The use of DNA barcode to identifying Sarcophagidae species (Diptera, Muscomorpha) of forensic importance from Brazil

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INTRODUCTION

Many Sarcophagidae species (Diptera, Muscomorpha) become important in forensic field due to their biology and behavior [1]. However, identification of immature forms is difficult and, in some cases, also of adults, due to diagnostic characters restrict to male genitalia and the scarcity of taxonomic keys [2]. The use of molecular markers rises as a helpful alternative to solve this kind of taxonomical issues [3].

So, we tested the viability of the use of cytochrome oxidase I (COI) DNA barcode for the identification of eight flesh fly species commonly found in Brazil.

MATERIAL AND METHODS

Species of *Oxysarcodexia avuncula* (Lopes, 1933), *O. paulistanensis* (Mattos, 1919), *O. riograndensis* (Lopes, 1946), *O. thornax* (Walker, 1849), *P.* (*Sarcodexia*) *lambens* (Wiedemann, 1830), *P.* (*Pattonella*) *resona* (Lopes, 1935), *P.* (*Squamatodes*) *ingens* (Walker, 1849), and *Microcerella halli* (Engel, 1931) (Diptera: Sarcophagidae) were collected in natural areas of Campinas and Botucatu, municipalities of São Paulo state, Brazil. Morphological identification of males were performed based on taxonomical keys [2; 4].

Genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen). COI region was amplified using primers proposed by Folmer et al. (1994), PCRs were made with final volume of 25µl and purified using QIAquick PCR Purification Kit (Qiagen). Samples were sequenced by *Dye terminator* method.

Sequences were edited manually and aligned using Bioedit software. PAUP software was used to calculate a distance matrix under the evolutionary model Kimura 2 parameters and construct a phylogenetic tree by Neighbor-Joining method with 1,000 bootstrap replications to calculate branches confidence values.

RESULTS AND DISCUSSION

Final consensus sequences of 665 base pairs were obtained.

Intraspecific divergence (less than 3%) was smaller than the interspecific (varying between 8 and 15%) (Fig. 1). According to the distance matrix, an efficient species identification could be achieved using COI molecular marker.

Support values found for phylogenetic tree branches (Fig. 2) were higher than 50%. Haplotype variation in *O. thornax* and *O. paulistanensis* species suggests extensive diversity within these taxa, whereas *P.* (*P.*) *resona* species seems taxonomic well defined.

Our study must be expanded in order to obtain the best knowledge of haplotype diversity and to ensure the efficiency of identification of flesh flies using DNA Barcoding.

FINANCIAL SUPPORT

CAPES

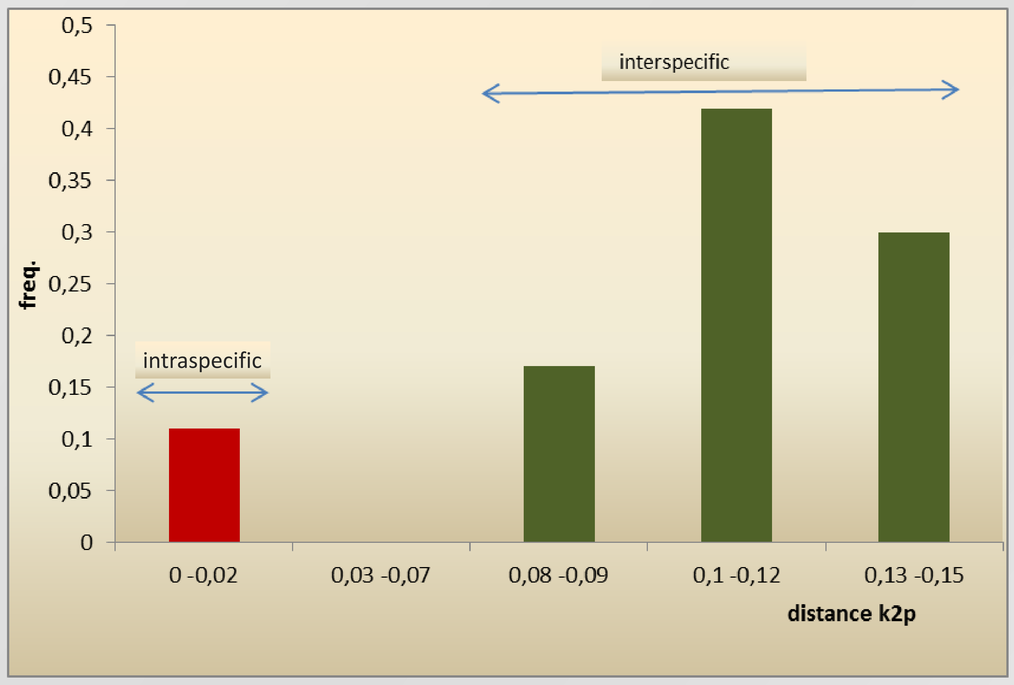
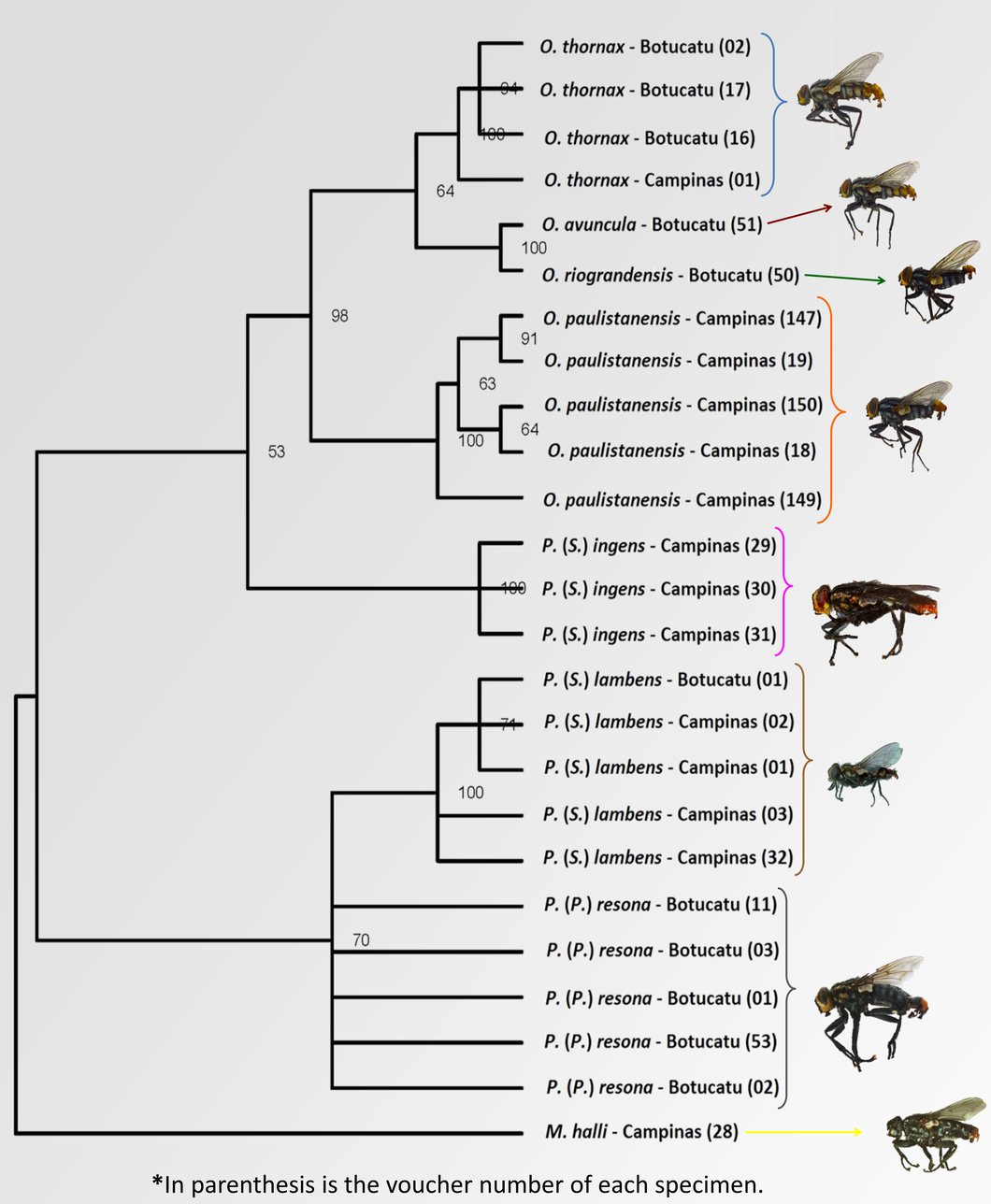


Figure 1. Intra and interspecific distance obtained of a distance matrix calculated under Kimura 2 parameters evolutionary model for *M. halli*, *O. thornax*, *O. paulistanensis*, *P.* (*P.*) *resona*, *P.* (*S.*) *lambens* and *P.* (*S.*) *ingens* species.



*In parenthesis is the voucher number of each specimen.

Figure 2. Phylogenetic tree constructed using PAUP software under the evolutionary model Kimura 2 parameters and Neighbor-Joining method with 1,000 bootstrap replications for branches confidence values.

REFERENCES

[1] Byrd, JH; Castner, JL. 2001. Insects of Forensic Importance. *In: Forensic Entomology* – The utility of arthropods in legal investigations. CRC Press, USA, pp. 43-80.

[2] Carvalho CJB & Mello-Patiu CA. 2008. Key to the adults of the most common forensic species of Diptera in South America. *Rev Bras Entomol* 52: 390–406.

[3] Wells, JD; Stevens JR. 2008. Application of DNA-based methods in forensic entomology. *Annu Rev Entomol* 2008. 53:103–120.

[4] Vairo KP; Mello-Patiu CA; Carvalho CJB. 2011. Pictorial identification key for species of Sarcophagidae (Diptera) of potential forensic importance in southern Brazil. *Rev Bras Entomol* 55: 333–347.