross-reactivity in diagnostic assays has been demonstrated, since soluble proteins in both *T. cruzi* and *T. rangeli* epimastigotes are commonly used as antigens and share epitopes [11–13]. On the other hand, the use of trypomastigote forms as a substitute for epimastigotes reduces the cross-reactivity, suggesting that the recognition of *T. rangeli* by antibodies from chagasic patients is phase-dependent [14]. Therefore, the discovery of *T. cruzi* and *T. rangeli* trypomastigote-specific antigens could improve the specificity of serological diagnosis and reduce misdiagnosis of Chagas' disease [15].

Surface proteins (SPs) are involved in different tasks, including recognition, adhesion, and/or penetration into host cells, in addition to regulation of nutrient transport and cell signaling, making these proteins important virulence factors [16,17]. More importantly, SPs are major targets for serodiagnosis because of their potential to induce host immune response [18]. The glycosylphosphatidylinositol (GPI)-anchored proteins are the most abundant glycoconjugated proteins on the surface of such pathogenic trypanosomatids as *T. brucei* [19], *T. cruzi* [20,21], and *Leishmania major* [22]. For example, in *T. brucei*, the variant surface glycoproteins (VSG) play an important role in the parasite's mechanism to evade the host immune system; moreover, several *T. cruzi* GPI-anchored proteins are involved in the adhesion to and invasion of host cells [23], while in *Leishmania*, these proteins are reported to promote the promastigote complement-mediated lysis resistance, inducing macrophage phagocytosis and amastigote survival in macrophage phagolysosomes [24].

In *T. cruzi*, several GPI-anchored proteins are categorized into main families such as mucins (TcMUC and TcSMUG families), *trans*-sialidases (TS) (e.g., gp90, gp85 and gp82), mucin-associated proteins (MASP), metalloproteases (e.g., gp63), amastin-like and mucin-like proteins [20,21,25–30]. So far, only a few homologous genes to these *T. cruzi* GPI-anchored proteins have been identified in the *T. rangeli* genome, some of which have transcriptomic data to support the findings [31,32].

While mucins and MASP have not been described in *T. rangeli* [33], its sialidase (TrSial) [34] is a protein homologous to the *T. cruzi trans*-sialidase. However, TrSial does not have the ability to translocate the sialic acid from the host cell surface to the parasite surface [35]. Translocation is a key factor in parasite attachment to and penetration of host cells. Another member of the *trans*-sialidase family described for *T. rangeli* is the 85 kDa glycoprotein (gp85), a protein required for the establishment of the infection on triatomine [36,37].

The trypanosome SP (surfaceome) is important for parasite biology, for known protein variability, and for immunogenic potential [28]. We therefore performed a comprehensive comparative proteomic study of *T. rangeli* and *T. cruzi* surfaceomes. This first high-throughput *T. rangeli* proteomic analysis, conducted with gel-free (LC–ESI-MS/MS) and gel-based (GeLC–ESI-MS/MS) proteomic approaches, resulted in the identification of several novel *T. rangeli* proteins. Furthermore, immunoblotting assays revealed potential clinical diagnostic markers, that may increase the spectrum of specific targets for unambiguous diagnosis of *T. rangeli* and *T. cruzi* infections.

2. Material and methods

2.1. Parasites

T. rangeli (Choachí strain) and *T. cruzi* (Y strain) epimastigotes were cultivated in LIT medium, supplemented with 10% fetal calf serum (FCS) at 27 °C after cyclic passages in mice-triatomine-mice. In vitro-derived *T. rangeli* trypomastigotes, were grown as described by Koerich et al. [38], with minor modifications. Briefly, 1.2×10^8 epimastigotes were harvested in the exponential growth phase. They were washed three times with PBS (1500 ×*g*) at room temperature (RT) and then transferred to DMEM medium (pH 8.0) that contained 5% FCS and 6 mM L-glutamine. After eight days at 27 °C, we collected approximately 1×10^9 parasites (98% representing trypomastigotes), which were washed as described above and stored at −80 °C until use.

Cell-derived *T. cruzi* trypomastigotes were obtained as previously described [39]. Briefly, semi-confluent Vero cell

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