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TEM microscopy confirms that parasitic protist *Leptomonas pyrrhocoris* does not tolerate elevated temperature conditions

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Trypanosomatids are single-celled obligatory parasites. They can have one host (monoxenous) or switch between different hosts during life cycle (dixenous). Second host is usually a vertebrate, which above all else requires the thermal tolerance. Dixenous species evolved mechanisms to infect the warm-blooded host and cell morphology is intimately linked to the host change [1]. Complex genetics is involved in such transformation, which is still a subject of study. But what is even more intriguing monoxenous trypanosomatids have been reported as co-infecting agents which can at least survive in warm-blooded host [2,3]. Taking into account that transition to dixenous parasitism has happened independently at least 3 times during evolution of *Trypanosomatidae*, it is extremely interesting to catch the early stages of this process in monoxenous kins [4,5]. Of note, monoxenous species comprise the majority of trypanosomatids [6].

Previously it was shown that monoxenous trypanosomatid *Leptomonas seymouri*, which is often recovered from co-infections in leishmaniasis patients, can tolerate elevated temperatures (35^{o}C) and parasite's cells undergo minor morphological changes. In contrast, high temperature conditions inhibited growth of closely related *Leptomonas pyrrhocoris* [7].

In current work we complemented comparative genomics analysis with transmission electron microscopy (TEM) to prove that *Leptomonas pyrrhocoris* cells do not tolerate elevated temperature growth conditions. Cells were grown in three different nutrient-rich media (not repeating exactly the experimental conditions used here [7]), but lost their viability after two days cultivation in elevated temperature conditions (33^{o}C, light microscopy showed no moving or dividing cells). We prepared samples for TEM from cells taken from LB + 0.5 BHI + mixture of vitamins medium (in which cell mobility was retained for the longest period of time) and precisely investigated the possible morphological changes including cell shape, flagella length, kinetoplast DNA localization. All these structures are usually dramatically altered when dixenous parasite switched to warm-blooded host. Few examples of microphotographs for culture in normal (24^{o}C) and high (33^{o}C) temperature conditions are present on Figure 1. We did not detect any difference in any of parameters listed above, but confirmed lots of cells with disrupted cell membrane in 33^{o}C sample. These data suggest that in contrast to *L. seymouri*, *L. pyrrhocoris* cells do not display any signs of morphological transition.

We traced orthologs of 324 genes that are up- or down-regulated in *L. seymuori* [7] cells in high temperature conditions. We found only 181 direct orthologs in *L. pyrrhocoris* genome and this number is significantly lower that an average expected number of orthologs found for random sample of 324 genes (1000 bootstrap replications, average 231 orthologous genes). This result suggests that *Leptomonas pyrrhocoris* lacks significant portion of genes involved in thermal tolerance in *L. seymouri*, while in other aspects these species are rather closely related.

These findings can point out possible genes involved in morphological alterations of cells that underlay the adaptation of dixenous species to warm-blooded hosts.

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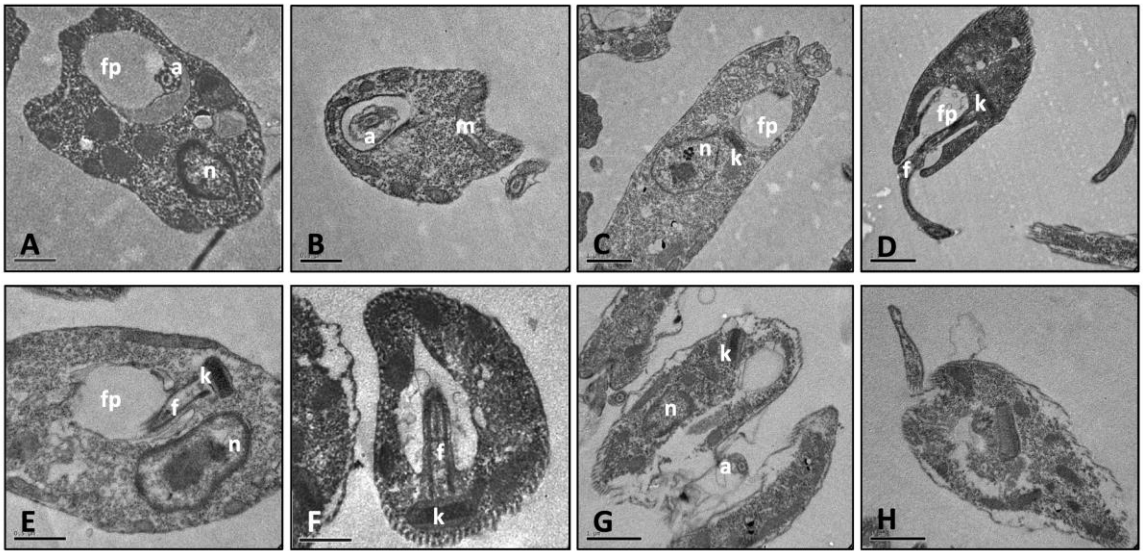

Figure 1. Transmission electron microscopy of *Leptomonas pyrrhocoris* cells in different temperature conditions. Cells were grown under normal conditions at 24°C (A-D) and under elevated temperature conditions at 33°C before fixation for 2 days (E-H). The longitudinal sections reveal typical features of trypanosomatid such as nucleus (n), kinetoplast (k), mitochondrion (m), flagellum (f), flagellar pocket (fp) and axoneme (a).

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