Co-amplification of Hymenoptera parasitoids' sequences during DNA Barcoding assessments of mycophagous drosophilids.

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Introduction

Drosophilidae have been the target of several studies in many areas of biological knowledge, mainly due to the performance of *Drosophila melanogaster* Meigen, 1830:85 as an effective model organism (Jennings, 2011). However, some basic aspects of the biology of the family remain unknown. One of these topics concerns the frequency of parasitism by Hymenoptera species (Fernandes *et al.*, 2009). In fact, few studies described the association of drosophilids with Hymenoptera parasitoids (Wertheim *et al.*, 2000; Marchiori *et al.*, 2003; Guimarães *et al.*, 2004; Yorozuya, 2006; Fernandes *et al.*, 2009). Fernandes *et al.* (2009), for example, found *Ganaspis* sp. (Figitidae, Eucoilinae) parasitizing an unidentified species of frugivorous drosophilids, that was hypothesized to be *Zaprianus indianus* Gupta, 1970 due to the length of the pupae. In addition, Wertheim *et al.* (2000) identified 673 individuals of hymenopteran drosophilids' parasitoids associated with 9,305 specimens of Drosophilidae in mushroom emergence evaluations.

Trying to enhance studies with mycophagous drosophilids, Machado *et al.* (2017) tested and proved the effectiveness of DNA Barcoding as an important way to identify and discover species in this poorly explored Drosophilidae group. In that study, the authors analyzed cytochrome oxidase subunit I (COI) sequences of 218 mycophagous drosophilids collected at 20 sites in the Brazilian Amazonian, Atlantic Forest, and Pampa Biomes, and revealed a high number of potential new species, some of which have already been described (Junges *et al.*, 2016). However, an unexpected result obtained by Machado *et al.* (2017) refers to the co-extraction and co-amplification of Hymenoptera parasitoids' DNA sequences. So, the aim of the present study is to report the presence of Hymenoptera parasitoids associated to at least two individuals of the obligatory mycophagous species *Mycodrosophila projectans* (Sturtevant, 1916).

Materials and Methods

Adult flies were first collected from macroscopic fungi fruiting bodies using an entomological aspirator (Machado *et al.*, 2014) and then fixed and stored in absolute alcohol. The two individuals whose DNA Barcoding sequences indicated the presence of Hymenoptera parasitoids were collected in Floresta Nacional de Caxiuanã, in Melgaço city (coordinates -51.457129, -1.737762), and in Horto Botânico UFPel, in Pelotas city (coordinates -52.431944, -31.816111), both located in the Brazilian Amazonian and Pampa biomes, respectively. The flies were identified as *M. projectans* based in male genitalia (Wheeler and Takada, 1963).

Total DNA was extracted using phenol-chloroform protocol (Sassi *et al.*, 2005), and COI fragments were amplified using primers HCO1490 (Folmer *et al.*, 1994) and C1N2329 (Simon *et al.*, 1994) or LCO2198 (Folmer *et al.*, 1994). The PCR products were purified and directly sequenced using an ABI 3730XLs automatic sequencer with the same primers used in PCR. The electropherograms were assembled using the Staden Package Gap 4 program (Staden, 1996), and each contig was checked for adequate sequence quality. To confirm species DNA identity, Nucleotide Blast (Altschul *et al.*, 1990) searches were performed using Gap4 consensus sequences as queries. Identity inferences were based on the returned hit with maximum score value, whose E-value and percentage of identity was taken as an indication of identification accuracy. The

obtained result was further confirmed through a search performed in the Bold system (Ratnasingham and Hebert, 2007), whose system is interconnected with GenBank. The sequences were deposited in Genbank under the numbers MF615211 and MF615212.

Results and Discussion

Due to the low number of Hymenoptera parasitoids' DNA sequences deposited in GenBank and Bold systems, accurate identification of the parasitoid species was not possible. In this sense, despite the divergence of 13% between both parasitoid sequences, Blast tool recovered for them a maximum identity of 87% to the same *Hymenoptera* sp. (GenBank access number JF271365.1) from Papua New Guinea, which is a parasitoid of caterpillars (Lepidoptera) (Hrcek *et al.*, 2011). Given that COI is the animal molecular marker most frequently used (Pentinsaari *et al.*, 2016), this result highlights the poor knowledge related to Diptera parasitoids taken as a whole.

Moreover, our results also suggest an apparent low infection rate among wild caught mycophagous drosophilids, given that only two cases of infection were detected among more than 200 analyzed specimens. Considering that some other infections may not have been adequately detected by our indirect approach, this infection rate is in agreement with other studies addressing this subject in mycophagous drosophilids. In this sense, Wertheim *et al.* (2000) reported for this group a Hymenoptera infection rate of 8% in Dutch woodland, and Yorozuya (2006) found for them a constant annual parasitism rate ranging between 6.7% and 9.8% in Japan. Nevertheless, this last author observed some seasonal variations in these values.

Interestingly, Fernandes *et al.* (2009) found a similar infection rate [8.3% - by *Ganaspis* sp. (Figitidae, Eucoilinae)] among frugivorous drosophilids colonizing fruits of *Coffea arabica* in Brazil. This suggests that the low infection rate is probably a characteristic of the family, independent of the resource explored, although some species could be more prone to parasitoid infection than others. In this sense, among the 54 different species which had their COI sequences characterized by Machado *et al.* (2017), the two cases of infection were detected for *M. projectans*, in populations distant by more than 3,000 kilometers. Thus, this report calls attention to the need for more studies in this unexplored group, whose diversity is largely misunderstood and whose impact in drosophilids dynamics must be further evaluated.

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