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Diversity of Trypanosomatids (Kinetoplastea: Trypanosomatidae) Parasitizing Fleas (Insecta: Siphonaptera) and Description of a New Genus *Blechomonas* gen. n.

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To further investigate the diversity of Trypanosomatidae we have examined the species present within the flea (Siphonaptera) population in the Czech Republic. Out of 1549 fleas, 239 were found to be infected by trypanosomatids. Axenic cultures were established from 90 infected specimens and 29 of them were further characterized. Molecular phylogenetic analysis of the SL RNA, gGAPDH, and SSU rRNA genes revealed a striking diversity within this group and analyzed isolates were classified into 16 Typing units (TUs) of which 15 typified new species. In addition to one *Trypanosoma* species, two TUs grouped within the sub-family Leishmaniinae, two clustered together with *Herpetomonas*, wheras 11 TUs formed a novel clade branching off between *Trypanosoma* spp. and remaining trypanosomatids. We propose to recognize this clade as a new genus *Blechomonas* and a new subfamily Blechomonadinae,

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Abbreviations: gGAPDH, glycosomal glyceraldehyde-3-phosphate dehydrogenase; kDNA, kinetoplast DNA; SL, spliced leader RNA gene repeats; SSU, small subunit ribosomal RNA; TU, typing unit.

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and provide molecular and morphological description of 11 TUs representing this genus. Our finding of such an ancient host-specific group sheds new light at the origin of Trypanosomatidae and the roots of dixenous parasitism. The strict host restriction of *Blechomonas* to Siphonaptera with adult fleas' dependence on blood meal may reflect passing of parasites from larvae through pupae to adults and implies potential transmission to the warm-blooded vertebrates.

Key words: Trypanosomatidae; phylogeny; Siphonaptera; *Blechomonas*; host specificity; co-evolution.

Introduction

Monoxenous (=one host) trypanosomatids constitute a very large and diverse group of protists of the family Trypanosomatidae Doflein, 1901. Prevalence of these intestinal parasites exceeds 20% in some insect host populations (Maslov et al. 2007; Votýpka et al. 2010, 2012b) Also united in this family are dixenous (=two hosts) flagellates of the genera *Trypanosoma, Leishmania* and *Phytomonas*, causative agents of serious diseases in vertebrates including humans, and plants (Maslov et al. 2001). Dixenous parasites spend a considerable part of their life cycle in the insect host which is utilized as a vector for their transmission (Schaub 2006). For many years monoxenous species were contemplated as dull cousins of the dixenous trypanosomatids, but recently they started to attract attention due to the extensive biological diversity, virtual omnipresence and potential impact on their insect hosts (Maslov et al. 2013).

The enduring taxonomical system of Trypanosomatidae was established about 50 years ago based on morphology and differences in life cycle traits (Hoare 1966; Wallace 1966). However molecular techniques have unambiguously proven this system to be artificial (Borghesan et al. 2013; Merzlyak et al. 2001; Votýpka et al. 2010; Yurchenko et al. 2008). Recent analyses also demonstrated that for most trypanosomatid species morphological features cannot be used for taxonomical purposes as they widely vary within a given population or even differ in the invertebrate host and the respective axenic culture (Votýpka et al. 2012b; Yurchenko et al. 2006a; Zídková et al. 2010). Correspondingly, the taxonomy of Trypanosomatidae is being redefined using molecular data (Maslov et al. 2013).

The set of genetic markers routinely used for molecular phylogenetic reconstructions of kinetoplastid flagellates is based on the small subunit ribosomal RNA (SSU rRNA) (Hollar et al. 1998; Maslov et al. 1996), the glycosomal glyceraldehyde-3-phosphate dehydrogenase (gGAPDH) genes (Yurchenko et al. 2006a) and the spliced leader (SL) RNA gene repeats (Croan et al. 1997; Maslov et al. 2007; Westenberger et al. 2004; Yurchenko et al. 2000) but can also include other genetic elements (Yurchenko et al. 2000). The first two markers are instrumental in revealing the major subdivisions within the Trypanosomatidae but fail to resolve inter- and intra-species relationships among and within these groups (Noyes et al. 2002a). The SL RNA gene repeat combines conserved and variable sequences, including the hypervariable intergenic region useful as a high-resolution molecular marker for phylogenetic reconstruction. Due to the dearth of information resulting from its small size, the SL RNA gene is not suitable for addressing relationships between phylogenetically distant groups, but may serve for barcoding organisms at the level of species or populations. This nucleotide sequence-based approach allows substitution of a species with its operational proxy – a typing unit (TU), representing potential new species. Based on previous studies of different *Leishmania* and other Leishmaniinae species, the threshold of 90% sequence similarity was set up to distinguish individual TUs (Maslov et al. 2007; Votýpka et al. 2012a).

The SL-based approach has been tested in a series of studies addressing diversity and host-parasite relationships of insect Trypanosomatidae in true bugs – Heteroptera (Maslov et al. 2010, 2013; Votýpka et al. 2010, 2012b; Yurchenko et al. 2006b) and flies – Diptera (Týč et al. 2013). As a result of these studies, many new TUs of trypanosomatids were described reflecting considerable diversity of these organisms. One of the major factors defining biodiversity is the number of available biological niches. The previously widely adopted "one host – one parasite" paradigm (Wallace 1966) is no longer applied for species description because many trypanosomatid species exhibit wide host specificity (Maslov et al. 2013; Votýpka et al. 2012b). Nevertheless, it is safe to postulate that host specificity still plays an important role in expounding biodiversity, as the parasite must evolve a molecular fit to its host to establish a stable infection. Prominent examples of high