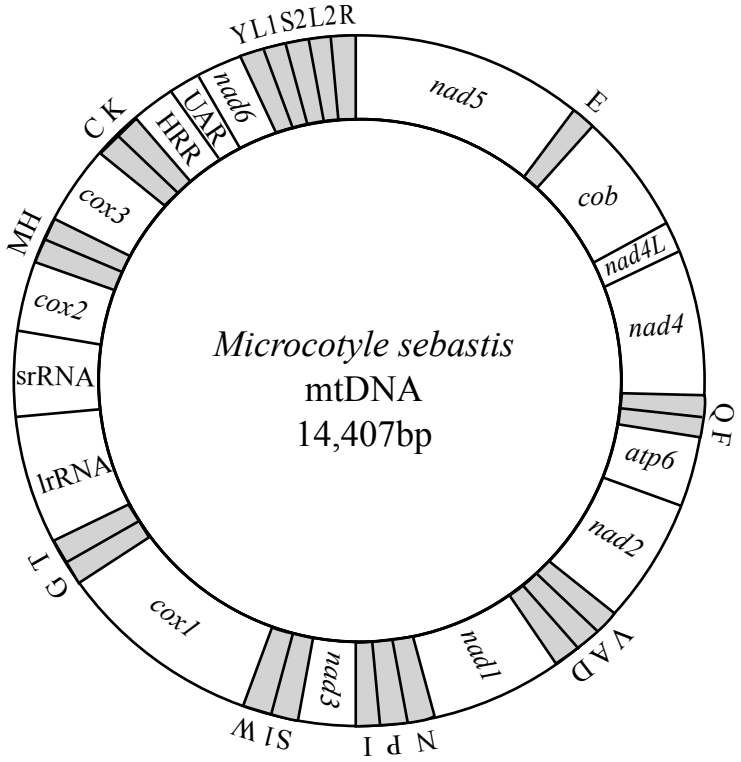**Additional file 1**